STUDIES IN THE

Osteopathic Sciences

Cells of the Blood

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STUDIES IN THE OSTEOPATHIC SCIENCES

Volume I. Basic Principles

Volume II. The Nerve Centers

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PREFACE TO THE FOURTH VOLUME

This group of books is devoted to the discussion of such subjects as seem to be of especial significance in osteopathic diagnosis and therapy. The importance of a good circulation of good blood in the preservation of health and in recovery after injury has been emphasized from the first teachings of Dr. A. T. Still to the present time. Since Harvey first proved the circulation of the blood there has been a constantly increasing appreciation of the importance of this fluid in physiological economy. More definite knowledge of the cells of the blood during normal and abnormal conditions is, therefore, greatly to be desired.

Very early in these studies it became evident that the problems presented by the changes in the blood cells under abnormal conditions are associated with various ontogenetic and phylogenetic problems of the blood cells. The cells of the blood of human subjects through embryological, fetal, immature, adult and senile periods were studied and were compared with the cells of the blood under various abnormal conditions of the human race. Similar studies were made of the blood cells of fetal, immature, adult and senile rabbits, white rats, cats, dogs and guinea pigs, and these findings compared with those secured by human studies. The studies made for animals were normal when this was desired, but the studies made for human embryos and fetuses were, of course, nearly always somewhat abnormal in some respect. Studies were made of the cells of the blood of all the other common genera of domestic, laboratory and wild animals of Southern California and in many cases several periods of life were studied for these animals.

During the years from 1903-1914 the blood cells of patients in the clinics of The Pacific College of Osteopathy were examined as a part of their routine examinations. During these years specimens of normal human blood and of various laboratory animals were studied in the laboratories of that college as a part of the educational program. During the years 1914-1930 the cells of the blood of patients examined in the clinical laboratories of The A. T. Still Research Institute have been subjected to careful study and the animals in the experimental laboratories of the Institute have had blood examinations at various periods of life and during the progress of the abnormal conditions associated with various bony lesions. More than twenty-six thousand records of these findings have been studied in the preparation of this book. In all this work the greatest emphasis has been placed upon osteopathic relations. Very much work remains to be done before the

problems presented by the variations in the structure of the blood cells can be solved, but this report is made at this time because much of the information secured has a useful place in the osteopathic sciences.

This work has been made possible only by the cordial co-operation and support of the Trustees and the staff of The A. T. Still Research Institute and of The Pacific College of Osteopathy, and by the helpfulness of many osteopathic practitioners in Chicago and Los Angeles. The facilities offered by the Bondies Sanitarium, in South Pasadena, have been especially valuable in securing blood specimens from patients under controlled conditions. Much of this work has been possible only because of gifts of money from the Osteopathic Women's National Association, the California Osteopathic Association, the Women's Osteopathic Club of Los Angeles, the Delta Omega Sorority and the constant generosity of the American Osteopathic Association.

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CHAPTER I

EFFECTS OF BONY LESIONS

The direct effects of bony lesions on blood cells are chiefly due to changes in the red bone marrow. Since the ribs include the largest area of red bone marrow in the body, lesions of the ribs and the thoracic vertebrae cause more marked changes in the development of the erythrocytes and the granular leucocytes then do lesions elsewhere in the body. Some review of the structural relations is necessary for adequate discussion of the relation of the lesions mentioned and the development of blood cells.

Structural Relations

The cells concerned in hematopoiesis are described in the chapters on the development of erythrocytes, neutrophiles and other granular leucocytes in this book. The innervation of the red bone marrow is of interest in this connection. The nutrient arteries, veins and nerves enter together at the nutrient foramina of the bones. The vaso-motor nerves are derived from the lateral chain of sympathetic ganglia, and the cells of these are controlled by the nerve centers in the lateral horn or homologous areas of the spinal cord. The segment from which the vaso-motor control of any given bone is governed is that segment most closely associated with the anlage of the bone during early embryonic development. Since there is considerable shifting of the embryonic skeletal structures and since the metameric nerve relations are maintained during the shifting the adult innervation of the bones presents many puzzling features. The problems are solved, however, by a study of the embryologic relations of bones, nerves and muscles.

Innervation of Red Bone Marrow

The vaso-motor nerves follow the blood vessels, chiefly the arteries and arterioles, to every ramification within the red bone marrow, and they terminate in nerve endings like those upon other blood vessels of the body. The endings are most abundant upon the arterioles, and are present less plentifully upon the venules, arteries, veins and capillaries in order. Another group of nerves terminates in fine, brush-like endings which branch freely among the hematopoietic cells of the red marrow. All these nerves are nonmedullated.

Medullated nerves are afferent, and are derived from the posterior root ganglia of the spinal segments from which the efferent nerves arise. The central prolongations of the cells of these ganglia enter the spinal

cord and are distributed to the gray matter of the posterior horn and the lateral horn, where this is present. Throughout the thoracic spinal cord the costal nerves are of especial interest since they innervate the areas of bone marrow of the ribs, the most important hematopoietic tissues of the body. The sensory nerves from the red bone marrow are not intimately connected with the brain centers, and very serious disease of bone marrow may be present without causing discomfort. After the disease has extended into the periosteum there is usually some dull pain in the affected bone. The functions of these sensory nerves are not well understood but it is certain that they are associated with the local vaso-motor centers and that the impulses carried into these centers from the bone marrow modify the circulation through all the tissues controlled by those centers.

Pathogenesis of Lesions

Lesions affect the functional relations of the hematopoietic tissues in much the same manner as that noted in the relations of bony lesions with the viscera generally. A lesion of a vertebra or a rib is a disturbed relationship of that bone with its fellows of such a nature that a persistent strain is produced without causing any actual rupture of ligaments. The position which the bones assume is that which may be normal under some circumstances; for example, the vertebrae have one relation with one another when the spinal column is flexed and another relationship when the spinal column is erect. If any two vertebrae maintain the relations normal to the flexed spinal column when the column has returned to its erect position, those two vertebrae are not in normal relations; this is a vertebral lesion. Ribs are approximated when the body is bent to one side; if any rib maintains that same position when the body returns to the erect position, that rib is lesioned. In either case there is a persistent strain upon the articular surfaces and the ligaments are subject to abnormal tension on one side, and to abnormal loosening on the other side of the joint.

When such a lesion is present the surrounding tissues become edematous; the tissue fluids become less alkaline than normal and there is a slight but persistent local congestion with some retention of the waste products of katabolism and some lack of normal oxidation processes of the cells of the immediate vicinity of the lesion. These changes are always present when a costal or vertebral lesion has been present for a few days and they remain present as long as the lesion remains uncorrected. The nerves passing through the intervertebral foramina are subjected to the abnormal pressure of the edematous tissues and to the effects of the abnormal tissue juices.

The sympathetic ganglia which lie near the costo-vertebral articulations are subject to these same pathogenic influencees. The non-medullated nerves leaving the sympathetic ganglia are somewhat more seriously affected than are the medullated nerve fibers, because the medullary sheaths of the latter protect the nerve fibers in some degree from pressure and from the tissue juices. The nerve fibers which enter the nutrient foramina of the bones, to be distributed to the blood vessels and to the hematopoietic cells of the red bone marrow, are subject to the effects produced by both the pressure of the edematous tissues and the abnormal chemical composition of the tissue fluids around the lesion. When the lesion is limited to one or two spinal segments the area of bone marrow affected may be small and only a few abnormal blood cells be produced. When lesions involve several segments or when the ribs fail to move properly in respiratory activity the blood contains many abnormal cells. The anemia thus produced may be slight or it may be profound, according to the manner in which other areas of red bone marrow in the flat bones, the small bones and the ends of the long bones compensate or fail to compensate for the costal hematopoietic deficiencies.

Any lesion of any bone in the body affects the circulation and the innervation of the red bone marrow of that bone and usually of one or two adjacent bones. A few abnormal blood cells are the result of such an abnormal state of the local red marrow, and it is possible to find abnormal cells in the blood of an individual with such lesions provided the blood smears are carefully studied. When the abnormal area of red bone marrow is very small the abnormal cells are few.

The bone marrow of the ribs of human subjects is subject to certain disturbances due to the habits of mankind.

Anemia of Civilization

It must be remembered that the nutrient blood vessels and nerves lie in a groove along the lower edge of each rib and that the largest nutrient foramen is near the angle of the rib. Animals walking on four legs have the ribs hanging downward from the spinal column, and as the animal walks or runs the spinal column and the ribs are moved back and forth gently; the intercostal tissues are constantly relaxed and there is rarely any abnormal pressure upon the nutrient vessels and nerves. Mankind walks on two legs and the spinal column is held more or less erectly. The ribs may be allowed to droop, in which case the intercostal tissues are subjected to some slight but persistent pressure. The thorax may be held erect, by means of muscular ac-

tivity along the spinal column, in which case also the intercostal tissues are subjected to some pressure. If the respiratory movements are normal alternate relaxation and contraction of the intercostal muscles secures fairly normal innervation and circulation of the red marrow of the ribs. But if the respiratory movements are impeded, so also is the circulation of the rib marrow.

Those members of the human race who exercise little or no self-control secure excellent circulation through the rib marrow by occasionally yawning, sobbing, laughing, panting, coughing, sneezing and by making many vigorous emotional respiratory movements. Persons who exercise freely also secure efficient respiratory activity and good circulation through the rib marrow. Persons who are restrained or repressed do not breathe deeply nor do they laugh, sob or yawn freely; if they do not secure respiratory exercise in some less natural manner they become anemic because of the lack of normal circulation through the rib marrow.

Neuroses and Anemia

Persons suffering from certain functional neuroses show abnormal thoracic rigidity with resultant anemia. The thoracic rigidity due to the neurosis or psychosis is very much like a lesion in its effects on the development of the blood cells. The tension of the intercostal tissues and the lack of respiratory movements leads to inefficient circulation and innervation of the red marrow with later development of typical costal anemia.

In these persons there are other pathogenic factors which increase the anemia indirectly. The rigid thorax fails to give the heart the normal stimulation due to variations in the intrathoracic pressure; a weakened heart muscle and low blood pressure are common results. The venous return to the heart is also impeded by the lack of the normal variations in intrathoracic pressure.

Inefficient diaphragmatic respiratory movements prevent the stimulation of the liver, the spleen and the pancreas due to the normal alternate contraction and relaxation of the diaphragm. Cholemia and increased rapidity of red cell destruction is a result of the abnormal hepatic condition thus caused. Toxemia and malnutrition are increased by the effects produced upon the stomach, spleen and pancreas by the diaphragmatic immobility and the blood cells are thus indirectly effected.

The conditions responsible for the functional neurosis may add other pathogenic factors to the anemia or not, but the respiratory inefficiency due to the neurosis itself must be considered an important factor in the anemia always present in these patients.

Specific Lesions and Their Effects on Blood Development

Certain local lesions exercise indirect effects upon the development of blood cells. Lesions of the tenth thoracic vertebra and of the tenth ribs affect the circulation of the blood through the liver and cause a slight but constant decrease in the tonicity of the muscular walls of the bile ducts. Lesions of the eighth, ninth and eleventh thoracic vertebrae and the corresponding ribs exercise a similar but somewhat variable influence upon the same tissues. As a result of the slight congestion and the slight edema always associated with these lesions together with the slight accumulation within the bile ducts, the bile passes into the venous blood and the lymph and thus into the general circulation. Bile pigments are slightly destructive to the blood cells, both red and white, and the bile salts are definitely destructive. Bile pigments may be recognized with sufficient accuracy for clinical purposes by the Gmelin test of the serum. Chemical tests for bile salts are difficult to make without using too great an amount of the blood, but their presence may be suspected when the surface tension of the blood plasma or the blood serum is lowered, and when bile pigments are known to be present. In such cases the erythrocytes are fragile and blood shadows may be found in the smears and on the warm stage. The protoplasm of the neutrophiles and the hyaline cells show frayed and irregular outlines. The eosinophiles do not show this effect. The nuclei are not so seriously affected and naked nuclei or masses of nuclear material without recognizable protoplasm are abundant in the blood smears.

Lesions Affecting the Thyroid Gland

The small basophilic or amphophilic cells, often called mast cells, are found in normal adult human blood in very small numbers; often none can be found in a differential count of five thousand cells or more, or one or two might be found in a count of five hundred cells. These are increased in certain forms of leukemia and other pathological conditions. They are also slightly increased as a result of any abnormal condition affecting the thyroid gland.

Bony lesions of the first or second thoracic vertebra and of the third cervical vertebra affect the circulation through the thyroid gland, cause an edema and diminished alkalinity of the tissue fluids of the gland, and increase the mast cells in the blood. These lesions and, occasionally, lesions of adjacent vertebrae, usually tend to increase basal metabolism, though hypothyroidism occasionally follows later. When mast cells are present in increased numbers or when only a few are present but these present evidences of immaturity of structure, a

determination of the basal metabolism of the patient is indicated. Correction of the lesions found on examination is followed by return of the basal metabolism to normal and by the disappearance of the excess of mast cells, if the thyroid pathology has not progressed to tissue destruction of considerable extent.

Bony Lesions Affecting Hyaline Cells

The hyaline cells show the effects of bony lesions by way of the changes due to the circulation through the spleen, so far as the lymphocytes are concerned, and by way of circulatory disturbances elsewhere in the body so far as the less common forms of hyaline cells are concerned.

Bony lesions affecting the spleen include the ninth thoracic vertebra and the ninth ribs, especially. The seventh, eighth and tenth thoracic vertebrae and the eighth and tenth ribs, especially on the left side, also affect the splenic circulation. When such lesions are present the blood smears show an increase in the number of large hyaline cells and of splenocytes. The large lymphocytes and the splenocytes are derived from lymphoid trabeculae and pulp. When such cells are found abundantly in the blood smears, with no evidence of leukemia or other gross pathology of the hematopoietic tissues, some abnormal circulatory condition of the spleen or of some other large area of lymphoid tissue is strongly suspected.

Pelvic Lesions and Eosinophiles

Eosinophiles of myelocytoid or immature structure are found in the blood stream whenever there is marked congestion of the ovaries or the testes. After sexual excitement such cells are normally present in the circulating blood; they disappear within twenty to forty hours. They appear in the blood of women at about the menstrual period. During adolescence and the climacteric they occasionally appear in the blood of either sex without evidence of any abnormal condition.

When there is any abnormal congestion of either testis or ovary these blood cells are found and they may be of importance in diagnosis.

Lesions of the eleventh thoracic or of the second lumbar vertebra cause a slight but persistent congestion of the reproductive glands. Myelocytoid eosinophiles are usually fairly abundant in the circulating blood under such circumstances. Correction of the lesion permits return to normal circulation and function of these glands, provided there has been no actual tissue destruction, and within a few weeks the peculiar eosinophiles are no longer found in the blood except under the physiological conditions mentioned above. (Plates V, VI)

Secondary anemias due to malnutrition are often due to bony lesions. Lesions of the fifth thoracic vertebra are especially associated with occasional hyperchlorhydria and a tendency to gastric ulcers; the anemia due to these conditions may be very severe. Lesions of the seventh and eighth thoracic vertebrae and the associated ribs are commonly associated with gastric atony, gastroptosis, and hypochlorhydria; the anemia due to these conditions is not usually so profound as that due to fifth thoracic lesions.

Anemia of Renal Origin

Lesions of the eleventh and twelfth thoracic vertebrae (and of the thirteenth and fourteenth thoracic, in animals) cause marked disturbance in the circulation and the secretion of the kidneys. The mild but persistent nephropathy thus produced causes a slowly developing secondary anemia which is very intractable until the lesion has been corrected. If the lesion has been present for more than a year, in laboratory animals, there is produced a permanent nephropathy. In human subjects the correction of the lesion within a few years after it has been produced results in an apparently complete recovery. It must be remembered that the human renal equipment is much greater than is necessary under normal and mildly abnormal circumstances. Any considerable amount of renal tissue capable of normal functioning is able to meet all ordinary and even quite heavy unusual demands upon the kidneys.

Summary

Bony lesions cause anemia directly, by affecting the circulation and innervation of the red bone marrow, and thus preventing normal blood cell regeneration.

Bony lesions cause anemia indirectly, by causing toxemia with resultant excessive blood cell destruction.

Bony lesions cause anemia indirectly also by interfering with normal nutrition and preventing normal blood cell regeneration.

CHAPTER II

ERYTHROCYTES

The word "erythrocyte" means "red cell," and these structures are often so called. By transmitted light, in thin layers, they are not red but a pale yellowish green tint. By reflected light, and in great masses, they appear a brilliant scarlet color, varying in tint according to several physical conditions.

Variations in the color of the red cells, and hence of the blood itself, are due to modifications in the chemical relations of the hemoglobin. Venous blood contains a greater amount of the purplish hemoglobin; arterial blood contains a greater amount of the more brilliant scarlet oxyhemoglobin. The peculiar cherry-like tint of the blood of persons who are suffering from severe acute carbon-monoxide poisoning is easily recognizable. A very much less pronounced cherry-like tint is present in the blood of persons who suffer from mild, chronic carbon-monoxide poisoning.

Functions

Adult human erythrocytes, or red blood cells, occupy a peculiar place in biology. They have no nuclei during their most important functional activity and they are unable to take up nutrient materials from their surrounding plasma or to give off katabolites while they are most active in carrying oxygen. They have neither motility nor power of reproduction. These cells consist only of a very delicate stroma which holds the hemoglobin in its meshes. Although the erythrocytes seem almost structureless they display some characteristics of living cells. They take up and give off oxygen with greater facility than can be explained on the supposition of a purely physical basis for their functional activities. Solutions of hemoglobin have not yet been made to perform the reactions with such facility as do the normal erythrocytes. The manner in which they may be affected by pathological conditions greatly resembles the manner in which these, or similar, abnormal states act upon other cells. It is true that all of these facts might be explained by the extreme delicacy of the stroma, the finely balanced relations of the osmotic tensions of cell and plasma, and other physical conditions. These considerations apply equally well to all forms of living structures. The erythrocyte is peculiar in that it performs its most useful function,—that of carrying oxygen,—after it has become, cytologically, a senile cell. Having lost its nucleus, its power of taking up nutrition and its ability to reproduce itself, it has left only its special activity,

due to its hemoglobin content, of taking up oxygen when the oxygen tension of its environment reaches a certain pressure, and giving off oxygen when the oxygen tension of its environment diminishes below a certain point.

In some lower animals the oxygen-carrying pigments are carried in solution in a circulating fluid. Red blood cells are present in the blood of all vertebrates except amphioxus, but not in the blood of invertebrates. Invertebrate blood cells occasionally show a trace of hemoglobin or of other oxygen-carrying pigments, but these are too few and the amount of pigment too scanty to give any tint to the blood or the other fluids of the invertebrate body, or of the amphioxus. The practice of carrying the oxygen-carrying pigment within circulating cells increases the efficiency of that pigment very greatly.

Structure

The form of erythrocytes has been rather exhaustively studied. When carefully examined upon a warm stage, under as nearly normal conditions as possible, they are seen to be shaped like a shallow bowl, with a very thick rim and a very thin bottom. The smear as ordinarily taken shows erythrocytes of the biconcave form usually described. At various times a cell wall or a peripheral limiting membrane has been described: this is a delicate condensation of the stroma. (Plate I)

When moving blood is watched under the microscope, especially on the warm stage, the erythrocytes are seen to change in shape remarkably. They elongate to a surprising extent, often attaining a length of more than twice their ordinary diameter in passing through very narrow places, and regaining their normal form immediately upon reaching a widened space upon the slide. This elasticity is due to the lipoid structure of the stroma and it facilitates diapedesis through the walls of the blood vessels and within the tissue spaces of the body.

The form of red cell found in human blood is the most efficient form known, so far as the transmission of oxygen is concerned. The factors which maintain this peculiar structure are not well understood. It is known that abnormal environmental conditions may so affect the red blood cell as to cause it to change shape very quickly.

A typical normal adult human erythrocyte has a diameter of about eight microns, with variations of about one micron in each direction. The constant variations in the reaction of the blood and its carbon dioxide content cause variations in the size and the form of the red cells. The thickness of the heavy rim is about 1.7 microns while the central area has a thickness of less than one micron. The surface area has been

estimated by several observers and the figures differ somewhat, as is to be expected since the form and size of the red cells differ according to so many conditions. The figures given for the surface area range between 98 square microns and 128 square microns.

The volume of an average normal adult erythrocyte is somewhat less than 100 cubic microns. Welcker estimated the volume as 72 cubic microns, Ponder as 110 and Wintrobe as 70 cubic microns.

The surface area of the blood cells provide about 4,000 square meters for an average human body and of this about 81 square meters of surface pass through the lungs each second. Evans computed 3,500 square meters and Bailey 4,500 square meters of erythrocyte surface for an average human adult blood volume.

Henderson estimated roughly, that a liter of normal adult human blood presents a two-phase system in which the blood cells form one phase of about 500 square meters area and about one micron in thickness, with the plasma as the other phase of about equal extent and thickness. The relations between cells and plasma are more easily visualized and the oxygen-carrying functions of the red cells more easily computed by considering the blood as a two-phase system than in any other method of study.

Individual Differences In Red Cells

The red cells vary slightly in different individuals, with no apparent relation to physiological conditions. Individuals who are developmentally imperfect have greater variation in the size and form of their red cells during health than do persons who have normal structure of body. Conspicuous abnormalities of structure are rare in healthy persons. Individuals with oval blood cells have been reported in several instances.

Illustrative Cases Reports

Mrs. L., patient in the obstetrical clinic of The Pacific College of Osteopathy had oval cells almost exclusively. Only occasionally was it possible to find a circular erythrocyte in her blood. The long axis was about one and one half times the short axis; the relation varied slightly. No nucleated forms were present. Seven blood examinations were made during four months, giving identical findings. She was in good health, passed through normal pregnancy and labor, bore a normal child and regained her strength as rapidly as is usual. Her hemoglobin varied slightly, from 82% to 88%, but she never showed any evidences of anemia. Her family history was excellent and the family records were complete for several generations, both direct and collateral. With the

good health common to nearly all members of the family there was no history of blood examinations having been made. The examinations were made twenty-two years before this history is written, and she is still in excellent health.

In one monkey (rhesus) in the Chicago laboratory of The A. T. Still Research Institute oval erythrocytes were found present in the peripheral blood in considerable numbers. This monkey was in as good health as monkeys ever are under artificial conditions. The cells resembled the cells of the patient whose history has just been given.

Climatic Variations

Races living in the tropical zone have red cells about 0.5 micron smaller than races living in temperate zones and they have slightly lower counts of red cells. Races living in very cold climates have red cells of about the same size as those of temperate zones, but their red cell count is somewhat higher.

The red cells vary in size and in form during the day, following variations in the reaction of the blood. The amount of hemoglobin in each cell does not vary, however. During activity and the diminished alkalinity of moderate fatigue, the red cells increase in size. Forced breathing with the associated slight increase in alkalinity causes the red cells to shrink slightly. Increase in the carbon dioxide content of the warm stage specimen causes cells previously shrunken to return to their normal size and form. Diluting fluids which are slightly more alkaline than normal blood cause the red cells to crenate more rapidly than normal, while diluting fluids less alkaline than the blood cause them to become more globular, then to swell.

At very low carbon dioxide tension this gas is carried almost exclusively by the blood plasma, and in such blood the cells crenate rapidly. With higher carbon dioxide tension the gas is carried by the plasma and the red cells in about equal amounts, and under such conditions the red blood cells do not crenate so rapidly. Resistance of red cells to lowered osmotic tension of the plasma, caused by adding water, is increased by raising the carbon dioxide tension. The form of the red cells depends to some extent upon carbon dioxide tension and this is, no doubt, the reason for so many different descriptions of these cells.

Experimentally, substances which dissolve the lipoids of the stroma cause the cells to assume forms which are almost or quite spherical. The presence of bile acids in the plasma of the circulating blood is frequently associated with the presence of spherical or spheroidal red blood cells.

Variations Due to Age

The erythrocytes of newly born healthy infants range from 3.3 to 10.0 microns in diameter, with an average of 8.6 microns. During childhood the cells become more and more nearly equal in size until the adult type is reached at the age of about fourteen years. Fetal blood shows even more marked variations in size than does infant's blood.

During childhood the red blood cells show extravagant variations in size and form upon relatively slight provocation, so that even mild cases of secondary anemia may occasionally cause the red blood cells to show the anisocytosis and megalocytosis characteristic of pernicious anemia if they should appear in adult blood. It is necessary to keep this characteristic of immature blood in mind, else erroneous diagnoses of pernicious and other very severe anemias may be made for children with secondary anemias.

In old age the red cells become somewhat smaller. This seems to be due to the increased alkalinity of senile blood.

Variations Due to Disease

Conditions which interfere with the nutrition of the red bone marrow cause the appearance of abnormal forms of red blood cells. These conditions include bony lesions, which interfere with the innervation of the red bone marrow and the circulation of the blood through it; disturbances in the nutrition of the entire body, as by starvation or by disease of the digestion tract; diseases which cause severe toxemia and other conditions which cause secondary anemia.

The abnormal forms found among the red cells in anemia are sometimes due to abnormal development of these cells, but are often due to their abnormal fragility. In certain forms of anemia the cells are so fragile that it is extremely difficult to prepare them for examination without injuring them, yet, if the cells are very carefully prepared, nearly all of them are found to be of normal size and form.

In the primary anemias the cells are often more stable in structure than they usually are in the blood of normal persons, and in certain forms of secondary anemia the resistance of the red cells to abnormal environmental conditions may be considerably increased.

Abnormal Endoglobular Structures and Artefacts

The red cells of normal adult human blood present only the simple structure described,—a fine and very delicate stroma of lipoids holding in its meshes the hemoglobin. The delicacy of this stroma permits its easy modification by various fixing agents and stains, so that many and

varied intracellular structures have been described. Immature and abnormal forms present rather complicated structures and these have been variously described by different observers.

Living red cells do not take stains. Fixed and dead adult red cells are acidophilic. Immature red cells and abnormal forms are often feebly basophilic.

Polychromatophilic or basophilic cells take basic stains. This term is not properly applied to basophilic reticulation. Basophilic red cells are usually larger than normal; have less marked concavity, and are often poikilocytes.

Very young red cells are strongly basophilic and usually show nuclear remnants. Degenerating red cells may become basophilic, as in severe anemias, especially when these occur in old people.

Golgi described a "reticulo-fibrillar apparatus" which he found in red cells stained by two methods, one using mercuric chloride with potassium bichromate, the other using gold chloride with picric and osmic acids. These methods produce a fibrillar or reticular structure. In our laboratories these appear very distinctly to be artefacts, produced by the action of these agents on the stroma of the red cells. Petrone used a lead-impregnation method and produced an "endoglobular body" which also seems, in our slides, to be an artefact.

The "differentiated inner body of Lowit" has a somewhat fibrillar structure; it is found only in immature forms and is supposed to be a remnant of the nucleus. Nucleoids are remnants of the nucleus, in cells not yet quite mature.

Morris' granules are single, rather large sharply circumscribed basophilic granules lying near the center of the cell, giving with all stains the reaction characteristic of nuclei; they are very probably nuclear remnants. These are normally present in embryonic blood and in adult blood during rapid hematopoiesis.

Vaughan nuclear remnants are basophilic masses which lie near the center of the cell; they are probably nuclear remnants and are increased in conditions associated with rapid hematopoiesis. They may occasionally be seen in normal blood.

Granulations

Several kinds of granulations appear in the red cells. In malaria fragments of the chromatin of the parasites appear as granules; in old, atypical cases of malaria these may present considerable difficulty in diagnosis. Fragments of distintegrating nuclei are found in the peripheral blood during rapid blood regeneration. Various precipitation

forms of abnormal erythrocyte protoplasm are occasionally found. Under abnormal conditions erythrocyte protoplasm presents atypical structures which may be granular. Platelets and other plasma constituents often adhere to erythrocytes and appear to lie within them. The various granules, artefacts and structural peculiarities thus produced within the erythrocyte have received many names; some of these names indicate definite conditions. In other cases different names are applied to the same structure; perhaps with a different appearance due to differences in staining technique. It is not now possible to clear up the problems presented by these endoglobular structures; it may be convenient to review very briefly the terms most commonly applied to them, though it is not possible to explain their presence.

Maragliano's "endoglobular degeneration" consists of areas of definitely basophilic protoplasm occasionally found within the red cell, which itself is normally basophilic during its youth. The persistence of this immature structure occurs in many anemias.

Cabot's rings are sometimes circular, sometimes oval, sometimes long, slender, almost band-like in form. Cabot thought them nuclear remnants. They are especially abundant in pernicious anemia.

Grawitz' basophilic degeneration is probably not a true degeneration but an indication of immaturity. Cells showing this granulation are often called "stipple cells." There are fine, dust-like or granular particles of basophilic material within the cells which are present in the blood in nearly all cases in which blood regeneration is proceeding rapidly, especially in pernicious anemia and in lead poisoning. They occur normally in the red bone marrow.

Ehrlich's hemoglobinemic degeneration seems to consist of denser particles of hemoglobin, probably of abnormal structure, within the cell, itself much paler than normal. The conditions resemble those which might be expected to occur if the hemoglobin were to be collected together in masses within the stroma of the cell.

Crenation

When red cells are placed in a fluid of increased or decreased alkalinity or of increased or decreased osmotic tension they undergo several changes. In hypotonic fluids with alkalinity slightly less or equal to that of the blood, the cells swell slightly and become more nearly or quite spherical. The hemoglobin diffuses out from the cells leaving only a "ghost" or colorless remnant composed chiefly of the lipoids which make up the erythrocyte stroma. This phenomenon is called "laking."

In hypertonic fluids with alkalinity equal to or slightly greater than that of the blood, the cells give off water, shrink and become more nearly spherical. The surface is thrown into rounded or sharp prolongations somewhat resembling a burr; this phenomenon is called "crenation." These prolongations may even become pinched off from the mass of the cell somewhat resembling a string of beads. Laking occurs under such circumstances much less rapidly than is the case when the cells swell in hypotonic solutions.

Extravascular Changes

When the blood is placed upon a slide at 100° F., which is about the normal temperature of blood, and the slide is covered and sealed to prevent evaporation, the red cells retain their normal size and contour for a long time. If the slide is too cool, at about 97° F., the cells shrink slightly and become more nearly spherical. If the slide is too warm, at about 105° F., the cells become fragmented. Most commonly the cells become somewhat more nearly spherical, then a constriction appears near the equator. This increases in depth and finally the cell is divided into two parts. The constriction may appear near one pole of the cell in which case two unequal masses result. Rarely the cell breaks into three or four masses at about the same time. The cells assume various bizarre forms; commas, kites, dumb-bells and many other strange shapes occur.

Abnormal Red Cells

Certain abnormal forms of red cells appear during the course of anemias and these may be briefly described. (Plates I, VII, VIII)

Normocytes are red cells whose diameter varies only within the limits normal to the age of the patient. This age question is of importance, because in children the normal variation is greater than in adults.

Microcytes are red cells less than six microns in diameter; they may be as small as three microns in diameter, and even smaller forms are sometimes found in stained dried smears.

Megalocytes and macrocytes are large red cells, and the terms are used interchangeably by many authors. Certain authors have restricted the term megalocytes to cells eleven to thirteen microns in diameter, and the term macrocyte to those of nine to eleven microns.

Schistocytes are fragments of red cells.

Gigantocytes are more than twelve microns, and may be as much as twenty microns in diameter, in the stained dried smear.

Chlorotic cells are edematous cells. They are not pathognomonic of chlorosis but may occur in almost any form of anemia.

Demilune bodies occur in red cells which have imbibed water. In such cells the stroma containing hemoglobin occupies a narrow area along one edge of the periphery of the swollen cell, the form suggesting that of the new moon. They are found in chlorosis and in severe anemias due to long malnutrition.

Sickle cells are peculiar comma-like or sickle-shaped red cells found in the blood in certain anemias of the negro race.

Poikilocytes are red cells of abnormal form.

Anisocytes are red cells of abnormal size. The term as generally employed is anisocytosis, that is, a state of the blood characterized by great variation in size of the red cells.

Nucleated red cells present similar variations with similar names.

Microblasts are nucleated microcytes.

Macroblasts or megaloblasts are nucleated macrocytes or megalocytes.

Normoblasts are nucleated normocytes.

Erythroblasts are nucleated cells containing hemoglobin, but rather larger than normoblasts; the term is commonly applied to cells intermediate between megaloblasts and normoblasts.

Poikiloblasts are nucleated poikilocytes.

Many other terms have been employed by different authors, but these are explained by the author who so employs them and require no further discussion.

To some extent these variations in the forms of red cells are due to their imperfect development; this condition is especially true in the primary anemias. Secondary anemias are characterized by cellular developmental imperfections after the red bone marrow has been seriously affected. In other conditions the red cells are only extremely fragile, being normal or about normal in size and form within the blood vessels, but becoming greatly distorted while the blood is being prepared for examination. In still other conditions the abnormal condition of the plasma causes changes in the form and size of the red cells, so that cells originally about normal become abnormal in size and form while still in the circulating blood.

Abnormal chemical relations within the red cells may cause marked variations in their affinity for stains; this condition is called polychromasia.

Poikilocytes and microcytes are especially abundant in the secondary anemias, chlorosis and sickle-cell anemia.

Anisocytosis, polychromasia and megaloblastosis are most common in pernicious anemia and in certain forms of secondary anemia, notably those due to lead poisoning, certain intestinal worms and late stages of cancer with metastases in the bone marrow.

Blood in which the average erythrocyte contains less than the normal amount of hemoglobin is said to be characterized by hypocytochromia, while blood in which the amount of hemoglobin in an average erythrocyte is above normal is said to be characterized by hypercytochromia. In hypocytochromia the color index is below one; as in most secondary anemias and notably in chlorosis. In hypercytochromia the color index is above one, notably in pernicious anemia and in certain anemias due to intestinal parasites. These red cells are always abnormally large.

Red cells appear to be extremely fragile; they change shape readily and are rather easily laked in vitro. Yet in many respects they present remarkable stability in form and functions.

Blood is often taken from one individual and placed in the veins of another who needs blood. This transfusion, as it is called, is part of the ordinary treatment in severe anemias and after severe hemorrhage. The transfused blood cells retain their form and their functional power for some days, and in one of our cases transfused cells were recognizable for three weeks after having been placed in the veins of the recipient. That the transfused cells are able to carry on their proper functions in the blood vessels of the recipient is shown by the immediate relief in symptoms which occurs after the transfusion, and also by the fact that the nucleated, reticulated and other immature erythrocytes often disappear from the peripheral blood of the recipient very soon after the transfusion of new blood into his veins.

Experiments with rabbit's blood have been reported which show that blood can be taken from the veins of one rabbit, kept just above freezing for three weeks or more, and then used in transfusion as successfully as if the blood had been taken directly from one rabbit and transfused immediately. These facts indicate that with all its delicacy of structure the erythrocyte is still a thing of remarkable stability.

Rouleaux

In slightly thick smears of blood there occurs a peculiar grouping of the red cells in such a way as to suggest a pile of saucers or bowls, or a roll of coins. This tendency seems to be a purely physical phenomenon, partly due to the peculiar shape of the erythrocytes, and partly to their stickiness and their lack of any true cell wall. Rouleaux once formed are not necessarily permanent. In slides examined on

the warm stage rouleaux often form normally, then the cells break apart and rearrange themselves, sometimes in islet-like piles, sometimes in other rouleaux. Sometimes the rouleaux formation is complete within one minute in normal blood; sometimes it is delayed for three to fifteen minutes after the blood is taken. Sometimes rouleaux are not formed at all, or are very short and include only a small proportion of the cells present.

Abnormal conditions often affect rouleaux formation, and this may occasionally be helpful in diagnosis. In ordinary anemias the rouleaux are subnormal; in pernicious anemia they are almost or quite normal. In pyogenic states they are usually normal; in cancer they are either very much delayed, are subnormal, or, being formed, break apart and the red cells form islets instead of rouleaux. Rouleaux are subnormal or absent in Hodgkins disease and are usually subnormal in the leukemias.

Resistance of Red Cells

The resistance of the red blood cells to hypertonic, hypotonic and hemolytic solutions has been studied with much care. In cases of doubtful diagnosis tests of the resistance of the blood cells may give pathognomonic facts.

The manner in which the hemoglobin is held in solution is not definitely known, but various agents which cause laking are recognized. Saponin acts by injuring the lipoid substances of the stroma. The resistance of red cells to solutions of saponin remains within normal limits in pernicious anemia, diabetes and exophthalmic goiter, and is considerably increased in the secondary anemias, in syphilis, tuberculosis, splenomedullary leukemia, polycythemia vera and in normal or abnormal blood after splenectomy.

The resistance of the red cells is considerably diminished in hemolytic jaundice, both to solutions of saponin and to hypotonic salt solutions.

Normal red cells show no hemolysis until the salt in a solution is diminished to 0.44% or less, and complete laking rarely occurs, in normal blood, until the salt is diminished to about 0.34%. In hemolytic jaundice, on the other hand, the cells begin to lake in salt solutions of 0.6% or sometimes 0.7%, and complete laking occurs at 0.4% or even 0.45%.

In blood which contains recognizable amounts of bile pigments but not bile salts laking does not occur on the warm slide, but blood which contains bile salts or acids as well as bile pigments shows laking on the warm slide within five to fifteen minutes.

Blood Grouping

When the blood of one person is transfused into the veins of another the results upon the recipient may be good or bad according to whether the two bloods are compatible, that is, whether there is any agglutination of the cells of either blood by the plasma of the other or not. Hemolysis may or may not occur with agglutination, but it does not occur without agglutination. The human race is divided into four groups according to the relative compatibility of the blood of different groups, and it is most important that transfusion is not employed until the group relations of recipient and donor have been established.

Very young infants are universal recipients, and it is usually safe to use transfusion for them without grouping. It is, indeed, impossible to determine to which group a very young baby belongs.

The four groups depend upon the fact that the serum contains one or both of two agglutinins, and that the red blood cells contain one or both of two receptors. It is not possible to find an agglutinin in the same blood with its corresponding receptor, since such a relationship would not permit life at all.

Group I, Jansky, or Group IV, Moss. This group includes about 40% of all Caucasians. The cells of these individuals are not agglutinated by the serum of other groups. The serum from this group agglutinates the red cells of all the other three groups.

Group II, Jansky, or Group II, Moss. This group also includes about 40% of all Caucasians. The serum of this group agglutinates the cells of Group III, Moss and Jansky, and of Group IV, Jansky or Group 1, Moss. The cells of this group are agglutinated by the serum of Group 1, Jansky, or Group IV, Moss; and by the serum of Group III, Moss or Jansky.

Group III, Jansky, or Group III, Moss. This group includes about 10% of all Caucasians. The serum of this group agglutinates the cells of Group II, Moss or Jansky, and of Group IV, Jansky, or Group I, Moss. The cells of this group are agglutinated by the serum of Group I, Jansky or Group IV, Moss, and by the serum of Group II, Moss or Jansky.

Group IV, Jansky or Group I, Moss. This group includes about 10% of all Caucasians. The cells of this group are agglutinated by the serum of all three of the other groups. The serum of this group exerts no agglutinating influence upon the cells of other groups. The following hypothesis explains the grouping fairly adequately:

Individuals are placed in groups according to the behavior of their cells with the serum of other groups. If the cells contain both types

of receptor the serum from all other groups must agglutinate those cells. Conversely, the serum of this group cannot contain any agglutinin, since life would be impossible under such circumstances. This person belongs in Group I, Moss, or Group IV, Jansky.

If an individual has serum which contains both types of agglutinin it is evident that his cells cannot contain either type of receptor, since such a condition would be incompatible with life. His serum agglutinates the cells in all three other groups, and he belongs in Group I, Jansky, or Group IV, Moss.

If an individual has serum containing the A type of receptor and the beta type of agglutinin his serum agglutinates the cells containing the alpha type of agglutinin; he belongs in Group II, Moss or Jansky.

If an individual has red cells with the B type of receptor and the alpha agglutinin is found in the serum of his blood, then he belongs in Group III, Jansky or Moss.

Life would be impossible for an individual with A receptor and alpha agglutinin, because the red cells would be immediately agglutinated. The same statement is true for an individual who should have B receptor and beta agglutinin.

The behavior of the red cells with reference to agglutination is a constitutional trait which follows Mendel's Law as a dominant characteristic. Babies under about ten weeks of age do not seem to possess agglutinins or receptors and are therefore included as universal recipients. After about three months of age the baby can be grouped and this grouping seems to be permanent throughout life, though there may be changes in the vigor with which the cells are agglutinated or with which the serum agglutinates other cells.

Other than Caucasian races show different group relations. Any primitive race usually belongs to a certain group, but different primitive races may belong to different groups. Mammalian species may be classified upon a basis of blood-grouping and this method promises good results in biological studies. The blood of fourteen chimpanzees was tested with the blood of human groups; all belonged in Group II of the human groups. Many interesting relations have been reported for different mammals.

The technique of blood transfusion is beyond the scope of this discussion. The underlying principles are of interest in any discussion of the red blood cells.

Transfusion of blood from an individual of any group to another individual of the same group is considered safe.

A recipient of one group may safely receive blood from a donor of another group provided the donor's cells are not agglutinated by the serum of the recipient. The serum of the donor is so greatly diluted that agglutination of the recipient's cells probably does not occur. Therefore the individuals of Group IV, Moss, or Group I, Jansky, are generally considered universal donors. The cells of this group are not agglutinated by the serum of any group.

Since the serum of Group I, Moss, or Group IV, Jansky, does not agglutinate any cells, these individuals may receive blood from any group; they are considered universal recipients.

Before transfusing blood both donor and recipient must be grouped. Even within the same group, occasionally, or when donor or the recipient are of a "universal" type, the two bloods should be cross-matched. This is because there are individuals which do not belong exactly into any one of the four groups, and also because of the possibility of error in the preliminary grouping. It is true that errors are rare, but their possibility must be considered.

If second or later transfusions are necessary the tests must be repeated even though the same donor be employed. This is because it occasionally happens that after the first transfusion the recipient may develop an anti-body for the blood of the donor, in which case serious reaction may occur after the second transfusion. In any case the blood should be given very slowly during the first ten minutes of the transfusion and if any adverse symptoms occur the procedure immediately terminated.

Number of Red Blood Cells

Normal adult human blood contains from 4,500,000 to 5,500,000 erythrocytes per cubic millimeter. Rarely the number may reach 6,000,000 per cubic millimeter; as, for example, in a young man of good physique who has been engaged in violent exercise. Welker's original estimate, in 1854, of 4,500,000 for women and 5,000,000 for men is still accepted generally as correct, though these figures appear to be too low by about a half million in each case. The figures vary considerably under normal conditions, and to a very great extent in abnormal conditions. Normal physiological conditions cause variations in the red cell count which may be considerable, and it is of great importance that blood counts should be made with proper control of these factors.

Sex

The blood of women is generally supposed to be about 500,000 red cells poorer than the blood of men. This is not really a sex trait,

however. It is due to the differences in the lives of the sexes from the beginning of puberty. Blood counts made of men and women of all ages, whose life habits are similar, show no differences which can in any way be considered due to sex. Students, teachers, clerks or bookkeepers whose lives are spent indoors and whose muscular activities are slight have about the same blood counts and hemoglobin percentages, whether they are men or women. On the other hand, counts made of women whose lives are spent in active, muscular work out of doors have blood counts which do not vary from those of their brothers. In Southern California, for example, women living alone or by twos or threes sometimes attend to the work of small ranches alone. Counts made of the blood of such women follow that usually found in men in similar occupations. Since women do, generally, live less active lives and are more closely confined within doors, a difference of about a half million erythrocytes to the cubic millimeter is expected. In estimating the percentage of the normals the occupation and not the sex are to be considered.

Age

Very few actual counts of early fetal blood have been reported. The number of red cells reaches its highest normal point very soon after birth, then diminishes until just before puberty. The count then rises gradually, modified by the other factors presently to be mentioned, until old age. The anemia due to senile disturbances then may cause a fall; but it is probable that old age in itself is associated with a diminution of the water, and thus with a continually rising count.

The following table is compiled from counts reported from several laboratories, together with the records from the clinics and laboratories of The Pacific College of Osteopathy in Los Angeles, and The A. T. Still Research Institute in Chicago and Los Angeles.

Children

Fetus, 7-9 months	6,500,000-7,000,000 per cubic millimeter
Placenta, after birth (maternal blood)	5,500,000-7,000,000 per cubic millimeter
Child, after birth	6,000,000-7,000,000 per cubic millimeter
1 year	5,500,000-6,500,000 per cubic millimeter
5 years	5,000,000-6,000,000 per cubic millimeter
10 years	4,500,000-5,000,000 per cubic millimeter
15 years	4,800,000-5,500,000 per cubic millimeter

Men and Women Engaged in Active Outdoor Occupations

20 years .....5,000,000-5,500,000 per cubic millimeter
30 years .....5,300,000-5,500,000 per cubic millimeter
40 years .....5,300,000-5,800,000 per cubic millimeter
50 years .....5,500,000-6,000,000 per cubic millimeter
60 years .....5,500,000-6,000,000 per cubic millimeter

Women and Men With Sedentary Lives

20 years .....4,500,000-5,000,000 per cubic millimeter
25 years .....4,800,000-5,000,000 per cubic millimeter
30 years .....5,000,000-5,200,000 per cubic millimeter
40 years .....5,000,000-5,500,000 per cubic millimeter
50 years .....5,200,000-5,800,000 per cubic millimeter
60 years .....5,500,000-6,000,000 per cubic millimeter

Variations in Cell Count in Different Areas

The red cell count may vary in blood taken from different parts of the body. Local hyperemia causes local rise in the red cell count. The count is higher in dependent parts of the body. If one hand is elevated and the other hand allowed to dangle, the count is higher in the blood taken from the dependent hand and lower in the hand which is held higher than the body. If the hand is supported in an elevated position the count is higher than if it is held upward by muscular activity. Exercise of any part of the body increases the cell count in that region. If the arm and hand are vigorously exercised, the blood taken from a finger shows a higher count than blood taken before the exercise. If one arm only is exercised, the blood taken from a finger on that side has a higher count than blood taken from a finger on the opposite side.

The constriction of a limb, as by an elastic band, causes increased red cell count. Any part of the body in which the circulation of the blood is delayed has a higher red cell count than normal areas.

Very vigorous exercise involving a large part of the entire musculature increases the red cell count, sometimes by 1,000,000 cells or even more.

Variations Due to Temperature Changes

Temperature changes have an effect only if somewhat prolonged, or if associated by marked changes in humidity. Generally speaking, the red cell count of nearly all normal individuals is about 500,000 higher

in winter than in summer, in climates with marked seasonal changes. Persons who leave cold regions to go to the tropics may lose a million or more of red cells per cubic millimeter; persons who go from very warm to cold climates gain red cells in about equal numbers.

No doubt this variation associated with changes in temperature is due, in part, to the same factors which cause variations due to altitude, that is, to changes in muscular activity, appetite, respiration, pulse rate, blood pressure and other functions which vary directly or indirectly as a result of diminished oxygen supply to the tissues.

Hot baths, especially with much sweating, increase the red cell count. Cold baths decrease the red cell count at first, but after the reaction the count may be considerably increased. In typhoid fever cold baths may increase the blood count by almost or quite 2,000,000 cells. This increase disappears within an hour, or two hours at most.

Local applications of heat and cold differ. Heat, cold or rubefacients which increase the caliber of the peripheral vessels increase the erythrocyte count in the affected areas; agents which lead to vaso-constriction diminish the erythrocyte count. These variations are due to the fact that the layer of plasma lining the capillaries remains fairly constant under normal conditions; thus, when the caliber of the vessels is increased, it is chiefly the cell-containing central area of the capillary which is affected. Since the blood taken for counting comes from the capillaries, the variations in the counting due to these conditions may reach 200,000 or even more.

Altitude

The person who travels fairly rapidly from lower to higher altitudes shows increased red cell counts, about 100,000 cells for each 2,000 feet in elevation. If he descends at once the count returns to its original number within one or two days. The change is so rapid that it seems impossible that increased hematopoiesis causes it, and there is no evidence of increased rapidity of blood formation in normal persons making the journey. Variations in the distribution of the blood, flushing of the rib marrow by rapid respiration, increased rapidity of evaporation of water from the skin and the lungs; fragmentation of the red cells with retention of almost normal structure; a shower of red cells from the bone marrow in answer to the lowered oxygen tension of the higher altitude and diminished efficiency of the heart's action are some of the factors which may be concerned in this temporary polycythemia due to increased altitude. The fragility of the cells is increased with the increasing cell count and the albumins and globulins of the blood are diminished at the same time. The person who remains at high altitude

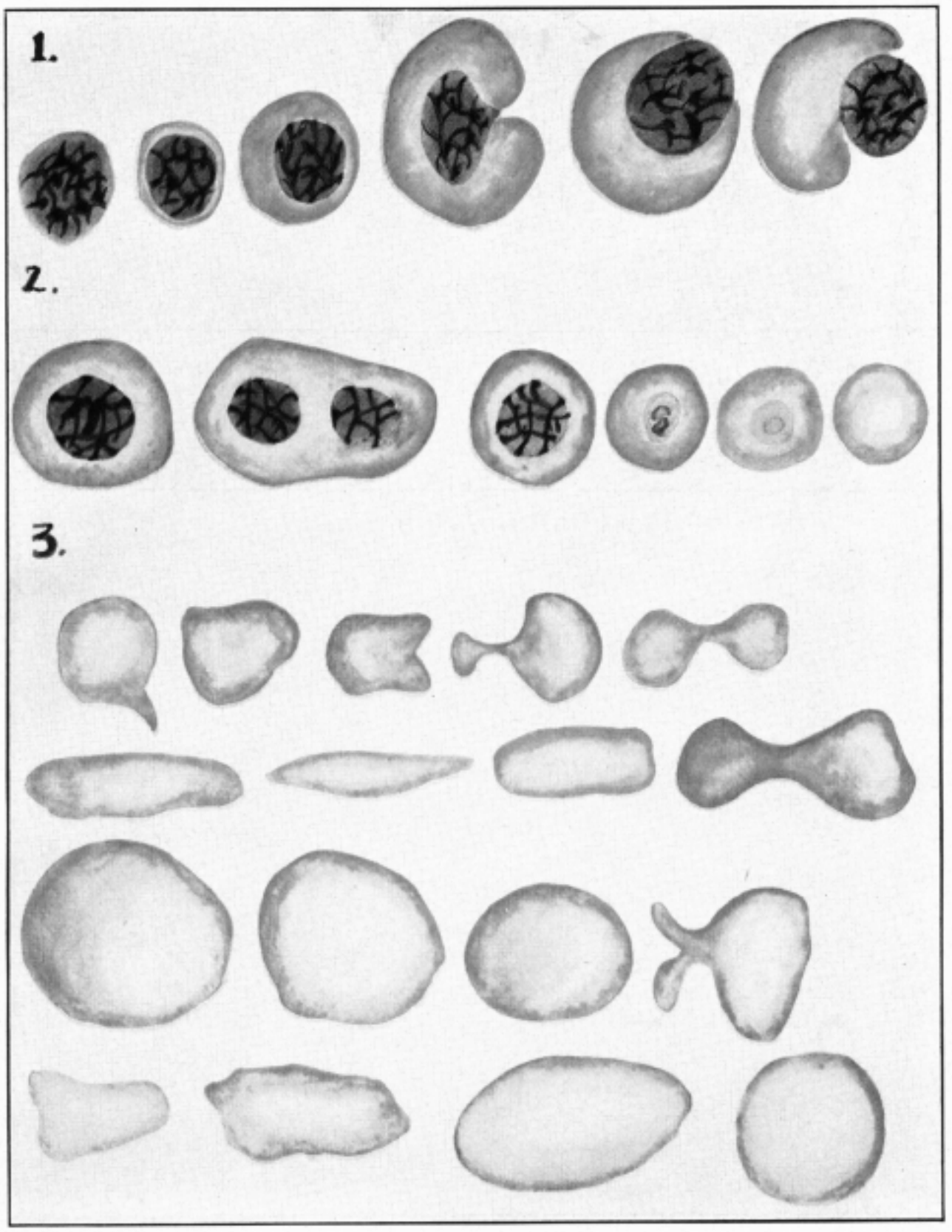


PLATE I

ERYTHROCYTOGENESIS AND CHLOROSIS

1. Extrusion of nucleus. The naked nucleus accumulates cytoplasm and this increases in hemoglobin content until the cell assumes the size of a normoblast. The nucleus then approaches the edge of the cell and is finally extruded.

2. The nucleated red cell divides by karyokinesis. The nucleus becomes progressively paler and finally disappears. Nuclear remnants may remain recognizable for a considerable time.

3. Poikilocytes in chlorosis. The patient had a color index of 0.23. Recovery complete.

usually shows a blood count normal to him at the lower altitude or only slightly higher, within a few months. However, the Indians in Mexico who live on high measa have habitual polycythemia, according to counts reported by F. Ocaranza. The air is very dry in that location.

In our laboratories blood examinations made at Los Angeles for people who have descended from higher altitudes show polycythemia during the first few days to two weeks. Decrease in the count is gradual and during the diminishing counts the blood shows the changes present during the absorption of abnormal katabolites. We have few counts made for normal persons under such circumstances, but many of these people suffered only from mild disorders, and in a few cases the only cause of ill health seemed to be an inability to live comfortably at the high altitudes.

A sudden rise to high altitudes gives the most marked variation; though the increase is certain no matter how gradually the rise is made.

The factors which seem to affect this rise are many:—

Increased evaporation of the water due to the high altitude is one factor, though this is now considered of minor importance.

Variations in the heart's action, due to the lowered oxygen tension, certainly modifies the peripheral count.

These two factors are probably responsible for the increase noted immediately after the rise; the actual increase in the number of erythrocytes in the entire blood is due to other factors as well.

The diminished oxygen tension of the higher altitudes is directly or indirectly responsible for the permanent and actual increase of the cells, and this acts upon the body in several ways:

Increased need of the body for oxygen leads to increased heart's action, and thus to increased cell counts;

Increased respiratory and cardiac activity means increased exercise; thus also increased cell counts;

Increased respiratory activity means increased rib movement, this means increased efficiency of the circulation through the ribs (which include a great proportion of the red bone marrow of the entire body) with resulting increased nutrition of the blood-forming organs;

Increased activity of the thorax with increased activity of the nerve centers associated with the respiratory and cardiac movements, causes increased activity of the nerve centers associated with the control of the hematopoietic marrow:

Increased respiratory movements, together with the increased activity and appetite, due to changes of environment, may often be partly responsible for increased nutrition and thus for increased erythrocyte

formation. This factor is not an important one in the variations noted under experimental conditions. It should be noted that in animals the variations in erythrocyte count are less marked and less constant than is the case with human subjects not under experimental conditions. Anemias due to hemorrhages, either experimental, accidental or pathologic, tend to more rapid recovery in high altitudes.

The indirect effects of mountain climates,—the increased activity, increased appetite and other effects of environmental change,—are worthy of consideration in estimating the effects upon the blood of nervous, over-tired or other patients who may be sent to elevated resorts for the sake of erythrocyte stimulation. In this connection it should be noted that the altitude does not always give increased powers of erythrocyte formation. Patients who suffer from primary anemias or serious nutritional or circulatory diseases may be harmed by changing to the higher altitude, which makes upon their hematopoietic organs a demand greater than can be met.

Pregnancy

There is some tendency for the red cell count to drop during pregnancy, and this condition may be so exaggerated, in women who appear normal otherwise, as to simulate pernicious anemia. We have records of one woman in whose case each of three pregnancies was associated with decrease of the red cell count to less than 2,000,000, with color index at one or slightly above one. After the birth of each child the count rose to normal within a few weeks. The children were apparently perfectly normal. Normal menstruation and normal lactation do not seem to modify either the number or the character of the erythrocytes.

Physiological Variations

Exercise, emotional states, excitement of various kinds, anything which raises the systemic blood pressure, increases the erythrocyte count through the filling of the peripheral vessels. Anger and fright do not increase the red cell count in animals or in human subjects after splenectomy, nor in animals after the solar plexus or the splanchnic nerves have been injured. Anger and fright cause increased contractions of the splenic capsule and this may account for the increase in normal subjects. Anything which abnormally increases the action of the kidneys or which increases the secretions of other glands increases the erythrocyte count by lessening the water content of the blood. Anything which increases the respiratory movements increases the erythrocyte count, both immediately and permanently. Probably the im-

mediate rise is in part due to rising blood pressure and increasing heart action, and partly to the flushing of the rib marrow and thus an increase in the rate at which the new cells are thrown into the general circulation. The permanent increase in the erythrocytes associated with habitual increase in the respiratory movements is partly due to the better nutrition, and partly due to the better circulation through the red bone marrow of the ribs.

Variations Due to Digestion

Eating a light meal may not modify the erythrocyte count perceptibly. After a heavy meal, the increase in the water thrown out with the digestive secretions lowers the watery content of the blood, and thus raises the erythrocyte count. Later, the increased absorption of the products of digestion causes a lowering of the erythrocyte count. A later rise may occur, due to either an increased formation or to a flushing out of the cells already formed. This later increase is not usually seen in the blood of normal persons but it may be quite marked after a period of starvation or other mal-nutrition.

Daily Variations

The red cell count is somewhat higher at about two o'clock in the afternoon in human subjects. This rise rarely exceeds 50,000 cells per cubic millimeter. It occurs without regard to fasting or to eating at noon. Persons who habitually eat nothing at noon, those who eat heartily at noon, those who habitually eat but who omit one noon meal, those who habitually omit the noon meal but who take food for one day only, all show about the same rise in red cell count within half an hour or so of two o'clock in the afternoon. A less marked rise occurs at about the same hour in the morning.

Variations Due to Concentration

Transient polycythemia may be caused by any condition which concentrates the blood or which causes marked capillary or venous congestion. Diarrhea, sweating of severe degree, prolonged vomiting, cyanosis, sudden cardiac inefficiency as in decompensation and several less common conditions associated with loss of fluid or with acute dilatation of blood vessels cause temporary increase in the red cell count of capillary blood.

Erythrocytosis

Erythrocytosis is a polycythemia which is a reaction to some increased demand for red cells, beyond normal limits. The term is analogous to leucocytosis. High altitudes to which the reaction is inefficient may cause such an erythrocytosis.

Heart disease is especially important; in congenital heart disease the count may be very high. In a case in the clinic of The Pacific College of Osteopathy a count of 8,000,000 cells per cubic millimeter was found in a boy eleven months old, with congenital heart disease. In adults cardiac disease sometimes causes very high counts. In one of our cases three days before death the count was 7,000,000 cells. Lung diseases usually increase the cell count, although if such diseases persist anemia supervenes later.

The red cells are increased during fevers, probably on account of the concentration of the blood. The red cells may be increased during rapid leucocytosis, as if the increased activity of the leucocytopoietic centers caused at the same time increased activity of the erythrocytopoietic centers in the bone marrow. This relation is not invariable. The red cells are generally increased in epidemic encephalitis and in many brain injuries. Rabbits which have been given brain lesions show increased red cell counts. In patients with rupture of the spleen, or with disease involving a considerable part of the spleen and in animals and in human subjects after splenectomy, the red cell counts may be very high, even to 10,000,000 per cubic millimeter or more.

Certain drugs and biological preparations increase the red cell count, but the repeated use of such drugs is always followed by a very persistent anemia. Other drugs diminish the red cells immediately and permanently. Radium and X-ray also diminish the blood cells.

Erythremia

The term erythremia, in a manner similar to the term leukemia, applies to a disease of the blood forming tissues characterized by very greatly increased red cell counts. This increase is not due to any demand on the part of the tissues of the body, so far as can be determined, but to a primary disease or developmental defect of the hematopoietic system.

Regeneration of Blood Cells

The red blood cells are normally being formed and being destroyed continually. They undergo fragmentation and probably hemolysis in the circulation. Phagocytosis of the fragments and of elderly entire red cells occurs continually in the endothelial cells of the liver, spleen and bone marrow. Regeneration occurs normally only in the red bone marrow, in the adult. It is very evident that if regeneration exceeds destruction, though only by a narrow margin, polycythemia must inevitably occur. If destruction exceeds regeneration by even a narrow margin, anemia must occur as inevitably. Normally the balance is maintained by increased regeneration when any unusual de-

struction of red cells occurs, and by diminished regeneration and by increased phagocytosis when the number of red cells tends to become too great. Only under distinctly pathological conditions does the number of red blood cells vary beyond the limit of efficiency of their oxygen-carrying function.

The rate of development of red blood cells in the human adult has been variously estimated. Since the bile and urinary pigments are derived from hemoglobin, the measure of these pigments in the urine and the feces should indicate the number of red cells destroyed each day, and thus the number of new cells formed. Estimations based upon such data vary greatly, as is to be expected. About one-tenth to one-fortieth of the blood cells are destroyed each day according to different computations. Studies made after transfusion indicate that transfused blood cells may live for three months or more within the blood vessels of the recipient, though this does not seem to be the usual term of life.

Women are able to lose from fifty to five hundred grams of blood at each menstrual period, yet they show little or no increased erythrocytopoiesis before the menstrual period and no erythrocytopenia afterward. This loss, then, must be relatively negligible when compared with the normal formation of new cells. After single hemorrhages amounting to five hundred grams of blood no abnormal conditions are recognizable in the peripheral blood. The removal of one hundred to three hundred grams of venous blood daily except Sundays for three weeks, for experimental purposes, caused no recognizable changes in the hemoglobin or the red or the white cell counts and no appearance of immature red or white cells, in one case in our records. After comparing all the reports accessible with our own records it seems that many normal women must form about ten thousand cells each second all the time in order to provide menstrual blood alone.

Various normal and abnormal conditions increase the rate of blood formation, and thus the number of cells in the peripheral blood.

Development of Red Blood Cells

In early embryonic life the first red blood cells appear in the extra embryonic mesoderm. There is a possibility that entodermal cells emigrate to this location. Groups of cells which are sometimes called "blood islands" appear, and in the midst of these there are certain cells which become flattened and somewhat elongated. These form the endothelium of the blood vessels. The cells within these embryonic vessels develop into red corpuscles. The cells without the

vessels ultimately form leucocytes and adventitial cells, and the muscular walls of the blood vessels. After the first cells are formed in the extra embryonic mesoderm, other tissues within the embryo begin to form blood cells, and during embryonic life both red cells and white cells seem to be formed almost anywhere. Later the liver and the spleen are sites of abundant hematopoiesis and finally the red bone marrow assumes the most important place in the manufacture of red cells. After birth for a short time the spleen still provides some red cells, but the red bone marrow is pre-eminently the location of the hematopoietic tissues after birth.

In normal adult human life the red cells are all formed in the red bone marrow. The origin of the normoblast, in normal adult human blood, presents several problems. In the red bone marrow are found the earlier cells of the erythroblast series. These are cells which resemble small lymphocytes in some degree, but which contain a small amount of hemoglobin within their protoplasm. The nuclei are vesicular and the chromatin is in small and rather scanty masses; there may be one or two nucleoli. This cell, when stained after Sabin's method, may show several mitochondria, usually of somewhat elongated form. This cell divides into two daughter cells and these into others; within one to a few divisions the daughter cells show more abundant hemoglobin, smaller nuclei with denser chromatin and this arranged in typical "cartwheel" form; nucleoli are usually absent and mitochondria very scanty or absent. This nuclear structure is characteristic; the chromatin is arranged in rather dense masses which are especially abundant around the periphery of the nucleus. This causes the "cartwheel" appearance of the cell as seen in a thin smear of blood. The megaloblast nucleus has rather dense masses of chromatin but they are not quite typically of the cartwheel structure. The erythroblast nucleus shows the cartwheel arrangement clearly, as does the normoblast nucleus and the naked nucleus found in the normal adult red bone marrow and in the blood of pernicious and certain other very severe forms of anemia. The erythroblasts and the normoblasts contain fairly abundant hemoglobin, and this increases with the maturity of the cell until the typical erythrocyte hemoglobin concentration is reached.

Loss of the Nucleus

The manner in which the nucleus of the normoblast is lost has been the subject of much discussion. In the laboratories of The A. T. Still Research Institute studies have been made of the hemocytopoietic tissues of human embryos of ten days, three weeks, ten weeks, four

months and later development; of embryos of rabbits, guinea pigs, cats, rats, moles and gophers; of the red bone marrow of human subjects dying of acute diseases and of aplastic, pernicious and several forms of secondary anemia, and of the red bone marrow of adult rabbits, dogs, cats, rats, guinea pigs, gophers, and horses. The following method of discarding the nucleus seems certainly to be characteristic of human erythrocytogenesis. In other mammals it has been found present also, though in these the relations have not been quite so thoroughly studied as in the human. (Plate I)

The normoblast has its spherical nucleus and its deep rim of hemoglobin-containing protoplasm. This nucleus being apparently efficient with its deeply-staining chromatin and its typical structure, is to be extruded. The nucleus shrinks slightly and assumes an eccentric position in the cell; it approaches the periphery of the cell gradually; the protoplasm becomes thinner and thinner over the nucleus and finally shrinks away from it altogether, so that the nucleus is left altogether outside of the protoplasm, which is then an adult red cell, ready for its functional existence. Cytologically, of course, it is really in a senile state. The naked nucleus then swells slightly and soon it shows a thin rim of basophilic protoplasm around it. This protoplasm increases in thickness and develops hemoglobin within it. After a time the nucleus is again at the center of a normoblast and again the nucleus may be extruded in the same manner. It is not possible to say how many times a single nucleus may undergo this series of changes.

In another series the nucleus of the normoblast has a less definite structure. It stains less vigorously; the chromatin masses are not quite typically cartwheel in arrangement; their outlines are less sharply marked and the nucleus is a trifle larger. The nucleus swells somewhat, its chromatin masses stain less and less vigorously; the nucleus loses its sharply defined outlines; there is a clear area in the adjacent protoplasm; the protoplasm accumulates hemoglobin most abundantly at the periphery of the cell and finally the nucleus seems to dissolve within the red cell, leaving some of the nuclear remnants called nucleoids or various other names already mentioned in connection with the intracellular structures of the erythrocytes. The extrusion of the vigorous nucleus and the digestion of the senile nucleus offer two parallel modes of conversion of the normoblast into the erythrocyte.

Abnormal Development

During severe anemias the red cells do not follow the normal course of development. The megaloblast may pass directly into the circulation. It may lose its nucleus prematurely and the large cell thus

formed has scanty hemoglobin and is a megalocyte. The normoblast nuclei may fail to grow before division and microblasts be allowed to reach the blood stream; or the microblast may lose its nucleus and the microcyte pass into the general circulation. Protoplasm may be budded off from red cells, under abnormal conditions, and microcytes thus be produced. The cells may be so poorly made that they undergo distortion with facility; many poikilocytes and poikiloblasts are so formed. Others are improperly formed in the marrow and pass into the blood stream unchanged. In pernicious and other very severe anemias and in the leukemias, the immature blood cells may be so abundant as to cause the blood smear to resemble a smear made from red bone marrow or from pus.

Under very abnormal conditions in the adult, red blood cells may be formed in other tissues of the body, as in embryonic life.

The spleen, the hemolymph glands, areas of endothelial cells and connective tissues associated with areolar and fatty tissues, even the secreting glands of the body, occasionally show structures resembling red bone marrow, in which hematopoiesis is seen to be very active.

Metastases from the red bone marrow occur in certain leukemias and in pernicious anemia, and it is often difficult to determine whether any given area of hematopoietic tissue found in some aberrant location is a metastasis or is an instance of metaplasia.

Red cells which have passed their period of usefulness must be quickly removed from the circulation, because it is only very rarely that such cells are found in normal blood. There is a form of basophilic degeneration which occurs in cells which are presumably too old to function properly. In blood kept in vitro the red cells become fragmented, laked or swollen according to environmental conditions but they do not become basophilic. In the circulating blood in cases of jaundice and of intestinal toxemia fragmentation occurs more abundantly than in normal blood. Fragments and abnormal cells of various types are taken up by the phagocytic endothelial cells (Kupffer cells of the liver; endothelial cells of the spleen, lymph nodes and bone marrow) and by these cells they are digested. The processes are not yet well understood, but the hemoglobin is certainly broken into an iron-free pigment (bilirubin, hematoidin or some related compound) and this pigment is eliminated in the bile and the urine. There is good reason for believing that the reticular cells perform the major part of the work, and that the true hepatic cells pass the pigments onward into the bile, perhaps after elaborating them in some manner. Much further work must be done before the steps of this procedure can be

described, and the problems presented by the dissolution of the red cells and the conservation of the iron-containing molecule are very complicated.

Relations of Red Cells and Hemoglobin

The amount of hemoglobin carried by each red blood cell, the amount carried by a given amount of erythrocyte stroma, and the functional efficiency of the hemoglobin are subjects of some interest.

These factors are determined by a study of the color index, the volume index and the saturation index of the red blood cells.

The Color Index

This is the fraction obtained by dividing the hemoglobin percentage by the erythrocyte percentage. The hemoglobin and the erythrocyte count should be made, and the percentage of the normal for the age of the patient computed. The color index expresses the relative amount of hemoglobin carried by an average red cell of the patient's blood.

For example, a young baby should have 150 to 200 grams of hemoglobin per liter, according to its age; it should have 5,500,000 to 6,500,000 erythrocytes per cubic millimeter. If these figures were given by a baby's blood its color index would be 1., which is normal.

A child five years old should have about 112. grams of hemoglobin per liter, and about 5,000,000 erythrocytes per cubic millimeter. These figures being given, its color index is 1., which is normal.

An adult should have about 140. grams of hemoglobin per liter, and 5,000,000 erythrocytes per cubic millimeter. These figures give a color index of 1.

An adult who has suffered a severe hemorrhage from accident, may, immediately after the loss of blood, give 60% hemoglobin and 60% erythrocyte count; 60%÷60%=1. which indicates that each erythrocyte is carrying the normal amount of hemoglobin, though it is evident that the number of the erythrocytes is diminished.

The next few weeks usually alter the erythrocytes, if the hemorrhages are repeated. The same person may then have 55% hemoglobin and erythrocyte count of 110%.

55%÷110%=0.5 which is the color index characteristic of secondary anemia. The erythrocytes are then normal, or above normal, in number, but they carry about half the normal amount of hemoglobin. The blood-forming organs are reacting to loss of blood by manufacturing increased numbers of erythrocytes, but the hemoglobin is insufficient to form perfect erythrocytes; hence the low color index.

In chlorosis extremely low color index is the rule. One case of ours gave an erythrocyte count of 120%; hemoglobin percentage of 30; 30%÷120%=0.25, the color index.

In pernicious anemia, the color index is high, though the hemoglobin is very low. One case gave hemoglobin, 20%, erythrocyte count 15%. 20%÷15%=1.33, the color index. The color index may reach 3.2 in pernicious anemia.

Nearly all secondary anemias show a low color index, usually below 0.7 and sometimes below 0.4. Anemias due to intestinal worms, especially bothriocephalus latus, often show high color index. Lead poisoning and most cases of pernicious anemia are characterized by high color index. Rarely in these cases the color index may be below unity.

A patient with pernicious anemia or with lead poisoning may suffer from some cause of secondary anemia, with resultant leucocytosis and increased red cell count with low color index. The blood picture may be very puzzling in such cases. After the acute condition has passed the pernicious anemia type of blood picture recurs.

During an intermission in pernicious anemia the color index may be normal or low and other factors in the blood picture may fail to suggest anemia of pernicious type.

Volume Index

The red cells make up approximately one half the total volume of the blood. The volume index is the fraction secured by dividing the volume per cent of red cells by the red cell count expressed in per cent of normal. For example, if a patient has 5,000,000 red cells per cubic millimeter, or 100%, and if the hematocrit estimation of the blood cell volume is one-half the total blood voluume, or 100% of the normal, the volume index is 100% ÷ 100%, or 1, which is normal. If the patient has 4,000,000 red cells per cubic millimeter, or 80% of the normal, and has a cell volume of four-tenths of the total blood volume, or 80% of the normal volume, then also his volume index is 1.(80% ÷ 80%). If the red cell count is 4,000,000 and the volume of cells is three-tenths the total blood volume, or 60% of normal, then the volume index is 60% ÷ 80%, or .75. In other words, each red cell is about three-fourths of the normal volume or size, and this is the condition in ordinary secondary anemia. If the red cell count is 2,000,000 or 40% of the normal, and the cells make up one-fourth the total volume of the blood, or 50% of the normal, then the volume index is 50% ÷ 40%, or 1.25. This is the condition characteristic of pernicious anemia, in which the red cells have an average volume of considerably more than normal.

The volume index has about the same clinical significance as the color index.

Saturation Index

The volume of the red blood cell is never over-saturated with hemoglobin. In other words, a given amount of stroma never contains more than a normal amount of hemoglobin. The saturation index is the fraction formed by dividing the color index by the volume index. The normal saturation index is 1.0 and under no abnormal condition is it increased. In all anemias it is diminished. The saturation index has not yet been found of any value in practice nor in research work.

Hemoglobin

The color of the blood is due to hemoglobin. This remarkable substance is greenish yellow by transmitted light, and red by reflected light. The particular shade of red depends upon the chemical relations of the hemoglobin. The hemoglobin of venous blood, reduced hemoglobin, has a purplish tint within the veins or in a glass vessel into which it has been drawn without being exposed to the air. Oxyhemoglobin has the hue of arterial blood. Venous blood immediately takes up oxygen when it is exposed to the air, thus becoming oxyhemoglobin and assuming the characteristic brilliant scarlet tint. On exposure to carbon monoxide the hemoglobin unites with this gas, forming carbon monoxide hemoglobin and assuming a bright cherry-red color.

The hemoglobin is carried in the erythrocytes. The amount carried by each red cell varies somewhat in health, and varies within wide limits under abnormal conditions. For example, in chlorosis the color index may be as low as 0.2, which means that the average red cell is carrying about one-fifth as much hemoglobin as normal red cells carry. In pernicious anemia, on the other hand, the extremely large red cells may carry more than three times the normal amount of hemoglobin.

Hemoglobin makes up about 95% of the dried mass of the erythrocytes. The stroma, within whose meshes the hemoglobin is held, is made up chiefly of cholesterin, lecithin and other substances related to the lipoids, with a small amount of protein substances, various inorganic salts, sugar and traces of many other substances whose presence is merely adventitious.

Relations of Stroma and Hemoglobin

The manner in which the hemoglobin is held within the meshes of the stroma is not definitely known. The stroma at the periphery of the cell is in a condition of slight tension, barely perceptible by delicate methods of examination. This peripheral area has been called a cell-wall though

this is really a misnomer. Fragmentation of the erythrocytes does not cause laking, hence the peripheral tension is not the essential factor in keeping the hemoglobin within the stroma. The meshes of the stroma are rather coarse and the framework is open, so there is no actual mechanical restraint placed upon the hemoglobin. The hemoglobin within the erythrocyte is combined with sodium or potassium (sodium hemoglobinate; potassium hemoglobinate) and in this form it is much more soluble than in other forms. There is much reason for supposing that a very labile form of chemical union exists between the stroma and the hemoglobin, though just what the nature of this combination may be is not yet known. The study of hemoglobin is best made after the hemoglobin has been freed from the stroma. This process is called "laking".

Laking of the Blood

The combination of hemoglobin and stroma is very stable under all conditions normal to the body, and even under many conditions which are distinctly abnormal. Certain abnormal conditions, not always in themselves very serious, may cause the hemoglobin to be set free within the blood vessels, thence to be eliminated by the kidneys, chiefly, and to some extent by the liver.

Infections of several types may be directly or indirectly hemolytic. Infectious foci due to any type of the hemolytic streptococci may be apparently small and of negligible pathogenicity, yet the products of the activity within these foci may cause severe anemia.

The malarial parasite causes fragmentation of the red cells which have been invaded and under certain circumstances not yet well understood considerable laking of the blood ooccurs. The hemoglobinemia causes hemoglobinuria, the "black-water" of older writers. Syphilis may cause hemoglobinemia and hemoglobinuria, indirectly.

Jaundice causes hemoglobinuria which may be severe but is often unrecognized on account of the associated choluria. The bile salts lower the surface tension, dissolve the cholesterin, destroy the structural relations of the stroma and set the hemoglobin free in the plasma. Bile pigments have less marked effects but seem to be somewhat hemolytic.

Diseases of metabolism occasionally are associated with hemolysis. Scurvy causes abundant hemorrhages, which in turn cause anemia and hemoglobinuria. Intravascular laking occurs in severe cases of scurvy. Severe frostbites are often followed by hemoglobinemia and hemoglobinuria. Extensive burns and insolation are also often followed by intravascular laking. The local destruction of the blood cells is not

enough to account for the degree of laking, and there is good reason to suppose that the poisonous products of the injured tissues destroy the red cells within the blood vessels.

Paroxysmal Hemoglobinuria

Paroxysmal hemoglobinuria is a peculiar condition in which hemoglobin is excreted occasionally in the urine, very often without any recognizable cause. Persons so affected do not seem seriously injured by the excretion of this important constituent of the blood. They may be somewhat weak after an attack, and occasionally an unusually severe attack may be followed by transient anemia. Attacks may occur after exposure to moderate variations in heat or cold; they are often precipitated by chilling of the surface of the body. Pre-existent hemoglobinemia is rarely demonstrable. The red cells of persons so affected are somewhat less resistant to the action of various hemolytic agents in vitro than are normal erythrocytes.

Hemolytic Toxins

Many poisons are hemolytic. Generally speaking, those poisons which are lipolytic are also hemolytic, and they act upon the blood cells by dissolving or injuring the meshes which make up the stroma. There are other poisons which are distinctly hemolytic but are not known to be lypolytic. Many drugs used in the treatment of disease are hemolytic, and the habitual use of such drugs is a cause of much chronic anemia. Tincture of iodine, potasium chlorate, arsenic, carbolic acid, napthol, ricin (in castor oil), benzol, lead compounds, sulphuric acid, hydrochloric acid and many of the coal-tar derivatives are a few of the poisons sometimes given as drugs which cause some degree of laking within the blood vessels. The venom of certain snakes and the poisonous substances of certain morels (toadstoools) are also hemolytic.

Transfusions of unfit blood may cause hemolysis. This accident does not occur since the practice of studying the blood of the donor in connection with the blood of the recipient has become common.

Experimental Laking

Laking of the blood is easily caused in vitro. The addition of distilled water, ether, any one of several salts in varying proportions, and many other substances cause the hemoglobin to be set free from the stroma. The stromata may be separated from the solution of hemoglobin by centrifugation and the hemoglobin removed for study. The stromata may be washed by adding water or other solvents to the sediment and centrifuging the mixture; the process can be repeated until the hemoglobin and such extractives as may be desired have been carried away by the supernatant fluid.

Structure of Hemoglobin

The solution of hemoglobin has been studied in many laboratories. Hemoglobin is a conjugated protein with a formula which varies somewhat according to different chemists, but is about C_{758} H_{1203} N_{195} Fe S_6 O_{218} with a molecular weight of about 16,669. Recent analyses seem to indicate that there are four atoms of iron instead of one atom in each hemoglobin molecule.

Hemoglobin can be crystallized from the solution and the crystals so obtained vary for different animals. Related animals show some similarity of hemoglobin crystals, and this fact can be used as a basis for the classification of animal species. There are so many variations in the crystals due to variations in technique that medico-legal questions are not properly decided upon such evidence.

Various analyses have been made of the hemoglobin of different mammals. The exact figures vary, as do the figures given for human hemoglobin, according to the methods of analysis which are employed. The molecular weight of hemoglobin in the blood of many animals reported varies from 14,780 to 18,370, with one atom of iron in each molecule in each case.

Derivatives of Hemoglobin

Hemoglobin is easily broken down into two molecules, hematin and globin. Hematin makes up about four per cent and it contains all of the iron of the hemoglobin molecule. The globin makes up about ninety-six per cent and it contains all of the sulphur of the hemoglobin. Both these substances can be broken down into simpler molecules and these are often of physiological and pathological interest.

Hematin is found in the spleen and the blood of patients with malaria. Hematin, injected into the veins of an animal, lowers the blood pressure, causes chills followed by fever, and causes petechial hemorrhages in the kidneys.

Hemin crystals are formed by treating blood with glacial acetic acid; the procedure varies according to the amount of the crystals desired and the use which is to be made of them. Hematin can be made from the hemin crystals by treating them with alkalies. Hemin has the probable formula of C_{33} H_3 O_4 N_4 C_1 Fe. Hematin has a probable formula of C_{32} H_{30} N_4 O_3 Fe.

Hematin can be treated with sulphuric acid and thus the iron is removed from the molecule. This iron-free pigment is known as hematoporphyrin, which has a deep purple color in solution. Its presence in the decomposed blood around bruises or other hemorrhagic tissues pro-

duces characteristic purple tints. Hematoporphyrin has the formula of C_{34} H_{38} N_4 O_6, and is isomeric with bilirubin. Both hematoporphyrin and bilirubin are present in the blood plasma and in the urine in traces, normally, and either or both may be increased under certain pathological conditions.

Hematoporphyrin combines to form several closely related compounds. These are found in the urine of patients with rheumatism, several disorders of the liver, tuberculosis, malaria, and, occasionally, in other high fevers. The use of certain drugs, such as trional, sulphonal, veronal, the salicylates and many of the coal-tar products is sometimes followed by hematoporphyrinuria. Hematoporphyrin has a peculiar property of increasing the sensitivity of the skin to light. Patients with hematoporphyrinemia may be seriously injured by exposure to ordinary daylight and may suffer serious skin lesions as a result of exposure to sunshine.

Hemofuscin is an iron-free brownish pigment derived from hemoglobin. The name has been applied to several quite different compounds by different investigators and hence has fallen into disuse.

Hematoidin is an iron-free pigment derived from hemoglobin. It is often present in old accumulations of blood within the body. Its presence indicates that hemoglobin can be broken down by the cells and fluids of the tissues in various regions of the body, the iron-containing moiety being carried away to be used again and the hematoidin left with the clot, useless and not seriously injurious. Hematoidin is probably identical with bilirubin.

Methemoglobin is isomeric with hemoglobin. It is produced spontaneously in blood which has been allowed to stand for some weeks, or it can be produced in vitro by the action of potassium ferricyanide or potassium permanganate. Methemoglobin has a brownish color and it is occasionally found in old hematomata.

Hemochromogen is prepared from reduced hemoglobin by the action of caustic alkalies. This is an iron-containing pigment which can be combined with any one of a long list of protein molecules to form hemoglobin, and the hemoglobin so prepared can be induced to take up or to give off oxygen in much the same manner as that shown by the original hemoglobin under similar conditions. Hemochromogen in solution has a bright cherry-like tint somewhat resembling that of carbon-monoxide hemoglobin. This tint also appears occasionally around bruised or hemorrhagic areas of tissues.

Hemosiderin is a yellowish brown pigment containing iron, formed from hemoglobin within the body. It is deposited as yellowish granules

which are soluble in the body fluids only with difficulty and therefore are eliminated very slowly. Hemosiderin is found in the Kupffer cells of the liver and in the phagocytic cells of the blood very scantily under normal conditions but in considerable amounts in any severe anemia. Hemosiderin is especially abundant in the Kupffer cells of the liver and in the endothelial cells of the spleen during exacerbations in pernicious anemia.

Bronzed diabetes is a peculiar disease characterized by the deposit of hemosiderin and an iron-free pigment which is probably hemofuscin in the tissues of the body. The skin assumes a peculiar bronze-like tint not found in other diseases. Sugar metabolism is greatly disturbed and typical symptoms of diabetes mellitus may occur.

Sulphemoglobin may be formed within the erythrocytes by allowing hydrogen disulphide gas to pass through the blood whose coagulation has been prevented. The anemia which is associated with intestinal putrefaction is said to be due to the injury of the red cells by the hydrogen disulphide produced by the putrefaction of the protein of the food in the small intestines.

Analogues of Hemoglobin

Myohematin is the pigment of muscle cells. It is not quite identical in structure with hemoglobin but it performs the same function. It provides the muscle cells with nascent oxygen and thus facilitates the rapid oxidation processes essential to muscular activity. Myohematin forms a stable compound with carbon monoxide, as does hemoglobin, and is thereby rendered useless. The weakness and the marked fatigue on relatively negligible exertion which characterize chronic carbon monoxide poisoning are in part due to this fixation of myohematin. The digestion and absorption of the molecules thus destroyed is associated with considerable toxemia.

The myohematin of the meat in food is a valuable source of the globin necessary for the formation of the hemoglobin of new blood cells. It must be remembered that the globin of senile blood cells is not saved, as the iron-containing part of the molecule is, and that fresh supplies of globulins are essential to the formation of the new supplies of erythrocytes constantly required by the body. The copper, magnesium, potassium and other inorganic elements needed for the synthesis of hemoglobin are also present in lean meat used as food.

Several oxygen-carrying pigments are present in the body fluids of invertebrates. Hemocyanin or oxyhemocyanin occurs as a group of related compounds present in the blood or other body fluids of molluscs, cephalopods and crustaceans of fresh water and of salt water. Com-

pounds of the hemocyanin group are biological precursors of hemoglobin, though there is not any reason for supposing that hemoglobin was derived from any one of the group. In all the hemocyanin group copper has the place occupied by the iron in the hemoglobin molecule. The different compounds of this copper-containing oxygen-carrying pigment differ chiefly in the effects produced in their solutions by various reagents. For example a solution of hemocyanin derived from the snail becomes yellow on standing or after aeration with nitric oxide. A solution of hemocyanin derived from the lobster does not form the yellow compound even after long aeration with the same gas. Other animals produce hemocyanin which gives other reactions, hence there must be at least several different but closely related compounds included as hemocyanin. Copper does not seem as efficient as iron in the transportation of oxygen.

Chlorocruorin is a green pigment found in worms and a few marine animals. This pigment contains iron and it performs the respiratory functions characteristic of hemoglobin in higher animals.

Substances Resembling Hemoglobin

Both animals and vegetables contain many pigments which are concerned in the oxygenation and the oxidation processes of living cells, and in producing colors which have various functions.

Chlorophyll and chromophyll are colored substances occurring widely in the vegetable kingdom and in the bodies of certain animals of very simple structure. Chlorophyll is especially efficient in the utilization of the energy of the sun's rays in the manufacture of certain glucosides which in turn form starches and sugars in the sap of living plants. These substances, in their pure form, do not contain iron, but iron-containing molecules are always intimately associated with them and it is by virtue of the iron-containing molecules that the cholorophyll is able to build up the glucosides from carbon dioxide and water under the influence of sunshine. The iron of these vegetable compounds is an important factor in the synthesis of the hemoglobin of animals. Sulphur and magnesium are other very important atoms associated with chlorophyll and hemoglobin.

The Sources of Hemoglobin

The total amount of iron in the whole body is only about six grams, and of this only about four-fifths are present in the hemoglobin. This iron is used very economically, over and over, in the formation of new blood cells. Very minute amounts of iron in the daily diet are sufficient.

Restricted diets often fail to provide the globulins necessary for the synthesis of hemoglobin but it is rare indeed that a diet is too restricted to provide enough iron.

Diets rich in hemoglobin and myohematin provide the most efficient source of hemoglobin, and such diets best facilitate regeneration of blood in animals made anemic by bleeding, by a long period of iron-free diet or by the administration of drugs which produce anemia. Liver, kidneys, pancreas and muscle substance seem to be the most useful foods under such circumstances. Liver and meat have been found the most useful diets for promoting the regeneration of hemoglobin in human patients with nearly all forms of anemia. Hemoglobin and myohematin are derived from the flesh of animals.

Diets rich in the globulins and albumins but relatively poor in iron are also efficient in supplying the elements required for regeneration. A diet of milk or of casein has little iron but animals are able to regenerate hemoglobin rapidly on such a diet, and patients with secondary anemia often recover speedily on a diet exclusively of milk. This fact is due to the presence of a fairly abundant supply of iron in the patient's own tissues, which he is unable to utilize because of a lack of globulins and albumins. The milk diet provides these necessities in a form which is easily digested and absorbed and which avoids toxemia in considerable degree. Milk is not good as an exclusive diet for more than a few weeks because it does not contain all the substances necessary for the formation of hemoglobin nor for the maintenance of the needs of the body. Even in infancy an exclusive milk diet does not best provide for the needs of the growing body for more than a few months, at most, and babies seem to do better if other foods are given them at an early age. The anemia which occurs in children kept too long on an exclusive milk diet disappears rapidly when fruits and vegetables are added to the diet even though these may not contain any great amount of iron. The importance of adequate amounts of copper, sulphur, magnesium and potassium for the building of red cells should always be kept in mind.

At birth the Kupffer cells of the liver are abundantly supplied with iron derived from the maternal blood. The blood, bone marrow and spleen of newly born babies all have a high content of hemoglobin and of other iron-containing compounds. This supply of iron is enough to maintain a normal amount of hemoglobin and myohematin for several weeks or months; that is, during the period normally associated with an exclusive milk diet.

Except as animals eat other animals, the iron must be derived from vegetables. Chlorophyll and chromophyll are iron-free vegetable pig-

ments which are closely associated with iron-containing molecules in vegetable cells. Without the iron the chlorophyll is unable to utilize the rays of the sun, carbon dioxide and water in the synthesis of the glucosides. Chlorophyll is formed around the pyrrole ring, which also is an important part of the hemoglobin molecule. Vegetable cells containing iron, sulphur, copper, magnesium and chlorophyll are an important source of the hemoglobin and myohematin of the animal body.

Biology of Iron

Vegetables derive their iron from the earth, in the form of ferric or ferrous salts. These are sufficiently soluble to be carried in the sap and to be utilized by the vegetable cells, especially the leaves, in the synthesis of those substances necessary to the activities of the chlorophyll granules. The oxygen-carrying function of the iron in these compounds is as important in vegetable economy as is the oxygen-carrying function of the iron of the hemoglobin of the animal. In the case of the vegetables the iron is necessary to the utilization of the energy of the sunshine in the synthesis of the glucosides. Iron does not seem necessary, however, in the further synthesis of the sugars and the starches from the glucosides.

Even in the earth, before the iron is taken up by the vegetables, its oxygen-carrying activities are economically useful. There is a constant series of changing chemical relations of iron in the earth. Varying combinations of iron with oxygen are especially noteworthy. For example, ferrous silicate may be decomposed by carbonic acid into silica and ferrous carbonate; this absorbs oxygen, liberates carbon dioxide and forms ferric oxide. If decomposing organic matters are at hand, these take up oxygen from the ferric oxide and ferrous carbonate is formed, to be again oxygenated and decomposed. Sulphur and iron both act as oxygen carriers in other groups of chemical reactions by means of which the oxidation of organic matter is facilitated. Copper undergoes a similar series of oxidation and reduction processes. As a result of these alternating oxidation and reduction processes, nascent oxygen is continually being set free in the earth. Nascent oxygen is especially active in promoting oxidation. The growth of bacteria and plants is thus facilitated, and the decay of organic animal and vegetable matter is hastened.

Variations in Hemoglobin

Variations in the amount of hemoglobin follow the variations in the number of red cells rather closely. Variations said to be due to sex are really variations due to habits of life. Men and women who are

healthy and accustomed to active exercise or to hard work in the fields have blood with high hemoglobin content. Men and women who lead sedentary, indoor lives have blood with a low hemoglobin content. Since women usually are less active and usually spend more time indoors, the hemoglobin content of the blood of the average woman is about ten per cent less than the blood of the average man.

Variations Due to Age

Variations in hemoglobin due to age follow variations in the red cell count to some extent. The concentration of the blood immediately after birth is associated with very high hemoglobin content; from 180 grams per liter to 213 grams per liter, as reported from various laboratories. The red cell count is also very high at this time. The amount of hemoglobin diminishes gradually until about the fifth year, when the child may show only 110 to 115 grams per liter of blood. This is associated with a red cell count of about 5,500,000 per cubic millimeter. The red cells are smaller during childhood than they are in babyhood or during adult life. In any study of the blood of a child it is necessary to keep this peculiarity in mind, and to base the percentages and the color index upon the figures normal for the age of the patient.

The following table gives the number of grams of hemoglobin per liter of blood found in Los Angeles in normal and practically normal individuals of different ages. The sexes are about equal. The determinations were made for fifty or more individuals in each group.

Age	Grams hemoglobin per liter of blood
5 years to 10 years	112-115
11 years to 14 years	114-120
15 years to 20 years	120-130
20 years to 30 years	130-140
30 years to 60 years	135-150
60 years to 80 years	130-145

Individuals between sixty and eighty years were considered normal if no evidence of organic disease was noted. Naturally these persons were subject to some infirmities due to old age.

Diurnal Changes

Daily variations follow variations in the red blood count. In order to secure logical statistics for comparison of the hemoglobin of different individuals or of the hemoglobin of different individuals or of the hemoglobin of the same individual under different circumstances it is

necessary to examine the blood at the same time of the day. When the blood is examined at different hours the error due to this fact may be as much as ten per cent of the reported amounts.

Variations Due to Altitude

Variations in elevation cause variations in hemoglobin similar to the variations in the erythrocyte count due to the same conditions. On ascending to high altitudes the hemoglobin increases less rapidly than do the red cells. On descending the hemoglobin diminishes as rapidly as do the red cells. The color index thus is low during the few days following elevation and this is probably due to the rapid formation of new red cells. The color index remains unchanged during the few days after descent because new red cells are not formed rapidly.

The increase in hemoglobin occurs in animals subjected to diminishing oxygen tension, and in human subjects for whom the same tests are made. For each fall of 100 millimeters of mercury in the atmospheric pressure the hemoglobin increases about ten per cent.

Conditions Necessary for the Development of Normal Hemoglobin

In order that hemoglobin may be properly synthesized several conditions are necessary. The last steps in the building of hemoglobin take place within the erythrocyte stroma, and this stroma is essential to the process. It is not essential that the red blood cell be itself of normal size or form but it seems to be necessary for the meshes of the stroma to be at least approximately well built. The cholesterin, lecithin and other lipoids of the stroma are derived chiefly from the meat and other animal proteins of the food. Vegetable proteins are utilized by most people with some difficulty.

Hemoglobin differs from chlorophyll in its relations to sunlight. Without sunlight chlorophyll is not developed. Hemoglobin can be developed in the entire absence of sunlight. It is true that moles, gophers, ground squirrels and other burrowing animals are exposed to the sunlight at least for short periods of time. Horses, mules and burros which are born in mines and live all their lives in darkness have normal hemoglobin averages. There are mines in which horses have lived for several generations in darkness which is complete except for the candles of the miners, and yet they have normal hemoglobin.

The food must include some of the iron-containing molecules, and must contain an abundance of the protein suitable for providing the globin. It should be remembered that the iron-containing part of the hemoglobin forms only about four per cent of the hemoglobin mole-

cule, and that the iron is preserved to be used over and over again; the globin, on the other hand, forms about ninety-six per cent of the hemoglobin molecule, and this is used as food for the body and as a source of various secretions; it is not, apparently, utilized even a second time in the building of hemoglobin. The necessity of an abundant supply of the proteins from which the globin part of the hemoglobin molecule can be synthesized is evident.

The protein foods must be properly digested. In ordinary conditions this presents little difficulty. In pernicious anemia the lack of hydrochloric acid in the gastric juice prevents adequate digestion of the proteins, and this seems to be an important factor in the pathogenesis of the disease.

The severity of disease of the digestive tract does not bear any direct relation to the degree of anemia produced by the disease. Very severe ulcers of the stomach or the duodenum may be present in patients whose hemoglobin is almost or quite normal; in other cases either a small or a severe ulcer may be associated with marked anemia. Occasionally very severe anemias, presenting marked resemblance to pernicious anemia, may be associated with ulcers of the stomach which seem to be negligible in extent. This relation does not always depend upon the presence or the absence of normal hydrochloric acid secretion. Since patients with pernicious anemia lack hydrochloric acid in the gastric juice, it is not always possible to determine whether the gastric disease is the cause of an atypical anemia resembling the pernicious type, or whether the condition is really Addisonian anemia with the related achlorhydria. There is no reason for difficulty in diagnosis when the blood picture is definitely megaloblastic or is definitely of the secondary type.

Bony Lesions and Hemoglobin

Lesions affecting the digestive tract often cause a severe anemia of the secondary type. Lesions of the fifth thoracic vertebra, neighboring vertebrae and ribs are usually associated with hyperchlorhydria and ultimately with gastric ulcers. Anemia is not usually severe in these cases. Mild bleedings are always present in the stomachs of laboratory animals with the lesions mentioned but they do not usually become anemic. Probably the extravasated blood is digested and absorbed in these cases.

Lesions of the seventh thoracic vertebra and neighboring vertebrae and ribs are associated with hypochlorhydria, gastric atony and malnutrition. Anemia is usually present in these cases but is rarely very severe. In animals it is always of the secondary type, but in hu-

man patients in whom the lesions have been present a long time there may be factors in the blood picture which suggest pernicious anemia.

Lesions of the tenth thoracic vertebra and neighboring vertebrae and ribs affect the circulation through the liver. The bile pigments and the bile salts are mildly toxic in small amounts. The bile salts cause laking of the blood in small degree even while the blood is circulating through the vessels. The hemoglobin thus set free is eliminated by the kidneys. The anemia due to this elimination of hemoglobin is not usually severe and is of the secondary type. In none of our cases has there been any evidence of a megaloblastic type of anemia associated with cholemia.

Rib lesions are associated with disturbed nutrition of the local areas of red bone marrow. Without good circulation of the blood through the hematopoietic tissues, the hemoglobin is not normally provided for the red blood cells. When any considerable area of red bone marrow is affected by lesions the blood shows low hemoglobin, very low color index and many immature forms of red cells and of granular white cells. After correction of the lesions the red blood cells soon contain normal amounts of hemoglobin and the immature forms disappear.

The relation of certain of the internal secretions to the synthesis of hemoglobin has not yet been explained. It is, however, true that patients suffering from certain forms of goiter and from diseases affecting the ovaries or the testes often suffer from severe grades of secondary anemia, and that with recovery of normal conditions of these glands the hemoglobin returns to normal. The circulatory disturbances of these glands, due to bony lesions, are often associated with anemia which seems very much more profound than is warranted by the relatively mild pathological changes in the tissues affected. On correction of the lesions the hemoglobin returns to normal.

Fate of the Hemoglobin

As the red cells become senile and inefficient they are removed from the circulation. They may become fragmented as they are being carried in the blood stream, and they are easily though not frequently recognized in smears made from the capillary or the venous blood. When there is increased destruction of the red cells, for any reason, the fragmented forms may be quite abundant in the capillary blood. The fragments of erythrocytes are ingested by the endothelial cells of the liver, spleen and other tissues, the hemoglobin is broken down into an iron-containing moiety, which is carried by the blood to the red bone marrow, and an iron-free pigment, which is transformed into bilirubin

and related pigments. The Kupffer cells in the liver are especially efficient in the secretion of the bile pigments but the cells of the reticulo-endothelium system anywhere perform this function normally. When the liver of the human subject is diseased, or when the liver of a laboratory animal has been removed, the cells of the other parts of the reticulo-endothelial system may handle the hemoglobin metabolism with apparently perfect efficiency.

The pigments of the urine and the bile are chiefly, if not altogether, derived from the iron-free pigment of the hemoglobin. These pigments are easily replaced from the food materials ingested.

Hemoglobin set free in the blood plasma by the disintegration of the red cells is excreted from the body as such. The hemoglobinuria sometimes associated with severe forms of malaria is due to the destruction of the red cells by the malarial parasite with resultant hemoglobinemia. Laking of the blood in the circulation as a result of poisoning by certain reptiles or by certain drugs is also associated with hemoglobinuria.

Hemoglobin freed from the cells in stagnant blood, as in old hemorrhagic areas or in hematomata of long standing, undergoes various changes into methemoglobin, hematoporphyrin and other pigments. Ultimately these are absorbed by the blood and the lymph if there is any circulation of these fluids through the bloody accumulations, and they are excreted in the urine and the bile. Cells of the reticulo-endothelial system ingest the cells of extravasated blood in their immediate neighborhood, change the hemoglobin into an iron-containing and an iron-free moiety, transform the iron-free moiety into bilirubin or some related compound and allow the iron-containing molecules to be carried away by the plasma and the lymph, ready to be utilized again in the production of hemoglobin in new red cells in the bone marrow. This economical utilization of the iron-containing part of the hemoglobin is not perfect, and there is a constant, though minute, excretion of iron in the bile and the urine. The iron found in feces is chiefly a part of the food which has not been absorbed into the body at all; the traces of iron found in the bile are excreted with the feces from the body.

CHAPTER III

LEUCOCYTES

General Discussions

The cells of normal adult human blood include few forms, easily classified and with few or no intermediate types. Immature and abnormal specimens of blood present a great many forms of blood cells often classified only with difficulty and including many atypical and intermediate forms.

Normal adult human blood cells include two chief groups, hyaline and granular. The hyaline cells all have hyaline basophilic protoplasm in which no granules are found by the usual methods of preparation, while the protoplasm of the granular cells contains granules which present typical characteristics in size and in staining reactions.

Classification

Hyaline cells are further divided into lymphocytes, which make up the largest number of these cells, and monocytes. In embryonic and abnormal blood there are also endothelial cells and myelocytes which have hyaline basophilic protoplasm. The lymphocytes are further divided into small, medium and large. Extremely large hyaline cells and atypical forms are found frequently in the blood of lower animals, in embryonic human blood, and in adult human blood under abnormal conditions associated with other reversionary traits.

Granular cells are divided according to the nature of the granules into neutrophiles, in which the granules are small, abundant and feebly eosinophilic or neutrophilic; eosinophiles, in which the granules are larger and are intensely eosinophilic, and the rather scanty basophiles, in which the granules are large and are intensely basophilic. Amphophiles, in which the granules are large and may take eosin or basophilic dyes according to the concentration or reaction of the staining solutions, or can be so stained as to show eosinophilic and basophilic granules in a single cell, are extremely rare in normal adult human blood. They are often found in the blood of lower mammals, in embryonic human blood, and in adult human blood under certain abnormal conditions. (Plates III, V, VI)

The lymphocytes of the blood are formed in the lymphoid tissues of the body; there are a few which arise from other areas. The granular cells are formed, during normal adult life, in the red bone mar-

row. During embryonic and fetal life and under certain abnormal conditions the white cells may be formed in other areas of the body including the spleen, hemolymph glands, and in other viscera, and even in the fatty tissues of the mesentery and omentum. The extent to which this process of leucocytopoiesis may occur in the various organs of the body has been the subject of much discussion. Under those conditions in which such extramedullary blood formation occurs, there is a great amount of infiltration of the various tissues with white cells. Cells undergoing division in the circulating blood are fairly common under the same circumstances. It is quite possible that in such cases leucocytopoiesis occurs wherever and perhaps whenever the cells are permitted to find rest and food, and that accumulations of such cells result whenever they are not speedily swept away. In vertebrates below mammals and in certain of the lower mammals the leucocytes are formed in extramedullary tissues throughout life.

Functions

The functions of the leucocytes include many activities. By virtue of their powers of ameboid movement and phagocytosis they are concerned in the absorption of fats and probably of protein and sugars from the intestinal tract. They serve as reserves for proteins and carbohydrates in the body. They ingest foreign particles and render them harmless to a surprising degree. They neutralize toxic substances whether these are produced by the cellular activities of the body itself, are produced within the body by parasites, are taken into the body with food or injected as therapeutic measures. They are not always able to do this perfectly, as is easily apparent, but their efficiency is surprising in many instances. They ingest small particles of foreign materials, bacteria, micro-organisms of several kinds, and the cells derived from malignant neoplasms and from diseased tissues. It is not possible to say to what extent life may be preserved by these activities.

When any disease associated with profuse drainage of pus is present, the number of leucocytes lost in a day may be more than the number present in the total blood stream at any one time. Yet this rapid formation and loss does not seem to exert any very serious drain upon the blood forming tissues; these must, therefore, be fitted to produce daily a number not very far below the total number in the circulation at any one time, normally or abnormally. The dissolution of these white cells must add considerably to the protein content of the blood, and there is good reason to suppose that this elaboration of the nitrogenous elements of the food is one of the functions of the white

cells. No doubt the storage of these protein substances within living cells, thus maintaining a normal level of the soluble proteins of the blood, is a very important function of the cells.

Oxidase Reaction

It is possible to determine with a fair degree of accuracy whether the cells arise from bone marrow or not, by the use of the oxidase reaction. This test is rarely of value in diagnosis but it has given some interesting information about blood cells.

Smears stained with alpha-naphthol and dimethylparaphenylene-diamine show this reaction. By means of an oxidizing ferment, present in all cells which are formed in the red bone marrow by the myelocytoid cells, this stain produces indophenol blue. This reaction is not given by cells derived from lymphoid tissue, even that which is located within the bone marrow. Hence the reaction has a certain value in determining the origin of non-granular cells and of atypical granular cells under certain conditions. The granular basophilic cells (mast cells derived from the tissues) also fail to give this reaction.

Large hyaline cells with large nuclei, sometimes showing karyokinesis, may be found under any circumstances associated with increased activity of the blood-forming organs. They may give the oxidase reaction by which they can be distinguished from the large hyaline cells of the splenic pulp and inflamed lymphoid tissue. Immature hyaline myelocytes do not give the oxidase reaction.

Large hyaline cells, with protoplasm showing some affinity for acid stains, not giving the oxidase reaction, may be found in the blood of pregnant women, at about the fifth month especially. These are probably derived from the placenta.

Tumor cells may occasionally appear in the blood. They are rarely so large as the large hyaline cells, though they may be even larger. They have deeply staining nuclei, usually round, sometimes showing abnormal karyokinetic figures. Their protoplasm is acidophilic. They do not give the oxidase reaction. They cannot be considered of marked significance unless they are present in considerable numbers, show abnormal karyokinetic figures, and are associated with other symptoms characteristic of tumor.

Normal Count

Normal adult human blood contains a variable number of leucocytes, generally between 5,000 and 8,000 per cubic millimeter of peripheral blood. The small lymphocytes are the smallest of these, and the large mononuclear hyaline cells the largest. The neutrophiles are most abundant.

Leucocyte Counts, Normal

In the climate of Los Angeles we find average normal counts of 7,500, varying from 4,800 to 9,500 in persons apparently in excellent health.

Neutrophiles	60% to 70%	5,000 per cu.mm.
Small hyaline cells	18% to 33%	2,000 per cu.mm.
Large hyaline cells	4% to 8%	400 per cu.mm.
Eosinophiles	.5% to 2%	100 per cu.mm.

The total leucocyte count, the differential count and the actual numbers of the various cell groups vary almost continually both in normal and in abnormal persons. The granulocytes seem to be thrown into the circulation, normally, in showers. The utilization and destruction of these cells progresses fairly steadily. Either the formation or the destruction of these cells can be hastened or diminished by various normal physiological conditions. In disease many factors may be active which increase, diminish or modify these physiological factors. Increased formation or increased destruction may be due to the action of factors not present in health, or, at least, not concerned in blood formation and blood destruction in health.

The manner in which the white cells are thrown into the general circulation varies greatly for different persons. In one family studied in our laboratories the showers of neutrophiles appeared at intervals of about two weeks. The period during which the heaviest shower of leucocytes was passing into the peripheral blood was a time of physical and psychological depression, not marked but definitely recognizable. In this family the hyaline cells remained constant, varying only slightly from time to time, as in other people.

Normal Variations in Number

Daily variations have been determined by several workers. Two high tides have been described, one occurring in the early afternoon, the other soon after midnight. There is some difference of opinion as to the exact hour at which the tide is highest. Rises of 1,000 to 2,500 cells have been reported for this tide. The tides occur with reasonable promptness no matter whether food, rest, exercise, sleep and other physiological conditions remain regular or whether these habits are subjected to considerable modification. A low blood pressure has been reported for these same hours. The afternoon tide should be taken into consideration when blood counts are made for the sake of diagnosis at that time of the day. The rise which occurs after midnight is less important because it is only in emergencies that counts are made at

that hour. In emergencies differences of 2,000 white cells are not apt to be important. Still, the fact that a high tide of white cells does occur at that time of the night should be kept in mind. For accurate work the cell counts should always be made at the same time of the day for each patient, and, as nearly as is practicable, during the early afternoon hours for all chronic patients.

The distribution of the white cells within the peripheral blood of different parts of the body may be changed considerably by varying the vasomotor control of the blood vessels. For this reason it is necessary to be very careful to avoid irritation of the skin when preparing to take the blood for a count. The use of rubbing to redden the skin in order to secure blood more easily, or of irritating, heating or chilling solutions for the sterilization of the skin may cause a variation of 2,000 or more cells per cubic millimeter of blood. Vasoconstriction of a part causes diminished white cell count; vasodilatation of a part causes increased cell count.

Leucocytosis

The term leucocytosis applies to a temporary and marked increase in the leucocyte count, and it is commonly used with respect to the neutrophiles only. Increase in the lymphoid elements is called lymphocytosis or lymphemia and increase in the eosinophiles is called eosinophilia. General or neutrophilic leucocytosis, lymphocytosis or eosinophilia may be caused by physiological or pathological states. The physiological causes of leucocytosis should be thoroughly understood in order that pathological variations may be properly interpreted. Increase in the number of any one cell type usually is associated with at least a slight rise in other cells, though this is not invariably the case. Marked increase in any one group of cells naturally diminishes the percentages of other cell groups. For this reason it is not wise to draw conclusions from a differential count alone.

Active leucocytosis (Ehrlich) is an increase in the neutrophiles or phagocytic cells, ameboid cells which respond to chemotaxis.

Passive leucocytosis (Ehrlich) is an increase in hyaline cells, supposed to be passively washed out of lymphoid tissue.

These terms are not now in general use. The hyaline cells are often ameboid and emigration of these does occur.

Mixed leucocytosis is a term which has been used in different ways. It is now used to indicate leucocytosis in which many myelocytes are present. The condition is most marked in young children. In any severe or prolonged leucocytosis myelocytes usually appear, especially after severe hemorrhages.

Pseudoleucocytosis (Emerson) is a term applied to certain changes in white cells not associated with increase in blood count but which have the same significance as true leucocytosis. These include iodophilia, degenerations of leucocytes, increase in the relative number of neutrophiles, presence of myelocytes and other immature forms, fragmentation of nuclei, fragmentation and erosion of protoplasm and irregularities of staining.

Increase in the total leucocyte count without variations in the percentages of cell types occurs under several physiological conditions. Dilatation of the capillaries (as, for example, by too great rubbing in an effort to clean the skin before making the prick to secure blood) increases the total leucocyte count without causing any recognizable or constant change in the cell relations. The leucocytoses associated with digestion, pregnancy, cold bathing and massage are of this general type.

Digestion Leucocytosis

Digestion leucocytosis occurs after a meal heavy in protein food, taken after a short fast or after a few days of vegetarian food. This form of leucocytosis does not appear in vegetarians, nor after a meal of low protein content; it does not appear in any case in a person habitually on high protein diet. It is often noted in diabetics. It does not appear in gramnivorous animals. It occurs markedly in babies placed on cow's milk for the first time. Persons suffering from habitual constipation do not usually show this digestion leucocytosis. Digestion leucocytosis fails to occur if the gastric juice contains very little or no free hydrochloric acid, in which case the digestion and absorption of protein foods are delayed. From all these facts it has been supposed that digestion leucocytosis is a reaction to the foreign protein absorbed from the intestinal tract. The leucocytosis thus caused is often general in type, but a moderate neutrophilia is fairly common. This leucocytosis appears within the first hour, increases for two to four hours, sometimes to one and one-third the normal count, then gradually diminishes until the normal count is regained, about six hours after the test meal.

Individuals who are constantly over-fed with an abundance of protein foods usually show a total leucocyte count above 10,000 per cubic millimeter in this climate.

Leucocytes in Fasting Blood

The first day or two days of fasting or starvation is associated with a moderate rise in the leucocyte count, chiefly due to increase in the neutrophiles. After the second or third day the neutrophiles fall rap-

idly for a day or two, then slowly for as long as the fast is maintained. The hyaline cells fall slowly and steadily after the second or third day. The eosinophiles are relatively increased but not absolutely affected during the first week or so. During the absorption of muscle, in a long fast, the eosinophiles are absolutely as well as relatively increased.

Pregnancy

Pregnancy is usually associated with a mild leucocytosis, especially in primiparae. In our records of women who had had several counts during girlhood, before marriage, after marriage before pregnancy and during pregnancy, a slight increase in the total count was observed before menstruation and soon after the beginning of pregnancy. This leucocytosis has usually been general in type in normal cases, but in a few apparently normal cases and in most cases with mild complications the increase in cell count has been mostly due to increase in the neutrophiles. Moderate eosinophilia usually precedes menstruation and may be rather marked during pregnancy, especially at about the third and fourth months. At these times some congestion of the ovaries is probably present. Leucocytosis of normal pregnancy rarely exceeds 15,000 cells per cubic millimeter and usually remains below 12,000. Abnormal conditions often cause variations which are more marked than would be expected in non-pregnant women under the same circumstances.

Leucocytosis increases during labor, sometimes very considerably, and diminishes rapidly so that in uncomplicated cases within two weeks or less the leucocyte count is about normal. During involution there may be quite a marked increase in endothelial cells.

Exercise and Leucocyte Count

Very heavy work causes leucocytosis. After strenuous exercise with almost complete exhaustion counts of 14,000 to 25,000 leucocytes have been reported. The neutrophiles are more greatly increased than are other cells, though absolute eosinophilia is always present in some degree. Increase in the lipase of the circulating blood, most abundant in the lymphocytes, has been reported during fatigue.

Altitude

The leucocytes are increased by travelling to high altitudes, at the rate, approximately, of 1,000 cells for each 1,000 feet of elevation. The large mononuclears are more rapidly increased than are the neutrophiles. The count returns to normal within about ten days if the subject remains at the high altitude. If he descends within a few days

the leucocyte count drops to normal within about ten days. The person who lives at a high altitude, then descends to sea level shows a drop of about 1,000 cells for each 2,000 feet, and the count returns to normal within four or five days, if he remains at the low altitude. The leucocytopoietic tissues accommodate their activities to the demands of the individual without regard to altitude.

Leucocytosis due to the agonal state is now supposed to be of rare occurrence. Conditions which cause death are often associated with leucocytosis. With slowing of the circulation there is an accumulation of the white cells in the peripheral vessels.

Reaction Leucocytosis

Leucocytosis, usually mild in degree, follows tissue injury whether or not hemorrhage occurs. Post-hemorrhagic leucocytosis varies considerably, and bears no relation to the extent of the hemorrhage. Mild leucocytosis may be present after either severe or moderate hemorrhages, and marked leucocytosis, even to 20,000 or more, may be present after moderate or severe hemorrhages. This leucocytosis has been supposed to be due to a flow of tissue juices into the vessels, in which case there should be a lymphocytosis. Probably the flow of tissue juices into the vessels is associated with even more marked flow of the marrow cells into the circulation, in which case neutrophilic leucocytosis would, and actually does occur. Hemorrhages into the serous cavities (dural, peritoneal, pleural) cause leucocytosis amounting to two or three times the leucocyte count already present. This increase in leucocyte count begins almost immediately after the hemorrhage and continues more and more slowly for eight to twelve hours, when the numbers begin, at first very slowly, to recede. The normal number is reached during the succeeding three to five days, in uncomplicated cases. In doubtful cases two or three successive blood counts made during the six hours following the suspected hemorrhage indicate the leucocyte curve and may verify or eliminate the diagnosis of hemorrhage.

Post-operative leucocytosis commonly varies according to the amount of tissue injury sustained and may reach 20,000 or more. This leucocytosis bears no relation to the fever curve and it disappears within a day or a day and a half. Changing the packing on a wound also causes leucocytosis, mild and transitory unless there is considerable irritation of the tissues. This leucocytosis must not be confused with infectious leucocytosis. Post-operative leucocytosis disappears at about the time an infectious leucocytosis begins, and the infectious leucocytosis is almost always associated with fever and with other indications of infection.

Chloroform often causes transient and usually mild leucocytosis, but ether causes leucopenia more frequently.

Leucocytosis in Disease

Most acute pyogenic infections or febrile diseases cause leucocytosis. Low or absent leucocytosis may mean either very mild infection or very low resistance on the part of the individual. Diseases vary in their quality of arousing leucocytosis, as in their quality of arousing fever. In other words, the hematopoietic tissues react differently to different infectious agents and to different etiological factors.

Leucocytosis depends upon the amount of toxin absorbed, as a rule; with drainage of an abscess the count drops quickly in uncomplicated cases. Formation of exudate rich in pus cells is associated with higher counts than formation of non-cellular exudate, generally.

The height of the count alone bears no certain relation to the severity of the infection. A small boil may cause marked leucocytosis if the infectious agent is of marked malignancy and if the reaction of the patient is good. In one of our records a moderately acute exacerbation of a chronic appendicitis caused a rise of the leucocyte count from 11,000 to 32,000 cells within three hours. In another case of appendicitis with gangrene the count diminished from 25,000 to 3,000 cells within two days; death occurred two days later. Leucocytosis follows infection or other states usually causing leucocytosis only when the leucocytopoietic tissues are able to react to the demands made upon them. When the insult is too profound, or when the tissues are for any reason unable to react efficiently, then adequate leucocytosis does not occur. It is evident that in such conditions the prognosis is more gloomy for that reason. That the absence of leucocytosis is due to inadequate reaction and not to the mildness of the infection is suspected when there is iodophilia, increased nuclear average, fragmentation of the neutrophile nuclei with many nuclear pseudopodia, relative or absolute neutropenia, and the presence of many myelocytoid and endothelial cells in the blood.

It should be noted that the conditions mentioned as affecting leucocytosis also affect the temperature reactions to infection. In the following conditions leucocytosis parallels the temperature curve; pyogenic infections generally, erysipelas, empyema, renal abscesses, pulmonary abscess, acute bronchitis, pneumonia.

In the following infectious diseases leucocytosis is high in typical cases but does not parallel the temperature; acute cerebrospinal meningitis, acute follicular tonsillitis, acute poliomyelitis, cholera, pleurisy with effusion, anthrax, fetid bronchitis, tubercular meningitis.

Leucocytosis is not always present but usually develops at some time during the course of the following diseases: small-pox, typhus, rabies, endocarditis, pleurisy, gonorrheal arthritis, renal colic, gout, bronchiectasis and certain cases of influenza.

These various conditions, not primarily infectious, usually show marked leucocytosis with atypical characteristics.

Metastases from malignant neoplasms may invade the peritoneum, pleura or red bone marrow. In the last case the blood picture may resemble that found in the leukemias or may resemble that of pernicious anemia. Malignant neoplasms occasionally cause neutrophilic leucocytosis, more often lymphocytosis, and this usually disappears after removal of the tumor. Lymphocytosis, rarely neutrophilic leucocytosis, often follows treatment of cancer by radium. Leucopenia often follows this treatment, and usually follows treatment by X-rays. Ovarian cyst with torsion of the pedicle causes marked leucocytosis; this may be of diagnostic value. The myxedema which sometimes follows thyroid operations (less commonly nowadays) may be associated with leucocytosis reaching 50,000 or more.

Leucocytes are increased in patients with gastric hypersecretion, and diminished in patients with hypochlorhydria. Leucopenia is associated with achylia and leucocytosis with hyperchlorhydria, even in fasting subjects.

Acute intestinal obstruction causes leucocytosis, usually of the neutrophilic type in adults. Lymphocytosis occurs frequently in children with obstruction. This should not be above 20,000 in uncomplicated cases. If the neutrophiles are relatively increased on the second or third day, or if the count continues to rise, infection and gangrene are suspected.

Drugs used in the treatment of disease or inhaled or absorbed as an occupational condition may cause leucocytosis. Camphor is especially active in this connection. Leucocytosis follows administration of epinephridine hydrochloride. Immediately after injection all white cells rise, then the lymphocytes increase more rapidly, later the neutrophiles increase more rapidly. The reaction is due to the spasm of arterioles, venules and splenic capsule due to the drug.

Other drugs cause leucopenia; the most common of these are the coal-tar derivatives, lead, mercury, benzol, the chlorates generally, and arsenic. Counts below 1,000 are not rare in patients who have been treated with these, or with certain other less common drugs, or whose occupation causes such drugs to be absorbed or ingested with food.

Leucopenia

The term leucopenia is applied, usually, to counts below 4,000 cells per cubic millimeter of blood.

Leucopenia is characteristic of many cases of influenza; is present in typhoid fever after an initial leucocytosis; in peritonitis during the rapid formation of purulent exudate; in tuberculosis of the lymph glands and in miliary tuberculosis, and in pernicious and aplastic anemia. Leucopenia is also present in many cases of severe pyogenic infections to which the hematopoietic tissues are unable to react efficiently. Such cases are extremely serious.

Agranulocytic angina is a peculiar disease characterized by extremely severe infection of the throat and sometimes of other mucous membranes, in which the neutrophiles almost disappear from the peripheral blood. The hyaline cells which are increased at the same time seem completely unable to meet the emergency and the condition is almost inevitably fatal.

Radiation treatments given for leukemia, Hodgkins disease and other abnormal connditions may reduce the number of white cells to a dangerously low level, even to less than 500 cells per cubic millimeter of blood. Since the neutrophiles are so important in immunity their reduction may have serious results. Deaths from pneumonia during the course of radiation treatment for leukemia are not infrequent. Careful use of radiation therapy should prevent extremely low leucopenia.

Iodophiles

Granules which stain with iodine and which may be either glycogen or some other substance, as peptone or amyloid-precursers, are sometimes found in the leucocytes and in the plasma. Normal blood contains, sometimes, a few of these iodophilic granules, but they are rare. They occur most frequently in the polymorphonuclear neutrophiles, but may be found in any of the leucocytes, possibly in the platelets, and occasionally they lie free in the plasma.

Immature Iodophilia

Blood smears from a three-months fetus show iodophilic granules in the erythrocytes but not in the leucocytes. They are rarely present in the nucleated red cells, and, when present, they are round, very scanty, and lie near the nucleus. In the normoblasts of this fetal blood in which the nucleus is undergoing solution several rod-shaped iodophilic masses lie near the nuclear remnants. In erythrocytes which have lost their nuclei rod-shaped and round iodophilic granules may lie anywhere in the protoplasm.

Iodophilia in Disease

The chief diagnostic significance of iodophilic granules is their presence in considerable numbers in septic conditions, especially when the process is well developed. They are not found in the earlier stages of infection.

Iodophilia is found in the following diseases: Abscesses, septicemia, empyema, pneumonia, septic peritonitis, pyonephrosis, tonsillitis, gonorrhoeal arthritis, gonorrhoeal or streptococcal salpingitis, purulent conditions anywhere in the body; and in gangrene, especially that of hernia or intestinal obstruction.

They have been reported also for pernicious anemia, myelogenous leukemia, ovarian cyst with torsion of pedicle (in which case there is leucocytosis but not infection), and in cerebral abscess. They are not present in cerebral tumor, nor in diphtheria or tuberculosis unless secondary pyogenic infection occurs. They are absent in early typhoid but may be present after the second or third week.

They are present in cases in which there is absorption of degenerating nitrogenous substances within the body, and they may be of use in differentiating abscess and benign tumor; or syphilitic from other forms of secondary anemia; or in the recognition of obscure pyogenic foci. After drainage of the pus or resolution in pneumonia the granules disappear very rapidly.

Probably normal leucocytes contain glycogen in transient and varying amounts, according to the sugar requirements of the body. Under the abnormal conditions mentioned, however, the granules become more stable and are easily found in the blood smears.

Normal blood stains a diffuse, pale yellow with iodine. The nuclei are lighter in tint. Rarely iodophilic granules are found in the plasma or platelets of normal blood. A positive reaction is shown by deep, diffuse brownish color in many of the leucocytes, and by a recognizable number of brownish granules in the leucocytes, platelets and plasma.

Endothelial cells in the blood and fixed cells of the reticulo-endothelial system contain glycogen or some related substance in granules and also in a more diffuse form. There is no apparent relation between the degree of iodophilia or the number of iodophilic cells and the amount of sugar in the blood.

Fragmented Cells

These cells have received various names, — smudged cells, basket cells, fractured or fragmented cells. In smears which are carelessly made many of the cells are injured; they may be spread out and smeared over the slide, or may be broken into fragments varying in

size. They are of no significance under such circumstances. If the slides are scrupulously clean and the smears properly made, such cells are found only in very small numbers in normal blood. In the blood of persons who are very weary they are increased. Blood taken late in the afternoon shows a larger proportion of such cells than does morning blood, and the number in abnormal blood increases during the day and under the influence of fatigue as is the case in normal blood.

Phagocytosis and Gregariousness

Many of the functions of the white blood cells are performed through the medium of their ameboid movements. A flowing movement of protoplasm is one of the most common of vital characteristics, and by means of this property white blood cells ingest and carry fat and glycogen particles and protein molecules or radicals, thus facilitating absorption and transmission of food into the body tissues from the intestinal tract, and from one part of the body to another. By means of ameboid activity bacteria and other foreign substances are ingested, to be digested and rendered harmless. By means of the ameboid activity of blood cells, plasma cells or histiocytes, wounds are repaired, and there are other activities whose relations are not yet well understood in which phagocytosis seems to be important in physiological and pathological conditions. (Plate II)

Phagocytosis and diapedesis are associated in the reactions to foreign bodies and infection. By means of diapedesis the white cells accumulate around foreign bodies and in the immediate vicinity of various abnormal tissues. The nature of the attraction exerted by irritants is not well understood. Irritation of the sensory nerve endings in the skin or mucous membranes causes considerable accumulation of white blood cells, though the affected tissues may not be reddened. The accumulation of white cells in congested areas is no doubt partly due to changes in the caliber of the blood vessels and partly to changes in the rate of blood flow, but the accumulation of white cells which occurs as a result of sensory irritation, with no recognizable vascular change, is very puzzling. Inert foreign substances inserted beneath the skin with every precaution against infection and against sensory nerve irritation of more than the faintest degree, cause their speedy congregation. Irritating substances such as croton oil or capsicum cause very rapid assembling of leucocytes in the vicinity. Injury to the cells of the body also call them together; the greater the sensory disturbance and the more complete the destruction of the tissue cells the more rapid and abundant is the gathering together of the white cells. Pathogenic bacteria, especially pyogenic bacteria associated with de-

struction of the tissue cells, attract them mightily. It is not to be inferred that the attraction of these substances exerts an influence over any great distance; it is enough only that the cells passing through the capillaries in the immediate vicinity of the irritant be stopped and led to pass into the tissues to secure quite rapidly a considerable crowd of white cells. Very much work needs to be done before the nature of this chemotaxis and the manner in which diapedesis occurs can be explained satisfactorily.

To some extent phagocytosis is a function of all living protoplasm, since it is only by the ingestion of food materials that cells maintain life longer than for very short periods. Phagocytosis begins with the manifestations of life, in most cells, and it continues as long as cell life persists. This may be beyond the life of a multicellular body. Ameboid movements of white blood cells, for example, have been watched for five hours on the warm slide after the blood has been removed from the body. For several days after death ameboid movements of leucocytes and the waving of cilia occur in the cadaver.

The term phagocytosis as employed in the study of the white cells of the body usually refers to their activities in ingesting foreign substances, and, indirectly, to such procedures for the sake of the organism as a whole in protecting the body against bacteria and other parasites, and against the effects of foreign substances or injured tissue cells within the body.

Remarkable things are accomplished by means of this activity. Protection against infection is only one of the factors concerned. Malignant neoplasms often meet some such protective agencies, and large hyaline cells, often giant in size, ingest, digest and dissolve masses of tumor cells in many cancer cases. It is true that this activity is not sufficiently efficient to meet the situation, and that tumor growth seems to be unimpeded by the performance. It is manifestly impossible to determine whether early cancers or early metastases may be overcome by these agencies.

Foreign materials of considerable size are often ingested and carried away. In one of our cases a bullet, two years within the brain, was being slowly absorbed. Minute particles of lead were found within the endothelial cells of the capillaries and the neutrophiles of the blood at a considerable distance from the bullet. In another case a small whitish mass in sputum was examined; a small bristle, probably from a tooth-brush, was surrounded by phagocytes and the surface of the bristle was eroded rather deeply on all sides by these tiny scavangers.

Polymorphonuclear neutrophiles resemble amebae, especially proteus, rather closely. In both cells the protoplasm is finely granular and feebly eosinophilic, and in both a very delicate intergranular hyaline protoplasm is present. The nucleus of the ameba is single and almost round while the nucleus of the leucocyte is variable in form. The nucleus of the ameba changes in form during its activity so that the resemblance is quite marked despite the more persistent nuclear polymorphism of the leucocyte. The ameba normally shows a considerable and varying amount of vacuolization, while vacuoles do not appear in the normal leucocyte. With increasing fatigue and impending death the leucocytes become vacuolated.

Both amebae and leucocytes move by means of extruding masses of protoplasm called pseudopodia. In the ameba and the neutrophile, the fine hyaline protoplasm flows out first, then the granules follow, flowing into the pseudopod much as marbles might flow from one part of a loose bag into another part if the bag were pulled about over an uneven surface. In both ameba and leucocyte the nucleus remains centrally placed and always covered by a layer of protoplasm. Only under very abnormal conditions is the nucleus ever freed from its covering of protoplasm. If the temperature of the slide is raised to an abnormal degree, simulating hyperpyrexia, the protoplasm may flow away from the nucleus of the leucocyte, leaving it naked for a few seconds. Even in the presence of impending death of the cell from excessive heat, however, the protoplasm flows around the nucleus almost at once, so that the cell taken from almost or quite normal blood and subjected to excessive heat or to other very abnormal conditions on the warm stage shows almost always a rounded form in death. Naked nuclear masses sometimes are found in the blood of sick persons but it is not yet known whether these are nuclei from which the protoplasm has been carried away or has been digested, or whether they have been produced in some other manner.

Upon meeting any obstacle the leucocyte ceases moving for one to several seconds. A pseudopod then is protruded laterally, the granules, the rest of the protoplasm and the nucleus flow along in the same direction and this direction is maintained until some other impediment or some force changes the direction of movement. There is no recognizable tendency for the leucocyte to turn to the left or the right. If any impediment offers a slanting surface to the advancing leucocyte the pseudopod usually is protruded along the surface of the impediment. It is possible to induce the cell to turn either to the right or to the left by obstructing its pathway by a slanting surface. No matter how often

such an impediment is so placed as to cause movement to the right there is no increasing tendency for a movement to occur to the right when the cell meets a surface at a right angle to its pathway; nor is it possible to cause the cell to tend to right-handed movement by repeatedly obstructing its movement. In several unicellular organisms repeated obstruction of the movement to the right is finally followed by a marked tendency to turn to the right. No such modification of the leucocyte can be secured by conditions so far studied. The direction in which the leucocyte turns when it meets an obstruction placed directly across its path seems to depend upon the forces inherent in the cell at the instant of meeting the obstruction. If the larger moving area happens to be on the right side (the right side of the cell from the view-point of the observer) this mass tends to flow to the right forming a pseudopod turning to that direction. If, however, there happens to be an active mass flowing on the left side of the cell, there is a distinct tendency for the cell to turn in that direction. If a very small object, such as a grain of carmine or a bacterium, lies directly in the path of the leucocyte, the protoplasm is apt to send out two masses, usually unequal in size, and these surround and finally engulf the foreign substance. In other words, the leucocyte is positively thigmotactic. This quality is further indicated by the tendency which leucocytes have of apparently hiding beneath masses of red blood cells, on the warm stage. When the moving leucocyte encounters masses of erythrocytes it flows along the firmer surface presented by the red cells, and when the leucocyte encounters an opening between fairly firm masses of red cells and the slide or cover-glass it flows along the adjacent surfaces, thus evading further observation.

Their activity is increased by increasing temperature. At a temperature of 99° F. the average speed of a neutrophile is sufficient to carry it about five feet in a year. At 103° F. the speed is sufficient to carry it twelve feet in a year; this speed is erratic and non-purposive, however.

The rate of leucocyte motion varies in different individuals even though they are apparently in normal health. In the same individual the movements vary for different times of the day. The cells move most rapidly and begin the motions most speedily after being placed upon the warm stage in the early morning. The movements become much less active toward night and it is sometimes difficult to find any active cell in blood taken at ten or eleven o'clock at night. People who are tired or who are in any way enfeebled have white blood cells which move less freely than do normal cells.

These variations in the activity of the phagocytes of the blood may be one important factor in variation in immunity. Leucocytes increase in activity with increasing temperatures up to about 103 degrees F., but after that increasing temperatures decrease their activity until they die.

The activity of leucocytes can be diminished or prevented by the presence of any substance not normal to the blood plasma, or by varying the concentration of the substances normal to the plasma. Increased amounts of the salts normal to the blood, or of carbon dioxide, or of urea or other non-protein nitrogenous substances diminish the activity of the leucocytes. Diluting the plasma with distilled water also diminishes or prevents leucocytic activity. The presence of oxygen is essential to normal activity. Excess of oxygen increases the activity of the white cells but dimishes their period of vitality on the warm slide. Moderate degrees of light increase their activity but direct sunlight causes speedy death, with no recognizable change in activity.

Certain substances in abnormal adult human blood plasma affect the activity of the leucocytes variably. The leucocytes of an individual suffering from exophthalmic goiter, for example, show excessive activity. Certain toxic conditions are associated with abnormally increased leucocytic activity; others with diminished speed of movement. Further study is necessary before these relations can be accurately described.

The ability of the neutrophiles, especially, to ingest and destroy bacteria is affected by the opsonins of the blood plasma and this ability can be studied for various bacteria. The efficiency of phagocytosis for any individual at any certain time seems to depend upon many factors, —the presence of the opsonins already mentioned, the presence or the absence of fatigue toxins, of other toxic substances, of suitable nutritive materials in the blood plasma, of useful internal secretions, and probably many other factors. Immunity is, at least partly, dependent upon phagocytosis, and any factors which diminish the efficiency of phagocytosis must necessarily diminish immunity.

Factors which diminish phagocytosis include almost all kinds of toxemia and poisoning, including the presence of active drugs and the products of abnormal metabolism. The few forms of toxemia which cause increased leucocytic activity diminish the vitality of the leucocytes and lead to their speedy death on the warm slide. That such poisons also lead to speedy death within the circulatory system of the body is shown by the presence of naked nuclear masses and of partially

destroyed neutrophiles in blood smears made for patients with such forms of toxemia.

Bony Lesions and Phagocytosis

Bony lesions which interfere with the normal nutrition of the hematopoietic tissues and with those viscera which maintain a normal quality of the blood, and a normal circulation of the blood, also affect phagocytosis. Lesions of the third cervical, sixth cervical and the second thoracic vertebrae affect the circulation through the thyroid gland and seem also to affect its nervous control, thus affecting its internal secretion. If hypothyroidism results, the white blood cells become inert but their vitality is not perceptibly affected while they are on the warm slide. If the basal metabolism is increased, the white cells show markedly increased activity and they die speedily, on the warm stage. The cells in such blood protrude two or several pseudopodia at the same time, often in different directions, and the cells behave in an erratic manner, moving different pseudopodia rapidly in different directions in turn but producing little or no efficient activity at any time. The spectacle offered by these erratic movements is very interesting. Various forms of toxemia produce similar but less marked changes in the ameboid movements of leucocytes. Fatigue of mild degree tends to increase leucocytic activity. Fatigue carried to the point of exhaustion diminishes the rate of motion of the white cells. In either case the cells die speedily on the warm slide.

Lesions of the ninth and tenth thoracic vertebrae and adjacent ribs affect the circulation through the liver and permit bile pigments and sometimes bile salts to enter the circulating blood. These substances diminish the activity of the white cells and also shorten their lives on the warm slide. That such substances shorten the lives of white cells in the circulating blood also is shown by the great number of fragmented and senile cells and by the number of naked nuclear masses found in the blood in cholemia. (Plate IV)

Other lesions affect ameboid movements of the white cells chiefly by indirect means. That lesions diminish immunity in a general way and for several types of infection has been demonstrated many times in the laboratories of The A. T. Still Research Institute, by the speedy death of lesioned animals whenever an accidental infection gains entrance into the animal houses.

Activity of Hyaline Cells

Hyaline cells are somewhat less active than neutrophiles. Their protoplasm is chiefly hyaline, though under abormal conditions adult human blood may show granules in the protoplasm of its hyaline cells.

These granules are usually of the azur type, though occasionally they may be basophilic, neutrophilic or very feebly eosinophilic, and they do not ever show any activity themselves; they act like the deutoplasm of unicellular organisms in being apparently inactive and definitely apart from the life of the cell itself. The protoplasm of the hyaline cells forms very short, blunt, heavy pseudopodia sometimes, especially if the heat of the warm slide is increased to 101 degrees F. or so. At 103 degrees F. the hyaline cells become quite active.

Large hyaline cells are phagocytic for malarial plasmodia and for certain other organisms of this general type. The histoplasma capsulata of Darling is found almost exclusively within these cells. It is easily recognized by its peculiar structure and by the clear space in the hyaline protoplasm around the parasite. Large hyaline cells are phagocytic for a few bacteria, such as the bacilli of tuberculosis and typhoid, though they seem much less efficient as bacteriolytic agents than are the neutrophiles. The small hyaline cells are rarely found containing either animal or bacterial parasites. (Plates IX, X, XI)

Activity of Eosinophiles

Eosinophiles present most interesting forms of activity. These cells have abundant large granules in their very scanty hyaline protoplasm and they are conspicuous objects on the warm slide. The granules usually obscure the thin, hyaline, intergranular substance. The granules are the active agents in their movements. The hyaline material merely follows them. The eosinophile cells alternately rest and become active and in the resting stage are round or roundish in outline. Then, for no recognizable reason, one granule rolls along the protoplasmic mass, then another and then two or three, then several, until almost the entire mass of granules is rolling along, forming pseudopodia which may become two or three times as long as the diameter of the resting cell. The nucleus is often completely left behind and it may become completely naked. But the granules do not maintain their distance for more than a few seconds; then one granule rolls toward the nucleus, then two or three, then several, and then the entire mass rolls back to the nucleus and surrounds it again. Then the granules usually rest for a few seconds, then the cell begins its activity again. Sometimes the granules flow more slowly and in a steadfast sort of manner; the nucleus then is carried along with the granules and the cell may thus travel over a considerable distance, though the eosinophiles are not at all likely to travel as far or as rapidly as neutrophiles do. Plate II.

Eosinophiles do not seem to be phagocytic for any kind of infectious agent, nor for foreign materials left within the body. They do surround foreign materials within the body, together with the more abundant neutrophiles, and they die in great numbers around certain foreign particles, but particles of foreign matter or bacteria or other abnormal substances are not found within eosinophiles. They are probably concerned to some extent in immunity, but this function does not seem to be associated with phagocytosis.

The different kinds of white cells do not react in quite the same manner to variations in the quality of the blood plasma. Conditions which cause increased activity of the eosinophiles may cause diminished activity of the neutrophiles, and other conditions may cause increased neutrophilic activity with normal or even subnormal activity of the eosinophiles. The hyaline cells may show increased activity with no evidence of increased activity of the granular cells. Further study of the activities of these cells under various physiological and abnormal conditions is necessary before these relationships can be adequately explained.

Gregariousness

This phenomenon is visible only when the smears are correctly made so that no mechanical grouping of the cells occurs. Normally the leucocytes are not grouped in any kind of order; if two or more are close together it is accidental and their nearness due to co-incidence. Under certain abnormal circumstances the blood cells are definitely grouped together, either in groups of cells classified about as shown by the differential count, or as groups of cells of a single type. This grouping is often quite intimate, so much so that the cells may seem on the point of conjugating or of separating after cell division. More commonly the cells merely lie close together in the smear or on the warm slide.

It may be noticed while watching the movements of the cells on the warm stage that there seems to be an attraction of one cell to another cell and that these tend to approach one another. Usually this appearance of mutual attraction disappears before the cells come in contact; occasionally they become closely approximated and exert more than the orginal attraction for other leucocytes which are in the immediate locality. This attraction is not universal, for often one cell will fail to attract a certain cell but will definitely attract a third or fourth cell. Two cells of the same class may be attracted or two cells of different classes. The eosinophile and the endothelial cell seem to exert little at-

traction for other cells and are not often attracted by them. Eosinophiles are often grouped together and endothelial cells may be arranged in definite masses. Neutrophiles are most commonly found in groups, though this may be partly on account of their relative numbers. Lymphocytes are concerned in grouping, but only with neutrophiles and within their own class.

In the stained smears more definite information is secured because the smears are more even than is the case with warm slide preparations. Here the cells may be arranged in quite large groups either of the same or of different classes. The cells are usually not very closely approximated but they lie in definite groups. Ooccasionally they are arranged in close groups somewhat resembling a microscopic bit of tissue. These groups are especially interesting and abundant in the leukemias and in late leukemia they may resemble bone marrow. Such groups indicate an extremely grave prognosis.

The cause of this grouping is not known. It is not due to any particular stickiness of the cell protoplasm because the cells do not merely adhere, they are definitely attracted and move toward one another on the warm slide. They are rarely actually in contact, which also precludes the idea that increased stickiness of the protoplasm is a factor in causing the condition. Cells which are gregarious seem to be less active in phagocytosis than normal cells. Bacteria, foreign bodies and pigment granules are less commonly found within them than within cells of the same types but not showing this peculiar phenomenon of gregariousness.

Gregariousness adds gloom to the prognosis in any condition with which it is associated. In pneumonia the appearance of definite gregariousness indicates that the heart is becoming affected. In any acute infection, the sudden appearance of gregariousness suggests cardiac inefficiency. In cardiac diseases some degree of gregariousness is always present, and a sudden increase in the grouping of the cells indicates impending failure.

Gregariousness of mild degree may be present in mild circulatory disturbances, such as might be caused from cardiac neuroses or from local vasomotor disturbances. Bony lesions cause local circulatory disturbances in certain viscera, and some mild gregariousness due to such lesions is often found in blood which shows no evidence of organic disease. The cells return to their normal relations within ten days or two weeks after the correction of the lesions. The location of the area of inefficient circulation may sometimes be suggested by factors associated with the gregariousness. For example, if gregariousness of mild degree

is present in the blood of a patient with no definite symptoms of organic disease and without any definite increase in the red or white cell count, or any considerable variation from the normal differential counting, serious organic disease is not indicated, but some area of disturbed circulation is very strongly suspected. If in such a case there should be a trace of bile pigments in the plasma, beyond the very faint trace which is probably normal, then some circulatory disturbance of the liver is suspected. If the blood in such a case contained a slight excess of eosinophiles, and if these were definitely myelocytoid, with rather marked intergranular, basophilic, hyaline protoplasm, then it is suspected that the pelvic tissues (ovaries, testes, prostate) suffer from disturbed circulation. If the blood in such a case shows no evidence of anemia, and still contains occasional normoblasts or reticulated erythrocytes, or if there are many immature or myelocytoid granular cells, then it may be concluded that there is some considerable area of red bone marrow concerned in the circulatory inefficiency.

If gregariousness occurs in blood which shows also a rather high red and white cell count, then cardiac weakness is strongly suspected The heart may not be organically diseased, but there is certainly some abnormal condition affecting the cardiac function in such a case. Lesions of the third or fourth thoracic vertebrae cause cardiac weakness in laboratory animals, and the blood of these animals shows gregariousness which is chiefly marked in the hyaline cells.

When marked gregariousness occurs in the blood in any of the chronic diseases, or when there is a sudden appearance of gregariousness in blood which previously has not shown this characteristic, then an exacerbation of the disease or impending cardiac failure is to be expected. Sudden appearance of marked gregariousness in the blood of any person indicates the onset of some serious state, and if that person is already ill it often indicates impending death. This is especially noticeable in cases of leukemia, pernicious anemia, heart disease and pneumonia.

Leucocytes in Toxemias

The leucocytes are variously affected by toxemias. Typical changes occur in acidosis, alkalosis, fatigue, cholemia, senility and disturbances in protein katabolism.

Acidosis

In acidosis associated with excess of carbon dioxide in the blood stream there is a slight but definite swelling of all the blood cells. The erythrocytes are larger than normal by about one-half micron in diameter. The leucocytes are enlarged by one to four microns in diameter

as they appear on the warm slide. All the blood cells lie flatter on the slide, and they seem flabbier than normal. The nuclei take stains with less avidity and they show a grayish tint instead of the normal bright blue in eosin-methylene blue stains. The nuclei are somewhat larger than normal and they are occasionally vacuolated. The chromatin masses are less distinct and are somewhat smaller than normal. These findings apply to all the nuclei of the white cells in the blood of a patient with acidosis.

The neutrophile protoplasm stains less vividly than normal, and the granules show greater variation in size than in normal blood. The edges of the neutrophiles are often ragged and frayed in appearance. The limiting layer of the cell seems to be in solution so that the protoplasm merges into the plasma without definition. The eosinophiles stain with less than normal avidity, and the eosinophile granules seem less definitely spherical than normal. The hyaline cells stain less vividly than normal and their edges also are indistinct, though the fraying is less marked than in neutrophiles.

The leucocytes move sluggishly on the warm slide and they die within half an hour at most.

Alkalosis

In alkalosis the nuclei stain with unusual brilliance. In Giemsa staining the nuclei show a more distinct purplish tone than is the case with normal blood. The nuclei are slightly shrunken and they seem to have a thickened nuclear membrane. The chromatin masses are large, distinct, and deeply stained.

All the cells are diminished in diameter and all seem to be more definitely spherical than normal. The cell outlines are distinct and very often there is a peripheral condensation of protoplasm suggesting a definite cell wall. The granules of the neutrophiles are brilliantly stained in eosin-methylene blue preparations and they take eosin more avidly than do normal neutrophiles. The eosinophile granules stain vividly and they seem to have a higher refrangibility than in normal blood.

The cells move slowly and feebly and they begin to die within twenty minutes or so.

Fatigue

In severe acute fatigue the changes are less marked than in chronic fatigue. Fatigue is usually associated with some degree of acidosis and the changes already described for acidosis are present in fatigue. To these are added other changes. The granules of the neutrophiles are smaller than normal and may be fine and dust-like. Their outlines are very indistinct. The nuclei are more swollen in severe fatigue than in acidosis and the nuclear outlines are more irregular. Very often con-

siderable areas of the nuclear periphery are frayed out into a fringe-like margin. The outlines of the hyaline cells are more ragged than is the case in acidosis and they show considerable variations in staining reactions. Monocytes are usually increased in fatigue. After very severe acute fatigue there may be considerable numbers of endothelial cells present.

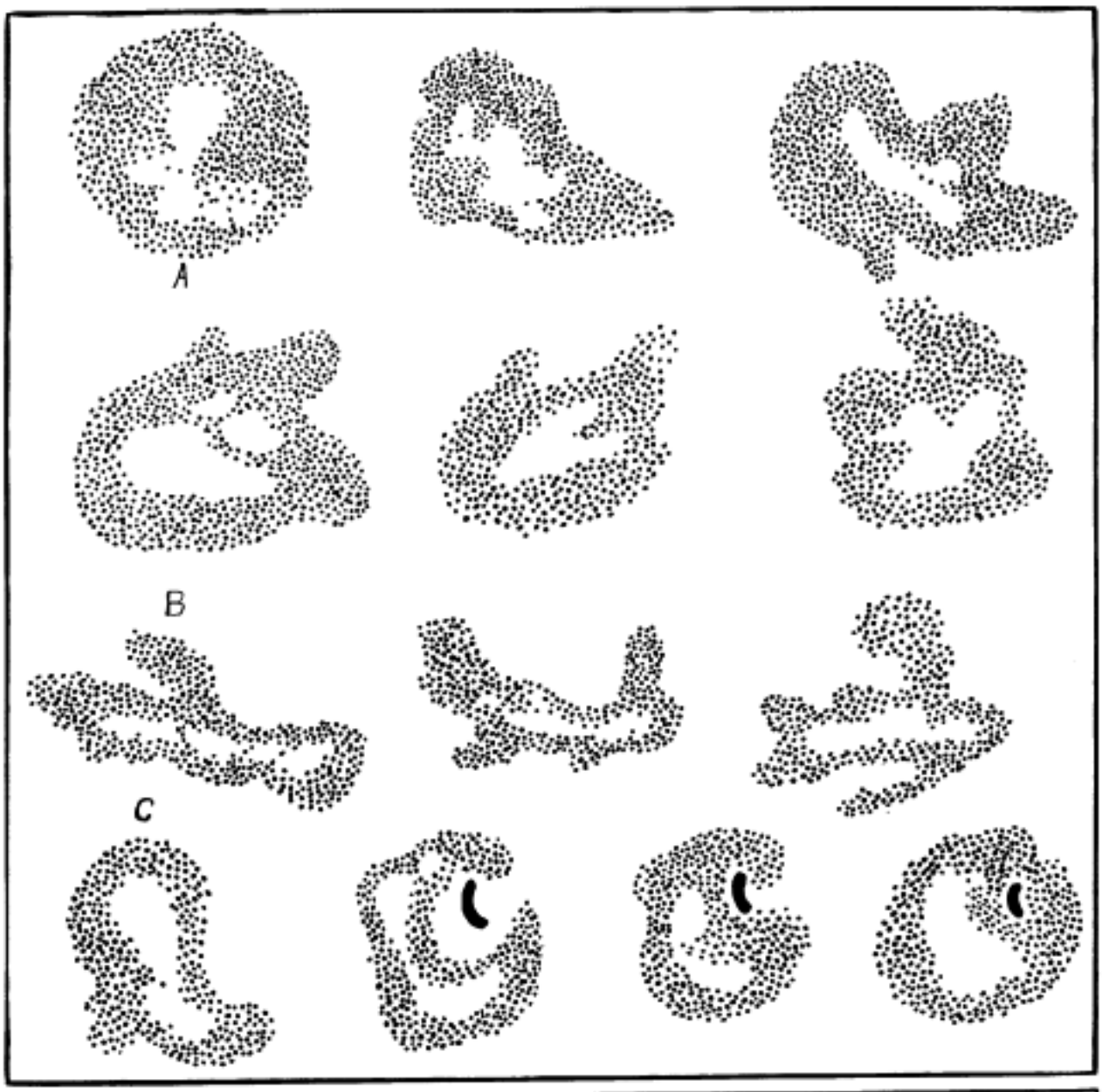
In severe chronic fatigue there may be many naked nuclear masses present in the blood smears, and these may occasionally retain a few very small masses of protoplasm which identifies the nature of the original cells. Such nuclear masses seems to be derived from all the leucocyte groups as a result of the disintegration of their protoplasm.

The cells show little movement on the warm slide, in severe or chronic fatigue, and they often begin to die almost at once. In mild acute fatigue the cells begin to move at once and show remarkably increased activity. Their pseudopodia are long and sprawling and are protruded and retracted in different directions, in a peculiarly purposeless manner. They begin to die within ten to thirty minutes.

With rest the cells regain normal activity. It is impossible to determine whether cells once seriously affected regain normal functions or not. Since the lifetime of the leucocytes is certainly very short it may be that the leucocytes once affected by the toxins present in acute fatigue perish and are replaced by others which develop in the rested body and are, therefore, unaffected. In chronic fatigue the newly developed cells are also affected by the toxins constantly present in the blood plasma.

Cholemia

The presence of bile salts or acids in the blood stream exerts a destructive effect upon all blood cells. The red blood cells show speedy laking on the warm slide, and the stained smears show many fragmented erythrocytes. The red cells are fragile and inelastic. Blood shadows (red blood cell stromata from which the hemoglobin has been dissolved) are present, sometimes in considerable numbers. The protoplasm is often disintegrated to such an extent that it may be impossible to make a satisfactory differential count in severe cases of cholemia. Vacuoles are often found in the protoplasm and in the nuclei of the neutrophiles, and in the nuclei of the eosinophiles. The eosinophile granules are not affected. The staining reactions of the nuclei are not affected. The hyaline cells are affected less seriously than the neutrophiles, but their protoplasm is often ragged around the edges if the cholemia has been present for some weeks. (Plates III, IV, XIII)



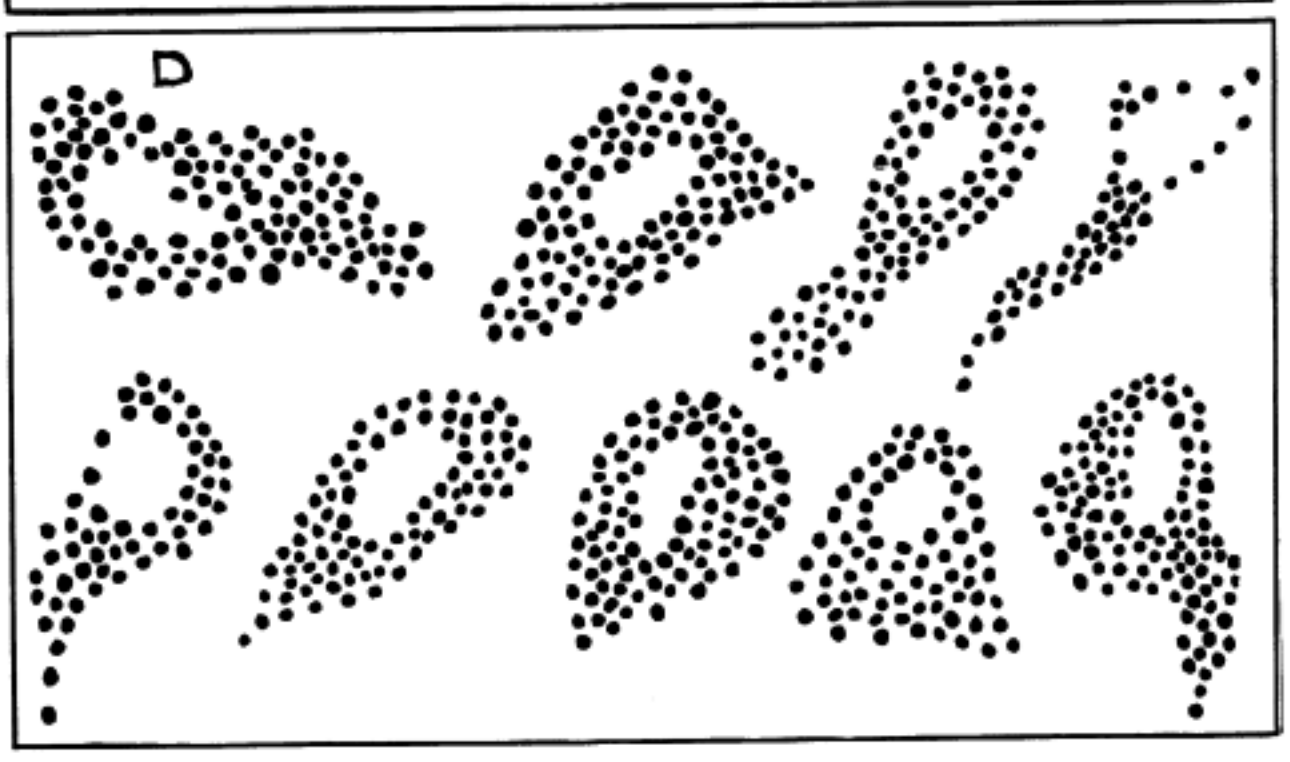


PLATE II

PHAGOCYTOSIS

A. Normal movements of neutrophile at temperature of 99° F. Camera lucida drawings at intervals of ten seconds.

B. Increased activity at temperature of 104° F. Camera lucida drawings at intervals of ten seconds.

C. Phagocytosis of bacilli of tuberculosis on warm slide. Camera lucida drawings at intervals of thirty seconds.

D. Movements of normal eosinophile on warm slide. Camera lucida drawings at intervals of ten seconds.

Disturbances in Protein Katabolism

The toxic products of abnormal degeneration of protein substances may be absorbed into the blood and may affect the blood cells quite seriously. The red cells show little or no ill effects of this form of toxemia unless anemia supervenes.

The neutrophiles show the most marked effects. Their nuclei present extremes of polymorphism and small aberrant masses of nuclear substance may occasionally be found in the protoplasm near the periphery of the cell. The nuclei themselves remain near the central area of the cell. The edges of the nuclei are definite and distinct, as in alkalosis. From this edge there are peculiar prolongations somewhat resembling pseudopodia except that the ends are often broadened out into knob-like structures. These nuclear pseudopodia have distinct outlines and do not in the least resemble the fringelike frayed edges of the nuclei found in fatigue or acidosis. Vacuoles are not found in the nuclei in typical cases.

The nuclei of the eosinophiles and of the hyaline cells rarely show the nuclear pseudopodia which are so conspicuous in the neutrophiles, but they often are divided into two or more definite masses. The lymphocytes often contain two nuclei but it is extremely rare that evidences of cell division are found in these cells. The monocytes are increased in numbers and they often contain granules which seem to be deutoplasmic and are probably formed from ingested protein materials derived from the degenerating tissues or fluids.

The fibrin threads appear upon the warm slide more abundantly and more speedily than they do in normal blood. The threads are irregular in outline, are often beaded and they may form peculiar, radiating, net-like or felt-like structures. In the absorption of pneumonia exudate and during the very early stages of pneumonia the threads are very long, very abundant, very quickly formed, regular in contour and they form heavy, felt-like masses. In carcinoma they are more often radiating and the threads are much more irregular in contour.

Small, highly refractile granules are usually abundant on the warm slide and they stain in different ways according to their chemical nature. Some of these are lipoid or fatty, and they stain with Sudan III or osmic acid; these granules are present only when there is some degeneration of protein substances. Others give a brownish, reddish or bluish color with iodine; these also are present when protein substances are being broken down into glycogen or some related substance. Other granules do not stain by ordinary methods; they include several different compounds such as granules from disintegrated

leucocytes, tissues, fluids and other protein substances. The Sudanophilic and iodophilic granules are recognizable within the hyaline cells and the neutrophiles.

Toxemia of this type is always associated with some absorption of abnormal protein katabolites. Individuals with any kind of intestinal putrefaction and who eat excessively of high-purin foods often absorb toxic substances which produce this blood picture. The stage of resolution and absorption after pneumonia may be characterized by the same findings. A degenerating benign neoplasm may also cause sufficient toxemia to bring about these same changes. The most marked form of this type of toxemia occurs during the rapid growth of a malignant neoplasm or the absorption of fluids from a cyst or from peritoneal or pleural exudates. Carcinoma produces more definite findings than any other neoplasm.

Senility

In old age or in premature senility the neutrophiles show characteristic changes. The granules are fine, often dust-like, and they stain feebly. The nuclei of all leucocytes stain feebly. Vacuolated swollen nuclei and nuclei with frayed outlines are abundant. Polymorphism of the nuclei of neutrophiles, eosinophiles and large hyaline cells is pronounced. The lymphocytes often contain two nuclei.

A peculiarity of the nuclei of the leucocytes of senility is the presence of shrunken nuclei in which the chromatin forms large masses which stain with unusual avidity.

Gregariousness is often marked in senile blood.

CHAPTER IV

LEUCOCYTES

Cell Types

The relations between the different classes of white blood cells are not yet satisfactorily determined. The simple cellular relations of the classes of leucocytes found in normal, adult, human blood are quite different from the complicated relations of the blood cells in abnormal conditions of adult blood and in normal conditions of early embryonic development.

In normal, adult, human blood the white cells are divided into two distinct groups, the granular cells and the hyaline cells. The granular cells are derived from red bone marrow, during adult life. They are distinguished by the presence of granules within the protoplasm. The hyaline cells do not, normally, show any granules within their protoplasm by ordinary methods of staining. A very few granules may sometimes be brought into view by certain staining methods, but these are scanty and are not distinctive by ordinary staining of normal blood. The hyaline cells are mostly derived from lymphoid tissues and are called lymphocytes for that reason.

Granular cells include neutrophilic, eosinophilic and basophilic, named according to the staining reactions of the granules. Hyaline cells include large and small, named according to size. Even in normal blood a few hyaline cells of intermediate size may be found, and these are increased under abnormal circumstances.

The various kinds of white cells are best described separately.

Neutrophiles

Neutrophiles are white blood cells whose protoplasm is filled with fine granules which are neutrophilic or feebly eosinophilic, and whose nuclei are of extremely variable form. They are about ten microns in diameter in the living state. They have a very delicate intergranular hyaline protoplasm, and this is feebly basophilic. Neutrophiles make up sixty to sixty-six per cent of the leucocytes in normal, adult, human blood. Their number is subject to marked variation under varying physiological states, and still more marked variation under abnormal conditions. They are normally present only in the blood stream and bone marrow, not in the tissue spaces. (Plates III, IV)

Neutrophiles are formed only in the red bone marrow, during normal, adult, human life. Under certain abnormal conditions they may be formed in the spleen and, probably, in other lymphoid tissue.

Cytoplasm

The granules of neutrophiles vary somewhat in their staining reactions. Even in a single cell some granules are less eosinophilic than others. In a smear certain cells may be more or less eosinophilic than other cells, and in smears made from the blood of the same normal person at different times there may be greater or less affinity for various stains. As a rule, the physiological conditions of fatigue, hunger and lowered blood pressure cause increased affinity for eosin and similar stains (usually called acid stains) while exercises, hot baths, deep breathing and other conditions which cause moderate physiological leucocytosis cause also diminished affinity for eosin and increased affinity for methylene blue and other stains usually called basic. The intergranular protoplasm may become definitely basophilic under such circumstances. Since younger forms of neutrophiles show greater basophilia, it is quite probable that the increased basophilia under such conditions is due to the associated leucocytosis.

The thin, hyaline, intergranular protoplasm remains present but is not usually visible in the normal adult stained cell.

This thinner material seems to be the active part of the cell in ameboid movement, and the pseudopodia as first formed are composed of it. The granules follow in a massive sort of motion, and as they move they maintain a sort of united relationship. (One observer said that they looked as if they were chained together as they marched along.) In this respect the activity of the neutrophile differs from the activity of the eosinophile.

In the younger forms a centrosome is occasionally present. The younger forms also often contain mitochondria; these disappear gradually with increasing senility of the cell.

The abnormal neutrophilic cells found in leukemia contain peculiar rod-like masses of irregular contour; these may be mitochondria. They may take neutral, acid or basic stains. The relations between these bodies and the true neutrophilic granules have not yet been determined.

The granules are very small in normal neutrophiles, but in excessive fatigue and under pathological conditions larger granules may be found within the neutrophiles; these may be intensely basophilic, eosinophilic or amphophilic, or they may retain their normal neutrophilic or feebly acidophilic affinities. These atypical granules are never found in normal blood.

Nuclear Structures

The nuclei of neutrophiles present great variations even in normal, adult, human blood. Mononuclear neutrophiles have, as the name indicates, a single round or roundish nucleus. This nucleus is centrally placed and it may be slightly indented or reniform. The granules are finer than those of the polymorphonuclear neutrophile and are definitely neutrophilic, very rarely even feebly eosinophilic or acidophilic. The intergranular protoplasm is rather more definitely basophilic and is often more abundant than in the polymorphonuclear forms. Intermediate forms exist between the mononuclear neutrophiles and the polymorphonuclear neutrophiles, and there is some reason for believing that the mononuclear cells are immature forms of the polymorphonuclear forms. Nuclear structures take the basic stains with avidity. One or two nucleoli may be present.

The nucleus of the polymorphonuclear neutrophile presents great variations both in normal and in abnormal blood. Neutrophiles with deeply notched or saddle-shaped nuclei are grouped with the polymorphic forms. Nucleoli are rarely found in the younger forms, and are never found in older types. Apparently with increasing age of the cell, the nucleus becomes more and more slender, finally approaching the form of a ribbon or a cord, folded back and forth in a very irregular manner. This ribbon-like structure may be variously indented and the wider portions may be connected only by fine threads of nuclear substance. Rarely these lobules may be completely separated; at least, no nuclear substance connecting them can be demonstrated by ordinary staining methods. In normal blood the number of distinct lobules rarely exceeds four; in abnormal blood there may be six or even ten or more distinct lobules in a single cell.

These nuclei do not show such distinct structure as is found in the mononuclear forms. Cells with the greatest number of nuclear lobules show the greatest senility in the character of the nuclear structures. The chromatin is in large irregular masses and is avidly or feebly basophilic. When marked toxemia is associated with neutrophilic proliferation some of the cells may show abundant senile traits while other cells show marked evidences of immaturity or even of atavism.

The staining reactions of the nuclei as well as the number of nuclear lobes or lobules vary with changing physiological conditions. In pathological conditions these variations may be extreme. When there is increased outpouring of neutrophiles from the red bone marrow under physiological conditions, such as the increase which occurs as a result of rapid travelling to high altitudes, many young forms are found in the

peripheral blood. These young forms show larger nuclei with fewer lobes, simpler forms and more delicate nuclear structure than do the older forms. The cells thus thrown into the peripheral circulation are so speedily produced that they must have been already formed and held in reserve in the sinuses of the bone marrow. They do not differ from the younger forms of the cells previously in the circulation, the only difference being that there are many more of the younger forms than is ordinarily the case. Within a day or at most a few days the cells become older and the blood picture is practically that of the individual before his journey.

Immature Forms

Under certain abnormal conditions the neutrophiles are manufactured more rapidly than is normal, and very immature cells may be thrown into the general circulation. These include myelocytoid forms and myelocytes. The nucleus is very large and occupies more than half the entire area of the cell; it is round or roundish, is eccentric in its position and is often bare of protoplasm upon one side. It has one to three nucleoli which stain deeply. The chromatin is fine and deeply staining and is arranged in irregular masses. These cells are abundant in cases of myeloid leukemia and may be found in considerable numbers in cases of severe, acute, pyogenic infection.

With still more rapid formation of leucocytes, especially when the hematopoietic areas approach exhaustion, atavistic types are occasionally found. These cells are not present in normal adult human blood or marrow. Atavistic forms are occasionally found in abnormal embryonic human blood and in abnormal adult marrow, but they are characteristic of the cells of mammals below human, or of vertebrates below mammals. The neutrophiles of human blood are not exactly like those of other mammals, and no cells which can properly be called neutrophiles are present in the blood of animals below mammals. For this reason it is easier to recognize atavistic forms among the neutrophiles than it is to find them among the hyaline cells, because the latter are found in about the same forms in all mammals and in nearly all vertebrates. (Plates V, VI, XII).

Effects of Vertebral Lesions

Bony lesions affect the structure of the neutrophilic cells recognizably. Any bony lesion is associated with a disturbance of the circulation through the red marrow of the bones concerned in the lesion and, in the case of small bones, those in the immediate vicinity of the bones actually concerned in the lesion have also some circulatory disturbance.

For example, when one rib is lesioned there is some edema of the tissues around the joint or joints concerned in the lesion. This edema frequently extends beyond the nutritive foramina of neighboring ribs as well as those of the rib which is lesioned and often also to the foramina of adjacent vertebrae. The nerves and blood vessels passing through these nutrient foramina are subjected to the pressure due to the edema. The nerves, especially those which are non-medullated, are subjected to the chemical changes in the edematous tissue juices. Because of these physiological relations, the red marrow of one or several bones has some circulatory disturbance whenever there is a bony lesion anywhere in the body. The red marrow thus affected produces blood cells which are not quite normal in structure; such cells are usually immature or myelocytoid in type; the nuclei are roundish, somewhat vesicular in form, show larger and paler chromatin masses and occasional nucleoli. Their protoplasm shows granules of irregular size and irregular staining reactions. The intergranular hyaline protoplasm is more abundant. These cells flatten to a thinner layer on the slide than do normal cells, and their pseudopodia are less regular in form, are protruded from two or several parts of the circumference of the cell simultaneously and are less efficient in the ingestion of bacteria or of foreign particles than are normal pseudopodia.

The presence of these immature or myelocytoid forms, in blood which shows no other cause for this abnormality, indicates the existence of some conditions causing inefficient circulation through the red bone marrow somewhere in the skeleton. Bony lesions are the most common cause of such nutritional defect of the red marrow.

Arneth's Index and the Nuclear Average

Arneth's index is the result of computations based on nuclear structures. It is supposed that those neutrophiles which have a single nucleus, round or only indented, are the younger forms. Those with two lobes are younger than those with three lobes. In the older neutrophile the nucleus is almost or quite divided into several lobes. Arneth's index is a method of estimating the relative age of the neutrophile by means of a study of these variations in the nuclear structure. While the nuclear lobulations may not be altogether accurate as a basis for the calculation of age, still this study gives useful results under certain conditions. In our laboratories Arneth's index has been superseded by a study of the "neutrophilic nuclear average" which we find much more useful. (Plates XIV, V, IV)

Arneth divided the neutrophiles into five groups, Class I having a single nucleus which is round or indented; Class II having two nuclei,

and so to Class V which has five or more nuclei. Each class is subdivided according to the form of the nucleus. In Class I are three groups, M cells which are really myelocytes and are not found in normal blood; W cells which are mononuclear neutrophiles or myelocytes and have nuclei which are slightly indented or reniform, and T cells which have nuclei rather deeply indented but which are still definitely single in structure. The W cells may or may not be found in normal blood and, if present, are very scanty. T cells may make up 5% of the total neutrophile count. Class II, III, IV, and V are subdivided into different groups according as the nuclei show definite knob-like lobes, or are ribbon-like with grouping of chromatin in such a manner as to form lobules. Both forms of nucleus may be present in a single cell, so that Arneth describes in Class II cells with two knob-like projections, cells with two S-shaped masses, and cells with one S-shaped lobe and one knob-like lobe. Class II has, altogether, about 35.5% of all neutrophiles, in normal blood. Class IV has four subdivisions, based on different combinations of the knob-like and the S-shaped nuclei, and this class makes up about 36% of all neutrophiles of normal blood. Class IV has five subdivisions and makes up about 2% of all neutrophiles in normal blood. These percentages are given from various laboratories and they are practically identical with our own figures. According to Arneth, the neutrophiles are greatly increased during infections and hence immature forms are found in the circulation; the index is then said to "shift to the left," that is, there are many more cells in the first and second classes than is the case with normal blood. After an infection has been present the younger cells are no longer being formed so rapidly, there is an accumulation of older forms, and an increased number of cells in the fourth and fifth classes. However, the effect of toxins on the white cells also increases the number of nuclei, causes the nuclei to become pyknotic and the chromatin to become arranged in irregular masses. Fragmentation, vacuolization and cloudy swelling of the protoplasm, with a loss of the distinctive granules, lead to great difficulty in estimating Arneth's index in many cases, and especially in those cases in which the diagnostic value of the method is of most importance.

Modifications of the method of determining the index of Arneth have been devised by Cooke, Schilling, Ponder and others. After considerable study of normal and abnormal bloods we have discarded all of these in favor of the study of the neutrophilic nuclear average. In making this computation true myelocytes are disregarded and only typical

mononuclear and polymorphonuclear neutrophiles are included in the count. The myelocytes, if present in any considerable numbers, are separately estimated. In this method the myelocytes which are present as a result of imperfect circulation through the red bone marrow, or of developmental abnormalities, or in the blood of leukemic patients, are not allowed to affect the nuclear average. Thus the nuclear average represents only the relative ages of the neutrophiles. In this connection it must be remembered that toxemias often increase the nuclear average and cause, in other ways, an appearance of senility in neutrophiles.

Arneth's index shows marked shift to the left (predominance of youthful and immature forms) when there is efficient reaction to acute infections; pneumonia and pyogenic infections generally cause extremely marked shifting. During an infection to which the myeloid tissues do not make adequate response, toxemia is severe, the neutrophiles show abundant senility and there are relatively very few young cells being thrown into the circulation; the index is shifted to the right in such a case. After an infection and during recovery there is also a shift to the right, due to the fact that young cells are not being thrown into the circulation and the cells already present are somewhat affected by the toxemia of the infectious processes. There is a marked shift to the right in jaundice, in toxemias due to malignant neoplasms, the absorption of old pyogenic foci or degenerating benign neoplasms, in intestinal toxemia due to putrefaction and in many other conditions associated with disturbances in putrefactive products anywhere in the body.

The changes in the nuclear average are of similar import. The normal adult neutrophilic nuclear average varies from 2.45 to 2.55. During the early stages of an acute infection this decreases to below 2 and occasionally to 1.5. The nuclear average is below 2.3 in normal young children and may be below 1.3 during an acute infection in childhood.

In chronic toxemia and during convalescence from an acute infection the nuclear average often exceeds 3.0 and in senility it is normally above 2.7.

In severe infections to which hematopoietic tissues are unable to react efficiently, there is no leucocytosis and may be leucopenia; in such cases the nuclear average may be 3.5 or even 4.0.

The lower the neutrophile nuclear average, the greater the proportion of newly-formed, young neutrophiles; the higher the average, the greater the proportion of worn-out and elderly neutrophiles.

Functions of Neutrophiles

The functions of the neutrophiles have not been adequately determined. That they are important factors in protecting the body against certain types of bacteria is evident, though they are not efficient in other infections. They ingest and digest and render harmless injured tissues and many foreign substances within the body. The fact that they increase and diminish so rapidly at different times of the day and, under certain circumstances, during the digestion of food leads to the view that they may have a nutritive function.

In their own metabolism they take up nitrogenous substances and, probably, certain forms of carbohydrate related to glycogen. When they die and are digested and absorbed and, perhaps as a result of their own living metabolism, these substances are given off again into the blood plasma. They represent a storehouse of food materials and are very efficient in this relation, since substances so stored do not affect the composition of the blood plasma, and since they are so thoroughly scattered over the body that they are immediately at hand for every demand. They carry tiny globules of fat in their protoplasm, and this also is an efficient method of storage.

They elaborate an enzyme which is efficient in the digestion of necrotic tissues resulting from injury of the body cells and from exudates, transudates or hemorrhages within the body. After pneumonia, for example, the processes of resolution are due chiefly to the activities of the leucocytes and of the enzymes formed by them.

Development of Neutrophiles

The neutrophiles are normally formed, during adult life, only in the red bone marrow. From the "stem cell" arise myeloblasts which show differentiation by the development of individual cells and as a result of the unequal division of the mother cells. The myelocyte of the neutrophilic series is a large cell with its nucleus occupying much more than half the mass of the cell. The protoplasm is very finely granular and many contain mitochondria; it is feebly basophilic. With further differentiation the nucleus becomes smaller and the protoplasm more abundant; the nucleus loses its nucleoli and becomes lobed; the protoplasm loses its mitochondria and developes more abundant granules which become more and more definitely neutrophilic. The protoplasm becomes more and more motile. With increasing differentiation the cell is pushed or grows toward the endothelium of the blood vessels of the marrow, and finally it enters the blood vessel through the endothelial wall. In human marrow there are many spaces in which no endothelial wall can be seen; the cells are simply pushed out into the blood stream.

Under normal circumstances only the myelocytes of almost adult type seem to be concerned in reproduction; at any rate karyokinesis is seen only in these cells in normal, or almost normal, human marrow. In the leukemias and in pernicious anemia karyokinesis is abundant among the earlier myeloblasts and also among the "stem cells".

Life History

The length of life of the neutrophiles cannot be estimated in any satisfactory manner. The rise of the nuclear average during a few hours after an acute leucocytosis due to acute infections would seem to indicate that the development of young to senile forms is rather a hasty process under such conditions, which are, of course, distinctly abnormal. Neutrophiles containing carbon particles may be found several weeks, even several months after such particles have been injected into the veins, but these same particles may have been ingested and left behind at the death and digestion of many cells in the interim.

Cells which appear to be senile are found constantly in normal blood. They fail to show normal movements on the warm slide; they do not stain distinctly; they have swollen and fragmented nuclei and swollen and fragmented protoplasm. Naked or almost naked nuclear masses of distinctly neutrophilic type are found and the occasional ragged fragments of protoplasm which may occasionally be found clinging to such nuclei are of neutrophilic structure. In the blood of a normal young woman such cells were found to be present, in the early morning, 25 senile cells or naked nuclear masses to 230 normal neutrophiles. At nine o'clock at night 25 senile cells or naked nuclei were present in 150 normal neutrophiles. Other estimations of this relation in several other apparently normal persons gave similar results; in some cases the differences between morning and evening blood were much greater. Based upon these studies, the life of the neutrophile in circulation can scarcely be more than a week. Estimations based upon the increase present in infections give one day or two days as the probable life period of the neutrophiles.

Disposal of Leucocytes

Fragments of leucocytes are found in the endothelial cells of the spleen and the liver, and, to some extent, of the bone marrow. These are, no doubt, utilized as food by these and perhaps other cells of the body. The molecules of which they are composed seem to be very well adapted to serving as food for other cells, and it is quite probable that in building up the food materials brought into the body into these more complicated molecules they serve their most important physiological function.

Eosinophiles

Eosinophiles are, as the name indicates, intensely eosinophilic or acidophilic. A typical eosinophile is about 9 microns in diameter on the warm slide. From .5% to 1.5% of the total leucocyte count are eosinophiles in normal adult human blood. In children the number is greater both relatively and absolutely. Under certain abnormal conditions of adults and children the number may be very greatly increased, even to 80% of the total leucocyte count. Nearly all animal blood contains higher eosinophile counts than human blood, and young animals show higher eosinophile counts than do older animals.

Eosinophiles show hyaline protoplasm filled with very large granules. These are conspicuous objects on the warm slide. When they are stained with eosin they are very brilliant and noticeable. In younger cells and in eosinophilic myelocytes the intergranular, hyaline, basophilic protoplasm is visible. In older cells this protoplasm can sometimes be demonstrated by very delicate staining methods. The granules seem to be definitely alive; they are the active elements in the movements of the cell. Very often no other protoplasm is visible even with the most careful staining of smears and the most exhaustive study of living cells.

In lower mammals the granules are often definitely oval or rod-shaped. The rod-shaped form occasionally appears in human blood when other atavistic characteristics are present. The oval form has not been reported in human blood.

The nuclei vary in form, but are always roundish in outline. Reniform and saddle-shaped nuclei are common forms. They are never ribbon-like, nor are they so markedly polymorphic as are neutrophile nuclei. The nuclei have rather a coarse structure. The chromatin masses and net-knots are larger than in hyaline cells and are not so deeply basophilic. Dividing forms are not seen in normal human blood, but under abnormal conditions in human blood, in the blood of embryos and in lower vertebrates dividing eosinophiles are occasionally found. Nuclear pseudopodia are never seen in normal blood, either animal or human, and are rarely found in abnormal blood.

Activity of Eosinophiles

Eosinophiles move with considerable celerity on the warm slide. They die within twenty or thirty minutes. The large granules are the active element. One granule usually begins to roll over; this may be near the nucleus or at the periphery. It rolls over and over, between or over other granules but not necessarily causing these to move. Other granules then begin to move and these tend to follow one another in curving

lines. Very often these curved lines of granules are discernible in the stained cell. The hyaline part of the protoplasm, when it is visible, usually follows the granules. Occasionally, especially in the blood of fasting patients or those with severe malnutrition from any cause and in very young or animal blood, the hyaline substance may precede the granules and the granules may even, sometimes, seem to be carried along by the streaming protoplasm without displaying any intrinsic activity of their own. The nucleus follows the protoplasm sometimes, and it seems to be carried along as if it were heavy or reluctant. In many cases the nucleus does not move at all; the granules flow away in long masses, sometimes leaving the nucleus almost or quite naked, then they return to surround the nucleus almost evenly; they may then flow out in another direction only to repeat the process. This phenomenon is most commonly seen in blood which has been taken from a feverish patient, or in blood which has been under observation upon a slide whose temperature is one or two degrees above that of normal blood. Phagocytosis has not been observed in eosinophiles though their ameboid movements are so rapid. (Plate II)

Functions

Very little is known of their functions, and a study of the conditions associated with their increase and their decrease add little to our understanding. The granules contain iron and copper and they seem to be concerned in iron and copper metabolism. They are very abundant in the sputum under certain conditions. The formation of Charcot-Leyden crystals and crystals of the seminal fluid is usually associated with their degeneration.

Eosinophiles are diminished in nearly all uncomplicated acute pyogenic infections. A differential count of 5,000 leucocytes made for one of our cases with lobar pneumonia included no eosinophiles.

After the crisis in pneumonia and after the fall in fever in most acute infections the eosinophiles increase rapidly, often considerably exceeding the normal numbers.

Pyogenic and other acute infections in which the eosinophiles remain in almost or quite normal numbers, or perhaps somewhat above normal numbers, are osteomyelitis, ovaritis, orchitis, prostatitis, measles, scarlet fever and acute articular rheumatism. In malaria they are increased the day after the chill.

Eosinophilia, or marked increase in the eosinophiles, occurs under many different physiological and pathological conditions. Eosinophiles above 2% should be considered a relative increase, and above 200 per cubic

millimeter an absolute increase, but the term eosinophilia is usually employed only when the eosinophiles rise above 3%, relatively, and above 300 per cubic millimeter, absolutely.

Eosinophiles are increased physiologically during and for a day after physiological congestion of the reproductive tissues, that is, after sexual excitement in both sexes, and before menstruation in women or at the time of heat in female animals.

They are also increased as a result of vertebral lesions which cause congestion of the reproductive glands; this is more marked in the female with congestion of the ovaries produced by lesions than in the case of the male with congestion of the testes as a result of lesions. Correction of lesions concerned is followed by decrease in the eosinophiles within three days, in human subjects.

They are increased in diseases of the spleen, including the congestion of the spleen due to lesions of the ninth thoracic vertebra, and, in less marked degree, of neighboring vertebrae and ribs. Diseases of other lymphoid tissues do not cause eosinophilia in the same degree, nor is it always present. After splenectomy and after destruction of considerable splenic tissue by neoplasms eosinophiles are increased also. In cases of splenomegaly the eosinophilia may reach tremendous heights. Counts exceeding 80% of the total leucocyte count and 130,000 per cubic millimeter absolute count have been reported by several workers.

Diseases of the bone marrow, including myelogenous leukemia, usually show high actual counts of eosinophiles, often exceeding 20,000 per cubic millimeter. In osteomyelitis, osteomalacia, sarcoma and carcinomatous metastases and in less common diseases of the bone marrow the eosinophiles may be greatly increased. In pernicious anemia they are relatively increased and they may be absolutely increased even though leucopenia may be extreme.

The absorption of the toxic products of degeneration of animal proteins causes eosinophilia, but the absorption of degenerating vegetable proteins does not. In intestinal putrefaction the eosinophiles are increased if the patient is on a diet high in meat or eggs, but not if he is on an exclusively vegetarian diet. Mild eosinophilia is present in malaria, syphilis and during the absorption of exudates, transudates, and pus, and during resolution after pneumonia. It is often present when degenerating benign neoplasms or rapidly growing malignant neoplasms are present in the body. Eosinophilia may be marked during the fever of scarlet fever, measles and acute articular rheumatism. In cases of mumps with ovaritis or orchitis the eosinophiles are in-

creased, and this rise may precede recognizable symptoms of the spread of the inflammation to these tissues by a day or even two days. The value of this reaction is evident.

Eosinophilia may be marked after the use of any of the animal substances used in the treatment of disease, such as tuberculin, antitoxin and vaccines. They are not increased after the injection of vegetable proteins unless anaphylaxis or definite irritation of the skin is associated with the injection. They are occasionally diminished immediately after the injection of animal products, then rise to a surprising degree within a few hours; counts of 40% and of 45,000 per cubic millimeter have been reported.

Eosinophilia in bronchial asthma is useful in differentiating bronchial asthma from symptomatic dyspnea. It must be remembered that the eosinophiles are also usually increased in emphysema. In uncomplicated bronchial asthma eosinophilia is always present, usually above 8% of the total leucocyte count and above 1,000 per cubic millimeter of blood in our cases.

Diseases affecting the sympathetic nervous system may cause marked eosinophilia; this is especially noted in cases in which the solar plexus is invaded by carcinomatous metastases.

Any irritation of the skin causes increase in the eosinophile count, whether this be mere mechanical or chemical irritation or whether inflammation of the skin is present (as by poison oak). Even rather small burns may cause marked eosinophilia; it may be that the absorption of the proteins from the injured tissues is a factor in this case. Counts up to 60% of total leucocyte count, and to 4,000 eosinophiles per cubic millimeter of blood have been reported in urticaria; with relief of the hives the eosinophile count returns to normal within a few days. Any skin disease associated with itching or other sensory irritation increases the eosinophiles to some extent, and this increase may be surprisingly high for mild cases. There seems no relation between the area of skin involved, the severity of the disease or of the sensory irritation and the height of the eosinophilia.

Animal parasites usually cause definite or high eosinophilia. Amebic infections frequently cause no eosinophilia at all, and in uncomplicated cases there is never more than a slight increase in eosinophiles. Filaria sanguinis hominis usually is associated with mild eosinophilia. Darling's histoplasma capsulata caused slight eosinophilia in the one case studied in our laboratories. Hydatid cysts of the liver may cause little or high eosinophilia, usually less than 20% of the total count. Tri-

chinae cause eosinophilia which may be of diagnostic value in obscure cases of muscular pain.

Worms in the intestinal tract often cause very high eosinophilia, to 70% or even more, and to 8,000 eosinophiles per cubic millimeter of blood; commonly the count runs from 15% to 25% for the ordinary pinworms, round worms and tapeworms.

Several drugs may cause eosinophilia, usually moderate and transitory. Camphor is of interest in this connection and persons whose occupation causes the inhalation of camphor fumes may show persistent eosinophilia. Diagnosis is sometimes difficult in such cases.

It is evident from this review of the changes in eosinophile counts that the biology of these peculiar cells is extremely complicated and that much further study must precede our satisfactory understanding of their behavior under normal and abnormal conditions.

Fate of Eosinophiles

Normal human adult blood occasionally shows eosinophiles in which the nucleus stains feebly and the granules are separated widely. These cells are undoubtedly disintegrating. Such cells are much more abundant in abnormal blood.

Fragments of these cells can be found within the cells of the spleen and the liver, and in other areas of the reticulo-endothelial system. In abnormal blood they are occasionally found within the endothelial cells.

There seems to be no doubt that these cells die in the circulation, are ingested by phagocytic cells and are used as food by the tissues of the body.

Basophilic Granular Cells

Basophiles, or mast cells, are scanty in normal, adult, human blood, but are more common in embryonic blood, in the blood of animals and during the course of certain diseases, especially the leukemias. In normal adult human blood one or two mast cells may be found in a differential count of 2,000 to 5,000 cells. Occasionally a specimen of normal adult human blood is found which contains no basophiles at all, even when several students make simultaneous differential counts of the same blood taken at the same time, each student counting 500 to 1,000 cells. If the basophiles exceed 1% or 100 per cubic millimeter of blood, some explanation of the excess must be sought.

A basophile is eight to ten microns in diameter on the warm slide. It has a nucleus which may be roundish but is usually lobate. The nucleus stains feebly and chromatin masses are usually very dimly visible. There

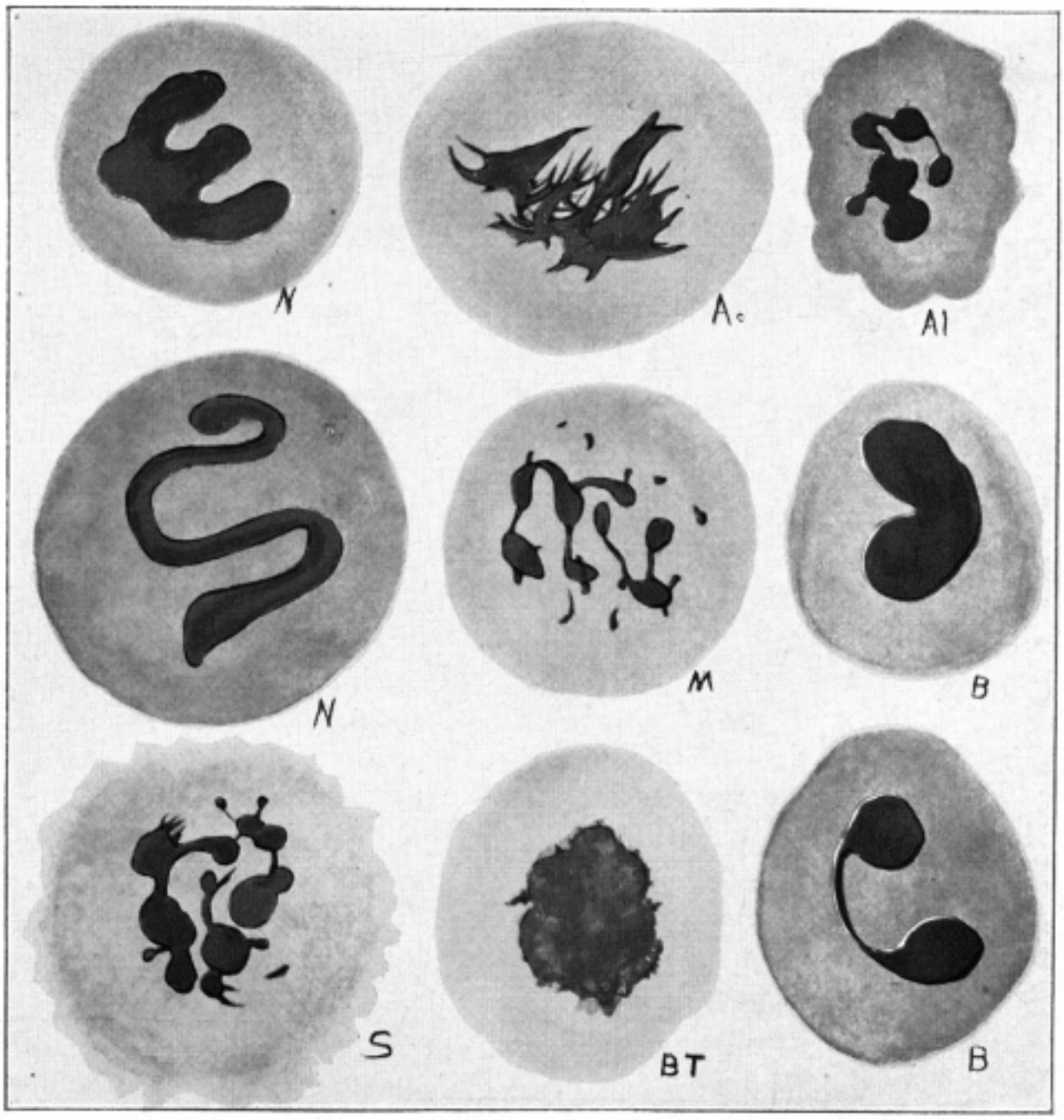


PLATE III

NEUTROPHILES

N. N. normal neutrophiles.

Ac, Neutrophile from blood showing acidosis and toxemia of the fatigue type.

Al, Neutrophile from blood with high alkalinity and toxemia due to excessive use of soda as a drug.

M. Neutrophile from blood of patient with late cancer.

B. Immature neutrophile from patient with developmental anemia.

S. Senile neutrophile.

BT. Immature neutrophile showing effects of acidosis.

is a very scanty hyaline, feebly basophilic protoplasm which may not be visible at all in normal blood but is usually visible in leukemic blood. The granules are variable in size usually including a few which are very large. Like eosinophile granules, these seem to be the more active part of the protoplasm when ameboid movements occur. In living blood basophiles are recognized with difficulty, being distinguished from eosinophiles chiefly by the larger and more varying size of the granules and their less marked activity. The granules are absolutely basophilic and are left unstained by all acid dyes. Those found in normal blood are not easily soluble in water, so that they stain easily with suitable dyes in watery solutions. Those found in leukemia are more easily soluble in water, so that watery solutions of stains are not suitable for them unless they have received special methods of fixation.

Mast cells, or basophiles, give the oxidase reaction. They are formed in the red bone marrow. Cells which are indistinguishable from these except that they may not give the oxidase reaction, are present in the tissues of the body, and these are increased during inflammatory states, especially those of traumatic origin. These cells are known to enter the blood stream and it seems very probable that they wander into and out of the blood vessels according to the changing tensions of the various gases and other substances in solution in the blood plasma and in the tissue juices. They are more abundant in the tissues when absent in the blood, and vice versa.

It is not yet known whether they have any functions different from those of other granular cells or not. They are increased in the leukemias. In gonorrheal pus and in pleural exudate they are often very abundant; these abnormal accumulations of basophiles differ somewhat from the basophiles found in normal blood and also from those of leukemia.

Amphophiles

In abnormal blood amphophilic cells are sometimes found. Only rarely do they appear in the blood of healthy individuals. These have usually a single round, vesicular, eccentric nucleus, often lobate, rarely notched or polymorphic, feebly staining with ordinary nuclear stains, and often very eccentrically placed. The granules are vividly stained, some with basic and some with acid stains. These, lying over the paler nucleus, offer a brilliant spectacle. By washing the smear with a feebly acid or feebly alkaline solution, the amphophilic granules can usually be induced to stain as eosinophiles or as basophiles at will. In general appearance the amphophile resembles the basophile, with which it is usually associated.

The Hyaline Cells

Hyaline cells form from one-fourth to one-third of the white cells of normal adult human blood. Of these the lymphocytes are the most numerous. Monocytes are present in small numbers. Under abnormal conditions plasma cells, hyaline myelocytes and a few other less common forms may appear. In the discussions of these less common forms there have been great differences in the terminology employed by different authors. The developmental relations of different types have also been a matter of considerable discussion, and many disputed points must await further study. In this connection the differences of opinion between the unitarians, who hold that the "stem cell" is the progenitor of all blood cells, and the dualists, who believe that there is a limitation of the developmental potentialities of the stem cells, is of some temporary interest. With further study the actual facts will terminate the discussions.

The development of certain forms of hyaline cells from certain other forms is well known. Defibrinated blood containing no plasma cells, placed in tubes and kept in moist air in an incubator, was found to contain plasma cells after five days, in our laboratory. In other laboratories cultures of blood cells have shown various instances of a changing cell type, apparently due to metaplasia. Studies made of inflamed tissues indicate that certain cells may become transformed into other forms under abnormal circumstances.

Lymphocytes

Normal adult human blood contains small lymphocytes, which make up about 25% of the white cells; large lymphocytes, which make up about 4% of all the white cells; and, occasionally, a cell is found which is intermediate in size between the large and the small lymphocytes. In the lymph nodes there are hyaline cells of very much larger size, the progenitors of the lymphocytes of the blood. These may enter the blood under abnormal conditions and they are then called large endothelial cells or giant lymphocytes according to their stage of development. Lymphocytes are found in relatively larger numbers in the blood of young individuals, whether human or other mammalian blood is studied; and the lower mammals have larger proportions of lymphocytes than the higher. Normal adult human beings have a smaller percentage of lymphocytes than is to be found elsewhere among mammales. (Plates X, XIII)

Functions

The functions of the hyaline cells are not well known. The large hyaline cells are phagocytic for certain pathogenic organisms, especially for protozoa (malarial parasites; histoplasma capsulata). Since they

are increased during certain infections it may be supposed that they exert some protection against either bacterial invasion or bacterial poisons.

The phagocytic activity of the lymphocytes seems to be an important factor in the absorption of fats from the intestinal tract. In this way particles of fat are carried into the lymph spaces without the necessity of first being broken up into soluble substances. They also are concerned in providing conditions necessary for growth and repair. This last function is not well understood. They may neutralize poisons or they may elaborate needful nitrogenous materials for building or they may perform other services.

Lymphocytes and other large hyaline cells from the blood are able to live and to multiply in blood serum, in vitro. Fibroblasts are unable to grow in blood serum alone, but when cultures of lymphocytes and large mononuclears grow near cultures of fibroblasts, the latter become able to live and to multiply. Apparently the lymphocytes produce from the serum albumins or globulins the substances required by the fibroblasts for their nutrition. An important function of the lymphocytes is the utilization of proteins absorbed from the intestinal tract in the manufacture of the peculiar proteins necessary for the feeding of the other cells of the body.

Development

The lymphocytes of normal adult human blood are formed in lymph nodes and in the spleen, tonsils and other lymphoid structures. Those derived from the red bone marrow are from small lymphoid masses within the marrow and not from those areas of the marrow which produce the red cells and the granular cells.

A short review of the structure of the lymphoid tissue explains the manner in which lymphocytes are formed and are enabled to enter the blood stream.

In the spleen, tonsils and all other lymphoid tissues the structures present similar characteristics. There is a delicate framework of connective tissue trabeculae, and associated with this are the reticular cells. These are stellate, oval, spindle-shaped or elongated cells with a nucleus which is roundish, oval or elongated, and has scanty, feebly basophilic chromatin. These reticular cells are abundant in many organs of the body; they are phagocytic and moderately motile. They are part of the reticulo-endothelial system and share in the general functions of that system.

Associated with the reticular cells is a group of cells, usually syncytial, with smaller, paler nuclei, less differentiated protoplasm, and

no apparent motile or phagocytic activity. Between the reticular cells and the syncytial cells are all intermediate forms, and the syncytial cells seem able to change into reticular cells or to produce lymphocytes on demand. They are not found in the circulating blood in typical form. The connective tissue trabeculae with the histiocytes and the syncytial cells form the reticulum of the lymphoid tissue. In the interstices of this reticulum there are abundant masses of lymphocytes and a varying number of reticular cells (histiocytes or macrophages) which have become removed from the immediate vicinity of the trabeculae. The lymphocytes include several sizes.

Cells apparently identical with the large lymphocytes of the circulating blood are present. These have been called lymphoblasts or lymphoidocytes in this situation. They are most abundant in proliferating lymphoid tissue and are rather scantily found in resting lymph nodes. They divide by karyokinesis and thus are formed the small lymphocytes.

Cells identical with the small lymphocytes of the blood stream and of the tissue spaces generally are most abundant in the lymph nodes. These do not divide. They may be the only cells present in the spaces of the reticulum of a resting lymph node.

Very large lymphocytes, never found in circulating normal human blood, apparently are the daughter cells of the lymphoblasts grown to several times their original diameter. They often show karyokinetic figures and their daughter cells are smaller in size; small lymphocytes may be derived from these cells also, especially in rapidly proliferating lymphoid tissue. These very large lymphocytes are never found in circulating normal human blood but under abnormal conditions may reach the blood stream. They are not identical with the giant lymphocytes of the blood of certain lower mammals, sometimes found in the atavistic blood of morons or other imperfect human beings.

Cells showing karyokinesis are not found in the blood stream normally, but when lymphoid tissue is seriously inflamed or in lymphatic leukemia dividing forms may be found in the circulating blood. Cell division of lymphoid tissue is always by karyokinesis. Direct division and budding have never been reported for them.

The tonsils and spleen as well as other lymphoid tissues show peculiar pale, roundish areas called nodules or germinal or germ centers. The general structure of these areas is about the same as that already described, but these centers undergo marked variations in activity, both normally and in answer to unusual demands made upon them by toxic or inflammatory conditions.

Each such nodule has its own blood supply and this may be the only visible indication of such a nodule in the resting lymph node. No such nodules are present at birth or for some weeks thereafter; they increase rapidly during childhood and youth, then diminish very slowly until, in old age, they disappear altogether. They show considerable variations in activity even under normal conditions, and they react to toxic and inflammatory states with very rapid proliferation.

Active Phase of Lymphoid Tissue

During a period of increased proliferation the blood supply becomes increased. Vasomotor nerves for these tissues have not been demonstrated nor are secretory nerves known to exist for them. The impetus to increasing proliferation seems to be in the character of the blood or the lymph reaching the nodes. The mode of stimulation is not yet understood. The outlines of the nodule become very definite and they are paler in tint than the rest of the section of a lymph node in all ordinary methods of staining. The reticulum is relatively less abundant but it has the same structure as in other parts of the section. The lymphocytes are usually chiefly of the lymphoblast type and these show abundant karyokinesis. The very large lymphocytic cells are also present; very frequently these are grouped in threes to sixes; rarely in larger groups. Many intermediate sizes are present. Small lymphocytes are occasionally very abundant. Very large reticular cells, macrophages containing fragments of blood cells and lymphocytes, are often present; these are derived from the reticulum in this location as in other parts of the lymph node. They may reach a diameter of twenty or more microns, especially in the lymphoid proliferation due to acute pyogenic inflammation.

A comparison of the resting lymph follicle with those in various stages of activity indicates that the reticular cells in the immediate vicinity of the arteriole supplying the nodule first show karyokinesis and that the cells so produced show consecutive changes and differentiation with succeeding divisions and growth until the lymphoblasts and the lymphocytes are developed abundantly. As the small lymphocytes are produced they tend to assume positions at the periphery of the nodule. As they are small cells with relatively large, deeply basophilic nuclei they form a dark ring which seems to surround the nodule. They pass from this peripheral location into the lymph stream and the venous blood. As they increase slightly in size in this passing they become the adult small lymphocytes of the blood and the tissues. As the period of activity wanes the peripheral zone of small lymphocytes

forms a smaller and smaller ring around the diminishing numbers of larger, actively dividing cells until these have almost disappeared and the nodule again assumes its resting stage.

Small Lymphocytes in the Blood

The small lymphocyte has about the size of an erythrocyte, in life, but it may spread out to eight or nine microns in diameter in a thin, stained smear. The protoplasm as seen in the living cell is almost or quite structureless. By special methods of staining a few azur granules and a delicate cytoplasmic reticulum can be made visible. The cytoplasm varies from deeply to faintly basophilic. There is no cell wall and the limiting layer is of the sol type. In many cells there is so thin a layer of protoplasm that the nucleus may seem to be almost or quite naked. Mitochondria are small and lie near the nucleus. The protoplasm is somewhat more abundant upon the side of the cell opposite the crease in the nucleus.

The nucleus has a limiting layer which is quite distinct, and a nuclear wall is sometimes discernible. The nuclear structures are deeply staining. One rather large and two or three smaller nucleoli are usually visible. The chromatin is arranged in rather large irregular masses presenting something of a tiger-skin arrangement. Wheel-like arrangements are rare. The nucleus usually shows a shallow or deep groove or infolding upon one side.

Large Lymphocytes

The large hyaline cells of normal, adult, human blood are ten to twelve microns in diameter, in the living state, and they make up from four to eight per cent of the white blood cells. They are relatively increased by those physiological conditions which diminish the neutrophiles, but are not actually increased by physiological conditions. Under certain abnormal conditions they are considerably increased. They are relatively decreased by those conditions which cause actual increase in the neutrophiles and other cell elements but are not actually diminished appreciably under any normal conditions.

In large lymphocytes the protoplasm varies in basophilia and granules are stained with more difficulty. The cytoplasmic reticulum is more easily demonstrated. The edges are even more definitely of the sol type and the periphery of the cells seems to pass indefinitely into the blood plasma in cells which seem to be older. These cells are phagocytic and they ingest animal parasites of the blood with avidity. The nucleus is not often moved about by the ameboid activity present on the warm slide. The cytoplasm contains azur granules and occasionally a few

basophilic granules of varying degrees of basophilia. Mitochondria are somewhat more abundant than in small lymphocytes. On the warm slide the protoplasm may form bud-like pseudopodia which become detached from the cell completely.

The nucleus is almost spherical and it has a fold or crease across one side. In some cells this fold may divide the nucleus into two almost separated parts; in other cells the nucleus is only notched, creased, reniform or saddle-shaped. The chromatin masses are arranged much as is the case with the small lymphocyte nuclei, and they are about the same size; there are clear spaces between the chromatin masses and fine linen threads pass between the chromatin masses. In cells which appear to be older nuclear vacuoles and canaliculi are often present; these are filled with a clear fluid which does not take any of the ordinary stains.

Variations in Lymphocytes

The proportion of hyaline cells is increased under many pathological conditions. Physiologically, the hyaline cells are relatively increased by fasting and relatively diminished by the factors which cause an increase in the neutrophiles. They are actually increased at fairly regular intervals corresponding to the periods of increased proliferation during the active phases of lymphoid tissues.

Effects of Sudden Emotional States

Emotional states suddenly produced increase the leucocytes, especially the hyaline cells, temporarily. This reaction does not occur in animals or human subjects who have undergone splenectomy. Animals which have been subjected to injury of the splanchnic nerves or the solar plexus do not show the reaction. Under the influence of emotions, especially fright and anger, the splenic capsule contracts, thus forcing many hyaline cells into the blood stream. The biological significance of this increase in the hyaline cells lies in the fact that these cells have, as one of their important functions, the healing of wounds.

Lymphocytosis

Lymphocytosis, or abnormal increase in the lymphocytes, occurs more frequently in children than in adults. It is usually marked in ordinary gastro-intestinal diseases of children, in rickets, mumps, whooping-cough, bronchopneumonia and cervical adenitis. The lymphocytes are increased also during the reaction to tuberculin used for diagnosis. In severe pyogenic infections to which the hematopoietic tissues are unable to react efficiently there may be a great increase in the hyaline cells. They are increased in several adult diseases, such as malaria, typhoid, relapsing

fever and a few less common conditions. The large hyaline cells and especially the monocytes are greatly increased in agranulocytic angina, kala azar, Malta fever and also in severe pyogenic infections when ordinary granular leucocytosis does not occur. The large hyaline cells are increased in tetrachlorethane poisoning. Chronic diseases which are usually characterized by lymphocytosis are syphilis, whether acquired or inherited; tuberculosis of all parts of the body except the meninges and brain, and several other chronic diseases. During the digestion of a meal rich in carbohydrates and in patients whose diet includes an excessive proportion of carbohydrate and hydrocarbon foods, the lymphocytes are relatively and absolutely increased. During starvation the lymphocytes are relatively and sometimes absolutely increased as long as the glycogen and fats are being utilized as foods. During lysis in pneumonia and during convalescence in scarlet fever the hyaline cells are increased. After injury to the spleen or to any great area of lymphoid tissues the large hyaline cells are usually considerably increased.

Relative or absolute lymphocytosis is usually present in scurvy, pernicious anemia, chlorosis, general debility, late typhoid, hemophilia, exopthalmic goiter, and sometimes associated with malnutrition.

In lymphatic leukemia the increase in hyaline cells may be tremendous. The small hyaline cells are most abundant in the less malignant and chronic lymphatic leukemia, the larger forms in the more malignant acute lymphatic leukemia and in the terminal stage of the chronic type. In the terminal stage of myeloid leukemia the hyaline cells may predominate and may seem to be exclusively present, but in this case the hyaline cells are really of the myelocytoid type and are derived from bone marrow. The oxidase reaction may or may not be present in these hyaline myelocytes. (Plate XIII)

In the presence of inflammation involving lymphoid tissues the hyaline cells are increased, and this increase is associated with many structural changes in large and small lymphocytes and endothelial cells. The small lymphocytes then show less distinct nuclear structures, the nuclear limits are less well defined, there is less intensely basophilic protoplasm, the protoplasm is more abundant, and basophilic granules appear occasionally in the protoplasm. The large lymphocytes include some which are smaller than normal, though still distinctly larger than the small lymphocytes. These show less intensely basophilic protoplasm and occasional basophilic granules. The large hyaline cells occasionally contain a few fine granules which are neutrophilic or feebly eosinophilic, and their protoplasm is occasionally vacuolated and at the periphery of the cell the protoplasm presents a frayed-out or a ragged outline. These

large lymphocytes then have more irregular nuclei; notched, reniform, saddle-shaped and occasionally double or even polymorphic forms are much more abundant than are the normally rounded nuclei. In severe inflammations of lymphoid tissue the large and the small lymphocytes may often show karyokinetic figures and dividing forms are not rare.

The endothelial cells present nuclei larger than normal, with less marked affinity for basic stains. The protoplasm presents a swollen appearance and it flatttens out on the warm slide very noticeably. The cell outlines are indistinct and often ragged. Granules do not appear, though sometimes there may be inclusions of fragments of other cells, of bacteria or of foreign particles.

Lymphopenia

Lymphopenia is of much less common occurrence. During the digestion of fats and proteins, in some cases of tuberculosis and in some cases of lymphosarcoma the lymphocytes may be reduced to a few hundred in number per cubic millimeter of blood. They are relatively diminished in all conditions associated with marked neutrophilic leucocytosis but are often actually slightly increased in these diseases.

Effects of Bony Lesions

Direct effects of vertebral lesions upon lymphoid tissue have not been observed, but indirectly very marked changes in lymphoid tissues may be due to such lesions. Space permits only a few references to these conditions.

Lesions of the cervical and upper thoracic vertebrae affect the circulation through the mucous membranes of the head and throat. A mild, chronic congestion of these tissues results. With this congestion, there is a moderate but persistent edema and some lessening of the alkalinity of the tissue juices. The lymph from tissues so affected passes into the lymph nodes, and these are stimulated into increased activity. The small normal lymphoid tissues of the nasopharyngeal region are often so affected and adenoid growths result. Moderate non-infectious enlargement of the tonsils is another such result of cervical or upper thoracic lesions. Enlargement of the cervical chain of lymph nodes results from longer existence of the same lesions. Immunity is diminished in all tissues affected by lesions as well as by the engorgement and hyperplasia of the lymphoid tissues. Infection of the tonsils is a later result of the lesion. Tubercular infection of the cervical lymph nodes is partly due to local lowering of the immunity of these glands and partly to systemic conditions, themselves often due to mid-thoracic lesions. The common infectious agents are almost universally present.

Lesions of the ninth thoracic vertebra cause dilatation of the splenic blood vessels and decreased tone of the muscles of the splenic capsule. With the congestion thus produced there is inevitably some hyperplasia of the splenic lymphoid tissue.

Lesions of the lower thoracic vertebrae cause mild chronic congestion and some loss of tonicity of the intestinal walls. With the resulting mild, chronic stasis, there is a mild but constant edema and a constant mild poisoning due to the stasis. Hyperplasia of the lymph nodes of the intestinal tract is very common under such conditions.

Lesions of the third lumbar and neighboring vertebrae cause mild and chronic congestion of the uterus. With this congestion there is edema and diminished alkalinity of the tissue juices. The weight of the edematous membranes increases with the persistent congestion though this may not be severe in degree at any one time. A serous polyp may result or an area of lymphoid tissue in the immediate vicinity of the edematous region may proliferate and form a lymphoid polyp of the cervix. In other cases there may be marked connective tissue proliferation and a fibrous polyp is produced. The character of a polyp produced by upper lumbar lesions may pass through these three stages in order, in experimental animals, and a similar progression is quite possible in the human subject.

In many areas of the body similar relations in pathogenesis may be observed. Such relations are suspected when the blood cells include immature or other abnormal forms of lymphocytes associated with a few granular myelocytes and other evidences of disturbed circulation through the red bone marrow of the bones concerned in the lesion.

Other Hyaline Cells

A few other large hyaline cells may be found in very small numbers in normal adult human blood; these are found in considerable numbers under abnormal conditions. Turck cells are somewhat like ordinary large hyaline cells. The origin of Turck cells is not certainly known; they are increased under certain pathological conditions, and they are considered identical with plasma cells by several writers.

Turck cells are hyaline cells with intensely basophilic protoplasm and deeply staining nucleus. The spongioplasm is usually distinctly visible and vacuoles are frequently present in the cytoplasm. Fatty material may be stored in vacuole-like areas, thus producing the "foam" cell type. The protoplasm presents a somewhat granular appearance but contains no real granules. These cells are ten to fifteen microns in diameter. They are ameboid and phagocytic, and often contain fragments of red cells. This is particularly true in those diseases associated with blood de-

struction, such as malaria. The nucleus is often eccentric and may be altogether free from a cytoplasmic covering upon one side. They are certainly concerned in the healing of wounds, and they seem to be derived from the adventitial cells.

Plasma cells are like Turck cells except that they are often angular on account of pressure conditions; the nucleus is eccentrically placed, and very often the nucleus contains more definite chromatin masses which may show a wheel-like arrangement. The nucleus often lacks any recognizable protoplasmic covering for half or even more of its surface. The protoplasm is often vacuolated in the vicinity of the nucleus. The protoplasm is intensely basophilic at its periphery and very rarely can fine neutrophilic or basophilic granules be demonstrated in the region farthest from the nucleus. These cells are not found in normal blood. They may be abundant in inflammatory conditions and seem to be concerned in regeneration. They are derived from small lymphocytes, probably those of adjacent tissues.

Myeloplaxes are very large hyaline cells which are very rarely found in the circulating blood. They are identical with the megakaryocytes of the red bone marrow.

During pregnancy a few cells derived from the placenta may appear in the maternal blood. These are very large hyaline cells, with feebly eosinophilic or basophilic protoplasm and round nuclei, not deeply staining and without marked nuclear structures. They are often not found at all though repeated examinations of the blood are made, and they may appear in considerable abundance in other cases. They are of little diagnostic significance for this reason, and also because the diagnosis of pregnancy is usually easy before the placental cells can be expected. In three of our cases in which pregnancy had not been suspected the finding of these cells led to further study of the patient with this possibility in mind, and, in one case, an X-ray plate showed a normal fetus which went on to normal birth. In another case proposed surgical interference for uterine fibroids was postponed and a normal baby was born. In one case the fetus was dead at the time the pregnancy was first suspected. The diagnosis was made as a result of finding these cells in the blood.

Hyaline myelocytes are not found in normal blood. They are present in very large numbers in late stages of chronic or in rapid and severe acute leukemias. They may be found in small numbers when the bone marrow is invaded by malignant disease, especially in cancer metastases. They are then associated with other types of myelocytes and myeloblasts.

Hyaline myelocytes have very large, spherical nuclei, usually feebly staining, and a very narrow rim of protoplasm, usually with very marked affinity for basic stains. No granules are found by the ordinary staining methods. They vary in size, the smaller being distinguishable with difficulty from small lymphocytes and the larger having a diameter of forty microns or more. Every size between these may be found in sever cases of either lymphatic or myeloid leukemia. (Plates XII, XIII)

The Fate of Hyaline Cells

Disintegrating lymphocytes are not found in normal human blood, which seems to serve them chiefly as a means of transportation. They emigrate into the tissue spaces and to the surfaces of mucous membranes in great numbers and many are lost to the body in the feces and in various excretions. They undergo destruction in the tissue spaces and are utilized as food by the tissue cells. Their period of life is probably less than one day under normal conditions. When there is any inflammatory condition present in the body they are formed and destroyed in great numbers.

The fate of the other hyaline cells is probably similar. They become disintegrated in the blood or in the tissues spaces, are digested as a result of the enzymes of the tissue juices, dissolved, and probably are used as food by the active tissues of the body.

Further study of these various cell-types is urgently required. A clearer understanding of the actual condition of the patient would be possible were we able to recognize the variations in these cells under normal and abnormal conditions.

Phagocytic Monocytes of the Blood

The relations of the cells of the reticulo-endothelial system to the large phagocytic monocytes of the blood has been the subject of much study, yet many problems remain unsolved. That certain of the phagocytic monocytes found in the blood stream under abnormal circumstances are derived from the endothelium of the sinuses of the bone marrow, spleen and other lymphoid tissues, and probably from the liver and from certain glands of internal secretions is fairly well demonstrated by experimental and by pathological findings.

Large, hyaline, mononuclear, phagocytic cells are found in the blood stream, a few normally, and many under abnormal conditions. Their presence in more than 1%, or in abnormal forms, indicates some definitely abnormal condition in the body. If they are merely increased in number but present no definitely abnormal forms, it may safely be concluded that there is some circulatory disturbance affecting the spleen and

the liver. If they show vacuoles or inclusions, the significance depends upon the nature of these abnormalities. If they are immature and are derived from lymphoid tissue, it may be concluded that there is a chronic infectious process present in the body and that this affects a considerable area of lymphoid tissue. If many myelocytoid forms of granular cells or if a few immature red cells are present without other evidences of anemia, then there is some marked disturbance of the red bone marrow; if there is no evidence of serious disease of the red marrow the disturbance may be merely circulatory. If excessive numbers of the endothelial cells are present, with high red cell and rather high white cell counts, without other evidence of visceral disease, then it may safely be concluded that there is some very considerable area of the vascular bed in which the circulation is inefficient. Cardiac inefficiency is suspected in such conditions.

The endothelial cells which normally remain within the lymphoid tissues are found in the circulating blood when there is any chronic inflammatory state which affects these tissues. These cells also may be found in the blood stream of an individual who suffers from the effects of ninth thoracic or neighboring lesions; in this case the cells are derived from splenic sinuses. The cells are often called splenocytes because they are so commonly derived from the spleen but they may be derived from any lymphoid tissues which have been affected by inflammatory conditions. They are not commonly derived from lymphoid tissues other than the spleen as a result of the direct effects of bony lesions, on account of the anatomical peculiarities of the tissues concerned.

Large mononuclear phagocytes containing fragments of erythrocytes and particles of hemoglobin or of hemoglobin derivatives are found in the circulating blood under several quite different conditions. Such cells are often found in the blood after an attack of pneumonia, and are undoubtedly derived from the adventitial cells of the lungs. Occasionally such cells are found in the blood of persons with mitral lesions; in this case they are also from the pulmonary adventitial cells and they are a result of the excessive pulmonary congestion. They may also be found, less frequently, in persons with bony lesions which affect the heart and thus the pulmonary circulation, or which interfere directly with the pulmonic vasomotor centers in the upper thoracic spinal centers.

Endothelial cells containing fragments of erythrocytes or the brownish granules of one of the hemoglobin derivatives are usually present in the blood during an attack of malaria and for some time afterward. These same cells ingest and probably destroy the malarial plasmodium. The organism of histoplasma capsulata was found within endothelial

cells and large lymphocytes in the blood of one of our patients, and the blood of inoculated guinea pigs contained many endothelial cells containing the organism. (Plates X, XI)

During the course of pernicious anemia the endothelial cells of the sinuses of the spleen, lymph nodes, bone marrow and hemolymph glands ingest great numbers of the abnormal red cells, and the endothelial cells found in the blood stream which contain fragments of red cells are probably derived from those areas. Whether the Kupffer cells commonly gain entrance into the blood stream is not known; that they do pass into the blood stream under experimental conditions seems definitely verified by Sabin and others. The Kupffer cells are abundantly filled with fragments of red blood cells during relapse in pernicious anemia, and this is true also of sickle-cell anemia and of other diseases characterized by abnormal fragility or abnormal structure of the red blood cells.

Endothelial cells carrying granules of melanin are often found in the circulating blood when melanotic tumors are present anywhere in the body, and are more abundant when there are metastases in the liver, lungs, spleen or bone marrow.

The amyloid substance is first found in the Kupffer cells of the liver when that gland undergoes amyloid degeneration. Endothelial cells containing granules of amyloid or a precursor of that substance are often found in the circulating blood under such circumstances.

Endothelial cells often carry iodophilic granules and such endothelial cells are rather abundant when there is an abnormal condition of the liver. When the neutrophiles as well as the endothelial cells of the blood carry iodophilic granules some disturbance of sugar metabolism is suspected and a determination of the sugar tolerance curve is indicated. While patients with marked iodophilia occasionally show no further evidence of disturbances in sugar metabolism, yet the relation is sufficiently common to warrant further investigation.

The large phagocytic cells carrying iodophilic granules are also found when there is a marked disturbance in protein katabolism. Such conditions occur during the absorption of chronic pus foci, during the period of absorption after pneumonia, during the absorption of the products of degeneration of a tumor of any kind or of the absorption of any degenerating tissues of the body, or during rapid loss of weight after the fat has been absorbed during fasting, or even when a patient with intestinal atony is for some time on a heavy protein diet.

The endothelial cells seem to have some especial affinity for fat, and droplets of fat are occasionally found in these cells in the blood stream.

When there has been any injury to the shaft of a long bone, or when the fatty marrow has been invaded by infectious processes or by metastases of any malignant neoplasm, fatty globules are often found within the phagocytes of the circulating blood, including the endothelial cells. Fatty globules of small size may also be found free in the plasma under these conditions. Endothelial cells containing fatty globules are also found, occasionally, in disease or abnormal circulatory conditions affecting the liver, even though the hepatic disorder may not be very severe. When the fat of the body is undergoing rapid disintegration endothelial cells carrying fat are present; this is significant in patients who are reducing weight too rapidly. During the rapid absorption of pus, rapid degeneration of tumors, rapidly growing cancer, rapidly progressing pulmonary tuberculosis, and similar states these fatty inclusions within endothelial cells are occasionally found. When the cells are stained by ordinary methods the fatty globules appear as vacuoles. When stained by osmic acid or Sudan III their fatty nature is clearly shown.

Metallic poisons are often stored in the endothelial cells and occasionally these cells are found in the blood stream. Mesotherium is so stored, and this may be one cause of obscure anemias. Lead particles also are so stored and these have been found in the circulating blood in cases of chronic lead poisoning. In an obscure case in our laboratory in which pernicious anemia was strongly suspected the finding of metallic particles in several large phagocytic monocytes in the blood smears led to the correct diagnosis of chronic lead poisoning.

The endothelial cells of the peripheral blood are increased after emotional excitement, especially after fright and anger. Human beings who have undergone splenectomy do not show this reaction. From these and other related facts it may be concluded that during emotional states the contractions of the spleen force these cells out and into the blood stream. After removal of the spleen or of all or part of the sympathetic ganglia from the abdominal region the reaction does not occur in animals.

Human subjects and animals with lesions of the ninth thoracic vertebra (and, in less marked degree, neighboring vertebrae and ribs) show higher than normal numbers of large hyaline cells in the peripheral blood; no doubt this is due to the chronic slight congestion and the chronic slight atony of the spleen due to the lesions.

Under experimental conditions the cells derived from the reticuloendothelial system may be found in the circulating blood. By injecting certain colloidal or particulate substances into the blood of an animal

the endothelial cells may be stained in such a manner as to be easily recognizable wherever they are found. Blood examinations made at any subsequent time, after any selected manipulations planned to show some chosen relationship either show or do not show the stained endothelial cells in the blood. By selecting stains for which certain endothelial or reticular cells have special affinities, it is possible to determine whether those cells do or do not gain entrance into the blood stream. To some extent these experimental procedures involve relationships which may occur during pathological or physiological processes and the finding of cells from the endothelial system reported by various hemotologists is thus justified.

A very brief review of the studies made of the reticulo-endothelial system may be useful. The discussions by Aschoff first aroused general interest in the subject. Very many histologists and pathologists have made reports; unfortunately the cells have received many names and many descriptions because the methods employed have produced so many reactions on the part of cells which were, essentially, of the same class and of similar, if not identical, structure.

The descriptions of the phagocytes of the blood and the tissues given by Metchnikoff are classical. In his reports the neutrophiles of the blood are called microphages and the larger cells of the tissues macrophages. The macrophages are phagocytic and are usually found lying free in the meshes of the connective tissues around the smaller blood vessels and the capillaries. Because they are especially abundant in that location they have been called adventitial cells. Because they were observed by Ranvier to present peculiar budding processes he called them clasmatocytes. With ordinary staining methods they are not distinguished from the fibroblasts of the connective tissues.

The sinuses of the bone marrow, the spleen and other lymphoid tissues, the hemolymph glands and perhaps the tissue spaces of certain of the glands of internal secretions are lined with cells which resemble the endothelium of ordinary capillaries to some extent, but which are definitely phagocytic and which are able to store both particulate and colloidal dyes in such a manner as to prove them distinctly different from ordinary endothelium. The Kupffer cells of the liver, the podasteroids of the brain, the adventitial cells of nearly all the body, the plasma cells of the loose connective tissues and of the omentum as well as the cells of the sinuses of the bone marrow, spleen and other lymphoid tissues may, under certain physiological or pathological conditions be carried from many viscera in the blood stream. They may reach the

blood by way of the lymph channels and the thoracic duct under certain circumstances. They can be cultivated in vitro and their development watched. There is very good reason for supposing that the words tissue macrophage, blood monocytes, clasmatocyte, fibroblast, hemohistioblast, wandering adventitial cell, endothelial cell and other terms less commonly employed are really different names for the same cell which presents somewhat different appearances under differing circumstances. These cells are capable of developing from one form into certain others according to the needs of the body at any given time. Their developmental potentialities are still a subject of considerable discussion and of intensive experimentation. The endothelial cells are important in the protection of the body against foreign substances and infections and they are concerned in the development of tubercles, together with the lymphocytes. In tuberculosis the blood often contains a marked increase of these cells. The blood of children with tuberculosis usually shows these cells abundantly.

Further study of these cells should be made. With a definite understanding of their functions more adequate methods of diagnosis and therapy should become possible.

CHAPTER V

THE BLOOD PLASMA, PLATELETS AND FIBRIN

The fluid part of the blood is not less important than the cells which are carried therein. The constituents of the plasma change constantly, as the constituents of living cells change constantly, and, like living cells, the blood plasma maintains always a fairly uniform chemical structure. This uniformity is not, apparently, maintained by any vital activity of the plasma itself. The tissues which are bathed by the fluids derived from the plasma vary their activities according to varying plasma conditions, and the result of such variations in the physiological activities of normal tissue cells is the maintenance of a chemical and physical equilibrium of blood plasma, tissue fluids and lymph. So long as the circulation of the blood remains normal, and the various tissues of the body not too badly injured, the blood plasma and the fluid derived from it remain at nearly the same level of water-content, chemical structure and osmotic tension. The various salts and organic substances vary slightly according to varying diet, exercise and other physiological conditions but even these variations are only slight and transitory, so long as the circulation of the blood is normal and the tissues reasonably normal in structure.

The blood plasma serves as a method of transportation. The waste products of katabolism are removed by the blood plasma and are carried to the various emunctories to be eliminated from the body. Katabolites which can be utilized again are carried in the plasma to the tissues which need them. Hormones are katabolites of one tissue which serve a useful purpose in some other tissue; they are carried in the blood plasma from the places of their manufacture to the places of their functional activity. Various gases are transported in solution by the plasma. Food materials are carried from the intestinal tract to the liver and other tissues, to be elaborated into the compounds required by the living cells of the body, and from these various organs to the tissues which need food.

The blood cells themselves are fed by the plasma and they give off katabolites to the plasma. The plasma transports the newly formed blood cells from their sites of origin in the bone marrow and the lymphoid tissues over the entire body; the plasma feeds them through their

lives within the blood stream, the plasma receives their dead bodies and disposes of them.

The plasma is a medium of communication between distant tissues, by means of the hormones and enzymes which it carries and by the manner in which it is affected by varying physiological states. For example, if the blood plasma carries an excess of carbon dioxide certain groups of nerve cells are thereby so affected that they cause increased respiratory activity. Many such reactions occur constantly and the entire body is enabled to act as a unit because the circulating blood plasma as well as the delicate nervous tissues maintain always these systems of inter-communication between distant tissues.

The varying states of the blood plasma are best studied by means of the changes which occur in the blood cells, and by chemical analysis of the blood. The latter subject is beyond the scope of this book. The changes which occur in the blood cells as a result of changes in the circulating plasma is a matter of much interest.

Total Plasma Volume

The importance of determining the total plasma or blood volume is evident, yet at this time there is no known method which is sufficiently accurate and simple to be used in ordinary circumstances. In hospitals with a large and efficient laboratory staff it is possible to determine the total blood volume with reasonable accuracy. From published reports of work in this field it is now known that the total blood plasma per unit of body weight or body surface varies only moderately in nearly all diseases, and that so far as practical clinical experience is concerned the studies made of any given amount of blood are sufficiently accurate for diagnosis in most cases.

Much work has been done in an attempt to determine the normal amount of the blood in the entire body.

Welcher's findings were based upon a study of decapitated criminals. The blood was received into vessels, and the veins were then washed out with water. The hemoglobin was determined for the fresh blood and also for the washings mixed with blood. He estimated the total blood weight as 7.7% and 7.2% of the body weight for two different men.

Haldane and Smith allowed the patient to inhale a measured amount of carbon monoxide and after a time the amount of carbon monoxide hemoglobin was determined. This method gives figures approximately identical with those secured by the vital red method.

The best method now known for the determination of the total amount of blood in the human body is that employed by Rowntree and his associates of the Mayo Clinic. It consists, briefly, of the injection of a known amount of some harmless dye into one cephalic vein, then the withdrawal of a known amount of blood from the opposite cephalic vein three or six minutes later. The total amount of blood in the body can be estimated from the dye present in the withdrawn blood.

According to Denny and also to Bock the plasma volume of the blood remains at a definite level per unit of body weight in nearly all normal and abnormal conditions except in those associated with severe dessication of all the tissues. Changes in the total blood volume are due to changes in the corpuscle volume. Blood volume is increased during pregnancy but returns to normal within about a week after delivery. Blood volume is increased in certain anemias; the increase is in the plasma alone. Increased blood volume occurs in chlorosis, polycythemias and a few other diseases. Decreased blood volume occurs in dessication, after very severe hemorrhages and after severe diarrheas and severe sweating.

The total blood volume remains remarkably constant under varying physiological conditions.

Terminology

Rowntree advises the use of the terms normovolemia, hypovolemia and hypervolemia to express a normal relation between blood volume and body weight, abnormally low blood volume per kilo of body weight and abnormally high blood volume per kilo of body weight. When the blood cells are relatively increased the condition is called polycythemic normovolemia, polycythemic hypovolemia or polycythemic hypervolemia, according to the increase in cells in blood of normal volume (relative to body weight), or in blood of diminished or increased volume. Similarly, when the cells are relatively low and the serum relatively high the condition is called oligocythemic normovolemia, oligocythemic hypovolemia or oligocythemic hypervolemia according as the diminished cell count occurs in blood of normal, increased or decreased volume per body weight.

Normal Volume Relations

Rowntree has taken the figures which he has derived from an average of normals as a criterion; for blood, 87.7 cubic centimeters per kilogram of body weight; for plasma, 51.2 cubic centimeters per kilogram of body weight; for blood cells, 36.5 cubic centimeters per kilogram of body weight. This means that approximately one-eleventh of

the body weight of normal individuals is composed of blood. Findings for normal persons vary by 10% or more from these figures, just as some perfectly normal persons may have a basal metabolism rate of 10% more or less than 40 calories per hour per square meter, or a temperature slightly above or below 98.6°F. or a red blood cell count of four million or of five and one-half million, per cubic millimeter, and so for every other condition in which a definite normal figure is generally accepted.

Abnormal Blood Volume

Under several abnormal conditions Rowntree found significant variations in plasma volume and in blood volume.

In obesity without edema the amount of blood in the body is considerably increased, while the amount of blood per unit of body weight or per unit of body surface is considerably diminished.

In pernicious anemia the blood volume per unit of body weight or body surface is diminished. In secondary anemias the blood volume may be increased, decreased or unchanged, according to the causes of the anemias.

In polycythemia vera the total volume of blood is increased both actually and per unit of body weight and body surface. In secondary erythrocytosis the total blood volume remains almost or quite unmodified.

In edematous states there is little or no variation in the blood volume except in glomerulonephritis. In this renal disease the total blood volume may not be affected, and if it is affected at all it may be increased or diminished. Cardiac edema shows no significant changes in the total blood volume.

In Banti's disease and in splenomegaly without anemia, with or without cirrhosis of the liver, the blood volume is somewhat increased. In all forms of leukemia the total blood volume is increased considerably. Hypertension is associated with a normal blood volume per unit of body surface and body weight.

Alkalinity of the Blood

The blood has feeble alkalinity due to the presence of alkaline carbonates and alkaline phosphates. These bases are in feeble combination with the blood proteins, including hemoglobin, under ordinary conditions. With increasing carbon dioxide content the bases are set free, combine with the carbon dioxide and the essential neutrality of the blood is preserved. The presence of these bases in this loose chemical combi-

nation provides the "buffer action" which is of such great importance in maintaining the power of the blood to carry oxygen and carbon dioxide to and from the active tissues of the body. The alkalinity of the blood is commonly measured in terms of its ability to carry carbon dioxide, and variations in the carbon-dioxide-combining-power of the plasma are identical with variations in the alkalinity of the blood.

The average reaction of the blood of a healthy young man, at rest, as reported from several laboratories, is as follows:

Arterial blood plasma.....	pH	7.443
Arterial blood cells.....	pH	7.152
Venous blood plasma.....	pH	7.416
Venous blood cells.....	pH	7.134

The average reaction of the blood of a healthy young man, after vigorous exercise lasting for about an hour, is as follows:

Arterial blood plasma.....	pH	7.375
Arterial blood cells.....	pH	7.062
Venous blood plasma.....	pH	7.277
Venous blood cells.....	pH	7.026

The alkalinity of the average young healthy woman is somewhat lower, the variation being in harmony with the lower hemoglobin and the lower specific gravity of the blood of women. These variations are not primarily sex characteristics. Women who live active lives show the specific gravity, cell count, hemoglobin content and alkalinity characteristic of the blood of men who live active lives, and men who live sedentary lives show the specific gravity, hemoglobin content, cell count and alkalinity characteristic of the blood of women who live sedentary lives. Since more women than men are inactive, and more men than women live active lives, the proportions given as characteristic of the sex variations in blood are fairly accurate, so far as averages are concerned. It must always be remembered, however, in making blood examinations for women who are athletic, and for men who live quiet and inactive lives, that figures normal for the habits of the patient must be taken as normal without regard to sex.

The alkalinity of the blood varies slightly and temporarily as a result of many physiological conditions. Increase in the amount of carbon dioxide of the blood results in increased respiratory activity, the elimination of the carbon dioxide and return to normal resting alkalinity. Increased intake of alkaline substances results in the elimination of the excess, chiefly by the kidneys.

Changes in the alkalinity of the blood as a result of disease may be considerable. In diabetic coma there are several organic acids present in the blood plasma. Henderson gives the following figures for a patient in diabetic coma:

Arterial blood serum	pH	7.140
Arterial blood cells	pH	7.075
Venous blood serum	pH	7.028
Venous blood cells	pH	6.955

The alkalinity of the blood is diminished in all diseases in which the aeration or the circulation of the blood is impeded, as in pulmonary tuberculosis, pneumonia, endocarditis and other cardiac diseases. The alkalinity of the blood is also diminished in renal disease. Henderson gives the following figures for a moribund nephritic:

Arterial blood serum	pH	6.994
Arterial blood cells	pH	6.987
Venous blood serum	pH	6.969
Venous blood cells	pH	6.970

The fact that so little difference exists between venous and arterial blood cells and blood serum is of interest in this connection.

The alkalinity of the blood is increased by increased atmospheric pressure. Menten gives the following figures:

Atmospheric pressure	mm Hg	762 or more
Reaction venous blood serum	pH	7.72
Atmospheric pressure	mm Hg	730 or less
Reaction venous blood serum	pH	7.40
Menten's figures are, for normals	pH	7.50

Menten gives also the following figures for abnormal states:

Malignancy (60 cases)	pH	8.00 to 8.44
Malignancy (5 cases)	pH	7.50 to 7.65
Active syphilis	pH	7.90 to 7.94

Henderson gives the following figures for a patient with myxedema:

Arterial blood serum	pH	7.506
Arterial blood cells	pH	7.228
Venous blood serum	pH	7.458
Venous blood cells	pH	7.214

Henderson gives for a patient with pernicious anemia:

Arterial blood serum	pH	7.450
Arterial blood cells	pH	7.100
Venous blood serum	pH	7.398
Venous blood cells	pH	7.092

Accurate determinations of the reaction of the blood can be made by means of a potentiometer or a voltmeter. These methods do not require an unreasonable amount of blood. Determination of the carbon-dioxide combining power of the blood serum gives results of practical value but this method requires rather more blood than it may be advisable to remove from the veins of a very sick person.

Methods based upon the use of reagents are much less accurate but they may give results of clinical value under certain circumstances.

The effects produced upon the cells of the blood by variations in the reaction of the blood plasma are of considerable interest.

In acidosis due to the presence of abnormal acid substances in the blood stream, such as may be found in diabetes mellitus, the cells show no marked variations in their staining reactions.

In acidosis due to renal disease differential staining is difficult because the chemical differences between nucleus and protoplasm, and between the hyaloplasm, deutoplasm and granuloplasm of the cells are less marked than in normal blood. In slides stained with eosin and methylene blue the nuclei take a dull, grayish blue, the neutrophilic granules a grayish lavender, the eosinophiles show a dull, bluish hyaloplasm with grayish, pink granules, the hyaline cells show a dull, bluish protoplasm in which irregularities of staining often cause a semblance of granulation. The various structural changes in the cells due to disturbances in protein katabolism are usually present also.

In acidosis due to circulatory disturbances, such as are found when cardiac inefficiency supervenes in other diseases, the changes in staining are similar but are usually less marked. In addition there is considerable gregariousness of the cells, and the neutrophiles and the monocytes show marked irregularities in contour; often they have their edges quite ragged and frayed. Concentration of the cells of the peripheral blood, with red and white cells counts considerably above that normal to the individual are also usually conspicuous factors in the acidosis due to cardiac inefficiency.

In the blood of persons with alkalosis the cells show increased avidity for differential stains. In slides stained with eosin and methylene blue

the nuclei are a very vivid and brilliant blue. The hyaline cells also show unusual brilliance of staining and they frequently contain azur granules of large size and a royal purplish blue. The neutrophiles are definitely lavender in hue and the eosinophiles shine with even more than their normal brilliance. All the cells of the blood are a trifle smaller than in normal blood and the red cells crenate very quickly on the warm slide.

These variations are recognizable only if the methods employed in studying different specimens is uniform, and if the stains can be varied to meet varying conditions of the blood cells. If the conditions usually associated with acidosis are found, the stain should have a crystal or two or three of sodium bicarbonate added to the solution. This should show an approach to normal staining if acidosis is really present.

If the conditions characteristic of alkalosis appear, a stream of carbon dioxide should be passed through the stain before it is used, or a drop of weak acetic acid may be added to the stain. This should cause the cells to swell slightly and to give more nearly normal staining reactions, if the cause of the staining peculiarities is really alkalosis.

Pigments of the Blood Plasma

Variations in the color of the plasma or of the serum are often noted in pathological blood. Normally the blood plasma and the serum alike present a rather pale straw color due to the presence of urobilin and other bile derivatives. Abnormally several other pigments are present and these may be of importance in diagnosis under certain conditions. Incorrect readings of hemoglobin may be made as a result of discoloration of the plasma in severe cases of cholemia or carotinemia.

Hemoglobin and its derivatives may tint the plasma under those conditions which disintegrate the erythrocytes. Malaria is a common cause of hemoglobinemia; the malarial parasite causes fragmentation and stroma injury of the red cells and laking occurs easily. The hemoglobin is finally excreted in the urine and the bile. The pigment within the parasite is probably a derivative of hemoglobin. The death of the parasite sets this free in the plasma. The kidneys and the liver finally eliminate it from the body. The leucocytes and the cells of the reticuloendothelial system ingest the fragments of erythrocytes which have been ruined by the malarial parasite, and the iron-free moiety of hemoglobin is finally transformed into bilirubin or some related pigment. This pigment also is set free in the plasma. As a result of these various reactions the plasma often becomes definitely tinged in cases of malaria.

Hemoglobin may be set free from the erythrocyte stroma by several other conditions. Saponins and certain other glucosides, several types of venom, certain bacterial products and the bile salts all have the property of laking the blood. Excess of heat and cold, especially alternating heat and cold, may also cause laking. This may occur, though rarely, after severe frost-bites and after some part of the body has been frozen. Hemolytic streptococci may cause very severe anemia which is associated with a dark-colored blood plasma. Infection by the blastomycetes hemolytica causes hemoglobinemia like that produced by malaria; both conditions being due to the fragmentation of the erythrocytes by the organism and the ultimate laking of the fragments with destruction of the hemoglobin. (Plate IX)

Carbon Monoxide Poisoning

The blood of patients with chronic mild carbon monoxide poisoning shows some traces of methemoglobin derived from the carbon monoxide hemoglobin of the erythrocytes injured by the gas. The erythrocytes whose hemoglobin has been combined with carbon monoxide remain for a time in the circulation but are finally destroyed. The pigment derived from these blood cells includes methemoglobin and some related compounds which tinge the plasma a peculiar brownish color. Such plasma does not give a reaction for bile pigments by any of the usual tests.

Carotinemia

Persons with lesions of the lower thoracic vertebrae and the related ribs suffer somewhat from renal and hepatic disturbances, and such persons are often unable to eliminate the coloring matter of vegetables adequately. These persons are usually somewhat emaciated and anemic, quite nervous and irritable and show other symptoms of mild, chronic toxemia. Unfortunately a diet rich in the colored vegetables is often advised for such patients. Being unable to eliminate the carotin speedily this pigment accumulates in the blood plasma. The plasma and also the serum in such cases presents a peculiar greenish tint which is quite distinctive, and it does not give any reaction for the bile pigments.

Carotinemia is not an uncommon condition in those countries in which colored vegetables are freely eaten. In order to correct the condition it is necessary that the patient should eat only colorless vegetables until the blood plasma has returned to its normal color and the symptoms of toxemia have disappeared. Unless the patient receives adequate osteopathic treatments he must always refrain from eating more than a small amount of colored vegetables.

Illustrative Case

Mr. P. C. This patient was a young man with early pulmonary tuberculosis. He had been advised to go to Southern California and to live largely on vegetables. He earned his living by working in a small roadside lunch room. Being persuaded of the great value of spinach and other colored vegetables as blood-building materials he ate spinach three times each day, and ate other colored vegetables and fruits freely. He ate no cereals or starchy vegetables, no meat, eggs, milk or milk products.

The lesions usually present in tubercular subjects were found on osteopathic examination. The blood cell examination showed moderate secondary anemia and the usual evidences of mild toxemia and malnutrition. There was also a greenish tint of the blood serum. The serum did not give any of the ordinary reactions for bile pigments.

For three weeks no osteopathic treatments were given, in order that the dietetic condition might be tested. Mr. P. C. was given a balanced diet which included all good wholesome foods except colored vegetables or fruits. He was permitted a reasonable amount of colorless vegetables and fruits. As a result of this diet alone the symptoms of toxemia and malnutrition diminished, the blood serum regained its normal color and the general condition improved. Some malnutrition and toxemia persisted, and the symptoms of tuberculosis showed no change. After the carotinemia had disappeared the usual osteopathic treatment for the vertebral lesions was given and ultimately Mr. P. C. recovered.

Cholemia

Almost any disease of the liver is associated with some abnormal absorption of the bile pigments into the blood. The bile acids and salts are also often absorbed. The plasma which is tinged with the bile pigments presents a somewhat opalescent and greenish appearance. There are several tests for bile pigments and for bile salts which can be used for blood plasma. Patients with lesions of the tenth thoracic vertebra always show some degree of cholemia. After the lesion has been corrected the blood returns to its normal tint within a few weeks.

Melanemia

Abnormal pigments are found in the blood plasma in certain cases of Addison's disease of the adrenals and in cases of melanotic tumors, especially when metastases are abundant. Occasionally when the solar plexus is invaded by a tumor or by a severe chronic infection there may be marked staining of the plasma, as of the skin, by a pigment whose nature has not yet been determined.

BLOOD PLATELETS

Blood platelets, or "third corpuscles" are small masses of protoplasm found in the blood stream. They vary in size with an average diameter of three microns. They have no recognizable cell membrane and no true nucleus, though the center of the mass often takes nuclear stains in a feeble and atypical manner. They are of varying forms, being roundish, oval, rod-like or spindle-shaped. They disappear very quickly after the blood has been drawn and special methods of technique must be employed in order to count them. A few are still visible in nearly every smear preparation. They have a peculiar sticky consistency and they adhere to glassware very closely. They are concerned in the coagulation of the blood.

Theories of Origin

Views concerning the origin of the platelets are interesting. Engel, Maximow, Preisch and others considered them the extruded nuclei or remnants of the degenerated nuclei of the normoblasts. Hayem thought them immature red cells. Wlassow considered them fragments broken from red cells. Schultze believed them to be fragments of broken down leucocytes. Lowit denied their actual existence and thought them merely artefacts. Bizzozero, Osler and Deetjen considered them truly cells, in the sense that the erythrocytes are cells. Cole found that certain agglutinins which affect the platelets do not affect the red blood cells. Kemp found evidences of hemoglobin in the platelets. Marchesini considered erythrocytes grouped into three classes, stable, partly stable and unstable. Platelets are formed from fragments of the third class. De Govaerts and several others described bacteria found within platelets, and supposed this form of phagocytosis of bacteria and other foreign objects to be a factor in immunity. It is now thought that reports of this kind rest upon the presence of fragments of white cells containing bacteria, and that these have been mistaken for platelets. By means of more recent methods of staining, such fragments can be differentiated from platelets, and this source of error eliminated.

Platelets arrange themselves in groups in shed blood and these groups may be the center from which radiating threads of fibrin arise. Disintegration occurs rather rapidly unless some methods of preserving them has been employed and during the process of degeneration various peculiar appearances occur, these have been called "mucoid degeneration" and "viscous metamorphosis;" various other terms have been applied to pseudo-structures produced during the degeneration of the platelets.

The platelets are now known to be formed by budding from the protoplasm of the megakaryocytes in the red bone marrow. Other sources have been described; none has been definitely proved, but there may be several other methods of development of platelets than from the megakaryocytes. The platelets in mammalian blood are analogous to the thrombocytes in the blood of birds and reptiles.

Numbers

The normal number of platelets seems to vary between rather wide limits at different times of day for the same person, and for different normal individuals. The normal number of platelets in normal human adult blood has been estimated at from 200,000 to 350,000 per cubic millimeter with variations of 50,000 in either direction for the same person at different times.

They are physiologically low in the new-born and in senility. The blood of animals which live in darkness and of young born of such animals is low in platelets. Persons whose diet contains little or no vitamin A, and animals kept without vitamin A have low platelet count. In such cases the platelets can be increased by exposure to sunshine or, less efficiently, to radiations from a mercury lamp. Increase of the foods containing vitamin A facilitates the return of the platelets to normal, though these foods are less efficient without sunshine. The sunshine seems to affect the development of platelets from tissues of the body, since platelets are increased during starvation if the animal, previously bred and maintained in darkness, is placed in sunny quarters. They are greatly increased after hemorrhages whether these are due to accident or to the effects of disease.

They are increased in all secondary anemias, in chlorosis and in some cases of myelogenous leukemia. They are diminished in typhoid fever, idiopathic purpura, aplastic anemia, pernicious anemia, lymphatic leukemia, and in almost any very severe anemia with inefficient regeneration of blood. In sudden acute fevers the platelets first diminish, then increase; the changes parallel the changes in the leucocyte count. In acute fevers of somewhat longer course the platelets do not follow the leucocyte changes but diminish during the early weeks, then increase as the strength of the patient diminishes. Rapid decrease of platelets is of ominous significance during the course of the slower acute fevers, especially typhoid fever.

In most cases of acute, severe, high pyrexia the platelets are very low; in severe pneumonia and in severe malaria it may be difficult to find any platelets at all, even by the most careful methods. After crisis in pneumonia, and after sudden decrease in any fever, the plate-

lets may suddenly rise far above normal, and then within a few days return to normal numbers. In erysipelas, septicemia and acute articular rheumatism, however, the platelets are considerably increased.

Platelets are diminished in the circulating blood during the formation of a thrombus; this fact may be useful in the diagnosis of thrombosis in the early stages. In one case of ours the diagnosis made upon this fact, in a case of doubtful nervous disease, was quite important.

The platelets remain unchanged in most hemorrhagic diseases, and in most other forms of secondary anemia the platelets are considerably increased. This fact is sometimes useful in diagnosis.

Fate of Blood Platelets

These particles of protoplasm seem to undergo dissolution in the circulating plasma, and probably serve as food materials for various tissues. The spleen and other areas of the reticulo-endothelial system, and the monocytes of the circulating blood all ingest and destroy them.

Their length of life has not been well studied. Probably they live only a few days at most. Indeed the term "life" is scarcely applicable to their feeble activities of the time of their functional value.

THE FIBRIN RELATIONS OF BLOOD

The relations of the fibrinogen of the blood, the phenomena of clotting and the mechanisms by means of which the blood is maintained in a fluid state under ordinary conditions present a group of interesting problems.

The functional value of coagulation is evident. Upon even a slight injury to the blood vessels the blood coagulates and thus plugs the bleeding vessels. The formation of a coagulum in wounded areas serves several useful purposes. The injured cells are cemented together and held in place. The peripheral layer of adult cell protoplasm is subjected to the tension normally present in embryonic cells and the pressure thus exerted upon the cell contents initiates cell division, thus leading to replacement of the cells destroyed by the injury. Further bleeding is prevented and conditions adapted to rapid repair of the wound are presented. The repair of wounds by first intention is often surprisingly rapid and this is due to the fact that the coagulum provides these necessary conditions for the recovery of injured cells and the replacement of destroyed cells.

Theories of Coagulation

The mechanism of coagulation has been studied carefully by many workers but the problems are not yet solved in any satisfactory manner. It seems fairly well demonstrated that coagulation of the blood can

occur only when there are present fibrinogen, platelets, calcium salts and extracts derived from injured cells, either of the blood itself or of the tissues. The relations between these factors and the steps by which each substance reaches the final clot have been variously described by different students.

Howell's theory is the basis upon which more recent investigators have developed many ingenious descriptions. According to Howell, normal circulating blood contains fibrinogen, prothrombin, calcium salts and anti-thrombin. The function of the last named substance is to hold the prothrombin in combination and to prevent the formation of thrombin within the blood vessels. When blood escapes from the vessels, or when certain abnormal conditions occur within the vessels, the platelets disintegrate and thromboplastin is set free; this combines with the antithrombin, which in turn sets free the prothrombin. The prothrombin becomes thrombin in the presence of calcium salts; this acts upon the fibrinogen and transforms it into fibrin, which is the substance which forms the clot. In the blood of birds and lower vertebrates the thromboplastin cannot be formed from blood alone, which is due to the fact that these animals have no true blood platelets. For coagulation to occur in the blood of nearly all birds there must be some substance derived from tissue cells.

Thromboplastin is a substance related to the phosphatids and it can be extracted from injured tissues by means of ordinary fat-solvents.

The Morawitz theory assumes that thrombin exists in the circulating blood in an inactive state (prothrombin; thrombogen). Thrombokinase is produced by the action of calcium salts on some tissue extract or some product of injured living cells. Or, by the simultaneous presence of calcium salts and cell products without chemical relationship, the same effect is produced. This thrombokinase acts upon the thrombogen (prothrombin) causing it to become transformed into thrombin. The cell product is called a thromboplastic substance. Soluble calcium salts must be present in order that the thromboplastic substance and the prothrombin may form thrombin, but it is doubtful whether the salts enter into chemical relationship with the thromboplastic substance or not.

Several variations of these two theories have been offered, but they are based upon one or the other of the two just described. It will be noticed that they differ only in the points affecting the development of thrombin.

Circumstances of Coagulation

Coagulation occurs under several different circumstances and the essential nature of the process must vary to some extent with these circumstances. Artificial and experimental methods as well as the conditions which occur under pathological conditions must be considered. The coagulation which occurs within the blood vessels, that which occurs upon the surface of a wound and within the meshes of injured tissue, that which occurs outside the body in whole blood and that which occurs when blood is coagulated after various experimental procedures present different phases of activity.

Intravascular Clotting

Intravascular clotting or thrombosis occurs under different circumstances. Extracts from injured cells are always concerned in thrombosis, and it is rare that injured blood cells themselves are the important factors. When the blood vessels are injured, the endothelial cells produce the extracts needed for clotting. Such a clot begins at the site of injury, gradually fills the vein and may extend toward the heart until the clot has reached the next branch of the vein; the clot may continue past one or several branches of the veins and these also become filled with the clot. Fragments may be broken from the clot and pass in the blood current as emboli, with further pathogenic influences.

Thrombus formation presents several peculiarities. The part of the thrombus first formed contains a great abundance of platelets. These apparently have agglutinated for some reason. In certain cases it seems that some change in the platelets themselves facilitate abnormal agglutination. In other cases it seems that some abnormal current of blood causes mechanical grouping of the platelets with resultant adhesion and agglutination. The grouping of floating elements of like weights and sizes in the eddy of a stream illustrates the method of grouping of platelets at the site of an aneurysm or in vessels which are irregularly dilated. The presence of a foreign substance, an injury to the vessel wall, or an inflammation of the intima may initiate accumulation and agglutination of the blood platelets, and the thrombus follows inevitably. In many cases of thrombosus it is impossible to determine the cause of the intravascular clotting.

Blood found in the serous cavities at operation is usually free from clots, though it may have been outside the blood vessels, so far as can be determined from the symptoms, for several hours. Absorption of such blood can occur. From experimental evidence it seems that many cells become degenerated, but that some of them may be absorbed by

the peritoneal lymphatics. The plasma seems to be absorbed chiefly by the capillaries and veins. In other cases of peritoneal hemorrhage blood clots are found at operation. It is not known whether this is due to some difference in the rate of bleeding, in the quality of the blood or in some condition of thrombokinase, antithrombin or fibrinogen. There is no reason for supposing that there is any difference in the calcium content of the blood which coagulates in the peritoneal cavity and that which does not clot under apparently identical circumstances.

Foreign substances in the blood stream may cause coagulation. It may be that the blood cells injured by the foreign substance provide the necessary cell extract, but in nearly all cases it is the injured endothelium which produces this substance. The floating foreign particle rarely, if ever, forms the nucleus of a clot. It is quite possible that this is due to the fact that the particle ceases to float as soon as it is surrounded by coagulum. An embolus derived from a thrombus serves as the starting point of other thrombi; in this case the thrombus is itself a foreign substance.

Experimental Methods

Intravascular coagulation may occur as a result of the injection of solutions containing tissue extracts, such as may be derived from testis, thymus, lymph nodes, spleen, liver, and other tissues rich in nuclei. Extracts may be prepared from any of these tissues which cause speedy coagulation of the venous blood when they are injected in a vein. It seems to be nucleoalbumin or some related phosphoprotein which causes the clotting, and its manner of action is not clearly understood. In terms of Howell's theory, such a substance neutralizes the antithrombin. In terms of the Morawitz theory the extracts provide a thrombokinase. If the animal is poorly nourished the clot is confined to the vein injected. The well-fed animal, under the same circumstances, produces clot through all the veins.

If, however, instead of a single mass injection of the tissue substance is made, a series of injections of very small amounts of the same substance are given an animal the coagulation time may be greatly prolonged. By careful manipulation of the extracts and of the nutrition of the animal the coagulability of the blood may be completely destroyed. It is not possible to act upon blood in vitro in such a way as to secure such changes and the repeated injections of small amounts of the cellular extracts must cause a reaction on the part of the living cells of the body somewhat similar to that caused by mild infections or by the absorption of the products of infectious processes within the body.

The stroma of mammalian erythrocytes from which the hemoglobin has been washed facilitates clotting. If any considerable amount of this stroma is made into a suspension and injected into the veins of an animal, even of the same animal from which the erythrocytes were derived, the blood clots within the blood vessels within a few minutes. This stroma is not related to fibrinogen but is composed chiefly of cholesterin or some similar lipoid.

Origin of Fibrinogen

Fibrinogen is present in the circulating blood and in various tissue fluids, such as lymph, chyle and certain transudates and exudates. Fibrin may be found in the sputum, urine, various inflammatory exudates and occasionally in the contents of cysts. Fibrinogen may occur in these fluids also, in which case coagulation can be produced by the addition of thrombin, calcium or tissue extracts, according to the lacking factor in the fluid being examined. Fibrin in sputum and in certain inflammatory products and certain cyst contents presents a gross resemblance to mucus. The differentiation is made by chemical and staining reactions.

Fibrinogen in the circulating blood is not very abundant. It is a globulin which is constantly being utilized as a food by nearly all the cells of the body. It is formed chiefly in the liver but is also formed, to some extent, by the intestinal walls, the spleen, the bone marrow and, possibly, by certain leucocytes of myeloid origin. The lymphocytes seem unable to form any fibrinogen at all, and the endothelial cells of the blood are very inefficient as manufacturers of fibrinogen.

The amount of fibrinogen in the circulating blood is greatly diminished in human subjects suffering from any disease of the liver except abscess and cancer. Phosphorous poisoning, acute yellow atrophy and any form of cirrhosis of the liver are associated with extremely small fibrinogen content. In such cases the coagulation time is not greatly increased but the resultant clot is very soft; in some cases the blood may not form a recognizable clot at all.

In animals the removal of the liver from the circulation by any operative procedure (or its destruction by poisons) prevents the development of fibrinogen after this substance has been removed from the blood vessels.

Both human and animal subjects with bony lesions affecting the circulation through the liver show similar but less marked effects. The clot is formed almost or quite within the normal time but it is soft and the clot does not retract readily. After the correction of the lesion the

fibrinogen returns to its normal amount within a few weeks or months, according to the size of the animal and according to the diet and the nutritive condition of the human subject.

Under certain physiological conditions the blood varies in coagulability without regard to the fibrinogen content.

Increase in the epinephrine content of the blood hastens coagulation. This occurs normally whenever an animal becomes angered or frightened, and there is much reason to believe that the same reaction occurs under emotional excitement in man.

The place of adrenal secretion in modifying coagulation is of importance. The experiments of Cannon and others are enlightening. As a result of fright or anger the adrenals secrete increasing amounts of epinephrine, and this increases the coagulability of the blood. The biological significance of the reaction is apparent; the speedy closing of the wounds anticipated in battle is thus facilitated. The contraction of the peripheral blood vessels under similar circumstances has also the effect of preventing serious hemorrhage from superficial wounds. Epinephrine added to blood in vitro does not hasten its clotting, and the addition of epinephrine to the blood of animals does not affect its coagulability unless the circulation of the blood through the liver remains unimpeded. The diminished coagulability of the blood of humans with atrophy of the liver or with certain serious degenerations of liver cells is of interest in this connection.

Repeated massive doses of epinephrine may delay coagulation of the blood of dogs, and may even destroy coagulability altogether. By varying the amounts it is possible to hasten or delay coagulation time or to cause prolonged or delayed changes in coagulability.

The Splanchnic Centers

Direct stimulation of the splanchnic nerves increases coagulability. This reaction does not occur in animals whose adrenals have been removed, which suggests that the nervous stimulation of the adrenals might be responsible for the effects of splanchnic stimulation.

Human subjects with lesions affecting the splanchnic spinal centers show diminished coagulability; this more commonly displays itself in a soft clot with little or no retraction than in increased coagulation time. Such persons have almost always a low blood pressure, weakened heart action and some visceroptosis, all of which indicates diminished activity of the adrenals. However, the presence of bile pigments in the blood of these persons suggests also diminished hepatic activity, and

since the liver is the chief source of fibrinogen the lack of this substance may be the most important factor in the effects of splanchnic vertebral lesions. Further work must be done before these relations can be explained.

Defibrination of Drawn Blood

The fibrin can easily be removed by beating and stirring freshly shed blood with any slender rough rods, such as metal wires or wooden sticks. The fibrin is deposited upon the foreign substance and thus can be removed easily, leaving the serum and the cells in a fluid state. The fibrin holds some red cells, many hyaline cells and nearly all the granular cells within its meshes. A differential blood count made of the fluid blood therefore shows an undue proportion of hyaline cells. Normal blood which has been defibrinated is useful for a study of the changes occurring in vitro in the cells of shed blood. The injection of defibrinated blood into the veins of the person from whom the blood was taken, or into the veins of anemic persons, has been used in therapy.

Experimental Prevention of Coagulation

Animals differ somewhat in the coagulability of blood. The vein of a horse can be tied in two places, enclosing any convenient length of vein between the ligatures. The vein then can be severed above the highest and below the lowest ligature and it will then retain the blood in a fluid state for a long time, sometimes for several days. If this bag be kept quiet, the corpuscles sink to the lowest part and the supernatant plasma may be poured off into another vein or into a glass vessel which has been coated thoroughly with paraffin or some other perfectly smooth surface. If the plasma is poured into glass or other vessels, not specially treated, coagulation occurs almost immediately. Horses' blood received into a prepared vessel at 0° C. and kept at that point does not coagulate.

The blood of a bird coagulates very quickly after ordinary wounding. But the blood can be removed from a vein by means of an oiled or paraffined canula, avoiding contamination of the blood by any substance derived from the tissues, and coagulation may not occur for several days, if all dust and foreign matter be kept away. Such blood can be centrifuged and the plasma and cells secured separately.

Several salts can be added to the blood which prevent coagulation. Human blood to be used for chemical tests is usually taken from a vein by a sterile syringe and immediately thrown into a vessel containing a few crystals of sodium citrate or sodium oxalate. The oxalate precipitates the calcium. One part of oxalate to 1,000 parts of blood is sufficient. Rabbits, cats, dogs, guinea pigs and other laboratory animals

have speedier clotting time. In taking their blood for chemical tests we use a syringe which has been rinsed in oxalate or citrate solution, and put the blood into a vessel also rinsed with the same solution, in order to prevent clotting. The corpuscles settle out of oxalated blood or they may be thrown down more quickly by centrifuging. Oxalated blood or plasma can be made to clot by adding some suitable calcium salt.

The citrate has a somewhat different action. This salt does not precipitate the calcium but it enters into the formation of a double salt, sodium calcium citrate, in which the calcium is in the anion; that is, the calcium is associated with the acid radicle while the sodium is the kation. Coagulation does not occur, even in the presence of a soluble calcium salt, unless the calcium is ionized as the kation. This condition is present in calcium chloride and calcium sulphate. Either of these salts added to either oxalated or citrated plasma or whole blood is followed by almost immediate clotting.

Salted Plasma

The addition of one part of a 25% solution of magnesium sulphate to four parts of blood prevents coagulation in the blood of any mammal for an indefinite length of time. Magnesium sulphate precipitates the thrombokinase but this reaction proceeds slowly. If the blood is centrifugated immediately the plasma is clear and fluid, but it coagulates within a short time after it has been diluted to about the normal specific gravity. But if the magnesium salted plasma of whole blood is allowed to remain for one to several days then neither dilution nor the addition of tissue extracts causes clotting; evidently this is due to the precipitation of the thrombokinase by the salt.

The addition of one part of a half-saturated solution of sodium sulphate to one part of blood also prevents coagulation of the blood of any mammal for an indefinite time. Either the whole blood or the plasma freed from the cells coagulates at once after dilution with water to about the original specific gravity of the blood.

Organic Substances Which Prevent Coagulation

Certain animal extracts prevent coagulation in vitro or when injected into the veins of the animal.

Hirudin is an extract made from the anterior part of the body of a leech. The prolonged bleeding time of wounds produced by the bites of leeches has long been known. The efficiency of leeches in old-time methods of treatment by bleeding depends upon the fact that a peculiar albumose derived from the buccal glands of the leech was injected into the tissues of the wound, and this prolonged the bleeding time. Blood

received into a vessel containing a solution of hirudin does not coagulate. Hirudin injected into the veins of an animal prevents coagulation within the blood vessels after death. Blood drawn either before or after the death of the animal injected with hirudin does not coagulate.

Since hirudin is an antithrombin, blood which has been prevented from clotting by its use needs only to have thrombin added to it in quantities beyond the efficiency of the hirudin still present. Clotting then occurs.

Peptone Reactions

A solution of peptone injected into the veins of an animal prevents the coagulation of the blood both within the vessels after death and in vitro whether the blood is removed before or after the death of the animal. Peptone does not prevent coagulation if it is added to the blood in vitro, however. The nature of the physiological relations concerned is puzzling. An animal which has received an injection of peptone and has thus had the coagulability of its blood diminished or destroyed cannot be used for a second similar test for several weeks. At any time within a few days a second or later injection of peptone solutions has little or no recognizable effect on coagulation. There is no other recognizable change in the physiological condition of the animal under such circumstances.

A solution of peptone perfused through an extirpated liver causes the appearance of an anti-coagulating agent in the hepatic veins. This agent seems to act by neutralizing the fibrin ferment but its nature is not yet known.

Peptone is much more efficient as an anti-coagulant if the animal to be employed has fasted for a few hours before the experiment is begun, and if the peptone be injected rather slowly. An anticoagulin is formed within the liver and this may be antithrombin.

Peptone plasma can be induced to clot by adding an extract from tissue cells to it, or by passing a stream of carbon dioxide through the vessel containing it. The addition of ordinary amounts of fibrin ferment to peptone plasma does not cause clotting, but very large amounts of fibrin ferment may induce coagulation in peptone plasma or peptone whole blood.

Venoms

Venoms from snakes and other lower animals produce different effects on coagulation. The venom of the cobra inhibits coagulation whether it is injected into the blood in minute amounts during life or is placed in a vessel into which the blood is to be received. Other snakes (for example the pseudechis porphytaceous) produce a venom which causes

abundant coagulation within the vessels of a living body, such as might follow the injection of tissue extracts into the blood stream. It is not known to what constituents of the venom this effect is due.

Intestinal Parasites

Certain intestinal parasites produce a substance which prevents coagulation. Wounds made by them in the intestinal wall continue to bleed for a long time and the entire blood may show greatly diminished coagulability because of the absorption of this substance. The severe anemia due to the hook worm, the dibothriocephalus latus and other intestinal worms is thus explained.

Coagulation At Various Periods of Life

There is little change from birth to senility in the coagulability of normal blood. The coagulation time has been determined for newly born infants by many authors. The figures vary slightly from four to ten minutes, with an average of seven minutes; this is longer than the average coagulation time of adults. Newly born infants with hemorrhages show greatly prolonged bleeding time and increased coagulation time. Intramuscular or intravenous injection of paternal or other whole blood frequently provides the necessary substances and after coagulation of the blood has been established the hemorrhages may cease.

Menstruation

For a day or two before menstruation begins the coagulability of the blood is slightly diminished for about two-thirds of the women examined. The old idea that menstrual blood does not coagulate is untrue; any blood mixed with mucus coagulates with difficulty or not at all. The presence of mucus and of degenerated endometrial cells prevents coagulation to some extent. Pure menstrual blood coagulates as quickly as does blood derived from the veins of any part of the body.

Pregnancy

During pregnancy the coagulability of the blood is somewhat diminished; possibly the developing embryo or fetus needs the serum albumins and globulins. Before labor an increased coagulability of the blood is occasionally noted. The biological relations of these facts are self-evident.

Embryonic Blood

The blood from embryos shows low coagulability. In pig embryos from 100 to 250 millimeters in length the average coagulation time was found by Emmel and others to be twenty-three minutes. The adult

pig's blood coagulates within about three minutes. Addition of adult pig platelets or of extracts of adult cells to the embryonic pig's blood caused coagulation to occur within three to four minutes. Calcium is higher in the blood of embryonic pigs than in the blood of adult pigs.

Senile Blood

In normal old persons the coagulability of the blood is normal or only slightly hastened. Various diseases affect senile blood rather more seriously than younger blood but there is no quantitative variation in the effects after adult life has been reached, so far as coagulation is concerned.

Coagulation In Disease

Variations in the coagulability of the blood in certain diseases present even more puzzling problems. The abnormal conditions of coagulation include (a) delayed clotting (b) imperfect nature of the clot (c) hastened clotting. The causes of these different abnormal conditions of coagulability may differ considerably.

There may be a lack of fibrinogen in the circulating blood. This causes the formation of a soft and imperfect clot which permits prolonged bleeding from wounds which may be almost negligible. Lack of fibrinogen is known to occur when the liver is seriously injured, as in cases of poisoning by phosphorous or chloroform, or in cases of hepatic cirrhosis or acute atrophy, or in fulminating cases of certain infectious diseases. There is not any delay in coagulation in uncomplicated cases of deficiency of fibrinogen.

Morawitz considers lack of thromboplastin (prothrombin) an important factor in cases of delayed coagulation. It is difficult to see how there could be a lack of a substance derived from injured cells, especially as injured blood cells may produce it. It is possible that a disturbance in the metabolism of the cells, necessarily of developmental and constitutional nature, may so affect the end-products of katabolism that the thromboplastic substances are inefficient in the transformation of prothombin to thrombin.

Deficiency of calcium salts was at one time considered important in the etiology of delayed coagulation. That the blood always contains enough of the soluble calcium to provide for coagulation seems definitely proved. Increased calcium intake does not hasten coagulation in any useful degree, though certain cases of delayed coagulation in obstructive jaundice seem to be somewhat improved by increased calcium intake. Deficiency of prothrombin is present in cases of melena neonatorum. In these cases intramuscular infusions of whole blood or intravenous

injections of blood serum or of whole blood are usually efficient in relieving the condition, by adding the necessary prothrombin to the blood of the infant.

Excess of antithrombin may prolong coagulation. Certain chemical agents, such as hirudin, act as antithrombins and coagulability may be completely destroyed by such substances injected into the blood stream.

Lack of blood platelets diminishes coagulability, since these structures are the chief source of prothrombin, probably also of thromboplastin.

Rapid Coagulation

Increased rapidity of coagulation may occur as a result of increased amounts of epinephrine, as already noted, under experimental conditions. It does not seem to occur as a result of disease. Increased amounts of antithrombin occur in pneumonia and this fact is useful in diagnosis. In septicemia and in miliary tuberculosis there may be excess of antithrombin. The biological significance of increased coagulability in these diseases is evident.

Hemophilia

Hemophilia is a puzzling disease which is characterized by a marked tendency to continued bleeding from wounds apparently insignificant. The blood coagulates in vitro within a normal time, but the clot is soft and fails to show retraction present in a normal clot. In this disease the platelets are normal in number but they do not agglutinate properly, and it seems that they lack the ability to form prothrombin. The nature of this functional defect is not known, but the hereditary character of the disease indicates that the defect is inherent in the germ plasm. The blood of a hemophiliac contains normal amounts of fibrinogen, calcium, salts and platelets; the blood cells are normal in both actual and differential counts. A normal clot is formed in vitro from the blood of hemophiliacs if kephalin is added to it. If the tissue of the bleeder is bruised or if the blood flows over injured tissues after leaving the vessels, the blood clots and the clot retracts as in normal blood. These various observations indicate that the platelets defect is essential in the disease. Another peculiarity of the blood platelets of hemophiliacs is their failure to agglutinate at the site of bleeding points, as do the platelets of normal blood.

Deficiency of platelets may prevent normal coagulation. Excess of platelets does not cause abnormally rapid coagulation nor the formation of an abnormal clot. In certain hemorrhagic diseases such as the leukemias, and in certain infectious diseases, such as "black" smallpox and "black" diphtheria, the platelets are tremendously diminished. As a re-

sult of leukemia the leucocytopoietic centers crowd out the megakaryocytes, thus preventing normal replacement of the platelets. As a result of the exhaustion of the bone marrow, in extremely malignant infections, the megakaryocytes share in the atrophic changes. In these cases it is occasionally impossible to find even one platelet by the most careful methods of taking the blood. In these diseases the coagulation time remains almost or quite normal but the clot is very soft. The bleeding time may be prolonged almost indefinitely.

Coagulation In Hepatic Diseases

The diminished coagulability of the blood in persons with hepatic disease has long been recognized. Several factors are concerned in this relationship. The place of diminished fibrinogen formation as a result of hepatic disease has already been mentioned.

The mixture of bile pigments, and especially of bile salts, with blood in vitro diminishes coagulability. With hepatic diseases cholemia is very common; the diminished coagulability may be, in part, due to the cholemia. The bile interferes with the conversion of fibrinogen into fibrin, but the amount of thrombin is not affected.

Carbon monoxide poisoning delays coagulation. In lethal cases the blood may not coagulate at all within the vessels. In the chronic mild carbon monoxide poisoning which is so common in large cities the coagulation time is usually prolonged to ten minutes or more (normal by our methods, four to six minutes).

Summary

Deficiency of fibrinogen may prevent the formation of a normal clot. The coagulation time is not modified, if coagulation occurs at all, but the resultant clot is soft, does not retract and serves little useful purpose in closing wounds.

Deficiency in prothrombin is apparently the cause of melena neonatorum. Intravenous transfusions or intramuscular infusions of normal blood usually result in supplying the lacking factor to the infant's blood and recovery usually follows promptly.

Deficiency in thromboplastin has been emphasized by Morawitz. Since this substance is derived from injured cells of blood or tissues its presence would seem almost inevitable. Possibly there is some developmental defect in the cells of bleeders of this group.

Excess af antithrombin occurs under experimental conditions such as the injection of hirudin or peptone into laboratory animals. An excess of antithrombin is said to occur during the course of septic diseases, especially those affecting the lungs.

Fibrin Formation on the Warm Slide

The manner in which the fibrin threads appear on the warm slide which is practically a vital phenomenon, gives much useful information. The slide is kept at 99° F., to 100° F. usually by means of an electric appliance made for the purpose. The blood is placed on this slide directly from the exuding drop, is immediately covered with a warm cover glass and examined immediately. Hence the blood is under physiological conditions, except for the lack of circulation and the recurring variations of oxygenation, nutrition and so on. Vital phenomena occur under such circumstances almost normally. The presence of the foreign bodies, the slide and cover-glass, initiate reactions similar to those occurring within the body around a foreign body, if not identical with them. The manner in which fibrin is formed under such circumstances presents variations which are often very useful in diagnosis.

Normal blood placed on the warm slide begins to show fibrin threads after about ten minutes. If the slide is warmed to 103° F. fibrin threads may appear within five minutes. The threads are very fine, so that accurate measurements have not yet been found practicable under the circumstances of the warm slide preparations.

When threads of fibrin fail to appear upon the warm slide within about ten minutes, the condition is distinctly abnormal. In persons whose diet fails to include a proper amount of protein foods, but who are not utilizing their own muscles as a source of energy, the amount of fibrin may be extremely scanty and may appear only after fifteen minutes or more. The greatest delay and the greatest lack of fibrin upon the warm stage occurs in persons with serious hepatic disease, but not in cancer of the liver, either primary or metastatic, nor in abscess of the liver. The even caliber of the normal threads is noticeable. The threads vary in length. When first visible, they are from three to six microns long. During the fifteen minutes following their first appearance they increase in length, at first visibly, then more and more slowly. By careful watching it is usually possible to say that the fibrin formation ceases at a definite minute.

Abnormally fibrin may be present as soon as the slide is seen under the microscope, or it may not appear at all, or only after half an hour or more on the warm slide.

In normal blood the fibrin threads appear to have no relationship with one another, and rarely to any other blood structures. Occasionally they seem to originate from a group of platelets; this is in normal blood at correct temperature. The threads are straight and they may or may not lie across one another.

Abnormally many fibrin threads radiate from groups of platelets or from white cells; they form net-like arrangements which may be quite complicated in structure; they may be so related with phagocytes as to appear to be merely continuations of abnormally long and slender pseudopodia; they may be abnormally long and abnormally heavy, or they may present marked irregularities in contour or they may even have sharp variations in thickness so that they present a definitely beaded appearance.

The significance of these variations is not yet clearly understood. Much further work must be done before the problems presented by these most interesting reactions are solved in any satisfactory manner. But there are useful indications of diagnosis and prognosis to be gained from a study of fibrin formation as it occurs on the warm slide.

Fibrin develops very speedily under several conditions, and in many cases this is of great value in early diagnosis.

Fibrin in Pneumonia

In lobar pneumonia fibrin develops immediately and abundantly. The threads are long, heavy, fairly even in contour, not arranged in nets but often so abundant as to present a felted appearance. The fibrin is completely formed within a very few minutes or, occasionally, is completely formed at once, so that no increase in the length of the threads is visible. By the time the slide is placed under the microscope, very often, the abundant heavy threads are easily visible. This reaction is present in such degree in no other acute disease, and it occurs so early that a diagnosis of lobar pneumonia can often be made twenty hours before any other pathognomonic finding can be secured. During the course of the disease and for several weeks after recovery this rapid fibrin formation is present. It disappears gradually and the blood returns to the normal condition after some weeks,—the exact time for recovery of normal fibrin relations has not yet been studied.

In aborted cases of pneumonia this fibrin reaction also occurs and it is possible to determine whether or not an actual pneumonia has been aborted by a study of the fibrin at any time within a week after the initial symptoms have disappeared.

In ordinary colds, in cases of influenza without pulmonary involvement, in bronchitis without alveolar involvement and in other infections without lung disease but presenting symptoms that might be confused with early pneumonia, the fibrin formation is either normal or only feebly modified.

Fibrin in Malignancy

In active carcinoma the fibrin formation is very rapid, and in some cases is as rapid as in pneumonia. In malignancy the threads are uneven in contour and there may be so many and such marked inequalities that the threads present a definitely beaded appearance. The threads often radiate from a small group of platelets or from a white blood cell, usually a lymphocyte. In cases in which there is some difficulty of diagnosis, the presence of these irregular fibrin threads, appearing quickly and in great abundance, suggests carcinoma. In sarcoma the fibrin threads present less marked modifications, and the reactions are of much less significance.

Fibrin in Malnutrition

In malnutrition the fibrin threads are extremely fine, delicate and scanty. If the malnutrition is associated with any marked toxemia, the threads are apt to be uneven in contour. If there is little or no toxemia, the threads are even and regular, but are so very fine that it may be difficult to see them at all, except when the field is darkened or a dark-stage illuminator is used. The fibrin formation is considerably delayed in malnutrition, often to twenty or thirty minutes after the blood is placed on the warm slide.

When malnutrition is associated with malignancy or with early pneumonia, the fibrin threads become heavy, abundant, irregular and are formed very speedily, as in ordinary malignancy and pneumonia. In other diseases associated with moderate increase in the fibrin threads, the presence of severe malnutrition modifiies the fibrin reactions, so that it may often be difficult to determine the relationships of the fibrin studies. Fortunately most of the conditions in which there are complicating factors are those in which some other laboratory findings or some symptom complex helps in differentiation.

Fibrinolysis

The fibrinolytic ferment was first studied in the laboratory of The A. T. Still Research Institute in Chicago. Since that time it has been studied in other laboratories of the Institute and in several other laboratories, though the subject has not yet received the attention to which its importance entitles it.

Normal blood usually contains an enzyme which digests the fibrin of the blood clot but which does not digest the cells of the blood or the tissues of the body. The blood of approximately one-fourth of all persons, healthy or ill, fails to contain this ferment. The fibrinolytic fer-

ment is destroyed by heat above 108° F., and its activity is diminished at 104° F. and by temperatures below 96° F. Tests have not yet been made determining the low point at which the enzyme is destroyed. Fibrinolysis is decreased by the use of distilled water in the tests and by the presence of any appreciable excess of the salts present in tap water or spring water.

Roseman was able to precipitate a fibrinolytic substance from fibrin autolysate. A similar substance was extracted from the pressed juice of pneumonic lung. This substance differs from leucocytic trypsin in its greater thermolability and by the fact that it is not related to the leucocytic content of clot. The exudate from tubercular serositis markedly retards fibrinolysis. Roseman also later reported that the fibrinolytic substance of horses' blood serum is precipitable by alcohol, ammonium sulphate and zinc chloride. It is not dialyzable. Temperatures of 46° to 48° C. are destructive. Tubercular exudates inhibit fibrinolysis by this enzyme also. Human material gives the same findings, according to Roseman.

The function of this ferment in normal life is not known. Inasmuch as persons who lack the ferment show no evil effects referable to its lack, except as shown later in this chapter, its function may be altogether protective. Possibly other ferments may perform similar or identical functions under ordinary circumstances.

The function of fibrinolysis is most easily recognizable after the repair of wounds. When any tissue is injured, the blood vessels of the immediate vicinity are dilated. An increased amount of fluid passes into the tissue spaces. Usually the capillaries of the part are also injured so that there is some extravasation of blood (hemorrhage per rhexin of the older pathologists). If there is no frank bleeding the dilatation of the blood vessels permits some escape of blood from the capillaries (hemorrhage per diapedesin). The fluid derived from the blood plasma as well as the blood itself undergoes coagulation throughout the areas involved. The clot contracts very slightly and this reaction forms a fairly firm substance which exerts pressure upon the periphery of every living cell within the area.

This pressure exerts the same influence upon the cellular contents as that which is exerted by a cell wall. The mature cells of animal bodies do not have a cell wall, and their surfaces offer no resistance to the growth or the swelling of the cell. Mature animal cells continue to grow until the metabolic control of the nucleus is reached but they do not undergo division unless the cell contents are subjected to pressure. The coagulum exerts such pressure. The cells imbibe some fluid from

the surrounding edematous fluids which, in turn, are due to the vasodilatation. With increasing intercellular pressure the phenomena of karyokinesis are initiated in some of the cells and they divide. This division of the cells is necessary to the repair of the wounds. Not only the tissue cells themselves but also the various hyaline cells of the blood and of the tissue spaces begin to divide in the same way (plasma cells, macrophages, lymphocytes, monocytes and others.)

These processes follow a definite series of events which differ slightly according to the histological characters of the tissue which has been wounded but which always include the multiplication of several types of cells after a preliminary pressure due to the clotting and the swelling due to the edema. The ingestion, digestion and removal of the debris left by the injured tissues is also an essential part of the phenomena of repair.

When cell division is no longer required the clot must be removed. In persons whose blood contains the fibrinolytic ferment the digestion of the fibrin of the clot begins within about twenty-four hours and is complete within about fifty hours. If the wound is simple, without any serious bruising of the tissues and no infection; that is, if the wound is repaired by first intention, there is little or no need for further cell division after the first day or so. The digestion of the fibrin thus removes the impulse to karyokinesis and no further multiplication of the cells occurs. If the fibrinolytic ferment is absent the various microphages and macrophages must destroy the coagulum; this is a slower process and there is some reason to believe it less efficient than normal fibrinolysis.

After inflammations of any ordinary type, the presence of normal fibrinolysis facilitates the removal of the remaining coagulum. Persons recovering from pneumonia may show rapid and complete resolution if their blood contains normal fibrinolysis, or resolution delayed with a greater amount of cirrhosis if the blood lacks fibrinolytic ferment.

During high fever and under certain other conditions there is produced a non-specific proteolytic ferment in marked degree. This ferment is present in the blood of all persons in a very slight amount, and it may be very greatly increased during high temperatures. This non-specific proteolytic ferment facilitates the digestion and removal of coagulum even in persons without fibrin ferment, so that the lack of fibrinolysis is not a serious matter in those cases characterized by hyperpyrexia. Low grade inflammations do not initiate any great increase in the non-specific proteolytic ferment (or ferments), and persons whose blood lacks the fibrinolytic ferment show delayed resolution, a

greater amount of connective tissue hyperplasia and more serious adhesions after recovery from inflammations with mild pyrexia than do persons with normal fibrinolysis.

For example, of many patients with severe acute inflammatory rheumatism with high temperatures about one-fourth have no fibrinolysis while about three-fourths have normal fibrinolysis. All of these patients develop an abundant supply of the non-specific proteolytic ferment during the high fever. The coagula of the inflamed areas are digested and absorbed, and there is no recognizable difference between the two groups of persons so far as recovery is concerned. On the other hand, of a considerable number of persons with some low-grade arthritis characterized by little or no fever, about one-fourth have no fibrinolytic ferment while about three-fourths have normal fibrinolytic ferment. As a rule (not without exceptions) those persons without fibrinolytic ferments have greater hypertrophy of the affected joints with denser adhesions than do the persons with normal fibrinolysis. Of all persons with any type of chronic articular rheumatism about eight-tenths have no efficient fibrinolytic ferment. That is, persons with normal fibrinolysis have a partial immunity or else they recover more speedily.

The place of fibrinolysis in protection against malignant neoplasms has been studied with some care. Animals which have about the same cancer-incidence as human beings have about the same fibrinolysis-incidence, that is, about one-fourth of all individuals lack the ferment while about three-fourths show its presence in the blood serum. Animal families which seem to be immune to cancer all show normal fibrinolysis. Animal families which have no immunity to cancer have no fibrinolysis.

In human families in which cancer never occurs, all members have blood with normal fibrinolysis. In human families in which there are many cancers both in the paternal and maternal line of inheritance, the fibrinolytic ferment is absent in nearly all individuals. That is, many persons of the human race inherit an important factor of protection against cancer. Persons who do not have this factor of protection against cancer may still fail to develop cancer.

For the development of certain kinds of cancer repeated irritation seems to be necessary; for other kinds some chronic inflammatory processes seem to be necessary. Other types of cancers arise from some developmental defect. For all kinds of cancer the co-operative activity of two or several pathogenic processes seems to be necessary. The lack of the fibrinolytic ferment is one factor which is common to many cancer-producing conditions, among animals and human subjects alike.

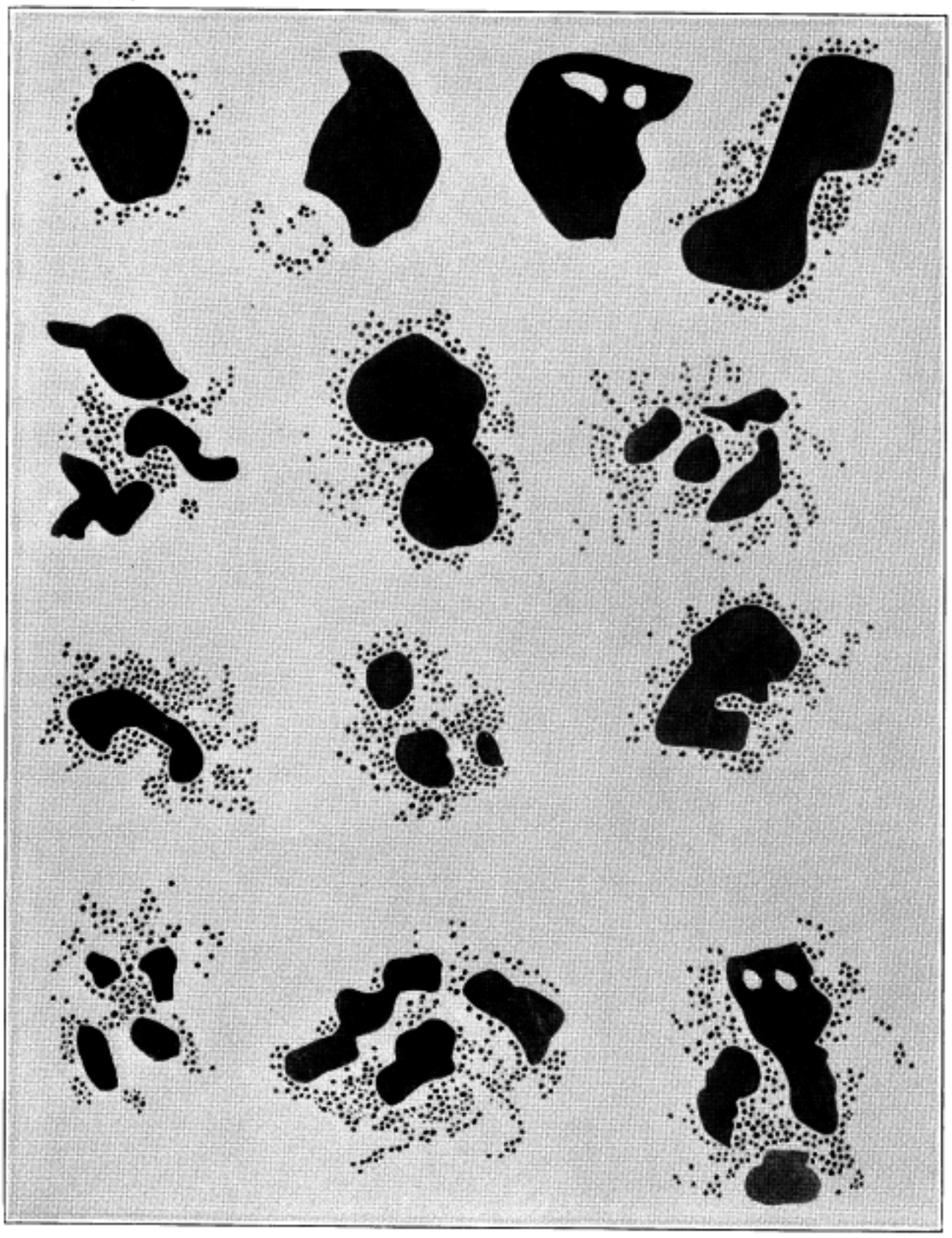


PLATE IV

Neutrophiles in severe cholemia. From patient with cancer of the liver, a few days before death. The protoplasm is eroded, leaving the nuclear masses.

Attempts have been made to produce the fibrinolytic ferment in persons not naturally provided with this substance.

Diets

Persistent vegetable diet does not lead to its development. This test was repeated in human beings because of the rather common idea that vegetarianism tends to diminish cancer development. In this connection it may be said that certain gramnivorous animals are very prone to cancer, and that other gramnivorous animals are almost or quite immune; that certain carnivorous animals are prone to cancer and other carnivorous animals are immune; that of any animal group certain strains or families may be immune while other strains or families may be unusually susceptible. The animals most throughly studied in this connection are all laboratory animals, kept under conditions as nearly normal as is practicable for animals in confinement.

Direct Addition of Fibrinolytic Blood

Persons known to have cancer, and in whose blood no fibrinolysis can be shown, have been treated by giving them infusions of the blood of persons whose blood is known to be well-provided with the fibrinolytic ferment. The number of cases so treated is too few to warrant definite statements. The patients so treated were those for whom recovery could not be expected under ordinary methods of treatment, and a few of these have recovered from the cancer and have lived without recurrence for ten years or more. Such persons have shown normal fibrinolysis for six years or more after the last administration of blood. Normal blood has been given to patients whose blood contained no fibrinolysis and who suffered from arthritis deformans with unusually dense adhesions; normal fibrinolysis was established in these cases and further adhesions did not occur in the joints.

About two cubic centimeters of blood were taken from the vein of a donor known to be in excellent health and free from any infection, and injected into a muscle of the recipient. Usually two or three such infusions at three to five days intervals resulted in the development of normal fibrinolysis on the part of the recipient. This method presents certain possibilities for cases otherwise hopeless but is not to be commended as a routine practice.

Bony Lesions

Tests have been made in an attempt to find that some especial organ or tissue produced the fibrinolytic ferment, in the hope of finding some

cause for its absence other than heredity. No tissue has been found to be solely or especially capable of producing it. Persons lacking this ferment do not show any particular lesions. Persons lacking it do not develope it after the most persistent osteopathic treatments, no matter what lesions were present before the treatments were begun. Persons normally provided with this ferment do not lose it, though the activity of the ferment is delayed or subnormal during the course of several abnormal conditions. Much more study is necessary before definite reports can be made as to the relations between abnormal conditions and the delay or inhibition of fibrinolysis in persons normally provided with the ferment. It is not now possible to say that there is any lesion or any disease which exerts a specific action upon fibrinolysis.

CHAPTER VI

DEVELOPMENTAL RELATIONS OF THE BLOOD CELLS

The manner in which the cells of the blood have been developed, both ontogenetically and phylogenetically, is of great interest. Many problems presented by the cells of the blood in the leukemias and in patients with abnormal inheritance are explained by a knowledge of the biological relations of the blood.

An accurate understanding of the structure and the function of the red bone marrow is especially desirable, and much work has been done in the study of this important tissue. Unfortunately we have only begun to pick up a few facts and these have not yet been properly classified.

The importance of the red bone marrow in physiological economy is indicated by the fact that this is one of the last of the tissues of the body to lose its blood supply as a result of bleeding or of any other cause of anemia.

Animals which have been bled to death retain a fairly adequate blood supply to the bone marrow as well as to the heart, lungs, digestive tract and respiratory muscles long after the muscles of locomotion, the skin, spleen and omentum have become apparently completely bloodless. In the anemias the bone marrow retains its blood supply long after other tissues become very pale. Yet the marrow is affected by many abnormal conditions and the effects of these may be very severe.

Bony Lesions

The marrow is seriously affected by vertebral and costal lesions which affect the innervation of the blood vessels and the cells of the hematopoietic tissues. Such lesions and their effects have been studied in osteopathic institutions.

When vertebrae or ribs are lesioned there is a condition of strain in and around the affected joints. The joint surfaces are abundantly supplied with sensory nerve endings and these are subjected to abnormal pressure due to this tension. The swelling and edema which always follow any strain of joints increase the synovial fluid, and this also irritates the sensory nerve endings.

The central relations of the sensory nerves distributed to the joints are interesting. Normally sensations from the joint surfaces are not carried to the cerebral centers, but they exert an important influence

upon the nerve centers of the spinal cord. Impulses derived from any joint surface affect the spinal nerve centers which control the skeletal muscles moving that joint and the viscera which were derived, embryologically, from the same somites. When vertebrae or ribs are lesioned the abnormal sensory impulses affect the centers which control the small, deep spinal muscles and the intercostal muscles. These muscles are stimulated constantly and are thrown into a condition of hypertonicity, which is called contracture by several pathologists. This constant hypertonicity of the spinal and intercostal muscles tends to perpetuate the lesion. Several interesting changes occur in muscles subjected to this abnormal form of stimulation and in time a condition of rigor is present. Still later fibrosis occurs and after this time complete recovery of the normal condition of the muscle is probably impossible. The visceral centers affected at the same time by the abnormal sensory impulses from lesioned articulations include the centers governing the red bone marrow of the bones concerned in the lesion.

Again, when vertebrae or ribs are lesioned there is some swelling or edema of the surrounding tissues. This edema is not yet well understood, but the effects produced by a strained ankle or a bunion are too well known for the facts of the case to be doubted. This edema is always present around lesioned vertebrae or ribs. Edematous fluids are not quite identical with normal tissue juices; in edema there is some acidosis, some accumulation of carbonic acid and other katabolites and other effects of the disturbed circulation through the affected area. The tissues around the thoracic vertebrae and the ribs are so constructed that in lesions of these bones the intervertebral nerves and the adjacent sympathetic ganglia are subjected to considerable pressure, due to the edema. The nerves passing through the intervertebral foramina to and from the sympathetic ganglia upon the heads of the ribs pass through the edematous tissues and are thus subjected to pressure and to the effects of the abnormal tissue juices. Medullated nerves are not quite so seriously injured as are the non-medullated nerves and the latter carry impulses from the sympathetic ganglia to the blood vessels and to the viscera innervated from the same spinal segment as that concerned in the lesion.

The nervous control of the red bone marrow is not yet well understood. The nerve fibers entering the bone marrow in any part of the skeleton include medullated fibers, which seem to be sensory, and non-medullated fibers derived from the sympathetic system. Of the sympathetic fibers at least two groups are present, vasomotor nerves which terminate in plates upon the walls of the blood vessels, mostly the arterioles, and other nerves which terminate in fine brush-like endings which

lie free among the intrinsic cells of the bone marrow. The sensory nerves are distributed chiefly to the periosteum and to the region of the red bone marrow nearest the bony walls.

In animals, a bony lesion affecting the circulation through any area of red bone marrow is followed by atrophy of the marrow cells of that area. Within two or three years after such a lesion has been produced, the interior of the bone shows pale, hardened areas in which bony spicules and connective tissue are associated with only a few pale areas of remaining marrow cells. These latter areas contain little or no evidence of active blood development, but are made up chiefly of blood vessels, connective tissue cells and a few atrophic remnants of blood-forming tissues.

The effects of various diseases and poisons upon the bone marrow have been studied in many laboratories, and the discussion of the facts in this connection is best associated with the diseases in which such marrow changes are of interest.

A brief review of the structure of the red bone marrow may be of interest.

Cells of the Normal Adult Bone Marrow

The blood of adult marrow includes all the cells present in fetal blood, but the proportion of younger cells is greater in the marrow from younger individuals and in lower animals. Since the bone marrow is the chief site for the manufacture of red cells and the granular white cells, it is easy to find immature cells of the two groups abundantly present in the red marrow.

Hyaline cells are abundant in the red bone marrow, especially in younger subjects and fetuses.

Cells which cannot be differentiated from true lymphocytes by any method of staining now in general use are abundant. Larger cells which resemble large lymphocytes are present in about the same relative proportions to the small hyaline cells as is the case in the blood. There is much difference of opinion as to the origin, functions and destination of these cells. (Plates V, VI.)

There are also hyaline cells which are definitely myeloid in origin and which seem to be derived from the reticular cells. These cells may or may not give the oxidase reaction. The hyaline myeloblast (non-granular marrow cells; lymphoid mother cell; undifferentiated leucoblast; hemocytoblast; lymphoid hemoblast; microlymphoidocyte; macrolymphoidocyte; stem cell; indifferent lymphoid germinal cell) is a cell which is rounded, occasionally somewhat irregular in form, with hyaline, basophilic protoplasm and a nuclear structure which is charac-

teristic. The myeloblast seems soft and has relatively great protoplasm. There are no true granules but the protoplasm is faintly and irregularly granular in structure. Vacuoles are found within the protoplasm frequently. The nucleus has a fine, delicate reticular appearance with one to several fairly large nucleoli. The lymphoblast differs from the hyaline myeloblast in being somewhat firmer in structure, and in having a smaller nucleus with coarser chromatin masses and a heavier nuclear membrane. Azur granules are sometimes found within the protoplasm of the lymphoblast but not the myeloblast. The oxidase reaction is of little or no value in distinguishing these primordial cells, because immature forms do not always give the oxidase reaction even though they are of the myelocytic group.

The nucleus of the myeloblast has a definite nuclear membrane which is very delicate and very thin; it is sometimes difficult to see this membrane but it can be shown by careful study in all cells of this type. The chromatin is rather evenly distributed throughout the nucleus, but may be somewhat denser around the nucleoli. The parachromatin is abundant and the nucleus generally appears pale on this account. Under pathological conditions the parachromatin is often relatively diminished so that the nucleus appears much deeper in color. The chromatin granules are distinctly demarcated from the parachromatin (oxychromatin) in these myeloblasts, in which respect they are differentiated from lymphocyte nuclei; in the latter the chromatin granules have indistinct outlines. In the myeloblasts which seem to be younger the chromatin granules present a stippled appearance. With further development the granules tend to arrange themselves in strands with node-like masses at the intersections.

From two to five nucleoli are present within the myeloblast nucleus. These nucleoli within the same nucleus present differences in staining reactions, indicating that they have different chemical structure. These are smaller than the nucleoli which may be found in sections of lymphoid tissue.

Variations in the structure of the myeloblast are found in the leukemias and under other pathological conditions. Such cells may be found undergoing mitosis in the circulating blood, especially in the leukemias. Cells with relatively more abundant protoplasm are sometimes found in the circulating blood in splenomedullary leukemia. Another cell found in this disease has its nucleus indented deeply in several areas, thus producing a lobulated appearance; this is called a "Reider cell." These cells are occasionally found in the circulating blood in sarcoma of the bone marrow. They are not found in normal

bone marrow at any time. The myeloblastic nature of the Reider cell has been called in question, chiefly on account of the presence of fine granules in its protoplasm.. In two of our fulminating cases of leukemia we have found two different cells of this type. In one fine azure granules were present and the chromatin was arranged in coarser masses; no doubt these were immature monocytes. In the other type no azur granules were present and the chromatin was arranged in extremely fine and delicate granules; these Reider cells were certainly properly included with the myeloblasts though the nucleus was deeply lobulated.

From the hyaline myeloblast all the cells of the bone marrow, and perhaps the lymphocytes are, or may be, derived.

From the myeloblast are developed myelocytes, from which are developed the adult neutrophiles, eosinophiles and basophiles of the circulating blood; megaloblasts, from which are developed the normoblasts and the erythrocytes and also the plasma cells, Turck cells and monocytes. They are increased under abnormal conditions. Under very abnormal conditions de-differentiation seems to occur; leucocytes appear to become transformed into myelocytes or myeloblasts. In one of our cases of aplastic anemia the cell-structure indicated this type of anaplasia. Lymphocytes which are apparently of normal adult type, sometimes dividing, are found in the circulating blood and the daughter cells are morphologically identical with the microlymphoidocytes, or smaller myeloblasts. This relation was noted very plainly in one of our cases of sarcoma of the bone marrow associated with marked anemia.

Whether the cells from which lymphocytes arise are identical with the myeloblasts or not has not yet been definitely determined. One group of hematologists, called unitarians or monophyletists, emphasizes the identity of the stem cell which is the progenitor of lymphocytes and other blood cells. The other group, called polyphyletists, recognizes the marked similarity of the primordial cell of the lymphocytes and the primordial cell of the myelocytic series but denies their identity. According to the latter group the cells derived from bone marrow elaborate oxidizing and, perhaps, other ferments, and these cells can be recognized by the oxidase reaction and by other specific methods of staining. Inasmuch as the argument is based on a study of acute or fulminating forms of the leukemias and other seriously abnormal conditions it is evident that the actual facts can be determined only with great difficulty.

More spherical than the microlymphoidocyte in the stained smear and somewhat more deeply staining, are other cells not more than

five microns in diameter. The protoplasm of these cells may be so thin as to be invisible; possibly it may be absent. These have been supposed to develope into normoblasts. Cells which resemble these, but which have a thicker rim of protoplasm, with a nucleus which is eccentrically placed and occasionally lobulated, are present also. These differ from ordinary lymphocytes in the eccentricity of the nuclear position and in the finer chromatin structure.

Megakaryocytes are hyaline cells with basophilic protoplasm. These have a single, long, ribbon-like, coiled nucleus of characteristic form. They may form red cells by budding off fragments of their peripheral protoplasm; probably this is not a normal source of red cells. There is much reason for believing that they form platelets by budding off very small masses of their protoplasm.

Osteoblasts are usually found in the bone marrow. They do not seem to be concerned in blood formation.

Large phagocytic cells are sometimes found. They disappear very quickly during the process of preparing the slides for examination. They ingest large numbers of senile or abnormal red cells and other debris. These phagocytic cells are especially over-filled in pernicious and in sickle-cell anemias. They have a single round nucleus and are not readily distinguishable from other monocytes unless they contain fragments of red cells. Similar cells contain droplets resembling myelin, glycogen or some related carbohydrate substance, and various other granules which seem to be deutoplasmic. These cells are supposed by Osler and others to develop into normoblasts.

Erythroblast is a term applied to several types of cell by different hematologists, but always with the understanding that the cell so named is intermediate between megaloblast and normoblast. These cells may be eight to twelve microns in diameter. The protoplasm stains rather feebly and the nucleus shows a small amount of chromatin which has a typical wheel-like arrangement. The protoplasm contains no granules and scanty or much hemoglobin. Intermediate forms are found in series between any type of cell described as an erythroblast and the cells which are commonly called normoblasts. (Plates VII, VIII.)

Normoblasts are nucleated red cells. They are very abundant in the red bone marrow. The protoplasm contains hemoglobin in varying amount; the less the amount of hemoglobin the greater is the basophilia of the protoplasm; with increasing hemoglobin concentration the cells show increasing avidity for eosin. The nuclei of the normoblasts vary greatly. Extrusion of the nucleus is common; so also is grad-

ual dissolution of the nucleus. Intermediate forms are abundant between normoblasts and adult erythrocytes. Normal erythrocytes are also found within the red bone marrow.

Normoblasts have been divided by Howell into mature and immature forms. The immature forms are a little larger than normal adult erythrocytes; they stain variably according to their degree of maturity, from basophilic to acidophilic; the chromatin fibers are arranged radially, and they divide by karyokinesis, very rapidly. Mature forms are eosinophilic, as are adult red cells; they are of the same size as adult cells; the nucleus is very small, is deeply staining, often vacuolated, without chromatin structure. Other cells present a peculiar rosette-like arrangement of chromatin within the nucleus. These nuclei are recognizable as erythrocytic even when they seem to have no protoplasm.

The bone marrow is very abundantly supplied with granular white blood cells in various stages of development. Eosinophilic forms are not very abundant except under pathological conditions. Eosinophilic myelocytes are larger than adult eosinophiles. They have a basophilic, hyaline, intergranular protoplasm with large round granules which are deeply eosinophilic. The large, round, pale nuclei are placed near the periphery of the cells and may be bare for half their circumference. Cells smaller than these, with nuclei which present indented, saddle-shaped and polymorphic shapes, are present in series leading to the normal adult eosinophiles which appear in the circulating blood. The basophilic, intergranular, hyaline protoplasm gradually diminishes with increasing maturity and is barely visible in the eosinophiles of normal adult human blood.

Basophilic granular cells are scanty in normal marrow. Cells which resemble polymorphonuclear neutrophiles except that the fine granules are definitely basophilic are present. Typical mast cells are rare; they may be mononuclear or polymorphonuclear.

Amphophilic myelocytes are present in small numbers. Atypical granules in any of the younger forms of basophiles, eosinophiles and neutrophiles are occasionally found even in normal marrow, and they may be so abundant under certain abnormal conditions as to render the differential count of the various forms almost or quite impossible.

Neutrophiles of adult forms, younger neutrophiles which are approximately of adult type, neutrophiles with larger and merely indented nuclei and all intermediate gradations are present in the bone marrow. The typical neutrophilic myelocytes are large, sixteen microns or more in diameter when fresh, and they may reach twenty microns when

spread out in a thin smear. Their nuclei are very large, pale, without marked chromatin structure and they often appear naked because of the thinness of the protoplasm around them. The protoplasm is scanty, filled with very fine granules which do not take any stain with avidity but are feebly neutrophilic. The very early and young myelocyte (Cornil's marrow cell) disappears quickly and it is rather difficult to find good stained specimens of this form. Many names have been applied to the intermediate stages of development of the neutrophiles, (metamyelocytes, promyelocytes,) but intermediate gradations between every stage and the next are present; there is no logical demarkation between the types. The mononuclear neutrophile with its round or slightly indented nucleus is often called, incorrectly, a "transitional" cell. It is more abundant in the normal bone marrow than in the normal blood, no matter at what age of the subject enumerations are made. These cells, which are usually included with the monocytes of the circulating blood, include several types which differ only slightly in their nuclear structure and which probably have somewhat different lines of development. Among these are cells which have a very fine and delicate arrangement of chromatin in a perfectly round, pale nucleus; other cells with larger masses of chromatin, a nucleus which is sometimes indented or even saddle-shaped, and still other cells in which the chromatin is in large masses connected with delicate fibrillae. Much further study must be made of the cells of normal human adult bone marrow before the relations of the different cell types can be accurately determined.

ATAVISM IN HUMAN BLOOD

Human adult blood cells show very peculiar and puzzling changes under abnormal conditions. Some of these changes suggest very strongly a condition of atavism or reversion to earlier forms. Atavistic traits are those qualities not normally present in an individual of a certain race or family, but present normally in some distant ancestor. For example, oval blood cells are not present in normal human blood at any stage of development, nor in normal human bone marrow. Oval nonnucleated blood cells are normally present in the blood of the camel and certain related animals, and are found, though rarely, in the blood of human beings. When they are so found in human blood this is considered atavism.

This condition of atavism, that is, reversion of human tissues to some ancestral form, is very puzzling. It was formerly supposed that atavism proved direct inheritance,—that is, that the occurrence of oval non-

nucleated cells in human blood, for example, proved that the human race descended from some ancestor whose blood contained cells of this type. It is now supposed that this is not necessarily the case but that the presence of oval red blood cells indicates an ancestry from a race in whose hematopoietic tissues a potentiality of oval forms existed. In other words, the reversion is to some primordial cell structure in which were present the possibilities of development of red cells of different forms, and for some unknown reason in certain human beings whose development has been adversely affected in some way the blood-forming tissues followed the line of development leading to oval red cells rather than the line leading to round red cells. While oval red cells are rare in human blood, several cases have been reported in which they are present, apparently as a developmental anomaly.

The ontogenetic development of the mass of the blood cells, as well as of the individual cells, follows the phylogenetic development in many respects. This statement applies to the relationships of the various classes of leucocytes, as well as to their absolute numbers, and it applies also to the variations in the blood cells under physiological or pathological conditions.

Under abnormal conditions of the circulation, the nutrition, or the metabolism of the body, the blood, as a mass, tends to revert to its primeval appearances. It is not possible to determine whether the cells themselves actually assume primeval appearances, or whether the formation of new cells, under the abnormal conditions, becomes imperfect and of a more or less embryonic type, and thus the cells found on examination present the characteristics of phylogenetically and ontogenetically immature cells. The latter view is inherently more probable, and it is further supported by the fact that certain irritants in the circulating blood seem to bring about first an increase in the relative numbers of cells showing the characteristics of old age, while the continued presence of the irritant is associated with increasing numbers of phylogenetically younger cells.

In one of our cases, a young woman apparently healthy, though not robust, had blood with oval cells. The first blood examination was made as a routine procedure in the clinic of The Pacific College of Osteopathy. The oval cells were recognized immediately. During the three years following the first examination her blood was examined many times and the cells always included a large proportion of oval forms. During most of that time she was in ordinarily good health. The oval blood cells showed no changes, and the condition was evidently

one of a developmental peculiarity; an instance of atavism. Other cases of oval red cells in human blood have been reported in various periodicals.

Developmental anomalies in the blood cells are often associated with developmental anomalies of several other parts of the body, especially those of the nervous system. The recognition of atavistic blood cells may differentiate a developmental and therefore an essentially incurable nervous or mental condition from an acquired neurosis or insanity with similar symptoms. Atavistic cells are present in the blood of paranoiacs, morons, idiots, feeble-minded children, and in various developmental or inherited abnormal states. These cells are not present in the blood of persons suffering from nervous or mental symptoms which are due to trauma, acquired diseases or bad training. It is to be remembered in this connection that even those neuroses based on abnormal development may be greatly improved by suitable methods of treatment.

Embryonic but not usually atavistic cells are present in the blood after extremely serious demands have been made upon the hematopoietic tissues, as, for example, after repeated hemorrhages, or long infection with virulent pathogenic organisms. Atavistic cells are often present in the leukemias and in erythremia, chlorosis, pernicious anemia and malignancy of the bone marrow. They are rare in aplastic anemia and are not commonly found in ordinary infections nor in ordinary secondary anemias, though these may be extremely serious.

A very brief review of some of the cells characteristic of the blood of certain lower animals, together with the conditions under which such cells are found in human blood, is interesting, though the relationships which are concerned in these instances of atavism cannot be explained at this time.

Analogues of Hemoglobin

Most invertebrates carry their oxygen-bearing chemicals in the blood plasma. Certain molluscs and tunicates have colorless globulin-like substances which hold oxygen in a loose combination and carry it to the tissues; these are called achroglobins. Other molluscs and certain crustaceans use copper instead of iron in a pigment called hemocyanin; this also carries the oxygen, feebly bound, to the tissues. Hemocyanin is blue in tint, in arterial blood, and is almost or quite colorless in venous blood. Certain worms carry oxygen by means of an iron-containing green pigment (colorless when reduced) which is called chlorocruorin. Echinoderms and certain other marine animals have a red pigment, also iron-containing, called echinochrom; this is colorless when reduced.

The hemoglobin percentage varies rather irregularly. In general, among all animals the hemoglobin increases in ascending scale of vertebrates, and from infancy to maturity. In most diseases affecting the blood the hemoglobin is decreased, though in certain diseases, in which the amount of water in the blood is diminished, the hemoglobin is relatively increased.

The color index is high in lower vertebrates, since these have extremely large erythrocytes. Among mammals, the color index increases generally from the lower to the higher forms, and from infancy to maturity. In most diseases the color index is lower than normal; in pernicious anemia the color index is high, because of the presence of many abnormally large red cells. The saturation index is never increased by any abnormal condition.

Red Blood Cells

The power of the erythrocyte cytoplasm to carry a relatively large amount of hemoglobin is thus one characteristic of the higher development, the more nearly perfect specialization.

Vertebrates carry hemoglobin in red blood corpuscles, and all carry small amounts of oxygen free in the plasma. The efficiency of the red cell as an oxygen-carrying structure increases with a fair degree of regularity, though many diversions from the direct line of ascent are found. Vertebrates below mammals have their red cells usually nucleated, though non-nucleated red cells are occasionally found in the blood of all those examined in our laboratories.

In all vertebrates below mammals the red cells have either a cell wall or a definite external limiting layer which can be demonstrated by careful staining. This cell wall is not present in normal adult mammalian blood though a delicate peripheral condensation of the stroma is present. In severe anemias, such as may be due to intestinal parasites, gastric ulcers or old, severe, chronic infections with some hemolytic bacteria, human red cells show a marked thickening of the stroma at the periphery and this may somewhat resemble a cell wall.

Erythrocytes in the blood of fishes are usually round or roundish, though long ovals are characteristic of some species. Except for occasional cells all are nucleated. The nuclei vary from round to rod-shaped, but are never polymorphic and are rarely lobate. The cells vary greatly in form and in size, and may be as much as five times the diameter of the normal human red cell. The counts are low and the oxygen-carrying efficiency is always far below that of human blood.

Cells apparently identical with the megaloblasts of human blood in pernicious anemia are abundant in certain normal fishes, such as the minnow and the cod, but in other fishes, such as the perch, these large red cells are absent or very scanty. The hemoglobin is often present as granules within the red cell protoplasm, in the blood of fishes.

The development of the red cells in the fish is of interest. The first stage is a small round cell resembling a lymphocyte but with characteristic wheel-like nucleus. Between this and the large nucleated hemoglobin-carrying erythrocyte there are all intermdiate forms, including hyaline cells with more abundant protoplasm than that of lymphocytes, then basophilic granules or basophilic reticulation with thickenings at the intersections appear, then hemoglobin appears, at first very scantily, then more and more abundantly until the adult form is reached. The nucleus grows progressively smaller but remains present and apparently active throughout the life of the red cell.

Amphibian blood contains erythrocytes which are oval in nearly all genera and in other genera are round or roundish. In some species the red cells are large enough to be visible to the naked eye. They are nucleated in all species though non-nucleated individual cells may occasionally be found in any blood. Irregular non-nucleated masses of hemoglobin-containing protoplasm are occasionally found. Nuclei are usually oval, occasionally irregular in outline, usually deeply staining. The nuclear structure of all amphibian erythrocytes presents one peculiarity,—the linin network arises from the nuclear membrane in masses somewhat resembling feet. Threads from these masses form the linin network of the nucleus with meshes rather regular in size and form except where the nucleoli and chromatin masses interrupt the continuity of the netlike arrangement.

This peculiarity is rarely found in human blood. In our laboratory two cases have been found,—both during pernicious anemia—occurring in men between forty and fifty years of age, both of whom presented rather plentiful stigmata of degeneracy and both of whom had been of subnormal mentality always. Cells resembling the megaloblasts of human blood in pernicious anemia and related conditions are extremely rare in amphibian blood. Polychromasia is almost universal.

Seasonal variations in blood formation are more marked in amphibia than in other animals. Blood counts taken from the same animal at different seasons varies more widely than does blood from different genera at the same season.

Reptiles have only oval red cells, biconvex and nucleated. They are smaller than amphibian cells, generally. Their nuclei show a linin network which touches the nuclear membrane in slender, rather pointed processes. They carry the hemoglobin chiefly around the edges of the red cells, and in masses near the ends of the ovals. Cells resembling human megaloblasts are scanty and usually have nuclei which stain less avidly than do human megaloblasts. Seasonal variations in blood formation are generally less marked than in amphibia.

Birds have oval, biconvex, nucleated red cells. Rarely round or roundish, non-nucleated red cells may be found. Birds have much larger red cells than do reptiles, and, generally speaking, their erythrocyte protoplasm is more efficient as an oxygen-carrying mechanism. The hemoglobin is carried mostly at the periphery of the cell.

Cells with very scanty hemoglobin located mostly in the periphery of the cell were found in one of our cases of aplastic anemia, a baby seven months old. None of the red cells was nucleated, and no immature or myelocytoid cells, either red or white, were found in this blood.

The red cells of birds present rather long ovals and the nuclei are oval of about the same general form as the cells. The cells are more uniform in size, form and staining than is the case with lower vertebrates. The genesis of avian red cells differs somewhat from that of red cells in other vertebrates. In the red bone marrow of nearly all species of birds the capillary walls are entire, and the capillaries are greatly dilated into sinus-like spaces. In these spaces the red cells are formed from mother-cells which lie next to the capillary walls. As the cells assume progressively more nearly adult traits they are pushed toward the lumen of the sinus and are washed out into the blood stream. In the red bone marrow outside the sinus walls there also are active masses of marrow cells, and from these masses the cells enter the sinuses by diapedesis between the endothelial cells. The endothelial cells themselves seem to form red cells by dividing into daughter cells of two different characters,—one is an endothelial cell, the other develops into a typical red cell.

The tendency of endothelial cells to form red blood cells is not present in normal mammalian tissues, but after repeated hemorrhages in experimental animals this form of red-cell development has been reported, denied and again reported. We have found no evidence of this form of development in our cases of abnormal blood.

Normal adult mammalian blood has only round, non-nucleated red cells, except that in the case of the camel and related species the red

cells are oval and non-nucleated. The finding of oval cells in human blood has already been discussed in this chapter. Oval nucleated red cells have been described in human embryonic blood, but these have not been found in any of the human embryos studied in our laboratories.

Among mammals, the lower forms generally show the more distinct stroma, with greater irregularities in size, shape, and staining, and of greater instability under slightly abnormal conditions, as well as after removal from the vessels. Cabot's rings and other structures abnormal to human blood are often found in the blood of lower mammals.

The erythrocytes of the lower mammals become distorted more easily after removal from the vessels than do those of the higher forms, and the erythrocytes of the younger individuals, both human and animal, are more easily distorted. Under abnormal conditions affecting the nutrition of the human body, the erythrocytes become more fragile. The remarkable variations of form found in sickle-cell anemia (a peculiar developmental anomaly of blood cells found especially in negroes or in persons with some negro inheritance) should be considered in this connection.

Among the lower animals also and among the young in any genus, slighter nutritional variations, such as fatigue, poor nutrition and starvation, affect the appearance of the erythrocytes more seriously than is the case in older individuals. In human children comparatively slight metabolic disturbances produce very marked changes in the appearance of the erythrocytes; conversely, in the presence of anemias appearing severe, even slight improvement in nutrition is followed by very speedy improvement in the blood picture.

Spindle Cells

Spindle cells are peculiar structures not found in the blood of mammals. They are very abundant in the blood of birds and are present in small numbers in the blood of reptiles, amphibia and fishes. They seem to function in much the same manner as do the platelets of mammalian blood.

A spindle cell, as its name indicates, has a spindle, almond, or elliptical form, with the ends often rounded. It is four or five microns in length by two or three microns in width, and it has usually a thickened area near its center around its nucleus. The protoplasm is faintly fibrillar with the fibrillae arranged in irregularly concentric rings around the nucleus. In some animals a faintly granular appearance is visible. The protoplasm contains no hemoglobin. The nucleus stains feebly and contains chromatin in fine masses. The network is delicate and has

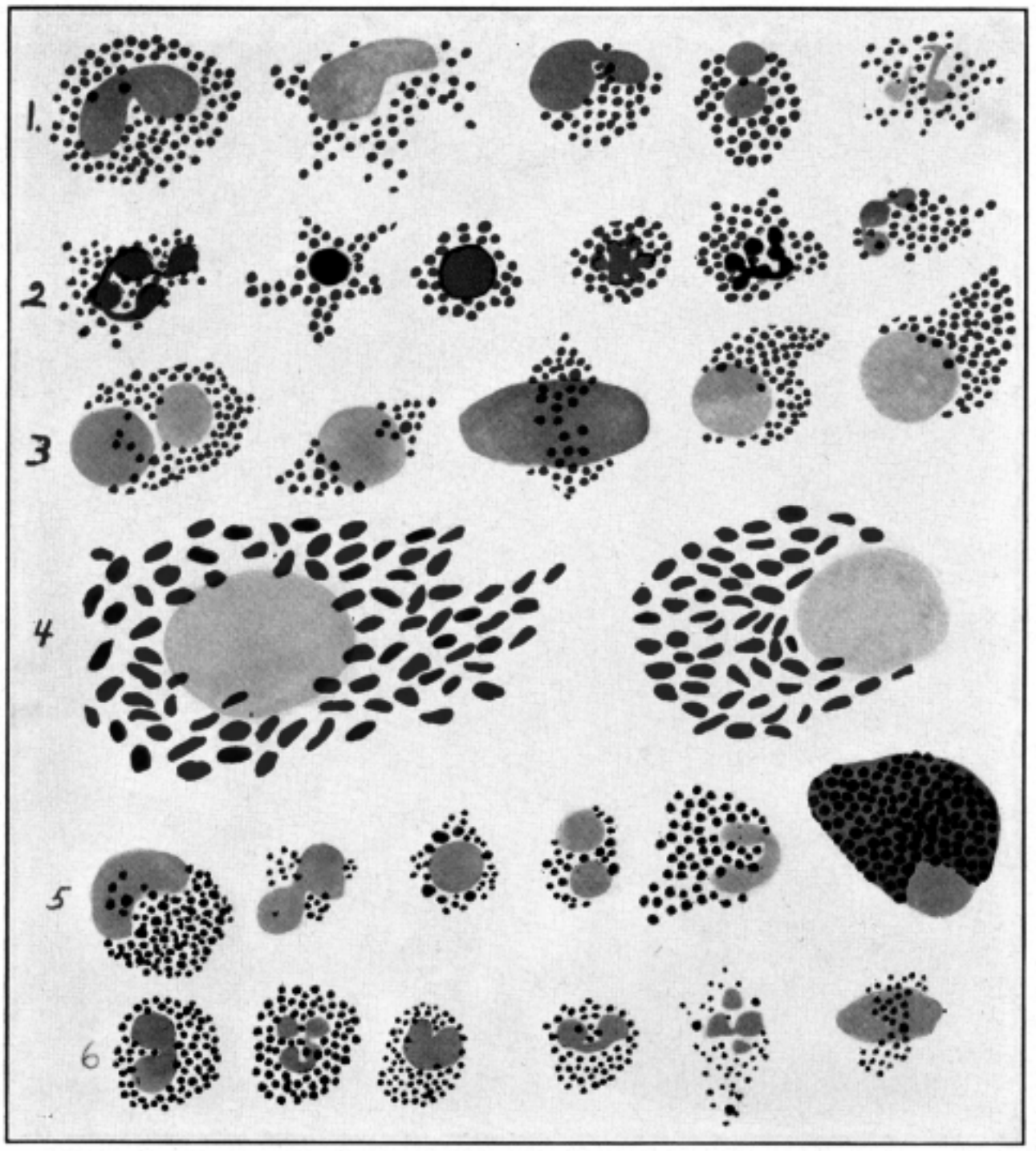


PLATE V

ATAVISTIC CELLS

1. Eosinophiles from blood of rabbit, normal.

2. Eosinophiles from blood of guinea pig, normal.

3. Eosinophiles from blood of patient with many stigmata of degeneracy and mentality of imbecile.

4. Eosinophiles from blood of horned toad.

5. Basophiles from blood of gopher.

6. Basophiles from blood of human fetus of three months development.

slight thickenings at the intersections. After the blood leaves the vessels the spindle cells tend to become rounded, to throw out fibrin threads and to form net-like masses. Their development is still uncertain, though they are known to be formed in the bone marrow.

These cells have not been reported for any human blood. In our laboratories they have never been found in any mammalian blood.

Leucocytes

The total number of leucocytes per cubic millimeter of blood varies more for different individuals, and for the same individual at different times, among lower mammals than among members of the human race. Seasonal variations and daily variations are more marked among lower mammals. The same environmental and pathological conditions cause the same general changes in the total leucocyte count among lower mammals, and these changes are more pronounced than in adult human subjects.

Children's blood shows more extravagant reactions to pathological and environmental changes in both the leucocyte and the erythrocyte counts than is the case with adults. The maintenance of a fairly constant level of leucocyte count under varying physiological states is thus a function of adult and highly developed hematopoiesis. This relationship may be compared, in a very general way, to the ability to maintain a constant temperature level under varying environmental conditions. Both characteristics are present in efficient degree only among mammals.

Variations in the cells themselves and in the relative numbers of different cell groups present many peculiarities which suggest atavistic relations.

Hyaline Cells

The earliest form of blood cell is a hyaline, basophilic, mononuclear cell bearing some resemblance to the small lymphocyte of normal adult human blood. It does not give the oxidase reaction. This has been called a primordial cell or a stem cell. It has been found in the blood or the tissues of nearly all forms of metazoa. Even such highly developed invertebrates as crabs and caterpillars have only this form of blood cell though it occurs in varying sizes. The bone marrow of marsupials contains only this type of cell, and from it other forms of blood cells are developed. Wandering cells capable of differentiating into these hyaline cells and also into tissue cells are present in the tissues of nearly all metazoa, even those as low as sponges.

Crustaceans have a mass of cells near the stomach in which large hyaline cells are abundant, and are rapidly dividing. This mass seems to be the chief hematopoietic tissue of animals of this type.

In all vertebrate blood are found plasma cells, large and small hyaline cells resembling human lymphocytes, and large mononuclear cells resembling human endothelial cells.

In fishes the small hyaline cells have a delicate spongioplasm which is more finely meshed at the periphery of the cells, so that the cell often seems to have a cell wall. The protoplasm is scanty, the nucleus relatively large, but it is practically always possible to find some enveloping cytoplasm. Cells with extremely thin cytoplasmic covering of the nucleus, so often seen in human lymphocytes, are not found among the small hyaline cells of fishes. Granules which vary somewhat in staining are sometimes found, very scantily, in the hyaline protoplasm. The nucleus is always round or oval, and never shows any marked irregularity of contour.

Large, mononuclear, basophilic, hyaline cells which are phagocytic are important in the blood of fishes. They surround foreign objects and they digest and utilize as food suitable foreign substances within the body. These cells often contain scanty granules which are feebly basophilic, feebly eosinophilic or amblychromatic. These cells sometimes contain lobed nuclei and are probably the precursors of the granular phagocytes of higher forms of life.

Both large and small hyaline cells are occasionally spindle-shaped or oval in certain genera of fishes and in these cells the nucleus also is oval. In the cod, the triton and certain related forms a large mononuclear cell is present which is irregularly triangular, with well rounded angles. The nucleus is relatively small and occupies one corner of the cell. The cytoplasm shows a web-like structure which presents some faint resemblance to granulations. This cell is very feebly basophilic and does not contain true granules. Cells presenting identical structure are occasionally found in human blood as a developmental anomaly.

In ganoid fishes the hyaline cells resemble human lymphocytes but they possess a very distinct nuclear membrane. Lymphocytes, or similar cells, with distinct nuclear membranes were found in one of our cases of late lymphatic leukemia, a few days before death.

Amphibian blood contains several types of hyaline basophilic cells, and these are generally rather more primitive in type than is the case in the blood of fishes. Azur granules are occasionally found in the cytoplasm, sometimes arranged at the periphery, sometimes near the nu-

cleus. Amphibian blood also contains extremely large hyaline cells with deeply basophilic protoplasm and feebly staining nucleus which almost entirely fills the cell. The chromatin is in peculiar radiating masses. Cells of this type are often found in the blood of human beings as a developmental anomaly.

A rather small hyaline cell shows unusually eccentric nuclear site; this permits half or two-thirds of the nuclear outline to be perfectly naked while there is an amount of cytoplasm almost or quite equal to the nuclear area upon the opposite side of the nucleus. This cytoplasm is feebly basophilic and it does not contain granules. This cell is not found in normal human blood or marrow at any stage of development, but it does occasionally appear in human blood after long and severe infectious processes.

Amphibians react to infectious processes by producing abundant large and small hyaline cells. This reaction is often present in human children, especially those poorly nourished. The peculiar disease of adults called agranulocytic angina is characterized by a similar type of reaction to infection, except that the cells are never so abundant as is the case with amphibians.

Reptilian blood contains many small and large hyaline cells, and also the extremely large cells with very large nuclei which have been mentioned for amphibian blood; they may be thirty or even forty microns in diameter. The large hyaline cells are the most important phagocytes of reptilian blood.

The blood of birds includes all three sizes of hyaline cells described for reptiles and amphibia, and also a large cell with scanty, deeply basophilic protoplasm and a large round nucleus with large masses of chromatin which show marked avidity for stains; these resemble the Turck cells of human blood. These large hyaline cells are the most important phagocytes of avian blood. Birds also carry large mononuclear cells resembling the endothelial cells of human blood, especially during the active stage of some inflammatory process. Birds possess very little lymphoid tissue in their bodies and the hyaline cells are chiefly produced in the red bone marrow and in the spleen.

All mammalian blood contains hyaline cells very like those of human normal blood, except that very large hyaline cells with large round nuclei, such as are present in the blood of birds and lower vertebrates, are present in some of the lower mammals . (Plates V, VI, XIII)

Granular Leucocytes

Progressive differentiation is more marked among granular cells than among hyaline cells. Reversionary traits are, for this reason, somewhat more easily recognizable. Basophiles (mast cells) are not found at all in the blood of invertebrates or of fishes. In amphibia and in higher vertebrates several varieties of basophilic granular cell are found. One type, especially, seems to be a precursor of the human neutrophile. The granules are abundant and are very fine, and they are sometimes only feebly basophilic. The nucleus of this type of basophile may have two lobes though usually a single nucleus is round or roundish. These cells are especially abundant in amphibian blood. Another type of basophile has a rather small nucleus, deeply staining, and a scanty, basophilic, hyaline, intergranular protoplasm. The granules are very large, very deeply basophilic and are arranged mostly around the periphery of the cell. The granules of this type of cell are so large, so deeply stained and so brilliant that they present an unusual and vivid picture. Other types of basophile show different forms of nucleus and different arrangement of granules; many intermediate forms are present. These forms appear in the leukemias of human beings.

Reptilian blood contains a basophile with a large nucleus which occupies at least two-thirds the area of the cell, abundant, deeply staining basophilic granules of moderate size, and scanty, hyaline, intergranular, feebly staining basophilic protoplasm. The nucleus of this cell stains rather feebly with ordinary dyes. This type of cell is rarely found in the blood of birds, and is never found in normal embryonic or adult human blood or bone marrow. It occurs in human subjects with late lymphatic leukemia, though rather rarely. In our records such cells have been three times reported,—one man with very late lymphatic leukemia, one woman with an atypical leukemia following typical Hodgkins disease, and one late myeloid leukemia in which reversionary traits were unusually abundant.

Basophiles of the type found in normal, adult, human blood are not found in the blood of lower mammals, and are scanty in any blood except human.

Eosinophiles

Eosinophiles are not found in typical form in the blood of invertebrates. The blood of fishes contains a few atypical eosinophiles. The granules are usually round, they stain feebly and are most abundant near the center of the cell.

Reptilian blood contains eosinophiles abundantly. The granules vary considerably in size and in form in different species, and are sometimes definitely polygonal. Extremely large nuclei which stain feebly are present in many eosinophiles. In others there are smaller and sometimes bi-lobed nuclei containing definite chromatin masses which take stain with avidity. Rather smaller round nuclei with indistinct chromatin are found in other species. Polymorphonuclear forms are also present; these cells are phagocytic and ameboid, and they behave much as human neutrophiles do. In certain snakes eosinophiles occur which have rather scanty, rod-like granules and very noticeable large nuclei in which the chromatin material is arranged in tigroid masses. The nucleus is placed at one side of the cell and often is completely bare of protoplasm for almost half its periphery. It has an abundant, intergranular, basophilic, hyaline cytoplasm. This cell has not been found in any human blood or marrow. The blood of certain other reptiles contains eosinophile granules which are quite long and rod-shaped, and these rods are arranged in the basophilic cytoplasm in radiating lines forming a star-like structure. The nucleus is smaller and tigroid markings are less marked than is the case with the cells just described. Similar star-like arrangement of rod-shaped eosinophile granules has been found in one case of aplastic anemia, in our records. Many other stigmata of degeneracy were present in that case.

In the blood of birds eosinophiles are less abundant. Two forms are rather common. The type most abundant contains granules somewhat finer than human eosinophile granules, and the nucleus is often bi-lobed. These cells divide by mitosis while circulating. Another form is less abundant. The granules are rod-shaped and are closely and densely packed together; no hyaline protoplasm is visible. The granules are intensely eosinophilic and the nucleus varies from round, notched and bilobate to polymorphonuclear. These cells are ameboid and phagocytic, and they seem to be rather closely related to the neutrophiles of human blood. They increase during inflammatory conditions, as do human neutrophiles, and they surround foreign bodies.

Among mammals the eosinophiles are often very large or very small, oval and rod-shaped granules are frequently found. These oval granules are never found in normal embryonic or adult human blood or marrow, but sometimes in human blood after long and severe infectious processes with evidences of exhaustion of the hematopoietic tissues the granules of the eosinophile become rod-shaped.

Precursors of Neutrophiles

Really typical polymorphonuclear neutrophiles are found only in human blood. Very similar cells are found in the blood of primates, and cells which are polymorphonuclear but whose granules are feebly basophilic, feebly eosinophilic or feebly amblochromatic are present in the blood of nearly all vertebrates. Amphophilic cells which are phagocytic and ameboid, which are developed from the small hyaline cells previously mentioned, are found in many of the lower metazoa and are present in the blood of many vertebrates.

Fishes have peculiar granular cells which are spindle-shaped or round. The granules seem to be concerned in the coagulation of the blood. They are acidophilic and stain deep red with Giemsa's stain. The granules are much smaller than eosinophile granules of human blood. Granular cells in the blood of fishes often show vacuoles, and there is much reason to believe that these cells have some sort of secretory function. The granules are formed within the cell in increasing numbers so that the nucleus may be crowded into a very eccentric position. The granules then seem to dissolve, leaving vacuoles, and these seem to discharge their contents into the blood plasma. The cell again fills with granules, and the process is again repeated. In some fishes the cells of the lymphoid tissue form granules very abundantly during the digestion of food, and these are lost during sleep. These cells do not seem related to any human cells and have not been reported for any human blood or marrow.

Amphibians have many amphophilic, basophilic and eosinophilic cells. All three forms of granular cell are ameboid and phagocytic, and they ingest many forms of pathogenic bacteria. These cells include both mononuclear and polymorphonuclear types. In these cells the granules are often rod-like or oval rather than round.

Birds have also eosinophilic, basophilic and amphophilic ameboid and phagocytic cells. True neutrophilic granules are not present in their blood.

Among mammals fairly definitely neutrophilic granules are occasionally found. Apes' blood contains neutrophiles something like those of human blood. With certain stains neutrophilic granules of varying sizes are found in the polymorphonuclear leucocytes of the goat, dog, mouse and a few other animals, though with other stains these granules are eosinophilic or amphophilic. The polymorphonuclear leucocytes of the rabbit have amphophilic granules which include a few rather large neutrophilic granules. The granules of the polymorphonuclear leuco-

cytes of the guinea pig are very fine and are faintly eosinophilic. The granules of certain polymorphonuclear cells of the horse are neutrophilic and are extremely fine. The polymorphonuclear cells of the cow, pig, rat and sheep contain faintly eosinophilic granules.

Under many conditions of exhaustion of the hematopoietic tissues and in the leukemias, the neutrophilic granules diminish or disappear and amphophilic, feebly eosinophilic or feebly basophilic granules appear in the polymorphonuclear cells of human adult blood.

Special Study of the Blood of Monkeys

Differences in the numerical relations of the different classes of blood cells of mammals are of interest in this connection. In the laboratory of The A. T. Still Research Institute in Chicago the blood of normal monkeys (macacus rhesus) was studied during the autumn and early winter months. Ten examinations were made of the blood of each of ten monkeys, all in good health, all about two years old. The hemoglobin was determined by means of Dare's hemoglobinometer, and this was checked with the Meisner modification of Fleischl's instrument. For the differential count a modification of Wright's stain was used and for each count 1,000 cells examined. The actual counts were based on cells found in 200 small squares, for the red cells, and 4,000 small squares, for the white cells. The blood was taken about four hours after the last meal, in each case, and at about ten o'clock in the morning. The following findings were thus secured:

	lowest	highest	average
Hemoglobin	73%	82%	75.4%
Red cells	4,030,000	6,000,000	4,643,000
White cells	8,290	12,650	11,200
Polymorphonuclears	32%	50%	42%
Eosinophiles (coarsely granular)	.01%	12%	3.7%
Basophiles	0	.6%	.24%
Monocytes and large hyaline	0	2.7%	.8%
Small hyaline	63.3%	37.7%	53.6%

Summary

Instances of atavism are often noted in connection with various deformities and developmental anomalies, in many tissues of the body. The blood cells share in this tendency of abnormal development to follow the line indicated by some remote progenitor. When atavistic cells occur in the blood of a human being during the course of some disease it may safely be concluded that the hematopoietic tissues of that individual were not quite properly developed during his embryonic life. Immature forms may appear in the circulating blood of any person as a result of disease affecting the bone marrow, but true instances of atavism occur only in human blood when other tissues of the body also show evidences of abnormal embryological development.

The presence of these reversionary forms is to be included with other stigmata of degeneracy. The frequent occurrence of atavistic forms of white blood cells during the course of the leukemias suggests the possibility that these diseases have a developmental origin. In pernicious anemia atavistic forms are fairly common. The developmental abnormalities associated with pernicious anemia are discussed elsewhere. The study of the atavistic cells occurring during the course of other diseases may show that abnormal developmental conditions may be important factors in lowering immunity and in delaying or preventing recovery from accidental injuries or poisonings.

CHAPTER VII

THE PRIMARY ANEMIAS

Primary anemias are those forms of anemia for which no adequate cause is known. With increasing knowledge of the relations between structural abnormalities, hygienic improprieties and the formation of the blood cells, the cases called primary or idiopathic are slowly diminishing in numbers. Pernicious anemia, chlorosis and aplastic anemia are the most common of the primary anemias.

Simple primary anemia is already known to be a misnomer. This is by definition a form of anemia without known adequate cause, which progresses through many remissions, with no intermissions, until death occurs from asthenia or from some relatively mild intercurrent disease. The cell count in these cases is rather high, the hemoglobin low, the leucocyte count almost or quite normal with relative excess of the small hyaline cells. This form of anemia is due to malnutrition and to rib lesions, and is, therefore, really a form of secondary anemia.

It is evident that no abnormal condition can occur without adequate cause. In the primary anemias there is some congenital defect. Very often individuals with such defects are able to live normal lives so long as no excessive demand is made upon the hematopoietic tissues, provided always that environmental factors remain good. When these persons fail to receive the best of food, adequate rest, sufficient sunshine or fresh air, or when any infection or other cause of disease occurs, the defective hematopoietic tissues are speedily overcome by the increased burdens. Fatal or very serious anemias supervene unless the etiological factors are speedily recognized and removed.

The causes of the developmental defect have not been well studied but are probably identical with the causes of many other aberrancies of embryological life. Experiments made upon animals in many laboratories have shown that embryological development can be easily modified. The breathing of fumes of alcohol, ether, chloroform, mercury, illuminating gas and any other abnormal volatile substance by either male or female guinea pigs, rats and other laboratory animals causes the appearance of many deformed young in the progeny. Exposure of incubating eggs to such fumes and to excess of carbon dioxide increases the number of deformed chicks, sometimes to 90% or more. Addition

of abnormal substances to the water containing gametes of any living creatures increases the number of deformed ova or embryos. Both male and female germinal cells are affected by such poisons.

Drugs administered to either males or females before mating increases the number of deformed young, and this applies to mammals and to submammalian genera.

Exposure to the fumes of alcohol or other drugs or the administration of drugs to females early in pregnancy increases the number of deformed young and the number of still-births.

Human embryos found at operations for tubal pregnancy include a larger proportion of deformities than are found in uterine pregnancies; this is undoubtedly due to the abnormal environment of the developing embryo.

In the laboratories of The A. T. Still Research Institute the progeny of animals with bony lesions always show some developmental defects.

From all these findings it may be concluded that developmental defects may be due to some abnormal condition affecting either the sperm cell or the ovum cell before union; abnormal conditions affecting the ovum after fertilization and before its fixation in the uterus, and abnormal conditions affecting the embryo and fetus as a result of maternal disease or poisoning.

APLASTIC ANEMIA

Aplastic anemia is a rather rare condition characterized by aplasia of the red bone marrow. The cause is not known, but the nature of the findings on blood examinations and autopsies strongly indicates a developmental basis for the disease. In adult life a similar condition occurs, apparently as a result of exhaustion of blood-forming tissues which are unable to meet some excessive demand.

Osteosclerotic anemia is a very rare disease in which there is an increasing growth of bone or of dense fibrous tissue into the red bone marrow. The anemia is of the aplastic type after the disease has become well established. Albers-Schonberg disease is a form of osteosclerotic anemia occurring in childhood. The bones become hard, solid and brittle and fracture easily or spontaneously. The red marrow is invaded by bone or fibrous tissue and the anemia is of the aplastic type. Differential diagnosis between these various anemias of the aplastic type is difficult. Biopsy of a fragment of rib may determine the true nature of the disease.

True aplastic anemia occurs in young people and children, usually after some disease which has affected the blood. The disease is char-

acterized by marked anemia with no evidences of regenerative activities on the part of either leucocytopoietic or erythrocytopoietic tissues, and by a rapid progress to an inevitably fatal ending. Hemorrhages are frequent. The skin and the fat of the body are very pale. At autopsy the red bone marrow is scanty and may seem to be completely absent, the cavity of the bone being occupied by dry connective tissue fibrillae within whose meshes a few small, pale, dry areas of atrophic bone marrow may occasionally be found.

The differential diagnosis between pernicious anemia and aplastic anemia may be indicated by the following comparison:

	Pernicious anemia	Aplastic anemia
red cells fall	gradually	rapidly
color index	high	low
basophilic stippling	abundant	absent or scanty
poikilocytes	abundant	few or none
normoblasts	abundant	few or none
microblasts	many	few or none
megaloblasts	many	few or none
at autopsy		
fat	deep orange	very pale
bone marrow	hyperplastic, dark red	scanty or none, very pale

Illustrative Cases

The following case is not quite typical because the color index was very variable and occasionally high and because nucleated red cells were found in the blood before death.

E. S. A young man eighteen years old, never robust but always well until he was sixteen years old, when he suffered a rather mild attack of malaria. Thereafter he became progressively weaker and more emaciated.

At the first examination the blood count was as follows:

Hemoglobin 26% (33.6 gms. per liter, vonFleischel's apparatus)

Red cells 2,320,000 per cu.mm. (46%)

Color index .56

Poikilocytes, microcytes, megaloblasts, present in small numbers.

Poikilocytosis more marked than anisocytosis.

Normoblasts	40 per cu.mm.
Microblasts	20 per cu.mm.
Megaloblasts	100 per cu.mm.

Basophilic stippling present in a few cells.

Blood platelets greatly diminished.

Red cells laked quickly on the warm slide. Polychromasia marked. Leucocytes began to move at once and were more active than normal. They began to die within ten minutes and all were dead within twenty minutes.

Leucocytes 5,000 per cu.mm.

Large hyaline cells	2.4%	120 per cu.mm.
Small hyaline cells	68.6%	3430 per cu.mm.
Mononuclear neutrophiles	1.0%	50 per cu.mm.
Polymorphonuclear neutrophiles	25.8%	1290 per cu.mm.
Eosinophiles	.8%	40 per cu.mm.
Basophiles, none in 2,000 white cells		
Myelocytes, hyaline	.6%	30 per cu.mm.
Myelocytes, granular	.8%	40 per cu.mm.

Nuclear average 1.86

All classes of leucocytes include many immature forms.

Iodophilic and Sudanophilic granules, none

The treatment given after this count included osteopathic manipulations planned for the purpose of securing better circulation through the rib marrow and the spleen. The diet remained unchanged, since it was already very good. After eight days the following blood findings were given:

Hemoglobin 25% (33.2 grams per liter, von Fleischl's apparatus)

Red cells, 1,660,000 per cu.mm. (33%)

Color index .79%

Poikilocytes, microcytes and megalocytes about as before.

Normoblasts	100 per cu.mm.
Microblasts	50 per cu.mm.
Megaloblasts	140 per cu.mm.

Polychromasia more marked than at previous examination. Basophilic stippling less marked.

Blood platelets 20,000 per cubic millimeter.

Leucocytes 6,500 per cu.mm.

Large hyaline	3.2%	208 per cu.mm.
Small hyaline	76.4%	4966 per cu.mm.

Mononuclear neutrophiles	.4%	26 per cu.mm.
Polymorphonuclear neutrophiles	18.4%	1196 per cu.mm.
Eosinophiles	.6%	39 per cu.mm.
Basophiles, none		
Myelocytes, hyaline	.4%	26 per cu.mm.
Myelocytes, granular	.6%	39 per cu.mm.

Nuclear average 1.78

Uranalysis this date and at later dates showed no abnormal findings.

The treatments were useful in that the patient seemed stronger, suffered less dyspnea and showed some color in the skin. The course of the disease was not perceptibly modified, and this was not expected. Four weeks after the first examination the blood findings were as follows:

Hemoglobin 15 grams per liter (10.5%)

Erythrocytes 754,000 per cu.mm.

Color index .69

Poikilocytes, microcytes, megalocytes, polychromasia, basophilic stippling, unchanged.

Normoblasts 120 per cu.mm.

Microblasts 12 per cu.mm.

Megaloblasts 60 per cu.mm.

Platelets, none found

Leucocytes 6,000 per cu.mm.

Large hyaline	4.6%	276 per cu.mm.
Small hyaline	67.5%	4050 per cu.mm.
Mononuclear neutrophiles	.5%	30 per cu.mm.
Polymorphonuclear neutrophiles	26.1%	1566 per cu.mm.
Eosinophiles	.3%	18 per cu.mm.
Basophiles, few		
Myelocytes, hyaline, few		
Myelocytes, granular	1.0%	60 per cu.mm.

Nuclear average 1.45

Immature forms more abundant in all leucocyte groups.

Gregariousness pronounced.

Patient very weak, dyspnea marked.

After this, blood transfusion gave some slight improvement in the symptoms. Two days later he seemed quite comfortable and walked

around in his room. On the fourth day after transfusion he suddenly became weaker, dyspnea increased, a condition of almost complete coma followed and death seemed imminent.

On this day, five weeks after the first examination, the following blood findings were noted:

Hemoglobin 24.8 grams per liter (18%)
Erythrocytes 674,000 per cu.mm.
Color index 1.33
Poikilocytes, microcytes, megalocytes, polychromasia, basophilic stippling, all considerably increased.

Normoblasts	257 per cu.mm.		
Microblasts	72 per cu.mm.		
Megaloblasts	216 per cu.mm.		
Leucocytes	9000 per cu.mm.		
Large hyaline		1.2%	108 per cu.mm.
Small hyaline		78.6%	7074 per cu.mm.
Mononuclear neutrophiles		.6%	54 per cu.mm.
Polymorphonuclear neutrophiles		18.6%	1674 per cu.mm.
Eosinophiles		1.0%	90 per cu.mm.

Large hyaline	1.2%	108 per cu.mm.
Small hyaline	78.6	7074 per cu.mm.

Leucocytes 9,000 per cu.mm.

Mononuclear neutrophiles	.6%	54 per cu.mm.
Polymorphonuclear neutrophiles	18.6%	1674 per cu.mm.
Eosinophiles	1.0%	90 per cu.mm.

Basophiles, none
Myelocytes, hyaline, few
Myelocytes, granular, few
Malarial parasites, few

Small hyaline cells include many myeloblastic and "stem cell" types.

Death occurred the next day. Necropsy about an hour later.

Body very pale and thin. Fat scanty and very pale.

Heart; size normal. Muscle very soft, flabby and pale. Valves all normal. Pericardial fluid about 3 c.c., clear, pale.

Lungs; very much distended with air; moderate pneumokoniosis; no evidences of tubercular or other infection; no pleural or pericardial adhesions.

Stomach; contains about 300 c.c. of partly digested blood; no evidence of ulceration; hemorrhage apparently per diapedesis.

Duodenum; contains partly digested blood and some bile.

Jejunum and ileum contain blood in diminishing amounts until at ileo-cecal valve no blood is present. Gas and bile present in small intestine. Small intussusception, evidently post-mortem, in jejunum. Cecum, appendix and colon all normal. No parasites; no ulceration anwhere.

Liver pale, increased in size about one-fourth. Spleen pale, about three times normal size. Pancreas pale, otherwise normal. Abdominal lymph nodes slightly enlarged. Adrenals slightly enlarged, slightly congested. Kidneys very pale, otherwise normal.

Tissues made into microscope slides; no abnormal findings except as indicated by gross examination.

Bone marrow atrophic throughout entire body. Cavity very dry, containing only very pale, fine connective tissue trabeculae, with microscopic areas resembling atrophic bone marrow.

B-7. Boy, 13 months of age. Child seemed normal at birth and until age of seven months. At that time he had mumps, and thereafter he became progressively paler and weaker, with no other definite symptoms.

The amount of blood in the toes was so scanty that the actual count and the hemoglobin could not be determined without making larger incisions than we considered justified under the circumstances. The drops secured were so pale that the liquid was not recognizable as blood. Smears showed occasional red cells normal in form but very small. No nucleated red cells were found in 2,000 red cells examined. White cells extremely few. An examination of six smears, including about 500 fields, discovered only a few hyaline cells of normal appearance, two polymorphonuclear neutrophiles and one large hyaline cell of normal appearance.

Death occurred three days later. No necropsy.

PERNICIOUS ANEMIA

(Progressive pernicious anemia; hemolytic anemia; Addisonian anemia; cryptogenic anemia; primary progressive anemia).

Pernicious anemia is a disease of unknown origin, characterized by its chronic and intermittent progressive weakness to death; hypertrophy of the red bone marrow, and blood showing low hemoglobin, high color index and the presence of megaloblasts and other immature blood cell forms.

Etiology

Many factors have been discussed as probable causes of pernicious anemia. That there is some adequate cause of every abnormal condition is axiomatic, but for this, as for several other very serious diseases, no such cause has yet been found. Infection by hemolytic bacteria; the presence of hemolytic products of abnormal katabolism; proliferative activity of the bone marrow resembling the proliferative activities of malignant neoplasms; the lack of some food material necessary to the manufacture of normal red cells, and the presence of some developmental defect in the hematopoietic tissues have all been considered etiological factors. Perhaps several of these factors are essential to the development of the disease; perhaps some other condition, as yet unsuspected, will be found the essential cause of pernicious anemia. The occurrence of intermissions, during which the health of the patient and his blood picture seem perfect, is one of the most puzzling features of this disease.

In one of our cases the last remission lasted for eleven years, after which relapse was extremely rapid and quickly fatal. Even longer intermissions have been reported. The blood cells include many embryonic and occasionally atavistic cells; the color index is always high, usually exceeding unity, and leucopenia is always present in typical cases.

Forms of secondary anemia resembling pernicious anemia are found in patients suffering from bothriocephalus latus; in this case a lipoid substance extracted from the worm is distinctly hemolytic. Infection by streptococous hemolyticus sometimes causes a blood picture of the pernicious type. When cancer metastases invade bone marrow the blood may resemble that found in pernicious anemia. There are several less common conditions in which the diagnosis between pernicious and extremely severe and atypical secondary anemia is very difficult. In one of our cases a balantidium coli infection caused blood findings typical of pernicious anemia. Sprue and other tropical diseases may also cause a blood picture like that of pernicious anemia.

True pernicious anemia may begin during pregnancy. In many cases a form of anemia resembling pernicious begins during pregnancy, and this is associated with a mild leucocytosis. It disappears soon after labor. In other cases the anemia may not seem to be more severe but it does not disappear after the termination of pregnancy. In one of our cases an anemia with hemoglobin of 45%, color index of 1.2 and typical

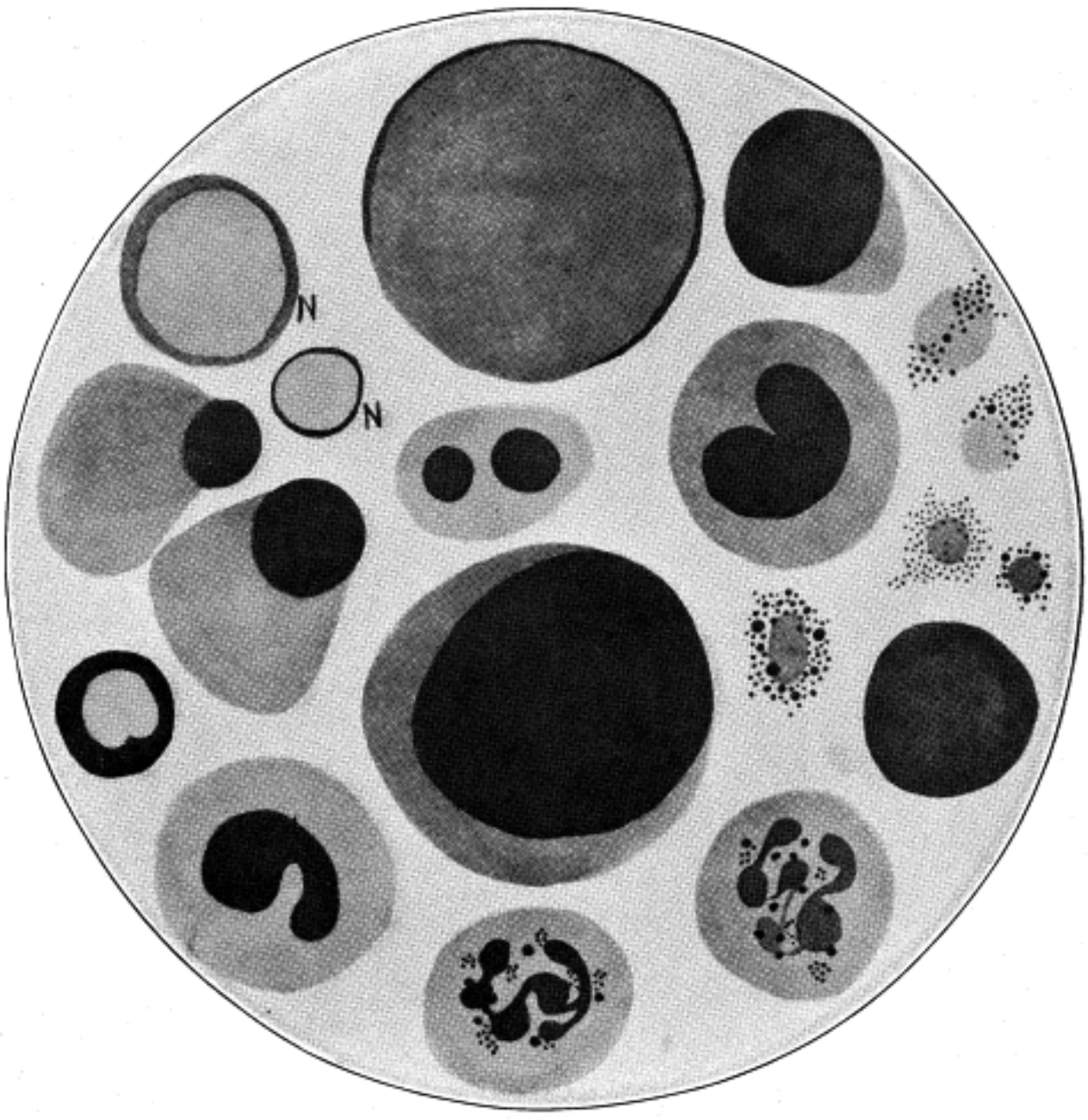


PLATE VI

ATAVISTIC CELLS

N, N, Lymphocytes from normal adult human blood.

Other cells from blood of moron with many stigmata of degeneracy. These cells are identical with cells found in the blood of lower vertebrates.

megaloblastosis was found at the fifth month of pregnancy. Within three weeks after the birth of the child the hemoglobin had reached 85%, with a color index of 0.7.

Pernicious anemia rarely occurs in children, and in the few cases reported achlorhydria has also been present, as is the case with pernicious anemia in adults. Children kept too long on milk, especially on goat's milk, may develop an anemia which resembles the pernicious form very closely. In this case the hydrochloric acid of the gastric juice is normal, and recovery follows the establishment of a normal diet, though sometimes convalescence is prolonged and stormy.

Typical cases of pernicious anemia have been reported in wild mammals kept in captivity, by Fox and others.

The symptoms can be given only briefly in this discussion. The patient is rarely emaciated; the skin presents a typical lemon-yellow tint; achlorhydria is always present in typical cases and indigestion due to this factor is almost always noted; gastric pain and persistent diarrhoea are common.

Glossitis, often with ulceration, and pyorrhoea alveolaris are usually present. Weakness, palpitation, headache, dyspnea, vertigo, edema of the extremities and the face, neuritis and various forms of tingling, numbness and other nervous phenomena are very common. Late in the disease degeneration of different areas of the central nervous system causes symptoms which may or may not be related to the site of recognizable degenerative processes. Degenerative areas of considerable extent may occur without causing symptoms; this is true also of other degenerations within the central nervous system.

The course of the disease varies greatly. Sometimes death occurs within a few weeks after the first symptoms are noted. More commonly, several periods of intermission or remission occur before the terminal attack; very often death occurs from some intercurrent disease.

Blood Picture

Diagnosis is usually easily made from the blood picture. In one of our cases, and in many reported cases, the nervous symptoms have been misleading and blood examinations postponed until the pallor or the characteristic skin tint led to further investigation. In atypical cases the diagnosis may present great difficulty. In selected cases it may be advisable to remove a bit of a rib for a study of its marrow, in order to determine the essential nature of the disease.

Chemical examination of the blood has not given useful information. The specific gravity is low, often below 1.027. The osmotic tension remains normal or increased. The high osmotic tension with low specific gravity is due to the fact that iron and the large organic compounds are diminished while the smaller inorganic molecules are increased. The blood sugar may be increased or may remain unchanged. The nitrogenous wastes are increased, as is inevitable with nephritis due to the anemia. The alkaline reserve is frequently diminished. According to Rowntree, the total blood volume in pernicious anemia is lower than normal, hypovolemia. Of nine cases only one showed normovolemia. The average was 76.4 cubic centimeters of whole blood and 58.8 cubic centimeters of plasma per kilogram of body weight.

The blood serum is deeply pigmented, but there is no marked excess of bile pigments. The urine is paler than normal in most cases.

Platelets are greatly diminished and it may be impossible to find any. The coagulability of the blood is decreased, and there may be no clot formed at all. The viscidity is very low and the blood seems to be as thin as water. The water of the blood is greatly increased and may exceed 90% of the weight. The albumin of the blood plasma is not modified, though the albumin of the cells is greatly diminished. In this respect the blood in pernicious anemia differs from the blood in equally severe secondary anemias. The cholesterin is greatly diminished and the fats of the blood substantially increased. Fatty globules are frequently found in the blood smears, both within the neutrophiles and lying free in the plasma.

The hemolytic aspect of this disease has been emphasized until recently, but studies made during the last few years tend to show that the destruction of the red blood cells is the natural result of the excessive and imperfect formation, and that the latter is primary in pathogenesis. The possibility that some lack of suitable materials for the manufacture of the red cells may be the essential factor in etiology must be considered. As a result of this lack, imperfect red cells are developed; these are destroyed and ultimately the hypertrophy of the erythrocytopoietic tissues results. The complete story of the pathogenesis of pernicious anemia remains to be written.

The red cells show characteristic changes, though these are not absolutely pathognomonic. Hemoglobin may be diminished to 10% of the normal for the age of the patient. Red cells are diminished more than the hemoglobin, and the color index is always high in typical cases without recent transfusion. Nucleated red cells of many types

are present. Cells showing various endoglobular structures, nuclear remnants and basophilic stippling or reticulation are abundant. Poikilocytosis is less marked than anisocytosis and the megaloblasts more numerous than normoblasts in typical active cases. Edematous cells are frequently found. (Plate VII)

Erythrocytes

The red cell count may be very low, and is usually about 2,500,000 at the first examination, in our cases. Counts as low as 500,000 are found and patients with these low counts may be comfortable and reasonably active. The hemoglobin may be as high as 18% with a red cell count of 9% of the normal for the age and sex of the patient.

The volume index is always above one in typical cases, but the amount of hemoglobin carried by a given volume of erythrocyte protoplasm is less than normal; the saturation index is low.

Anisocytosis is more marked in pernicious anemia than in any other disease. Microcytes (from 2 microns to 6 microns in diameter) are abundant. They may be very dark in color; this is due to their sperical form. Macrocytes and megalocytes (9 microns to 16 microns in diameter) are abundant. They may be spherical and appear very dark in color, or they may be flattened and seem to be very pale. Gigantocytes exceed 16 microns and may reach 20 microns or more in diameter. They often appear during the last few days of life. Both microcytes and the very large cells often show a tinge different from that found in normal cells, as if some abnormal pigment might be associated with the hemoglobin.

Poikilocytosis is less marked than anisocytosis but is abundantly present. Sausage-like forms are especially abundant, though all the ordinary types of poikilocytes may be found.

Polychromasia is abundant. Basophilic reticulation and stippling occur in young cells. Degenerative forms may also be basophilic. They are most abundant in the severe cases.

Megaloblasts, gigantoblasts and megakaryocytes are found in the blood in order according to the severity of the case. In typical cases megaloblasts exceed normoblasts. Erythroblasts are usually more common than megaloblasts in mild cases. Megakaroycytes and gigantoblasts usually appear only in very severe cases approaching death. Microblasts, poikiloblasts and normoblasts vary considerably in the same patient at different times; their numbers do not seem to bear any definite relation to the severity of the disease.

Crises

Blood crises are fairly common occurrences. The bone marrow undergoes suddenly increased activity and a shower of blood cells is thrown into the circulation. These cells may be almost or quite normal and an intermission or a remission occur. The cells may include great numbers of nucleated and immature cells, in which case a more rapid progress of the disease is to be anticipated. If normoblasts exceed megaloblasts the prognosis is better than when great numbers of the very large nucleated forms are present at the time of a crisis. The more abundant the large nucleated cells, the more gloomy is the prognosis.

Leucocytes

The leucocytes are always diminished in typical cases, and a high leucocyte count should lead to very careful search for some definite etiological factor. A diagnosis of pernicious anemia is not warranted when leucocytosis is present until some complicating infection or other leucocytogenic factor has been found. The leucopenia has reached 3,000 cells in many of our cases, and has reached 1,000 several times. Counts as high as 6,000 occasionally occur in otherwise typical cases. The leucopenia is due to diminished neutrophile count; the hyaline cells usually remain normal in actual numbers and in cell characteristics. With the changing count of granular cells, the hyaline cells show varying percentages, but their actual numbers remain almost or quite steadfast throughout the entire course of the disease. In those cases in which the blood is highly toxic, there may be mild absolute as well as relative lymphocytosis. In cases characterized by marked malnutrition, with wasting, the lymphocytes may be absolutely increased.

The neutrophiles show nuclei of a peculiar rosette-like structure. These nuclear rosettes are not so abundant nor so perfect in any other disease. The nuclear average is much higher than normal, reaching 3.8 in one of our cases, and usually exceeding 2.7 in typical cases.

The eosinophiles are somewhat increased both relatively and absolutely. Moderate increase in eosinophiles is often associated with beginning remission. Sudden diminution in the eosinophiles is of grave significance. Mast cells are usually increased. Myelocytes are always present and they may be abundant; these include neutrophilic, eosinophilic, basophilic and hyaline in the order mentioned. Extremely large myelocytes in the peripheral blood usually presage speedy death.

With increasing hypertrophy of the erythrocytopoietic tissues, the leucocytopoietic tissues may be variously affected. There is usually atrophy of the latter, so that leucopenia increases steadily. In other cases there may be some hypertrophy of the leucocytogenic areas of the red bone marrow also. The white cells thus thrown into the circulation include great numbers of myelocytes and the blood picture may resemble that of leukanemia.

Nutrition

The weight of the body remains constant or increases moderately, in typical cases. The fat is not diminished and there is a varying amount of edema of the tissues. Patients who lose steadily in weight should be subjected to very careful examination in the hope that some adequate cause of the anemia can be found and removed.

Nervous Changes

Degenerative processes affect the central nervous system after the disease has been present for some time, and this time varies greatly. It is not rare for the patient to seek relief for some nervous disorders before the anemia is suspected. In other cases no nerve degeneration appears at all, though the patient may die after several severe relapses. Nervous symptoms do not always bear a definite relation to the anatomical location of the degenerative processes. At autopsy areas of the nervous system may seem to be structurally normal after the symptoms have apparently indicated definite local degeneration, and, on the other hand, areas of marked degeneration may be found at autopsy although no symptoms referable to the affected areas were noted during life. The degenerated areas do not seem to follow any definite anatomical or physiological course.

Gastric Juice

The constant lack of hydrochloric acid in the gastric juice of patients with pernicious anemia is of interest. The achylia precedes the anemia and persists during remissions without change. The very frequent lack of hydrochloric acid in patients with malignant neoplasms is of interest in this connection. The achlorhydria of pernicious anemia is not like the hypochlorhydria due to lesions of the sixth and seventh thoracic vertebrae, since in the latter cases the correction of the lesion results in a gradual return of the normal gastric secretion. In pernicious anemia and in the achlorhydria associated with malignant neoplasms of developmental origin, no treatment causes a return to normal gastric secretion. There is much reason for believing that this

form of achlorhydria is due to some developmental defect whereby the oxyntic cells fail in proper differentiation. Persons showing other evidences of imperfect development, mentally or physically, very often suffer from achylia, achlorhydria or hypochlorhydria.

Pathology

Autopsy findings are confusing. If death occurs during an intermission there may be nothing found anywhere to indicate pernicious anemia. The bone marrow may be of normal structure and normal location; the fatty marrow may occupy its normal position in the long bones. Liver and spleen may be normal in gross and in microscopic structure. If death is due to accident, the entire body may appear to be perfectly normal in every way. If death results from intercurrent disease, the autopsy finding may be only those of the fatal disease, with nothing to indicate pernicious anemia.

More commonly death occurs from some other disease during relapse or remission, in which case variable indications of pernicious anemia may be found. In one of our cases death occurred from pneumonia, and the autopsy findings included those of pneumonia as well as those of pernicious anemia. In this case the anemia had just begun to grow more severe, after a remission of moderate degree lasting several months.

When death occurs during the progress of the disease the autopsy findings are distinctive and easily recognizable, but they do not explain the nature or the cause of the disease. Although the blood is so deficient in iron, this metal is stored abundantly in the liver, both in the Kupffer cells and in the hepatic cells; the reticulo-endothelial cells of the bone marrow and of the spleen also contain iron more abundantly than normal.

The red bone marrow shows marked hyperplasia and extends into the areas normally occupied by the yellow marrow, which it may completely replace. The solid parts of the bones may be so seriously invaded that they break spontaneously; this is especially true of the ribs.

Areas resembling the erythrocytopoietic tissues of the embryo may be found in the spleen, liver and other glandular and lymphoid tissues of the body. Careful examination usually shows these cell groups to be composed of masses of endothelial cells, some of which are ingesting the abnormal red cells. They are, therefore, destructive and not constructive so far as the red cells are concerned. Phagocytosis of the red cells by the endothelial cells is much more abundant in post-mortem

than in biopsy material. This indicates that at least part of the phagocytosis is a terminal or a post-mortem phenomenon. The moderate degree of fatty degeneration, the moderate desquamation of the Kupffer cells, their moderate hypertrophy and their phagocytic activities in pernicious anemia do not seem greatly different from similar conditions found in severe infectious or toxic diseases. Hence these phagocytic activities do not seem to be the most important factor in pathogenesis.

The fat of the body is deep yellow, sometimes definitely orange in color. The blood may be so pale as not to be recognizable as blood when removed from the vessels. All the organs are extremely pale and they may seem almost completely bloodless. Areas of degeneration may be found within the central nervous system, even in cases in which no nervous symptoms are presented before death. Other organs show degenerations due to the long-continued anemia and toxemia. The blood volume is not greatly modified, and the apparent bloodlessness of the tissues is due to the pallor of the blood.

Therapy

Methods of treating pernicious anemia have changed frequently with changing views of the etiology and the pathogenesis of the disease.

Since no definite structural lesions have been found responsible for the disease, there is no specific osteopathic treatment for it. Patients with pernicious anemia are as subject to the evil effects of lesions as are other persons, and such lesions as are found on examination should be corrected. Very often such corrections are followed by marked relief of the symptoms. In this, as in almost all other diseases, it is the actual condition of the patient and not the name of the disease which determines the most efficient therapeutic methods.

Since the hydrochloric acid deficiency is of developmental origin, little can be done to correct the condition; it is probably impossible to further developmental progress in persons old enough to suffer from typical pernicious anemia. Attempts have been made to provide artificial gastric juice for these patients. Occasionally the administration of hydrochloric acid, with or without pepsin, has been followed by marked and prolonged remission or intermission. In a patient for whom we made many blood examinations, the administration of an artificial gastric juice was associated with an intermission of eighteen years. At the end of that time the anemia suddenly became worse and he died within a few weeks.

Splenectomy was at one time a favorite method of treating pernicious anemia. This was because an abnormal spleen was supposed to destroy the fragile red cells too rapidly. After splenectomy the red cells become more resistant and the anemia often diminishes for a time. The progress of the disease is only temporarily delayed at best, and no good results are visible at all, in many cases.

Toxemia is always present in patients with pernicious anemia, and any rational methods of therapy must include careful attention to this phase of the disease.

The administration of liver and of certain other nitrogenous foods containing considerable amounts of nucleins causes speedy intermissions with return of the blood to almost or quite the normal picture, in many cases. If patients die from some intercurrent disease during such an intermission, there may be slight or no indications that the patient had ever suffered from pernicious anemia. The intermissions may be prolonged almost indefinitely by persistent careful feeding. Yet, on relatively slight carelessness, the pernicious anemia blood picture may supervene with extreme rapidity and the patient may die within a few weeks from pernicious anemia despite the most careful feeding and care.

The rapid improvement in the blood picture and in the general condition of the patient as a result of feeding these particular foods may lead to the inference that pernicious anemia is really a deficiency disease. A pre-existing cause of severe toxemia is almost always present in pernicious anemia, and while these conditions do not cause pernicious anemia except in certain people, yet there is much evidence that the toxic products of pyogenic foci and of intestinal putrefaction have some etiological relation to the disease. The fact that some developmental defect is necessary for the occurrence of typical pernicious anemia has been mentioned. When the developmental defect (or constitutional dyscrasia) is associated with toxemia of a hemolytic type and with the lack of some nutritional factor necessary for the development of normal blood cells, pernicious anemia becomes developed. Improvement often follows relief of the toxemia. Improvement often follows the administration of the lacking nutritional elements. Remissions may be greatly prolonged in either case, but since the constitutional defect is necessarily unmodified relapses are practically certain to occur.

Whether modern methods of dietetic treatment of pernicious anemia can prolong the intermission or remission indefinitely remains to be seen. Under older methods of treatment the remissions have lasted for many

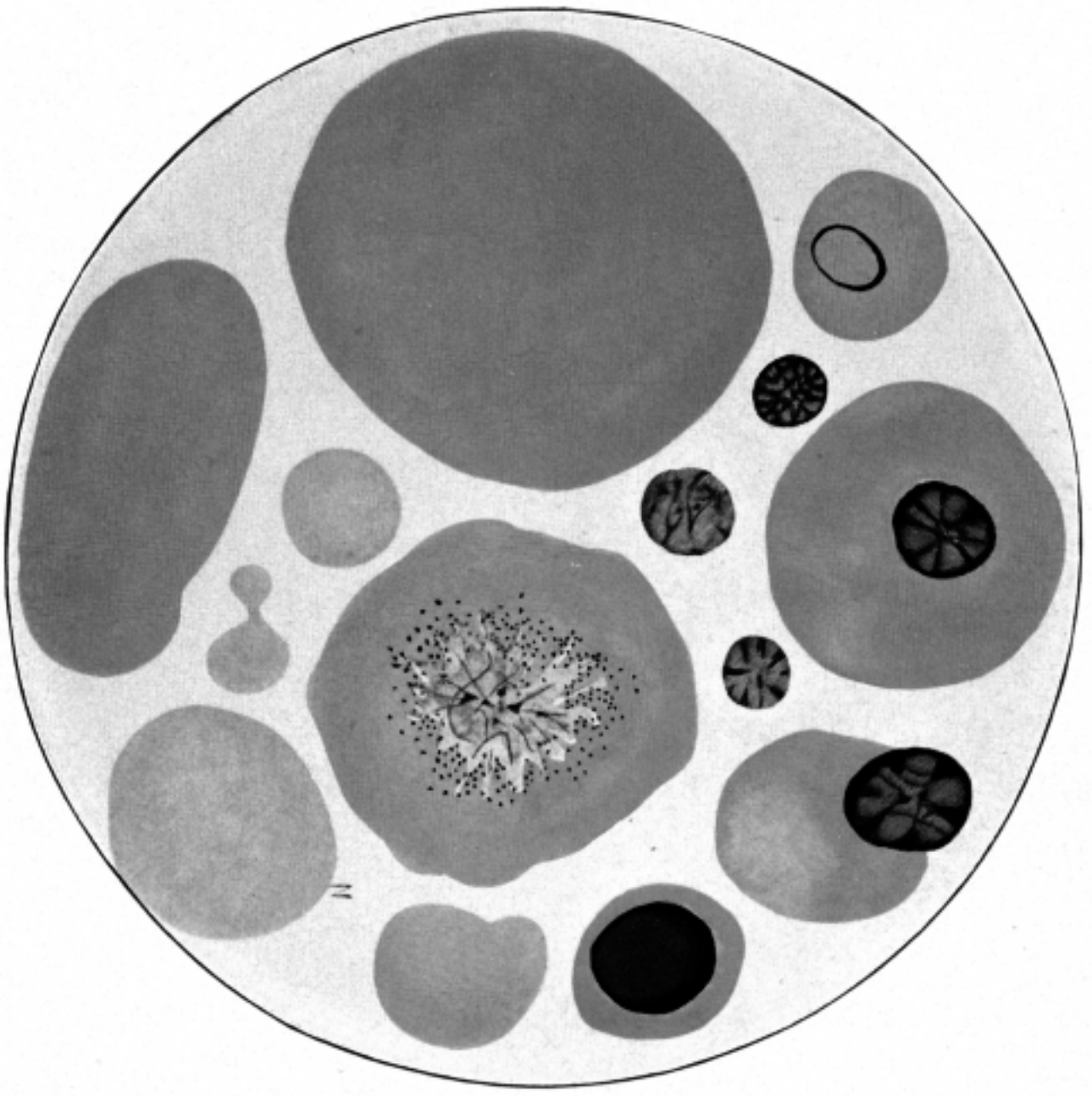


PLATE VII

CELLS IN PERNICIOUS ANEMIA

N, erythrocyte of normal size but spherical form. One cell shows the sausage-like form characteristic of pernicious anemia. One megaloblast and one megalocyte are present; the first shows a nucleus undergoing solution. Two microcytes, one micropoikilocyte with dumb-bell form and one microblast show typical forms. Two normoblasts are shown; one nucleus is being extruded. One cell shows a Cabot's ring. Three naked nuclei show different stages of development.

years, but unless some other disease or some accident causes early death pernicious anemia inevitably returns. The attacks tend to increasing severity and the patient finally dies from pernicious anemia.

Illustrative Case

K-9. Man forty-four years old. Symptoms indefinite, patient is weak and pale and skin presents peculiar lemon-like tint. At first examination the following blood report was made:

Hemoglobin 75.7 gms. per liter (vonFleischl-Miesher) 55% of normal, for age.

Erythrocytes 2,620,000 per cu.mm.

Color index 1.05.

Poikilocytes, microcytes, megalocyts, normoblasts and microblasts, all present. Megaloblasts, none.

Platelets considerably diminished. Anisocytosis more marked than poikilocytosis. Polychromasia marked. Basophilic stippling not present.

Leucocytes 3,000 per cu.mm.

Large hyaline	3.9%	117 per cu.mm.
Small hyaline	25.6%	768 per cu.mm.
Mononuclear neutrophiles	.8%	24 per cu.mm.
Polymorphonuclear neutrophiles	67.4%	2022 per cu.mm.
Eosinophiles	1.9%	57 per cu.mm.
Basophiles, very few		
Myelocytes, hyaline	.1%	3 per cu.mm.
Myelocytes, granular	.3%	9 per cu.mm.
Neutrophile nuclear average	2.17	

Leucocytes moved very feebly on the warm stage, and died within fifteen minutes (should live an hour or more in normal blood.)

During the six weeks after this examination osteopathic treatment was given which was intended to secure better circulation through the ribs. The diet and hygienic conditions were improved and the symptoms diminished rapidly. (This history antedated the use of liver in the treatment of pernicious anemia).

Six weeks after the first examination the following blood report was made:

Hemoglobin 100.5 grams per liter (73% of normal)

Red cells 3,500,000 per cu.mm., (70% of normal)

Color index 1.04

Abnormal forms of red cells, very few. Normoblasts, very few, Other nucleated red cells, none.

Leucocytes 4,000 per cu.mm.		
Large hyaline	7.0%	280 per cu.mm.
Small hyaline	38.4%	1536 per cu.mm.
Mononuclear neutrophiles	1.2%	48 per cu.mm.
Polymorphonuclear neutrophiles	50.0%	2000 per cu.mm.
Eosinophiles	3.0%	120 per cu.mm.
Basophiles	.4%	16 per cu.mm.
Myelocytes, hyaline and granular, few		
Neutrophile nuclear average	2.98	

The condition continued to improve, both symptomatically and hematologically, for three months. An attack of influenza then caused relapse with the following blood findings:

Hemoglobin	81.2 gms. per liter,	58%
Erythrocytes	2,816,000 per cu.mm.,	56%
Color index 1.04		
Abnormal red cells abundant. Nucleated red cells, none.		
Leucocytes 1,500 per cu.mm.		
Large hyaline	7.4%	111 per cu.mm.
Small hyaline	29.2%	438 per cu.mm.
Mononuclear neutrophiles	1.6%	24 per cu.mm.
Polymorphonuclear neutrophiles	58.8%	882 per cu.mm.
Eosinophiles	2.8%	42 per cu.mm.
Basophiles	.2%	3 per cu.mm.
Myelocytes, hyaline and granular, few		

The patient disappeared from observation during the next ten months, after which he returned with a history of varying degrees of weakness and dyspnea. No blood examinations were made during this time. One week later he contracted pneumonia and died within a few days. Necropsy twenty-four hours after death.

Lungs showed consolidated areas and pleural adhesions abundant, on both sides, all of comparatively recent date and characteristic of pneumonia.

Spleen about three times normal size, soft and friable. Liver about twice the mass and nearly three times the weight of normal. Other viscera showed the findings characteristic of pernicious anemia and of pneumonia. Red bone marrow showed great hyperplasia. Tibia completely filled with red marrow of type characteristic of pernicious anemia. Microscopic examinations of lungs, marrow and spleen. Death due to pneumonia with pernicious anemia also present.

W-2. Man about fifty years old. May 15, blood findings as follows:

Hemoglobin	62 grams per liter,	45%
Erythrocytes	2,000,000 per cu.mm.,	40%

Color index 1.11

Poikilocytes, microcytes, megalocytes, polychromasia, all present in moderate numbers. Anisocytosis more marked than poikilocytosis.

Normoblasts	50 per cu.mm.
Microblasts,	10 per cu.mm.
Poikiloblasts, few	
Megaloblasts	30 per cu.mm.
Leucocytes	5,000 per cu.mm.

Large hyaline	6.8%	340 per cu.mm.
Small hyaline	41.0%	2050 per cu.mm.
Mononuclear neutrophiles	.4%	20 per cu.mm.
Polymorphonuclear neutrophiles	48.6%	2430 per cu.mm.
Eosinophiles	3.0%	150 per cu.mm.
Myelocytes, hyaline and granular, few	.2%	10 per cu.mm.

Neutrophile nuclear average 2.75

Iodophiles, none.

Two weeks later the red cells were as follows:

Hemoglobin	47 grams per liter,	34%
Red cells,	1,400,00	28%

Color index 1.21

Normoblasts	80 per cu.mm.
Megaloblasts	120 per cu.mm.

Microblasts and poikiloblasts, very few

Three weeks after the first examination, the following findings were reported:

Hemoglobin	27.5 gms. per liter,	20%
Red cells	1,000,000 per cu.mm.	20%

Color index 1.

Leucocytes, 1,000, per cu.mm., with differential about as before.

Four weeks after the first examination, the following blood report was given:

Hemoglobin	23 gms. per liter,	17%
Red cells	612,000 per cu.mm.	12%

Color index 1.5

Abnormal and nucleated red cells abundant, with many megaloblasts.

Leucocytes 800 per cu.mm.

Large hyaline	3.0%	24 per cu.mm.
Small hyaline	38.5%	308 per cu.mm.
Mononuclear neutrophiles	1.0%	8 per cu.mm.
Polymorphonuclear neutrophiles	57.5%	460 per cu.mm.

Eosinophiles, basophiles, myelocytes, iodophiles, all absent.

Neutrophile nuclear average 2.66

Death occurred two days later. Bone marrow only examined. Ribs and long bones filled with red marrow, paler than usual, of microscopic structure characteristic of pernicious anemia.

Sickle-Cell Anemia

A very peculiar and interesting phenomenon occurs in the blood of certain negroes and persons of partly negro descent, characterized by the appearance of red blood cells having a sickle-shaped form. The cells vary in appearance, and may resemble half-moons, commas, oat-grains, and irregular parts of a circle, as well as the typical sickle. The cells seem to include many normal forms, in the circulation, but they are very delicate of structure and soon tend to the characteristic forms when the blood is prepared for examination. The changes which occur after the blood has been taken under conditions suitable for accurate examination were studied with especial care by Levy in 1929. In our laboratories we have had only a few cases and these were not suitable for special study.

The tendency to sickle-cell structure of the red cells is a trait associated with imperfect development of the erythrocytopoietic tissues. Normoblasts, megaloblasts, myelocytes and cells showing basophilic reticulation or punctate basophilia are usually present in the circulating blood.

Anemia may be slight or profound. In typical cases the red cell count is between two and three million, and the hemoglobin between 40% and 75%. The color index is between 0.8 and 1.2, in typical cases. The white cell counts vary considerably. Usually there is some leucocytosis. It is difficult to find uncomplicated cases because the anemia itself is rarely the condition which causes the patient to come under observation. Immature and myelocytoid forms are abundant.

The condition is rather common among the negroes in Southern States and in the negro districts of New York and other Northern cities. It has been described in a very few cases of white people whose

family history seems to preclude possibility of negro blood. This peculiar condition seems to be hereditary as a dominant trait according to Mendel's Law.

The abnormal condition of the red-cell formation is present throughout life. When some disease affects the individual the cells are very seriously affected. Abnormal red cells increase in numbers and they are rapidly ingested by the cells of the reticulo-endothelial system. Anemia becomes extremely severe upon relatively slight nutritional disturbances or upon relatively mild diseases. Death is usually from some intercurrent disease, though deaths from the anemia itself occasionally occur.

At autopsy the spleen and liver are distended with the abnormal red cells and their fragments. In some cases an abnormally small size of the spleen and the liver with moderate hyperplasia of the red bone marrow has been reported. Fatty degeneration of the heart, liver and other organs has been reported. These are apparently due to the anemia itself.

Since the condition is evidently developmental no treatment could affect it in any adequate manner. As in all other conditions associated with imperfect development, it is necessary to avoid all causes of malnutrition or ill-health very carefully in order that the unfortunate individual may have as good a life as is possible under the circumstances. In such a case as this the importance of keeping the best possible circulation and nutrition of the red bone marrow is apparent. No cases have been reported under osteopathic observation and treatment.

Chlorosis

In this country chlorosis has almost disappeared during recent years. Chlorosis is a peculiar form of anemia which occurs in girls during adolescence and which has its basis in a developmental anomaly.

The aberrant development is indicated by an abnormally small heart and by narrow blood vessels; these conditions are sometimes found only at autopsy, but are always present in typical cases. The heart, originally small, is usually slightly dilated and hemic murmurs are present. The "bruit de diable" is present in typical cases; this is a sound like the humming of a giant top, heard over the base of the great vessels of the neck. These circulatory conditions are due to the abnormal structure of the heart and blood vessels with compensatory phenomena.

Other developmental anomalies associated with chlorosis include congenital weakness of the abdominal muscles and the walls of the abdomi-

nal and pelvic viscera. Dilatation of the stomach and intestines and ptoses of the abdominal and pelvic viscera are always present in typical cases. The gastric juice shows low or absent hydrochloric acid, and this defect also is developmental. The chest is rigid and rather narrow and the diaphragm is rather high. The respiratory excursion is always very small. These conditions are primarily developmental but are exacerbated by habit.

The adolescent phase of the disease is indicated in the abnormal menstruation and the cystic ovaries,—always present in typical cases. In this connection the occurrence of ovarian cysts and of other developmental anomalies of the ovaries in the female progeny of lesioned animals in the Sunny Slope Laboratories is of interest. In the human race other causes of developmental aberrations are often present, as well as bony lesions in the parents. During the years since about 1890 there has been increasing interest in the care of pregnant women, and osteopathic views as to the importance of bony lesions in the etiology of gynecological and obstetrical complications have been increasingly recognized. The diminishing occurrence of chlorosis is, no doubt, associated with the diminishing occurrence of other developmental anomalies among the more intelligent members of the human race.

During the same years also there has been increasing interest in diet, and improved transportation facilities have made possible a supply of fresh vegetables and fruits throughout the entire year. These conditions may be important in the diminishing occurrence of chlorosis.

Menstrual disorders are sometimes of the type usually called nervous. Amenorrhea is the most common condition and this is often thought to be a result of the anemia. Dysmenorrhea is also very common. Menorrhagia and metrorrhagia are much less common in chlorosis, and the anemia associated with these conditions is typically secondary in nature. That the absence of the internal secretion of the ovaries is not the essential cause of chlorosis is evident from many facts,—chlorosis does not occur before the onset of puberty nor after the menopause under any normal or abnormal conditions; in the days when ovariotomy was a common method of treatment for young women with neuroses or with dysmenorrhea chlorosis did not result (though other serious symptoms did occur), and in laboratory animals the removal of the ovaries does not cause any of the characteristics of chlorosis.

With further development of the chlorotic girl, abnormal phenomena of the nervous system are inevitable in typical cases. Behavior becomes

increasingly aberrant. Abnormal cravings become manifest; the girl eats clay, charcoal, chalk and other improper articles; she sleeps poorly and wanders around at night; she is furiously in love or as furiously antagonistic to her associates, her teachers, and members of her own family, whether male or female. This abnormal behavior has its basis, no doubt, in developmental anomalies of the nervous system as well as in the abnormal state of the internal secretions of the ovaries and related glands. Headache, neuralgia, cold hands and feet, irregular feverish attacks, and constipation are common symptoms. Dermatographia and other hysterical phenomena occasionally appear.

The chlorotic girl is usually fatter than normal. This condition is often only apparent and is due to edema of the tissues. There is, however, some excess of fatty tissue due to deficient oxidation. Imperfect oxidation is inevitable with the low hemoglobin and inefficient cardiac activities associated with chlorosis, but the abnormal state of the internal secretions, especially of the ovary and the thyroid, are also concerned in the abnormal tissue metabolism.

The greenish tint of the skin is characteristic. The cause of this color has not been determined. The urine is not of abnormal tint and the conjunctivae are never dark in color in typical cases.

The eyes are commonly of great brilliancy, with sclerae rather bluish white. In some cases considerable dilatation of the blood vessels of the skin masks the greenish tint. The lips and the lobes of the ears are yellowish and somewhat waxy in appearance in typical cases.

The blood changes are pathognomonic. The hemoglobin is very low, usually between 20% and 40% of the normal for the age of the girl. The red cell count is high, often exceeding the normal. The color index is lower in chlorosis than in any other disease, often being below 0.4, and occasionally reaching 0.25 or lower fractions.

Viscidity, alkalinity, specific gravity and osmotic tension are lower than normal. The blood coagulates more rapidly than normal, and this causes a peculiar phenomenon which is rarely present in other conditions. When a drop of blood is allowed to fall upon filter paper, as in making the hemoglobin estimation by means of the Tallquist scale, the plasma spreads into the paper leaving a drop of brilliant red blood, often clotted, in the center of the moistened circle. This increased coagulability of the blood is probably responsible for the thrombosis and sudden deaths which occasionally occur in chlorosis.

The total blood volume is somewhat increased. The cell volume is diminished.

Poikilocytosis is more marked in chlorosis than in any other disease. Large, pale, edematous cells are conspicuous in the warm-slide examination. True megalocytes are not present. The red cells are very unstable, so that poikilocytosis is much less marked if the blood is taken with great care to avoid distortion; it is very difficult to avoid some distortion of the red cells even in the warm-slide preparations. Immature forms of the cells are abundant. Polychromasia, basophilic reticulation and stippling and normoblasts are common. Megaloblasts and erythroblasts are not found in uncomplicated cases. (Plates I, V, VI)

The leucocyte count is normal or low. High counts suggest some complicating disorder. Chlorotic girls react to relatively mild causes of anemia or leucocytosis rather extravagantly. In uncomplicated cases, the lymphocyte count is relatively high and immature forms are common; this relation is of developmental type. Atavistic cells are rare. Neutrophiles are often fragmented; they are fragile and easily distorted in preparing the blood for examination. Edematous forms are often noted. Staining reactions vary considerably in different cells in the same smear. The nuclei usually stain feebly. Eosinophiles with abundant, hyaline, basophilic, intergranular protoplasm, large round nuclei and rather large scanty granules are often found. Coarsely granular basophiles and basophilic myelocytes are often abundant.

The course of the disease varies. Usually recovery is slow even with correct osteopathic treatment. Under old-time methods improvement is apparently due solely to the development of the girl during the later years of adolescence.

Osteopathic treatment has given excellent results. Such treatment is devoted to the correction of the thoracic and upper lumbar lesions usually present, and to securing as nearly normal hygienic conditions as possible.

During adolescence some correction of developmental defects seems possible. In many cases compensatory hyperplasia of the heart and increased tone of the muscular walls of hollow viscera are associated with increased hemoglobin content of the blood and practically complete recovery as a result of osteopathic treatment with wise dietetic and hygienic teachings.

Girls who do not receive such treatment may seem to outgrow the disease during the adolescent years but the blood continues to show chlorotic traits as long as they live. During their later life any conditions causing anemia causes also a return to the chlorotic type of blood.

In one of our cases chlorotic blood appeared in a woman of thirty-five after labor. She had had chlorosis during her adolescence, had apparently recovered from this within a few years and had married at the age of twenty. She had borne three children with no abnormal symptoms. She had borne no children for nine years at the time of the birth of her fourth child. There had been a rather severe post-partum hemorrhage, and the secondary anemia due to the hemorrhage was typically chlorotic in nature.

Another patient was forty-six years old. She gave a history of "green sickness" during her adolescent years, with recovery during the early twenties. She did not have the exact years at which the disease begun or ended. After the age of twenty-four years she was well and showed no evidence of anemia. Blood examinations made during the thirty-sixth year, at which time she was in the hospital for total hysterectomy following the discovery of multiple fibromata, showed no evidence of anemia. When she was forty years old she suffered a severe attack of acute articular rheumatism and after that she became anemic. At the age of forty-three she came to the osteopathic clinic for examination, and the blood showed a typical picture for chlorosis.

CHAPTER VIII

SECONDARY ANEMIAS AND POLYCYTHEMIA

Blood-forming tissues depend upon other tissues of the body for nutrition, removal of katabolites, nerve impulses and probably certain hormones. Without the various influences derived from normal activities of other tissues of the body the hematopoietic tissues become unable to produce normal blood. In this respect the bone marrow, spleen and other tissues concerned in the metabolism of the blood cells and plasma share in the mutual dependence which is shown by all parts of living metazoa. Secondary anemia is, as the term indicates, an impoverishment of the blood due to some recognizable cause. The term is commonly applied to deficiency of hemoglobin, whether this is due to a lack of red cells or to a lack of hemoglobin within the red cells. Secondary anemia may be due to any one or more of a long number of causes, and its characteristics vary according to the etiological factors.

The symptoms are not very definite and are often masked by the symptoms due to the cause of the anemia. Palpitation, pallor, dyspnea, malaise, vague headaches, weakness, insomnia and excessive fatigability are common. Edema of the feet and legs, loss of appetite, petechial hemorrhages of the skin or mucous membranes, epistaxis, retinal and uterine hemorrhages occur rather frequently. The pallor is often masked by excessive dilatation of the arterioles of the face, which gives a ruddy tint to the cheeks, especially. In certain cases sallowness or mild jaundice mask the pallor.

Character of Blood Cells in Secondary Anemia

Secondary anemia is associated with a typical blood picture though this may be somewhat modified as a result of varying individual reactions to disease or varying pathogenetic influences. The typical blood picture in secondary anemia shows little or no diminution in the number of red cells but the hemoglobin is considerably reduced, usually to less than 70% of the normal for the individual. The color index is low, usually less than 0.7 and often being below 0.4. The resistance of the red cells to saponin solutions is increased. The platelets vary but are usually diminished to half the normal number, or even less. The white cells are often increased, and the differential count as well as the actual leucocyte count very often indicates the nature of the primary disease. The blood volume may or may nor be affected; this depends upon the

cause of the anemia. The relations between cell volume and plasma volume also show variations in certain cases. Fibrin threads are scanty and slowly formed. Viscidity, specific gravity and usually osmotic tension are low. Rouleaux are subnormal and are slowly formed. Other factors vary with the nature of the primary disease causing the anemia.

Occurrence

Anemia is universally present as a result of diseases which affect the blood-forming tissues. All vertebrates which are subject to nutritional, developmental, toxic or parasitic diseases are subject also to secondary anemias. The blood changes are similar, varying only according to the varying types of blood cells found in different groups of animals. The manner in which the organs of the body react to causes of anemia varies for different animal groups also. In the lower mammals, in birds and in reptiles extra-medullary hematopoiesis is abundant after hemorrhages. Instead of neutrophilic leucocytosis many of the lower animals show mononucleosis or lymphemia. Increase in the eosinophiles occurs in certain birds, after infections.

Wild birds and mammals in captivity suffer from secondary anemia due to parasitic infections, osteomalacia, gastrointestinal inflammations of various etiology, nephritis and other conditions which cause anemia in human subjects. The severe secondary anemia of kittens infested with fleas is very common.

Further study of the manner in which the lower vertebrates react to causes of secondary anemia promises to add very useful information and thus lead to more efficient methods of handling atypical cases of secondary anemia.

Treatment

In the treatment of patients with secondary anemia the most important factor is, of course, the removal of the cause of the anemia when this is possible. After the removal of cause, and in those cases in which the cause of the anemia is beyond control, it is necessary to secure the most adequate possible renewal of the blood cells and the hemoglobin. For this the first and most important requisite is to secure correct structural relations of the body and all its parts.

Since new blood cells are developed in the red bone marrow it is essential that the bones have good circulation of good blood, and unimpeded nerve pathways to and from the central nervous system. Since the ribs contain so great a proportion of the hematopoietic areas these must receive especially careful attention. The thorax must be kept

flexible and the movements of the ribs in breathing must be free and as extensive as is practicable. Rib lesions must be corrected and the patient taught to breathe properly, if he is not already using correct respiratory movements. The skull, innominates, scapulae and other flat bones, the vertebrae and the short bones of the bone also contain fairly extensive areas of red bone marrow, and lesions affecting these should be corrected.

Since the cells of the liver and the spleen play an important part in the metabolism of hemoglobin and blood cells, these organs must be kept as nearly in normal condition as possible. Lesions of the ninth thoracic vertebra affect the spleen, especially, and lesions of the tenth thoracic vertebra affect the liver; lesions of adjacent vertebrae and ribs also affect these organs and such lesions may prevent the best possible restoration of the blood after the cause of the anemia has been removed, or they may prevent adequate reaction to persistent causes of anemia which are essentially not serious in pathogenesis.

Other tissues may be important in the control of the hematopoietic organs, and anything in the body which interferes with the general health should receive attention. But the most important factors by far, in dealing with the secondary anemias, is, first the removal of the cause of the anemia when this is practicable, and second the osteopathic treatment of all incorrect structural conditions which might prevent adequate functions of the red bone marrow, the spleen and the liver. After this attention should be given to incorrect structural relations of the other tissues of the body, and to any unhygienic conditions that may be found. The diet should receive attention, though any ordinarily wholesome and varied meals provide all that is necessary for the manufacture of good blood.

Diets

Much experimental study has been given to the place of various therapeutic agents in hastening recovery from anemia. In these experiments a group of animals is made anemic by repeated or single bleedings, by the administration of hemolytic drugs, or by the omission of iron or of some other necessary factor for the manufacture of hemoglobin. Animals thus made anemic are then divided into smaller groups, and each group is then given special diets, drugs or other therapeutic measures and the speed with which hemoglobin is regenerated in the animals is considered a measure of the potency of the therapy under consideration.

Classical medicines have received much attention. The administration of iron in inorganic form has been studied with especial care. In animals with moderate anemia the giving of inorganic iron does not seem to facilitate regeneration of hemoglobin efficiently but the giving of foods rich in iron in the organic form, especially in connection with foods containing the piperidin ring, greatly encourages the formation of new hemoglobin.

Those foods containing molecules closely resembling hemoglobin have been found best adapted to the formation of new blood in nearly all the animals tested. Lean meat, liver, sweet-breads and other glandular viscera, and blood itself all provide the best foods for promoting hemoglobin regeneration. Vegetable foods containing chlorophyll and chromophyll are also useful. It must be remembered that other elements than iron are important in hemocytopoiesis. Dried apricots, peaches and other fruits have been found useful in the regeneration of anemic animals and in the diet of anemic human beings.

The exclusive milk diet has been found useful in selected cases of secondary anemia and in chlorosis. It cannot be employed permanently for obvious reasons. The value of an exclusive milk diet for a few weeks seems to lie in the fact that milk provides an abundance of easily digested and easily absorbed proteins. Indirectly the fact that large amounts of fluid are thus given may be important in dietetic therapy. An exclusive milk diet for too long a period of time causes an anemia of persistent type. In one of our cases a child of two years became anemic because he suddenly refused any food except milk after he had been on a normal mixed diet for nearly a year. In this case no cause for the sudden aversion for ordinary foods could be explained, except that a fall had produced a lesion of the fifth to the seventh thoracic vertebra. The strength and weight diminished considerably after the accident. The correction of these lesions improved nutrition slightly but the abnormal appetite persisted. Finally he was given no milk during the daytime and was starved into eating proper foods within about four days. During this time milk was given him at bedtime and in this way he was able to sleep normally and to maintain reasonable strength. Within a week he was taking normal foods and he soon regained his normal weight and strength. After fifteen years he is robust and normal. There is still more than an average appetite for milk, though no abnormal aversion for other foods is recognizable.

Many authors have found that the administration of inorganic iron in any considerable amounts depresses the hematopoietic tissues and thus delays recovery from these experimental anemias.

Inorganic iron has been found to be utilized in the regeneration of hemoglobin in extremely severe experimental anemias. After several generations on completely iron-free diets certain laboratory animals become extremely anemic though they retain life and even continue to breed. If some soluble inorganic iron is then added to their original iron-free diets, these animals develop hemoglobin and finally show almost normal blood. After extremely severe anemia has been produced in other animals by prolonged and repeated bleedings, together with the use of an iron-free diet, the animals seem to utilize some inorganic iron in regenerating blood. The possibility that inorganic iron, administered under extremely unusual experimental conditions, can be utilized in the regeneration of hemoglobin seems certain. That inorganic iron has a really useful place in the treatment of the secondary anemias seems extremely doubtful. There is no question at all that the iron which is in combination with the other molecules and radicles which go to the formation of the hemoglobin molecule is by far the best food for persons with secondary anemia.

In connection with the diets best fitted for anemic individuals, it must be remembered that copper, phosphorus, magnesium, sulphur, lime and several other minerals are necessary for the manufacture of good blood, and that these also are best given in foods which contain them in chemical union with organic molecules. Foods rich in nuclei, such as liver, sweet-breads and kidneys contain these minerals in excellent combinations for blood-building. These foods also tend to toxemia unless they are carefully given in connection with other foods; it is often necessary to guard against toxemia with great care when high purin foods are used abundantly. Lean meats and blood contain copper, phosphorus, magnesium, sulphur, iron and other minerals in a form which facilitates blood-building. They are somewhat less apt to cause toxemia than are glands used as food.

Diet which is chiefly carbohydrate fails to facilitate regeneration of blood. Starving animals regenerate blood more rapidly than do animals on a high carbohydrate diet. This is, of course, due to the fact that starving animals are really on a meat diet.

Animals which have undergone splenectomy regenerate hemoglobin as rapidly, but not more rapidly, than do animals with normal spleens.

Animals in high altitudes are made anemic with greater difficulty than are animals near the sea level, whether the anemia is produced by repeated bleedings or by the administration of hemolytic drugs. Animals regenerate hemoglobin more rapidly in high altitudes.

Experimental animals made anemic in any manner regenerate blood and hemoglobin more rapidly in sunlight or under the influence of ultra-violet rays than in darkness. This seems to be due to the effects of sunlight on metabolism directly. Animals which have lived in darkness for many generations have normal hemoglobin.

These diets have been found most useful also in promoting recovery from secondary anemias in human subjects, after the cause of the anemia has been removed.

Classification

A classification based upon causes of the anemia plus the effects produced upon the blood cells is helpful in diagnosis.

Secondary anemias may be classified primarily as nutritional, hemorrhagic, developmental, toxic and parasitic. Any given case may be due to the effects of two or more of these causes but it is possible in nearly every case to determine some one factor in etiology which is of most practical importance. For example, that individual who has a developmental imperfection affecting the blood-forming tissues is more easily affected by disturbed nutrition than is one whose structural relations are normal. In such a case the nutritional disturbance alone can be successfully treated, though the developmental cause may be of greatest importance so far as etiology is concerned. Serious developmental anemias are usually called primary anemias.

Each group presents certain factors of resemblance and the information gained from a study of the blood cells is useful in diagnosis and in determining the most efficient methods of treatment.

Nutritional Anemias

In the anemias called nutritional the essential factor is the lack of a sufficient supply of food for the hematopoietic tissues. Many factors are concerned in providing the bone marrow and the lymphoid tissues with proper nutrition and a failure of any one of these factors may cause anemia.

When an insufficient amount of food is eaten, the bone marrow maintains its nutrition and circulation long after the other tissues of the body show serious effects of starvation. The most common dietetic defect concerned in anemia is a lack of food containing the globulins

from which hemoglobin can be synthesized. Only an extremely restricted diet fails to provide enough iron and other inorganic salts to meet the daily requirements, for this amount is very small. But the requirements for globulin are considerable, and there is no provision made for preserving the globulins for further use. It will be remembered that the iron-containing moiety of hemoglobin is saved to be used again while the iron-free pigment is excreted from the body after some stay in the plasma. The globin part of the molecule is probably used as food for other tissues, but it is not known to be preserved as far as the formation of new blood is concerned. The supply of the globulins and other proteins in the food must be adequate and must be steadily maintained if anemia of the nutritional type is to be prevented.

Partial Starvation

The anemia due to a lack of proper food intake presents certain definite and usually recognizable peculiarities. The red cells are present in almost or quite normal number but the hemoglobin is considerably diminished. In fifty cases of partial starvation associated with reducing diets the red cell counts varied between 4,200,000 and 5,400,000 per cubic millimeter; hemoglobin between 40% and 86% of the normal for the age and sex of the patient; color index between 0.45 and 0.78. In all these cases the food intake had been kept extremely low and in all cases acidosis had been prevented by the use of very large amounts of citrus fruits, baking soda or magnesia. Weight reduction in these cases was severe, varying from sixty pounds in two months for a very fat man to five pounds in one month for a woman already emaciated. There was no known organic disease in any of these patients before the beginning of the weight-reduction, and in fifteen cases there had been a fairly careful physical examination with ordinary laboratory tests, before the special diets for the reduction of weight had been begun. In the other cases the patients themselves had carried on the diet with no professional advice. The changes in red cell count and hemoglobin given above are characteristic of anemia due to partial starvation.

In such cases the white cells show characteristic changes. The granular cells are diminished both absolutely and relatively. The leucocyte count varies between 3,000 and 4,500 in typical cases, with the neutrophiles between 40% and 55%. Small hyaline cells are relatively increased but the actual numbers remain almost or quite unchanged. The large hyaline cells show a slight increase both relatively and actu-

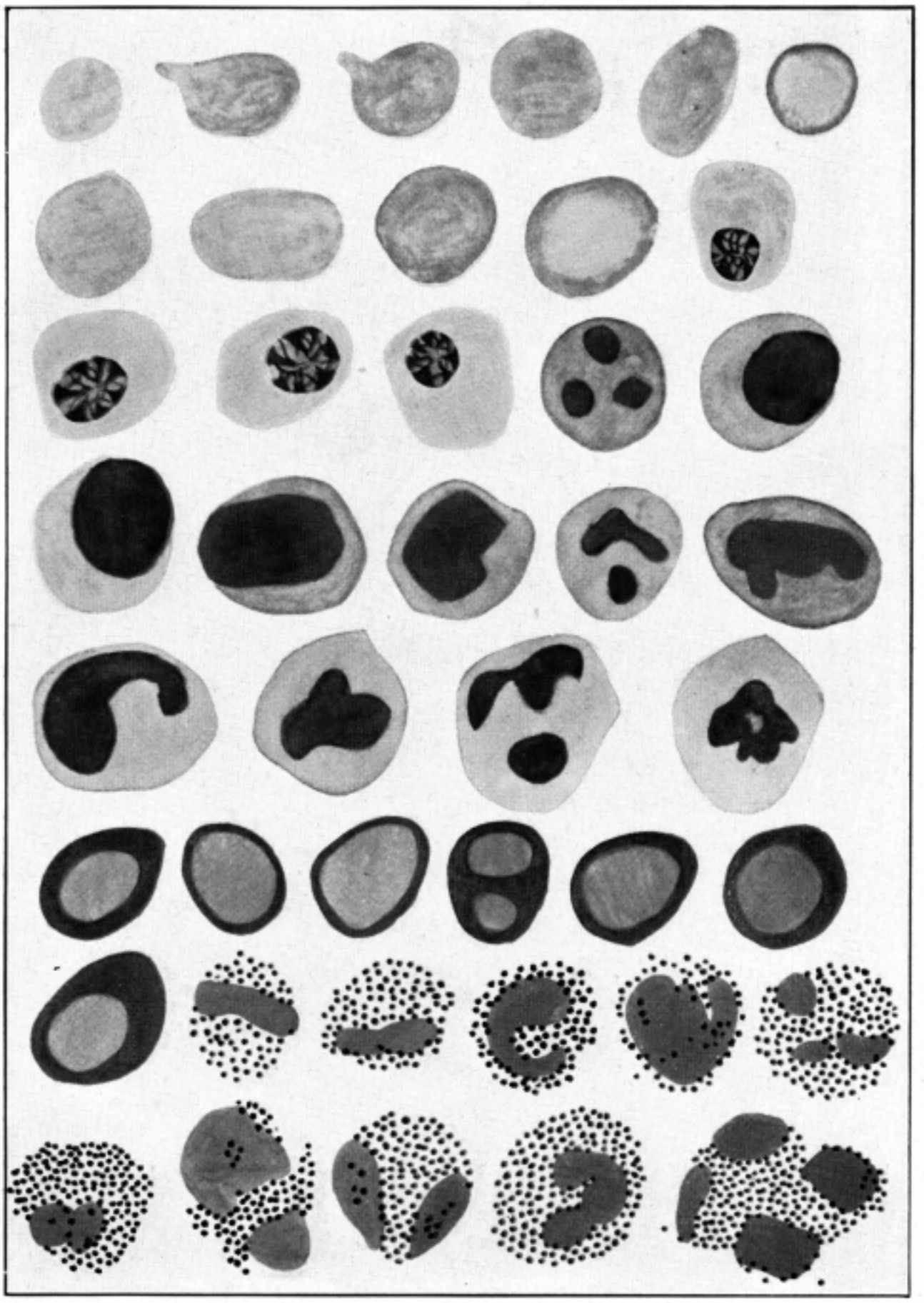


PLATE VIII

BLOOD CELLS IN SECONDARY ANEMIA

Patient suffered from effects of Fallopian abscess of long standing. Upper right corner contains normal red blood cell. Poikilocytes and normoblasts are present. Neutrophilic myelocytes indicate beginning exhaustion of the leucocytopoietic tissues. Hyaline cells show effects of toxemia. Eosinophiles include immature and myelocytoid forms, and also several fragmented cells with eccentric nuclei.

ally. The eosinophiles are unaffected actually in those cases in which the protein intake remains fairly adequate. If the protein intake is too low the patient is on a high meat diet (his own muscles) and the eosinophiles are somewhat increased. Myelocytoid and immature forms are present in moderate numbers. (Plate VIII)

If the special diet includes too small an amount of water intake the blood may be concentrated and the red cell count then exceeds the normal number while the hemoglobin remains at about the normal amount or is only slightly reduced. The white cells show an increase, sometimes to 12,000 per cubic millimeter. The lack of water is associated with toxic symptoms and there is neutrophilic leucocytosis. The hyaline cells may show a slight actual increase in this condition; they do not show relative increase when the toxemia is recognizable.

If the special reducing diet is based on scanty proteins and there is no provision for avoiding acidosis, the red cells are often above normal in number with very low hemoglobin. Three such cases are recorded. The diet included only one slice of thin, white toast without butter, three cups of water or weak tea with sugar but no milk or cream at each meal and an allowance of one ounce of hard candy during the day, to be eaten when desired. This diet was to be used for ten days, then the subject was allowed a day of food ad libitum. In each of these cases the red blood cells exceeded 5,000,000 with hemoglobin below 50% of the normal for the individual. Toxic symptoms and acidosis were marked. The granular cells were 1,400 per cubic millimeter and the hyaline cells above 4,000 per cubic millimeter in each case. In all three cases the water intake was insufficient for physiological requirements. The loss of weight was negligible in one case, and in the other cases was twenty pounds in two months for a fat woman, and five pounds in three months for a lady already rather thin. The fat lady became diabetic within six months; whether the starvation diet had any etiological significance is unknown.

Starvation

Absolute starvation and complete fasting affect the blood less severely. The toxemia associated with complete fasting causes a moderate neutrophilic leucocytosis. With complete starvation or complete fasting the subject utilizes his own body to meet physiological requirements. After a few days of using the glycogen of the liver and muscles and a few days of fat feeding the muscles begin to be used as food and the patient is on a high meat diet. The blood cells are about

normal in size, hemoglobin content and numbers. The white cells show the effects of toxemia, due to the high meat diet and the fact that the excretory organs are usually somewhat inactive under these conditions.

The leucocytes show markedly increased activity on the warm slide and they usually begin to die within fifteen minutes. The eosinophiles show especially rapid motion.

Fibrin is formed within two or three minutes during the time the muscles are being used as food and the threads are fine, short, of irregular contour and often arranged in radiating star-like groups or in an irregular net work of somewhat vaguely formed meshes.

Fibrinolysis is normal in about three-quarters of all cases until the wasting of muscles has become extreme. An undifferentiated proteolytic ferment is found in the blood of many patients during rapid wasting of muscle tissues, and this masks fibrinolysis because it digests fibrin as well as cells in less time than is required for normal fibrinolysis.

During the days of high fat feeding, in complete starvation, there are fine dust-like globules of fat in the plasma and the hyaline cells often contain small globules of fat. The fat globules are found in the blood after all evidence of heavy fat feeding disappear, and they are probably derived from the disintegrating muscles of the body. This is not certain, because even after death from starvation some fat remains in the body.

Effects of Incorrect But Abundant Diets

Diets proportionately too high in carbohydrates cause moderate secondary anemia with moderate neutrophilic leucopenia. The hyaline cells are relatively high and are often absolutely moderately increased. The blood plasma is paler than normal. Bile pigments are not present in these cases unless there is an associated disturbance in the circulation through the liver.

Diets proportionately too high in proteins show the effects of moderate toxemia of the type associated with abnormal protein katabolism. Neutrophilic leucocytosis is moderate, rarely exceeding 9,500 cells per cubic millimeter in typical cases. The neutrophiles show the effects characteristic of intestinal toxemia but the findings are less pronounced; basophiles and eosinophiles show moderate increase. Red cells and hemoglobin are normal or only slightly diminished. The color index is never very low and usually remains at about unity.

Diets proportionately too high in fats are usually associated with moderate secondary anemia with a low color index. These diets, when

habitual, are nearly always associated with some evidence of hepatic disorder and bile pigments are often found in the blood plasma. The white cells show marked evidences of the effects of cholemia.

Diets which include too high an amount of colored vegetables frequently cause carotinemia. Persons with renal disorders or with lesions of the eleventh and tenth thoracic vertebrae are especially apt to show this discoloration of the plasma with carotin. The white cells show the effects of moderate toxemia, usually of the fatigue type. The red cells and the hemoglobin are normal or only slightly decreased. The color index does not vary far from unity in typical cases.

Anemia Due to Digestive Disorders

Starvation may occur if an adequate and correct diet is provided but indigestion and absorption are inadequate.

In stenosis of the esophagus the food cannot be properly ingested. It sometimes occurs that sufficient food is swallowed but with such difficulty that digestion is disturbed and starvation is much more serious than can be accounted for on the basis of the actual food intake. Anemia is of the starvation type.

In gastric ulcer the quality of the blood changes depends upon the amount of hemorrhage. With hyperchlorhydria a moderate increase in the alkalinity of the blood is suggested by a slightly increased basophilia of the leucocytes, most noticeable in the hyaline cells. A well developed ulcer is associated with an increase in the mast cells and Turck cells are frequently found. The eosinophiles are slightly increased. Red cells may be diminished, rarely below 80% of the normal for the individual affected. Hemoglobin shows greater reduction, usually below 75% of the normal for the individual. The color index rarely exceeds 0.7 in cases without hemorrhage. Cases not associated with tendency to cancer show normal fibrinolysis and normal fibrin development on the warm slide. The leucocytes show normal activity and live well on the warm slide.

The occasional relation of cancer and ulcer is significant. Fibrinolysis is absent in about twenty-five per cent of all human beings, who thus lack at least one cancer-protective factor. In terms of the fibrinolysis hypothesis, these individuals would be especially prone to develope cancer at the site of an ulcer in the stomach. Surgical intervention should be considered when gastric ulcer is found in persons with absent or deficient fibrinnolysis.

Gastric cancer is associated with the blood changes noted in other forms of malignancy. If hemorrhage is considerable the blood often

assumes the characteristics of pernicious anemia. The red cells and the hemoglobin are reduced in approximately equal proportions in most cases. Gastric cancer shows a greater tendency to the development of undifferentiated proteolysis than is noted in other forms of cancer, and in quite early cases of cancer of the stomach the diagnosis may be suggested by the presence of this ferment in the blood. Eosinophiles are slightly increased and eosinophilic myelocytes are more abundant than in other forms of cancer. With invasion of the red bone marrow by the metastases from cancer of the stomach myelocytes increase steadily in numbers in the circulating blood. Cancer cells undergoing abnormal karyokinesis are found, though very rarely, in the blood of patients with wide-spread metastases in the bone marrow.

Fibrin is formed abundantly and speedily on the warm slide. The threads are long, heavy, irregular in contour or definitely beaded, often arranged in radiating or net-like masses and often containing highly refractile particles entangled in the fibrin threads.

Gastric atony due to lesions of the seventh thoracic or of adjacent vertebrae is often associated with abnormal hunger and thus with habitual overeating or overdrinking, or both. The passage of the food through the stomach is slow. Hypochlorhydria is a very common associated condition. Fermentation often occurs in the stomach. Proteins are not acted upon properly by gastric juice of subnormal acidity and intestinal putrefaction is thereby facilitated. Intestinal flora are affected; the putrefactive bacteria increase much more rapidly than do the fermentative bacteria. Various abnormal products of fermentation, putrefaction and imperfect digestion are absorbed into the blood stream.

Achlorhydria is often a congenital defect. In such cases the blood cells include a few immature or reversionary types. The evidences of nutritional anemia are then superimposed upon a developmental type of anemia.

If the food of these patients consists of excessive carbohydrates, the toxemia associated with the secondary anemia is characterized by low fibrinogen content of the plasma, delayed fibrin formation on the warm slide, increased eosinophilia of the blood cells, increased lymphocyte count, subnormal hemoglobin content with almost or quite normal red cell count and a low color index. The nuclei of the granular cells are less basophilic than in normal blood, the nuclei have frayed or ragged edges. The nuclei of all the leucocytes are somewhat swollen, show no pseudopod-like projections and their chromatin masses are indistinct.

If the diet includes an excess of protein foods the leucocytes show atony, the apparent diminution of alkalinity is still more marked.

Cholemia is common in such a condition. Fine, dust-like globules of fats are often found in the blood plasma and within the hyaline cells.

If the diet include an excess of protein foods the leucocytes show pronounced evidences of toxemia of the type associated with abnormal protein katabolism. Fibrin appears quickly on the warm slide and the threads are heavy, irregular in contour, often beaded, and often arranged in an irregular network of indistinct fibers. Refractile bodies are abundant and these usually include iodophilic, Sudanophilic and unstained particles. The nuclei are irregular in shape but not often frayed; projections of nuclear substance into the protoplasm, often resembling pseudopodia, are abundant in the nuclei of neutrophiles.

Intestinal Diseases

Abnormal conditions affecting the intestinal tract cause different conditions according to the nature of the disorder. Diarrheas cause blood changes due to starvation, together with the effects of the parasites, poisons or other causes of the diarrheas. Chronic constipation without change in the intestinal flora causes little or no change in the blood picture. If purgative drugs are taken for the relief of the constipation some absorption of toxic materials may occur and this produces the changes characteristic of chronic intestinal toxemia.

Chronic Intestinal Toxemia

This condition produces the blood changes characteristic both of nutritive and of toxic anemia. The nutritive factors depend upon the diet of the patient.

Intestinal toxemia is a form of poisoning which is often obscure and which causes a fairly characteristic blood picture. The symptoms are not definite; pallor, weakness, malaise, irregular periods of constipation and diarrhoea, bad breath, headache, visual disturbances, somnolence with restlessness most marked at night and some slowing of the reaction time are the most common manifestations of this form of poisoning. Blood pressure varies, pulse is usually slow, respiration irregular.

The absorption of the products of putrefaction is the essential feature of the disease. Bony lesions which cause weakness of the musculature of the intestines, modify their circulation and cause related secretory abnormalities include the eighth thoracic to the second lumbar vertebrae. Rigidity of this part of the vertebral column is always present and a definite lesion is always shown by careful palpation or by stereoscopic X-ray plates carefully studied. The ultimate result of the lesion is an atonic area of considerable extent involving some part of the intestine

according to the location of the lesion, with mild chronic congestion and disturbed secretory activity of the same area. The mucous secretion is increased through the affected area while the water and the enzymes are diminished. The alkalinity of the intestinal contents is increased. All these conditions tend to encourage the growth of putrefactive bacteria in the intestinal tract, and these bacteria break down the nitrogenous moiety of the food into substances poisonous to the human organism.

The effects of these conditions on the blood cells are characteristic but not altogether pathognomonic. The red marrow of the lesioned vertebrae and ribs is affected and the cells produced in this area include many immature and reversionary forms. The poisonous substances absorbed act on all types of blood cells in some degree. (Plates III, IV)

Small hyaline cells are always relatively and usually absolutely increased. When the lesion includes the ninth thoracic vertebra the increase is most marked. Atypical hyaline cells are present; these include cells which are larger or smaller than normal. Immature forms are variable and they seem to appear in the blood in showers. The lymphocyte nuclei are often lobed or indented and they may be double.

Large hyaline cells are increased. Their nuclei are often lobed, indented, double or polymorphic. Large lymphocytes and endothelial cells are especially abundant. Plasma cells are rare or absent.

Eosinophiles are usually increased slightly, both relatively and absolutely. If the abnormal intestinal content happens to include many protozoa or any of the vermes the eosinophilia may reach very high figures.

Neutrophiles show serious changes. They are diminished relatively and usually absolutely. Immature and reversionary forms are occasionally present. Neutrophiles affected by the toxic substances show the changes characteristic of abnormal protein katabolism generally. The nuclei vary widely in their affinity for basic stains. The nuclear average is very high, often exceeding 3.50. Budding processes resembling nuclear pseudopodia and aberrant small masses of nuclear substance in the protoplasm are present. Nuclear forms are bizarre. The protoplasm in the immediate vicinity of the nucleus is often clear, free from granules, without any staining reactions.

The granules have usually increased affinity for eosin, though very often a few granules are definitely basophilic. The intergranular hyaline protoplasm is rather more abundant and more definitely basophilic than normal. The cell outlines are indefinite and often ragged or frayed in appearance. Fragmented forms are abundant.

The basophilic granular cells are not increased in uncomplicated cases.

It will be noted that the findings just described resemble those present in carcinoma. This is to be expected since the poisonous factors are much alike. The changes are usually less pronounced in intestinal toxemia. The blood of the person with intestinal toxemia whose diet includes considerable amounts of meat and highly nuclear foods, such as sweet-breads, liver, kidneys and brains, shows cells which are very like those present in carcinoma.

The alkalinity of the blood is usually increased in cancer, and is usually normal or low in intestinal toxemia.

In intestinal toxemia an undifferentiated proteolytic enzyme may be present, but it is much less marked than in carcinoma, as a rule. In early carcinoma this enzyme may be present in a patient with high protein diet. In intestinal toxemia fibrinolysis may be normal; this has never been found true in carcinoma. Patients with intestinal toxemia may not have normal fibrinolysis.

In intestinal toxemia the fibrin formation is considerably delayed in most cases and is scanty in amount; in cancer the fibrin threads are speedily and abundantly formed. In both intestinal toxemia and in cancer the refractile bodies may be increased; beaded, radiate and net-like forms of fibrin threads may be present or may be abundant.

It must be remembered that intestinal toxemia and carcinoma may be present in the same person at the same time. Either condition exaggerates the cell variations due to the other sources of toxemia, since both produce similar effects.

In order to differentiate between the blood cell changes due to intestinal toxemia and those due to cancer or to some other form of abnormal protein katabolism, it is best to have the patient under observation for several days. During this time his diet must be chiefly cellulose foods, such as fruits and vegetables, and the intestinal tract must be as thoroughly cleansed as is practicable. Thus the absorption of toxic substances from the intestinal tract is avoided. Within a few days thereafter the blood cells show decided tendency to normal structures if the toxemia is of intestinal origin.

Hemorrhagic Anemias

Very great loss of blood is possible. Osler reported a patient with hematemesis who lost ten pounds of blood during one week, and who recovered from the effects of the hemorrhages. Ehrlich reported a case in which twenty kilograms of blood were lost during six and one-half months, with later recovery from the hemorrhages.

Blood taken immediately after severe hemorrhage shows little or no change. Within a few minutes the depleted blood vessels take up liquid from the tissues and the blood shows the low red cell count and hemoglobin count characteristic of simple dilution. At first the color index remains unchanged, but within a few hours increased numbers of immature or imperfect red blood cells are thrown into the circulation from the red bone marrow, and the low color index characteristic of secondary anemia is found. Normoblasts appear in the peripheral blood within an hour or two after the hemorrhage has occurred. Poikilocytes and microcytes appear more quickly. These are frequently derived from red cells originally of normal size and form, but distorted or fragmented as a result of the plasma changes.

Leucocytosis often occurs within an hour to a few hours after severe hemorrhage; this is of the neutrophilic form in ordinary cases. In children and in adults with deficient activity of the hematopoietic tissues lymphocytosis may occur after a hemorrhage.

Repeated losses of blood result in increased activity of the hematopoietic tissues. If the losses do not exceed the powers of these cells to replace the blood, no harm follows. The condition is that present during adult womanhood, during which time a loss of blood which may be quite considerable is replaced habitually, with no evidences of anemia. Professional donors of blood for transfusion show no evil effects from the losses. Even in cases of gastric ulcer, moderate hook-worm invasion, moderately severe cases of amebic dysentery and other hemorrhagic diseases there may be no recognizable anemia. But if the losses of blood are habitually beyond the regenerative ability of the bone marrow, anemia is inevitable.

Anemia due to hemorrhages alone, before exhaustion of the bone marrow, is characterized by a red cell count reduced slightly or not at all; hemoglobin considerably reduced; leucocyte count usually above normal with a normal or almost normal differential count; increased activity of the neutrophiles on the warm slide with markedly increased activity of the eosinophiles; scanty fibrin threads slowly formed on the warm slide; subnormal viscidity of the blood; the formation of a very soft clot within normal coagulation time and, after the condition has been present for some months, the presence of normoblasts and myelocytoid leucocytes in the circulating blood.

If the excessive loss of blood persists until the hematopoietic tissues become exhausted the blood shows the characteristics of aplastic anemia. No normoblasts or myelocytoid cells are present in the blood and the lymphocytes alone remain normal in character and in actual counts.

The lymphoid tissues do not become exhausted as a result of hemorrhage no matter how severe or how frequent the loss of blood may be.

Illustrative Case

Mrs. Q., patient in the clinic of The Pacific College of Osteopathy. Cervical polyp which extended into the vagina was the apparent cause of persistent uterine hemorrhage. Age of patient, 33 years. History included no factors of interest except that the polyp and hemorrhage had been present for about seventeen months before examination.

Hemoglobin 12%; 16 grams per liter

Red blood cells, 1,400,000

Leucocytes, 2,500, with normal differential count.

Polyp removed under local anesthesia. No adverse symptoms associated with the operation.

Five weeks later she returned for examination.

Hemoglobin 63%, 84 grams per liter.

Red blood cells, 3,800,000

Leucocytes, 6,600.

One year, three years and five years later she returned to report her condition. No abnormal conditions had occurred during these years, except that she suffered a broken ankle once, and several times contracted ordinary slight colds which yielded at once to osteopathic treatment. The blood remained normal during these years.

Developmental Anemias

Probably all secondary anemias for which really adequate cause is not found have a developmental anomaly of the hematopoietic tissues. Well-made people become anemic only under extremely severe conditions. The serious forms of developmental anemia are included with the primary anemias. The actual etiological agency in the developmental anemias lies in some abnormal conditions affecting the life of the patient during embroyological or fetal existence, or the germ cells from which the embryo was developed. Heredity is sometimes the important factor. The germ cells, originally normal, may have been affected by some systemic disease of the parent. The embryo or fetus is, of course, subject to many abnormal conditions affecting intrauterine life.

The most rapid development of the blood-forming tissues and the most rapid development of the central nervous system occur at about the same embryological period. This fact explains why persons with neuroses and psychoses associated with developmental imperfections of the nervous system usually show also some developmental anemia.

The most marked form of developmental anemia is found in the rare congenital aplastic anemia. In this form the hematopoietic tissues, congenitally inefficient, fail to react to even slight demands. At autopsy the bones show only scanty and very pale areas of red bone marrow. The blood is watery, scarcely tinted; both red cells and hemoglobin are very low and the total leucocyte count is below 1,000 with 90% to 99% lymphocytes.

Mild forms of developmental anemia are always found in persons who show other stigmata of degeneracy. The red blood cells and the hemoglobin are both diminished; the hemoglobin shows the most marked reduction. The red cells are smaller than normal, show considerable variation in size and in form and are more frequently basophilic than in normal blood. Nucleoids or other nuclear remnants are common. Normoblasts and microblasts are found in small numbers. The white cells are usually normal in number but include a high proportion of hyaline cells while the granular cells include many immature or atavistic forms. Amphophilic micromyelocytes are common. Atypical granules are frequently found in the protoplasm of hyaline cells and neutrophilic granular cells. Fibrin threads are scanty and of slow development. The clot is formed within a normal time but it is soft and does not retract normally. Platelets are usually below normal in number and they fail to agglutinate properly. These persons are often bleeders, and usually they suffer also from achlorhydria. (Plates V, VI, VIII)

The achlorhydria might be expected to increase the severity of the anemia, though in ordinary cases those patients with mild developmental anemia with achlorhydria or hypochlorhydria do not display any lower hemoglobin than do those with normal hydrochloric acid in the gastric juice. On the other hand, the lack of hydrochloric acid in the gastric juice of patients with typical pernicious anemia must be considered in this connection.

The prevention of developmental anemia in the next generation should be reasonably easy. If the youthful members of this generation receive adequate osteopathic treatment, and if they live reasonably hygienic and sensible lives, there should be few or none of these cases among their children, and probably none among their grand-children. Even now it is difficult to find cases of this kind in the children of families accustomed to osteopathic attention.

The treatment of the developmental anemias is necessarily rather unsatisfactory to those wishing complete restoration to normal conditions. Very pleasing results are, however, sometimes secured. It is possible only to provide the imperfectly developed hematopoietic tissues with the

conditions best adapted to their conditions. Structural relations as nearly normal as is practicable, excellent hygienic conditions and the avoidance of all excessive demands upon the blood forming tissues often lead to the development of blood which is good enough for all practical purposes and thus to symptomatic recovery from the anemia. These unfortunate individuals must always avoid infections, fatigue, malnutrition and any cause of toxemia with much more than ordinary care.

Toxic Anemias

Nearly all forms of secondary anemia are associated with at least some toxemia. There are many drugs used in the treatment of disease which ultimately cause anemia. Diseases of several different viscera are associated with marked anemia and toxemia. The poisons associated with fatigue, the absorption of degenerating proteins of the body itself or of exogenous materials all cause anemia, if the process is long continued. These varying conditions produce varying changes in the blood cells.

Drugs

The history of the patient with reference to the use of drugs is usually sufficient for diagnosis, if the facts can be discovered. The habitual user of drugs is often unaware of the serious nature of his addiction. A patient in the clinical laboratory of The A. T. Still Research Institute suffered from such a toxemia. He denied using drugs except "some small homeopathic doses once in a long while, for cold or toothache." But persistent questioning of the patient and, finally, of his wife, disclosed the fact that self-drugging had, in his case, reached an extreme of habitual poisoning.

The effects produced vary with the character of the drugs which are used. Habitual use of purgative drugs causes changes in the blood characteristic of chronic starvation. The use of thyroid extracts causes increase in the mast cells in the circulating blood. Nearly all of the coal tar derivatives and the salicylates cause a mild but persistent hemolysis with the blood changes of typical secondary anemia. Camphor and irritating fumes cause eosinophilia together with the usual characteristics of secondary anemia if the use of the drug is persistent.

Chronic lead poisoning causes secondary anemia which is often confused with pernicious anemia. The color index is near unity. Megalocytes, megaloblasts, basophilic stippling, low leucocyte count, high nuclear average, all are frequently found in chronic lead poisoning and may cause confusion in diagnosis if the history of the patient does not explain the findings. (Plate VII)

Lead poisoning causes loss of normal elasticity of the red cells with increased fragility on the warm slide. The cells are more resistant to changes in the osmotic tension of diluting fluids than are normal cells; this is probably due to the fact that the lead seems to affect only the periphery of the erythocytes. The blood is less viscid than normal and the cells lose their normal stickiness. The rouleaux are extremely scanty, and in many cases none are formed in an ordinary warm-slide preparation.

Fatigue

Fatigue toxins cause fairly constant changes in the blood cells. In chronic fatigue the usual indications of secondary anemia are present in the blood cells, together with indications of toxemia of a peculiar type. The red cells are softer and more fragile than normal. On the warm slide they are easily distorted by the pressure exerted by other cells carried along in the currents of plasma, and after such distortion they do not regain their original form for some seconds after the pressure is released. If the currents are rapid the cells frequently become fragmented, forming two to several very small masses which become rounded within a few seconds. These cells resemble microcytes, and in many instances cannot be distinguished from the microcytes found in the dried smears of blood. The hyaline cells vary in size, and include a few which are larger and a few which are smaller than those found in normal blood. Atypical granules are often found in these cells. Neutrophile nuclei are swollen and have ragged or frayed outlines, but pseudopod-like processes and aberrant nuclear masses are absent or rare. The neutrophilic granules include many which are larger than normal. Eosinophiles frequently show three or four nuclei, and these often present ragged or frayed outlines. Mast cells are increased slightly. Myelocytoid forms are present, though rarely in any considerable numbers. Differential staining is more difficult than in normal blood. In severe fatigue many masses of pale nuclear material without any recognizable cytoplasm may be found lying free in the plasma. Occasionally a few granules in the immediate vicinity of such nuclear masses indicate the type of cell which has been destroyed. More often there is no identifying structure. These nuclear masses become thinner and more irregular in outline, then less definitely basophilic, and finally seem to dissolve completely. Fibrin threads are scanty and are formed after ten to twenty minutes. Refractile particles are abundant; these include Sudanophilic and iodophilic granules. Rouleaux are formed slowly and are often imperfect. This blood picture is sufficiently distinct to differentiate fatigue from certain definite diseases. When fatigue com-

plicates some chronic disease the blood findings may be puzzling or even misleading. For this reason the patient with a chronic disorder who is weary should be allowed to rest for an adequate time before the blood is taken for examination. In acute diseases the effects of the fatigue toxins are usually masked by the effects of the acute disease.

Treatment of secondary anemia due to fatigue is difficult. There is very often a developmental basis for the anemia because normal people do not exert themselves to such an extent, except under extremely severe and unusual environmental circumstances. Normal people react to even serious fatigue and anemia is absent or temporary. Neurotic persons find rest difficult, especially after fatigue becomes profound. Persons with developmental anemia are often neurotic and often lack the self-control essential to rational alternations of work and rest. For these reasons treatment is difficult.

In order to secure recovery the treatment for ordinary forms of secondary anemia must be modified. Complete rest is sometimes best; in other cases a moderate activity is essential to good nutrition and good circulation of the blood through the red bone marrow. Various methods of elimination of the toxins may be helpful; these include adequate drinking of water, fruit juices or milk, according to the general condition of the patient; hydrotherapy is occasionally useful. The most important factor in these cases, as in other forms of secondary anemia, is the maintenance of a good circulation of the blood through the ribs and other fields of hematopoiesis.

Toxemias Due to Protein Degeneration

The products of putrefaction or degeneration affect the blood cells seriously. Malignant neoplasms, degenerating pyogenic foci, degenerating cystic masses, degenerating benign tumors, resolution and absorption in pneumonia and other circumstances characterized by the absorption of the products of abnormal protein disintegration cause the blood cells to show certain traits which are quite characteristic. Chronic intestinal toxemia has already been described.

The red cells may be affected slightly or seriously, according to the nature of the toxic substances. Many of these are definitely hemolytic, such as the products of certain malignancies, the poisonous substances produced by the activities of the hemolytic streptococci and the substances absorbed from the intestinal tract containing an excess of putrefactive bacteria acting on a diet rich in purins. In these cases the red cells are very pale, include a large number of poikilocytes and the count may be low or normal. The hemoglobin is always low. The hyaline

cells are usually normal in structure and are normal or only slightly increased in numbers. The eosinophiles are somewhat increased and they include many myelocytoid forms. The neutrophiles show nuclei which stain deeply, have definite and sharp outlines and which show peculiar processes which resemble the pseudopodia of the protoplasm of active cells. Very small masses of nuclear material lie in the cytoplasm, often near the periphery of the cell. The entire nucleus is occasionally very eccentric. The nuclear average is high, often reaching 3.5 or more.

Fibrin threads are abundant and are formed speedily. They are often irregular in outline and may be definitely beaded. They seem to radiate from groups of platelets. Often the fibrin threads are rather flattened and form an irregular mesh-like structure on the warm slide. Refractile granules are more abundant than normal; they rarely include any Sudanophilic or many iodophilic granules; unstained granules are very abundant.

THE POLYCYTHEMIAS

Cases characterized by abnormal increase in the red blood cells are much less common than are the anemias, but they do occasionally occur. The simplest form of polycythemia occurs as a reaction to some abnormal environmental condition, or is produced by some abnormal abstraction of water from the blood.

Erythrocytosis

Erythrocytosis is an abnormal increase in the number of red cells per cubic millimeter of blood, due to some physiological or pathogenic condition. Premature infants show polycythemia during the first few days of life, as do normally born babies.

Physiological erythrocytosis occurs in mild degree during the early afternoon and the early morning hours, usually about two o'clock in each case. At this time the red cells may be as much as 1,000,000 per cubic millimeter above the counts taken at about eight o'clock but usually the variation is less than 500,000 cells. The hemoglobin varies with the varying red cell count, so that the color index remains practically unaffected. In making successive counts for the same patient and in comparing counts made for different patients it is necessary to make due allowances for this source of variation. In our laboratories it is a custom to make as many of the counts at about two o'clock as is practicable, and when this cannot be done we make successive counts for the same patient at the same time of day. This early afternoon erythrocytosis was attributed to the changes due to the digestion of food, in earlier days, but it has been found that the rise occurs

whether the patient has eaten or not. Persons who do not eat during the day at all, persons on a long fast, and persons who eat ordinarily heavy meals at noon, habitually, all show about the same erythrocytosis during the early afternoon hours. Only when some person who has been fasting or has been eating very abstemiously eats a meal heavy in proteins is there a recognizable digestion erythrocytosis; this condition goes with the digestion leucocytosis in extent and, very probably, in cause.

Persons who ascend to high altitudes rather suddenly may show an increase in red cells sufficiently marked to be called erythrocytosis. In many such cases the blood cells include immature forms, and normoblasts may be abundant. Erythroblasts and megaloblasts are occasionally found, and this condition should be classed as pathological rather than physiological.

Many abnormal conditions may cause erythrocytoses just as certain abnormal conditions may cause leucocytoses. Heart disease causes erythrocytosis which may be extreme; in an osteopathic clinic a child with congenital mitral lesion had a red cell count of 8,500,000 cells. Other cases with counts of 9,000,000 cells have been reported for congenital heart cases. Disease of the mitral valve causes more constant and more marked erythrocytosis than do diseases of other valves. Adherent pericardium and chronic pericarditis without adhesions also cause erythrocytosis. Functional cardiac disorders cause less marked increase in the red cell count; rarely the count exceeds 6,500,000 in our records. The total blood volume remains unchanged. The functional cardiac inefficiency due to lesions of the fourth thoracic vertebra (less commonly the third or the fifth thoracic vertebra) causes an increase in the red cell count, rarely to more than 6,500,000. Cardiac weakness due to malnutrition or to toxemia also may increase the red cell count; in these conditions the hemoglobin is usually quite low and the color index may be 0.5 or less. Lowered oxygen tension in the tissues is one factor causing this erythrocytosis.

Chronic pulmonary diseases may cause puzzling increase in the red cell count. Especially those diseases characterized by severe attacks of coughing often show high red cell counts, and these cells may present a remarkably normal appearance. The act of coughing causes increased circulation through the red bone marrow of the ribs and no doubt this is one factor in the erythrocytosis present in certain cases of tuberculosis, chronic bronchitis and other pulmonary diseases. Diminished oxygen tension in the hematopoietic tissues leads to increased development of red cells.

Septic cases usually show some erythrocytosis during the acute stages. Pneumonia also shows erythrocytosis during the early days, and this may persist through the course of the disease and for some weeks after recovery. No doubt the stimulus to increased leucocytogenesis caused by pyogenic infections affects the erythrocytogenic areas of the bone marrow also. In chronic pyogenic infections, anemia and leucopenia are often present; this is due to exhaustion of the hematopoietic tissues. Occasionally this exhaustion terminates in anemia resembling the pernicious form; more often the terminal anemia is of the aplastic type.

The erythrocytopoietic areas of the bone marrow may be stimulated by certain poisons. Such stimulation may affect all the bone marrow areas, thus increasing both red and white cells, or may affect the erythrocytopoietic tissues alone, thus causing erythrocytosis but not leucocytosis. Medicines derived from coal-tar, such as acetanilid, cause erythrocytosis with later anemia; phosphorous poisoning causes an erythrocytosis which is somewhat more prolonged. Chronic mild carbon monoxide poisoning causes a mild erythrocytosis which may persist for several years; the count rarely exceeds 6,000,000 red cells per cubic centimeter. The color index remains at about one in the cases examined in our laboratories.

Vomiting, diarrhea, excessive sweating and other conditions which abstract water from the blood cause mild increase in the red cells which rarely rises to a degree warranting the term of erythrocytosis. The blood quickly takes up water from the tissues to replace the loss, except in cases of profound desiccation, and the erythrocytic rise is usually transitory. Profound desiccation such as follows long exposure to severe and very dry heat may cause erythrocytosis to 7,000,000 or more; this occurs in persons lost in the desert or in high mountains.

Erroneous diagnosis of erythrocytosis may be made if the technique of taking the blood is not accurate. If the area to be pricked is handled too much or is washed with any irritating substance so that the capillaries are dilated, the red cell count may be increased by as much as 2,000,000 cells per cubic millimeter. If the correct count is 5,000,000 or more, such an error in technique might easily lead to mistaken diagnosis of erythrocytosis or erythremia.

Erythremia

(Polycythema vera; Splenomegalic polycythemia; Vasquez's disease; Osler's disease; Gaisbock's disease).

This is a disease for which no adequate etiology is known. No definite bony lesions have been found with apparent etiological signi-

ficance. It is characterized by abnormally increased formation of red cells, just as leukemia is characterized by abnormally increased formation of white cells. The spleen is always somewhat enlarged and it may be as large in erythremia as in lymphatic leukemia, though it rarely approaches the enormous size occasionally found in splenomedullary leukemia. A lesion of the ninth thoracic vertebra is occasionally present, and the correction of this exercises a temporary effect upon the size of the spleen, but in many cases no such lesion is found, and the effect produced by its correction is never permanent.

The disease usually appears during middle life or early old age.

The symptoms are not pathognomonic. Cyanosis is the most common symptom and this may be severe; an incorrect diagnosis of cardiac or pulmonary disease is easily made if the blood is not examined. The mucous membranes and the skin may show a peculiar brilliant cherry-like tint. In one of our cases there was an odd bronze-like pallor which suggested disease of the adrenals. The systolic and the diastolic blood pressure may both be considerably increased, and the pulse pressure is usually slightly below normal. Gross or petechial hemorrhages of the mucous membranes are fairly common and the cyanosis is relieved when these occur. Therapeutic bleeding diminishes the symptoms temporarily and diminishes the danger of excessive spontaneous hemorrhages. Apoplexy frequently occurs and is a common cause of death.

Increased pressure of the cerebro-spinal fluid is a common condition in erythremia and this causes various symptoms of vertigo, tinnitus, headache and peculiar disturbances in the sense of time, personality and space-relations. These symptoms are all temporarily relieved by removal of a very few cubic centimeters of spinal fluid, and are usually relieved by the occurrence of a spontaneous hemorrhage or by therapeutic bleeding.

The blood picture is characteristic. In three cases examined in our laboratories the counts were 7,400,000, 7,700,000 and 8,200,000. Counts of 13,000,000 and more have been reported by Osler and others. Counts below 7,000,000 should not be included unless other findings are pathognomonic. The color index does not fall much below one, and is almost never above one. The hemoglobin varies from 120% to 150%, and in our cases was 150%, 135% and 130%, Dare.

Normoblasts and erythroblasts are common; megaloblasts are rare. Basophilic cells and polychromasia are common; Cabot's rings, Jolly bodies and other endoglobular structures are rare.

The increased activity of the hematopoietic tissues is usually but not always limited to the red cells. Associated leucocytosis is rare, and is always limited to the granular cells and myelocytes. Even when no leucocytosis is present a few myelocytes can be found on careful search. McAlpin reported a case of polycythemia rubra with a red cell count of 9,400,000 and hemoglobin of 128% in which the white cells rose to 96,000 before death. The spleen was extremely large in this case. The leukemia was of the spleno-medullary type.

Lymphocytes remain normal in actual numbers, though the percentages vary with the varying counts of granular cells.

Leucopenia occasionally occurs; this also is due only to diminished numbers of the granular cells. The actual number of the lymphocytes remains constant.

The total blood volume is always considerably increased. Fifty cases of polycythemia vera were studied by Rowntree and his associates at the Mayo Clinic. The erythrocyte counts in these cases were not excessively high, varying between 5,000,000 and 7,660,000 cells per cubic millimeter of blood. The hemoglobin varied between 171 grams and 290 grams per liter of blood. In all cases a polycythemic hypervolemia was present. The total blood volume varied between 121 and 246 cubic centimeters, and the plasma volume between 51 and 88 cubic centimeters per kilogram of body weight. (Rowntree's normals are 87.7 cubic centimeters of total blood volume and 51.2 cubic centimeters of plasma volume per kilogram of body weight.)

No treatment gives permanent relief. Bleeding relieves the symptoms but the effect is transitory. X-ray treatment of the skeleton diminishes the speed of red cell formation for a few weeks. Correction of lesions affecting the spleen give temporary relief, if such lesions are found on examination.

The course of the disease is like that of chronic lymphatic leukemia. Death occurs from apoplexy, hemorrhages, heart failure or some intercurrent disease.

CHAPTER IX

PARASITIC AND SPLENIC ANEMIAS

The anemias associated with certain abnormal conditions of the spleen and those due to parasites are not logically associated, except that in the latter group of anemias the spleen is often concerned in the pathology, and thus the final condition is partly parasitic and partly splenic in nature. This relation is particularly noticeable in malaria and in kala azar, though no cases of the latter disease have been studied in our laboratories.

The Splenic Anemias

Several related diseases are included in this group. Originally the term was applied to all anemias in which the spleen was enlarged but with further study many of these abnormal conditions have been explained by a recognition of definite etiological factors.

Several chronic infectious diseases cause enlargement of the spleen with some degree of anemia; tuberculosis, syphilis, malaria, kala-azar and hemolytic blastomycosis may be associated with splenic enlargement, anemia and no other prominent symptoms for long periods of time. In certain cases of cirrhosis of the liver, cardiac inefficiency and Hodgkins disease, enlargement of the spleen and anemia may be the most conspicuous symptoms for a time.

Splenic enlargement is conspicuous in several of the leukemias and anemia usually occurs after the leukemia has been present for a time.

The term splenic anemia should be limited to those cases in which anemia and splenomegaly are associated with normal or low white cell count, and with no other recognizable cause of either the anemia or the splenomegaly.

Effects of the Experimental Removal of the Spleen

The place of the spleen in the metabolism of blood cells has been studied by noting the effects of splenectomy in animals and by observing human beings with various abnormal conditions of the blood and of the spleen itself.

Splenectomy in normal laboratory mammals is followed by marked decrease in the red cell count, usually to about two-thirds the number normal to the animal. Leucocytosis to about three times the normal white count follows the operation within a few hours to a few days; this diminishes gradually within ten days or so to about twice the nor-

mal leucocyte count. The anemia and the leucocytosis gradually diminish and a normal count is usually present within two months in small animals, and within six months in large animals. Young animals show return to normal more quickly than do older animals. The reactions in man are complicated by the disease which was supposed to require splenectomy.

The red blood cells become more resistant to hemolytic agencies as a result of splenectomy. Urobilin in the urine is diminished, indicating that the red blood cells are less rapidly destroyed.

The Spleen and Immunity

There is diminished immunity to certain infections, in animals which have been splenectomized. This has not been definitely shown for human subjects but is probably true. Vertebral lesions affecting the circulation through the spleen and the liver are known to diminish immunity to the infectious diseases of childhood.

Treatment of both normal and abnormal human beings which cause increased rapidity of the blood flow through the liver, spleen and pancreas increase the opsonic index and the leucocytic index for tubercle bacilli (Whiting). After splenectomy hyperplasia of other tissues of the reticulo-endothelial system occurs.

The enlargement of the spleen which occurs during the course of nearly all acute infectious diseases suggests its functional value in reaction to infections. It should be remembered that this enlargement may sometimes be due to infection, abscess and other pathological conditions of the spleen itself, due to the disease or to some complication.

Spleen and Red Blood Cells

The place of the spleen in the control of the erythrocytic level of the blood is not yet well understood. Certainly the beneficial effects of splenectomy in splenic anemias with severe hemolysis suggests the possibility that an abnormal spleen may exert some abnormal inhibitory influence upon the erythrocytopoietic tissues. The increased resistance of the red blood cells to variations in osmotic tension and other test conditions after splenectomy is of interest in this connection. For this reason splenectomy was formerly employed as a therapeutic agent in pernicious anemia.

Abnormal conditions characterized by splenomegaly and anemia with no other etiological or other associated disease are rare, and they are usually of developmental origin.

BANTI'S DISEASE

This disease has no known etiology. It is characterized by progressive anemia, enlargement of the spleen, hemorrhagic tendencies and cirrhosis of the liver with marked ascites.

Enlargement of the spleen first occurs and this may antedate the anemia by months or even by several years. Rarely the spleen may become greatly enlarged; usually it is not more than two or three times its normal size.

Anemia is of the aplastic type with normal or low color index, few or no reticulated cells or normoblasts and neutrophilic leucopenia. The lymphocytes usually remain normal in actual numbers. Platelets show moderate reduction. The red cells do not show diminished resistance to hypotonic solutions.

Eighteen cases of Banti's disease studied by Rowntree usually showed moderate degrees of oligocythemic hypervolemia. The findings varied from 67 cubic centimeters to 112 cubic centimeters of whole blood, and from 50 cubic centimeters to 83 cubic centimeters of plasma per kilogram of body weight. After splenectomy the blood volume diminished, with an average decrease of 7.4% in the whole blood and 17.5% of the plasma.

Hemorrhages which are apparently spontaneous may occur from any of the mucous membranes. Hematemesis is especially common and this may lead to an incorrect diagnosis of gastric ulcer.

Portal cirrhosis occurs rather late in the disease, sometimes twelve years or more after the splenic enlargement is first noticed, and is usually associated with ascites. Jaundice is slight or absent. Cachexia develops rapidly after the cirrhosis occurs and death may be due to this or to severe hemorrhage from stomach or intestines.

At autopsy the spleen shows characteristic changes. The increase in size is partly due to the great amount of blood which it contains in its greatly dilated veins. The vasa brevia are enormously dilated. Great blood sinuses are found which connect the spleen with the stomach and the diaphragm. These increase the difficulty of splenectomy. Moderate hyperplasia of the bone marrow appears to be due to a reaction against the anemia.

Fibrosis of the Malpighian bodies and of the trabeculae are the most important microscopic changes in the spleen.

Splenectomy is the only adequate treatment and the results of this operation are often satisfactory. The mortality is higher than in many

other operative cases, and the percentage of deaths increases the longer the operation is postponed. After hepatic cirrhosis is recognizable splenectomy is of little value.

HEMOLYTIC JAUNDICE

In this disease there is a definite relationship between the enlarged spleen and the anemia, though the red blood cells are themselves of abnormal form and quality. Two types of the disease are generally recognized.

Familial hemolytic jaundice (Chauffard-Minkowski type) follows Mendel's law of heredity as a dominant type, though cases of the disease in which there seems to be no doubt of the diagnosis have been reported with no history of the disease in ancestors or in collateral relations. The acquired form (Hayem-Widal type) occurs during adolescence or later, and is somewhat milder in degree. No family history of the disease can be found.

The disease is characterized by splenomegaly, hematogenous jaundice, anemia and a greatly diminished resistance of the red blood cells to salt solutions. The splenomegaly may be slight or extreme, sometimes approaching the size of the enormous spleens sometimes found in leukemias. The jaundice exists in mild degree constantly but crises occur once to several times each year in which there is sharp increase in the size of the spleen with marked and sudden jaundice. The skin presents an orange or brownish tint rather than the green of ordinary obstructive jaundice. The serum, urine and stools contain greatly increased amounts of pigments. Sharp pains around the gall-bladder often suggest gall-stone colic. Gall-stones are frequently present in patients with hemolytic jaundice and symptoms due to the stones may cloud the diagnosis. The colicky pain may be very severe in cases with no gall-stones.

Anemia is not typically of the secondary type. The color index remains at about unity. The red cells are peculiarly globular in form so that they appear smaller and of deeper tint than normal. While normal blood withstands hemolysis in salt solutions as low as 0.44% or even less, these cells show hemolysis in salt solutions of 0.7% to 0.5%. The red cells are very fragile also on the warm slide. This peculiarity of the red blood cells is pathognomonic of the condition, and is not found in so marked a degree in any other disease.

Reticulocytes and normoblasts are more common than in ordinary secondary anemia of the same degree. Megaloblasts, megalocytes, microcytes, poikilocytes are all rarely found in typical cases.

The anemia is rarely severe. The red cells may be diminished to three millions per cubic millimeter but may be almost or quite normal in number at times. The hemoglobin varies with the red cell count.

During and for a few days after a crisis there may be a neutrophilic leucocytosis. At other times the leucocyte count is actually about normal but there is a moderate relative lymphemia. Platelets remain normal during the disease.

The disease is rarely fatal and life seems hardly to be shortened by it. The crises occur one to several times a year, and they may last a few days to two or three weeks. During this time the patient is acutely ill with malaise, headache, fever, vomiting and sometimes sharp pains resembling gall-stone colic. The jaundice may be mild or very severe. There is no itching such as commonly accompanies obstructive jaundice. Recovery from the attack is slow. After the attack is over many patients seem to be in excellent health. Others are jaundiced, weak, anemic and subject to gall-bladder discomfort nearly all of the time.

The accepted treatment is splenectomy in the familial cases and in those acquired cases for which no pre-disposing other disease can be found. The results of this operation are usually excellent in the familial cases, and are occasionally good in the idiopathic acquired cases. The anemia, jaundice and crises usually cease at once and the patient rapidly becomes well and remains so. The red cells are always fragile and of spherical form, but they remain whole in the circulation and carry oxygen adequately. It is usually best to remove gall-stones at the same operation. The surgical work should be done during an intermission, not during a crisis.

The treatment of those acquired cases in which some other disease seems to have a predisposing influence is that of the predisposing condition. Such diseases include malaria, tuberculosis, sepsis, dysentery, hookworm and other parasitic infections, syphilis, carcinoma and several others. Cholelithiasis has been considered predisposing, but it must be often true that the jaundice antedated the cholelithiasis. These diseases are so common while hemolytic jaundice is so rare that some predisposing factor, probably developmental, must be present in all, or nearly all, of the so-called acquired cases.

The acquired cases are much more serious, as a rule, than the congenital. The crises are more acute and more frequent; the intermissions may not occur at all, and remissions be characterized by more or less severe symptoms. The anemia is more severe; sometimes the red cells reach less than one million red cells per cubic millimeter.

The jaundice is less marked and resistance of the red cells to hypotonic salt solutions is more nearly normal than is the case in familial jaundice. The acquired form is not transmitted to the children.

At autopsy, in both acquired and congenital cases, the spleen shows hyperplasia and thickening of the capsule, and it is greatly engorged with blood. The endothelial cells of the spleen contain great numbers of red blood cells. The Kupffer cells of the liver show marked siderosis. Both the erythroblastic and the leucoblastic areas of the red bone marrow show abundant hyperplasia. The gall-bladder often contains stones and its walls are thickened. The kidneys usually show some nephropathy and their cells show siderosis in many cases.

VON JAKSCH'S ANEMIA

This anemic leukemia of infants (anemia pseudoleukemica infantum) is probably not a distinct disease. There seems to be some developmental basis for the inability of these babies to react to infectious processes in a normal manner. This disease follows or is associated with some severe nutritional or infectious disease and is characterized by anemia of increasing severity, splenomegaly and myeloid leucocytosis, together with the symptoms of the underlying disease. It is possible that symptoms of severe malnutriton ordinarily supposed to be the cause of the anemia, may really be the earlier smptoms of the disease itself, as an entity due to developmental defect in the spleen and red bone marrow. That some developmental defect is an essential factor in etiology is suggested by the remarkably abundant myelocytes with relatively scanty leucocytosis, by the great diversity and the prevalance of the diseases considered to be essential predisposing factors and by the rarity of Von Jaksch's anemia among sick babies. The fact that several children in the same family may have this rare disease also suggests a developmental fault. The autopsy findings suggest a developmental basis for the peculiar blood and splenic changes.

The red cells are greatly reduced, sometimes to less than half a million per cubic millimeter. Reticulocytes, normoblasts and various nuclear remnants within red cells are rather abundant; megaloblasts are rare. The blood picture may resemble that of pernicious anemia, which is, however, extremely rare in young children. The hemoglobin is considerably reduced and the color index is usually below 0.8. The leucocyte count may reach 50,000 or even 150,000 per cubic millimeter. Neutrophiles and neutrophilic and eosinophilic myelocytes predominate; hyaline cells include hyaline myelocytes and these may dominate the blood picture, thus suggesting lymphoid leukemia.

The spleen is enlarged, sometimes slightly, more often very considerably. The liver shows some enlargement.

The disease has a prolonged course. The predisposing disease passes into the anemic phase gradually. The splenic enlargement may be the first indication that anything more serious than delayed recovery from the underlying illness is present. Pallor becomes more marked, weakness and prostration increase, and the child often bleeds at the nose, or coughs or vomits blood, or blood may be found in the stools. Unless the nutritive condition improves, the child may die of inanition within a few months. There is a tendency for improvement if conditions permit. No doubt with the development of the child the persistent embryological relations tend to diminish and disappear. About fourth-fifths of all cases recover within a year or two at most.

Treatment is devoted to the underlying disease, plus an attempt to secure improved nutrition and better muscular tone. Lesions causing atony of the muscles of the splenic capsule have been reported in several cases under osteopathic treatment, and the course of the disease seems to be shortened by the correction of these lesions.

At autopsy the spleen is found enlarged and great areas of erythrocytopoietic and leucocytopoietic areas are found. The red bone marrow is hyperplastic and may intrude upon the yellow bone marrow quite extensively. The liver and the lymph nodes may also contain large areas of hematopoietic tissue.

GAUCHER'S DISEASE

This is a disease of childhood, probably due to some developmental error, characterized by enormous splenomegaly, peculiar graying or bronzing of the skin, hemorrhagic tendencies and moderate anemia. The disease occasionally occurs in several members of the same family but it is not directly inherited, for obvious reasons.

The enlargement of the spleen is greater than in any other disease. The enlargement of the liver seems to be secondary to the splenomegaly. The anemia is not severe until late in the disease. The bronzing of the skin often suggests Addison's disease. There is often pain in the bones and this may be extremely severe and persistent; it may be due to hemorrhages but is probably due to the pressure exerted by the tumor-like masses of cells within the marrow. Hemorrhages are rarely severe. Bruising of the skin occurs upon slight provocation; hematemesis and epistaxis occasionally may be quite severe. The disease has a slow progress but is inevitably fatal.

At autopsy the spleen is found to be firmer than normal, with grayish, yellowish, whitish and brownish mottling. The whitish areas are composed of great masses of endothelial cells, twenty to forty microns in diameter, often arranged in alveoli but sometimes showing no definite arrangement at all. The protoplasm of these cells is crowded with vacuoles filled with some lipoid-like substance, probably a cerebroside. This trait gives them their name "foam cells." The Malpighian bodies of the spleen show atrophy apparently due to the pressure of these cells. The brownish and yellowish areas of the spleen show hemorrhages in various stages of absorption. Cysts due to the degeneration of hemorrhagic areas are common.

The liver, bone marrow and occasionally the lymph nodes show masses of these cells. They are occasionally found in the peripheral blood. At autopsy the developmental basis of the disease is strongly suggested by the finding of other developmental anomalies. Horseshoe kidney, cystic ovaries, uterine malpositions, cystic kidneys and various other developmental abnormalities are very common autopsy findings in these cases.

The nature of the disease precludes any successful therapy. Splenectomy seems to give some relief and to prolong life in some cases. In other cases splenectomy has been followed by rapid increase in the size of the liver and speedy death.

Neimann's Disease

This resembles Gaucher's disease somewhat. The two may be simply different types of the same disease. In Niemann's disease the foam cells are filled with a substance which gives the reactions for fats and the blood serum is definitely turbid from the presence of fat-like globules. The viscera, thymus, lymph nodes, bone marrow and sometimes the connective tissues show a peculiarly brilliant yellow color. There is no satisfactory treatment and death is inevitable from the nature of the conditions present. The child rarely lives to be more than two years old, or more than three months after the first symptoms are noted.

PARASITIC ANEMIAS

Anemia due to parasites varies according to the location and the nature of the agents. Cats and dogs with abundant fleas suffer a severe anemia which cannot be differentiated from the anemia due to starvation, except that the eosinophiles are considerably increased. Human beings afflicted with lice and other parasites upon the skin also show secondary anemia of the starvation type, plus moderate eosinophilia.

Parasites within the intestinal tract are generally associated with blood showing the typical picture of starvation anemia, plus eosinophilia which may be slight or extremely marked. The hookworm and certain other intestinal parasites cause slight but chronic intestinal hemorrhages; in such cases the anemia is of the hemorrhagic type. Several flagellate unicellular organisms may infest the intestinal tract and these frequently cause an anemia which is definitely of the pernicious type. Bothriocephalus latus is a tapeworm, fortunately rare in this country, which causes a condition resembling pernicious anemia in almost every respect.

Trichina infection causes secondary anemia which is rarely severe. Eosinophilia is usually extreme.

Parasites of the blood itself include several very different forms, and these diseases of the blood require especial attention.

BLASTOMYCOTIC ANEMIA

(Blastomyces hemolytica)

This form of anemia was first described for patients in the clinic of The Pacific College of Osteopathy. The organism was found later in patients studied in the laboratories of The A. T. Still Research Institute in Chicago and in Los Angeles.

Infection by blastomycetes has been reported many times in medical literature. In every case so reported the disease was fatal. The organisms vary somewhat, and all those previously reported have been almost or quite as large as ordinary yeast and have been pyogenic. The blastomycotic forms which produce this chronic anemia are not pyogenic and are not directly fatal. This organism (blastomyces hemolytica) is very much smaller than ordinary yeasts. Both large forms and small forms of yeasts have been found in several malignant neoplasms and have been reported as having etiological value by several authors. They may, possibly, be concerned in producing the irritative influences which have some etiological value in certain forms of sarcoma and carcinoma but there is no reason, at this time, for supposing them important in the etiology of any kind of neoplasm.

Blastomyces hemolytica can be isolated from the blood of persons infected and from scrapings from the tumor-like masses and the dry sores which are characteristic of the disease. Cultures made from these materials are of very slow growth and require from ten to forty days to become visible. They are facultative anerobes. The best culture media include boullion agar mixed with defibrinated blood; various gelatine

preparations mixed with blood or with ascitic fluid, and defibrinated human blood alone. Culture media with pH of about 6.5 give better growth than those with pH of 7 or higher.

Cultures are easily made of the organism in defibrinated blood alone; in these cultures the organisms attack the erythrocytes. Both the organism and the injured erythocytes are phagocytized by the large hyaline cells and the neutrophiles in much the same manner as occurs in the circulating blood of the infected person. (Plate IX)

Guinea pigs inoculated with the blastomyces hemolytica show the symptoms characteristic of the infection in man and cultures from their blood show the same characteristics as is the case with cultures made from human material. These cultures produce the same symptoms in other guinea pigs inoculated with them.

Symptoms

The most pathognomonic symptom is the dry sore upon the skin. One or many such sores may be found. Occasionally the sores disappear in which case it may be difficult to secure a history of the initial skin lesion. The sore is characteristic. Usually a small lump is first noted just below the skin. This increases in size and the skin becomes eroded over the tumor. A dry scab forms, this drops off, only to be followed by another scab. These may be successively larger until an area of an inch or so in diameter may be concerned in the lesion. Rarely the sore exceeds an inch before the scabs begin to become successively smaller and finally the skin is healed over the area leaving no discoloraton; if no pyogenic infection occurs there is no scar. Scrapings made from the tissues beneath the scab or from the tumor before the skin has become eroded show the characteristic yeast-like organism. Usually there is a secondary infection with staphylococci or other organisms after erosion occurs. The tumor-like mass beneath the skin contains only the blastomycotic organisms.

The anemia is of the secondary type generally, except that on examination of many smears the organism can be found within the red blood cells, the large hyaline and, occasionally, the neutrophilic cells. It is not easily recognized free in the plasma because of its small size and because it is often associated with the platelets in rather large groups.

Fragments of red cells of characteristic forms may be found, and these should suggest the disease. The rim of the erythrocyte with its inner edge eroded in such a manner as to leave a scalloped outline is a very common finding in these cases. Cultures of blood showing these peculiar fragments may contain the parasite even though it may not be

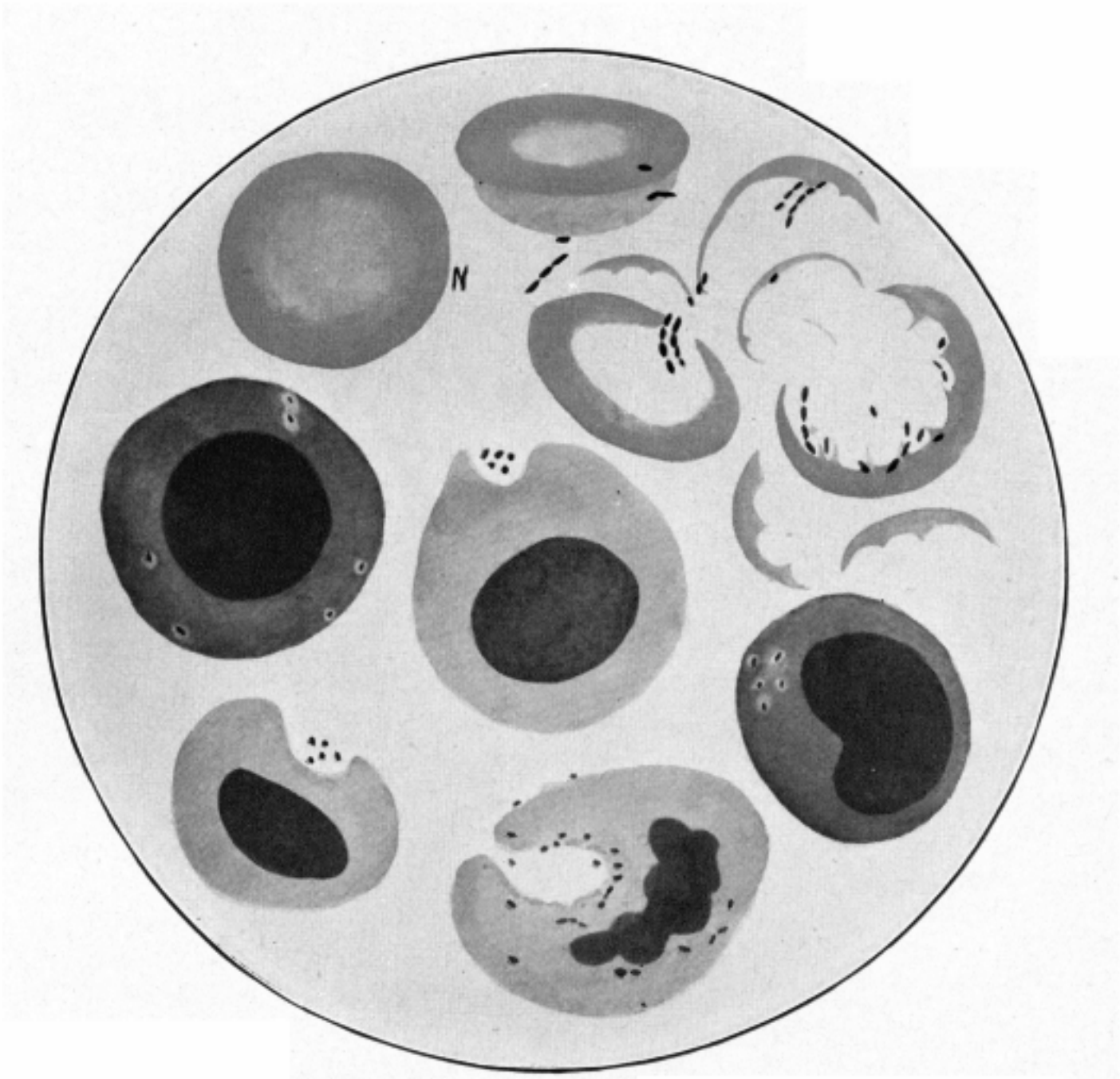


PLATE IX

BLASTOMYCES HEMOLYTICA

Blood cells scraped from a tumor beneath the skin of a human subject. N, a normal erythrocyte. Just above and to the right is an erythrocyte showing the normal bowl-like outline of living red cells. Several of the yeast-like organisms lie upon this cell. Other red cells show different stages of destruction by the parasite, and the peculiar, scalloped, semilunar remnants of the invaded erythrocytes.

Two large hyaline cells are beginning to ingest blastomyces; two have ingested parasites and are digesting them; one is losing the battle and seems to be about to die from the evil effects of invaders.

recognizable in blood smears. Inoculation of these cultures into guinea pigs produce typical symptoms of blastomycosis, and the autopsy findings are characteristic. The pigs must be killed; they rarely die as a result of inoculation of the blastomycetes alone.

The lumps cause discomfort and dull or acute aching which varies according to the nerves of the area affected. They may appear anywhere, with no regard to the exposure of the skin to light or to irritation by clothing, or to the thickness of the skin; there is no difference between flexor and extensor surfaces or between the skin of the trunk and of the extremities.

The lungs are occasionally infected, in which case the symptoms resemble those of pulmonary tuberculosis. Rales are usually more marked than in tuberculosis. The temperature curve is erratic and shows greater variations than is the case in tuberculosis. Night sweats are usually very severe. The euphoria characteristic of tuberculosis of the lungs is replaced, in blastomycosis, with a gloomy tendency which may approach actual melancholia. The organism can be cultured from the sputum. Inoculated guinea pigs show typical pathology.

Weakness, pallor, muscular atony, anorexia, nervous and emotional instability and motor restlessness suggesting hyperthyroidism are common symptoms. Basal metabolism is not affected, however.

Prognosis

The infection has not, so far as has been reported, been the direct cause of death. The anemia and the pulmonary infection lower the resistance to other etiological factors and thus are predisposing factors.

Mode of Infection

The manner in which the infectious agent gains entrance into the body is not known. In some study of yeasts used as foods in 1914-1915 in Chicago, similar organisms were found in beer and in commercial yeasts used for baking. Animals inoculated with these organisms did not show any symptoms of the dry sores nor of anemia, and cultures made from their blood did not grow. The tests were not completed and further work remains to be done before the organism can be described in detail and its origin definitely determined.

Treatment

Since the organism grows best on faintly acid media, increase of the alkalinity of the blood and the tissue juices of the patient is indicated. This is best done by means of diets including foods with alkaline ash

and by avoiding an excess of those with acid ash. The circulation through the red bone marrow must be kept normal because the development of red cells to take the place of those destroyed by the blastomycetes must be encouraged. The food must provide a normal amount of iron and other minerals in the form of hemoglobin, myohematin and cholorophyll-containing vegetables for the same reason. Since the lack of oxidation results in the formation of sub-alkaline or acid katabolites the patient must have good air and must breathe properly. No method of destroying the organism directly without injury to the patient has been found.

The scabs may be scrubbed off from the sores and the bleeding area washed with alkaline lotions. Weak carbolic acid lotions are often soothing and may exert some antiseptic influence. Any comfortable applications may be used.

Typical Cases

Mrs. H. History of peculiar dry sores following small lumps which appear beneath the skin. Occasionally blisters occur instead of dry sores. These become purulent within a few days. This patient lived in a distant city. Hemoglobin reported as being 68%; no other blood examinations made. Smears were taken from the pus, blood and serum from a non-purulent blister and were sent, under aseptic conditions, to the laboratory of the Institute. Cultures were made and a growth of blastomyces hemolytica occurred. The cultures were used for several experiments.

Cultures from pus were added to normal human blood which had been defibrinated by beating and the mixture incubated for thirty minutes at 38 C. Smears were then made and examined. The erythrocytes showed invasion by the blastomyces. The large hyaline cells had phagocytosed both the parasite and fragments of erythrocytes. A few neutrophiles also phagocytosed a few of the parasites and had ingested the erythrocyte fragments abundantly. Cultures from the blood of Mrs. H. were added to normal human blood which had been defibrinated, and the mixture was incubated for 24 hours at 38 C. The erythrocytes were abundantly invaded and the large hyaline cells had ingested many of the organisms; the neutrophiles had ingested the fragments of red cells abundantly, and also a few of the parasites.

These tests were repeated for different temperatures and for different period of incubation.

Normal human blood in normal salt solution was mixed with a culture of the blastomyces and a hanging drop preparation was watched

for several hours. The yeast cell adhered to the erythrocyte and as it grew the substance of the red cell disappeared very slowly in the immediate vicinity. The yeast cell then grew into the red cell and began to divide.

Defibrinated blood from a normal guinea pig did not provide a good culture medium. The cultures from the blood of Mrs. H. were mixed with defibrinated guinea pig blood in various proportions and the mixtures incubated at several temperatures. The yeast attacked the red blood cells of the guinea pig but the leucocytes did not ingest the organism. The yeasts did not multiply in guinea pig blood during three weeks incubation.

Inoculation of the ear of a guinea pig left a small mass which slowly diminished in size and finally disappeared, leaving the pig apparently uninjured by the infection. The pig was kept under observation for four weeks and no symptoms appeared. Observation was then neglected although the pig was still kept isolated. Ten days later the pig died. At autopsy a dry sore was found at the site of the inoculation. The body was emaciated and the lungs were congested. No hepatization was present. Smears from the sore on the ear, from the lungs and from the blood showed the blastomyces present in each case. Pneumococci were also found abundantly in the smears from the lungs, so the blastomyces was almost certainly not the direct cause of death. No other guinea pig in the place had pneumonia.

Mrs. B. History of dry sores following subcutaneous tumors of very small size. She complained also of weakness. Anemia was not present in her case. The coagulation time was diminished to 2½ minutes (normal for our method, five to eight minutes). Red cells, 4,996,000 per cubic millimeter. Hemoglobin 98% (Dare). Leucocytes, 8,300 per cubic millimeter with differential count as follows:

Large hyaline cells	1.9%	158 per cu.mm.
Small hyaline	38.2%	3170 per cu.mm.
Mononuclear neutrophiles	.6%	50 per cu.mm.
Polymorphonuclear neutrophiles	55.2%	4582 per cu.mm.
Eosinophiles	2.8%	232 per cu.mm.
Basophiles	.8%	66 per cu.mm.
Amphophiles	.5%	42 per cu.mm.

Some organism, nature not certain, present within the red cells and within a few of the large hyaline cells. Cultures were made of the blood and these were used for further tests. (Plate IX)

On physical examination considerable edema was found; the skin was definitely purplish in tint, and a cardiac murmur indicated a mitral lesion. Sphygmogram verified this diagnosis.

No history of syphilitic infection was secured. Patient is a widow with four children, all normal, no history of miscarriages or of still-births. Patient is janitress and works ten hours each night in an office building.

Cultures made from blood of Mrs. B. were added to normal blood which had been defibrinated, and the findings already described in the case of Mrs. H. were repeated.

Guinea pig inoculated in right ear. Four weeks later a dry sore appeared at site of inoculation. Pig became thin and anemic; was killed by ether anesthesia five weeks after inoculation. Smears from the lungs, liver, blood and spleen showed the characteristic organism. Smears from the spinal fluid, peritoneal fluid, pericardial fluid and from the brain did not show any of the parasites.

Four puppies inoculated in the right ear with the cultures from the blood of Mrs. B. Three showed no symptoms whatever though they were kept under observation for three months. The fourth puppy developed the typical dry sore at the site of the inoculation, became thin and pale, and was killed by ether two months after the inoculation. The intestines were full of small round worms and this no doubt was partly the cause of the emaciation and anemia. Blastomyces hemolytica was abundant in the smears from the lung and the blood. Cultures made from these cultures developed the usual symptoms in guinea pigs.

Note. In the case of Mrs. B. the concentration of the blood associated with the heart lesion probably masked the anemia.

Mr. S. History of progressive weakness, emaciation and pallor. Occasional small dry sores reported. Blood examination made in another laboratory reported as follows:

Hemoglobin	72%
Red cells	3,800,000
Leucocytes	9,400

Cultures were made from one of the small lumps which he reported as antedating each small scab-like sore. The characteristic blastomyces invading red cells appeared in the smears and the culture growth was characteristic. Inoculations into guinea pigs produced the typical symptoms but not the death of the pig, which was finally killed at the end of the fifth month, with ether. Smears from the lungs, blood and

spleen showed blastomyces. Cultures from these tissues produced similar symptoms in other pigs. Smears and cultures from the brain, peritoneal fluid, kidneys, cerebro-spinal fluid and gall-bladder were all negative.

HISTOPLASMOSIS

Histoplasma capsulatum is a parasite somewhat resembling a certain stage in the development of the Leishman-Donovan bodies of kala azar, and producing a disease called histoplasmosis. This parasite was first described in the Isthmus of Panama by Samuel Darling, in 1906. Since that time several cases have been described in the southern part of Mexico and Yucatan; one case was described in Wisconsin, in a patient who never had been in any other state or country. We had one case studied in the laboratory of The A. T. Still Research Institute in which the diagnosis seemed definite and one case in which the diagnosis was probable but in which no careful study was possible. Both these patients probably contracted the disease in Mexico.

The symptoms include those due to cirrhosis of the liver, splenomegaly, invasion of the endothelial cells of the intima of the blood vessels and of the epithelial cells of the intestines and the lungs. Fever is occasional and may be very severe. Thrombosis produces nervous symptoms. Invasion of the endocardium is common.

The parasite does not attack the red blood cells directly. It is engulfed by the hyaline cells of the blood but is not ingested by the neutrophiles. It is occasionally found free in the blood plasma.

In one of our cases the symptoms included feverish attacks, hepatic symptoms resembling those of cirrhosis, painless splenomegaly of moderate degree, and a peculiar paralysis which seemed to be due to thrombosis. The parasite was found in the large hyaline cells of the blood, but no further study could be made of the case. The man is still alive after three years. In another case some further study was made, but no autopsy was permitted.

Illustrative Case

H 13 age twelve years. At first examination was apparently very ill, with constant fever varying between 99° F, and 102° F. Spleen and liver much enlarged and painful on pressure. The spinal tissues between the fifth thoracic and the third lumbar segments were tense and hypersensitive. Cardiac sounds indicated valvular lesions involving the mitral valve and at least one other valve, probably the tricuspid. Pulse varied between 102 and 120, respirations irregular, from 78 to 32 per minute.

History of her childhood was uneventful, except as follows. Her parents took her to Mexico when she was three years old, and she lived there for eighteen months. She was then taken to Chicago. She was never robust but did not seem definitely ill. At the age of seven years she had measles and this was followed by attack which was thought to be acute articular rheumatism of atypical form. This left her with a valvular heart lesion. (Instead of having acute articular rheumatism of atypical form she may have suffered from histoplasmosis at that time). No careful study was made of the condition. During the next three years she improved in general health and the cardiac symptoms diminished in severity.

This last attack began a few weeks before she was first examined. There was first increased severity of the cardiac symptoms followed by increasing fever, increasing size of the abdomen and severe headaches. Blood and urine examinations were made soon after the first physical examination, with results as follows:

Blood examination.
Hemoglobin 70 grams per liter, 54% of normal for age.
Erythrocytes 3,870,000 per cubic centimeter, 77% of normal for age.
Color index 0.7.
Poikilocytes, microcytes, normoblasts present.
Leucocytes 12,000 per cubic millimeter

Large hyaline	24.2%	2904 per c.mm.
Small hyaline	38.2%	4584 per c.mm.
Mononuclear neutrophiles	2.4%	288 per c.mm.
Polymorphonuclear neutrophiles	32.8%	3936 per c.mm.
Eosinophiles	1.2%	144 per c.mm.
Basophiles	.2%	24 per c.mm.
Myelocytes	1.0%	120 per c.mm.

Myelocytes included hyaline, neutrophilic and eosinophilic forms.
Neutrophile nuclear average 2.12
Malarial parasites, none

A parasite which most nearly resembles the histoplasma capsulatum of Darling is present within the splenocytes and the large hyaline cells of the blood. (Plate X)

Uranalysis
Chemical
Total amount in 24 hours, 800 cubic centimeters
Specific gravity 1016
Total solids 31.3 grams

Acidity percent	72
Urea	14.4 grams
Phosphates	2.5 grams
Chlorides	12.0 grams
Sulphates	0.7 gram
Uric acid	.3 gram

Indican, slightly increased
Albumin, trace
Globulin, trace
Sugar, none
Bile pigments, trace
Bile salts, faint trace
Other tests, negative
Microscopical
Kidney cells, few
Casts, hyaline and granular, present
Pus cells, few
Leucocytes, few
Erythrocytes, few
Calcium oxalate crystals, present
Amorphous urates, none
Other crystals, none
Bacteria, none in fresh specimen

A few parasitic organisms like those found in the blood are present within the renal epithelium and within certain large hyaline cells apparently derived from the blood.

Inoculation of Urine Into Guinea Pig

Late in the afternoon of May 4, three drops of urinary sediment were injected into the peritoneal cavity of two guinea pigs and two drops were injected into the ear veins of the same animals. No ill effects followed until the morning of May 9, when it was noted that the pigs were inactive. Both were feverish with rapid pulse and respiration. Both died during the afternoon of that day and were examined at once. The findings were identical for both.

All viscera were intensely congested and hemorrhagic areas were abundant. Smears were made of the blood, urine, and saliva. Smear preparations were made from the lungs, pericardial sac, endocardium, peritoneum, spleen, pancreas, liver, kidneys, adrenals and thymus.

Smears from the saliva and the pancreas did not show the organisms. All other smears showed endothelial or other hyaline cells con-

taining from one to five parasites like those found in the blood of the patient. The smears from the blood and the urine also showed these organisms. (It may be stated in this connection that our guinea pigs do not show the Kurloff bodies in their blood, and that the organism in question does not resemble the Kurloff bodies). (Plate XI)

Inoculation of Blood Into Guinea Pig

On May 19, blood from the patient was inoculated into the right ear vein of a guinea pig. No effects were noted until May 22 when the pig was inactive and feverish with rapid pulse and hasty labored respiration. Blood smears were prepared from the skin of the left ear. Parasites were found in 1.4% of all the hyaline cells. No parasites were found within the granular cells. The pig did not die but remained feverish and ill. On June 10 chloroform was given and the pig quickly succumbed. Viscera were less severely congested and less abundantly hemorrhagic than in the pigs inoculated with urinary sediment. Spleen considerably enlarged, to approximately three times the normal size. Tricuspid and aortic valves showed evidences of acute inflammation. Smears were made from viscera as in the pigs with urinary inoculation, with same findings, except that the parasites were about three times as abundant.

Both the urinary sediment and blood from the patient, and urine, blood, scrapings from various viscera and the cerebro-spinal fluid from all three of the guinea pigs were inoculated upon and into various culture media but none showed any sign of growth. These same materials were inoculated into ten other guinea pigs, but none of them succumbed to the disease and the parasite was not found in the cells of these pigs at any time. They were killed and the tissues examined at intervals of one week for two months.

This parasite differs from that described by Darling in form. Darling's descriptions and the photographs made of cells containing the parasite show it to be oval or roundish, while this parasite, in the hyaline blood cells and the epithelial cells of the urine, was somewhat angular, with fine processes extending from the central body almost or quite to the wall of the capsule. The parasites are so much alike, however, that they should be tentatively considered identical.

A diagnosis of histoplasmosis was made and the prognosis was gloomy. Treatment was chiefly symptomatic and devoted to the relief of the pain with rest for the heart. Cardiac symptoms were followed by left hemiplegia with symptoms suggesting embolism and death

occurred about thirty days after the first examination. No autopsy was permitted. No other member of the family showed any evidence of the disease.

MALARIA

Malaria or paludism has been known for many centuries. Both names suggest the older idea that malaria is due to the emanations from marshy land. These emanations were supposed to be most dangerous at night and the dangers of "night air" were seriously discussed. The discovery of the plasmodium malariae by Laveran in 1880 paved the way for adequate study of the parasites which cause different forms of malaria and the final control of the disease through sanitation.

The complicated life history of these parasites, the manner in which they are transmitted from human host to mosquito, and from mosquito to human host and the remarkable rigors and fevers which are caused in the human host by the physiological events of the life of the parasite while it is in human blood make up one of the most fascinating chapters in biology.

Cases studied in the laboratories of The A. T. Still Research Institute, in Chicago and in Los Angeles, include chiefly old, atypical malaria. Recent acute attacks are not found often because there are no anopheles mosquitoes in the vicinity of these cities.

Many cases of old and atypical malaria are found and these present typical changes in the blood. The patients suffer from vague symptoms of malaise and headache, dull aching in the region of the spleen and the liver, and irregular attacks of chilliness or feverishness or both. These symptoms are often so vague and so atypical that malaria is not suspected until the blood examination shows the presence of the characteristic organisms or of the cell relations usually associated with chronic malarial infection. Very often several examinations of blood taken at different times are necessary before the organisms can be found. The characteristic cell relations are present always.

Malarial Organisms

Plasmodium malariae, the smallest of the malarial parasites and the one which is less commonly found, is most easily recognized by the rosette-like arrangements of the schizonts within the erythrocytes. Plasmodium vivax is not easily differentiated from plasmodium falciparum in smears taken from these old, atypical cases. The history of the early disease usually indicates which of these organisms is present. Plasmodium malariae causes the quartan type of fever, the plasmodium

vivax the tertian and the plasmodium falciparum the aestivo-autumnal or malignant type of malarial fever. Quotidian fevers are due to double infection by the vivax or triple infection by the malariae.

The intervals between the attacks are measured by the time required for the completion of the asexual cycle. In old cases the period required for the asexual cycle seems to vary for different organisms in the blood at the same time, so that instead of the definite chill followed by the definite fever there are irregular attacks of chilliness or feverishness or both, with varying symptoms of malaise, headache and perhaps some dull pain or aching in the region of the spleen.

Blood Changes

In these late cases the blood changes may be quite significant. The large hyaline cells and the intermediate sizes of hyaline cells called splenocytes are considerably increased, both actually and relatively. These cells often contain fragments of red blood cells or granules of pigment derived from the hemoglobin of cells previously ingested and destroyed. The blood serum may be stained brownish and this is due to methemoglobin or some related compound. The granular cells are often diminished both relatively and absolutely. They rarely contain erythrocyte fragments. They are often irregular in outline and have swollen nuclei with frayed edges. The neutrophile granules are often rather irregular in size, especially in those cases which are characterized by a rather large spleen. Fragments of red blood cells may be found, and these may suggest the diagnosis. The malarial parasite is occasionally found, and the diagnosis is then definite.

Diagnosis

In many instances patients suffering from vague and indefinite symptoms show blood with these traits, and the search for the malarial organisms should then be very thoroughly carried on. It may be necessary to study blood specimens taken at different times of the day and on different days in order to secure a specimen in which the parasites can be found. If any chilliness or feverishness occurs, the blood should be taken just before such attacks are expected, or at as nearly this hour as is practicable. Vigorous osteopathic treatment, of a kind planned to cause rapid blood flow through the spleen, often causes the parasites to appear in the peripheral blood. Such treatment exercises a definite therapeutic effect upon the patient, no doubt partly because the parasites are thus driven into the peripheral blood, and through many tissues where they are subjected to the various parasiticidal agencies of the body.

The length of time during which the malarial organisms can remain in human blood is not known. In our clinics patients have been found with malarial organisms in the blood who had shown no recognizable malarial symptoms for twenty and even for fifty years. Many of these patients had not lived in a malarial country for many years, and have not known that they had been exposed to the possibility of infection during that time. It is, however, easily possible that re-infection occurred during some journey to malarial districts. Re-infection cannot be certainly excluded. It is significant that nearly all of the patients in whom old, atypical malarial parasites have been found are those who have suffered very severe malarial attacks in early life.

Protective Agencies

Recovery from malarial invasion depends upon several factors which differ somewhat from those which protect the body against bacterial invasion. It should be remembered that in bacterial diseases the neutrophiles ingest the bacteria, that various agglutinins, opsonins and precipitins are developed within the body, and that these seem to be of considerable value in immunity. In malarial invasion and in other forms of animal parasites the body cells do not react in the same manner. The neutrophiles are phagocytic only very inadequately, if at all, for animal parasites. The large hyaline cells, the epithelial cells and the splenocytes are the chief phagocytes for malaria and other animal-like parasites of the blood. The only physiological reaction of the body as a whole is the development of the fever. The high temperature often present during a malarial fever is fatal or at least very harmful to the parasites, and this is one important factor in preserving human life in malarial countries. It is, of course, frequently an inadequate reaction, and the fever itself may destroy the more delicate cells of the body. But it does help to destroy the parasite and it does facilitate the oxidation of the wastes of katabolism.

The liver and the spleen are the most efficient of the protective agencies within the body against malaria. These organs remove the fragments of the injured red cells from the blood and transform the various substances derived from them into harmless materials to be excreted, or into substances fit to be utilized again in the manufacture of new cells. The spleen provides a constant supply of active hyaline cells which are able to ingest and destroy the malarial parasites. The increased activity of these organs during a malarial attack is evident. The enlargement of both is a very common symptom. This increase in

size is in part due to the increased activity caused by the blood destruction, and is in part a reaction to the invading organisms and the products of their activity.

Treatment

In order that the liver and the spleen may react efficiently it is necessary that they have a normal circulation of good blood through their tissues. The most serious cause of circulatory disturbance of the spleen and the liver is the presence of osteopathic lesions of the seventh to the ninth thoracic vertebrae and the related ribs. Patients who have such lesions are unable to react efficiently to malarial attacks, because these lesions disturb the circulation through the liver and the spleen. They also disturb the normal control of the non-striated muscle fibers of the splenic capsule, the gall bladder and the ducts of the liver.

The red bone marrow must renew the supply of the blood cells in order that anemia may not follow the destruction of so many erythrocytes by the parasites. This means that the circulation and the innervation of the red bone marrow must be kept as nearly normal as is possible. Suitable food is also necessary; any ordinarily varied, wholesome diet, rich in proteins, provides the materials necessary for the renewal of the red blood cells and the hemoglobin.

The adequate treatment of malarial patients must include the correction of those lesions which interfere with normal circulation of the blood through the liver and the spleen, and with the circulation and innervation of the cells of the red bone marrow. This treatment prevents excessively high temperatures in all but the most overwhelming malarial invasions. Moderate degrees of pyrexia are undoubtedly helpful in destroying the parasites, both directly and by facilitating phagocytosis by the hyaline cells of the blood and the tissues.

Illustrative Case Report

Mrs. A., a woman fifty-four years of age, suffered a rather mild attack of influenza. She did not recover properly and for some months complained of irregular attacks of feverishness and chilliness, with varying headaches, malaise and discomfort in the region of the spleen. At the age of seven years she had suffered for several months from malaria. At that time the spleen was considerably enlarged, so that her dresses had to be made over and she felt humiliated by the large size of the abdomen. She had received enormous doses of quinine at that time, until the ears were seriously affected. The quinine was not given after the ear symptoms became serious, and the malarial attacks did not cease until cold weather came, which was nearly two months after the cessation of the quinine.

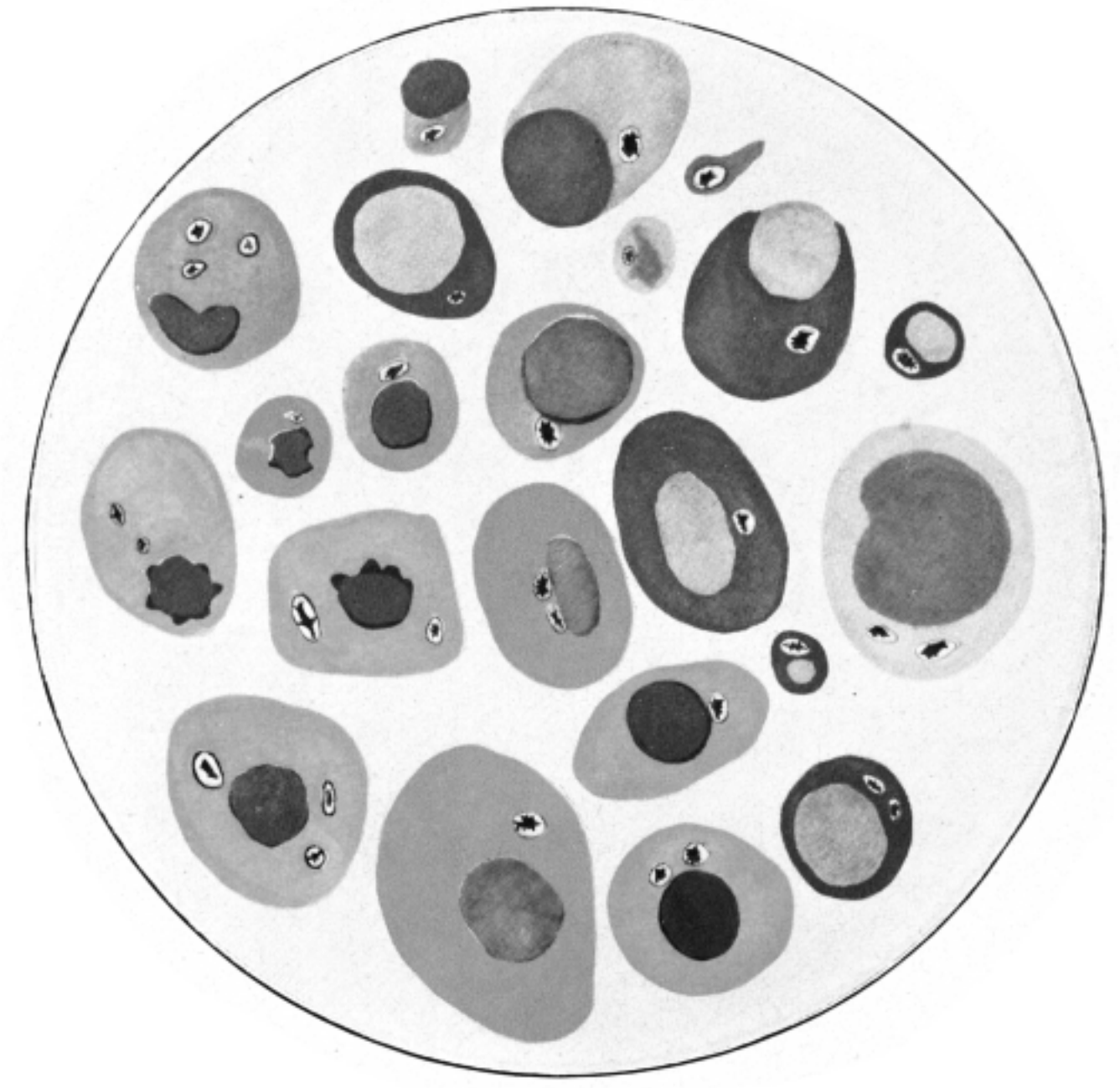


PLATE X

HISTOPLASMA CAPSULATUM (?) IN HUMAN BLOOD

Hyaline blood cells, stained blue, and epithelial cells, stained orange, from the urine of child with histoplasmosis. The large hyaline cells of the blood contained the same organism.

During the seventh to the tenth years of her life she had malaria every summer and well into the autumn. After her fourteenth year she had excellent health with only occasional accidental illnesses,—one or two attacks of food-poisoning, injuries from falls and strains, and one broken arm.

On physical examination some weeks after acute influenza at the age of fifty-four years, the spleen and the liver were both found somewhat enlarged, hypersensitive to pressure, and firmer than normal. The skin was sallow and the conjunctivae yellowish. There was an area of marked spinal rigidity extending from the sixth to the twelfth thoracic segments.

Blood examination made the day after a feverish attack showed no malarial parasites. Other smears were made at different times, until a smear was secured just as an attack of chilliness was beginning. These smears contained many parasites recognizable as plasmodium vivax. The white cell count was 4,500 per cubic millimeter. The differential count gave the following figures, based on a count of 500 cells:

Large hyaline cells	18 %
Small and intermediate cells, hyaline	29 %
Eosinophiles	3.5 %
Basophiles	2.5 %
Neutrophiles	47. %

In counting 500 white cells there were found 20 red cells containing the plasmodium.

The hygienic conditions and the diet of the patient were satisfactory and no change was directed.

Treatment was devoted to the anatomical conditions. The spinal rigidity was treated by the usual osteopathic manipulations. The circulation through the liver and the spleen was brought to normal by suitable manipulations which provided more adequate venous and lymphatic drainage. The spinal rigidity and the symptoms diminished after the first treatment. The liver and the spleen returned to normal size during the first week. After the third day no chills or feverishness appeared.

During the next six years the blood of this patient was examined at irregular intervals, twenty times in all. Usually no parasites were found in the blood. After a time of pronounced over-work with marked fatigue, after an attack of measles, and during a period of marked depression following a death in her family the organisms reappeared in the peripheral blood, and vague, indefinite symptoms of chilliness, feverishness and headache were noted.

TRYPANOSOMIASIS

Trypanosomes are flagellate unicellular organisms which usually live alternately upon vertebrate and invertebrate hosts. Many of these are pathogenic for man and for animals. Pathogenic trypanosomes are not endemic in this country but they may be brought from the tropics by infected persons. In such cases the symptoms may be very perplexing and diagnosis difficult.

Sleeping Sickness

The sleeping sickness of Africa is caused by one of two trypanosomes, Trypanosoma gambiense, which is carried by the tsetse fly, or Glossina palpalis, and T. rhodiense, carried by G. morsitans. Another trypanosome which is transmitted by G. tachinoides has been called T. nigerense, but there is good reason to believe that this is really identical with T. gambiense.

T. gambiense has been divided into two groups, Castellanella gambiensis, which causes a more chronic and less severe form of sleeping sickness, and C. castellanii, which causes a more acute form of the disease in the human race, and which is speedily fatal to laboratory animals.

Another trypanosome, T. bruccei, causes the terribly severe disease of horses and cattle in Africa. It is carried by G. morsitans and is supposed by many authors to be identical with T. rhodiense found in human blood.

These trypanosomes all affect the blood of the mammalian host. All have a peculiar body called variously kinetonucleus, parabasal body, micronucleus or blepharoplast. This structure takes nuclear stains, is smaller than the true nucleus and lies near the blunt end of the organism. There is a tiny granule near the parabasal body and from this granule there arises an undulating membrane. The flagellum borders the undulating membrane and extends for some distance beyond this membrane at the end opposite the parabasal body. The flagellum and the undulating membrane may be absent during certain periods of the life of certain trypanosomes. The organisms vary from fourteen to forty microns in length, and are two or three microns in diameter. The nucleus is round, roundish or oval, and, in the oval forms, the long diameter of the nucleus forms a right angle with the long diameter of the body of the parasite. All of these structures are best studied in the organisms secured after experimental inoculation of rats. In laboratory animals T. rhodiense usually causes an acute disease, speedily fatal,

while T. gambiense inoculations are often unsuccessful and, in successful cases, cause a chronic disease of slow progress.

Transmission

The tsetse fly which feeds upon infected mammals may transfer the infection immediately to another mammal. After a few hours this direct transmission is impossible. The organism undergoes further development in the body of the fly and within about three weeks the organisms can be transmitted in the saliva of the fly to another mammal. The fly remains infective for the rest of its life, which rarely exceeds six months. The parasite is not transmitted to the pupae. Of all tsetse flies which feed upon infected mammals, only a few, not more than about one in twenty, become infective.

Symptoms

The first symptoms of sleeping sickness are fever and enlargement of the lymph nodes, usually of the neck. After about a week the fever diminishes or disappears and the lymph nodes return to normal size, or almost to normal size. There is no definite relation between the size of the lymph nodes and the height of the fever. After a few days or a few weeks these symptoms recur; the fever becomes remittent or intermittent and other lymph nodes enlarge. The lymphoid hyperplasia becomes chronic and variable. During this period the parasites may be found in the blood and in juice extracted from the enlarged lymphoid tissues. These recurrent attacks may persist for months or even for years. After a time invasion of the cerebrospinal fluid, meninges and the brain occurs, and the typical lethargy and mental inertia of sleeping sickness follows.

Various convulsive attacks resembling epilepsy may occur; the gait becomes shuffling and uncertain; fine tremors affect the skeletal muscles and the diaphragm, and sometimes the abdominal muscles. Actual sleep may be scanty but apathy is often very profound.

Recovery does not occur after the nervous symptoms appear. During the earlier stages symptomatic recovery is possible. The periods of intermission can be lengthened and the comfort of the patient increased by the maintenance of good structural relations of the body, good hygienic conditions and change of climate.

Diagnosis

When recurrent attacks of fever with enlargement of the lymph nodes occurs in an individual who has been in Africa or in Latin-

America, the blood and other tissues should be studied carefully in a search for the parasites.

Smears made from peripheral blood may show the trypanosomes on the warm stage, as was the case in one of our patients. This is not commonly the case. Thick smears may be made from peripheral blood and this permits them to be found rather more easily. They are easily recognized; the long, slender unicellular organism with its long, slender flagellum and the waving undulant membrane are distinctive. If the organisms are not found in smears from the peripheral blood, about ten to twenty cubic centimeters of blood should be taken from a vein in the elbow into an equal amount of citrate solution. The mixture is then to be centrifuged for five minutes, at a speed of about three hundred revolutions per minute. In the sediment the parasites are easily seen: they accumulate in the leucocyte layer, which lies between the red blood cells and the supernatant plasma. In some cases it is best to take several specimens of ten cubic centimeters each, centrifugalize specimen separately, remove the leucocyte layers, place all together into another centrifuge tube and centrifugalize again. In this manner the organisms from a considerable amount of blood are brought together in a few drops of leucocyte and plasma mixture.

In case this method does not demonstrate the parasites, one of the enlarged glands should be punctured, using a sterile and perfectly dry needle. A few drops of fluid from the gland is almost certain to contain the trypanosomes if they are the cause of the symptoms.

The blood or the gland extract may be used to inoculate guinea pigs, monkey or rats. The last named rarely succumb to blood inoculation but usually develope great numbers of trypanosomes when inoculated with material from a lymph node which is enlarged.

After nervous symptoms occur, the trypanosomes can usually be found in the spinal fluid. They are, very rarely, found in the saliva and the urine of infected human beings.

The blood cells show secondary anemia and little other change. During the fevers there may be slight leucocytosis. The increase in monocytes, which so commonly occurs in other diseases due to parasites in the blood, does not occur in trypanosomiasis, at least in any marked degree. The reticulo-endothelial reactions characteristic of malaria and kala azar are not noted in this disease. A slight increase in eosinophiles has been reported.

Protective Agencies

Trypanosomes have been studied chiefly by means of animal inoculations. The absence of any reaction on the part of the reticulo-endothelial system has been mentioned. Splenectomized animals do not succumb to inoculations more rapidly nor less rapidly than no normal animals, hence the spleen does not seem to be important as a protective agency. Trypanosoma bruccei, T. evansi and T. equiperdum are not pathogenic for human beings but are pathogenic for mice. Rosenthal and others have injected human blood serum into the veins of mice, and then have inoculated them with one of these trypanosomes, whereupon the mice did not succumb to the infection. But if serum from a human with serious liver disease is injected into the veins of the mouse, the inoculation with one of the trypanosomes mentioned causes the usual symptoms and, ultimately, the death of the mouse. Hence it is concluded that the liver produces the trypanocidal substances, whatever it may be. If the mice are injected several times with human serum, the latter loses its protective influence, so that it seems probable that the human serum activates some substance in the juices of the mouse. The serum of human beings does not kill the trypanosomes mentioned, in vitro. Human serum exerts little if any protective influence in splenectomized mice. Other experiments seems to indicate that the spleen produces the substance with which the human serum combines, to protect the mouse against the trypanosomes experimentally inoculated. These and other experiments seem to indicate that there is an indirect relation between the spleen and other areas of the reticulo-endothelial system in protection against trypanosomes, although there is no recognizable direct activity of the endothelial cells in this disease.

Treatment

There is no trypanosomicidal drug which is not even more definitely fatal to the tissues of the host. Patients in the earlier stages should be taken to a temperate or chilly climate, in order to prevent later infections and also in order to avoid transmitting the disease to the carrier insects. Hygienic conditions should be as good as is practicable. Osteopathic treatments which cause a free circulation of the blood through the liver and the spleen are indicated, and any lesions which affect the functions of these organs should be corrected at once.

Chagas Disease

In Brazil, and occasionally in other countries of South America, there is an organism which is sometimes called Trypanosoma cruzi and

later called, by Chagas, Schizotrypanum cruzi, which causes repeated fevers in children, and, later, marked enlargement of the thyroid gland. This parasite is carried by several insects of the vicinity, including bedbugs and ticks. These insects, and others, live in the cracks of houses. The parasites infect the bugs, and are discharged in great numbers with their feces. The latter are left upon the skin of the human host and gain entrance into the body through wounds in the skin; the wounds caused by scratching are especially dangerous. After a latent period which is probably several years the child suffers from increasingly severe feverish attacks, much pain in the muscles, emaciation and various nervous attacks, the thyroid enlarges, and the further course resembles that of other trypanosome infections.

During the acute fever the parasite can be found in the peripheral blood of the child, but during remissions blood examinations are futile. Schizotrypanum is about twenty microns long and about one micron in diameter. It is characterized by a larger parabasal body than is shown by other trypanosomes infesting the human body. The organisms undergo division in the voluntary muscles, the brain and spinal cord, and in many glands of the body, especially the thyroid. With abundant and rapid multiplication the young organisms form cyst-like structures; these break apart when the parasites become fairly mature and they then invade the blood. The location of the cysts determines the symptoms of the disease and accounts for the varied symptomatology associated with the fevers and thyroid enlargement.

FILARIASIS

Filariasis is a disease due to the presence of any one of several varieties of filariae in the blood. The most commonly found is Filaria Bancrofti, originally called filaria sanguinis nocturnis. The latter name is due to the fact that the embryos are found in the peripheral blood at night, in persons who sleep at night. They are most abundant at about midnight. In persons who work at night and sleep during the day the embryos are found in the peripheral blood only during the day, being most abundant at about eleven o'clock in the morning. There is some reason to believe that this distribution is due to changes in the blood pressure and in the varying amounts of blood in the skin and in the viscera during sleep and wakefulness. Much further study is necessary before the problem can be solved.

Filaria Bancrofti in the adult form lives in masses, often entwined inextricably. The females are fifty to sixty-six millimeters long and almost or quite two millimeters in diameter; they resemble transpar-

ent hairs. The males are forty millimeters or less in length and rarely more than one-tenth millimeter in diameter. The worms are ovoviviparous. The ova are oval or roundish and have no true shell; they are about fifty microns by about thirty-five microns in size. These are occasionally carried in the blood but being unable to pass the capillaries they occlude the smaller arteries and the arterioles. Normally the ova are retained until they reach the larval stage, the microfilariae of the peripheral blood. These are variable in length and may be somewhat longer or shorter than three hundred microns; their diameter also varies, but is not far from eight microns, the diameter of a red blood cell. They are able to pass through the capillaries with comparative ease and they are found in the capillary blood taken for an ordinary examination. Having gained entrance into the human body they probably remain indefinitely, undergoing their adult life and their sexual reproductive period in the viscera, especially the lymphatics and the lungs, and their larval period in the circulating blood.

The intermediate host is the mosquito; the culex fatigans is probably the most important. Other species of culex and certain species of anopheles also act as intermediate hosts. The mosquito bites the human host, the larval forms are taken into the stomach of the mosquito, pass into the muscles of the mosquito, undergo further development, and finally reach skin of another human being through the bite of the mosquito. Probably a very long time is required for development within the human host, for children show no symptoms of disease until they are five or ten years old, in countries in which filariae are abundant. The development within the body of the mosquito probably requires less than a month.

Symptoms

These worms may infest the human body for years without causing any noteworthy symptoms. The worms have been demonstrated in the blood of about one in four of the natives in certain countries and many of these persons seemed to be in ordinarily good health.

Chyluria or hematochyluria is usually the first symptom. The urine, usually otherwise normal, contains abundant fine fatty globules and this causes it to resemble a milky fluid, which may be pinkish from blood. On settling a reddish clot is sometimes noted; this is composed of blood. This condition may be present at intervals, or almost or quite constantly, and very often no other symptoms occur for many years. In one of our cases this was the sole symptom during the time the patient was under observation, about two years. Occasionally the clots are formed in the bladder and this may cause difficulty in urina-

tion. The worms or the parasites are not often found in urine, but the blood should be carefully examined at different times of the day and the night until the microfilariae are found. It must be remembered that chyluria may occur, though rarely, because of other etiological conditions.

Elephantiasis is commonly associated with filariasis, though it may never occur. The legs, scrotum, labia and, rarely, other parts of the body undergo progressive increase in size, sometimes becoming enormous. The enlargement may be gradual or may be sudden, and in this case is usually associated with fever.

The occlusion of the smaller arteries and the capillaries by the embryos and ova is the apparent cause of the lymphatic engorgement, and the latter is the cause of the tremendous overgrowth of the tissues concerned in elephantiasis.

Diagnosis

The finding of the worm in the peripheral blood is the most definite method of diagnosis. It may be found on making an ordinary blood examination when its presence has not been suspected. When it is suspected a thick smear should first be made and this examined with one-sixth objective; even a two-thirds objective with a one-inch or a half-inch eye-piece may be sufficient to allow them to be seen. They are noticed at first because of the turmoil of the red blood cells on the warm stage; these are whirled about by the lashing movements of the filaria. Smears may be dried and stained with thionin or hematoxylin for permanent use.

If the worms are not found in such a specimen the blood should be centrifuged. Take about one cubic centimeter of blood into a centrifuge tube containing about nine cubic centimeters of two per cent acetic acid. Mix thoroughly and centrifuge for about five minutes at about five hundred revolutions per minute. The red blood cells are destroyed by the acetic acid and the debris of these, with the worms and the white cells, are thrown down. Take a drop of this sediment and examine as before. This test may be repeated at several different times of day and night if necessary.

The filaria are more easily found in capillary than in venous blood. A fairly deep puncture is necessary in order to secure the rather large amount of blood. There is no harm in using antiseptics which dilate the blood vessels of the skin, in this test, and fairly vigorous rubbing of the skin may increase the number of embryos present.

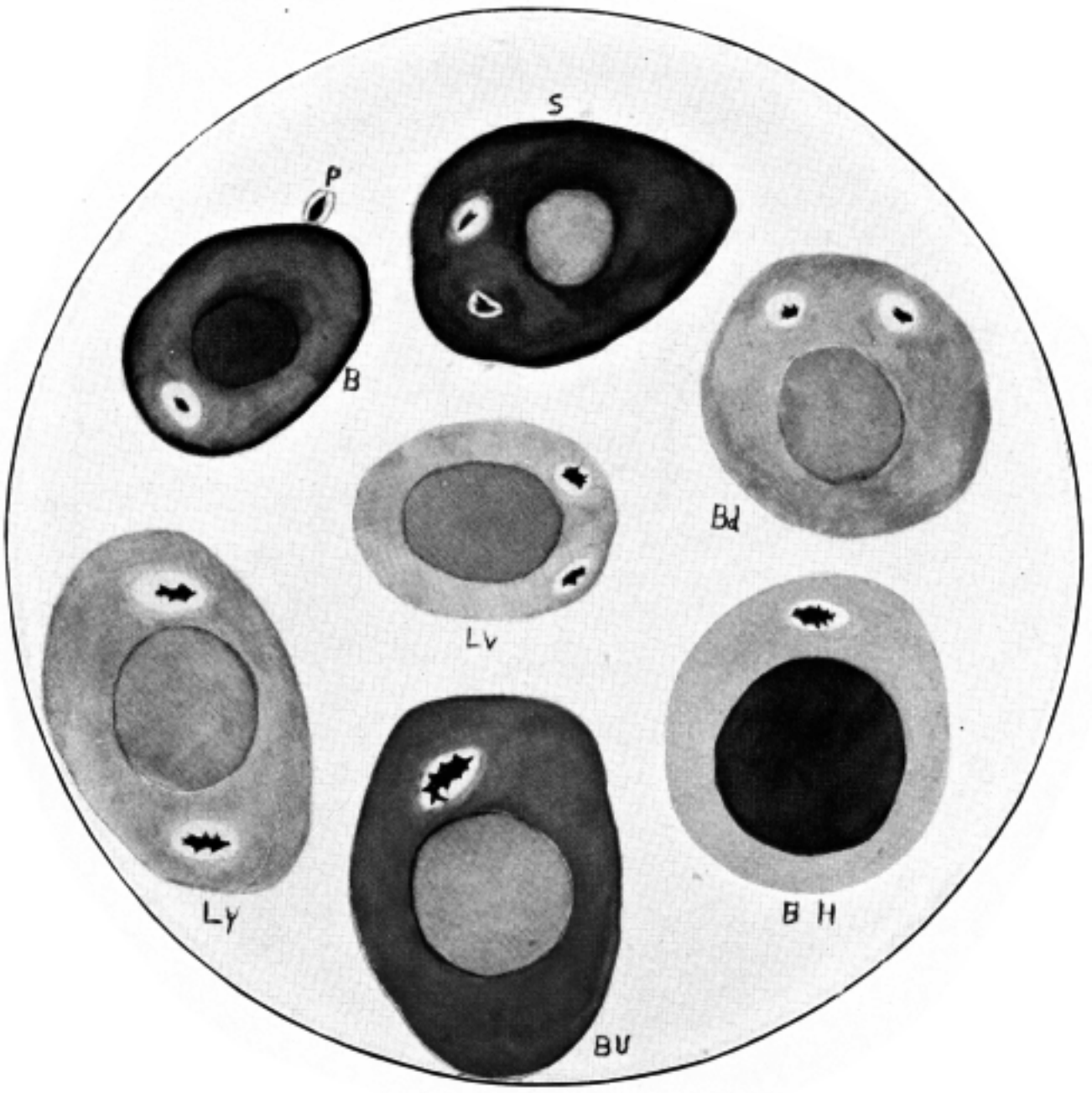


PLATE XI

HISTOPLASMA CAPSULATUM (?) IN CELLS FROM INOCULATED GUINEA PIG.

P. Parasite touching large hyaline cell of the blood.

B. Large hyaline cell containing parasite.

S. Cell from spleen containing two parasites.

Lv. Cell from liver containing two parasites.

Bd. Cell from scraping from bladder containing two parasites.

Lg. Cell from lung containing two parasites.

Bu. Cell from scraping from kidney containing parasite.

BH Cell from pleura containing parasite.

The blood shows moderate eosinophilia in practically all cases. Moderate degrees of secondary anemia are common. The actual and differential counts are otherwise almost or quite normal.

Treatment

Sanitary measures are important. Mosquitoes should be exterminated. Infected persons should be protected against mosquito bites. In countries in which filariae are present, all persons should be guarded against mosquitoes.

There is no adequate treatment for the disease. Any drug which exerts even a detrimental effect upon the worms injures the host more seriously.

Limiting the fats of foods diminishes or prevents chyluria and hematuria. In this way the discomfort associated with the passing of blood clots through the urethra is lessened or removed.

All measures which increase the nutrition and the general health of the patient should be employed. Since the manner in which the body protects itself against the worms is not known (if, indeed, there is any protection at all) it is impossible to say whether the maintenance of a good circulation of the blood has any parasiticidal effects or not. Certainly the maintenance of good nutrition and good circulation enables the patient to live more comfortably and efficiently than he could do otherwise.

SPIROCHETAL INFECTIONS OF THE BLOOD

Several forms of spirochetes, spirosomes or spirilla are known to cause diseases. Most of these are filth diseases, so-called because they are transmitted from one person to another through the intervention of lice, bedbugs, ticks, or other insects whose presence indicates lack of cleanliness in persons or in dwellings. Some species are transmitted directly from one diseased person to another and others are carried by mosquitoes. Methods of transmission differ for different species.

The biological place of these organisms is still somewhat in dispute. Schaudinn and others place them with the protozoa; Dobell and many others class them with the bacteria, while Doflein and many others beg the question by forming a separate class for organisms which are intermediate between protozoa and bacteria. However puzzling the question of classification may be, there is no question about the facts of the life history and the pathogenicity of these organisms.

There are very many spiral-like organisms which infect lower animals but only comparatively few which infect man, and of these not

every species, and not every infection of pathogenic species, causes recognizable symptoms of disease. This lack of pathogenicity does not seem to depend upon the development of parasiticidal factors in the human body, as is the case with certain pathogenic bacteria, but rather on a passive non-resistance on the part of the cells of the body of the host. This reaction, or, rather, lack of reaction, is one factor which suggests that the biochemical relations of these parasites are with the protozoa rather than the bacteria. There are other factors concerned, however, and the entire question of classification must await further study of these interesting and dangerous organisms.

RELAPSING FEVER

(Febris recurrens; famine fever; typhus recurrens; tick fever; African fever; Chinese fever).

This is a general term which includes several related diseases, all due to some spirochetal infection and all associated, directly or indirectly, with filthy habits, filthy surroundings and, usually, extreme poverty.

European relapsing fever has for its specific infectious agent the spirochete obermeieri (spirosoma obermeieri; spirillum obermeieri; borellia recurrentis; spirosoma recurrens). This spirillum varies from about ten to about fifty microns in length and rarely exceeds half a micron in diameter. It has from four to sixteen turns and is flagellated. The ends are sharply pointed. It is freely motile during the early stages of the fever, then diminishes in motility until only the barest waving is perceptible. It reaches its greatest abundance in the peripheral blood just before the crisis in the fever, and during defervescence it may be difficult to find any specimens in the peripheral blood. During intermissions it is almost impossible to find any specimens though occasionally such blood inoculated into monkeys causes great numbers of the spirochetes to appear in the monkey's peripheral blood within a few days to a week.

If death occurs during an intermission, the spleen and bone marrow show hyperplasia and the organisms are abundant in these tissues as well as in other lymphoid tissues and in the liver, kidneys and other glandular viscera.

Symptoms

The disease is characterized by repeated attacks of high fever lasting about a week. Nausea, vomiting, profuse sweating, and convulsions and other nervous symptoms may be extremely severe. Intermissions last ten days to several weeks. Later attacks of fever may be less and

less severe until recovery seems complete, or more and more severe until death results, usually from exhaustion or from intercurrent disease which may itself be apparently negligible. Death from relapsing fever is hardly to be expected except in unusually severe cases, but recovery is long delayed, convalescence slow and often interrupted, and even for many months after apparent recovery other attacks may occur.

Diagnosis

The organisms are best recognized in rather thick warm slide preparations of peripheral blood, and these must be made during the early stages of the fever. They move very freely among the red blood cells, which themselves are not disturbed by the moving spirilla. Later in the fever the spirilla become less and less motile and it may be necessary to make stained smears in order to find them. Dark stage illumination is best. They may be very abundant during the early stages of the fever, so that in a thick smear observed under the one-sixth objective as many as twenty or more may be found in a single field. They may be absent or scanty on the first day of the fever, and they increase until just before the crisis, when they begin to diminish in numbers rather rapidly. They may form ring-like bodies during defervescence, and at all times, even during intermissions, there may be abundant small highly refractive bodies in the blood which may be degenerative fragments of the parasites or may be spore-like structures.

The blood cells show the characteristics of secondary anemia. Leucocyte counts are usually high, but rarely exceed 15,000 per cubic millimeter. Large hyaline cells and eosinophiles are increased moderately. This eosinophilia suggests protozoal rather than bacterial infection.

Neutrophiles and large hyaline cells may contain fragments of the spirilla, and in this relation the spirilla resemble bacteria rather than protozoa.

The urinary changes suggest the renal complications always present in this, as in other high fevers. In extremely severe cases acute hemorrhagic nephritis may be present. These are the "blackwater" cases so feared in countries in which the disease is endemic.

Varieties

Several varieties of relapsing fever occur in different countries. Spirilla obermeieri causes the European type of relapsing fever and this has just been described. It seems to be carried especially by bedbugs, though other insects seem also to act as intermediate hosts.

Spirosoma or spirillum duttoni (Borrelia duttonia) causes the relapsing fevers of East Africa and West Africa. It is transmitted by ticks (Ornithodorus savignyi or O. moubata). The tick becomes infected by sucking human blood containing the parasites, and it transmits the infection to its young. The coxal fluid of the tick and the feces contain the parasites, and this mixture is left upon the skin of the human being next bitten by the tick. This material as well as the biting of the tick causes itching of the skin. On scratching the tick may be crushed upon the skin, and both the remnants of the tick and the mixture of coxal and fecal material are apt to be rubbed into the skin through the abrasions due to the scratching.

Spirosoma (or spirillum or borrelia) carteri causes the relapsing fever of India. It is thought to be transmitted chiefly by lice, and either the pediculus capitis or the P. vestimenti may carry these organisms. S. persica causes the Persian relapsing fever. It is carried by ticks, for the most part.

The relapsing fever of Northern Africa is caused by S. berberi or Borrelia berberi. It also is transmitted by lice.

S. novyi has been found in the United States and it caused a severe epidemic in several large eastern cities during the middle of the eighteenth century. It is about ten microns long and has two or three rather sharp turns. It is not now found in the United States. There is a form of relapsing fever in Panama which is carried by ticks.

Treatment

The elimination of the disease depends upon sanitation, and with cleanliness and the destruction of lice, bedbugs and ticks the disease soon disappears.

During the fevers the osteopathic treatments which control the circulation of the blood through the viscera are usually effective. The convulsions and other nervous phenomena are sometimes prevented by relaxation of the abnormally tense cervical tissues and the correction of lesions of the cervical vertebrae. Patients who do not have such lesions rarely show any nervous symptoms. The nausea and vomiting should not be treated until the stomach has been well emptied; after that if the vomiting persists inhibition of the upper splanchnic centers is all that is needed in most cases.

There is no adequate parasiticidal drug. Recovery depends upon the action of the normal parasiticidal activities of the body and upon the prevention of re-infection. Change of climate is advisable in order

to avoid re-infection and to promote the recuperative powers of the body as a whole.

Records of the osteopathic treatment of the disease are scanty and are limited to those cases which have come to the United States from other countries. In all these cases there have been complicating factors so that typical cases have not been well studied.

Other Spirochetal Forms

There are several other spiral-like organisms which infect the human body and may occasionally be found in the blood.

Leptospira icterohemorrhagica (spirochete icterohemorrhagica) is not ordinarily found in the blood on direct examination, but it can be successfully inoculated into guinea pigs from the blood of the patient or from his urine. This parasite causes infectious jaundice (Weil's disease). It is carried constantly by wild rats, in countries in which this form of jaundice is endemic, and human beings become infected by drinking water polluted by the urine of the rats, or by eating uncooked vegetables grown in ground polluted by the rats, or by some other accidental means. This spirochete is rarely more than fourteen microns in length and one-fourth micron in diameter. It stains a reddish or purplish color with Giemsa's stain but it does not take any of the ordinary aniline stains.

Leptospira icteroides (spirochete icteroides) causes yellow fever. It is transmitted by the mosquito, stegomyia fasciata. This insect becomes infectious about twelve days after it has bitten a human being during the first few days after the onset of an attack of yellow fever. The mosquito is an intermediate host. Leptospira icteroides rarely exceeds nine microns in length or one-fifth micron in diameter. It is sometimes visible by dark-field examination of fresh blood but does not stain readily. It can be cultivated or animals can be successfully inoculated from the patient's blood.

Leptospira hebdomadis causes a "seven day fever" in Japan. Wild mice carry the organism, and human beings are infected from the water and the ground polluted by the mice.

Leptospira morsus-muris causes rat-bite fever, which follows the bite of an infected rat. It is of relapsing type and resembles the ordinary form of that disease in many respects. It occurs only in Japan.

Spirochete pallida (treponema pallidum) is the cause of syphilis. It is rarely found in the blood, and blood cell examinations are not commonly employed for its diagnosis. The Wassermann test and the Kahn test are the most common diagnostic methods.

In syphilis the blood picture includes moderate eosinophilia with moderate lymphemia. Leucocytosis and the usual findings in secondary anemia occur under certain circumstances in syphilis.

Many other forms of spirochetae, spirilla, treponemata, and similar organisms are occasionally found associated with various ulcers and sores of the skin; they are often found in the healthy as well as in the diseased mouth, and harmless forms are isolated from many specimens of water and from the bodies of animals.

LEISHMANIASIS

Leishmania are parasites found in the blood and in the tissues during the progress of certain diseases. The organisms appear to be alike, but different strains or varieties causes different forms of disease.

The organisms are usually flagellated in cultures and in the invertebrate hosts, but are usually non-flagellated in the mammalian host. In the human host and in the canine host the Leishmanias are found within the large hyaline phagocytes of the blood and in the spleen and other lymphoid tissues. The reticulo-endothelial system seems to be of great importance in protecting the body against these organisms, as is the case with malarial infections. The organisms may divide rapidly within the phagocytic cells, and finally the cell resembles a small cyst, filled with the parasites. In other instances the parasites are digested and absorbed by the phagocytic cells, and it is in this way that the further injury due to the organisms is prevented. The organisms are also very abundant in the red bone marrow and in granulation tissue around the lesions caused by the Leishmanias.

In their mammalian hosts Leishmanias are about four microns long by about one and one-half microns in diameter. Somewhat larger and somewhat smaller forms may be found. Each cell contains an oval, round or roundish nucleus placed at about the center of the cell. There is a round karyosome associated with the nucleus. The nucleus stains avidly with ordinary nuclear dyes. Near the periphery there is a parabasal body or centrosome which takes nuclear stains quite deeply. The long axis of the parabasal body lies at right angles to the long axis of the cell.

The blood shows the usual findings of secondary anemia, except that leucopenia is frequent. The leucocyte count rarely exceeds three thousand cells per cubic millimeter and is often less than two thousand. The neutrophiles are both relatively and absolutely diminished and the large hyaline cells both absolutely and relatively increased. Lymphemia may be quite marked.

The parasite is not often found in the blood on ordinary examinations and some method of concentrating the parasites is necessary. For demonstrating the organisms take several cubic centimeters of venous blood into a centrifuge tube containing an equal amount of five per cent potassium oxalate, and centrifuge at about five hundred revolutions per minute for six minutes. The red cells are then at the bottom of the centrifuge tube, the plasma at the top, while the white cells form a thin layer at the top of the red cell column. Smears made from the white cell layer contain great numbers of leucocytes and the parasite is occasionally found within the large hyaline cells or in rather large masses resembling zooglea. They are best stained with one of the methylene-blue-eosin blood stains.

If the parasites are not found after several attempts have been made to find them in concentrated blood, puncture of the spleen or the liver, the removal of a small bit of a rib, or the removal of one of the enlarged lymph nodes, may be considered. All of these methods are somewhat dangerous, and fatal hemorrhages occasionally follow such operations. Smears made from the granulation tissue or from the firm base of the local sore usually contain the organism in great numbers.

The parasites which cause the different types of disease are much alike and diagnosis of the type of disease as a result of examination of the parasite is usually impossible. They differ somewhat in cultural characteristics, however.

Leishman-Donovan bodies (Leishmania donovani) cause the disease usually called kala azar, of Asia. It is most common in Assam, Indo-China, Ceylon, Syria, Burma and in some parts of India. It is transmitted by bedbugs, probably by other insects, and may be transmitted from one human host to another through feces. When sores are present in the intestinal tract the parasites are present in the feces in great numbers.

The symptoms are characteristic; a series of irregular attacks of fever is soon followed by enlargement of the spleen and sometimes the lymph nodes of the neck and elsewhere, and by progressive anemia and emaciation. Recovery is doubtful but the patient may live for many years.

Two cases have been examined in our laboratories. In one the diagnosis was fairly certain from the history and the symptoms. The parasites were not found in the blood, and no attempt was made to secure extracts from the liver, spleen, lymph nodes or marrow. The patient disappeared and his death was reported two years later.

In another case the diagnosis was not suspected before death. Microscopic examination of the spleen after death disclosed great numbers of Leishman-Donovan bodies.

Leishmania infantum was first found in Naples and Rome, and since then has been found in Tunis, Malta, Sicily, Egypt, Turkey, Arabia and other countries bordering the Mediterranean. Children and dogs are chiefly affected, and the disease is probably carried from one to the other, and perhaps from one child to another, by fleas.

Leishmania tropica causes the "Delhi boil", also known by many other names. It was first found in Oriental countries, especially India, Persia, Arabia and other tropical countries; later it was found in Egypt and other countries of Northern Africa, and in several Mediterranean islands.

The disease called espundia, in Brazil and other countries in Latin America, is probably the same disease.

No treatment has been found successful. Methods which increase nutrition, enforce cleanliness and freedom from insect parasites and which encourage good circulation blood through all the body are indicated.

Antiseptic washes and comfortable dressings should be used for the local lesions on the skin.

CHAPTER X

LEUKEMIAS AND RELATED DISEASES

A satisfactory definition of leukemia has not yet been devised. Barker's definition of the leukemic state as one "in which there is definite proliferation of leukopoietic tissues, either myeloid or lymphadenoid, and the appearance in the blood of immature white cells, usually in large numbers, the degree of whose immaturity is more pronounced the more acute the cases," has much to commend it, but it seems to exclude those cases in which the blood picture is atypical. It is true that diagnosis is very difficult when the blood picture is atypical.

There are many instances in which immature forms never are found in the blood, the white cell count is not found increased at any time, and yet at autopsy the pathological changes are definitely those of leukemia. It must be remembered that an aleukemic phase may occur during the course of the disease and also that in its initial stages the blood picture may remain unaltered for variable and sometimes for considerable periods of time.

Hodgkin's disease and other abnormal conditions of lymphoid and myeloid tissues included as pseudo-leukemias are properly excluded from the leukemias by the definition given. There is no rational basis for the attempt made by Piney and others to change the terms employed in discussing these diseases because of their inaccuracy; if the names of diseases were changed with each advance in our knowledge of their nature great confusion would be inevitable. The term leukemia as applied to this group of diseases is certainly not more objectionable than is the term malaria, as applied to a disease with no real relationship to bad air. There are many other conditions in which a name has been given to a disease or to a symptom complex before correct understanding of its essential qualities, yet these names persist because their use is convenient.

The relationship between hyperplasia of the bone marrow and the leukemias has not been adequately studied. That certain forms of leukemia develop after infections is certain; it is not certain that the infection is the sole cause of the hyperplasia of the bone marrow. That aleukemic leukemia follows an infectious process to which the system

appears unable to make adequate response is also frequently the case. But it is not at all certain whether the inability of the bone marrow to make adequate response to the infectious agent was or was not due to developmental imperfections or to some acquired etiological factor. The possibility that the inadequate response to the infection may be due to costal or vertebral lesions has received no investigation at all.

Fox reported a case of leukemia in the opossum (Didelphys virginianus) in which the autopsy findings were typical, though the diagnosis had not been determined before death. Birds in captivity and ordinary barnyard fowls occasionally suffer from leukemia, and in these cases the disease seems to be infectious. Corson-White reported a case of chronic lymphatic leukemia in a monkey.

Hyperplasia of the bone marrow occurs as a reaction to various infections and secondary anemias. Certain infectious agents arouse hyperplasia of the leucocytopoietic areas of bone marrow. Other infectious agents initiate hyperplasia of the lymphoid tissues. Very severe hemorrhages, repeated at intervals, lead to hyperplasia of the erythrocytopoietic areas. Hemolytic infections sometimes cause hyperplasia of the erythrocytopoietic tissues.

Exhaustion and atrophy of the bone marrow occur in rare cases, when the condition known as aplastic anemia supervenes if the erythrocytopoietic tissues are affected. The rare monocytic leukemia is associated with atrophy of the leucocytopoietic tissues, though it is not known whether the atrophy is the cause of the failure of the neutrophilic reaction, or the overwhelming infection is the cause of the neutrophilic failure.

The relations between the erythrocytopoietic and the leucocytopoietic tissues affected by disease are not yet well understood. In pernicious anemia the leucocytopoietic tissues seem to be inactive if they are not truly atrophied. Yet in occasional cases of pernicious anemia after several exacerbations the leucocytopoietic areas share in the hyperplasia and the condition called leukanemia is found. On the other hand in certain cases of leukemia the anemia becomes very severe, and in late cases the high color index, the megaloblasts and other findings characteristic of pernicious anemia are found. When the patient is first examined late in the disease it is often impossible to determine whether the red cells or the white cells were first affected. The unsatisfactory diagnosis of leukanemia is then unavoidable.

In all the leukemias there is a marked tendency for the cells characteristic of the disease to appear in exudates, transudates, pus, sputum and various abnormal secretions. Patients with leukemia fail to develop opsonins and agglutinins when they contract typhoid fever or when they receive typhoid vaccine. They are especially subject to pneumonia and to other acute infections. Immunity may still further be lowered as a result of ill-judged treatment of the leukemias by radium or by the X-ray. The leucopenia thus produced by unwise treatment seems to reduce several of the factors concerned in immunity to acute infections. If pneumonia occurs during the course of lymphatic leukemia, the small hyaline cells are abundant in the sputum. In cases of splenomedullary leukemia, when pneumonia or any other pulmonary infection occurs, the sputum contains great masses of myelocytes. Pus occurring anywhere in the body contains abundant small hyaline cells if the patient already suffers from lymphatic leukemia, abundant myelocytes if he has splenomedullary leukemia, large monocytes if he has monocytic leukemia. In cases in which the leukemia is chronic, mild and unrecognized at the time of the acute infection, diagnosis may be clouded by the finding of these unusual cells in secretions. Examination of the blood usually clears the diagnosis. (Plate XII)

Extramyeloid masses of tissues resembling bone marrow may cause atypical symptoms. In lymphatic leukemia especially the growth of masses of lymphoid tissue within the central nervous system may complicate the diagnosis very seriously. Chloroma and splenomedullary leukemia sometimes cause symptoms of brain tumor by means of these invading masses of cells.

The leukemias are classified according to the type of cell most abundant in the blood stream. Many intermediate and mixed forms are found, and in late cases it is very frequently impossible to determine the exact diagnosis.

SPLENOMYELOGENOUS LEUKEMIA

(Splenomedullary leukemia; myeloid leukemia; splenic leucocythemia; leukemic myelosis).

Splenomyelogenous leukemia is a disease of the hematopoietic tissues characterized chiefly by marked enlargement of the spleen and by the presence of considerable numbers of immature granular blood cells; usually also by great increase in the white cell count. The symptoms

are indefinite and usually include irregular feverish attacks, feelings of fatigue, various nervous symptoms and discomfort due to the increased size of the spleen. Irregular feverish attacks are very common. They seem to bear no relation to fatigue, constipation or infections. The temperature often remains below 101° F. and rarely exceeds 103°.

Subcutaneous and submucous hemorrhages are very common. Occasionally death follows severe bleeding from the nose, intestines or uterus in cases which have not shown serious symptoms before.

Priapism is a common symptom and is probably due to thrombosis. Ascites also is a common symptom; this is usually due to enlargement of the spleen. Visual disturbances due to the invasion of the retina by masses of leucocytes are not common, but may be an early symptom. Symptoms suggesting tumor of the brain may follow invasion of the brain by masses of leucocytes and myelocytes.

The changes in the structure of the spleen and the bone marrow and the changes in the blood picture are the most interesting features of the disease. Two forms are recognized, the acute and the chronic; these differ chiefly in the time necessary for the progress of the disease.

Etiology

Lesions of the ninth thoracic vertebra and neighboring bones have been found in every human with chronic splenomyelogenous leukemia. Every human subject with a lesion of the ninth thoracic vertebra has also some congestion of the spleen and the spleen is always palpable when such a lesion has been present for two years or more. These patients may not show any recognizable increase in the white cell count, but they always have a recognizable increase in immature and myelocytoid granular cells. Every laboratory animal with a lesion of the ninth thoracic vertebra has an enlarged spleen. Records of blood examinations of such animals are too scanty for any definite statements to be made as to the changes due to splenic enlargement or to the lesion itself.

Correction of the lesion has resulted in marked improvement in every case of human subjects with splenomyelogenous leukemia receiving regular and systematic osteopathic care.

Other etiological factors are unknown. Malaria, syphilis and chronic pyogenic infections have been mentioned but none of these always causes the disease and all are absent in many cases. Only the ninth thoracic lesion is always present, and this lesion invariably causes some of the

conditions characteristic of the disease. Not every patient with a ninth thoracic lesion develops typical symptoms or typical blood picture of leukemia, but all show some symptoms of the disease.

Pathology

Pathological changes in the spleen are those which might be expected to occur as a result of abnormal relaxation of the muscular fibers of the splenic capsule, together with those changes due to accumulation of leucocytes within the blood vessels. The Malpighian bodies show no evidence of being actively concerned in the pathological changes, but are affected by pressure changes and circulatory disturbances due to congestion and infarction. In early cases the sole change in the spleen is a profound congestion with atony of the capsule. Later the accumulation of white cells causes thrombosis, infarction and degeneration of the pulp cells.

In early cases there is no marked change in the bone marrow. After the disease has assumed its classical form the bone marrow shows characteristic changes. The fatty areas of the bone marrow are replaced by cellular masses in which leucocytopoiesis is progressing rapidly. The bony spicules are frequently absorbed. Grossly the marrow presents irregular areas of reddish, pinkish and whitish areas. Sometimes greenish areas are also present; the latter are due to degeneration of the red cells. After death this richly cellular material undergoes rapid digestion so that the marrow presents an appearance of being purulent. On microscopic examination it is seen that the cells are all myeloid in type and that no pus is present. Eosinophilic myelocytes are especially abundant, usually in definite areas, and associated with these eosinophilic areas are many Charcot-Leyden crystals (needle-like crystals, roughly octohedral, about 10 microns in length in this situation). Neutrophilic and basophilic myeloblastic cells are also abundant. Erythroblastic areas are scanty and may show atrophic changes.

Blood Changes

The blood picture is characteristic in typical cases. The white cell count varies very rapidly and may reach tremendous figures. In one of our cases the count dropped from 503,600 to 146,400 in twelve days, then increased to 546,000 in the next nine days. Counts of 250,000 per cubic millimeter are not rare, though those below 100,000 are more frequent. The increase is chiefly due to neutrophilic cells, and these include great numbers of myelocytes, myelocytoid forms and early myeloblastic types. Cells resembling neutrophiles but with very fine and feebly-staining eosinophilic, basophilic or amphophilic granules are

abundant in some cases. These are not normally present in human blood or bone marrow at any age, and are found normally only in certain lower vertebrates. Similar cells containing no granules and with amblychromatic protoplasm are occasionally found, and these may account for almost the entire increase in leucocyte count. The neutrophiles vary greatly in size, and in the same slide cells may be found which are 30 microns in diameter, 20 microns, and even 4 microns in diameter. Nuclear variations are mostly in the direction of the embryonic or the atavistic types. Senile types are occasionally found in chronic cases which are of slow development. Myelocytic and myeloblastic forms frequently show karyokinesis. Eosinophiles and basophiles are often greatly increased, varying between 2,000 and 15,000 per cubic millimeter but they may remain relatively or even absolutely normal in number. Both eosinophiles and basophiles sometimes show atavistic qualities, and immature, myelocytoid and myeloblastic forms are common. Ehrlich considered absolute increase in basophiles essential to a diagnosis of this form of leukemia. Blood in which the eosinophiles are abundant often develops Charcot-Leyden crystals on standing for an hour or a few hours. Small hyaline cells include varying numbers which give the oxidase reaction and are, therefore, really myeloid. Small lymphocytes are usually relatively diminished and actually increased. Large hyaline cells include many hyaline myelocytes and many endothelial cells. The large hyaline cell with intensely basophilic nucleus and cytoplasm containing fibrillae is rather common; the protoplasm is either feebly acidophilic or feebly basophilic. These cells are not found in normal embryonic or adult human blood or marrow and are normally found only in lower vertebrates. (Plate XII)

Examination of a blood slide alone may give the diagnosis in typical cases. Ewing considers the finding of eosinophilic myelocytes with granules of varying size, form and staining pathognomonic. The finding of any considerable numbers of myelocytoid forms, especially the finding of several basophiles within a comparatively small area, should lead to further study of the blood. In one of our patients receiving treatment for neurasthenic symptoms, the leukemia was not suspected until the examination of a smear of blood showed many myelocytes. A differential count alone usually gives more definite information in leukemia than the actual count alone. The actual count of white cells alone cannot be considered diagnostic, because the white cells reach such high counts in pyogenic infections and in pneumonia.

Atypical counts are not rare in leukemia. The actual count may drop to normal or even to a very low number. Under such conditions

the presence of immature forms and myelocytes, and an excess of basophiles and eosinophiles may cause leukemia to be suspected. The blood picture may become normal in every recognizable manner in some cases, and this condition may persist even until death. More frequently the low counts are followed by increasingly high counts which show the usual blood picture. Sometimes an intercurrent infection, such as influenza, typhoid fever or malaria, may lead to diminished actual count; in such cases the differential count usually remains indicative of leukemia. Occasionally the intercurrent disease is fatal, and in such cases the autopsy may show abundant evidences of leukemia, or may show no signs whatever that leukemia had ever been present.

With exhaustion of the hematopoietic tissues the granules disappear from many cells. The erythrocytopoietic areas also may become exhausted and the red cell picture may be that of aplastic anemia, or they may share in the hyperplasia and anaplasia in which case the red cell picture is that of pernicious anemia. The leucocytic blood picture remains almost or quite unchanged by the development of the anemic phase of the disease.

The red cell count usually remains fairly normal during the early stages of the disease, especially in the chronic types. Later there seems to be some increased destruction of the red cells, and always there is some anemia as the disease progresses. The hemoglobin may be as low as 30% of the normal for the age of the patient, but is commonly between 40% and 60% in chronic cases. In acute cases the hemoglobin may be 10% of the normal, or even lower. No doubt this is due to the effect of the abnormal leucocytopoietic areas upon the erythrocytopoietic areas in the bone marrow together with the effects of the toxic katabolites of the abnormal leucocytes. The hemorrhages which are so common in this form of leukemia also tend to produce secondary anemia. The seriousness of the anemia does not bear any recognizable relation to the seriousness of the leukemia, nor to the progress of the disease. Patients with quite low red counts and lower hemoglobin may feel very well and show considerable improvement in the white cell counts, while others with either normal or low red cell counts may be extremely weak and toxic and may die. Perhaps in no other disease does the symptom-complex bear less definite relations to the actual progress of the disease.

Blood fibrin is increased. The threads appear on the warm stage at once or within two minutes at most. Beaded and irregular threads are abundant. Threads radiating in long strands from debris are fre-

quently found. Net-like arrangements of fibrin threads are less common.

Fibrinolysis is absent in all cases examined in our laboratories. The blood volume is increased. Ten cases were studied by Rowntree. In all polycythemic hypervolemia was present. The mean values were 109 cubic centimeters of whole blood and 69 cubic centimeters of plasma per kilogram of body weight. The findings varied between 92 cubic centimeters and 125 cubic centimeters of whole blood, and between 65 cubic centimeters and 78 cubic centimeters of plasma per kilogram of body weight.

Atypical Forms

Monocytic leukemia is a form of splenomedullary leukemia in which monocytes are greatly increased in numbers. Usually this is a temporary condition, and ordinary myelocytes soon cause the usual findings of splenomedullary leukemia.

Eosinophilic leukemia is a form of splenomedullary leukemia in which many eosinophiles and eosinophilic myelocytes are present.

Basophilic or mast-cell leukemia is very rare. The large granular basophiles or mast cells are present in large numbers. It is often a terminal stage in splenomedullary leukemia.

Plasma cell leukemia seems to be related to lymphatic leukemia. Many of the large hyaline cells are of the plasma cell type.

Aleukemic leukemia is probably always a stage in the uneven progress of splenomyelogenous leukemia. It should not be confused with aleukemic lymphoma.

Typical Cases

The following cases were selected because they illustrate the place osteopathic treatment has in the handling of cases of splenomyelogenous leukemia. Our records are too few for any definite conclusions, but they are suggestive, and they may point the way to methods far more efficient than those which have been previously employed. Perhaps we may find that many cases in the initial stages can be cured and that, after the disease has become advanced beyond a certain point, no treatment is of any avail. Since it has been fairly well proved, by reports from many laboratories, that one function of the spleen is the maintenance of a correct relationship between leucocyte development and leucocyte destruction, it is easy to see the steps in pathogenesis from a lesion of the ninth thoracic vertebra, abnormal relaxation of the splenic capsule, disturbed nervous control of the splenic blood vessels, disturbed functions of the spleen and disturbances in the control of white-cell destruction and development, with the leucocytopoietic tissues left completely uncontrolled.

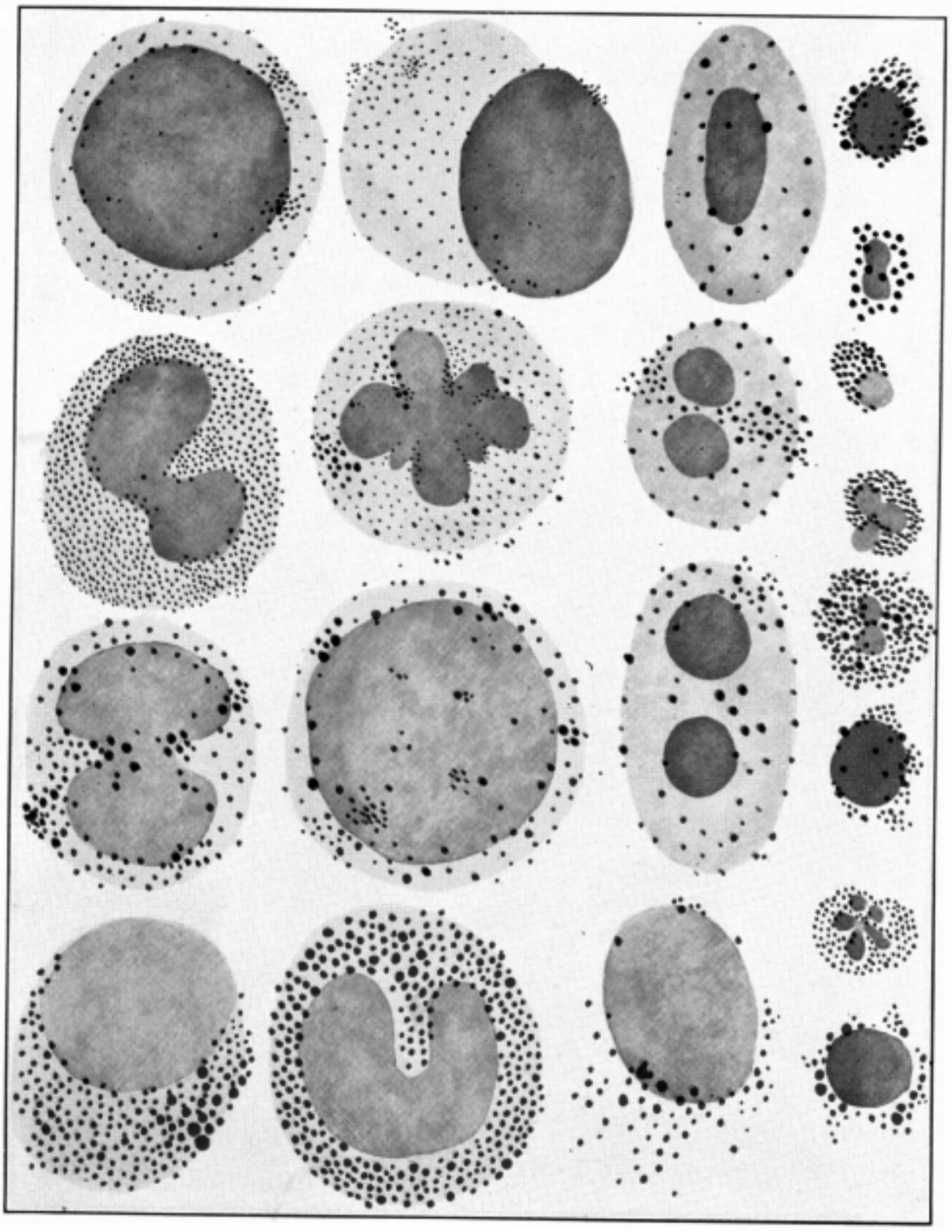


PLATE XII

MYELOCYTES IN LEUKEMIA

Megalomyelocytes and micromyelocytes containing eosinophilic, basophilic and amphophilic granules showing great variations in size. Blood taken from patient with splenomedullary leukemia of atypical nature, two days before death. Cells show distinctly atavistic characteristics.

The following case history is typical of what seems to be a very early case of splenomyelogenous leukemia. We have no reports of very early stages in this disease among the publications accessible, and only a very few in our own records.

Mrs. J., aged 48 years, six years past the menopause, had been ordinarily well until an attack of influenza during July and early August of 1921. She had received medical care during that attack and had been given an unusually severe course of drug therapy. She was unable to learn the identity of the drugs given, but she attributed various gastric, nervous and cardiac symptoms to the medication received, and many of these symptoms disappeared very shortly after she refused further medication. In November, 1921, she complained of insomnia, emotional instability and great weakness. The following blood findings were reported on that date:

Hemoglobin, 105 grams per liter; 89% of normal for age.

Erythrocytes, 4,480,000; 80% of normal for age.

Color index, .9

Normoblasts about 10 per cu.mm. Abnormal red cells few.

Coagulation time 11 minutes; (normal for technique, 5 minutes).

Leucocytes, 7,700 per cu.mm.

Large hyaline	12.8%	985 per cu.mm.
Small hyaline	28.4%	2187 per cu.mm.
Mononuclear neutrophiles	3.4%	262 per cu.mm.
Polymorphonuclear neutrophiles	49.0%	3773 per cu.mm.
Eosinophiles	5.0%	385 per cu.mm.
Basophiles	1.4%	108 per cu.mm.

Hyaline and granular myelocytes and endothelial cells, all present in small numbers.

Neutrophile nuclear average 1.86 (normal, 2.45 to 2.55).

Notes; Atypical granules abundant in hyaline and granular cells.

Immature and atavistic types abundant.

Physical findings were negative except that the spleen was easily palpable. A lesion of the ninth thoracic vertebra was present; no other definite spinal or costal lesions were found. The lesion was corrected; symptoms disappeared. During the next six years the blood remained normal.

In such a case as this the ninth thoracic lesion should be corrected as the most important factor in therapy. If the patient's dietetic and other habits include any unhygienic factors these should also be corrected.

Early Splenomyelogenous Leukemia

Mrs. O., aged 43 years, history contains nothing of significance so far as the leukemia is concerned. She appeared at the clinic of The Pacific College of Osteopathy complaining of weakness, insomnia, with "nervousness," which seemed to mean emotional instability in her case. A rather superficial blood examination was made Mar. 1, 1909, with the following findings:

Hemoglobin, 57% (Dare).

Erythrocytes, 4,920,000 per cu.mm.

Leucocytes 20,000 per cu.mm., with many myelocytes.

April 2, 1909, the second blood examination was made and the findings were as follows:

Hemoglobin, 50% (Dare).
Erythrocytes, 4,296,000 per cu.mm.
Leucocytes, 64,000 per cu.mm.

Large hyaline	3.6%	2,304 per cu.mm.
Small hyaline	16.4%	10,496 per cu.mm.
Neutrophiles	57.0%	36,480 per cu.mm.
Eosinophiles	12.4%	7,936 per cu.mm.
Basophiles	4.5%	2,880 per cu.mm.
Basophilic myelocytes	2.5%	1,600 per cu.mm.
Neutrophilic myelocytes	.8%	512 per cu.mm.
Eosinophilic myelocytes	.2%	128 per cu.mm.
Fractured and degenerated myelocytes	2.6%	1,664 per cu.mm.

Unrecognizable forms, many.

On physical examination the spleen was found enlarged to a point just below the umbilicus. Color somewhat pasty, no jaundice. Other findings negligible. Lesion of eighth to tenth thoracic vertebrae with rigidity. Many secondary lesions in which rigidity was not marked; the latter apparently secondary. Diet and habits of living were found to be good and no change was advised. Correction of the lesion was advised and this was the only treatment given.

This treatment was carried on in the clinic for several weeks, during which time several blood examinations were made. A gradual improvement in the insomnia and the weakness was noted within a week, then the spleen began to diminish in size and the leucocyte count diminished gradually, with diminishing numbers of myelocytes. On May 11, 1909, the count was as follows:

Hemoglobin	56% (Dare)		
Erythrocytes	4,464,000 per cu.mm.		
Leucocytes	11,520 per cu.mm.		
Large lymphocytes		3.3%	380 per cu.mm.
Small lymphocytes		15.7%	1809 per cu.mm.
Mononuclear neutrophiles		.6%	69 per cu.mm.
Polymorphonuclear neutrophiles		75.0%	8640 per cu.mm.
Eosinophiles		2.1%	242 per cu.mm.
Basophiles		1.5%	173 per cu.mm.
Myelocytes (all neutrophilic)		1.8%	207 per cu.mm.

At that time the spleen was just easily palpable. Symptoms were nearly gone except for some weakness due, no doubt, to the low hemoglobin. The lesion had been corrected but it recurred after she had lifted some heavy articles at house-cleaning time. Correction of the lesion the second time was easy and required only a few treatments.

April 13, 1910, the blood count was as follows:

Hemoglobin 68%			
Erythrocytes	4,456,000 per cu.mm.		
Leucocytes	5,600 per cu.mm.		
Large hyaline		3.8%	213 per cu.mm.
Small hyaline		30.8%	1725 per cu.mm.
Neutrophiles		62.2%	3483 per cu.mm.
Eosinophiles		1.2%	67 per cu.mm.
Myelocytes		2.0%	112 per cu.mm.
Basophiles, very few (less than .1%)			

After this her history was uneventful until January, 1912, when she fell and broke both radius and ulna. Feb. 26, 1912, her blood count was as follows:

Hemoglobin	53% (Dare)		
Erythrocytes	3,576,000 per cu.mm.		
Leucocytes	7,100 per cu.mm.		
Large hyaline		4.8%	341 per cu.mm.
Small hyaline		35.0%	2485 per cu.mm.
Mononuclear neutrophiles		.2%	14 per cu.mm.
Polymorphonuclear neutrophiles		57.8%	4104 per cu.mm.
Eosinophiles		.9%	64 per cu.mm.
Basophiles		.6%	43 per cu.mm.
Myelocytes (all neutrophilic)		.7%	50 per cu.mm.

At this time the ninth thoracic lesion had not recurred, and the few myelocytes were attributed to the injury. There was some general

spinal rigidity. The spleen was not palpable and the splenic dullness was normal in size. Her diet had been somewhat deficient during the winter and she had been over-worked. With rest, improved diet, and the relief of spinal rigidity during the months of March and April her condition improved again.

May 10, 1912, the blood examination gave the following findings:

Hemoglobin	80% (Dare)		
Erythrocytes	4,476,000 per cu.mm.		
Leucocytes	9,100 per cu.mm.		
Large hyaline		3.6%	328 per cu.mm.
Small hyaline		32.6%	2967 per cu.mm.
Mononuclear neutrophiles		1.2%	109 per cu.mm.
Polymorphonuclear neutrophiles		62.0%	5642 per cu.mm.
Eosinophiles		.6%	55 per cu.mm.
Basophiles, very few			
Myelocytes, none			

During the years of 1912, 1913 and 1914 several blood examinations were made with about the same findings. During 1914 she left the city and no further blood examinations were made. In 1915 her health was not good, and no osteopathic attention was possible where she lived. She had no blood examinations made. She was able to be up, and helped with the housework and with house-cleaning on the last day of her life. That night she died in her sleep. After death hemorrhagic areas appeared upon the skin of the legs and arms, though no hemorrhages had ever appeared during her lifetime. The coroner signed a certificate of death from "heart disease."

Splenomyelogenous Leukemia; Aleukemic Stage

Miss C. Age 24 years. History contains nothing significant; no definite symptoms. While the diagnosis is not positive, the case is probably one of aleukemic leukemia. Spleen and cervical lymph nodes enlarged moderately. Four blood examinations made between June 30 and July 23. These tests were made while the patient was on a uniform milk diet, resting in bed, and the blood was always taken at the same time of the day. Red cell counts varied from 4,460,000 to 4,550,000, hemoglobin about 85%, with color index remaining at about .8 constantly. A few normoblasts and reticulated cells at each examination.

Leucocyte counts varied from 3,600 to 4,400 per cubic centimeter not varying with red cell count. The differential count presented

only slight variations. A typical report, based on the examination of 2,000 cells, is as follows:

Leucocytes, 4,000 per cu.mm.		
Large hyaline	4.4%	176 per cu.mm.
Small hyaline	24.6%	984 per cu.mm.
Mononuclear neutrophiles, less than .1%		
Polymorphonuclear neutrophiles	54.0%	2160 per cu.mm.
Eosinophiles	.8%	32 percu. mm.
Basophiles	.2%	8 per cu.mm.
Neutrophilic myelocytes	1.0%	40 per cu.mm.
Eosinophilic myelocytes, few		
Large hyaline myelocytes, including gigantoblasts	5.0%	200 per cu.mm.
Small hyaline myelocytes	10.0%	400 per cu.mm.

On examination the moderately enlarged spleen and cervical lymph nodes were found; also a lesion involving the ninth and tenth thoracic vertebrae associated with marked rigidity, and several other lesions in which the rigidity was much less pronounced. The time when the lymphatic enlargement began was not known, nor was there any injury which might have caused the lesion definitely known. The patient had had several falls while riding horseback, during the fifteenth to the seventeenth year of her life, and these might have caused the spinal condition.

She did not receive osteopathic treatments and her further history is not known.

Late Stages, Splenomyelogenous Leukemia

In the later stages of splenomyelogenous leukemia the myelocytes become so fragile that satisfactory counts of the white cells are very difficult and may be impossible. Fragments which are the remains of myelocytes undergoing degeneration within the blood vessels, and even more rapidly as the blood is withdrawn for counting, obscure other elements in the smear, and irregular nuclear masses as well as protoplasmic fragments obscure the fields in the counting chamber. Myelocytes cannot be satisfactorily classified because the hematopoietic tissues seem to become unable to develop granules; occasionally a very few, feebly staining granules may be found in cells with feebly staining, ragged protoplasm and with nuclei which present atypical forms and atypical staining reactions. Nearly all of the cells show scanty protoplasm of somewhat irregular consistency and staining surrounding roundish, rather large, feebly staining nuclei. Megakaryocytes are oc-

casionally found and these are easily recognizable. Normoblasts, megaloblasts, poikiloblasts, microblasts and erythroblasts are abundant, and the predominance of megalocytes and megaloblasts with the abundant fragmentation may strongly suggest the terminal stages of pernicious anemia. In one of our cases, seen the first time only a few days before death, it was impossible to secure either a white cell count or a red cell count because of the abundant debris. The examination of the smears gave findings equally suggestive of pernicious anemia, lymphatic leukemia and splenomyelogenous leukemia. A diagnosis of splenomyelogenous leukemia was made on the history of the case and the physical findings, together with the fact that the blood examination did not eliminate this disease.

LYMPHATIC LEUKEMIA

Lymphatic leukemia may be either chronic or acute. Both forms are characterized by the great abundance of hyaline mononuclear cells in the blood stream. Many of them really are lymphocytes. Others are myelocytes of atypical form while still others are reversionary types. Usually one certain type of cell predominates in each case. The most common form is a small hyaline cell with a round nucleus in which there is often a wheel-like arrangement of chromatin. The protoplasm of this cell is scanty, feebly or intensely basophilic and the edges of the cytoplasm are often ragged or frayed. While this cell predominates, in the most common form of lymphatic leukemia, it is by no means the sole abnormal cell. Other cells usually associated with this include many myelocytoid forms. A peculiar, large, hyaline cell with scanty protoplasm and feebly staining, round, or oval nucleus eccentrically located is frequently present. The protoplasm of this very large cell sometimes presents a rather granular appearance but no true granules are present. The cytoplasm may be feebly basophilic or feebly eosinophilic. In other cases a medium sized hyaline cell may predominate; in still others the most abundant cell is very large, round and of the endothelial type. (Plate XIII)

The leucocyte counts are not generally so high as in the splenomyelogenous type of leukemia, but they may exceed 200,000 per cubic millimeter. Usually they vary around 100,000 cells, of which from 80% to 99% may be small hyaline cells.

The neutrophilic granular cells are usually greatly diminished. Eosinophiles may be absent altogether. Myelocytes are not present in the blood in typical cases.

In lymphatic leukemia the blood volume is normal or increased. The findings varied between 87 cubic centimeters and 114 cubic centimeters of whole blood, and between 33 cubic centimeters and 46 cubic centimeters of plasma for each kilogram of body weight in four cases studied at the Mayo Clinic by Rowntree.

Periods often occur during which the white cell count drops to normal or very near normal. During these periods the differential count usually shows some relations suggesting the disease.

An intercurrent infectious disease may cause neutrophilic reaction on the part of the red bone marrow and the leukemia disappear. When death occurs during the intercurrent infection, there may be no post mortem evidence of the characteristic findings of the leukemia. When pregnancy occurs during chronic leukemia the blood may return to normal. Within a few weeks after labor the leukemia recurs. If death occurs during pregnancy or labor the necropsy may show no evidence of leukemia.

Pathology of Lymphatic Leukemia

The bone marrow shows about the same changes on gross examination in lymphatic and in splenomyelogenous leukemia. In the lymphatic form, however, the cells found on microscopic examination are chiefly small hyaline cells, mostly lymph cells. These apparently have invaded the bone marrow. However, the normal bone marrow contains small areas of true lymphoid tissue, and the hyperplasia of these areas may be the source of the cells found so abundantly in the bone marrow.

The lymph nodes in lymphatic leukemia are usually considerably enlarged. The increase in size is due to an overwhelming abundance of the cells predominant in the blood (usually small hyaline) and there is no associated hyperplasia of connective tissue. Occasionally none of the lymph nodes is recognizably enlarged and the sole site of lymphoid hyperplasia is in the bone marrow.

The spleen may or may not be greatly enlarged in lymphatic leukemia. In one of our cases the spleen extended into the pelvis on the left side, and beyond the umbilicus on the right; its dull area extended to the liver dullness. On X-ray examination the diaphragm was found to be pushed upward by the enlarged spleen, so that the lungs were subjected to considerable pressure. In other cases there is no recognizable enlargement of the spleen on physical or X-ray examination, though usually some slight enlargement is found at autopsy. The enlargement of the spleen, when this is present, is due to the same pathological changes as those found in the enlarged lymph nodes.

The pathogenesis of lymphatic leukemia is unknown. In fowls there is an infectious disease which causes either lymphatic or myeloid leukemia, and both these forms seem very much like the leukemias of human beings. There is no evidence that lymphatic leukemia is infectious in the human form. No vertebral lesions have been identified with the disease, and there is no evidence that the correction of such lesions as may be found present in the cases so far reported has exerted any effect upon the progress of the disease. As in other incurable diseases, very often the correction of such lesions as may be found on examination gives considerable relief to the patient and improves the health in general. Symptoms due to the disease itself are often relieved by correction of local tension of the tissues and other palliative manipulations.

According to Fox the lymphatic adenopathies may be classified according to the reaction of neutrophiles to radiation treatments. The leukemic group, including chronic lymphatic leukemia, sublymphemic adenosis and leukosarcoma, is characterized by a neutrophile count both relatively and absolutely low, and which decreases as a result of radiation. The sarcoma group, including aleukemic adenosis (aleukemic leukemia) and lymphosarcoma, is characterized by a neutrophile count which does not vary greatly from normal, and in which the neutrophiles are diminished only slightly or not at all by radiation. In the first group unwise radiation may so reduce the neutrophiles as to diminish immunity and prevent neutrophilic reaction to infections.

Since the publication of Fox's report our cases have all had radiation and other methods of treatment before our first counts, or have been unsuitable for study for some other reason.

Acute Lymphatic Leukemia

This is rather a rare disease. It occurs especially in children and is occasionally associated with tumors of the thymus gland. The disease usually begins with sore throat. Enlarged tonsils, moderate or high fever, pharyngeal ulcers, stomatitis, nausea, diarrhea, vomiting, subcutaneous and submucous hemorrhages, dyspnea, anemia, all follow in order within a few days. The serious nature of the disease is usually manifest at once. Very rarely improvement occurs and the disease becomes chronic.

The disease progresses very rapidly and death may occur within a few days of the first appearance of symptoms. Small hyaline cells may make up 99% of a total count of 20,000 to 1,000,000 cells per cubic millimeter. The red cells are greatly diminished, and often the red

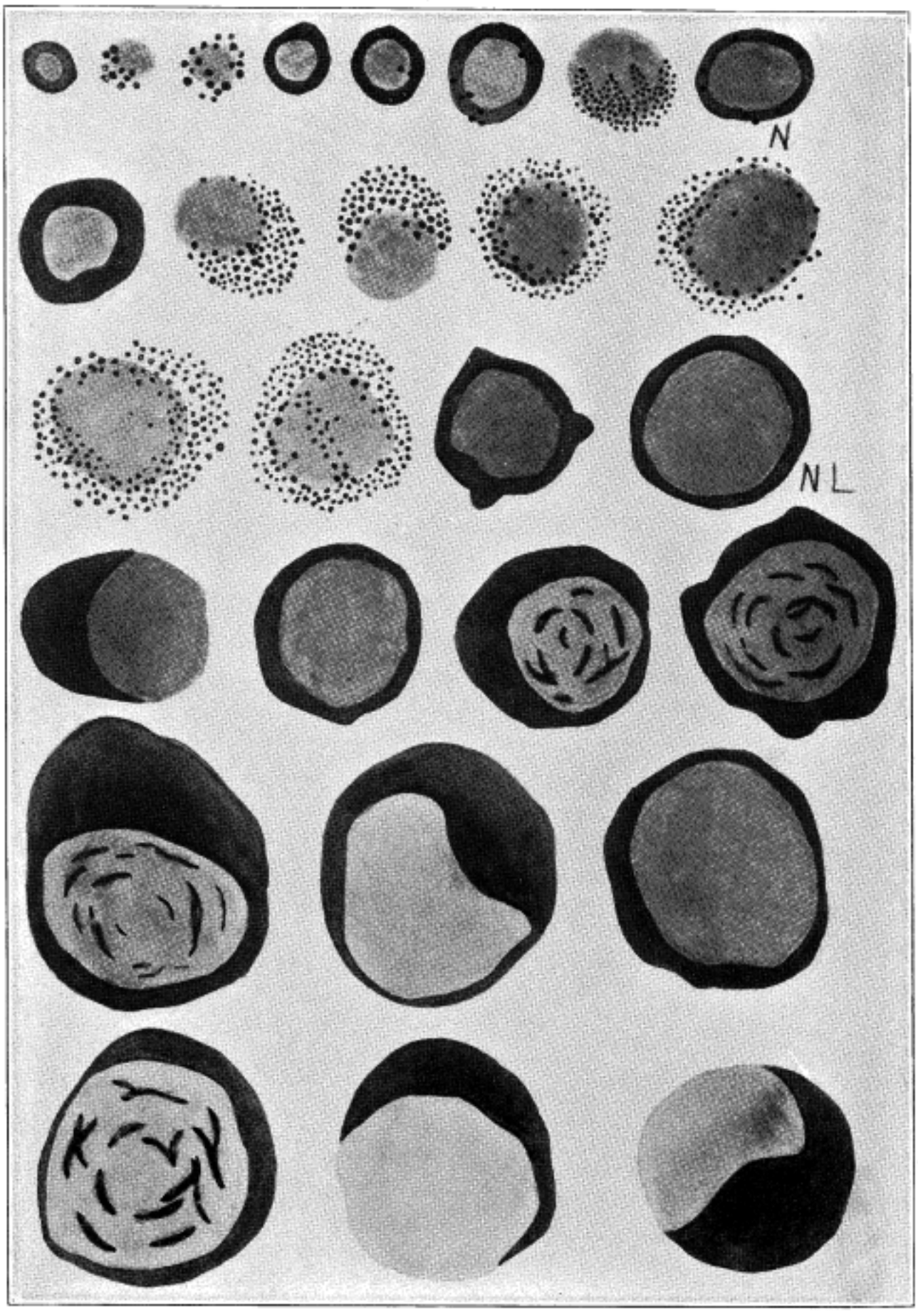


PLATE XIII

LATE LYMPHATIC LEUKEMIA

N, This abnormal cell is of the same size as normal small lymphocytes.

NL This abnormal cell is of the same size as normal large lymphocytes.

Note the great variations in size and in nuclear positions. This blood was taken ten days before death from gastric hemorrhage.

cell count is less than the white cell count. Nucleated red cells are abundant. Falling white cell counts precede death. No cases under osteopathic treatment have been reported.

Death usually occurs within three months at most after the first symptoms.

Recoveries have been reported for cases of acute lymphatic leukemia occurring in adults. Treatment has included many drugs. Spontaneous recoveries have been reported; also recoveries following acute infectious processes. Since the diagnosis is usually not made until the patient seeks medical attention it is not possible to say how many cases recover without such treatment.

Chronic Lymphatic Leukemia

Chronic lymphatic leukemia is a disease of the blood characterized by insidious onset, vague and indeterminate symptoms, enlarged lymphatic glands and the presence of greatly increased numbers of lymphocytes in the circulating blood. Pigmentation, urticaria and attacks of severe itching often occur. Irregular fevers and a tendency to neurotic symptoms are frequent.

One noticeable feature which appears after the disease has become well-established is the presence of miliary tumors which arise from the enlargement of the subcutaneous lymphoid masses, normally only microscopic in size. These may break down into ulcers. The tonsils are usually enlarged and are frequently inflamed. In the later stages anemia may become severe. Hemorrhages, usually ecchymotic, may be submucous or subcutaneous.

In the few cases which have been treated by osteopathic physicians the bony lesions and the reflex muscular contractions are so varied that no structural etiological factors can be given at this time. The cause of lymphatic leukemia is unknown.

Symptoms

The symptoms are rather vague and mild in the beginning of the disease. Sometimes the enlargement of the glands of the neck, axilla or groin is first noticed. Weakness, short breath or insomnia, pain in the limbs like sciatica, muscular rheumatism or brachial neuralgia are common early symptoms. This pain is localized according to vertebral lesions affecting the spinal segments from which the painful areas are innervated. The correction of these maladjustments exercises a palliative effect upon the pain.

The nausea, diarrhea and other gastro-intestinal symptoms bear little or no relation to the time of eating or the variety of food which is taken.

Lesions involving the mid-thoracic area are present when digestive symptoms are marked and the correction of the lesions as found relieves the symptoms. Permanent relief is not secured, however, and the patient returns with the same or other symptoms because of the progress of the disease itself.

The physical examination shows the enlarged lymphatics, sometimes pallor, rarely a slightly weakened heart-action. After anemia has been well advanced hemic murmurs may appear. A dull area over the sternum may be due to the presence of a persistent thymus, or to enlarged bronchial lymphatics. The spleen is usually slightly enlarged. The muscles of the abdomen are often flabby and intestinal atony is then present. Gastro-enteroptosis often is found early in the disease when lesions of the sixth to the tenth thoracic vertebrae are present.

The urine shows an increase in the uric acid in relation to the urea, sometimes slightly excessive indican. In the later stages albumin, casts, and kidney cells may appear. The blood contains high uric acid and creatinin.

Blood Picture

The blood in the early stages shows normal hemoglobin and erythrocyte count. The color index is likely to be low, even in the early stage, and normoblasts may often be found while there is still no sign of anemia. The small lymphocytes are at first relatively increased; later the increase is absolute and it may reach a total of more than a million small lymphocytes in a cubic millimeter of blood in the later stages. After the disease has become well-established the lymphocyte percentage is very high. A few cases are on record of 99% of lymphocytes. Even from the beginning there is a tendency to a return to immature types in the blood cells. This is shown by basophilic reticulation of the erythrocytes, various nucleated forms and somewhat more than the usual variations in size and shape of erythrocytes. A slightly increased number of mononuclear neutrophiles is present. The nuclear average of the polymorphonuclear neutrophiles is low. The eosinophiles include more than the usual number of mononuclear forms. Rarely true myelocytes may appear. (Plate XIII)

Fibrinolysis is absent. The fibrin forms normally on the warm slide.

The blood in the later stages of chronic lymphatic leukemia greatly resembles that of acute lymphatic leukemia. The hemoglobin and ery-

throcyte count are greatly diminished, sometimes to below 20% of the normal findings. The color index is always low, sometimes below 0.5. The white cell count is very high, often surpassing the red cell count, though the number of white cells is usually less than the number found in spleno-medullary leukemia. Great numbers of large hyaline, basophilic myelocytes are present as in the acute form of the disease.

The disease is rarely recognized in the early stages because blood examinations are not often made in general practice for patients suffering from the symptoms given above. In clinics and hospitals where the blood examination is made as a routine procedure the earlier stages of the disease often evade recognition because patients are not likely to come under care until the disease is well-established. A lymphocyte count of 5,000 per cubic millimeter or more, occurring in an adult, should arouse suspicion of beginning lymphatic leukemia. The blood should be watched in all such cases and the condition of the lymphatic glands investigated in order to make as early a diagnosis as possible. After the disease is well-established, with a high lymphocyte percentage and a high leucocyte count, the enlarged lymphatic glands and the progressing weakness, pain and gastro-intestinal symptoms usually make the diagnosis very easy. It is distinguished from Hodgkin's disease by the lymphocyte count; from splenomedullary leukemia by the high lymphocyte percentage, from the anemias by the lack of the pathognomonic erythrocyte changes and the presence of the lymphocytic increase; from acute lymphatic leukemia by the slow and insidious onset and milder symptoms.

Treatment

No rational treatment for the disease can yet be given. The symptoms can be relieved by the treatment adapted to each as it occurs. The correction of the vertebral lesions usually relieves the pain. Very gentle massage of the affected areas and rest of the painful joints may be helpful. For the gastro-intestinal symptoms the correction of the lesions is usually efficient, sometimes temporarily, sometimes permanently.

There is no one diet that is particularly suitable for these cases but every patient is a law to himself. Generally speaking it is better to limit the purins considerably. The rapid growth and destruction of the white cells fills the blood with nuclear derivatives, as is apparent from the increase in the uric acid and other purin bodies in the blood and urine. The pressure of clothing or other factors of irritation to the affected glands must be avoided. Generally speaking, the hygenic con-

ditions should be good and the surroundings pleasant, wholesome and comfortable in every way.

The insomnia is best met by osteopathic treatment for the relief of cerebral congestion. Baths of suitable temperatures, plenty of fresh air and such educational measures as are necessary to keep the mental state of the patient normal, are helpful. It is perhaps needless to insist that drugs are absolutely to be forbidden in the treatment of these diseases.

The use of the X-ray and of radium have been advised in the treatment of this as of other leukemias. A few recorded cases seem to indicate that these treatments are of some value; in others the progress of the disease seems to be more rapid under their influence than before the treatment was begun. Since the progress of the disease varies irregularly it is difficult to determine the usefulness of such treatments.

Prognosis

It is not yet possible to make any absolute statements as to prognosis under the osteopathic treatment. A few cases which have been recognized by osteopaths while the disease is yet in its early stages have seemed to improve as the result of the treatment as outlined above. None has been under observation long enough to warrant any conclusions. From the medical standpoint such cases are hopeless from the beginning. Nothing can exercise more than temporary delay in the progress of the disease. Surgically no help is to be expected. The removal of the spleen or of the enlarged glands in the neck, axilla and groin is usually followed by a more rapid hypertrophy of other glands in the body.

The disease is frequently complicated by kidney and cardiac diseases and by various pulmonary infections. Death often occurs from pneumonia, nephritis or some other intercurrent disease. The chronic form sometimes passes suddenly into acute type and death occurs within a few days to a few months.

The relation between chronic and acute lymphatic leukemia is probably of the same nature as that between fibroma and sarcoma or between adenoma and carcinoma. So long as the blood cells include only somewhat increased numbers of lymphocytes of the adult type no immediate danger is to be feared. But when the lymphocytes increase rapidly and when cells of the fetal or the myeloid type appear the terminal stages are imminent.

Typical Case

The following patient with chronic lymphatic leukemia terminating in the fulminating form had a fairly typical blood count. Mr. H., a man of sixty-three years, first noted that the glands of the neck were enlarged several years ago; the exact date was not remembered. The swellings appeared, then diminished, then reappeared; finally they persisted and increased. In December, 1921, the neck was not very noticeably enlarged though the glands were hard and easily palpable. X-ray examination showed the thoracic lymphatic nodes all enlarged. Small nodules were present all over the body in irregular masses. The spleen covered more than one-third the surface area of the abdomen. Feverish attacks occurred at very irregular intervals, so that some suspicion of atypical malaria had arisen. Except for these feverish attacks and some slight sense of weakness there were no subjective symptoms. The history was negative except as stated. The first blood examination was made Dec. 27, 1921, with findings as follows:

Hemoglobin, 77.3 gms. per liter, 72% of normal for age.
Erythrocytes, 3,600,000 per cu.mm., 56% of normal for age.
Color index .78
Poikilocytes, abundant
Microcytes present
Megalocytes, few
Normoblasts, 800 per cu.mm.
Poikiloblasts, few
Microblasts, few
Megaloblasts, 700 per cu.mm.
Hemoconien, increased considerably.
Leucocytes, 400,000 per cu.mm.

Large hyaline cells	.8%	3,200 per cu.mm.
Small hyaline cells	45. %	180,000 per cu.mm.
Mononuclear neutrophiles	.1%	400 per cu.mm.
Polymorphonuclear neutrophiles	3.8%	15,200 per cu.mm.
Eosinophiles	.2%	800 per cu.mm.
Basophiles	.1%	400 per cu.mm.
Myelocytoid forms, protoplasm degenerated	50. %	200,000 per cu.mm.

Fibrinolysis absent. Warm stage examination difficult because the small hyaline cells and myelocytes obstruct vision of the more active cells.

The second blood examination, Jan. 18, 1922, gave the following findings:

Hemoglobin 85.6 grams per liter; 75% of normal for age.

Erythrocytes 3,776,000 per cu.mm., 62% of normal for age.

Color index .83

All abnormal red cells less abundant than before.

Leucocytes		370,000	per cu.mm.
Large hyaline	3.0%	11,100	per cu.mm.
Small hyaline	70.0%	259,000	per cu.mm.
Mononuclear neutrophiles	.2%	740	per cu.mm.
Polymorphonuclear neutrophiles	6.5%	24,050	per cu.mm.
Eosinophiles	.3%	1,110	per cu.mm.
Basophiles, very few			
Myelocytoid and atypical	20. %	74,000	per cu.mm.

General appearance of the cells improved; less evidences of toxicity and a larger proportion of normal forms among both red and white cells.

Chemical tests made Jan. 18, 1922, were as follows:

			Normals
Total non-protein nitrogen,	40	mgs. per 100 c.c.	35
Urea	20	mgs. per 100 c.c.	18
Creatinine	1.9	mgs. per 100 c.c.	1.9
Uric acid	10.	mgs. per 100 c.c.	3-4
Sugar	130.	mgs. per 100 c.c.	100.
Carbon dioxid combining power	69.	mgs. per 100 c.c.	70-75

The third blood examination was made April 12, 1922, as follows:

Hemoglobin 77.0 grams per liter; 71% of normal for age.

Erythrocytes 3,552,000, 56% of normal for age.

Color index .8

Poikilocytes, microcytes, megalocytes, normoblasts, microblasts, poikiloblasts, megaloblasts, all present; megalocytes more abundant than microcytes; megaloblasts more abundant than normoblasts.

Leucocytes, 500,000 per cu.mm. Differential count difficult on account of the abundant reversionary and atypical forms, and on account of atypical staining reactions and the speedy degeneration of many forms of cells. Fragmented cells and naked nuclear masses were abundant. The following figures were secured with difficulty:

Large hyaline	.34%	1,700 per cu.mm.
Small hyaline	80.0 %	400,000 per cu.mm.
Mononuclear neutrophiles	.06%	300 per cu.mm.
Polymorphonuclear neutrophiles	.78%	3,900 per cu.mm.
Eosinophiles	.02%	100 per cu.mm.
Basophiles, very few		
Myelocytoid and atypical	18.8%	94,000 per cu.mm.

The cells degenerated very rapidly on the warm slide, but smears fixed immediately as the blood left the capillaries showed also a great abundance of degenerated cells, so that the processes of degeneration were evidently occurring within the blood vessels. In May, 1922, Mr. H. left the city. His death was reported in July of that year.

In this case no definite bony lesions were present. No adequate etiological factors could be found, either in the structural relations, the hygiene, or the clinical history of the patient to account for the condition. So far as history could be secured and so far as any laboratory diagnosis or physical examination could determine, there was only an ordinary history of the life of an ordinary farmer in good circumstances. His work had not been exhausting and his diet and his habits were wholesome.

Some few areas of slight rigidity were found on examination and the treatments were devoted to relieving these. Some slight dietetic changes were advised. These methods were followed by the slight general improvement noted at the second blood test. The inadequacy of these methods so far as affecting the progress of the disease was concerned was recognized by the osteopathic physician in charge of the case, and only symptomatic good results anticipated.

Until further study has clarified our understanding of the nature of such cases as this, palliative measures only can be employed.

HODGKIN'S DISEASE

This disease remains essentially unexplained. It presents the characteristics of a granuloma involving lymphoid tissues almost or quite exclusively. The cornybacterium Hodgkini was at one time supposed to be the etiological agent. Several other etiological agents have been described. It has been reported in young children, even in babies three months of age. These cases are acute and death is rarely delayed for more than a few weeks after the first symptoms appear. It has been supposed to be an aberrant form of tuberculosis of the lymph glands. Several authors have considered it to be really a neoplasm. None of these hypotheses has been verified.

Usually the lymph nodes of the neck are first enlarged, then other lymphoid tissues are affected in turn. Sometimes the enlargement appears first in other lymph nodes of the body, and occasionally several widely separated areas may be affected at the time. Always the lymphoid tissues are affected; metastases in tissues not containing lymphoid tissue are extremely rare, and usually the disease follows some chain of lymph nodes in its extension. Symptoms are indeterminate. There is no pain in the affected lymph nodes unless adjacent nerve trunks or sensitive tissues are subjected to the pressure of the enlarged lymph nodes. Weight is not usually affected. There is some weakness and late in the disease toxic symptoms occur. Remissions are common but death from the disease is inevitable unless life is terminated by some intercurrent acute disease or by accident.

Hodgkins disease may begin insidiously with no recognizable symptoms before the glandular enlargement is noticed. Often an attack of pharyngitis or tonsillitis or some other acute infection seems to initiate the disease. Itching of the skin is often very distressing. Bronzing suggesting Addison's disease often occurs. Cough and dyspnea may be due to intrathoracic lymphatic enlargement.

Acute Hodgkins disease may cause death within a few weeks. Chronic Hodgkins disease with atypical symptoms may not be recognized during life, and death be due to intercurrent disease. At autopsy the condition is recognized.

More commonly the disease is moderately chronic and death results from it within two to five years.

Pathology

The changes in the lymph nodes are characteristic, and in many cases only a biopsy of an enlarged lymph node determines the diagnosis. The microscopic picture varies with the progress of the disease in any given node, and if several nodes in different stages of involvement are examined the various stages of the disease can be recognized.

In the earliest stage the only change in the lymph node is an increase in the number of small hyaline cells. This stage does not indicate the nature of the disease since the same changes might be found in any chronic infection involving lymphoid tissue.

Next there occurs a great hyperplasia of the reticulo-endothelial cells with the development of masses of large round cells, very feebly basophilic, with large rounded vesicular nuclei. These cells are pathognomic. They are not found so abundantly in any other disease. Associated with these are the multinuclear and very large cells called

"Dorothy Reed" cells. These contain four to a dozen or more roundish nuclei crowded together; the nuclei are intensely basophilic. At this stage there are usually many mononuclear and polymorphonuclear eosinophiles. In no other disease of lymphoid tissue are the eosinophiles of the lymph nodes so abundant and so immature in structure.

The connective tissue cells of the trabeculae of the lymphoid tissue show increasing hyperplasia. In the later stages of the disease the connective tissue crowds out the other cells so that only a small mass of scar-like tissue remains at the site of the lymph node.

The spleen is rarely affected early in the disease. In later cases the spleen shows rather a patchy involvement so that on microscopic examination the cut spleen shows areas of a suet-like or fatty appearance in which the changes found in the lymph nodes are recognizable on microscopic examination.

The thymus, bone marrow, mesentery and other parts of the body in which lymphoid tissue is normally present may become involved in the disease.

Blood Changes

The blood picture is not distinct, but by repeated and careful examinations it is often possible to determine the diagnosis without biopsy of a lymph node. Eosinophiles are usually increased moderately and they may reach remarkably high numbers; hyaline cells are relatively increased; large hyaline mononuclears are considerably increased; endothelial cells are present in varying numbers and these are often of abnormal type, being derived from the cells of the affected lymphoid tissues. It is not often possible to find these abnormal cells in a differential count of the ordinary type, but on making a differential count of two thousand to four thousand cells the abnormal cells are apt to be found in sufficient numbers to warrant at least a tentative diagnosis of Hodgkins disease. The blood platelets are usually increased, sometimes to five hundred thousand per cubic millimeter.

Anemia occurs after the disease has been present for some weeks or months. This is usually secondary in type, but occasionally the anemia may resemble the chlorotic or the pernicious form.

Leukemia often follows or is associated with the later stages of Hodgkins disease.

Illustrative Case of Hodgkins Disease

Miss A., late stage with leukemic symptoms. Death in October. Blood count in March with a differential count based on examination of 3,000 cells.

Hemoglobin 87 grams per liter; 60% of normal
Erythrocytes 4,000,000 per cubic millimeter, 80% of normal
Color index .6
Poikilocytes abundant
Microcytes, abundant
Megalocytes, none
Normoblasts, 20 per cubic millimeter
Other abnormal red cells, none

Leucocytes		24,000 per cu.mm.
Large hyaline	4.0%	960 per cu.mm.
Small hyaline	6.0%	1,440 per cu.mm.
Mononuclear neutrophiles	3.3%	792 per cu.mm.
Polymorphonuclear neutrophiles	77.0%	18,480 per cu.mm.
Eosinophiles	9.7%	2,328 per cu.mm.

Basophiles and amphophiles, very few
Hyaline myelocytes and reticular cells, abundant
Granular myelocytes, very abundant
Endothelial cells, many
Degenerated forms, unrecognizable, few
Neutrophile nuclear average 1.47
Leucocytes began to move after three minutes on the warm slide. Neutrophiles moved very feebly. Hyaline cells showed no activity. Eosinophiles showed greater activity than normal.
Immature and reversionary forms abundant.
Fibrin threads long, heavy, formed completely at once.
Fibrinolysis absent
Serum tinged with a pigment which does not give reaction for bile and is probably a derivative of hemoglobin.
Gregariousness marked.

ATYPICAL AND UNUSUAL CONDITIONS

There are several conditions which show atypical symptoms or atypical blood findings. In some instances these may be merely symptom-groups, in others the same disease has probably received different names by different authors. No satisfactory pathogenesis is known. Studies made of these diseases in our laboratories have not resulted in any satisfactory understanding of the conditions. Very much more study is necessary in order that these various symptom-groups may be classified and their etiological and pathological relations explained.

Mixed Leukemia

Occasionally the blood picture in leukemia includes many small hyaline cells and also many myelocytes and myeloblasts. Basophilic and eosinophilic myelocytes are also present, but less abundantly than in typical splenomyelogenous leukemia. This form of leukemia has been found in early cases. Many myelocytes are frequently found in the late stages of lymphatic leukemia, and a diagnosis of mixed leukemia should not be made when the first examinations are made for a patient apparently near death, or in whom anemia is very profound. In late cases of splenomyelogenous leukemia many very small hyaline myelocytes are present. These are to be differentiated from lymphocytes only by the oxidase reaction; even when this test is employed there may be errors due to the fact that in exhaustion of the bone marrow the oxidizing ferment may be absent from many cells and especially from the immature forms. So a diagnosis of mixed leukemia should not be made in any case unless the first blood examinations are made before any evidences of marked anemia or of exhaustion of the bone marrow appear.

In children very acute leukemias are characterized by many hyaline and many myelocytic forms, so that it may not be possible, even at necropsy, to differentiate between the lymphatic and the splenomedullary types.

Leukanemia

Leukanemia is a disease usually secondary to some very severe infection such as severe malaria or pyogenic infections; or to some severe cachectic disorder, such as cancer of rapid growth; or to repeated injuries or hemorrhages which make great demands upon the hematopoietic tissues.

In late stages of either lymphatic or splenic leukemia there may occur an anemia of the pernicious type. In late stages of pernicious anemia there may be marked leucocytosis with abundant myelocytes. When the patient is first seen after the development of the conditions, a diagnosis of leukanemia may be the only one possible.

The red blood cells show the changes characteristic of pernicious anemia. The white blood cells show the changes typical of either lymphatic, splenomyelogenous or mixed leukemia.

Status Lymphaticus

Status lymphaticus is a congenital anomaly in which there is some developmental defect of the autonomic nervous system, certain ductless glands, the bones and the circulatory system. The thymus fails to

atrophy and the child is seriously injured by relatively insignificant poisoning, injury or nervous shock. Sudden deaths during anesthesia or as a result of comparatively trivial injuries are common among these children. The administration of serums is especially dangerous to them.

The blood shows relative and often absolute lymphocytosis, with increase in the number of large hyaline cells and endothelial cells. The hyaline cells reached 70% of the total leucocyte count in several of our cases. Red cells, hemoglobin and fibrin are normal in uncomplicated cases.

Chloroma

This is a form of leukemia in which there are metastases in bones, most commonly of the skull of the orbital region. Exophthalmos is a common symptom. The tumors produced by these metastases present a greenish tint, hence the name.

The blood shows the characteristics of splenomedullary leukemia. Cases with the findings of lymphatic leukemia have been reported, but these were probably late splenomedullary leukemia with a large number of hyaline myelocytes of immature type. The leucocyte count may be only moderately increased, or may reach 500,000 or more. The myeloblasts are more common than myelocytes in typical cases.

A peculiar tendency associated with the tumors of chloroma is an associated hyperplasia of the tissues invaded by metastases. The invasion of the skull by metastases may produce pressure on the brain with symptoms referable to the area affected. Metastases in the long bones may be associated with spontaneous fractures of the weakened bones.

The disease is usually acute and death occurs within a few months from the time of the first noticeable symptoms. No treatment has any satisfactory effect.

Aleukia

Aleukia is a peculiar condition associated with aplasia or atrophy of the red bone marrow. The granular leucocytes are greatly diminished. The red cells present the usual findings noted in aplastic anemia, with no evidence of regeneration. Blood platelets are greatly diminished. Subcutaneous and submucous hemorrhages are abundant and death often occurs from gastric or nasal hemorrhage. Death usually occurs within a few days after the first hemorrhage. No treatment has any good permanent effects. Transfusion of blood sometimes delays the inevitably fatal outcome.

Aleukemic Lymphoma

Occasionally there is enlargement of the lymph nodes with symptoms of lymphatic leukemia, but the blood itself shows no lymphemia. In such cases a careful study of many slides usually finds a few abnormal large hyaline cells.

Anemia of the secondary type is usually present. Fibrinolysis is absent.

Aleukemic lymphoma may be an early stage or an intermission in lymphatic leukemia, or it may be unassociated with leukemia at any time. Anemia of secondary type is usually present. Rarely the blood seems normal. The nature of the disorder is unknown.

Glandular Fever

This is a disease which is most common in children. It is characterized by irregular attacks of fever or feverishness, with swelling of the lymph nodes. The disease itself is chronic but the separate attacks are acute and may be quite severe.

The blood shows typical findings. The hyaline cells are considerably increased, both actually and relatively, and many atypical forms are present. These include—

Immature lymphocytes in which the chromatin is arranged in fine masses with a delicate network between them. Sometimes one to three nucleoli are present.

Abnormally large lymphocytes which are apparently derived from the germinal centers of the lymph nodes and the spleen.

Monocytes of atypical staining, containing azure granules in scanty protoplasm which is either very deeply or very feebly basophilic.

Hyaline myelocytes which sometimes contain a very few small, feebly eosinophilic granules and which give the oxidase reaction.

The red cells are normal. Hemoglobin may be slightly diminished. Fibrin is scanty. The coagulation time is somewhat prolonged.

The attack usually begins with a mild degree of malaise and feverishness. The lymph nodes of the neck may enlarge slightly. Other lymph nodes rarely increase in size. Moderate splenic enlargement occasionally can be found. The thymus often persists in children subject to glandular fever, sometimes into adolescence.

No vertebral lesions are found of etiological importance, except that such lesions lower resistance generally. The children subject to glandular fever usually do have vertebral lesions, but these vary from week to week. There is an associated weakness of the ligaments of the entire body, in many cases.

Osteopathic treatment devoted to such structural abnormalities as can be found often relieves the attack and hastens the ultimate disappearance of the attacks.

During adolescence the attacks diminish in severity and in frequency until they do not occur at all.

Agranulocytic Angina

This is a disease of early or late middle life, and is most common among women.

The disease is initiated by a high fever and the development of ulcers, resembling those of diphtheria, in the throat. Rarely the vagina, intestines, skin and cheek show the ulcers.

On blood examination the neutrophiles are found absent or present only in very small numbers. A high percentage of large mononuclear cells with finely granular, feebly basophilic or feebly eosinophilic protoplasm is found. The total leucocyte count rarely exceeds 4,000 cells per cubic millimeter of blood and counts of less than 1,000 cells are common. Lymphocytes are present in normal numbers absolutely but are relatively increased because of the low total count. The red blood cells are not perceptibly affected.

No cases have been reported under osteopathic care.

Death is to be expected within a few days after the onset of the symptoms.

By several authors this is not considered an independent disease. It has been considered identical with the glandular fever of children, with infectious mononucleosis and with monocytic leukemia of fulminating type.

Infectious Mononucleosis

It occasionally occurs that the red bone marrow fails to meet the demands made by any very severe infections. Instead of neutrophilic leucocytosis the neutrophiles diminish or even disappear; and there is a marked increase in the mononuclear cells of the blood. These cells are myelocytoid, have large, round, vesicular nuclei, and their protoplasm presents a peculiarly granular appearance though no true granules are present. The protoplasm is feebly basophilic or feebly eosinophilic, and in many slides it is possible to find both tints present in adjacent cells. The origin of the cells is not known. The disease has been identified with agranulocytic angina by several authors.

Ulcers are not always present but some very severe infection is invariably the circumstance which initiates the abnormal state of the blood.

No cases have been reported in osteopathic practice.

Recovery is not to be expected. Death usually occurs within a few days or a few weeks, at most.

Myelomatosis

Myeloma (Kahler's disease) is a tumor of bone marrow, very malignant, with abundant metastases. It usually begins in the vertebrae or the ribs and extends rapidly to other parts of the skeleton. Aching is the first symptom in most cases. Cachexia, severe pains, paralysis and death follow during a few months.

The blood shows secondary anemia with changes in the cells which seem to depend upon the character of the tumor. The blood may show the picture of pernicious anemia, of splenomedullary leukemia or of secondary anemia with only a few nucleated red cells and a few myelocytes. Eosinophiles are usually increased, as in any disease of the red bone marrow. The presence of the Bence-Jones albumose in the urine is an important factor in diagnosis.

Any malignant neoplasm may invade the red bone marrow and produce similar symptoms including the presence of the Bence-Jones albumose in the urine. In such cases the blood shows the picture associated with the malignant neoplasms plus an unusual number of myelocytes, eosinophiles and nucleated red cells.

Multiple myeloma has been reported for a gopher (Citellus grammurus) by Fox.

Lymphosarcoma

Sarcoma may arise in any lymph node. The cells show abundant and often irregular karyokinesis. The capsule of the node is invaded and the cells escape into surrounding tissues, as in any other malignant neoplasm. Metastases are abundant, and they tend to invade other lymphoid tissues rather than non-lymphoid areas.

When the cells are small, resembling small lymphocytes, the tumor is called malignant lymphocytoma, or small round cell lymphocytoma.

When the cells are large and have arisen from endothelial cells of the lymph node the tumor is called a reticulum-cell sarcoma or a large round-cell sarcoma.

A small round cell sarcoma, such as may occur in other tissues, sometimes arises from the connective tissue cells, usually of the trabeculae of the lymph node or the adventitia of its vessels.

The possibility that a lymphocytoma may arise from an aleukemic lymphoma has been shown by several pathologists.

In all these cases the blood may show only a secondary anemia with toxemia. Fibrinolysis was absent in all the cases so far examined in our laboratories.

When the lymphocytoma has arisen in the intestinal lymphatics the blood often shows lymphemia. In other cases it is usually possible to find abnormal hyaline cells in the blood smears. In cases of malignant lymphoma the cells may be differentiated from ordinary lymphocytes with difficulty. The finding of dividing small hyaline cells in the circulating blood, especially if the karyokinesis is irregular in type, should suggest the diagnosis rather definitely.

Endothelioma of the lymph nodes occurs rarely, and most commonly follows some chronic infection and granuloma. The blood shows secondary anemia but it is rarely possible to find typical endothelioma cells in the circulating blood.

Illustrative Case

Mrs. P. Childhood history and heredity present no unusual factors. An attack of acute nephritis at the age of sixteen years followed severe cold. The nephritis became chronic and persisted for three years. Treatment given for this disorder was chiefly dietetic. Within a few months after the symptoms of nephritis disappeared the lymph nodes of the neck enlarged. These soon disappeared. At the age of twenty a lump appeared on the shoulder, beneath the muscles. The location could not be definitely described. The tumor disappeared within a few weeks after several X-ray treatments. Tumors appeared in nearly the same region, over the left scapula, between the tip of the shoulder and the back of the neck and in adjacent areas during the years between the twentieth and the twenty-fourth years. Various methods of radium and X-ray treatment were employed, with relief of the pain and pressure symptoms and diminution in the size of the tumor. Her health was excellent during the twenty-sixth and twenty-seventh years and she married. No pregnancy occurred. Two years later the tumors recurred around the shoulder, and extended around the neck to the right side. Other small tumors appeared in the neck and within the abdomen. During the next year an attack of influenza was followed by rapid growth of the tumors and by increasing weakness, toxic symptoms, great pain apparently due to pressure, and by increasing nervousness and insomnia. The tumors vary in size from week to week with no recognizable cause therefor. The pain varies greatly, and is not always worse when the tumors are largest.

Wassermann reaction negative; five tests had been made during the progress of the disease.

Blood chemistry. Tests made after 12 hours fast.

Non-protein nitrogen	45.9 mgs. per 100 c.c. of blood
Urea nitrogen	23.0 mgs. per 100 c.c. of blood
Uric acid	4.0 mgs. per 100 c.c. of blood
Creatinin	2.3 mgs. per 100 c.c. of blood
Sugar	97.0 mgs. per 100 c.c. of blood

Uranalysis same date showed usual findings in mild chronic nephritis. Urea, sulphates, chlorides, phosphates, uric acid, creatinin all corresponded to the reported diet for two days before the collection of the urine and the day during which the urine was being collected.

Blood cell examination made the same day showed the following picture:

Hemoglobin, 91.0 grams per liter; 66% of normal for age and sex
Red cells, 3,300,000 per cu.mm., 73% of normal
Color index .9
Poikilocytes, microcytes, normoblasts, few
Other abnormal red cells, none
Hemoconien, increased
Platelets, 350,000 per cu.mm.
Specific gravity, diminished slightly
Osmotic tension increased slightly

Coagulation time, ten minutes (normal for method employed, five minutes)

Leucocytes		29,400 per cu.mm.
Large hyaline	4.6%	1,352 per cu.mm.
Small hyaline	8.0%	2,352 per cu.mm.
Monocytes,	.6%	177 per cu.mm.
Polymorphonuclear neutrophiles	86.0%	25,284 per cu.mm.
Eosinophiles	.8%	236 per cu.mm.

Hyaline myelocytes, abundant
Granular myelocytes, abundant
Endothelial cells, abundant
Neutrophile nuclear average 2.04
Iodophilic cells and iodophilic granules, abundant
Malarial and other parasites, none

On the warm stage the leucocytes began to move at once very rapidly. They grouped themselves into fours to nines, usually each type

of cell together. They were dead within twenty minutes (should live more than an hour). Red cells inelastic, fragile. They were arranged in groups of twenty to one hundred cells, irregularly, with slight tendency to normal rouleaux arrangement.

Leucocytes included many reversionary forms. Anaplasia marked. Fibrin formed at once, very abundantly. Fibrinolysis absent.

During the next week seven X-ray treatments of unknown type were given with slight change in the size of the tumors and with moderate relief of the pain. The blood examination twelve days after the last X-ray treatment gave the following findings:

Hemoglobin 83 grams per liter
Red cells 3,520,000 per cu.mm.
Color index .77
Abnormal red cells and other general findings as before
Platelets, 100,000 per cu.mm.
Leucocytes 14,400 per cu.mm.

Large hyaline	6.0%	864 per cu.mm.
Small hyaline	7.5%	1,080 per cu.mm.
Monocytes	.3%	43 per cu.mm.
Polymorphonuclear neutrophiles	83.0%	11,952 per cu.mm.
Eosinophiles	3.2%	461 per cu.mm.

Neutrophile nuclear average 2.0
Anaplasia and reversionary forms more abundant than before.
Fibrin threads formed at once, more abundant than before.
Fibrinolysis masked by undifferentiated proteolysis.

Notes. In this case a diagnosis of sarcoma is indicated. The leucocytosis is due to extension of the metastases to the peritoneal cavity.

CHAPTER XI

RESUME OF THE SIGNIFICANCE OF CHANGES IN BLOOD CELLS

A brief list of the changes in the blood cells most commonly reported, with the significance of these, may be of some practical value. This list is suggestive rather than definite and it is given in the hope that it may serve to call attention to pathognomonic findings easily over-looked.

Reports of examinations of blood cells made in professional laboratories usually include some discussion of the significance of the findings, especially if the laboratory staff has been consulted in advance, and if the history of the patient and the findings on physical examination have been fully given. Ordinary commercial laboratories are not usually able to give this service, which is of considerable importance.

The osteopathic physician who wishes the most efficient assistance from laboratory tests must select his laboratory with care. Laboratory workers are specialists and consultants; if they are consulted about the patient's condition they can often advise the most useful tests to be made, and can give instructions concerning the best time for taking the blood and for preparing the patient. The changes which occur in the blood cells as a result of fatigue, digestion, dietetic peculiarities, changes of altitude and other physiological conditions have been described in previous chapters. In cases of severe acute or well-marked chronic states, such as acute infections or the leukemias, a diagnosis may be possible no matter when the blood is taken. But in those cases in which diagnosis is difficult, the patient must be rested, not recently fed, not menstruating, not recently subject to sexual excitement and not under the influence of any emotional or therapeutic circumstances. Under certain conditions it is best to have the patient come for blood-cell examinations just after an osteopathic treatment; this is especially true in cases of malaria and in early leukemias.

The indications are here given very briefly and further study is necessary in order to verify the correct diagnosis and to eliminate other possibilities.

Hemoglobin

Diminished hemoglobin suggests the anemias, malnutrition, dilution of the blood. Increased hemoglobin suggests dessication, concentration

of the blood, cardiac disease, certain pulmonary disorders, polycythemia, diabetes insipidus or mellitus. Abnormal tint of blood suggests carbon monoxide poisoning, cholemia, carotinemia, other more rare conditions.

Red Cells

Diminished red cell count suggests the anemias, malnutrition, dilution of the blood. Increased red cell count suggests cardiac or pulmonary disease, diabetes mellitus or insipidus, dessication, polycythemia.

Color Index

Low color index suggests secondary anemia or chlorosis, malnutrition, disturbance of the circulation through the red bone marrow. Present also in atypical primary anemias. High color index with low hemoglobin indicates pernicious rather than secondary anemia; is present also in certain poisonings and in secondary anemia due to certain parasites; occasionally also in malignancy.

Abnormal Red Cells

Poikilocytes are especially abundant in chlorosis, sickle-cell anemia, secondary anemias. Anisocytosis is especially marked in pernicious anemia. Normoblasts are abundant after hemorrhages and during rapid blood regeneration. Megaloblasts are especially abundant in pernicious anemia and lead poisoning; may be present in severe anemia of any type.

Aniscocytosis more marked than poikilocytosis, suggests pernicious rather than secondary anemia. Megaloblasts more numerous then normoblasts suggests pernicious rather than secondary anemia. Oval forms indicate long-continued secondary anemia of mild degree or a developmental anomaly. Basophilic reticulation indicates immaturity; present abundantly when blood is being rapidly regenerated. Basophilic degeneration indicates senility of blood cells, present in toxemia with alkalosis.

Blood Platelets

Increased in dessication and in polycythemia. Diminished in purpura, in the blood of certain "bleeders," in pernicious and aplastic anemias, malnutrition, and certain conditions affecting the nutrition of the red bone marrow. Diminished when food lacks proper amounts of vitamin A.

Coagulation

When coagulation time is increased, hemophilia, purpura, malnutrition, and certain developmental peculiarities are suggested. When the clot is soft and inefficient hepatic disease or malnutrition is indicated. Delayed or imperfect coagulation present in many abnormal conditions.

Leucocyte Count

Increased moderately during early afternoon and as a result of many physiological conditions. Increased in infections, especially pyogenic states, in dessication, cardiac and pulmonary disease, non-infectious irritation of peritoneum or pleurae, leukemias, secondary anemias, after surgical operations and after dressing of wounds.

Leucopenia suggests pernicious anemia, aplastic anemia, effects of radium or X-ray therapy or of certain poisons. Is usually present during recovery from infectious diseases.

Hyaline Cells

Never absolutely reduced excessively except by radium, X-rays or drugs. Relatively reduced when neutrophiles are absolutely increased. Absolutely and relatively increased in pernicious and aplastic anemias, certain deficient diets, fasting, toxemias, typhoid fever, pertussis, rickets, measles, paresis, tuberculosis, persistent thymus, at certain stages in many acute infectious diseases and in influenza, malaria, blastomycosis, lymphatic leukemia.

Lymphocytosis plus eosinophilia suggests syphilis, tuberculosis or malignancy; also complications of two or several pathogenic conditions.

Abnormal hyaline cells, especially immature forms, suggest chronic inflammatory processes involving lymphoid tissues. Hyaline cells of extravascular origin include those derived from the placenta, cancers and sarcomas.

Hyaline cells of normal structure increased, with no other findings abnormal, may be a developmental anomaly or may be due to excessive carbohydrate feeding.

Neutrophiles

Diminished after infection, after treatment by X-rays or radium, after use of certain drugs. Diminished in pernicious and aplastic anemias and in diseases of the red bone marrow, in measles, typhoid fever, influenza, malaria, splenic anemias and certain forms of tuberculosis. Relatively diminished in lymphatic leukemia.

Increased moderately in certain physiological states and in early morning and early afternoon hours. Increased in nearly all acute infections and in pyogenic processes. Increased very rapidly in pneumonia, meningitis, diphtheria, rheumatic fever, peritonitis and leukemia.

Neutrophiles of immature form including many myelocytes, total count from subnormal to great numbers, indicates splenomedullary leukemia. Immature and atavistic forms are a developmental anomaly in some cases; immaturity increases with severe acute or chronic disease.

Neutrophilia without eosinophiles, "septic factor," indicates pyogenic infection. Neutrophilia with eosinophilia suggests gonorrheal infection, tuberculosis, ovarian or tubal disease, complication of pathogenic agencies.

Nuclear Average

Below 2.4 in adults suggests rapid development of neutrophiles as in early infections or in the leukemias. Above 2.55 in adults, suggests rapid degeneration of neutrophiles, as in late infections or virulent infection with inadequate reaction, in toxemias, late severe fatigue and late severe malnutrition. Nuclear average is lower in children and the figures must be interpreted according to age.

Eosinophiles

Eosinophiles are diminished in all ordinary pyogenic infections. Leucocytosis plus normal or increased eosinophiles suggests that pyogenic infections may be gonorrheal.

Eosinophiles are increased usually after subsidence of pyogenic infections and during active infections of gonorrhea, scarlatina and nearly all infectious eruptive diseases. Nearly all animal parasites and all conditions causing irritation of the skin or the mucous membranes cause eosinophilia. Splenomedullary leukemia and bronchial asthma are associated with marked eosinophilia.

Eosinophiles of immature form and eosinophilic myelocytes indicate some disorder of ovaries or testes. These are present also when bony lesions affect the red bone marrow, ovaries, testes and spleen.

Basophiles

Basophiles are not abnormally diminished. Increased in the leukemias, diseases affecting the thyroid and pancreas, in Asiatic cholera and sometimes in hepatic disease. Increased when bony lesions affect the circulation through the red bone marrow.

Myelocytes

Neutrophilic myelocytes are especially abundant in splenomedullary leukemia. Present in acute infections, especially after long and exhausting infectious diseases. Present in pernicious anemia and in severe secondary anemias, and in lesions affecting the circulation and innervation of the red bone marrow.

Endothelial Cells

Inflammation is indicated when many endothelial cells are found. Abundant in certain leukemias. The location of the inflammatory process may be indicated by other findings.

Iodophilia

Iodophilic cells and granules indicate degeneration of protein materials, usually derived from pyogenic foci, tumors, or intestinal contents undergoing putrefaction. Present in severe anemias and acute leukemias.

Warm Slide Tests

Leucocytes inactive; toxemia, fatigue and malnutrition of long standing are indicated. Leucocytes show increased activity; early fatigue, recent, mild toxemia, early days of fasting or starvation suggested. Leucocytes die quickly in all these conditions.

Fibrin threads long, heavy, regular in outline, formed very speedily, usually at once. This suggests pneumonia or other acute pulmonary infections. Less abundant in other acute infectious diseases.

Fibrin threads formed at once, heavy, irregular in outline or beaded, radiating arrangements. Malignancy is suggested, may be some other protein degenerative processes.

Fibrin threads abundant, formed at once or later, arranged in vague, irregular net-like structure. Marked disturbance in protein katabolism, absorption of products of pyogenic foci or of degenerating tumors suspected.

Red cells fragile and lake quickly; cholemia, other toxemias, malnutrition, all of severe degree are suspected.

Rouleaux delayed or abnormal, malnutrition or toxemia of almost any form is indicated. Red cells form islet-like groups instead of rouleaux in severe toxemia, especially that due to disurbances in protein metabolism; malignancy suggested.

Pseudopodia long, slender, erratic in development, non-purposive; some irritant toxic substance is present. Hyperthyroidism suggested. Early fatigue, moderate fevers, irritating drugs might be considered.

Pseudopodia short, flat, blunt, inert; toxemia of long standing, late malnutrition. In marked inactivity, hypothyroidism suggested.

Gregariousness

Cells arranged in groups; suspect cardiac disease; some considerable area with disturbed circulation, bony lesions affecting the spleen, liver and red bone marrow.

Fibrinolysis

Normal fibrinolysis probably protects against development of malignant neoplasms. Absent fibrinolysis probably means lack of such protection. Fibrinolysis is masked by undifferentiated proteolysis in late malignancy, high fevers, severe malarias, severe intestinal toxemias.

Resistance of Red Cells

Red cells more resistant to saponin, weak acetic acid or hypotonic salt solutions. This suggests secondary anemias, syphilis, tuberculosis, myeloid leukemia, polycythemia; noted after splenectomy and in certain diseases of the spleen and the liver.

Red cells are normally resistant to such solutions in pernicious anemia, diabetes, exophthalmic goiter and most infections.

Red cells show diminished resistance to such solutions in high fevers and in jaundice or cholemia.

Serum

Very pale; secondary or aplastic anemias suggested. Starvation may be cause.

Brownish; anemia probably pernicious; cholemia, severe malaria, certain rare tumors are suggested.

Greenish; carotinemia or cholemia is indicated.

Reddish; chronic carbon monoxide poisoning, hemolytic infections; hemolytic drugs or certain rare poisons or hemoglobinemic diseases.

CHAPTER XII

TECHNIQUE OF BLOOD CELL EXAMINATIONS

Estimation of Hemoglobin

The amount of hemoglobin in the blood is estimated by means of some kind of color scale. Different instruments use different types of scale and different methods of preparing the blood. Older methods based upon chemical analysis for iron are much less accurate and are very cumbersome; they are not suitable for ordinary laboratory work in diagnosis.

The method of securing the blood is the same for all the newer methods of determining hemoglobin.

It is important to secure the drop of blood in such a manner as to prevent its changing from its condition within the vessels as little as possible. The lobe of the ear, the palmar surfaces of the fingers and the balls of the toes are almost devoid of sensory nerves. The skin at the sides of the fingers is thinner than that upon the heavier parts of the balls and is often less sensitive. In persons of certain occupations, such as piano or violin playing, the finger tips may have thick skin. It is necessary to avoid any area which is more than usually liable to injury after the prick is made, the violinist might find some difficulty in playing with the injured finger for a few hours after the prick has been made, for example. The lobe of an ear is the best site in adults, the side of a toe is best in babies.

Having selected the site of the puncture, the skin should be cleaned in any way that is convenient. Probably a good washing with clean water is best for ordinary conditions. Various antiseptics are sometimes employed, but these must be sufficiently dilute to prevent irritation to the skin; this means little or no antiseptic value. Any of the ordinary antiseptics strong enough to injure bacteria injure the tissues still more seriously. But a washing with anything which removes the dust and perhaps a few desquamating epithelial cells takes away the chief source of infection and does not prevent the most speedy repair. Care must be taken in the washing that the skin is not irritated enough to cause overfilling of the vessels since this modifies the hemoglobin content.

The first drop of blood must be wiped away, and the second or later drops taken up in the manner suitable for each instrument. The tissues must not be squeezed or handled roughly but the blood must ooze gently and freely from the puncture.

The puncture is best made with a sharp, angular lancet. The lancet which is provided with a spring makes a sudden thrust which is not painful at all. A pen which has been broken longitudinally in half, so that only one sharp point remains, is useful. A dozen or more pens can be prepared and sterilized and a new one used for each patient. Glove needles, being triangular, make a sharp, clean puncture which heals at once. Round needles are not quite so suitable because they cause more pain and leave a rougher puncture; the slight bruising which occurs may affect the concentration of the blood in the vicinity of the cut. The error due to this factor is negligible in most cases.

The blood drop as it wells up from the tiny wound made for the purpose gives a general idea of its hemoglobin content, by its color. When the blood is more viscid, this drop stands up round and high, and appears to be richer in hemoglobin than it really is, when it is of low viscidity it spreads out over the surrounding skin in a thinner layer, and appears to be lower in hemoglobin content than it is. The character of the skin modifies the height of the drop, also, as does the size of the wound and the rapidity of the flow.

The simplest, cheapest, and easiest method of estimating the hemoglobin is by means of the Tallquist Hemoglobin Scale. It consists of a scale of ten colored paper slips for comparison, each corresponding to a certain percentage of normal hemoglobin, and strips of filter paper of uniform thickness to take the blood. In using this scale the drop is secured by the usual means, and a strip of the filter paper laid gently and quickly over the drop, until the blood is soaked up into the paper over an area about one-fourth of an inch in diameter. This is held for a few seconds or until the first shiny look of the blood stain has disappeared, then it is compared with the color scale. Some of the scales have an opening in the center of each of the ten color-tint blocks, and the blood-stained paper is then placed beneath these openings, one after another, up and down the scale in succession until the matching tint of the scale is found. If the blood is apparently half way between two blocks as 70% and 80%, the hemoglobin may be considered as 75%. In this way, those who are experienced in the test can make estimations of the hemoglobin which are fairly useful though they cannot be considered accurate within more than 20% under the best of circumstances in the most skillful hands. The method is perhaps the most convenient of all, since there is no apparatus to wash, no special light requirements are necessary and the scale is so small that it can easily be carried in the pocket. Its lack of accuracy is the only factor against its use. The method is useful for a preliminary test or in emergency cases.

Next in ease of use is Dare's Hemoglobinometer. This instrument has a revolving color scale of glass, a telescoping tube for securing distinct vision, and two slips of glass which hold a measured film of blood between them. The slips of glass are placed in the holder and screwed closely but not too tightly together; the blood flows between them by capillary attraction. The holder then slips into its place at the back of the instrument. The telescope is pulled out until the edges of the two circles show very plainly and in good focus. The revolving color scale is moved by means of the screw at the upper part of the instrument, until the tint of the two circles appears exactly the same. The amount of hemoglobin is read off in percentages of the normal from a scale which is attached to the side of the glass circle, and which is seen through a tiny window at the side of the instrument.

In using the Dare's instrument it is necessary to have a yellowish light, not too brilliant, and to make the examination in a rather dark room. It is better to look with the eyes alternately, thus giving each eye an opportunity to rest, in turn. The correctness of the reading may be gauged by the fact that one is able to return to about the same figures several times in succession, after changing the position of the scale and resting the eyes. It is probably correct within 5% of the actual amount. This scale is made so that blood containing 137.7 grams per liter is considered as 100%, or normal. It is evident that in using this instrument correction must be made for the age of the patient.

Gower's hemoglobinometer is not now in general use. It consists of three tubes, one filled with gelatine stained with picrocarmine to be used for artificial light, one filled with gelatine stained slightly differently, to be used with daylight, and one to be filled with diluted blood, the latter graduated. The graduated tube is filled to a certain point with blood, then water is added until the tint exactly matches the tube filled with gelatine, using the one or the other according as the light is artificial (yellowish) or is daylight. The dilution is difficult; the gelatine tubes fade quickly if they are exposed to the light often (as they must be if they are used at all frequently) and the newer instruments are much more accurate and convenient.

Sahli's hemoglobinometer is somewhat like Gower's, but has certain advantages over the older instrument. It consists essentially of two tubes, one filled with a stock solution of 1% acid hematin, and one tube for the blood. Twenty cubic millimeters of blood are taken into the blood tube, and this is mixed with deci-normal hydrochloric acid.

(15 c.c. HCl: 1 liter H_2O is sufficiently accurate). The acid changes the hemoglobin of the blood into the stable acid hematin. The blood mixture is then to be diluted with water until it matches accurately the color of the stock solution in the other tube. The advantages of this instrument are that it can be used in any light; since both the stock solution and the blood mixture are colored by the same compound—acid hematin,—they are equally affected by different lightings, so that yellow light, daylight, dim lights or brilliant lights, all act upon both tubes in the same way. A moderate light, however, gives the most nearly accurate readings. This instrument is, in the hands of reasonably skillful observers, accurate within about five per cent. The readings are made in percentages of the normal, which is thus subject to the necessity for correction for the age of the patient. In this instrument 172 grams of hemoglobin per liter of blood are taken as 100%.

The most accurate, the least convenient and the most expensive hemoglobinometer is that of Fleischl as modified by Miescher. This has for its scale a long, slender, delicate glass bar which is thick at one end and thin at the other, so colored as to be spectroscopically identical in color with hemoglobin. This has a scale connected with it, and is set beneath a stage which is perforated in its center. A second perforation near the edge of the stage permits the scale to be read easily. The colored prism is moved across the central perforation. Beneath the prism is a reflecting dull white surface and a candle or other dim yellowish light is placed in front of this dull reflector.

The blood is taken in a pipette somewhat like those used for blood dilution for counting, except that the hemoglobinometer pipette is so graduated that dilutions of 1-200, 1-300, or 1-400 can be made. Dilution is made with 0.1% sodium carbonate; ordinary tap water is really better, in our experience.

If the blood appears very pale as it emerges through the puncture use the dilution 1-200. If it appears almost or quite normal in tint use a dilution of 1-400. For milder anemias use 1-300 dilution. In taking the blood into the pipette keep the lower end of the pipette within the blood drop but do not allow it to touch the skin. If by accident the blood should be drawn above the selected mark on the tube, or should not quite reach that mark, there are accessory graduations by means of which adequate correction can be made after the work is completed. Draw the diluting fluid into the pipette to the mark at the top of the bulb, and then immediately thoroughly shake the pipette from side to

side. The shaking must be gentle, and the pipette must not be shaken endways lest some of the blood be forced into the capillary portions of the pipette.

Two chambers, each divided into compartments, are used for the examination. Each chamber has a glass cover and a glass bottom, and a perforated dark metal cap to be placed over the cover glass. One chamber is 12 millimeters in depth, the other 15 millimeters, and readings are made with both chambers.

Put the glass bottom into the 15 millimeter chamber and fix it in place by means of the screw-like arrangement provided for the purpose. Fill one compartment with water or the solution used for dilution. By means of a rubber bulb blow out and discard four drops from the pipette used for diluting the blood. (This removes the fluid in the capillary bore of the tube, which presumably contains no blood.) Still using the rubber bulb, fill the other compartment with the diluted blood. Be very careful that both compartments are completely filled, that none of the clear fluid reaches the blood compartment, and that none of the diluted blood reaches the clear fluid. Cover with the glass; there must not be any air drops in either compartment. Place the metal cap over the cover in such a manner that two equal squares are visible, one of the diluted blood, the other of the clear fluid. Place the chamber in the perforation provided for the purpose in the stage of the hemoglobinometer in such a manner that the clear square is exactly over the colored bar. Arrange the candle and the reflecting surface so that a dim light is thrown upward through the colored prism and the clear fluid, and through the diluted blood.

Move the prism back and forth by means of the screw until the two squares have exactly the same tint. Use first one eye then the other. Do this quickly; if too much time is taken vision becomes less acute. Read and note the figures on the scale. Move the prism away from matching tint. Close the eyes for a few seconds, then repeat the test. In this way make ten different readings. If the readings do not vary more than five points the readings are sufficiently accurate.

Fill the 12 millimeter chamber in exactly the same manner and make ten readings in the same manner.

Make an average of the ten readings from the 12 millimeter chamber and make an average of the ten readings from the 15 millimeter chamber.

Divide the first average (from the 12 millimeter chamber) by 4 and multiply by 5, this should equal the average made from the ten read-

ings from the 15 millimeter chamber. If the figures thus secured do not agree within five points the work is not sufficiently accurate and should be repeated.

Compare the figures secured, corrected for the 15 millimeter chamber, with a scale provided with the instrument. This gives the grams of hemoglobin per liter of the diluted blood. If the blood was diluted 1-200, multiply the scale reading by 200, which gives the grams of hemoglobin per liter of whole blood. If the dilution was 1-300 or 1-400, multiply by these figures.

This instrument requires an absolutely dark room; it is too cumbersome to carry about easily; its use requires much time for the actual work and for the washing of the apparatus. Parts are easily broken and they cannot be easily replaced. It is by far the most accurate instrument on the market and for this reason has an important place in hospitals and research laboratories. We use it for checking up on other simpler instruments, and in cases in which unusual accuracy is required.

Sources of Error

The errors that may be made in the determination of the hemoglobin are not many. By the Tallquist scale, two errors are not infrequent; the drop of blood may be small, thus the area of stained paper is too small and the reading too low. Or, in the endeaver to secure a larger area of stained paper, from an insufficient amount of blood, the paper may be pressed again and again over the wound, until the same area of paper may be soaked several times in the blood; naturally, the reading is then very much higher than it should be. In severe anemias and in blood of very low viscosity, the serum soaks into the paper around the cells, leaving a rather dark center in a very pale ring. The reading is thus too high. This source of error is especially apt to occur in chlorosis, and it frequently occurs in pernicious anemia and in secondary anemias of similar type. Sometimes the examination is delayed, and the blood takes on a brown color, due, probably, to the formation of methemoglobin. When this occurs it is impossible to secure anything like an exact match of the tints of the scale; the resulting readings are certainly inaccurate, and they may be too high or too low.

In using Dare's hemoglobinometer, the slides may not be thoroughly dry, especially if it has been necessary to clean them after errors in technique. The slightest film of moisture upon the surfaces of the glass slides affects the reading by diluting the blood. Sometimes the slides are not screwed closely together; the film of blood is then too thick, and

the reading too high. Sometimes the slides are screwed too tightly together, whereupon they break.

The finger should be removed from the wheel which turns the scale, before the telescope-tube is removed from the eyes. Else, when the telescope is taken from the eyes, the fingers which have been turning the scale may still turn it slightly. The scale may thus be turned and the reading correspondingly inaccurate.

The use of Sahli's or Gower's instrument depends for its accuracy upon the care with which the blood is taken, is blown into the larger diluting pipette, and with which the readings and dilutings are performed. For if the drop of blood should not be removed from the end of the tube, after the requisite 20 c.c. have been taken, a part of this is almost sure to be drawn into the tube with the diluting fluid, and the determination is increased. If the tubes are moist, the blood is unduly diluted, and the readings are too low. If the blood is not thoroughly rinsed into the mixing pipette, the readings are thus too low. In adding the last of the water to the blood in the mixing pipette, it is best to read off the hemoglobin percentage, and write it down, before the later and decisive readings are taken. Then, if by accident or by choice, the dilution should be sufficient to carry the color past that of the control, the reading can be corrected accordingly.

In using the original Fleischl instrument sources of error are plentiful and for this reason it is not now used in many laboratories. The Miescher modification is subject to much less error but still it is necessary to use great care in making every step of the procedure. In very important cases for research work it is our practice to take the blood in two different dilutions and to compare these; discarding the results if the results do not agree within five points of the final computation, and making an average of the two final results if they do so agree.

For each method of determining hemoglobin accuracy is gained only by experience and carefulness; with these the hemoglobin can be determined reasonably well, within the limits of accuracy for the instrument employed, in any case.

The Actual Count of Cells

The enumeration of the cells by the use of a counting chamber is called the "actual" count, to distinguish it from the "differential" count, which is made upon a smear of blood. The actual count gives the actual number of cells per cubic millimeter of blood; the differential count gives the percentages of the different types of white cells, and the rela-

tive number of normoblasts, malarial corpuscles and any other items desired may also be determined. From the differential count and the actual count, the number of these various structures in an average cubic millimeter of blood can be determined. The actual count is not a complicated process but errors must be very carefully avoided or the findings may be false.

Apparatus Needed For The Actual Count

Provide, for the actual count, the following articles. Microscope with mechanical stage, iris diaphragm and condenser may be of the type used in ordinary laboratory work. The binocular microscope is far better for the eyes. Have the table, the microscope and the seat to be used by the worker correctly placed so that the worker sits perfectly squarely and at a comfortable height. Counting blood takes considerable time; if the head of the worker is held at a lateral angle, or if the microscope is too high or too low there is a strain upon the neck muscles; the cervical vertebrae are held in an abnormal position and the circulation through the eyes is affected. Strain upon the intrinsic muscles of the eyes is caused by the attempt to see plainly if the eyes are not placed at right angles to the planes of the lenses of the eyepieces. In this connection it should be stated very emphatically that the condition called "eye-strain" due to the use of the microscope for considerable periods of time is much more frequently "neck-strain" and is due to carelessness in the position assumed while the work is being done. There is no reason why the use of the microscope should injure the eyes any more than reading, except that a microscope occupies a fixed position and the worker tends to accommodate his position to the needs of vision without being careful to consider the comfort of his position. In order to avoid fatigue and injury to the eyes it is only necessary to be very careful to sit comfortably, squarely facing the microscope and to have suitable lighting and proper arrangement of the iris diaphragm and the lenses.

The lighting is important. In our laboratories a small electric light placed squarely beneath the condenser is used for ordinary work. A larger light placed in front of the small round reflecting mirror is good; this permits variations in lighting for low-power or high-power magnification in addition to the modifications secured by regulating the iris diaphragm.

Other articles needed are a lancet for pricking the skin, various solutions and containers, a counting chamber, pipettes for diluting the blood and sterilizers and other articles ordinarily present in a laboratory for clinical diagnosis.

Counting Chamber

Several types of counting chamber are in use. The best arrangement is made of a single piece of glass which is so cut as to form the chamber. Less useful and accurate is the chamber made by cementing pieces of glass together. The chamber in which the moat is H-shaped is more convenient than the older style in which the moat is circular. In any event the counting chamber is a glass slide which has upon its upper surface several parts. In the center there is a ruled area upon which the counting is done. Several arrangements of the lines are in use but all contain lines crossing at right angles, dividing the chamber into spaces of varying sizes. The "small square" is the unit space. This is formed by lines 1-20 millimeter apart, thus squares are formed 1-20 x 1-20 millimeter in size. In order to secure ease in counting an extra line is placed midway between these lines at regular intervals thus forming smaller squares 1-40 x 1-40 millimeter; these are of no practical value in ordinary work except that they help to keep the area being counted in easy vision. The central area of the counting chamber is occupied by 144 of the squares of 1-20 millimeter. Many of these are subdivided. Around this central area many chambers are provided with larger squares for the counting of the white cells; these are made by lines 1-5 millimeter apart, with lines 1-20 millimeter apart outlining the larger spaces; sometimes three lines 1-40 millimeter apart take the place of the two lines 1-20 millimeter apart, in the formation of the larger squares.

This ruled area is upon a part of the slide which is elevated above the rest of the slide; the exact amount of the elevation is of no consequence, and varies for different counting chambers. Around this ruled glass area, which is, properly, the chamber, there is a moat. This is a depression around the ruled area, itself surrounded by another elevation of glass. The glass around the moat is always 1-10 millimeter higher than the counting chamber, so that when the cover-glass is placed upon the slide a space exists between the ruled area and the cover-glass; this space is 1-10 millimeter in depth in all chambers in ordinary use.

The counting chamber requires care in cleaning because the ruled lines are delicate and the glass can be scratched easily. If the chamber is made by cementing different pieces of glass together, the chamber must never be washed or rinsed with anything but warm water. This is really all that is necessary, anyway, in ordinary cases. If the counting chamber is made of a single piece of glass, cut to form the various parts, it may be washed as any delicate glass-ware would be.

The ruled area must be treated with especial care, it must never be rubbed in drying, but only very gently mopped, or, better, left to dry after a final rinsing in warm distilled water.

Pipettes for Diluting the Blood

These are commonly of the Thoma-Zeiss types. They are composed of a glass bulb which receives the blood and the diluting fluid. Within this bulb is a small glass bead which facilitates mixing the blood and the fluid, and which also serves to remove deposits from the inside of the bulb in case of accident. A capillary tube receives the blood, and this is divided into ten equal parts. The entire content of the capillary tube of the erythrocyte pipette is one hundredth of the content of the bulb, and the content of one of the ten divisions is 1-1000 the content of the bulb. The fifth of these divisions is marked .5 and the division next the bulb is marked 1. At the top of the bulb another mark 101, is placed. Since the 101 includes the content of the capillary tube, and since the diluting fluid within the capillary tube is not mixed with the contents of the bulb, the dilution in the bulb can be made anything between 1 to 1,000 and 1 to 100. The most common dilutions for red cells are 1 to 100, or 1 to 200.

The tube frequently used for counting the white cells has a smaller bulb, or a larger bore in its capillary tube, so the bulb contains only ten times as much fluid as the capillary tube. This tube also is divided into ten equal parts. The fifth division is marked .5 and the upper division is marked 1 as in the red cell pipette, while the upper limit of the bulb is marked 11. Thus it is possible to secure dilution of 1-10, 1-20 or even 1-100 by drawing the blood to the 1 mark, to the .5 mark, or to the lowest mark, which is 1-10 the content of the bore of the capillary tube. Other markings are employed for the white cell pipettes occasionally; their significance is easily understood by a comparison of the markings with one another and with the red cell pipettes.

For both pipettes there is a dilated area above the constriction which marks the upper limit of the bulb; this receives any superfluous amount which may be drawn up beyond the proper limit, by accident. There is a rubber tube with a glass mouth-piece for each blood pipette, and it is best to have these rubber tubes a foot or more in length. The rubber tubes which come with the pipettes are rarely long enough to permit accuracy in vision or delicacy in manipulation of ascending columns. By having the longer rubber tubes it is easier to see clearly the ascending column of blood, and the increased elasticity due to the longer rubber tube allows greater accuracy in manipulating the columns as they are drawn upward.

Cleaning Blood Pipettes

After the pipettes have been used rinse them in clean water immediately. Rinse thoroughly in cold water, then in hot water, then run air through them until they are perfectly dry. When no water is visible, and the glass bead rolls around freely within the bulb, the bulb is dry. It must be remembered that while the bulb is still very wet the bead rolls around freely also, but in that case the water is easily visible. If there has been some staining of the inside of the bulb with the diluting fluid, this may be removed with sulphuric, hydrochloric or nitric acid, using the acid first in a very weak solution, then increasing the strength of the solution until the stain is gone.

If coagulation of blood should occur within the capillary bore or the bulb, this must be digested out. An artificial gastric juice containing both free hydrochloric acid and pepsin, or an artificial pancreatic juice containing trypsin and mildly alkaline in reaction, should be drawn into the bulb and the pipette left in an incubator over night. If the clot is not dissolved the process must be repeated, perhaps using some other artificial digestive fluid. After the clot has been digested the pipette is rinsed with cool water, then with hot water, then is dried in the usual manner.

A filter pump attached to the water tap cleans the tube very easily and efficiently. Attach the pipette to the intake of the filter pump, place the other end of the pipette in cold or hot water, or in the acid solution if this is to be used. Turn on the water and allow the cleaning solution or water to flow through the pipette as long as seems desirable. Then remove the lower end of the pipette from the water and allow air to flow through until the pipette is perfectly dry.

If the filter pump is not at hand, the pipettes must be cleaned by hand. Cold water, then hot water, then 95% alcohol, then ether are used in turn and then air is blown through the pipette until the tube is dry. Draw the fluids into the pipettes by suction through the rubber tube, then expel them by using a rubber bulb. Air is drawn into the pipettes and then is expelled by the rubber bulb. The final rinsing with alcohol and ether is intended to hasten the drying process because these fluids evaporate rapidly. (They are not necessary when the filter pump is used because the time question is not important to the filter pump, and hot water is not usually limited.) The cleaned pipettes may be kept in any place which is free from dust and moisture.

The Lancet

Several types of blood lancet are on the market. A glove needle, which is triangular in form, makes an excellent lancet for the purpose of securing blood. The triangular needle makes a cleaner puncture than does the ordinary round needle, though any ordinary sewing needle can be used in emergencies, after it has been sterilizd. A surgeon's needle is convenient and easily cleaned. Half a steel pen, properly cleaned and sterilized, is very good.

Lancets which are provided with a spring are rather less easily cleaned. They have the advantages of producing wounds of equal depth, and they can be set to make deeper wounds if the skin shows marked pallor, or shallower wounds if the patient is a bleeder or if the skin is very red. The needles without a spring depend upon the skill of the operator for the accuracy of gauging the depth of the wound.

Needles and lancets are best sterilized by rinsing in warm water, then dipping them into carbolic acid. After another rinsing in sterile water they may be dried on sterile cotton or left to dry.

Diluting Fluids

In order to count the blood cells it is necessary that the blood be diluted. For the white cell count acetic acid, in solutions of 3% to 8% is commonly employed. The red cells are destroyed by the acetic acid, which also causes the nuclei of the white cells to show more distinctly. Various stains may be added to the acetic acid solution and these stain the white cells. In our laboratories no stain is used; this is partly in order to allow any pigmented granules of the blood to be visible.

For the red cells many solutions are employed. In our laboratories normal salt solution is tinged with a few drops of methylene blue and this is used for diluting the blood for the red cell count. The cells remain in fairly normal condition for several hours, which is all that is required for ordinary cases. If the blood must be kept in the pipette for some time before it can be counted, Hayem's solution is excellent, and this is the one most used. The following is the formula:

Distilled water, 200 c.c.
Sodium chloride, 1 gm.
Mercuric chloride 0.5 gm.
Sodium sulphate 5. gms.

Another solution which is preferred by many workers is Toisson's fluid. Its formula is:

Distilled water, 160 c.c.
Neutral glycerine, 30 c.c.
Sodium sulphate, 8. gms.
Sodium chloride, 1. gm.

Methyl violet, 25 mgs., or just enough to give a faint purplish tinge to the solution. This fluid stains the white cells. It does not keep so well as Hayem's fluid, does not fix the cells appreciably, and we have found it less satisfactory than Hayem's fluid for keeping the blood for a long time (a day or two or three days) under experimental conditions.

Cleansing Solutions For the Skin

For ordinary cases, a very mild soap solution at room temperature followed by sterile tap water for rinsing is preferred as mode of cleansing. These are provided in convenient small bottles.

For cases requiring efficient sterilization the solutions to be employed are selected according to the nature of the infectious agent suspected. The methods of surgical procedure are followed, and this sterilization must precede the taking of the blood by an hour or by several hours, according to the manner in which the skin reacts to the sterilization process.

The use of alcohol, ether or any solution which reddens or which pales the skin, or which causes any sensory irritation worthy the name is bad and interferes with the accuracy of the count.

Cotton or gauze pads for applying the cleansing solutions should be sterile and at least five pads of gauze or bits of cotton should be ready.

Taking Blood For Counting

Very little blood is required for making ordinary cell counts, and this is, preferably, capillary blood. Capillary blood is easily secured, is alike all over the body, is modified only by vasomotor activity except as the entire blood picture changes, and the tiny wound made by securing the blood from the capillaries heals immediately.

The side of a toe is the selected site for taking blood from babies or small children, for the following reasons: Vaso-constrictor control is not abundant; the skin is thin; the foot is easily held, if the child is awake, and the toe is not apt to be irritated or infected afterward. If the child is asleep the process may not awaken him.

The lobe of the ear is selected for adults for the following reasons: The lobe of the ear is not visible and the patient cannot see the blood; the vaso-constrictor control is not abundant; the skin is thin, sensory nerves are scanty, and the lobe of the ear is not subject to later irritation.

In the case of an adult the worker stands at the left side and to the rear of the patient. The patient sits comfortably by the side of a small table upon which the necessary equipment is placed conveniently for the worker. The patient cannot see the blood being taken. If the patient is in bed he should turn the face away from the worker, thus permitting an ear to be accessible.

If really efficient sterilization of the skin should be necessary, this should have been done at least an hour before the blood is to be taken, and the skin protected by cotton or gauze in the interim. In most cases the washing which removes dust and some desquamating epithelium is all that is necessary. The ear lobe should be gently mopped with sterile water, dried, and protected against dust if any delay should occur. The lobe must not be rubbed or handled or caused to redden perceptibly by the cleansing. Such cleansing agents as alcohol are to be avoided because they dilate the capillaries and modify the counts.

If the patient has come in from the street a mild soap solution may be used for cleaning, then the ear washed with sterile water and dried with sterile cotton. If the cleansing should cause visible reddening of the skin the ear should be protected with sterile cotton and the patient allowed to rest for ten minutes or more, until the normal circulation has been well established in the ear.

Prick the skin with lancet or needle. Notice the emergence of the first drop, its size, color, and the manner in which it flows upward into a round drop or spreads around over the skin. Wipe away this first drop. Take the blood for the white cell count first. This is partly because the white cells stand longer without being modified, partly because the white cells are more quickly modified by the faint vasomotor change due to the slight irritation of the prick, and partly because a larger drop is used for the white cell count. (The first drops are usually rather larger than later drops of blood from so small a prick.) If the white blood cell pipette is used, draw the blood to the .5 mark, then very quickly draw the acetic acid solution to the 11 mark. Rotate the pipette gently while the solution is being drawn into the bulb. Close both ends of the pipette with thumb and finger and shake gently from side to side (not endways) to mix the blood with the solution

sufficiently to prevent clotting. Lay the tube aside, or give it to an assistant who will continue the shaking, gently from side to side. If the tube is shaken vigorously the cells may be fragmented and if it is shaken endways the cells may be forced into the capillary tube, or the mixture of cells and solution may be forced into the dilatation above the bulb; if the contents of the bulb were well mixed the latter accident would be of neglibible importance, but if part of the contents of the bulb were forced into the upper capillary tube of the pipette and the upper dilatation before the mixing is completed serious error would be present in the count.

In our laboratories an erythrocyte pipette is used for the white cell count. Draw the blood to the 1 mark on the pipette, then draw the acetic acid solution to the 101 mark; mix the contents of the bulb by gentle shaking in a sidewise direction, as already directed.

The advantage of this greater dilution lies in the absence of the debris caused by the destruction of the red cells; they are all completely destroyed by the greater amount of acetic acid and completely dissolved in the greater amount of fluid. The white cells are more easily recognizable. In cases of leukemia and leucocytosis, this method is necessary for accuracy, and we think it preferable in all cases.

Next, fill the red cell pipette for the red cell count. Draw the blood to the .5 mark on the capillary tube, then draw the diluting fluid for red cells to the 101 mark. Shake as directed for the white cell pipette. Lay the tube aside until the count is to be made.

The most frequent errors, in making the dilution, are these: The end of the pipette may be allowed to reach the air, either by being raised too high or by being pushed through the drop of blood. The entrance of air into the tube causes the "breaking" of the column of blood, or, if the air is admitted with the diluting fluid, bubbles are formed. In either condition a fresh drop of blood must be taken in a clean pipette and greater care observed. Sometimes the drop of blood is too small; if the blood has been secured with difficulty and if the column of blood reaches to the first mark below the .5, the count may be completed, and the correction made in the final computation. If it is practicable to secure another drop of blood this is a more satisfactory method. Sometimes the pipette is pressed against the skin too firmly, and the blood does not ascend into the tube no matter how hard the breath is drawn. In such a case, the tip is apt to be slightly lifted, and the blood is apt to ascend suddenly through the tube and into the bulb. When too great force is employed in the inspiration which draws the blood into

the tube, the blood is apt to rush too rapidly upward, fill the tube, and sometimes the bulb itself. The pipettes must be rinsed immediately else the blood may coagulate within them and cleaning become an extremely difficult matter.

Take next the blood for differential counts and for such other tests as may be indicated. The pipettes of diluted ordinary blood may rest for a time, even for an hour or two, without harm, but in cases of unusual fragility of the cells changes may occur more quickly. It is best in all cases to make the actual counts as quickly after the blood is taken as is practicable.

After the blood has been taken for the various tests indicated cleanse the ear lobe of the patient and note whether there is any indication of persistent bleeding. The wound should be closed by this time, if it is not handled. It may be kept open for five minutes or more by the slight manipulations necessary for wiping away the preceding drops of blood in preparation for further tests.

Use of the Counting Chamber

If the counting chamber has a circular moat, the following technique is to be employed.

Place the counting chamber on a perfectly level surface, and the cover-glass by the chamber. Close the ends of the pipette containing the red cells and shake again, using a side-to-side staccato movement, rather gently, about one hundred times. Avoid the endwise movement in shaking. Remove the rubber tube and attach a stiff rubber bulb to the upper end of the tube, force out about four drops of the mixture and discard this. This is in order that the diluting fluid in the capillary tube, which contains no cells or, at most, only a very few cells, may not be used for counting. Let the fifth drop begin to form at the end of the capillary tube, and touch this to the surface of the counting chamber, near the ruled area. Avoid allowing the end of the pipette to touch the surface of the counting chamber; if the glass should touch the ruled area the markings might be scratched. The amount of fluid necessary must be learned by experience; it is about half as much as would drop from the end of the capillary tube of the pipette if the pressure on the bulb should be increased. Holding the cover glass by its edges, lower it slowly over the counting chamber; if a bubble of air happens to be caught in the fluid clean the counting chamber and take another drop from the pipette. If the counting chamber itself is not filled with the fluid, clean the chamber and take

another drop of fluid. If the amount of fluid is too great, so that the moat is filled across, the cover glass cannot fit accurately and the count will be too high. In this case clean the counting chamber and take another drop of the fluid. Only experience can win accuracy.

If the counting chamber has an H-shaped moat, the procedure is somewhat less difficult. Place the cover glass on the slide first, and note that the cover glass fits the outer raised part of the chamber accurately. The test of this accurate fitting is best made by means of the phenomenon known as "Newton's rings." These are concentric bands of rainbow colors which appear when two glass surfaces are so closely in contact that the difference between their surfaces in not more than the distance measured by wave-lengths of light. When the slide containing the counting chamber, covered by the perfectly plane cover glass, is held slantingly to the axis of vision, in changing positions, these rainbow colors should be visible. This means that the fitting is fairly accurate. If the rings are not visible press firmly upon the cover glass; this may cause the necessary approximation of the two surfaces. If the rings are then visible, and remain so when the pressure is removed, the cover-glass fits accurately. If the rings disappear when the pressure is removed, there is some dust or moisture present; clean the cover-glass and the counting chamber and repeat the process. Occasionally a counting chamber is found upon which it is impossible to secure the rings. Such a chamber may still be accurate so far as the counting is concerned.

Another test for accuracy lies in the fact that two perfectly dry plane glass surfaces closely approximated adhere firmly. Having placed the cover glass in position turn the slide over gently; if the cover-glass adheres, the fitting is probably good. If the cover-glass falls off, the fitting is not good. Cleanse and dry the cover-glass and the slide and try again. This method of testing is less accurate than the finding of Newton's rings but it is useful in the use of certain chambers. A trace of moisture causes adhesion, separates the surfaces and seriously increases the count.

If Newton's rings cannot be produced on a certain counting chamber, the chamber may be tested by another in which these rings are produced. Take the same pipette of blood and make counts of the red cells on both chambers, in one of which Newton's rings have been produced, and in the other only the adhesion of the cover glass has been found well marked. If the counts made on the two chambers agree within the limits of accuracy permissible for the method (not more

than 3% of the total number of cells counted) then the second chamber is accurate enough for all clinical purposes and its use can be continued. If the two counts do not agree, the imperfect counting chamber should be returned to the maker.

Having secured the proper fitting of cover glass and counting chamber, shake the red cell pipette as before using at least 100 vibrations. Discard the first four drops, and touch the fifth drop to the edge of the chamber at the edge of the slide. The fluid runs beneath the cover glass and fills the ruled area at once, by capillary attraction. If any fluid runs out and fills the moat, thus lifting the cover glass and increasing the depth of the counting chamber, clean the cover glass and the counting chamber and take another drop from the pipette, after shaking it as before.

With both types of counting chamber, the later processes are alike. Allow the filled chamber to rest for three minutes or so in order that the cells may settle to the bottom of the counting chamber. The cells of normal blood settle quickly; those of anemic blood and blood which is from persons with certain diseases settle slowly. If any cells are still floating when the count is begun, this must be deferred for a time, until all the cells rest upon the bottom of the chamber. Accurate counts are not possible when any cells are floating, because they are not in focus at the same level.

Use a 1/6 objective and a one inch eyepiece on the microscope. Find the ruled area and select a large square containing five rows of five each of the small squares. Count all the cells in the upper row of five small squares and note the number found. In this count include every cell which touches the upper line and the left hand line, and the intersection of these; also at the intersection of the right line and the upper line; exclude from the count all the cells which touch the right line and the lower line, the intersection of these, and the intersection of the lower line and the left line.

Repeat for each of the five rows of small cells. This makes a column consisting of five numbers, each of which indicates the number of cells found in five small squares. The sum of this column indicates the number of cells in 25 small squares. Select another area containing twenty-five small squares arranged in the same way, and repeat. For ordinary work four such columns, indicating the number of cells in 100 small squares, is sufficiently accurate. In cases of anemia, count the cells in 400 to 800 small squares, using two or more counting chambers of diluted blood.

Compute as follows: Multiply the total number of cells counted by the dilution, and this by 4,000 (which is the cubic content of each square in terms of a cubic millimeter, since each small square is 1/20 by 1/20 by 1/10 millimeter in size). Divide by the number of squares counted. Example, 625 cells were found in 100 small squares, the blood being diluted 200 times. The computation is:

$625 \times 200 \times 4{,}000 \div 100 = 5{,}000{,}000$ cells per cubic millimeter.

Or, the average number of cells in each square is 6.25. Each square is 1/4,000 cubic millimeter in content, so that there must be 25,000 cells in each cubic millimeter of the diluted blood. Since the blood was diluted 200 times, the number of cells in one cubic millimeter of undiluted blood is 5,000,000.

The counting of the white cells follows a similar process. The same tests for accuracy of fitting the cover glass and for placing the drop of fluid in the counting chamber are employed.

If the white cell pipette was used, count the number of cells present in at least 800 small squares. If the red cell pipette is used count the number of cells present in 4,000 small squares. In either case the computation follows the same method,—total number of cells counted, multiplied by the dilution, multiplied by 4,000 and divided by the number of squares included in the count. For example, if the white cells pipette has been used, with a dilution of 1:10, and if 300 cells were found while counting 800 squares, the computation is:

$300 \times 10 \times 4{,}000 \div 800 = 15{,}000$ white cells per cubic millimeter.

If the red cell pipette was used, with a dilution of 1:100, and if 120 cells were counted within 4,000 small squares, the computation is:

$120 \times 100 \times 4{,}000 \div 4{,}000 = 12{,}000$ cells per cubic millimeter of blood.

Always count at least 100 cells, even if it is necessary to cover 16,000 or more small squares in the counting. In doubtful cases count 1,000 to 5,000 cells, using several pipettes if necessary.

Many counting chambers have large squares around the area of rulings for the small squares; the area of these large squares is easily understood by following the lines which form the small squares outward. By using these larger squares the white cell count is easily and quickly made, even though 8,000 small squares are necessary for accuracy. The computations are made upon a basis of the small square in all cases. This avoids any possibility of error due to the use of different units.

This actual count gives the number of red cells and the number of white cells per cubic millimeter of blood.

Sedimentation

Attempts have been made to substitute estimations of the blood cell volume for studies of the blood cell count, since it is often thought more important to know the total mass of hemoglobin-containing protoplasm than the manner in which this hemoglobin is arranged in cells. On the other hand, the manner in which the hemoglobin is divided into cells is an important factor in the oxygen carrying function of the hemoglobin, since hemoglobin arranged in a comparatively large mass with relatively small surface area is exposed to the air in the lungs less efficiently than an equal mass of hemoglobin divided up into smaller masses with relatively greater surface area.

The hematocrit is a form of centrifuge in which small tubes are placed in opposite arms; each tube has 100 equal divisions. The technique is simple:

Secure a large drop of capillary blood by the method outlined for taking the blood for counting. Place a rubber tube over one end of the glass tube; draw the glass tube perfectly full of blood. Cover a finger with vaseline, and cover the free end of the glass tube immediately. Remove the rubber tube; place the glass tube in the arm of the hematocrit. Place the other glass tube, filled with water, in the arm of the hematocrit, to balance the machine. If the blood of two patients is to be centrifugalized at the same time, make marks with a grease pencil upon the outside of each glass tube for identification. Start the centrifuge, gradually attaining the high speed within half a minute. Stop the centrifuge and note the height of the column of red cells at one minute intervals. When two successive examinations give identical findings, stop the centrifuge. Each division of the glass tube represents 100,000 cells, if the cells are approximately normal in size and in hemoglobin content. The column of cells is approximately equal to the column of plasma in normal blood. The blood plasma can be used for the determination of bile and other pigments. A very thin layer of fatty globules is occasionally seen at the upper end of the tube, in lipemia.

In the anemias the number of cells in each division of the tube may vary very greatly, so that in cases in which accuracy of cell count is important the method has no value. As a method of determining the actual volume of hemoglobin-carrying protoplasm the method is of value.

The time required for complete settling of the red cells has been studied by many workers, and this has been shown to vary greatly in different diseases.

Differential Counting

The differential count is made in order to determine the relative numbers of different types of white blood cells. These cannot conveniently be differentiated in the process of making the actual count of white cells. An attempt to use diluting fluids which give a differential stain in the counting chamber is not satisfactory, and in order to secure accuracy by thus combining the actual and the differential count it would be necessary to count the cells in several hundred chambers. The differential count is made of thin smears of undiluted blood, stained in some manner which affects different types of cells variably, according to their chemical constitution. By this means it is easy to recognize several different classes of white cells. Slides used for the differential count must be clean but they need not be sterile. Rather thick slides are more convenient.

New slides are greasy and must be well washed, first in warm soap solution, then in hot water. A second soapy washing is often necessary. They can be kept in acid bichromate solution made approximately as follows; exact proportions are not necessary:

Potassium bichromate	10 grams
Commercial sulphuric acid	10 grams
Distilled water	200 c.c.

In routine work take the drops which flow after the blood has been taken for an actual count. If only the differential count is to be made, prepare and prick the skin as directed for the actual count.

Have ready microscope slides which are perfectly clean and perfectly dry, at least eight slides for each patient. Touch one end of a slide to the top of a drop of blood, then touch this blood to the end of another slide. Allow the drop of blood to flow along the angle between the two slides for a second or two. Push the first slide along the surface of second slide, away from the drop of blood. The blood follows the moving slide and leaves a thin, even smear upon the second slide. Never push or pull the first slide along after the drop of blood, because thus many cells are injured and there is a tendency for some cells to cling to the first slide and thus to accumulate in groups. Repeat this process for each slide. If the amount of blood is scanty it may be necessary to take only six slides; if these cannot be secured prick the skin again. Never take less than six slides of blood for a differential count. If the condition suggests leukemia, severe anemia or the need for any special study it is best to take twenty slides or more.

As the smears are made lay the slides, blood side up, upon a flat surface until they are perfectly dry. Then put them into an envelope already marked with the name of the patient and the physician and the date and the hour of taking the blood. These smears keep almost indefinitely and they can be stained by different methods for special study of different structures or inclusions.

If the blood arranges itself in circular areas or rings, the slide was greasy. If the blood forms stripes or bands, the motion was jerky. It is necessary that the second slide be moved along the first in a steady, even, deliberate manner. If there are threads of fibrin or small thick places in the smear, the blood was partly coagulated. If the smear is too narrow and thick, the second slide was moved before the blood spread along the edge. If the smear is too thick and spreads over the entire slide, the drop of blood was too large. If it is too thin and spreads over too small an area, the blood drop was too small.

If two or more specimens are under observation at the same time it is necessary to mark each slide for identification. This is best done with a lead pencil. Using a pencil with a rather soft lead, write an initial or an identifying number upon one end of the smear, near the end of the slide, before the slide is stained. The pencil ruins the blood cells over which it passes and leaves a small amount of the lead; fixing the blood on the slide makes the lines so produced permanent. By making the marks at the end of the slide they do not interfere with the counting, since this is done in the central area.

Staining

Many different methods of staining blood smears for the differential count are in use. They include so many stains, each with so many modifications, that any satisfactory description of them all would be too long for this book. For example, about twenty different methods of using the eosin-methylene blue stain devised by Romanowsky have been described, and each method has its advocates. The method used in our laboratories is different from any of those described elsewhere, but it gives accurate and delicate staining of the structures included in an ordinary differential count, and it is easily modified so that good pictures can be secured of atypical blood.

Solutions required are:

Eosin yellowish, 0.5 gram in 100 c.c. methyl alcohol. This fixes the blood and stains the acidophile structures.

Methylene blue, 1.0 gram in 100 c.c. tap water, if the tap water is clean and reasonably pure, or

methylene blue,	1.0 gram
sodium bicarbonate	0.1 gram
sodium chloride	0.5 gram
distilled water,	100 c.c.

The methylene blue stains the nuclei and the basophilic structures of the protoplasm. It stains also the malarial and certain other parasites.

Take one of the slides already prepared and dried. Place the slide on a level surface, smear side up, and drop upon it several drops of the eosin solution. Let stand fifteen or a few more seconds; rinse gently in tap water. Drop upon it a few drops of methylene blue solution, enough to cover the smear very abundantly; let stand a minute or a little more; add a few drops of tap water and allow to stand on the slide about two minutes, rinse with tap water. Drain, allow to dry in air thoroughly, and examine, using oil immersion objective and one inch eyepiece. Or, after rinsing with water, mount in water under a thin cover glass and examine, using a dry one-tenth objective and one inch eyepiece. The one-eighth objective does not magnify sufficiently for the finer details of cell structure to be visible. For careful study of the cell structures, a one-eighteenth objective, oil immersion, is useful. In our laboratories the dry one-tenth objective is used for ordinary work and the one-eighteenth objective for careful study of selected cells in unusual cases. See also "Other Staining Methods," Page 325.

Make a general survey of the smear in order to note the type of blood cells present.

Counting

Have ready a sheet of paper with columns arranged for each class of blood cells,—large hyaline, small hyaline, mononuclear neutrophiles, polymorphonuclear neutrophiles, eosinophiles, basophiles, for ordinary blood. For abnormal blood other columns are required for normoblasts, megaloblasts, poikiloblasts, microblasts, reticular red cells, malarial parasites, and other peculiarities of the red cells which may be of interest in the particular case, and for myelocytes of each type found in the blood being examined. The general survey has indicated the columns required. As the count progresses other columns may be added at any time if other cell types are found.

Begin the counting at one edge of the smear, move the slide, by means of the mechanical stage, so that the field is brought toward the

observer (apparently) as far as the edge of the blood smear, and is carried as far to the right as the edge of the blood smear, or as far to the right as the limit of the mechanical stage permits. Then move the slide toward the left, noting each cell and making the notation in the column devoted to that cell type. When the slide cannot be moved further to the left, or when the limit of the smear in that direction has been reached, move the slide away from the eye the diameter of one field, so that some selected red cell which is barely in vision at the lower edge of the field is moved just beyond vision at the upper edge of the field. Then move the slide toward the right, counting and listing the cells as they appear in successive fields. Continue in this way, moving back and forth across the slide, until all the cells have been counted on the slide, or until the desired number of cells has been listed.

With practice it becomes easy to carry the counts in mind, and to make the notations in groups of twenty, ten or five, as the case may be. In our laboratories, neutrophiles are counted in groups of twenty cells, small hyalines in groups of ten, and other cells as units. These habits are made for the sake of accuracy, convenience and speed of counting. Each worker develops his own customs.

Count until the total number of cells counted is at least five hundred, in cases which present no marked variations from the normal and which show no marked irregularity of distribution of the cells. In abnormal cases at least one thousand cells should be counted, while in cases used for special study, in unusual cases, and in all the leukemias two thousand to twenty thousand cells or more should be counted. In one of our cases of leukemia, with an actual count of 250,000 leucocytes of which 80% were myelocytes of different forms, it was necessary to make a differential count of 50,000 cells in order to secure satisfactory accuracy.

The number of cells to be examined depends upon the fact that successive counts of any selected number give almost or quite identical results. In normal blood successive counts of one hundred cells each give approximately identical figures for lymphocytes and for neutrophiles, but may give very different figures for eosinophiles, while basophiles may not be found at all. Successive counts of 100 cells may give eosinophiles of ten per cent in one 100, and no eosinophiles at all for another 100. (Eosinophiles have a tendency to be in groups even in the best smears of blood.) Counts of successive five hundreds of approximately normal blood give satisfactory accuracy for such blood.

In very abnormal blood, counts of successive five hundreds may give results which vary greatly, even enough, in some cases, to affect the diagnosis. Hence counts of five hundred cells are not enough for satisfactory accuracy in such blood.

In acute cases in which diagnosis must be made quickly, as in suspected pyogenic processes probably requiring speedy surgical interference, a differential count of two hundred cells may serve the necessary purpose and enable treatment to be initiated quickly. But unless there is urgent need of haste, every count should include at least five hundred cells. Even when this haste is imperative, the count of five to ten hundred cells should be carried on later in order that accurate findings may be kept on record for later study and comparisons.

Computation of Differential Count

When the total number of cells in all columns reaches one thousand, if this is the number counted, add each column and divide by ten. This gives the percentages of each column. For example, if the neutrophile column has in it 678 cells, then there is 67.8% of neutrophiles in the patient's blood. If his actual count was 5,000 white cells per cubic millimeter, then he has 3,390 neutrophiles per cubic millimeter of blood. If there are 11 eosinophiles in that column, he has 1.1% eosinophiles, or 55 eosinophiles per cubic millimeter of blood. If the number secured by determining the actual number of any cell type from the percentage and the actual count gives a fraction of a cell, the nearest number is taken. For example, if a patient has an actual count of 5,700 leucocytes, and his large hyaline cells make up 4.3% of these, the computation gives 245.1 large hyaline. The report should indicate 245 large hyaline cells, because the limits of unavoidable error in this work are too large for us to report finding a difference of one cell in ten cubic millimeters.

Other Structures Counted With the Differential Leucocyte Count

While making the differential count of the leucocytes, certain other structures may also be counted. Columns may be arranged for red cells containing malarial parasites, for example, or for normoblasts, megaloblasts and other atypical red cells. With careful staining the reticulated red cells may also be enumerated. A column must be arranged for each structure to be counted. As the leucocytes are counted, such other structures are also counted and the figures placed in the column allotted

to them. They are not included in the sum of cells to be counted, however. Only leucocytes are to be counted in making the total of five hundred or a thousand or more upon which the percentages are to be computed. The total leucocytes must be 100%, and the other structures, not being leucocytes, must not be included. After the leucocyte count has been completed, as already directed, these other structures are considered. For example, in a patient with a total blood count of 3,000, there was 10% of large hyaline cells, that is, 300 per cubic millimeter. The differential count was based on the examination of 1,000 cells, so that 100 cells were in the column devoted to large hyaline cells. While these cells were being counted, 25 red cells were found which contained a malarial parasite, hence there were 75 malarial parasites within red blood cells per cubic millimeter of blood. That is, the amount of blood which contained 300 large hyaline cells also contained 75 cells containing malarial parasites within red cells. Extracellular malarial parasites were not included in this count.

In another case, with an actual leucocyte count of 2,500 cells, the neutrophiles included one half, or 50% of the total count. The differential count was based on 1,000 cells examined. While making the differential count thirty-four megaloblasts and twenty normoblasts were noted. That is, there were fifty normoblasts and eighty-five megaloblasts per cubic millimeter in this blood from a pernicious anemia patient, taken three days before his death.

In this same way, the number of several other structures can be computed on the basis of the differential leucocyte count, and much useful information gained thereby.

Special Methods of Counting

In making a differential count of the blood in certain leukemias and leucocytoses, when sometimes a great predominance of one type of cell is present, it may facilitate the process and increase the accuracy of the differential counting if the work is done in two stages. First, make a differential count of two groups only, the predominant type and all others. Examine and list 1,000 cells or more in this first stage. Stain another slide and make a differential count of all cells except the predominant type. Examine and list 500 or more of the cells for this count. Determine the percentage of the predominant type of cell by the first stage of counting, and of the other cells by the second counting. For example, in one of our cases of lymphatic leukemia the small hyaline cells made up 97% of the total blood count. The first stage of counting gave 970

small hyaline cells and 30 cells of all the others together. It is evident that this differential count of 1,000 cells could not give any accurate differential count of the 30 cells. The second stage disregarded the small hyaline cells altogether, and 500 cells of the remaining types gave accurate percentages of the granular cells and the large hyaline cells. Of the 500 cells examined in the second count, there were 40 large hyaline, 52 mononuclear neutrophiles, 200 polymorphonuclear neutrophiles, 120 eosinophiles, 88 basophiles, and when these percentages are taken for the 3% of "other cells" of the first stage of counting the final results were as follows (omitting the third decimal)

Total white cell count, 120,000		
Large hyaline	.24%	288 per cu.mm.
Small hyaline	97.00%	116,400 per cu.mm.
Mononuclear neutrophiles	.31%	372 per cu.mm.
Polymorphonuclears	1.20%	1,440 per cu.mm.
Eosinophiles	.72%	864 per cu.mm.
Basophiles	.53%	636 per cu.mm.

One megaloblast and three normoblasts were found in making the second stage of the count; these are too few to serve as a basis for accurate computation but they indicate that there is some beginning injury to the red bone marrow. They would not have been found at all in making an ordinary differential count. The figures thus secured are more nearly accurate than could be secured by making a differential count based on an examination of 30,000 cells using the ordinary technique and the time required for the counting was much less.

In cases of marked neutrophilic leucocytosis and in cases of monocytic angina this two-stage method of counting is very much more accurate and more convenient than the ordinary method.

Iodophilia

Iodophilia is of little significance when taken alone. When employed with other clinical and laboratory findings, it may give very useful information.

The older method of staining with iodin-gum preparations has been superseded by the staining with the vapor of iodine. A wide-mouthed, closely stoppered jar is kept for this purpose. About one gram of iodine crystals is placed in this jar.

Blood smears freshly made after the manner already described for the differential count are placed in the jar, smear side exposed to the vapor

of the iodine, and allowed to remain for five hours or more. The slides do not over-stain, and they may be left for several days without harm. One hundred leucocytes should be examined, and if iodophilic granules are not found, the reaction is negative.

Note whether the protoplasm of the white cells is diffusely stained and list such stained cells as iodophilic. Note whether granules are free in the plasma or are within white cells; if so, whether they are most abundant within the hyaline cells or the granular cells.

Nuclear Average

One of the slides prepared for the differential count can be employed. If the nuclei are not perfectly distinct, the slide should be floated with a watery solution of methylene blue for two minutes, then washed and again mounted in water. If the nuclei are still not distinct, the smear may be washed in N/100 solution of sodium bicarbonate, then the methylene blue stain repeated. The smear should be quite thin for accurate and convenient counting.

Have a sheet of paper with columns numbered from 1 to 5. Rarely columns 6 and 7 will be required. Begin at one edge of the smear, as in the differential count, noting the number of nuclei in each neutrophile, but disregarding all other blood cells. Count in this way the nuclei in 100 neutrophiles. If a cell contains two nuclei, place a mark under column 2, if it has four nuclei, place a mark under column 4, and so on, until 100 cells have been counted. (Plate XIV)

In counting the nuclei, a ring-shaped nucleus, even if slightly beaded in appearance, counts as a single nucleus. If the nuclear masses are united by a band, they should be counted as one. If they are united by a very thin filament of nuclear substance, they should be counted as two. If any cell has its nuclei piled one above another, so that it is impossible to determine the number of nuclei within it, it may be passed without counting; but if more than two or three such cells are found, the count must be repeated, using a thinner smear, for the higher counts will be those most often passed, under such circumstances, and the findings will thus be lower than the correct number.

Add each column. The sum of the cells of column 1, plus twice the cells in column 2, plus three times the cells in column 3, plus four times the cells in column 4, plus five times the cells in column 5 and six times the cells in column 6, if any, equal the sum of the nuclei in 100 cells. This divided by 100 gives the average number of nuclei for each neutrophile.

For example, in a certain specimen of blood there are:

10 cells having 1 nucleus; or 10 nuclei in all;
38 cells having 2 nuclei ; or 76 nuclei;
41 cells having 3 nuclei ; or 123 nuclei;
7 cells having 4 nuclei ; or 28 nuclei;
4 cells having 5 nuclei ; or 20 nuclei.

These 100 cells have, altogether, 257 nuclei; or an average of 2.57 nuclei per cell. The neutrophile nuclear average of this blood is 2.57. The nuclear average in normal adult human blood is between 2.45 and 2.55. In normal children the nuclear average varies from 2.00 to 2.4, according to age.

Special Methods of Staining

Endothelial cells. A stain for differentiating between mononuclear neutrophiles and monocytes supposed to be from the reticulo-endothelial system outside of the bone marrow is as follows:

Solution: 80 c.c. 100% alcohol
20 c.c. water, triple distilled
Warm gently to about 40° C
Add .2 gram alphanapthol (Merck)
.15 methyl violet 5 B (Grubler)
.2 c.c. hydrogen peroxide (must contain 3% of the gas)

Use dried blood films prepared as directed for the differential count. The films should not be more than a few hours old. Place the slide on a level surface and cover with six to eight drops of the solution. Allow to stain and fix for half a minute. Add an equal amount of distilled water and allow to stain for five minutes. Rinse several times with water. Cover the slide with basic fuchsin solution (0.01%) to counterstain for 2 minutes. Rinse with water; remove water with filter paper; dry in air; examine with oil immersion lens or mount in balsam.

Basophilic elements, including nuclei, basophilic granules, basophilic protoplasm, erythrocytes and platelets take various shades of red and pink. Eosinophile granules show a peculiar circular staining so that the granules look like rings. Neutrophilic granules and the finer granules of the endothelial cells show bluish tints. The difference between the neutrophiles and the endothelial cells lies in the characteristic nuclear structure and larger granules of the neutrophiles, and the characteristic nucleus and the smaller blue granules in the protoplasm of the endothelial cells. The stain is useful for its purpose.

Pappenheim's solution is adapted especially to a study of nucleated red cells.

Solution:—Take a saturated solution of methyl green, 30 c.c.
Add saturated solution of pyronin, 10 c.c.

This stain will keep for several days, in the dark.

Fix smears with heat, avoiding excess.
Flood slides with stain for five minutes.
Wash in water, dry, examine with oil immersion.

The nuclei of the normoblasts and nuclear fragments stain a clear blue, while basophilic granules within the red cells stain bright red.

Ehrlich's triacid stain is now little used. It consists of equal parts of saturated solutions of indulin, nigrosin and aurantia, mixed together after a difficult and tedious technique. It can be purchased in powdered form.

Ehrlich's triple stain also is somewhat difficult to prepare. It may be purchased ready made up, though the commercial preparations are not usually very successful. Grubler's stains are commonly used. The solution is made as follows:

Take a 100 c.c. graduate and measure the ingredients in the order given; do not rinse the graduate at all during the process. As each substance is measured pour it into a 500 c.c. flask and shake vigorously for one or two minutes.

Saturated aqueous solution orange G	13.0 c.c.
Saturated aqueous solution acid fuchsin	7.0 c.c.
Triple distilled water	15.0 c.c.
Absolute alcohol	15.0 c.c.
Saturated solution of methyl green (added drop by drop with frequent shaking of the flask)	17.5 c.c.
Absolute alcohol, (added drop by drop, with frequent shaking of flask)	10.0 c.c.
Glycerin (added drop by drop, with frequent shaking of flask)	10.0 c.c.

This mixture can be used at once, but it seems to improve during the few days following preparation. It deteriorates within a few weeks, more rapidly in the light or if the bottle is shaken.

To stain—Fix slides with heat and place on a level surface. Cover with solution taken from about the center of the bottle containing the stain, using a glass pipette for the purpose. Never shake the bottle.

Leave stain on slide for three minutes or more; the slides do not overstain if left twenty minutes. Rinse with water, remove excess water with filter or blotting paper, dry, examine with oil immersion or mount in balsam.

Erythrocytes stain yellow or buff. Normoblast nuclei stain a very dark green, almost black. Nuclei of leucocytes stain dark green but not so deep a color as the normoblast nuclei. Fine granules of the neutrophiles and the endothelial cells stain lilac or pale purple. Coarse granules of these cells and of the eosinophiles stain crimson. Basophilic granules do not stain. This stain is useful for distinguishing certain types of granules but it is not useful for general work. It is very difficult to secure good stains in cases of leukemia. Occasionally a patient appears whose blood refuses to take the Ehrlich triple stain, for no perceptible reason.

Leishman's stain is best purchased in powder form. For use make a solution of 150 mgs. of the powder in 100 c.c. of pure methyl alcohol. Smears are best made on cover glasses for this stain. Use the technique given for making smears on slides. Place a cover glass, smear side down, in a watchglass. Drop the stain into the watchglass until the cover glass floats. Allow to fix and stain for three minutes. Add an equal amount of distilled water, and allow to stain further for one minute. Remove the coverglass and wash in water, drain on edge until dry, examine with oil immersion lens. Or, mount in water and examine with dry one-tenth objective. The stain gives a fairly good picture when freshly made. After about ten days standing it gives a differential stain for the azur granules also.

Red cells take a coppery tint; in polychromasia some cells are pinkish. Nuclei are in shades of reddish purple or purplish red. Cytoplasm is bluish or blue. Eosinophile granules are coppery red. Neutrophile granules take a pinkish color. Basophile granules stain purplish or reddish purple. Azur granules are cherry red. The stain is fairly good for general differentiation. Leishman's stain has been simplified and modified in many ways.

Wright's stain has been developed from Leishman's stain. Its preparation is rather difficult and the resulting powder not always successful. The powdered stain, which is a precipitate formed by combining eosin yellowish with methylene blue under certain conditions, can best be purchased. This powder is to be dissolved in methyl alcohol, 1.5 gm. powder to 100 c.c. methyl alcohol. The solution keeps for a month

or more. Wright's stain is useful for general differentiation. The technique of staining is:

Place the dried slide on a level surface. Flood with the methyl alcohol solution, which fixes and stains the slide at the same time. Allow the stain to stand on the slide one minute. Add an equal amount of distilled water, and allow to stand for two or three minutes,—the longer period giving a deeper blue stain, but eosinophilic granules are more deeply stained in the shorter period of time. Longer standing than three minutes may cause a precipitate to be formed. Rinse in water for about half a minute. The thinner areas should be pinkish or yellowish in tint. Experience is necessary to determine the exact degree of differentiation which gives best results for each blood specimen. Mount in water and examine by means of a dry one-tenth objective, or dry and examine by means of an oil immersion lens. Red cells show pinkish or yellowish. All nuclei are blue or purplish blue, varying in shading for different types of cells. Neutrophile granules are pinkish or pale purplish in color. Eosinophiles are brilliant reddish pink or cerise. Hyaline cells show blue protoplasm which may be very dark or rather pale. Platelets are blue or purplish. Malarial parasites are blue with darker purplish, reddish or bright red chromatin. Mast cells show deep blue granules. The stain is fairly useful in general differentiation. Tap water used for differentiation increases the blue tints and this is often desirable.

Giemsa stain is much simpler than the methods described, and it gives excellent differentiation. The formula is simple and the stain constant in quality. The powdered stain may be purchased or it may be made up as follows:

Azur II eosin	3.0 gms.
Azur II	.8 gm.
Methyl alcohol, c.p.,	375. gms.

Grind up the stains in the alcohol, using a small amount of the alcohol first. When thoroughly mixed add,

Glycerine, c. p.,	125 gms.

The solution keeps for several months, and sometimes much longer.

The technique of staining is simple also.

Place slides on a level surface, flood with methyl alcohol for five minutes, drain but do not rinse. Put fifteen drops of the stain on the slide, then add ten drops of distilled water; stain for fifteen minutes.

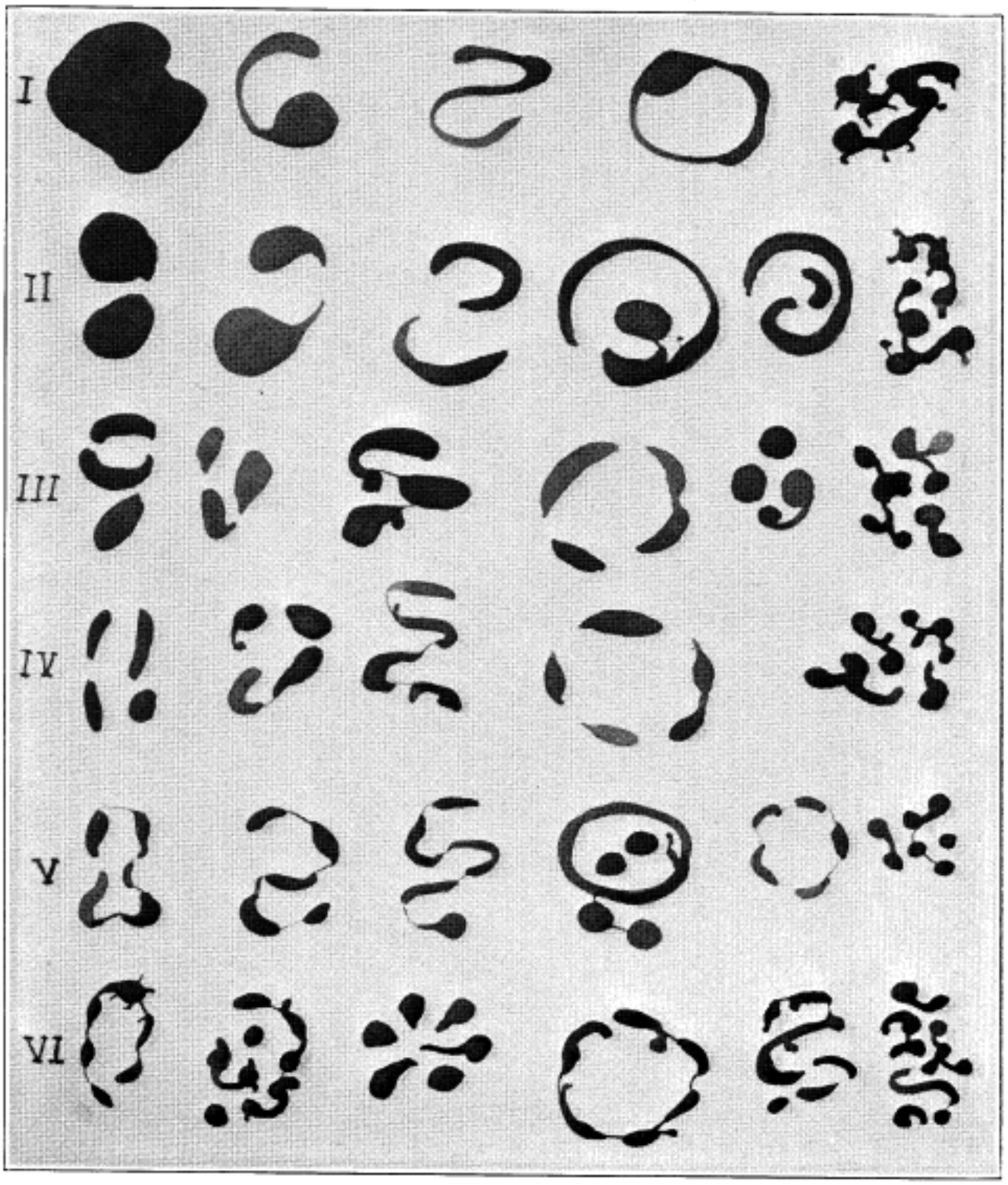


PLATE XIV

Determination of the neutrophile nuclear average. Nuclei only are shown.

I. Five single nuclei.

II. Six double nuclei.

III. Six triple nuclei.

IV. Five quadruple nuclei.

V. Six nuclei of five lobes each.

VI. Six nuclei of six lobes each.

Rinse, drain, mount in water and examine as usual. Or dry and examine with oil immersion lens. They fade quickly in cedar oil; paraffine oil may be used instead. One drop of half-saturated sodium carbonate increases the staining of the basophilic elements. The use of tap water instead of distilled water gives better differentiation in our laboratories.

Oxydase Reaction

This method of staining differentiates cells derived from lymphoid tissue from those derived from the red bone marrow. Three solutions are required:

A. 100 c.c. distilled water
5 drops saturated aqueous solution sodium hydroxide
1 gram alpha-naphthol

Boil this solution, cool, decant fluid from any residue which may be present. Allow to stand three days or more before using. This solution will keep a month or more.

B. 100 c.c. distilled water
.5 gram basic paraphenylenediamine

Mix without heat. Allow to stand at least twenty-four hours before using. This solution will keep a month or more.

C. 100 c.c. distilled water
5 c.c. formalin

Technique of staining. First mix together equal parts of A and B and filter. This mixture must be used within an hour or so.

Fix dried blood smear in solution C for five minutes.

Stain with the mixture of A and B for five minutes.

Rinse, mount in water and examine, using dry one-tenth lens, or dry and use oil immersion lens.

Cells derived from lymphoid tissue show no granules. Cells derived from bone marrow show blue granules.

The reagents are difficult to secure and preparation of the solution is cumbersome. It is rarely of value in diagnosis but it has given some good results in research work.

Merck offers simpler reagents; beta-napthol-sodium is sold in sealed glass ampoules as Mikrozidin. Solution A is a 2% solution of Mikrozidin in distilled water. He supplies also dimethylyaraphenylene-hydrochloride in similar ampoules. Solution B is a 1% solution of this in distilled water. Equal parts of the two solutions are mixed and the re-

sulting greenish precipitate filtered off. The further technique is the same as in the original method. The oxydase granules appear brownish or blackish by this method, instead of blue; the significance is identical.

The Bone Marrow

Examination of the cells from bone marrow is easily made. Take a piece of rib or of any bone containing red marrow; make a fresh break if the bone has not been removed immediately before the smear is to be made. With forceps press upon the bone just beyond the break until a drop exudes from the broken end. Very quickly make smears from this drop upon slides, following the method used for making blood smears. Dry in air, and stain after any of the methods used in the study of blood smears. Vital,—or supra-vital,—staining methods are employed in the same way. If much fat happens to be present it may be necessary to remove this by flooding the slide several times with warm alcohol, ether and alcohol, and ether alone until the fatty globules are washed away. Bone marrow from adults is usually very fatty, while bone marrow from still-born babies, human fetuses and certain laboratory animals is usually free enough from fat to stain readily and easily.

To demonstrate the nerve endings in bone marrow it is best to use histological methods, for which see any text-book on histology. The methods employed are too long for discussion in this chapter.

TECHNIQUE OF WARM SLIDE STUDIES

Very useful information can be secured from a study of the blood in the vital state; that is, during the lifetime of the blood cells on a warm slide. The conditions of the warm slide approach those of normal blood in the capillaries and the behavior of the cells and the formation of fibrin threads present pathognomonic variations in many instances. This study requires only a small amount of time and no expensive or complicated apparatus. The only difficulty is that the microscope and the patient must be brought together and that the examination must be made immediately after the blood is taken. If the patient is too ill to go to the laboratory the microscope must be taken to his bedside. Since there is no noise, odor or confusion associated with the work of warm-slide examinations it is not often annoying to the patient or to anyone else to have the work done at a small table beside the bed.

The technique is simple but considerable practice is necessary to attain skill in securing the correct amount of blood, speed in beginning the observations, accuracy in watching several factors at the same time and ability to distinguish between important and unimportant variations from the normal conditions.

Preparations

Have ready a microscope with a one inch eye piece and a dry objective of high power, preferably a one-tenth, though the one-eighth can be used fairly well. The light must be strong but the field must be well limited by the iris diaphragm. Electric lighting is steady and is usually accessible. Gas light or daylight can be used efficiently, and the person who makes these examinations should be constantly in the habit of using these lights interchangeably whenever there is any probability that it may be necessary to rely upon them for emergency work.

Several types of warm stage are on the market. These are commonly kept at the selected temperature by electricity. If electricity is not available at the bedside a heavy slide can be selected, warmed by water of the selected temperature, dried quickly and placed upon the stage of the microscope. The stage of the microscope can be warmed by placing any heated object on it for a few minutes before the examination is made. The cover-glass is best warmed by allowing it to lie upon the warmed stage of the microscope or by holding it between the palms for a few minutes. In using these make-shift methods it is necessary to be very careful to avoid too great heat.

Connect the warm stage and place it on the microscope stage several minutes before the blood is taken. Have the slide and the cover-glass perfectly clean; put them on the warm stage. There is some difference in the time required to reach the selected heat in different warm stages. Be sure that the slide and the cover glass are thoroughly warmed before the blood is taken. Have the light in place and be sure that the iris diaphragm is correctly adjusted for the study. The iris diaphragm requires delicate adjustment, because these living blood structures are not stained and are visible only on account of the variations in their refrangibility.

Cleanse the lobe of the ear of the patient, using only sterile water without any unnecessary handling. Prick the skin with the usual sterile needle and wipe away the first drop of blood.

Touch the flat side of the warm cover-glass very quickly to the top of a drop of blood just exuding from the wound, place this on the warm

slide, blood side down, put it on the warm stage and examine immediately. Let another person attend to the wound in the ear; if no other person is at hand allow the wound to remain untended until the first examination of the warm slide has been made. After a minute or so the observer may leave the microscope for the few seconds necessary to wipe off the lobe of the ear and thereafter the observations may be interrupted for a few seconds at a time if necessary.

In observing the changes on the warm slide many factors are to be kept in mind and all of these must be watched all the time.

Note the presence or absence of fibrin threads when the slide is first seen. These are lines of highly refractive material, best seen in the spaces between blood cells. If they are present when the slide is first examined note that fact and note any later time at which new threads are first visible. Note the amount of fibrin, the size and length of the threads and their contour. They may be even and regular, or swollen at long intervals or swollen at shorter intervals, presenting a beaded appearance. Note whether the threads lie in straight lines, apparently unrelated, or whether they radiate from cells or from groups of platelets. Note whether the fibrin is formed in net-like masses or in the normal straight threads. Note when the fibrin ceases to be increased in amount. With practice all these factors can be seen at a glance. Continue the observations until no further fibrin appears.

Normal blood shows a few fibrin threads within four to six minutes, and the fibrin continues to form, slowly, for about five minutes longer. The condition characterized by very scanty or absent fibrin is called hypinosis, and this is present during starvation and in certain forms of mal-nutrition. Excessive fibrin formation is called hyperinosis, and this condition is present during pneumonia, acute rheumatism and in certain other inflammatory states. Hyperinosis is usually marked in malignancy, and in this case the threads are formed almost at once, are very irregular, often beaded, radiate and arranged in irregular net-like tangles. In pneumonia the threads are very speedily formed, are heavy, long and abundant, and are regular in contour; they may or may not be radiating, and they do not form nets in typical cases. The test is very useful in making an earlier diagnosis of pneumonia than is possible by any other means.

Note the time at which the first movement is seen in a white blood cell, and note also the classification of the cell which first moves. Usually an eosinophile or a neutrophile moves first; rarely hyaline cells are first active. Eosinophiles are recognizable by their very large gran-

ules; neutrophiles by their fine granules and their irregular nuclei; hyaline cells by their glassy protoplasm and their round, central nuclei. No attempt should be made to distinguish between the different classes of hyaline cells in ordinary cases.

Note the manner of movements. Protoplasm may flow steadily or it may flow rapidly for a few seconds, then slowly or may cease moving for a time; note these conditions. Granules or intergranular protoplasm may seem to be the most active part of the cell; note these relations. Pseudopodia may be extruded slowly or rapidly; may flow from two or from several sides or angles of the cells and may seem to be purposive or purposeless and even antagonistic in their activity; note these conditions. The pseudopodia may be extruded and the protoplasm flow into them thus causing the cell to change its location on the slide. The pseudopodia may be extruded, some protoplasm may flow into them, then the protoplasm may flow back into the cell and the pseudopodia be retracted, or pseudopodia may remain present and again the protoplasm flow into them; these various activities may seem purposive or may seem erratic. Pseudopodia may vary in form being long, short, slender, broad, heavy, flat, active or inert, and may present many peculiarities of structure and of activity. All such peculiarities should be noted. The activity of the cells may change during the period the slide is under observation; cells at first normally active, inactive or excessively active may show increased or diminished activity within a few minutes, and all such variations should be noted.

Note the nuclear reactions. The nucleus may follow the protoplasm in its activity, or it may remain almost or quite immovable. Hyaline cell nuclei rarely move at all in normal blood and if they do move this is of interest. Neutrophile and eosinophile nuclei tend to follow the pseudopodia fairly quickly in normal blood; if they do not move at all or if they follow the protoplasm very quickly these facts should be noted.

The time when the leucocytes show diminished activity and the time when the first inactive cells appear should be noted. Pay no attention to inactive hyaline cells, since these very often fail to show activity at any time, in normal blood. Abnormally they may die with pseudopodia still visible.

Normal leucocytes live at least an hour and they may live several hours on the warm slide. Keep them under occasional observation at least forty minutes. If none, or only a few, are dead after forty min-

utes on the warm slide they may be said to "live well on the warm slide", which means that they have at least moderate vitality.

It is not necessary to watch the individual red cells. These undergo various changes on the warm slide. With evaporation of the watery content of the blood the red cells crenate. First there appears a bright spot on one edge of the cell, then another, then several and many such bright spots until the surface seems covered with thorns. Crenation may occur without evaporation, but it always occurs when the osmotic tension of the fluid surrounding erythrocytes is increased.

The stroma and the hemoglobin of the red cells may undergo various degenerative changes. Parasitic and bacterial inclusions may thus be imitated. Suitable staining methods quickly explain these structures, and it is best to disregard them while making the warm slide examinations.

Special Tests For the Warm Slide

Supravital staining. Interesting facts can sometimes be learned from a study of the blood cells which have received some stain which does not visibly affect their life and which have certain affinities for living granules or other cell structures.

The same technique is employed for all these stains. A few crystals of the stain may be mixed with the fresh blood, and smears made from this mixture, or a solution of the stain is allowed to dry upon a slide and the blood smear made upon this stained surface. The following stains are employed for supra-vital staining: Brilliant cresyl blue, Janus green, methylene blue, toluidin blue, thionin, Capri blue, Nile blue, paraphenyl blue, neutral violet, neutral red, pyronin, fuchsin and safranin. Each of these stains gives some particular reaction which may be of value in the study of cell structure or cell inclusions, but each stain is characterized by some inefficiency or some source of error for other studies than that for which it is most useful. In other words, there is no known stain which renders all cellular structures and inclusions simultaneously visible.

Brilliant cresyl blue and methylene blue give useful information in certain anemias and one of these should be used in routine blood examinations in order that immature red cells may be recognized. Either of these stains demonstrates the basophilic reticulation present in immature red cells, and the number of these is an important factor in the study of anemic bloods.

In our laboratories the following technique is used:

Prepare the slides in advance. Put a drop or a few drops of a solution of the selected stain on about the middle of a glass slide and allow it to dry, thus leaving a precipitate of the stain on the slide. If the stain is left in unequal masses or if there are clear spots left within the stained area the stain was not completely and perfectly dissolved or the slide was not quite clean; such slides should be cleaned, the solution investigated and the preparation repeated. These slides with the stained centers should be prepared in advance; they can be kept in slide boxes, well covered, for several days or indefinitely if carefully protected against dust and moisture. Just before the examination is to be made place the stained slide on the warm stage and take the blood as before, making such observations as are desirable in connection with the special stain being used.

Brilliant cresyl blue shows the reticulation of the red blood cells vividly and its chief value lies in this fact. The reticulum of the younger forms of white blood cells shows more plainly than is the case with older cells.

Methylene blue is useful occasionally. Very thin deposits are necessary for satisfactory work. Nuclear structures and basophilic granules are shown very clearly, and the cells retain life for a long time after being stained with methylene blue. Methylene blue may be decolorized and its color may be regained as successive oxidation and reduction of the stain occurs, and thus variations in the physiological activities of the cell may be studied. As the cells die the nuclei take the methylene blue present in small amounts with avidity not shown during life. If too heavy a deposit of methylene blue is present the cells do not show these reactions, but the nuclei are deeply stained at once and the cells die rather quickly.

FIBRINOLYSIS

The study of the fibrinolytic ferment is one of the newest and most interesting methods of hematological technique. This study in its present form is limited to osteopathic laboratories. The method is as follows:

Prepare fibrinolysis pipettes in lots of one to several hundred at a time. Have ready glass tubing of three millimeters diameter and about forty centimeters long. Heat to redness an area about five centimeters from the end of the tube and draw out to a capillary tube five to ten centimeters in length. Repeat this heating and drawing to a capillary tube, each time leaving a space of about three centimeters of the tube

unchanged in size. Continue until the entire length of glass tubing is changed into a series of small glass bulbs about three centimeters long and the diameter of the glass tubing, separated from one another by capillary tubes of five or more centimeters in length. Break the capillary tubes about half way between the bulbs. Seal the ends of the capillary tubes in flame and put into a clean dry box to keep ready for use. The interior of the pipettes is sterile, the glass having been heated to redness.

The exterior is easily sterilized by passing through a flame, when the pipettes are to be used.

Have ready also a supply of small vials or glass tubes. The tubes used for Wassermann tests are convenient. Small baskets in which these tubes may be supported are necessary. We use the small perforated aluminum baskets sold as coffee balls; the lids being removed they are of convenient size and shape. Each of these small baskets holds several vials or tubes, and also the identifying cards upon which notes can be written at each examination.

Incubator, microscope, slides, cover-glasses and ordinary laboratory equipment, cotton and boiled water for washing the skin and a blood lancet are also required for the test.

A supply of tap water which has been sterilized by boiling in a closed vessel on successive days is best for this use. Distilled water can be used but fibrinolysis proceeds best in water of the type ordinarily used for drinking,—either spring water, tap water or well water. If distilled water must be used, it should have added to it for each liter of water the following formula:

Magnesum sulfate	25 mgs.
Sodium chloride	50 mgs.
Sodium carbonate	25 mgs.

When ready to take the blood pass three pipettes through a flame, break off both sealed ends of the capillary tubes, leaving about one centimeter of the capillary tube at each end of the bulb of the pipette, cleanse the skin of the lobe of the ear if the patient is an adult or a large child, or the skin of the side of a toe or of the heel, if the patient is a baby or a small child. Prick the skin deeply enough to secure several drops of blood. Wipe away the first drop, using sterile cotton (or gauze), and touch the end of a capillary tube to the next drop which oozes from the skin. Fill the bulb about half full. Repeat until three pipettes have been filled in this way. Lay the pipettes upon a sterile plate and allow to rest for an hour.

Fill three of the small vials or Wassermann tubes with sterile tap water and boil for three minutes, allow to cool to about room temperature. Note on slip of paper the name of the patient, of the doctor, the date and the hour the blood was taken, the hour the blood clot was placed in the vials and into the incubator.

An hour after the pipettes have been filled the blood should be thoroughly coagulated and the clot should be separated from the serum. If coagulation is not complete, let the pipettes remain longer at rest. Break off the capillary tubes at the clear end of the bulb and break the bulb-like part which is filled with blood, just at its junction with the capillary tube which is filled with blood. This leaves both ends of the blood-clot free. Catch the serum on filter paper as it oozes from the end of the broken tube. Drop the clots, still within the bulb-like part of the pipette, into the vials or Wassermann tubes already filled with sterile tap water. Close the tops of the vials and shake them until the clots drop out of the pipettes. Ordinarily three to five fairly vigorous shakes are enough to allow the clots to fall out of the pipettes. If longer shaking is necessary to free the clots, or if the clots do not fall out of the pipettes completely, the clot is said to be adherent to the tubes. Place the vials in a small basket or on a rack, and incubate until the clots are dissolved, or until three days have passed without beginning digestion. Examine the vials at twelve hour intervals until fraying of the clot is noted, then at six hour intervals until the clot is almost digested, and after that watch the progress of digestion at shorter intervals until the clot is completely digested and dissolved in the water. Note the time of beginning digestion and of completed digestion.

When digestion begins in 20 to 30 hours, and is complete in 45 to 55 hours, fibrinolysis is normal.

Pour the contents of two of the vials into a centrifuge tube, fill another centrifuge tube with water to balance, and centrifuge at moderate speed for ten minutes. Pour off the supernatant fluid, and examine the sediment under a ⅙ or a 1/10 objective with 1 inch eyepiece. Note whether bacteria are present; if so, contamination has occurred and the findings while not actually negligible are less accurate. If any peculiarity in the process of digestion has occurred, the test should be repeated. A few bacteria do not militate against the accuracy of the test, but absolute sterility is much to be desired. It must be remem-

bered that the skin cannot be completely sterilized; that some bacteria are nearly always present in the deeper layers of the skin, and that the mixture of blood and water, at body temperature, gives excellent opportunities for bacterial growth.

Normally, the sediment contains debris derived from the digested fibrin; this is usually present in minute rounded masses. Normally many blood cells, both red and white, appear in the debris. When the blood cells are completely digested there is some undifferentiated proteolytic ferment present. Since this digests fibrin as well as blood cells, the report must be "Fibrinolysis masked by action of some undifferentiated proteolytic ferment."

If three days pass without recognizable fraying of the clot, the report must be "Fibrinolysis absent."

If the clots dissolve completely but more rapidly or more slowly than normal, by six hours or more, the reports must be, "Fibrinolysis delayed" or "Fibrinolysis hastened," giving the hours as noted at the different examinations of the clots.

One to three pipettes filled with the blood of a person known to show normal fibrinolysis should be used as controls for every test until the technique has been standardized.

The theory of fibrinolysis is discussed in chapter V of this book. Briefly, it may be said that, in terms of this theory, any person who has normal fibrinolysis has at least one factor in protection against the growth of malignant neoplasms; that any person lacking this ferment lacks this factor of protection; that non-differentiated ferments often occur during the progress of certain diseases, especially during the growth of cancer in the body, and these ferments which are not differentiated digest fibrin and blood cells together.

A report on the fibrinolysis test should include the following items.

Name of patient and doctor in charge of the case; date and hour of taking the blood and of placing in incubator; date and hour of beginning fraying and of completed digestion; or time of last examination if no digestion occurs. The report should state also either "Fibrinolysis normal," "Fibrinolysis absent," "Fibrinolysis delayed," "Fibrinolysis hastened," or "Fibrinolysis masked by some undifferentiated proteolytic ferment."

TESTS NOT COMMONLY USED IN ROUTINE EXAMINATIONS

Several tests are useful under certain circumstances, but are not necessary for all cases. These are not included in routine examinations unless statistics are being accumulated for research purposes. In our laboratories, these special tests are made whenever the condition of the patient indicates that useful information might be secured thereby. Whenever special studies are being made in which the information to be secured by any special test is thought to be useful, that test is added to the routine work for all patients for as long a time as is necessary to secure the statistics desired.

To secure all possible information from the study of every blood specimen examined in our laboratories is impossible. Some of the special tests require a considerable amount of blood, and it is not good for sick people to yield so much blood. The time required for making so many tests precludes their routine use. Many of them must be made very soon after the blood is taken, so that several persons must work at the same time if several of the special tests are done for the same patient on the same day. For these reasons it is best to limit the special tests to those which are indicated in each case, plus those which can be made for research purposes without too great demands upon the time of the laboratory staff and without any unnecessary demands upon the blood or the strength of the patient.

THE SPECIFIC GRAVITY OF THE BLOOD

Before the invention of the instruments which depend upon color changes for the estimation of hemoglobin, the determination of the specific gravity of the blood was the most important method of estimating the amount of iron in the blood. Modern hemoglobinometers give more accurate findings and the estimation of the specific gravity is now considered of little value in routine laboratory diagnosis. In research work it still has a place, and it occasionally occurs that useful information is secured by a study of the changes in the specific gravity of the blood under experimental or pathological conditions.

The specific gravity of normal blood varies almost exactly with the hemoglobin and the red cell count. Daily variations in red cell count, specific gravity and hemoglobin content of the blood form practically parallel curves in normal individuals; variations in the curves fall within the percentages of unavoidable error in technique.

Various methods and different students give somewhat different findings but all agree that the variations follow the blood count and the hemoglobin under normal conditions. Figures varying from 1.052 to 1.063 for healthy young people are given by different authors.

For normal young women in Southern California the specific gravity of the blood varies between 1.053 and 1.055; for normal young men the specific gravity varies between 1.055 and 1.059. Persons who seem perfectly normal occasionally show specific gravity as low as 1.052 or as high as 1.060. The blood of normal children has lower specific gravity than the blood of adults and may be as low as 1.048. At birth the specific gravity is much higher, corresponding to the high cell count and high hemoglobin present at birth and during the first few days of life.

Pathologically the specific gravity varies considerably, though this fact has not been found useful in diagnosis. In severe anemias the specific gravity may be as low as 1.025, and in high fevers or in jaundice as high as 1.070.

In certain forms of cachexia the specific gravity does not vary with the hemoglobin and the cell count; these may remain almost normal while the specific gravity of the plasma may be considerably lowered. In this case lowered osmotic tension is indicated by the changes which occur in the red cells during the actual count.

The specific gravity of the red cells is higher than that of the plasma. The specific gravity of plasma and serum vary together in health and in disease.

The higher specific gravity of the red cells in the plasma of lower specific gravity is associated with a common osmotic tension because the hemoglobin molecule is heavy and of large size. Since the osmotic tension varies with the number of molecules in a solution, the red cells, with heavy, iron-containing hemoglobin molecules of large size, are still isotonic with the plasma with smaller, lighter molecules of inorganic salts. When the osmotic tension of the plasma varies, the red cells imbibe or give off enough water to equalize the tension within and without the cell.

Accurate determination of the specific gravity is rarely needed in diagnosis. An estimation based upon the hemoglobin percentage, the color index and the osmotic tension of the red cells is sufficient for all ordinary clinical purposes. The specific gravity of the red cells varies with the hemoglobin, almost exactly, and the specific gravity of the

plasma varies with its osmotic tension. By comparing the hemoglobin, the color index and the osmotic tension it is possible to determine whether the specific gravity is normal or is increased or diminished from the normal, which is all that is useful in diagnosis.

The gravimetric method is quite accurate. This requires five cubic centimeters of blood, and the blood must be taken from a vein. This is measured accurately, weighed upon delicate chemical balances, and the weight and volume compared with the weight and the volume of distilled water, with corrections for temperature and height above sea level. The gravimetric method is of value only for scientific precision in research.

By using a pycnometer the specific gravity can be taken with accuracy, but a considerable amount of blood is necessary. In certain diseases the removal of ten to fifty cubic centimeters does no harm, and normal persons can give much more than this amount without recognizable effect. The technique is as follows:

Have ready the pycnometer and delicate chemical balances, also a syringe of twenty cubic centimeters capacity fitted with a medium needle, both sterile. Cleanse the skin over the vein of the patient's elbow, washing first with water and soap, then with alcohol, then dry with sterile cotton. Leave the cotton in place until the vein is to be pricked.

Weigh the perfectly dry, clean pycnometer. Fill it with distilled water at a temperature of 99° F. and weigh again. Dry the pycnometer.

Place a firm elastic band around upper arm of the patient. Remove the cotton and take blood from the median basilic vein, enough to fill the pycnometer. Cover the wound with sterile cotton. Immediately fill the pycnometer with the blood and weigh again.

Cleanse the skin of the arm from which the blood was drawn. If bleeding persists apply ice, or cotton moistened with alcohol, and keep under gentle pressure. Usually the wound is closed by the time the weighing is finished.

Computation: Subtract the weight of the empty pycnometer from its weight filled with distilled water, also from its weight filled with blood. Thus the actual weight of the water and of the blood are determined Divide the weight of the blood by the weight of the distilled water of the same temperature (and, of course, of the same height above sea level). This gives the specific gravity of the blood.

Aremetrical methods require considerable practice but only a little blood. Have ready several perfectly clean, dry glass tubes; those used for estimating the specific gravity of urine are convenient. Fill these with varying mixtures of chloroform and benzine, or of other suitable fluids. These have the following qualities,—they must not be miscible with blood but must be freely miscible with each other, and one must have a specific gravity above and the other below the specific gravity of any blood apt to be encountered.

Take five of the urinometers or other convenient tubes and fill them with mixtures of the selected fluids in proportions to produce specific gravities of 1.058, which is that of average normal blood; 1.068, which is the highest reading for normal blood; 1.051, which is the lowest reading for normal blood; 1.025, which is extremely low and 1.072, which is extremely high. Have these tubes properly marked and arranged in a row.

Take a glass tube of about three microns bore and about ten centimeters long, bent at right angles about one centimeter from its lower end. Attach a rubber tube at its upper end and draw blood from a capillary puncture upward into the tube for about two centimeters. Very quickly blow one drop of the blood into each of the urinometers at about one half the depth of the liquid. The drop will sink in the fluid whose specific gravity is lower than that of the blood and will rise in the fluid whose specific gravity is higher than that of the blood, and will remain stationary in the fluid that equals the specific gravity of the blood. If the drop does not remain stationary in any one of the fluids, prepare three other urinometers and fill with mixtures of intermediate specific gravities between that which causes the drop of blood to sink and that which causes it to rise. For example, if the drop of blood rises in the fluid with a specific gravity of 1.058 and sinks in the fluid with a specific gravity of 1.051, the fluids next selected should be of a specific gravity of 1.053, 1.055 and 1.057. If the drop sinks very slowly in the fluid with a specific gravity of 1.051, and rises rapidly in the fluid of 1.058, it may be best to prepare only one extra tube, at 1.053, for the second test. Practice in this work is necessary in order to determine what specific gravities to select for the second test, and to avoid unnecessary manipulations. If the drop of blood remains quiet in any fluid, that fluid has the specific gravity of the blood. If the blood rises in one fluid and sinks in another, some intermediate point is the specific gravity of the blood; that is, if it rises slowly at 1.055 and sinks slowly at 1.053 the specific gravity of the blood is 1.054.

Exton's immiscible balance is more convenient than the series of tubes. This instrument consists of a standard which supports a glass tube supplied with a tap at the bottom through which a fluid may be added to the contents of the tube from the bottom. The top of the tube is open. The tube is large enough to permit the use of a float for taking the specific gravity. The tube is filled with a mixture of varnolene (petroleum ether) and carbon tetrachloride in such proportions that the specific gravity of the mixture is that to be expected in the blood to be tested. The bulb which is connected with the bottom of the tube is filled with lighter fluid (varnolene) and a supply of the carbon tetrachloride is in a dropping bottle. The lighter fluid thus can be added from the bottom of the tube, the heavier at the top. Since the two fluids are freely miscible this process causes speedy mixing of the two fluids in the tube.

A drop of the blood to be tested is forced into the tube from the end of a glass tube, as in the method previously described. If the drop sinks, a drop or a few drops of the carbon tetrachloride are added at the top of the tube. If the drop of blood rises, a drop or a few drops of varnolene are allowed to flow into the tube at the bottom. The proportions are thus varied until the drop of blood remains at about the middle of the tube. The fluids are not miscible with blood and the blood drop should remain spherical and distinct during the small amount of time necessary for the manipulation of the fluids. The specific gravity of the fluid in which the drop of blood rests is then determined by means of a float. This float must be the one provided with the instrument because this is standardized for the surface tension of the fluids mentioned.

If considerable handling of the two fluids is necessary in order to attain stability of the drop of the blood, another mixture should be provided, of the specific gravity finally determined upon, and a fresh drop of blood added to this. Frequently this second drop of blood does not float freely in the mixture, because there is some slight change in the blood when it is allowed to remain for too long a time in the mixture of fluids. The second determination is more quickly made, for obvious reasons. Sometimes, of course, the specific gravity first determined is found to be accurate.

Platelet Count

There are several conditions in which the enumeration of the blood platelets gives useful information in diagnosis. These structures are so fragile that it is difficult to make counts which are as nearly accurate

as are those made of the red and the white blood cells, but even so the counts are useful.

The simplest method is that of Wright and Kinnicutt. Prepare two solutions as follows:

1. Brilliant cresyl blue	1 gram
Triple distilled water	300 c.c.

This may be kept in the refrigerator for some weeks.

2. Potassium cyanide	.1 gram
Triple distilled water	140 c.c.

This must be freshly prepared.

Immediately before using mix one part of solution 1 with three parts of solution 2, and filter.

Take the blood as in making ordinary red cell counts, preferably making a 1 to 200 dilution, and place in the counting chamber. Allow the diluted blood to stand in the chamber for ten or fifteen minutes, in order that the platelets may settle. Platelets are stained a lavender or lilac tint; red blood cells are almost invisible and the leucocytes have dark blue nuclei. Count the platelets in 200 small squares or more. The calculations are the same as in the case of the red cell count.

Because the platelets are so small it is desirable to use a higher power objective. In our work, however, an ordinary one-sixth objective with a one-inch eyepiece has been found quite satisfactory. By using a specially ground, very thin coverglass it is possible to use a one-eighth eye-piece. There is a coverglass sold which is of ordinary thickness but which has a thin area in the center; this is satisfactory but it is very easily broken.

Several indirect methods are in use, and these preserve the platelets rather better than is the case with the Wright and Kinnicut method. Different solutions are employed by different workers. The simplest is a 10% solution of sodium metaphosphate in water. This preserves the platelets but does not stain them.

Hayem's Fluid

Water	200 c.c.
Sodium chloride	1 gram
Sodium sulphate	5 grams
Potassium iodide solution	35 grams

(Potassium iodide solution is made of a 5% potassium iodide solution in water, to which have been added iodine crystals to saturation).

Picini's Solution

Distilled water	266 c.c.
Mercuric chloride	2 grams
Sodium chloride	4 grams
Glycerin	26 grams

Kemp's fluid is .9% sodium chloride in 2.5% formalin.

Determann's Fluid

Distilled water	160 c.c.
Glycerin	30 grams
Sodium chloride	1 gram
Sodium sulphate	8 grams
Methyl violet	25 milligrams

Method

Cleanse the skin as for any prick to secure blood.

Place one drop of the selected fluid upon the surface of the skin. Prick the skin through this drop, so that the blood mixes with the fluid as it emerges from the wound. When the drop is definitely a pink color make smears on glass slides. Dry in air, and stain by any of the usual methods for making a differential count. Count the red cells and the platelets on this smear and determine the ratio of platelets to red cells. By means of this ratio and the actual count of the red cells determine the actual number of platelets per cubic millimeter of blood.

For example, in a certain patient the red cells were 4,000,000 per cubic millimeter of blood. In the smears made from the fixed blood using the above method, the count differentiating red cells and platelets showed a ratio of one platelet to twenty red cells. Therefore there were 200,000 platelets per cubic millimeter of that blood.

Viscidity

There is little practical value in accurate estimations of the viscidity of the blood. Variations in viscidity follow variations in hemoglobin quite closely, and this is true for physiological changes and for abnormal conditions of the blood. Occasionally there is some need for the accurate determination of viscidity, and in routine reports the relative viscidity should be noted.

The hemoglobinometer slides may be employed for a rough estimation of the viscidity of the blood. When the slides are separated, one slide is drawn across the other, and the amount of stickiness noted. In normal blood this stickiness is enough to delay, slightly, the separa-

tion of the slides, but not to cause any appearance of threading. Blood which has increased viscidity shows a tendency toward thread-formation when the slides are separated. Blood which is subnormal in viscidity shows no recognizable stickiness. If the viscidity is in any way abnormal, more exact methods should be employed.

Viscidity is indicated by the manner in which the drop stands up as it emerges through the puncture. A normal drop of blood is practically hemispherical; blood which is deficient in viscidity spreads out over the skin; blood which is abnormally viscid stands up much higher.

A more nearly exact and very satisfactory method depends upon the size and rate of dropping. For this, a pipette of certain diameter is employed; the blood taken into the pipette and allowed to drop; the number of drops falling in five seconds or ten seconds is the measure of viscidity; as 2/5 or 3/10. The normal for the method must always be noted.

There is considerable variation in the viscidity of the same blood at different times of the day and as a result of dietetic changes.

Coagulation Time

A number of methods of determining the coagulation time of the blood have been employed, but none has been altogether satisfactory. The following methods have been employed in the laboratories of the Research Institute.

The blood taken in the slides of Dare's hemoglobinometer coagulates in about five minutes, normally. The slides are allowed to remain quietly for five minutes, and are then separated. If the coagulation time is normal, the blood is found completely coagulated but the serum has not yet separated. If the blood is not coagulated the coagulation time is increased, and more exact methods should be employed. If the blood clot has begun to separate from the serum, the coagulation time is diminished, and more exact methods may or may not be employed, according to the other blood findings and the general condition of the patient.

For more exact determination of the coagulation time, capillary tubes are used. These are made by drawing ordinary glass tubing out into fine capillary tubes, which should have a caliber of not more than the size of a dark hair and should be uniform in every respect. These tubes are broken into lengths of one inch. Ten of these tubes are filled for about half their length with blood, and allowed to lie for a length of time dependent upon the results of the observations made with the

hemoglobinometer slides. If these have shown delayed coagulation, the capillary tubes are allowed to lie for six or even ten minutes; then one tube is broken. If the blood falls out in a cast of the tube, coagulation is complete. If it is perfectly fluid, two minutes should elapse, when another tube should be broken. If it is still perfectly fluid, two or perhaps three minutes may elapse before another tube is broken. The coagulation time is given in the number of minutes required for complete coagulation as shown by the formation of a cast of the tube when the glass is broken. This is the most accurate and satisfactory method now known.

Howell's method requires more blood. Four cubic centimeters of blood are taken from the vein at the elbow, as usual, and immediately mixed with an oxalate solution (5 cubic centimeters of 1% sodium oxalate in ten cubic centimeters of 0.9% NaCl.) This prevents coagulation. The mixture is centrifugalized until the corpuscles have been thrown down. Three small tubes are provided, which contain one, two and three drops of 0.5% calcium chloride. Into each of these tubes five drops of plasma are placed: the time accurately noted, and the time of complete coagulation noted. By this method, Howell found normal coagulation time in nearly all conditions except hemophilia. Normal blood coagulates in nine to twelve minutes; hemophilic blood coagulates in one to five hours. Purpuric blood gave normal coagulation time by this test. It is evident that this method rules out all delayed coagulation due to disturbances of the calcium-content of the blood.

By the method of Lee and White about 1 cubic centimeter is drawn from the bend of the elbow, as usual, using a glass hypodermic syringe which has just been rinsed with .9 NaCl solution. The blood is quickly placed in a small glass tube, about eight millimeters in diameter, which also has been rinsed in .9 NaCl solution. The tube is then turned back and forth once each half-minute and the time noted at which the blood no longer flows. This method has little to commend it, for the friction modifies the coagulability.

When the coagulation time is normal but the clot is very soft, defective fibrinogen is indicated. This is usually due to some abnormal condition of the liver; occasionally it is due to deficient diet. These conditions are usually easily recognized by clinical phenomena. Cases of hemophilia may be associated with deficient platelet count, and this is easily determined by making an actual count of the platelets. In other cases of hemophilia the platelets fail to agglutinate properly, and this

can usually be determined by studying warm slide specimens made with rather thick smears of blood. In other cases of hemophilia there is deficient calcium in the blood, and this can be determined by the following method.

Calcium and Coagulation

Cleanse the skin of the elbow and cover with cotton or gauze wet with normal salt solution. Rinse a sterile syringe and needle with normal salt solution and leave all surfaces thoroughly wet with the solution so that the blood does not come in contact with air. Take two cubic centimeters of blood and place this in a small test tube containing one-half cubic centimeter of one percent of calcium chloride solution. Fill capillary tubes with this mixture and determine the coagulation time.

Compare the coagulation time of untreated blood with the coagulation time of the blood treated with the calcium solution. If the blood of the hemophiliac coagulates more normally after the addition of the calcium, the addition of calcium-containing foods to his diet is indicated. In such cases the para-thyroid glands may be abnormal and this aspect of the case should be studied.

HYGROMETRY

A study of the relations between the solids and the water content of the blood is sometimes useful in research work; it is not now known to have any value in diagnosis.

Have ready a perfectly clean and perfectly dry weighing glass; weigh this accurately. Put into the glass about five cubic centimeters of blood taken from a vein. Weigh accurately. Tilt the weighing glass so that the blood is spread over its inner surface, in order to increase the evaporation area. Replace the cover at an angle so as to permit evaporation. Place the glass in a thermostat at 60° to 70° C. for one or two days. Weigh again. Compare the weight of the whole blood with the weight of the dried blood. The dried solids make up about one-fifth the total weight of the blood, and in men the solids are a little greater than in women. That is, the water content of normal blood is about four-fifths the total weight. Figures given by various investigators vary from 19.58% to 20.53% for women and from 20.35% to 22.69% for men. The figures given are increased by those conditions which abstract water from the blood and decreased by hydremia. The test is not of value in diagnosis but it is occasionally useful in research work.

Electrical Resistance

The determination of the electrical resistance of the blood has been suggested as a speedy and accurate method of determining the blood cell count. It has been found that the electrical resistance varies as the amount of hemoglobin-carrying protoplasm plus the density of the blood plasma. It is a better indication of the cell-volume than of the cell count, though not very accurate at that. The technique is cumbersome and unsuited to ordinary laboratory diagnosis work. It has not, so far, been found useful in research work.

SEDIMENTATION TIME

The rapidity with which the red cells settle from blood whose coagulation has been prevented in some way varies in many conditions. The cells settle more rapidly in tubes which are placed at an angle than in tubes which are placed upright; no doubt this peculiarity is due to the Brownian movement of the cells.

Variations in the globulin content of the blood, the salt content, the relations between the electrolytes and the non-electrolytes of the plasma and the temperature at which the test is made all affect the sedimentation rate. Cells settle more rapidly in the blood of patients with malignancy, pregnancy, fevers and inflammatory states, after severe burns and, generally speaking, whenever there is tissue destruction or increased katabolism.

The technique is simple. The method of determining the blood volume by the hematocrit may be employed, except that the centrifuge is not used. The hematocrit tubes are placed at an angle of about 45° in a rack and the sedimentation of the red cells timed. Normally the cells settle within about an hour.

More nearly accurate findings are secured by taking a larger amount of blood (5 cubic centimeters or more) from a vein, citrating the blood or cooling it very quickly to 33° F., and placing it in very small tubes which are allowed to rest at an angle of about 45° until the cells have settled. If the cells settle within 20 minutes to 40 minutes, the sedimentation rate is increased; if the cells settle within 50 minutes to 80 minutes, the sedimentation rate is normal. We have not found any useful information resulting from the test and it has been discarded in our laboratories.

TOTAL VOLUME OF THE BLOOD

Many attempts have been made to find some adequate and practical method of determining the total amount of blood in the body, but none has been altogether successful. It is evident that only qualitative results can be secured from an examination of a small portion removed for that purpose unless the total amount of blood in the body is known.

The Haldane-Smith method consists in the inhalation of a measured amount of carbon monoxid, the removal of a measured amount of blood from a vein, and the determination of the amount of carbon monoxid hemoglobin by means of a spectroscope. The fraction of the carbon monoxid inhaled found as carbon monoxid hemoglobin in the measured blood indicates the amount of total blood in the body. This method is not suitable for ordinary work. Cells injured by carbon monoxid begin to be removed from the body quickly, and there is considerable unavoidable error in technique. Patients with even slight chronic carbon monoxide poisoning would give very erroneous findings.

Methods based upon the injection of non-poisonous stains into a vein, and the removal of a measured amount of blood from an oppposite vein, have been employed. The relative amount of the stain found in the measured blood indicates the total amount of blood in the body. The accuracy is diminished by the fact that such stains are partially removed from the blood by the liver, kidneys and other tissues of the body, and that complete mixture of the blood may not occur before this elimination has begun.

Rowntree's reports give interesting findings based on the use of Congo red and other harmless stains, injected into the blood. For the details of technique the original reports should be consulted.

Briefly, the method includes the following factors: The probable plasma volume is determined for the patient by multiplying his weight in kilograms by 50, because the average plasma volume is about fifty cubic centimeters for each kilogram of body weight. The figure thus secured is divided by 200, because a dilution of one part of stain to 200 parts of plasma is practically adequate. This quotient is the amount of the dye to be injected. The dye itself is a 1.5% solution of Congo red in fresh, triple distilled water.

Ten cubic centimeters of blood are taken from one elbow and the estimated amount of the dye injected into the same vein through the needle used in withdrawing the blood. Three to six minutes later 10 cubic centimeters of blood are taken from the other elbow. Both speci-

mens are oxalated and both are centrifugalized and the hematocrit readings recorded. The plasma from the two specimens is prepared for the colorimeter tubes and the readings give the amount of the dye in the plasma taken after the injection. By comparing the amount of dye injected with the amount recovered it is a simple matter to determine the amount of blood plasma in the entire body. By comparing the hematocrit readings with the total blood plasma the total amount of blood in the body can be determined.

The findings secured by Rowntree and his associates have been uniform and have given valuable information. The method is the simplest and the least harmful to the patient of the various methods proposed.

All of these methods have certain unavoidable errors and all are rather dangerous in the hands of unskilled persons. Many studies of the total volume have been made, for animals and for human subjects, and various fractions have been given as the relation between blood volume and body weight. These fractions vary from 1/13 to 1/20 for both men and women. For laboratory animals a fraction of 1/20, with a small increase during pregnancy, has been found by nearly all those making careful tests. That is, about 5% of the total weight of the body consists of blood. In obese animals the proportion is lower, as might be expected. In obese human subjects the proportion of blood to body weight has also been found lower. Pregnant women show some increase in the proportion of blood to the body weight.

The total plasma volume of the body remains remarkably constant in health and in disease, and the variations in total blood volume depend chiefly upon variations in the volume of the cells. In chlorosis, however, there seems to be an actual increase in the plasma volume, while in dehydration and also in edema, the plasma volume is diminished in nearly all cases. In edema the water is held in the tissues, not in the blood. It seems that so long as an adequate water intake is present, the blood plasma tends to remain constant under ordinary conditions. This being the case, ordinary methods of blood examination give quite accurate information, and the lack of a practical method of determining the total amount of blood in the body is probably not a cause of diagnostic errors.

STUDY OF BLOOD PARASITES

The presence of parasites in the blood may be suspected when symptoms are reported such as follow such infections, or when the blood picture is atypical and is associated with eosinophilia, especially if the

patient has been living in countries in which such infections are endemic. In making a differential count, fragments of parasites may be seen, or the large hyaline phagocytes may contain inclusions which arouse suspicion of parasitic infections. Inclusions found in the neutrophiles are not commonly noted but may be found occasionally. The red blood cells may show the effects of erosion due to blastomycetes, malaria or other infections. Fragments of the parasites or large hyaline bodies suggesting spores may occasionally be noted in the plasma while making a differential count, and these suggest more careful study of the blood with reference to the possibility of parasitic infection. Any findings which cannot be explained should always lead to more careful study of the blood in order that the puzzling factors may be interpreted, if possible.

Search for Unknown Organisms

The first method to be employed in the search for parasites is the study of a rather thick smear of fresh blood on the warm slide.

Take a warm slide on the warm stage of the microscope, cover with a warm cover glass, examine with the two-thirds objective and one inch eye-piece. Look over the entire drop of blood. Note whether the red cells show any unexplained movement; note whether any living worm-like organisms are present. Look over all thin areas with a one-sixth objective, then with one-eighth or one-tenth objective. If no organisms can be found discard the method.

Use next a method of concentrating the blood. Rub the lobe of the ear until it becomes distinctly reddened, using some alcohol or rather hot water or some other aseptic fluid. Dilating the blood vessels is, in this care, desirable. Have ready a centrifuge tube containing five cubic centimeters of five per cent acetic acid solution.

Prick the lobe of the ear rather deeply, and allow several drops of blood from the ear to flow into the centrifuge tube containing the acetic acid solution, mix thoroughly and centrifuge at about five hundred revolutions per minute for six to ten minutes. If the blood flows freely, two centrifuge tubes may be prepared in the same way. If the blood does no flow freely, the second tube, for balancing the first, should be filled with water to the same weight as the blood mixture. On removing the tube from the centrifuge the lower end will be filled with debris of the red cells amidst which the white cells and any parasites which might be present will be found. Thick smears from the upper part of this debris, and other thick smears from the lower part of the tube should be prepared and examined without staining. The parasites may be found in this manner, or thinner smears should be made, dried

and stained, using Giemsa's stain, thionin, some of the eosin-methylene-blue preparations and hematoxylin for one slide each, if the nature of the parasite is not indicated by the history or symptoms or by the findings on previous examinations.

If this method shows no parasites, venous blood must be used.

Take ten cubic centimeters of blood from a vein in the elbow, using the technique employed for determining blood chemistry, and put four cubic centimeters of the blood into a centrifuge tube containing six cubic centimeters of five per cent solution of sodium citrate, and six cubic centimeters of blood into a tube containing four cubic centimeters of the citrate solution. Each tube then contains ten cubic centimeters of mixtures of blood and citrate solution in varying proportions. Centrifuge ten minutes at about five hundred revolutions per minute.

The red cells then occupy the lower end of the tube, the clear fluid the upper portion, while there is a thin layer of white cells at the top of the red cell column. Take one thick drop from the white cell layer from each tube, for immediate examination. Make eight or ten smears from the same layer of each tube, lay these aside to dry for staining. Examine both the thick smears, unstained, using first the lower powers and then the high powers. Note evidences of living organisms in both. Stain the thinner smears, using several different stains, as before. Note parasites and also note whether there are any cellular inclusions in the large hyaline phagocytes or in the neutrophiles.

If these methods show no evidences of parasites in the blood, repeat with blood taken at different times of the day and night until the parasites have been found or until several examinations several times repeated have given negative results. Take blood at about midnight, after the patient has been three or more hours asleep at least two different times. Take blood immediately after a heavy osteopathic treatment, especially planned to cause increased rapidity of the circulation of the blood through the liver and the spleen, at least two different times. Take blood in the early morning, at about two o'clock in the afternoon and about two hours after a heavy protein meal, at least two times each, unless the parasites are found, or some explanation of the puzzling findings secured, before the group of tests has been completed. Whenever blood is taken for any of these tests, make two to six smears for differential count, using the usual technique. Save two or three of each of these smears, after they have been dried, for later study after the nature of the disease has been determined.

If there are any sores upon the skin, wash away the superficial debris and take smears from the granulation tissues or from the deeper floor

of the sore or the ulcer, and examine. If there are tumors beneath the skin, remove one of these for tissue examination. Make smears from scrapings of the cut surface of the tumor. These scrapings often show an infectious agent which is not shown by slides of the tissue. If there are enlarged lymph nodes, remove one for tissue examination, and make smears from scrapings from the cut surface of the node. Stain the scrapings with thionin, Giemsa's stain, hematoxylin and one or several of the eosin-methylene-blue stains, using the technique employed for differential counting. Make at least one thick smear and examine on a warm slide, for the recognition of living or unstained parasites.

If there are enlarged lymph nodes and the surgical removal of one for the sake of diagnosis does not seem desirable, some of the juices of the gland may be taken for examination. Take the syringe used for taking blood for chemical examination, have the needle sterile but dry, plunge the needle into the substance of the gland and withdraw the plunger enough to secure a drop or a few drops of fluid. From this material make one thick smear and several thin swears, on microscope slides. Examine the thick smear at once, unstained, and preferably on the warm stage. Dry and stain the thin smear using several different methods.

Inoculation of guinea pigs, white rats and rabbits from the blood of the patient and from the glandular extracts often determines or verifies the diagnosis. Use the technique employed for bacterial inoculations.

Search for Malarial Organisms

Examination of the unstained smears for malarial organisms is no longer a method in general use, which is, in some ways, unfortunate. The manner in which the organism moves within the cells, and the relations between cellular movements and the presence of the parasite are interesting, and in some cases are useful in the study of the efficiency of the parasiticidal activities of the phagocytes. The pigment granules of adult malarial organisms makes their recognition fairly easy. This pigment may not be present in the younger forms.

Stained smears give the most rapid and accurate diagnosis of malaria. Organisms may be noted in making an ordinary differential count and their number per cubic millimeter determined. The method is described in the paragraphs which give the method of making the differential count.

When malaria is suspected a thick smear may be used, in order to concentrate the organisms. Take three or four drops of blood in the center of an ordinary slide, spread the blood out over an area of about

one square inch, making the smear as even as is practicable. Allow to dry at room temperature; the slide must be thoroughly dry before staining.

Solutions necessary for this special method are simple.

1. Fixing fluid

Glacial acetic acid	1 gram
Formalin (40%)	5 cubic centimeters
Distilled water	100 cubic centimeters

2. Manson's fluid

Borax	5 grams
Methylene blue	2 grams
Tap water	100 cubic centimeters

Stand the dried thick slide in a jar containing at least fifty cubic centimeters of the fixing fluid for ten minutes. If the hemoglobin has not all disappeared by the end of ten minutes stand in a jar of tap water until all of the red or pink color has disappeared. Flood with Manson's fluid for half a minute, rinse gently in tap water. Mount in water and examine with dry one-tenth objective, or dry and examine with oil immersion lens. It is possible to make a fairly accurate determination of the number of parasites per cubic millimeter by counting the number of parasites in several fields, then counting the number of neutrophiles or the number of small hyaline cells in the same fields. The number of neutrophiles or of small hyaline cells per cubic millimeter having already been determined by the ordinary blood counts, the number of malarial parasites per cubic millimeter can be estimated quite easily.

In old cases of malaria the parasites can usually be found at almost any time. In cases in which the chills and fever follow a regular rhythm, as in tertian and quartan fevers, the smears should be taken, if possible, just before the onset of the chill. In autumnal fever the smears are most apt to show the parasite if they are taken eight or ten hours after the beginning of the fever.

The patient should receive an energetic osteopathic treatment planned to secure increased speed of circulation through the spleen just before the smears are taken, if this can be done. Such a treatment tends to diminish the severity of the attack, when it is given just before the onset of the chill. In cases with irregular attacks of fever such treatments increase the number of parasites in the peripheral blood, and they also hasten recovery from the malaria itself.

The different parasites which cause malaria may be distinguished as follows:

Plasmodium vivax (P. tertiana; Hemameba Laverani tertiana; P. malariae tertianum) shows rather active ameboid movements. The red cells infected are enlarged somewhat, are pale, and may show basophilic degeneration, but the non-infected cells do not show basophilic degeneration. The pigment is finely granular, yellowish brown, abundant, and it remains fine throughout the life of the parasites; it may be somewhat more abundant at the periphery but some fine granules are scattered over the entire organism. The young schizonts are ring-shaped at first, later they assume rather irregular forms. They grow until they fill the entire red cell, and they may become larger than the red cells of the infected blood. These segment into fourteen to twenty or even twenty-four merozoites, and these form a rosette which is often imperfect and of irregular arrangement. The extracellular forms are not easily distinguished from those of P. malariae but they do not resemble those of P. falciparum. The macrogametocytes are large and round with abundant, finely granular pigment which is scattered over the entire parasite. The protoplasm stains deep blue with ordinary methylene blue or thionin stains; the nucleus is poor in chromatin. The microgametocytes have less abundant pigment, the cytoplasm stains rather a greenish light blue and the nucleus is rich in chromatin.

Plasmodium malariae (Oscillaria malariae; P. quartana; Hemamebae malariae; H. laverani quartana; P. malariae quartana) shows only sluggish ameboid movements and may not move at all on the warm slide. The red cells infected do not swell nor do they become paler than normal. They may assume a brownish or brassy tint, apparently due to the change of hemoglobin into methemoglobin or some related compound. Basophilic degeneration may appear in non-infected as well as in infected red cells. Pigment granules are coarse, not abundant, and are mostly arranged near the periphery of the cell. Young schizonts are ring shaped, later becoming oval or round. They have a chromatin granule which soon becomes band-shaped, and these form equatorial bands. The schizonts do not fill the red cell completely and they divide into eight to fourteen merozoites, arranged in a rosette which is nearly always perfect. Extracellular forms are somewhat smaller than is the case with P. vivax, but they have almost or quite the same structure.

P. falciparum (Laverania malariae; tropical parasite; plasmodium precox; Hemamebae malariae precox; P. immaculatum) shows little ameboid motion but has a slight activity somewhat resembling pulsation. The infected red cells do not swell but may shrink slightly, and

they show the peculiar brownish or brassy color noted in red cells infected with P. vivax. The pigment granules are coarse and dark in color. Young schizonts are small and ring-like and they have one or two chromatin granules at the periphery. Occasionally two or even three parasites may be found within one red cell. Larger schizonts are not often seen in peripheral blood, and they rarely occupy more than half or two-thirds the area of the red cell. In larger forms the pigment granules are coarse, scanty and tend to clump together. The grown schizonts divide into seven to twenty-four merozites, and these form rosettes which are not always perfect. The extracellular macrogametes have a distinctive crescent-like form, becoming oval and round or roundish as the parasite increases in size. The cytoplasm stains deeply and the nucleus has scanty chromatin. The pigment granules are coarse and clumped near the center of the cell. Extracellular microgametocytes are also crescentic, and become oval or round with growth. The cytoplasm stains feebly and chromatin is fairly abundant in the nucleus. The pigment granules are coarse and are scattered over all the cell.

SEARCH FOR FILARIA

If filaria are suspected, the blood should be taken for examination at several different times of the day. Late in the afternoon, after the patient has rested, or better, has slept for several hours the blood may show the parasites of any variety. If the ordinary nocturnal form is present it may be necessary to take the blood at a time as near midnight as is convenient, after the patient has slept for several hours. The diurnal forms are most abundant at about noon. Other forms may be found at almost any time of day. Fresh specimens of blood are best examined. If the cover glass is ringed with vaseline the parasites may remain alive and active for several days.

If the parasites are not found in ordinary thick smears, the blood should be centrifuged in order to concentrate the worms. The skin may be reddened by rubbing with the aseptic solution, then pricked, and several drops, or even several cubic centimeters, collected. This may be mixed with either 2% watery solution of sodium citrate, or with 3% acetic acid, and the mixture centrifugalized at about 500 revolutions per minute for five or ten minutes. The worms accumulate in the leucocytic layer after centrifugalization and thick smears made from this layer show them usually quite active. The two-thirds objective is usually best for examining these preparations, though the dry higher power lenses are best for studying the organisms in thin smears.

The blood can be taken in small tubes and allowed to clot. The serum which separates contains nearly all of the worms. If a considerable amount of serum is collected this may be centrifuged and the sediment examined.

For permanent mounts the smears should be thinner and should be dried thoroughly in the air. They are then stained with hematoxylin, Giemsa's stain, or any of the ordinary blood stains. The hematoxylin-stained slides keep the best.

In temperate zones this infection is rarely seen. For the differentiation between the different forms a book on tropical diseases should be consulted.

SEARCH FOR LEISHMANIA

These parasites are found within phagocytic cells in the blood, especially within large hyaline phagocytes. In making ordinary differential counts they may sometimes be noticed as inclusions within the large hyaline endothelial cells. In these cells occasionally great numbers are found. It is only very rarely that they are seen free in the plasma or within neutrophiles. In slides stained with Giemsa's stain or with any of the eosin-methylene-blue preparations the cytoplasm of the parasites takes a pinkish or bluish lavender tint. The nucleus and the parabasal body stain dark blue. The parabasal body, with its long axis lying at right angles to the long axis of the cell is quite distinctive and makes the diagnosis clear when the parasites are well stained.

If the organism is not found by ordinary methods of examination the blood may be concentrated. Several cubic centimeters of venous blood should be well mixed with 1% or 2% solution of sodium citrate and the mixture centrifuged at about five hundred revolutions per minute for five or ten minutes. The leucocytic layer should then be collected and made into rather thin smears, and these stained after any of the usual methods for staining blood smears. The organisms are found within large hyaline cells.

Puncture of the spleen or of an enlarged lymphatic gland for the purpose of securing a few drops of the fluid for examination may be necessary for diagnosis. The removal of a small fragment of rib may be necessary in order that the red marrow may be examined. In either case the organism may be found within large hyaline cells or free in the tissue juices.

Material may be secured from a local lesion of the skin quite easily. If the skin has not yet become eroded the lesion should be punctured and the fluid made into smears. If the skin has become eroded the superficial debris and bacteria should be washed away, the floor of the

ulcer scraped, and deeper scrapings made into thick smears for immediate examination, and thinner smears for staining after several different methods.

The organisms which produce the different types of Leishmania cannot be differentiated from direct study of the smears, but cultures and animal inoculations may give differentiating features. For the differential diagnosis a book on Tropical Diseases should be consulted.

STUDIES OF THE FRAGILITY OF RED CELLS

In certain forms of anemia the red blood cells are more resistant to lipolytic solutions and to hypotonic solutions. In other forms of anemia and in certain other diseases the red blood cells are less resistant to such solutions than are normal red cells. For a study of the relations mentioned it is always necessary to determine the concentration of solution which just barely destroys normal red cells, then make a series of solutions of greater concentration and of less concentration than this. The blood to be tested is then placed in each of the varying solutions and the manner in which the red cells behave is noted. The technique for all these solutions is about the same, and may be illustrated by the method of determining diminished resistance to hypotonic solutions.

Normal blood plasma has an osmotic tension about equivalent to a 0.9% solution of sodium chloride in distilled water, and normal red cells begin to lake at about 0.4% solution of sodium chloride. In a 0.3% solution normal red cells are completely laked.

The technique of determining the resistance of red cells to hypotonic salt solutions requires two racks, each containing twelve graduated centrifuge tubes. One rack is for normal blood, as control, the other for the blood to be tested. Mark the tubes in each rack as follows:

.5%, .48%, .46%, .44%, .42%, .40% .38%, .36%, .34%, .32%, .30%, and .28%.

Fill the first tube to the line which indicates five cubic centimeters with 0.5% sodium chloride solution.

Fill the second to the 4.8 line with the same solution; the third to the 4.6 line, and so on, filling the last tube to the 2.8 line with the 0.5% sodium chloride solution.

Add distilled water to all the tubes except the first, to fill them to the line indicating five cubic centimeters. In this way the tubes all contain five cubic centimeters of solutions of sodium chloride of the strength marked upon them.

Both racks are exactly alike. Mark one with the name of the control and the other with the name of the patient. Cleanse the lobe of the ear, if the patient is adult, and make a rather deep prick, so that about two cubic centimeters of blood can be taken into a graduated pipette. Drop one tenth cubic centimenter into each of the twelve tubes of salt solution. Note the time. Repeat for the control. Note this time. Allow the racks to stand for half an hour, then observe. The red blood cells should have settled to the bottoms of the tubes within a few minutes after the blood was placed in them. After half an hour there may be a faint pink color in one tube and all the tubes with weaker solutions show darker tints. In one tube the red cells may have all disappeared, and in all tubes with weaker solutions the cells also have disappeared. If these conditions are not present at the end of half an hour leave the racks for a longer time until hemolysis has begun in the tubes on the rack of the control. This is usually within an hour, but occasionally two hours may be necessary.

Note the strongest solution in which hemolysis is just visible, in the control and in the patient's blood. Note the strongest solution in which hemolysis is complete (and the red cells destroyed completely) in the control and in the patient's blood.

The report should give these four findings, and also the time at which the blood was placed in the tubes and the time at which the last observation was made. Since the fragility occasionally varies during the day even in normal blood, the exact time should always be given.

TESTS FOR BILE PIGMENTS IN SERUM

Tests for bile are not, properly, included with these discussions of blood cells, except that it is often essential that the source of toxemias, which affect the blood cells, may be speedily determined.

The tests devised by Van den Berg depend upon the fact that fluids containing bile give the diazo reaction, provided the bile is not associated with the proteins of the plasma. The first is called the "direct reaction" and it is so called because the bilirubin is free in the serum.

The patient must not eat green or colored vegetables for two days beforehand.

Take five cubic centimeters of blood from a vein in the elbow, after proper sterilization of the skin. Put the blood into a suitable vessel until it clots and the serum becomes separated. The serum must not be recognizably stained with hemoglobin; it may be greenish, yellowish or brownish from the bile present. Measure one cubic centimeter

of the clear serum into a small tube, add one cubic centimeter of Ehrlich's diazo reagent. A brilliant reddish or purplish color may appear immediately, in which case there is a positive direct reaction. This indicates obstructive jaundice. Sometimes the color appears only after the mixture has been standing several minutes, in which case there is a delayed positive direct reaction, and this has the same significance.

The indirect reaction is so called because the bile pigment is combined with the proteins of the plasma in such a way that the reaction does not occur until these have been precipitated.

Take one cubic centimeter of clear serum, add two cubic centimeters of alcohol of about 96%. Place the mixture in a centrifuge tube, balance with an equal weight of water, and centrifuge at about eight hundred revolutions per minute for ten minutes. Remove one cubic centimeter of the clear supernatant fluid and to this add one-half cubic centimeter of alcohol and one-fourth cubic centimeter of Ehrlich's diazo reagent. The immediate appearance of reddish or purplish color indicates the positive indirect reaction, while the appearance of the same color after several minutes standing indicates the delayed positive indirect reaction. When this reaction is positive and the direct reaction negative, the jaundice is not obstructive but is due to liver injury or to blood cell destruction.

Quantitative findings can be secured. One unit of bilirubin is taken to be 0.5 milligrams in one hundred cubic centimeters of blood. One-half to one-tenth of a bilirubin unit is present in normal blood. For the quantitative estimation cobalt sulphate is used for the standard. Prepare a solution of 3.92 grams of crystalline cobalt sulphate in 100 cubic centimeters of distilled water and place in one cup of the colorimeter. In the other cup place the serum which shows the positive reaction. If the color of the serum is the same as the standard solution, the blood of the patient contains five units of bilirubin. If the color is lighter or darker than that of the standard, the cups are raised or lowered until the tints match, when the concentration can be read off on the colorimeter scale.

The Van den Bergh reactions are useful for distinguishing between obstructive jaundice and hemotogenous jaundice or the jaundice due to liver injury, but the reaction is not delicate and it is not useful for accurate determination of small amounts of bile.

Ehrlich's diazo reagent is made as follows:

Solution A	
Sulphanilic acid	5 grams
Hydrochloric acid, (conc.)	50 grams
Distilled water	1,000 cubic centimeters
Solution B	
Sodium nitrate	1 gram
Distilled water	200 cubic centimeters

These solutions keep well in darkness.

When the test is to be made take fifty parts of solution A and one part of solution B, mix and use immediately.

The icteric index is better for estimating small amounts of bile. A solution of ten milligrams of potassium bichromate in one liter distilled water is taken to have an index of 1, and this solution is used for a standard. The solution keeps well in the dark.

Take about ten cubic centimeters of blood from a vein of the elbow, place in a convenient vessel until the serum has separated from the clot. Take two or three cubic centimeters of serum, which must not be stained with hemoglobin, and mix with an equal amount of 0.9% sodium chloride solution. Place the diluted serum in one cup of the colorimeter, the standard solution of potassium bichromate in the other cup, and compare. The standard solution corresponds to an icteric index of 1., and normal blood has an icteric index of about 5. Icteric index of about 15, which corresponds to about two units in Van den Bergh's reactions, is present in cases of mild jaundice. An icteric index between about 6 and 15 is present in mild cases of cholemia, in which case the blood cells show more or less marked evidences of injury.

The Gmelin test is often positive for a small amount of serum. Allow the serum which is formed when the blood clot is ready to be placed in the incubator for the fibrinolysis test to soak into filter paper. Allow one or two drops of nitric acid which contains some nitrous acid to fall upon the filter paper and to touch the blood serum. Normal serum shows a brownish tint at the line of the acid, while cholemic blood gives a play of colors, including purplish or violet shades.

A more delicate test is made from oxalated blood. Take about five cubic centimeters of blood from a vein into a test tube containing about ten milligrams powdered potassium oxalate. Mix and centrifuge for about ten minutes. Remove the clear serum into another tube, and underlay this with nitric acid which contains a small amount of nitrous acid. A white coagulum will appear at the junction of the two fluids.

In this white band there will appear a blue-green color at once in severe cholemia, or within half an hour in less serious cases.

Serum from pernicious anemia blood may give this reaction though ordinary tests do not show bile pigments in such blood.

REPORTS OF BLOOD EXAMINATIONS

Reports of work done in the best clinical laboratories are always given to the doctor who orders the work to be done. It should be kept clearly in mind that laboratory work is done for the doctor, and that only the doctor is responsible to the patient. A carbon copy of the report is sent also, and the doctor usually gives this to the patient, unless there is some reason why the patient should not receive it. Reports are written in technical language and the doctor should explain to the patient or to some member of his family the significance of the various items. The laboratory worker should not give such explanations, unless requested by the doctor in charge of the case to do so. The doctor who has made the physical examinations and who has studied the symptoms and the history is the only one who is able to interpret the findings in a simple and practical manner, in the light of all the factors involved. Patients receiving copies of examinations should always be advised to keep them, and to show them to any other doctor who may be called to give treatments for any disease at a later time. This is especially important in cases of rare and chronic diseases. Much valuable time may be saved to the patient if he has such records ready at a time of later illness, in such cases.

With the reports of blood examinations there is sent, upon another sheet of paper, such notes and explanations as may be useful to the doctor in explaining the significance of the findings and in determining the best methods of treatment. While the laboratory worker is rarely in active practice, still there is much useful information which he can give in selected cases, provided he is doing his work upon a professional basis. The notes are not intended for the patient and should never be given to him or to his family.

The reports now being used in the clinical laboratory of The A. T. Still Research Institute are the most useful we have seen. The routine examination forms are printed upon pink paper and the special-test forms are printed upon light brown paper. This makes it easy to select from the files the reports which may be needed for special studies. The forms used for other work are of different colors; uranalyses forms are printed upon yellow paper, blood chemistry forms on green paper, and so on.

The following forms are those used for blood reports in 1930.

THE A. T. STILL RESEARCH INSTITUTE
Clinical Laboratory

Report of blood tests made.....

For Dr. .....
Address .....
Hemoglobin.....grams per liter.....%.....
Erythrocytes.....per cu. mm.....%.....
Color index .....
Poikilocytes .....
Anisocytes .....
Reticulocytes .....
Microcytes .....
Megalocytes .....
Normoblasts .....
Poikiloblasts .....
Microblasts .....
Megaloblasts .....
Degeneration types .....
Blood Platelets.....per cu mm.....
Osmotic tension .....
Coagulation time..... Minutes.....
Viscidity .....
.....
.....

Patient .....
Address .....
Leucocytes .....
Large hyaline .....%.....per cu mm.
Small hyaline .....%.....per cu mm.
Mononuclear neutrophiles .....%.....per cu mm.
Polymorphonuclear .....%.....per cu mm.
Eosinophiles .....%.....per cu mm.
Basophiles .....%.....per cu mm.
Myelocytes, hyaline .....
Myelocytes, granular .....
Endothelial cells .....
Degeneration types .....
Neutrophile nuclear average .....
Iodophilic cells .....
Iodophilic granules .....
Malarial parasites .....
Other parasites .....
Other tests .....
.....
.....

Warm stage examination.....
.....
.....
Remarks:

Signed.....

THE A. T. STILL RESEARCH INSTITUTE
Clinical Laboratory

Report of special blood tests made.....

For Dr. .....
Address .....
Blood taken .....
Fibrin threads .....
.....first appear .....
.....complete .....
.....radiate .....
.....beaded .....
.....
.....
Refractile bodies .....
.....
Fibrinolysis tubes filled.....
.....placed in incubator .....
.....clots .....
.....serum .....
.....digestion begun .....
.....
.....controls show .....
.....
Remarks.....
.....
.....
.....

Patient.....
Address.....
Specific gravity.....
Alkalinity .....
Abnormal pigments.....
.....
.....
Other tests.....
.....
.....
.....
.....
.....
.....
.....
.....
.....

Signed.....

ILLUSTRATIVE CASE REPORTS

Alkalosis

Miss R., aged twenty-eight years, presented an unusual history. Three years before coming to an osteopathic clinic for examination she had shown symptoms of pulmonary tuberculosis, and this had been treated by dietetic measures alone.

Blood examination showed the usual findings in alkalosis, and after more explicit questioning a detailed account of her diet for the tubercular infection was secured. She had been given foods of the alkaline-ash type exclusively. Three times each day she was given lemon juice and soda. Twice each day she was given a soda enema. The urine was analyzed twice each month, and if it was neutral or alkaline no change was made in the diet. If the urine was acid at any time the alkalinization of the food intake was increased.

Within a few weeks after the intensive alkalinization she began to complain of muscular cramps, most marked in the fingers. During the next three years the spasmodic contractions increased in frequency and in severity, and various types of paresthesia developed. During this time she consulted several other doctors of medicine. Without making any very careful study the condition was named either hysteria or acidosis by these men. Alkalinization was advised by every doctor consulted during this time. Colonic irrigations of soda and water were advised. The condition became gradually more severe.

When she came to the osteopathic clinic the contractions were tetanic in type. The muscles of the left hand were first involved and the "obstetrical hand" position assumed. The spasms then extended to the arm and shoulder, then to the neck, trunk and legs, until the entire body was involved in tetanic convulsions. Attacks occurred two or three times a week.

By a specially devised method the total blood alkalinity was found equivalent to 450 milligrams of sodium hydroxide per 100 cubic centimeters of blood. The diffusible alkali was much more noticeably increased than was the bound alkali.

The osteopathic examination showed the rigidity of the lower thoracic region which is always present in tuberculosis, and also a definite lesion of the fifth cervical vertebra. In order that the effects of dealkalinization might be studied, no osteopathic treatments were given during the first month.

The patient acknowledged an intense craving for hot white biscuits and beef-steak. These were given her for her first meal, in mod-

erate helpings. She was permitted other foods with acid ash until the urine became normally acid, then a wholesome, well-balanced diet was advised. The soda intake was stopped immediately.

The spasms were less severe the day after the soda was stopped. By the end of the month they appeared rarely. Some paresthesias of the hands were still noticed, and the relation of the fifth cervical lesion to the innervation of the arms and hands was explained to her. This explanation induced her to carry on the treatment which had been advised.

During the fifth week after the diagnosis had been made the cervical lesion was corrected and the spinal rigidity relieved. The lesions did not occur.

Six weeks after the lesions were corrected a second blood examination showed no abnormal findings. During the subsequent nine years she has been healthy and comfortable, except that she had colds several times, and was in an automobile accident once which broke an arm and caused cerebral concussion of mild degree.

G., a boy of nine years, suffered from attacks which somewhat resembled those of Jacksonian epilepsy. He had suffered from an attack of food poisoning at the age of seven years, and before that time had been in excellent health all his life.

The attack of food poisoning was treated by the administration of large doses of soda by mouth and he was given soda enemas at frequent, though irregular, intervals during the six weeks following the attack. After this time he was troubled with various gastric and intestinal disturbances, and for these increasing amounts of soda were given him.

On making the routine blood examination the findings characteristic of alkalosis were noted. The urine was found to be alkaline at three examinations on successive days.

The soda was stopped immediately. The spasmodic attacks disappeared within three days and never re-appeared. The gastric and intestinal symptoms persisted.

Osteopathic treatments were not given until after the urine became acid, because it was desired that the effects of de-alkalinization alone should be studied. The spasms had ceased by the time the urine became acid, and the osteopathic examination showed a definite lesion of the sixth thoracic vertebra. This was corrected at the third treatment. The gastric symptoms diminished gradually during the next

week, and the intestinal symptoms diminished during the two weeks following the correction of the lesion. No further symptoms ever appeared, and during the next ten years his health was excellent.

Mr. Q., thirty-nine years old, had suffered from diabetes at the age of thirty-two years. He had been given osteopathic treatment at that time, and had been given a diet list of foods exclusively of the alkaline-ash type. He avoided all white breads, all sugars, all plums, cranberries, prunes, meats, and, indeed, every article of food said to have any tendency to cause acid reactions. He did not use soda, but he did take alkaline laxative and purgative drugs, on his own initiative, and he tested the urine occasionally with litmus paper. When the urine was acid in reaction he worried terribly and hastened to take some alkaline medicines. The only common alkaline substance which he avoided was soda.

For two weeks before he came to the clinic for treatment he suffered from cramps in the muscles of his legs. These awoke him from sleep, and the pain was really quite severe. On making a blood examination the characteristic staining reactions and nuclear structures of alkalosis were recognized, and the blood was studied with reference to its alkalinity. At that time the technique of studying the reaction of blood was not so nearly accurate as is the case at this time, and it was only possible to determine that the alkalinity was considerably increased in the blood serum. In the urine the alkalinity varied, sometimes being only just recognizable, sometimes definitely alkaline on voiding.

Certain lesions were found and these were corrected before the blood study was completed. The lesions were not the cause of the cramping and the corrections did not affect the leg muscles. The lesions did cause some of the apathy and melancholy from which he suffered, and these symptoms were considerably relieved by the osteopathic treatments. The mental acuity was increased, and no doubt this permitted a better understanding of the conditions than might have been the case if he had not received those treatments at that time. Having the most extreme faith in the doctor who had prescribed the alkaline-ash diet, it might have been difficult to persuade him of the error of persisting in such a diet indefinitely.

When the relation of his diet to his symptoms was explained to him, he was willing to accept more nearly normal foods. He confessed to a craving for candy and white bread, and these foods were

given him in moderation. As soon as the urine became normally acid in reaction, a good wholesome diet was outlined and this he employed for several weeks. The cramps diminished gradually for ten days, then disappeared altogether. With this relief of his symptoms he disappeared from observation for seven years. At the end of that time he brought his little son for examination. He reported excellent health during the interim. He had a good position, had married a sensible wife and had not paid much attention to his diet during the past five years, because his table was well supplied with good, wholesome food from which he selected what he wanted to eat.

ACIDOSIS

Miss T., aged twenty-five years, complained of weakness, insomnia and occasional attacks of deep breathing associated with air-hunger. Acidosis was suspected from these symptoms. The routine blood examination showed the structures usually present in acidosis. Miss T. had suffered from an attack of inflammatory rheumatism, and the medical practitioner who attended her warned her against the use of any acid fruits. He specified especially that tomatoes, lemons, grapefruit and oranges were dangerous, and he advised a diet chiefly of toasted white bread, good red meat and whatever she liked, except sour things. She was very fond of candy and pastries. With increasing weakness she avoided exertion, but she did not gain in weight.

The alkalinity was diminished in the blood to the equivalent of two hundred fifty milligrams of sodium hydroxide per liter, and of this only fifteen per cent was of the diffusible type. (By the methods used twenty per cent diffusible of a total of three hundred milligrams is normal.)

The spinal column showed the irregularities usually present in acidosis. These were corrected during two weeks, with no change in the diet. The symptoms diminished considerably, and the alkalinity of the blood increased to the equivalent of nearly three hundred milligrams of sodium hydroxide.

The increase in the alkalinity of the blood after the osteopathic treatments, while the diet remained unchanged, was no doubt due to the fact that the correction of the lesions permitted normal circulation and innervation of the viscera, with resulting increased oxidation, the formation of katabolites more nearly neutral or, in some cases, definitely acid, and more nearly normal excretion of these from the body. In this case some exhaustive and interesting studies of the urine were made, but these are too long to be included in this report.

After this study of the effects of treatment alone, upon the reaction of the blood, the diet was changed materially. A good, wholesome diet which included rational proportions of fruits, vegetables and other foods was outlined. The alkalinity of the blood returned to normal and the symptoms disappeared completely. During the three years which intervened since that time, she has been normal and comfortable.

CHRONIC CARBON MONOXIDE POISONING

Mrs. N. 3. Symptoms included only persistent, dull headache and weariness for which no adequate cause could be found. On physical examination some vague tension of the cervical and upper thoracic spinal muscles was found, but no definite lesions and no recognizable evidences of visceral pathology.

On blood examination the peculiar cherry-like tint suggested carbon monoxide poisoning, and by the spectroscopic examination a small, but recognizable, amount of carbon monoxide hemoglobin was determined.

The patient had a closed car but drove only short distances and at intervals of several days. She used no gas for heating or cooking. Her home was in an old house on a quiet street. No manufacturing district or oil well was near. There seemed, at first, no possibility of the inhalation of fumes.

On studying the plans of the house in which she lived it was found that the house had been piped for gas. On further investigation one of these old gas pipes was found beneath her bed-room, and it was leaking steadily, though only slightly. Mrs. N. was having a new house built, and in this house her bed-room was on the second floor. As soon as she moved into the new home the headaches and weariness passed away, gradually, and within a few weeks her health was fairly good. She then received a few treatments for the abnormal tension of the tissues of the neck and shoulders, and she became perfectly well again. Several weeks later another blood examination showed no evidence of carbon monoxide hemoglobin.

In such a case as this it would be easy to infer that the worries inseparable from building a new home were the cause of the symptoms, and that the mental relief following the successful completion and occupancy of the new place were the cause of the recovery. The error of such an inference is obvious from the results of the blood tests.

Miss K. 11. This young woman, aged eighteen years, complained of languor with persistent dull headaches and some pain in the eye-

balls. Physical examination showed no cause for the symptoms. No bony lesions were found, and only some slight but persistent tension of the muscles of the neck could be discovered. Relief of this tension was followed by some slight relief of the discomfort, but the tension re-appeared, together with the aches, within a few hours. She was a solicitor for a wholesale cracker house, and she spent most of her time in her little, old, closed car, driving from one hotel, grocery or eating-house to another, taking orders. She rarely had the car windows open because the breeze disturbed her papers. She lived in a house composed chiefly of wire screening, on a quiet street, and no gas pipes were in the suburb anywhere.

Blood examination showed some evidence of chronic carbon monoxide poisoning and a trace of carbon monoxide hemoglobin was found on spectroscopic examination. The treatment is obvious. She exchanged the old, closed car for a new roadster, and she bought some convenient cases for her papers. Within a few days the symptoms diminished, and within a few weeks she was apparently as well as could be. A blood examination made three months later showed no evidences of carbon monoxide hemoglobin.

Mr. W., aged forty-three years, worked in a Los Angeles office in which smoking was habitual. His home was an hour's ride distant, and he smoked all the time, riding with friends in the smoker.

He had very severe headaches which did not yield to any treatment, and he complained of marked fatigue for which he could not find any cause. The blood showed the usual characteristics of carbon monoxide poisoning. He was persuaded to ride in the non-smoking compartment of the street-car and to have more fresh air in his office. He was also advised to diminish smoking to the lowest comfortable extent and to smoke in the open air. These changes were followed by considerable relief of the headache and the feeling of fatigue. There was recognizable improvement in the blood cells and in the color. But the discomfort of postponing smoking until he could be in the fresh air, and the lack of the usual conversations with his friends in the smoker proved too great. He returned to his old habits and accepted the headaches and other discomforts due to the bad habits. A few months later the bad habits, headaches and friendly relations were all terminated at once by a sudden attack of pneumonia.

Miss J. 2. This young woman was a student in a business college. She complained of increasing weakness with dull headache and apathy, and occasional insomnia. No adequate cause for these conditions could be found on physical examination. No bony lesions and no abnormal tension of tissues could be discovered even after very careful examinations.

Her school-work was done in well-ventilated rooms. No smoking was permitted in the school; she herself did not smoke, and no member of her family smoked. The house did not have gas pipes, and never had been piped for gas. She had a large, airy room and slept on a porch. No gas wells or manufacturing district was near her home. She had no automobile and rarely rode in a closed car.

She walked to and from school twice each day, and sometimes she made an extra trip at night. The distance was rather more than a mile. This walk took her through a tunnel in which traffic was very heavy. The cars were forced to stop and start at intervals of a few minutes. The air was full of the fumes due to imperfect combustion of gasoline and oil. The walk through the tunnel required about twenty minutes, which meant that she spent from an hour and a half to two hours every day breathing bad air. A small amount of carbon monoxide hemoglobin was shown by spectroscopic examination of the blood, after the usual findings had been noted in the routine blood tests.

The treatment was indicated by these facts. The walk through the tunnel was discontinued. The symptoms diminished gradually and two months later the blood showed no abnormal conditions.

Mrs. D. 4. This woman of thirty years complained of being unable to live at high altitudes. Her home was in a city nearly nine thousand feet above sea level and her husband's business compelled their residence there for at least ten months each year. During the two months spent at sea level and for the two weeks or so following her return home she was well. At other times she was weak, inert, with dull headaches and constant fatigue.

Blood examinations made just before her return to her home, after two months at sea level, showed no abnormal findings. Blood examinations made just after her return to sea level, after ten months at her home, showed the usual evidences of chronic carbon monoxide poisoning. No abnormal findings could be discovered on physical examination.

In her mountain home she enjoyed tinkering with her car. At that altitude the use of a gasoline engine presents certain problems and she enjoyed solving them in the most satisfactory manner possible. At

that altitude, also, there was much cold weather, so that her work was done within the garage. She had some ventilation in the garage, but this could not be very satisfactory.

On changing her habits of living, substituting other interests for the car, and returning to her home after the usual two months at sea level, she found herself able to go through the winter with no ill health at all. It was not the high altitude which affected her health, but the hours spent with her automobile in a poorly ventilated garage. This case illustrates the danger of superficial diagnosis. Changed environment forced changed habits, and these caused the recovery. People who are fairly normal can live where other normal people can live, but nobody can be well who persistently breathes air which contains much carbon monoxide.

CHOLEMIA

Patients who suffer from lesions of the eighth to the tenth thoracic vertebrae or the corresponding ribs often show moderate degrees of cholemia. This condition disappears within a week or ten days after the lesions have been corrected.

Miss Y., aged twenty years, complained of dull headaches, nausea, some itching of the skin. She was very slightly jaundiced.

The blood cells showed the effects of some hemolytic agent and the serum contained a moderate amount of bilirubin. The surface tension of the serum was diminished. The urine contained bile pigments but no recognizable amount of bile acids. There were no other abnormal conditions of the blood or the urine.

A lesion of the eighth thoracic vertebra was present and the seventh and eighth right ribs were approximated. No other cause for the cholemia could be found. The lesions were corrected by three osteopathic treatments given at three day intervals. Ten days after the last treatment the blood and the urine were normal. The symptoms disappeared after the third treatment.

Mr. F., aged twenty-nine years, a worker in an osteopathic laboratory, was in excellent health. He used his own blood in some experimental work in the technique of testing blood serum for bile pigments and bile acids, and found the condition normal. After three days of this work he helped to lift some heavy boxes from beneath a table, and thus strained his back. Thus he produced a lesion of the seventh to the ninth thoracic vertebrae which caused pronounced discomfort but no really serious symptoms. He continued his tests for bile acids and bile pigments, and on the third day after the strain he found that his own blood contained five times the normal amount of bilirubin, ac-

cording to the method he was using, and that the reactions for bile acids were definitely positive. No bile pigments or acids were present in the urine.

The lesions were then corrected by means of a single, rather heavy, osteopathic treatment. Three hours later the bile acids and the bile pigments were perceptibly increased in the blood, and both bile acids and bile pigments were present abundantly in the urine. The next day, sixteen hours after the lesions had been corrected, no bile acids were present in the blood serum, the bile pigments were within normal range, and the urine was normal.

The lesions did not recur and no further ill effects were noticed.

FATIGUE

Miss J., a teacher, aged thirty-nine years, complained of increasing weakness and persistent, though slight, loss of weight. Pulmonary tuberculosis was suspected.

On blood examination no evidences of tubercular or other infection could be found. There was no excess of hyaline, eosinophilic or endothelial cells. The neutrophilic cells showed the changes associated with fatigue and moderate acidosis. Immature red cells and white cells of all classes were numerous, though no anemia was present. Myelocytoid forms were also abundant, especially among the neutrophiles.

A diagnosis of fatigue plus bony lesions affecting a considerable area of red bone marrow was made. This diagnosis was accepted by the osteopathic physician in charge of the case.

When she was told the findings she acknowledged that her hours of work were too long. She was trying to care for an invalid mother during the nights, and was writing short stories to earn more money, while still teaching every day.

She had an unusually rigid thorax; both ribs and vertebrae were involved. The treatment was evident when these facts were known. In order to study the effects of rest alone, and partly because her circumstances prevented her receiving osteopathic treatments for six weeks, she was persuaded to cease the extra writing, to have help with the invalid mother by night as well as by day, and to secure as much rest as possible in every other way. Her diet was already wholesome and well balanced.

With rest alone the symptoms diminished considerably. The blood cells lost the evidences of toxemia but the immature forms persisted unchanged.

Six weeks later she was able to have good osteopathic treatments. With increasing flexibility of the thorax, better breathing habits, plus the continued rest already begun as soon as the diagnosis was definite, she began to gain in weight and to regain her normal physical condition. A blood examination made six months later showed no abnormal cells.

PNEUMONIA

Mrs. L. 7, aged sixty-four years, suffered from what seemed to be a mild attack of influenza. Fever was slight but she seemed weaker than she should be if this were the correct diagnosis. A blood examination was made for this reason. There was a moderate neutrophilic leucocytosis with low nuclear average and many endothelial cells were present. The fibrin was formed abundantly and immediately upon the warm slide, and the threads were long, coarse, regular in outline, arranged in a dense felt-like mass. A diagnosis of early pneumonia was made upon these findings, though clinical symptoms were negative. Treatment for pneumonia was initiated promptly and she made a good recovery. During her convalescence the sputum was scanty and contained rusty streaks; there were abundant pneumococci in the sputum. There was only a small recognizable area of consolidation, and the speedy recovery was no doubt due to the early diagnosis.

Mr. N. 5, aged seventy-three years, suffered from an attack of influenza and pneumonia was feared. No symptoms of cardiac involvement had been noted. Blood examination showed a moderate leucocytosis with low nuclear average; abundant endothelial cells were found. The blood was moderately concentrated and the leucocytes were grouped on the warm slide and in the smears for the differential counts. Splenocytes were abundant. The serum contained a trace of bilirubin. Fibrin was formed abundantly and immediately.

The probability that pneumonia was complicated by cardiac inefficiency was mentioned in the notes which were sent with the report. Treatment was planned for pneumonia plus cardiac inefficiency, and the patient recovered, though rather slowly.

Two months later he was killed in an accident. The autopsy showed some remaining hepatization in the lower right lung, and the mitral and the tricuspid valves of the heart showed the effects of an old, severe endocarditis.

INCORRECT DIET

Miss F. 11, aged seventeen years, was brought for examination because she was losing weight, and because she was becoming more and more irritable. The blood examination showed moderate secondary

anemia with very slight evidences of toxemia of the type usually associated with malnutrition. The blood platelets were very low; 50,000 per cubic millimeter.

The possibility that there was a lack of Vitamin A in the diet was suggested to the doctor in charge of her case. On investigation it was found that the patient displayed a marked aversion to eggs, and that other foods containing Vitamin A were avoided.

Correction of the diet was followed by moderately increased weight and by the disappearance of the nervous symptoms and the irritability.

The osteopathic physician reported that no definite bony lesions were present but that there was some abnormal tension of the spinal tissues in the upper lumbar region. This disappeared with the correction of the diet, with no further attention.

PREGNANCY

Mrs. R., aged thirty-nine years, married twenty years, no children, had been advised to submit to an operation for a rapidly growing tumor. The ordinary symptoms of pregnancy were absent. Certain atypical cells were found in the blood smear during the progress of the differential count, and on further study these appeared to be derived from the placenta. Surgical work was postponed, and a few days later an X-ray plate showed fetal vertebrae and other bones. The boy born after a rather stormy pregnancy and labor was normal, and is now nearly fifteen years old.

It is rare that the placental cells are useful in diagnosis, yet such cases have been found several times during twenty-eight years of blood cell study.

LATE MALIGNANCY

Mr. L. 22, aged fifty-two years, complained of certain vague gastric symptoms. Gastric analysis showed absent hydrochloric acid but no other important findings. Roentgenologist's report showed some delay in the emptying time of the stomach but no evidence of gastric ulcer or cancer.

Blood examination showed immediate and abundant formation of fibrin on the warm stage, with threads of irregular length and contour, often beaded, often arranged in radiating lines with a group of platelets or a lymphocyte at the center. Refractive granules were abundant, and these included iodophilic, Sudanophilic and unstained particles. Rouleaux were scanty and the red cells arranged themselves in masses.

Leucocytes showed the evidences of toxemia of the type associated with disturbances of protein katabolism. Hyaline cells and eosinophiles were increased, both relatively and absolutely. The eosinophiles often showed abundant, basophilic, hyaline, intergranular protoplasm.

Fibrinolysis was masked by undifferentiated proteolysis. The probability that late malignancy was present was noted in the extra report sent to the doctor. Surgical interference was decided upon, and an inoperable cancer was found around the pyloric region of the stomach. At autopsy, two months later, this was found to be completely surrounding the pyloric antrum. It was of the scirrhous variety, and there was no gross ulceration of the gastric mucosa. These facts explained the erroneous roentgenological report.

EARLY MALIGNANCY

Mr. O. 2, aged sixty years, suffered from severe pain in the stomach with occasional nausea; neither the pain nor the nausea seemed to bear any relation to the taking of food nor its quality.

Blood examination showed the cell findings reported in the case of Mr. L. 22. Gastric analysis showed absent hydrochloric acid and also a few small masses of cells showing abundant and often irregular karyokinesis.

Fibrinolysis was absent and no undifferentiated proteolytic ferment was found.

Surgical interference was based upon these findings. A small cancer was found upon the anterior aspect of the pyloric antrum. Gastrojejunostomy was performed, and the patient is alive and well at this time, twelve years later.

NON-MALIGNANT TUMOR

Mrs. W. 17, aged forty-three years, showed a small, hard tumor in the left breast. This was associated with vague pain in the same general region, radiating along the intercostal nerves to the spinal column.

There was a history of an abscess in the place occupied by the tumor, which was present about twenty years before the tumor was noted. She was not in habit of paying very much attention to her own body, and had always been an unusually busy and active person.

Blood examination gave a normal blood picture. Fibrinolysis was normal and no abnormal findings were reported for any of the special tests.

Surgical interference was postponed, on these findings. The rib lesion was corrected and the pain disappeared. The tumor seemed unaffected by the treatment. It was kept under observation for several

years but no increase in size ever occurred. After her death, fifteen years later, this tumor was removed for histological examination, and it was found to be composed of scar-like threads of connective tissue with no evidence whatever of malignancy.

OVARIAN CANCER

Miss C. 5, aged fifty-three years, noted slight and repeated uterine hemorrhages. On blood examination the findings characteristic of malignancy were reported, and fibrinolysis was absent. There was no evidence of an undifferentiated proteolytic ferment.

Pelvic examination discovered a myofibroma of the uterus but no evidence of cancer. Uterine curettings and a bit of tissue from the region of a small cyst on the cervix were removed for microscopic examination but no evidence of malignancy was found. The uterine hemorrhage was repeated, and a second blood examination was made five weeks after the first examination. The evidences of malignancy were somewhat more marked and there was present some undifferentiated proteolytic ferment. At this time the patient complained of pain in the lower abdomen and the pelvis.

Because of the myofibroma and the pain it was decided to perform a hysterectomy. A tumor of the right ovary was found, and on microscopical examination this proved to be a papillary adenocarcinoma. There were several small metastatic tumors upon the adjacent peritoneum. Radium treatment followed the removal of the cancer and the uterus, she made an excellent recovery, and is still in good health, eleven years later.

NON-MALIGNANT UTERINE BLEEDING

Mrs. T. 4, aged fifty-eight years, suffered from slight but frequent uterine hemorrhage. Diagnosis of uterine cancer was made by an eminent medical surgeon, who advised immediate hysterectomy. This she refused, for the time being. She consulted an osteopathic surgeon, who found a lesion of the fifth lumbar vertebra. The patient explained this by a fall she had had about ten days before the first uterine hemorrhage. This osteopathic surgeon advised the correction of the lesion before operating. The pelvic examination discovered a heavy, edematous, congested cervix and uterus but no definitely marked tumor.

Blood examination showed normal fibrinolysis with no evidences of malignancy. The lumbar lesion was corrected, the uterine hemorrhage ceased. Pelvic examination three weeks later showed normal cervix and uterus, and no reason for surgery.

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