ACCESSORY NUCLEAR LOBULES ON THE POLYMORPHONUCLEAR NEUTROPHIL LEUKOCYTE OF DOMESTIC ANIMALS

by

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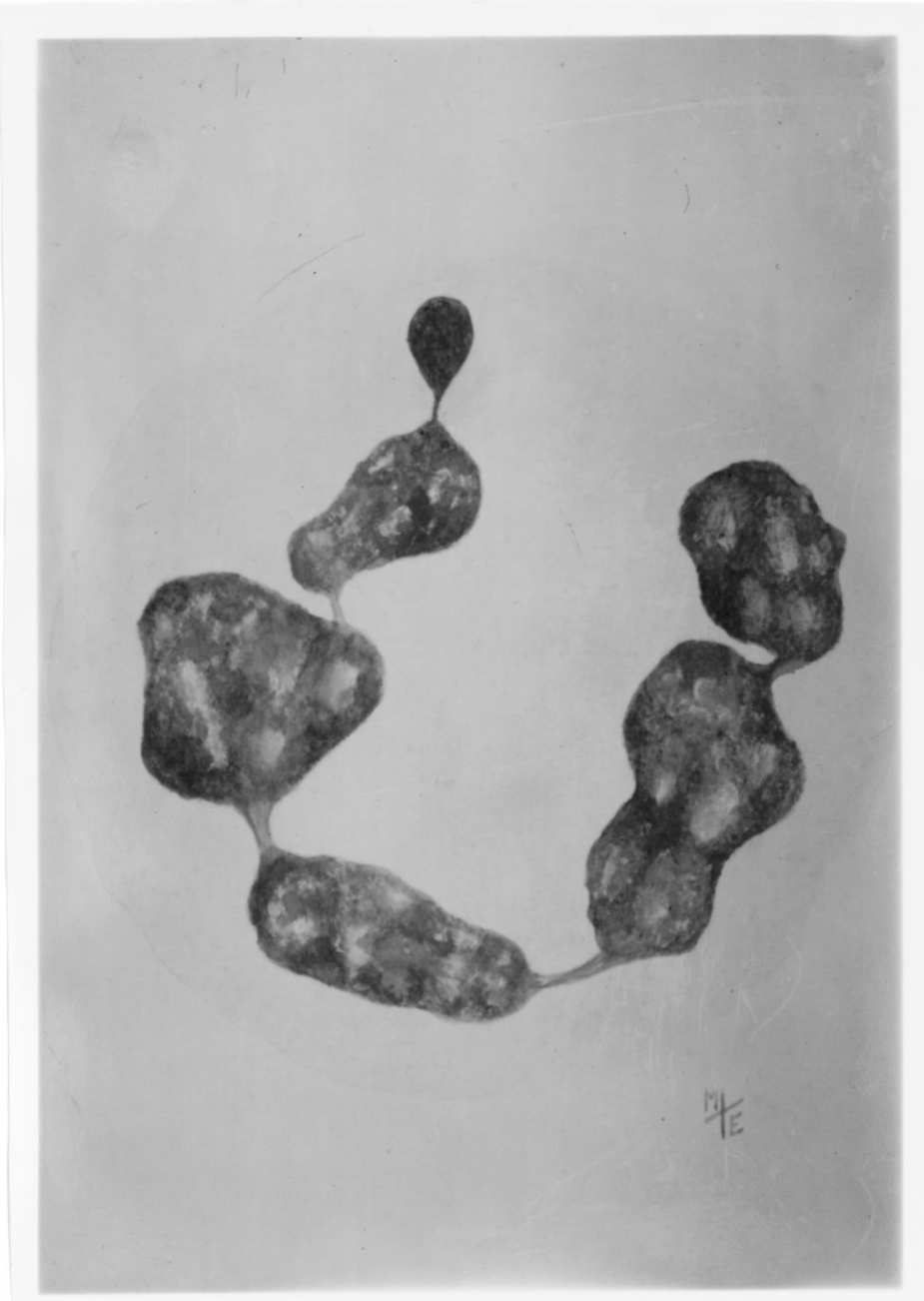
Frontispiece

An accessory nuclear lobule on a canine polymorphonuclear neutrophil leukocyte



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INTRODUCTION

The sexual dimorphism of cells was originally noted in the neurons of the cat (Barr and Bertram 1949). These studies have been extended to include many other types of cells in six orders of the class Mammalia.

In 1954, Davidson and Smith expanded the subject to include the polymorphonuclear neutrophil leukocyte of man. Smith and Calhoun (1956) reported similar findings in the leukocytes of domestic animals. With the exception of the dog (Porter 1957), very little information is available concerning their distribution.

The purpose of this investigation is, therefore, to verify the presence of a sex influenced chromatin mass in the polymorphonuclear neutrophil leukocytes of Bovis, Canis, Capra, Equus, Felis, Ovis and Suis. The data obtained should also indicate frequency of occurrence and, if possible, establish this procedure as a valid method of sexing these animals.

In the future, when the actual genetic basis for sexual dimorphism has been proven, it is hoped that this study will aid in the conclusions that may then be made. Until such time, this work may be of value to those interested in the domestic animals and the economics of breeding.

REVIEW OF LITERATURE

Painter's (1924) work with chromosomes in spermatogonia led to the study of sex chromosomes from marsupials to primates (Painter 1925) and a later investigation of their gene loci (Painter 1940). These observations were used in the study of at least one case of human intersex (Severinghaus 1942). Sexual differentiation on the basis of somatic heteropyknosis was employed in Lepidoptera by Smith (1944, 1945) to determine primary sex ratio.

The sexual dimorphism of resting cell nuclei was first observed by Barr and Bertram (1949) in neurons of the cat. Nuclei from female specimens contained a one micron intranuclear body adjacent to a larger nucleolus. This structure, which was not as well developed in the neurons of male cats, was termed "nuclear satellite".

Some doubt was expressed by Coidan (1951, 1952), who believed the nuclear satellite was identical to the perinucleolar basophil corpuscles (Saguchi 1930), paranucleolar bodies or accessory bodies (Ramon y Cajal 1933), and chromocenter area (Hyden 1943). However, in 1955, Lindsay and Barr observed these structures in nervous tissue which also contained the sex influenced chromatin mass.

In sections taken from adult female cats the satellite was most often situated very near the nucleolus and flattened on the nucleolar side. When free of the nucleolus it appeared rounded and no connecting strands were observed. Although the nucleolus varied in size

(10.5 cubic micra in the dorsal horn to 43.4 cubic micra in the ventral horn) the satellite volume remained extremely constant at 0.5 cubic micra. The position of the satellite was not related to the polarity of the neuron. Neurons in the male contained a nucleolus of comparable size, but the satellite was too small for accurate identification (Barr et al. 1950).

This same sex difference was observed in human sympathetic ganglion cells (Bertrand and Girard 1956) but was more readily seen in sections from the frontal cortex (Mylle and Graham 1954). The nucleolar satellite was more frequently observed flattened against the nuclear membrane, or touching both the nucleolus and the nuclear membrane (Barr et al. 1950).

Most investigators feel that this mass is a chromocenter derived from the heterochromatic portions of the X chromosome. It enlarges during nucleoprotein synthesis and moves toward the nuclear membrane. Within 36 hours after sectioning female cat hypoglossal nerves, the chromatin mass can be seen to move away from the nucleolus, returning to the normal position within two months. However, as the Nissl substance reappears the mass drifts away again returning in the eighth month after sectioning. When the axon reaction is induced by crushing the nerve, the same cycle results and is complete in 28 days (Crouch and Barr 1954). After prolonged electrical stimulation of the hypoglossal nerve of the cat the nucleolus enlarges as the Nissl material reappears in the cytoplasm (Lindsay and Barr 1955). The movement of the satellite toward the nuclear membrane during such periods may be due to an outpouring of material from the nucleolus, or to some factor of an electrical nature (Barr and Bertram 1951).

In adult human males the chromocenter is poorly developed in the sympathetic ganglion cells, more developed in the cerebral cortex and pyramidal cells of area four, and well developed in the cells of the cerebellum. These observations suggest a variation in nucleoprotein synthesis (Bertram et al. 1950).

Nerve cell nuclei were examined in five regions of the nervous systems in the orders Carnivora, Artiodactyla, Rodentia and Lagomorpha. The first two orders showed a characteristic nuclear morphology according to sex (Moore and Barr 1953). Klinger (1957) has been able to identify sex chromatin in motor neurons of female hamsters and rats. Prince (1952) and Prince et al. (1955) reported a sex influenced chromatin mass in all areas of the nervous system of the Rhesus monkey. A difference according to sex was also observed in the astrocytes and oligodendrocytes of the cat (Barr 1951). While the sex chromosome pattern is reversed in the pigeon, Brusa (1952) reported no difference in nuclear morphology.

The role of the nucleolus in relation to the chromatin mass has now been de-emphasized. The present hypothesis regards the chromatin mass as the actual expression of the intermitotic presence of heterochromic or heteropyknotic portions of the XX chromosomes of the female that are visible under ordinary magnifications with the light microscope (Barr 1955b). The absence of this structure in the male is thought to be due to the minute size of the Y chromosome, which in combination with one X chromosome is not resolvable. This theory has been substantiated by Klinger (1958), whose observations at higher magnifications show the mass to be actually "bipartite". Since it resembles the chromosome genetically, morphologically,

and histochemically he suggests that it represents the two heterochromatic rests of the XX chromosomes. A structure one-half this size was observed in male nuclei, but was hard to differentiate from the rest of the particulate chromatin. Thus the term "nucleolar satellite" was discarded in favor of "sex chromatin" (Nelson 1958).

In a study of Didelphys virginiana, Graham (1956) reported sex chromatin in the female nuclei of nervous and non-nervous tissue. She also observed a mass one-half this size in the same position in male nuclei.

Another piece of evidence that the XX chromosomes produce the sex chromatin has been offered by Hughes (1952) and Hsu and Pomerat (1953). They studied chromosomes of dividing cells in vitro by applying a hypotonic solution, thus dispersing the chromosomes before fixation.

The studies on species exhibiting male homogamety have been contradictory. Ashley and Theiss (1959) were not able to identify sex chromatin in domestic fowl, ducks, parrots, parakeets or snakes. Kosin and Ishizaki (1959) demonstrated a chromatin mass in the duodenal muscle and epidermis of growing feathers in the female Gallus domesticus.

Witschi (1956) studied these masses of chromatin with the electron microscope and reported them to be chromocenters of long chromosomes with each thread doubled. These heterochromatic regions show somatic conjugation. Thus, this mass actually consists of four short heterochromatic threads.

For these reasons, the term "genetic sex" is to be avoided until these theories have been definitely proven. Briggs and

Kupperman (1956a) and Barr (1956) suggested using "chromatin positive" or "chromatin negative" when considering test results.

Studies of non-nervous tissue also showed a chromatin mass in female nuclei that was not identifiable in the male. Graham and Barr (1952) identified sex chromatin in one-half to two-thirds of the nuclei from 20 representative tissues from female cats. They reported no morphological sex difference in pancreatic acini or parenchymal cells of the liver. Reitalu (1957) observed sex chromatin in the liver, cerebellum, kidney and gonads of man and stated that this may be due to the X chromosomes being heteropyknotic in the female cells but not in the male. Glenister (1956) also reported sex manifestation in liver cells from human embryos.

This same sex difference was noted in tissue from dog, mink, marten, ferret, racoon, skunk, goat, and deer, but a differentiation was not possible in certain species of rodents studied, namely guinea pig, rat, mouse, hamster and ground hog (Moore and Barr 1954). Hopkins and Whidden (1959) demonstrated sex chromatin in 86 percent of the liver cells from 11 female Chinchilla laniger. Walsh (1954) reported the presence of sex chromatin in certain cells of the golden hamster. The ameloblasts from one-day-old female rats have been found to contain sex chromatin (De Castro et al. 1956).

After the initial finding of sex chromatin in tissue other than nervous tissue, many workers routinely engaged in tissue study reported its presence. Moore and Barr (1955a) diagnosed the sex of 140 patients using smears taken from the oral mucosa. A mass of chromatin measuring 0.7 by 1.2 micra was seen at the periphery of the nuclei in 40 to 60 percent of the cells in oral smears from female

subjects. Marberger et al. (1955), Dixon and Torr (1956) and Dykstra (1958) have reported similar values. Aas (1957) suggested the nasal cavities as a site for mucosal scrapings. Marwark and Weinmann (1955) used biopsy material from the gingiva with equal success.

Tissue from four areas of the human eye was examined by Pedler and Ashton (1955). A chromatin mass was seen in 30 to 80 percent of the nuclei from female patients and in only 15 percent of those from male patients.

While examining normal human vaginal smears, Carpentier et al.(1956) noted sex chromatin in epithelial cells with clear vesicular nuclei. This was possible because of the fine granular chromatin in these cells. Sex chromatin may also be seen in urogenital epithelium stained with Papanicolaou's stain (Pilgaard and Riis 1956). Riis et al. (1956a) found samples of urine sediment unsatisfactory for cytological sex determinations.

Skin biopsies were first used to determine sex by Moore et al. (1953). A difference in nuclear structure according to sex in cells of the Malpighian layer of the epidermis was also reported by Hunter et al. (1954). Twenty-five to 54 percent of the nuclei of female epidermal cells showed such a chromatin mass. The percentage reported in male nuclei from the same area was zero to nine percent (Emery and McMillan 1954, Vitry et al. 1956). Marberger and Nelson (1954) observed sex chromatin in 69 percent of the nuclei in female cells and only five percent in male. They suggested the use of abdominal skin specimens. The scalp and sole of the foot are the least desirable sites for biopsies of this type (Lennox 1956). Both young and old spinosum cells yield the same results (Sachs and Danon 1956). Schleyer (1957)

stated that skin is still sexable for about the first week after death. He used 25 percent of the cell nuclei containing sex chromatin as conclusive. For this reason DeCastro et al. (1957) suggested its use in medicolegal cases. Studies on beaver skin were not satisfactory (Polani and Magnus 1955). Vague et al. (1956) believed the skin biopsy is most useful when studying endocrine disorders.

The manifestation of sex has also interested many embryologists. Graham (1954b) was the first to investigate sex chromatin in the embryo. She observed a chromatin mass in about one-half of the 19-day-old cat embryos studied and although this antedates any gonadal differentiation, these were assumed to be female. In the two- and four-cell stages the nuclei contain several small nucleoli making sexing before the 26 cell morula stage impossible (Austin and Amoroso 1957).

In human female embryos sex chromatin has been found in 30 to 50 percent of the nuclei examined. The youngest specimen to exhibit sexual differentiation was a 1.7 millimeter pre-somite embryo (Glenister 1956). Sex chromatin was seen at 12 days in the trophoblast and at 16 days in the embryo itself (Park 1957). Cells from the embryonic disk and cytotrophoblast were not suitable for testing (Glenister 1956). This has been substantiated by Witschi (1957) who suggested that all species be sexed before gonadal differentiation.

Exfoliated cells in the amniotic fluid show a definite sex chromatin mass in the nuclei. The incidence of chromatin positive cells in those cases associated with female fetuses is several times that of the male fetuses (Makowski et al. 1956, Serr et al. 1956). The epithelial cells seen are from the basal cell layer, the precornified or cornified layer

and the keratinized layer. The former two types are preferable for these studies (Sachs et al. 1956b). They originate from the gastro-intestinal, respiratory and genitourinary tracts, skin and umbilical cord (Shettles 1956b). Shettles (1956a) observed no chromatin positive cells in amniotic fluids associated with male offspring.

The use of amniotic fluid to determine the sex of human fetuses gained instant popularity among expectant parents. The puncturing of a placental membrane merely for curiosity's sake is not so widely accepted by the medical profession (Dewhurst 1956, James 1956, Shettles 1956c). The utility of this technic in veterinary medicine has been suggested by Sachs et al. (1956a) and Fuchs and Riis (1956).

The embryonic membranes of the cat contain 96 to 98 percent chromatin positive cells associated with females and only nine to twelve percent when the fetuses were male (Graham 1954a). In a study designed to prove that nuclear sex does not result from any hormonal influences, Klinger (1957) found the extra-embryonic membrane of human males exhibits no sex chromatin.

Sadovsky et al. (1957), using only placentas bearing male fetuses, suggested that the much disputed origin of the placental septa is maternal.

The nuclei of the mesenchyme cells in the supporting tissue of the chorionic villi may be used to determine the sex of a fetus (Stevenson and McClarin 1957).

Miles (1959) reported the presence of sex chromatin in human cells grown in vitro. These were noted in primary cultures and remained for ten weeks through nine subcultures. The Y chromosome has been demonstrated in dermal cells grown in tissue culture in a

case of male intersex (Hsu et al. 1953). The cells of mouse and hamster were not sexable in tissue culture (Miles and Koon 1960). The cells from female rats exhibited a chromatin mass in 20 percent of the nuclei and in only one percent of the male cells when grown in vitro.

Sex chromatin has been observed in inflammatory processes and neoplasms. The size and position of the chromatin does not differ in transneural atrophy (Cook et al. 1951). Sohval and Gaines (1955), in a study of hematoxylin and eosin stained routine pathological material, found chromatin positive cells in one-fourth of the specimens obtained from female patients. Moore and Barr (1955b) identified typical female nuclei in all specimens from female benign tumors. This difference is thought to be the result of more carefully prepared material stained by the Feulgen technique. In another study of benign tumors, sex chromatin was seen in the nuclei from 75 percent of the tissues from females (Moore 1955). DeLustig et al. (1958) found that six malignant neoplasms of the male breast contained chromatin positive nuclei. No morphological sex difference was noted in basal cell carcinomas (Weinmann et al. 1955).

Several studies of teratomas have shown that those occurring in females contain positive chromatin. In the male, approximately one-half of the cases studied also exhibited sex chromatin (Hunter and Lennox 1954). Tavares (1955), Cruickshank (1955) and Ruge (1958) reported the presence of chromatin positive nuclei in teratomas from male patients.

In 1954, Davidson and Smith, while investigating the arrest of nuclear lobing in the Pelger Huet anomaly, noticed a distinct nuclear

appendage occurring only in the mature neutrophil leukocyte of the female. In view of Barr's earlier work, the interpretation of these findings was evident. This appendage may be recognized by its deeply staining, chromatin-rich head about 1.5 micra in diameter attached to a lobe of the nucleus by a fine filamentous thread that may be up to three micra in length.

Davidson and Smith (1954) gave these projections the name "drumstick". Barr (1959) has used the term "accessory nuclear lobule" when referring to these appendages. It is the opinion of this author that accessory nuclear lobule describes this structure more scientifically.

Several other projections on polymorphonuclear neutrophil leukocytes have been described. The "sessile nodule", occurring more frequently in the female, may be an indication of sex. Small clubs, rackets, intermediate lobes and terminal nodules have been noted, but these are not concerned with the sex complex and have never been considered in such studies (Riis 1955).

Davidson and Smith (1954) reported the frequency of these accessory nuclear lobules in man as one per 38 neutrophils, the lowest being one in every six and the highest, one in 98 neutrophils. This was confirmed by counting five hundred neutrophils on smears from 250 females. The first cell to exhibit a typical accessory nuclear lobule was the thirty-sixth neutrophil counted. Nogues and Fisch (1958) reported six such appendages per 223 leukocytes counted. Hjelt et al. (1958) found an average of one per 32 cells in girls up to 15 years of age. This frequency, according to Davidson and Smith (1956), depends upon the degree of lobing in the neutrophils;

accessory nuclear lobules are not present in the Pelger Huet anomaly but occur in every thirteenth cell when the Arneth count nears three lobes per neutrophil. However, Riis (1955) reported the most accessory nuclear lobules on bi-lobed granulocytes. It was the practice of Davidson and Smith (1954) to find six accessory lobules in the first five hundred neutrophils. Using this criteria, Briggs and Kupperman (1956b) studied 47 normal persons -- finding no accessory nuclear lobules in males; while in females, including Negroid, Oriental and Caucasian, six were seen before five hundred neutrophils were examined. The incidence in cases of mongolism did not vary from that seen normally (Fiorio 1957). Briggs (1958a) suggested that the frequency of the accessory nuclear lobule is a characteristic of the individual. Studies are underway which tend to show a similarity among female members of the same family with respect to nuclear appendages, although this frequency has no relationship to any clinically measurable aspect of individuality. Caratzali et al. (1957) have suggested that the granulocytic appendages vary in females during the menstrual cycle due to the action of sex hormones which accelerate pyknosis and lobation of the sex influenced chromatin. In a later study of peripheral blood smears from one hundred student nurses, five hundred neutrophils in all maturing stages were examined and accessory nuclear lobules were found in every case. In five instances there were less than six such appendages per five hundred neutrophils. When the neutrophil lobe counts of these students' blood smears were plotted against the accessory nuclear lobule counts there was no evidence of any relationship between the two (Briggs 1958b). Kosenow and Scupin (1956)

and Kosenow (1956, 1957) statistically evaluated a procedure whereby the sessile nodules and accessory nuclear lobules are divided by the number of small clubs seen in counting five hundred segmented neutrophils. If this quotient is greater than 0.4 the diagnosis is female.

The sex influenced chromatin mass has been studied in the other cells of the peripheral blood. Riis (1955) observed seven accessory nuclear lobules on eosinophils in 8000 granulocytes. Murthy and ~~Emmerich~~ van Haam (1958) counted six per 189 eosinophils. Davidson and Smith (1956) and Cooper and Cranny (1958) also reported their presence in eosinophils. These appendages were not seen on basophilic granulocytes although the nuclear outlines were carefully studied. This is due to the coarse, intensely staining granules (Murthy and Emmerich 1958, Riis 1955). At this time Riis reported no morphological sex difference in the nuclei of lymphocytes and monocytes. It has been suggested that monocytes may exhibit an intranuclear mass comparable to that seen in epithelial cells (Davidson and Smith 1956).

In 1957, Riis published a complicated procedure whereby the buffy coat from cooled and centrifuged blood, incubated for 24 hours, showed morphological changes toward macrophages and fibroblasts. These cells then exhibited chromatin clumping in those specimens from female subjects. The percentages recorded were not as high as those for epithelial cells.

In a series of one hundred normal newborn humans, Halbrecht (1959) found the neutrophil morphology agreed with the phenotype in every case. Studying premature and newborn infants, Mosler (1957) found accessory nuclear lobules in the same proportions as reported for adults. Tenczar and Streitmatter (1956) set the ratio at 1:24

polymorphonuclear leukocytes in female infants. In 50 human fetuses studied by LaLorretta (1957), the incidence of accessory nuclear lobules was found to be less than in adults.

Ashley (1957) reported a heterochromatin mass on all types of nucleated blood cells in bone marrow except megakaryocytes, and late normoblasts where the nuclear chromatin is too dense. Accessory nuclear lobules were reported in sternal punctures by Dihlmann (1957). Romagnoli et al. (1958) observed a chromatin mass outside the nuclear membrane in female erythroblasts.

In studying chimeras, Davidson et al. (1958) found accessory nuclear lobules in five human twins of both sexes. A similar study on multiple births was done by Reiffenstuhl (1958).

Irradiated male rabbits transfused with female rabbit bone marrow exhibited six accessory nuclear lobules in less than five hundred heterophils within 33 days. The rabbit heterophil had previously been reported to contain a sex influenced chromatin mass (Luers 1956). This species seems the one of choice in research as 20 percent of the heterophils have accessory nuclear lobules (Carpentier et al. 1957).

Girod (1958) found accessory nuclear lobules in the neutrophil granulocytes of monkeys. Nuclear tags and sessile nodules were seen but no rackets were noted.

In guinea pigs there are accessory nuclear lobules on seven percent of the neutrophils in fixed smears and 30 percent in mobile preparations (Luycks 1958). Hinrichsen and Gothe (1958) showed no difference in the blood from rats and mice, according to sex.

Porter (1957) reported the incidence of accessory nuclear lobules as one in 22 neutrophils in the dog. He also found this lobule on the nuclei of eosinophils of female dogs. This was confirmed by Locatelli and Quarenghi (1958).

There are many advocates of the blood examination method of sexing. Aas (1957) believed blood smears have the advantage in ease of obtaining, transportation, and lack of alarming the patient. Hanicki and Hanicka (1957), in a survey of 250 patients, were able to correctly diagnose sex in every instance. These same results have been reported by Romatowski et al. (1955), Neimann et al. (1955), Sun and Rakoff (1956), Hienz (1957) and Santilli (1958).

Throughout these many investigations of both adult and embryonic tissue, including blood, there has been a constant search for the best staining procedure. Sex chromatin in nerve cells stains intensely with such basic dyes as hematoxylin, thionin, cresyl violet, the green constituent of methyl green-pyronin and is always Feulgen positive (Barr et al. 1950). No difference is noted in hematoxylins of Ehrlich, Mayers, Harris, Weigert or Delafield (Emery and McMillan 1954).

Carpentier et al. (1956) used the Papanicolaou technique for vaginal smears. Herrmann and Davis (1956) also employed this stain in a study of one hundred oral smears.

In 1957, Klinger and Ludwig reported a universal stain for sex chromatin using thionin with Michaelis' buffer on material previously hydrolyzed with 5N hydrochloric acid at room temperature.

Greenblatt et al. (1957) did a comparative study on smears of peripheral blood, oral mucosa and vaginal mucosa using pinacyanole

and Wright's stains. In a similar study Cuadrillero (1959) used a silver impregnation technique.

Schenk (1958) has compared five stains on peripheral blood. She has shown that either a Wright's or May-Grunwald counter-stained with Giemsa was most valuable when accessory nuclear lobules were to be examined. However, Carpentier et al. (1957) used Harris' alum hematoxylin in a study of rabbit blood. When fresh cells were studied under phase contrast the sex chromatin was visible without staining (Silva-Insunza 1957).

At the present time the most obvious application of these results is in cases of intersex (Browne 1955). The early diagnosis of congenital errors of sex development leads to corrective surgery and hormonal therapy before psychological disturbances arise (Shal 1957). Moore (1959), in a survey of 3,715 infants, calculated the frequency of female to male sex reversal in the 1,911 males to be .26 percent. In a study of 106 cases of abnormal sex development Barr (1955a), using skin biopsies, obtained results agreeing with the previous diagnosis in every case. However, Bettinger (1956) cautioned against its use with disregard for the individual personality and stated that chromatin sex should have no bearing on cases of intersexuality. Segal and Nelson (1957) stated that the physical situation of the patient is most important but that the physician should always have the genetic sex available.

Barr (1954a, 1954b) suggested that true hermaphrodites and female pseudohermaphrodites, not caused by adrenal cortical hyperplasia, may be either chromatin positive or negative. Greene et al. (1954) have reported a case of true hermaphroditism which they believe

has an XXY chromosomal pattern. The use of blood smears when hermaphroditism is suspected in infancy is urged by Wiedemann et al. (1956). They believe this test to be more practical and more reliable than a skin biopsy.

Klinefelter's syndrome may be diagnosed when chromatin positive material is found in a male (Briggs et al. 1958, Bradbury et al. 1956). Jackson et al. (1956) reported finding two cases where there was male nuclear morphology. Riis et al. (1956b) and Etcheverry and Wais (1957) employed blood smears to study this syndrome.

Turner's syndrome is thought to exhibit XX, XO, XY, or a mosaic of different sex chromosomes (Danon and Sachs 1956). Ovarian agenesis has been thoroughly investigated (Rannie and Erskine 1954, Ashley and Jones 1958b, Barr 1955b, Lennox 1956, Polani and Magnus 1955). The chromosomal male pattern in these cases results from the lack of testicular development in the fetus thus producing female genitalia and ducts in a chromatin negative individual (Wilkins et al. 1954).

In congenital testicular hypoplasia the subjects may have XX or XXY chromosomes (Plunkett and Barr 1956a, 1956b). A case of doubtful sex was male according to the neutrophil morphology and this finding was confirmed at necropsy (Bottura 1957).

Barr and Hobbs (1954) reported chromatin negative material from five cases of male transvestism. Dokumov and Papasov (1959) found chromatin positive nuclei in one case of homosexuality in a female.

Cantwell et al. (1958), in a study of swine intersex, found chromatin positive nuclei in all cases. The genetic femaleness of the bovine freemartin has also been proven by the finding of chromatin positive nuclei in the neurons (Moore et al. 1955, 1957).

There is little agreement among the workers in these various fields as to the merit of studying the sex chromatin in tissue, the accessory nuclear lobule in the blood and relating either or both of these to genetic sex and gametogenesis in the subjects investigated. Discrepancies have been reported at times in the literature (Ashley and Jones 1958a, 1958b, Bunge and Bradbury 1956, 1957, Raboch 1957, Platt and Stratton 1956, Manautou and Greenblatt 1957). Briggs (1958a), upon personal communication with these authors has been able to resolve any discrepancies between sex chromatin and accessory nuclear lobule. This leads to the belief that sex chromatin and accessory nuclear lobules are the same (Briggs and Kupperman 1957, Wiedemann et al. 1958). Carpentier et al. (1957) demonstrated a bipartite condition in the accessory nuclear lobule which, in conjunction with the work of Klinger (1958), tends to substantiate this theory.

Brum et al. (1959), in a study including Didelphys, Bufo, and Orthoptera, stated that sex chromatin is not due to the heteropyknosis of the XX chromosomes and doubts that it is even related to the sex complex. Ashley (1957) preferred to consider the tissue sex chromatin as the expression of the XX chromosomes but the accessory nuclear lobule only as a sex characteristic. He claimsto have observed a chromatin mass lying near the nuclear membrane in polymorphonuclear neutrophils that also showed an accessory nuclear lobule.

This intranuclear chromatin mass occurred in proportions agreeing with those of the bone marrow and epithelial cells. A well outlined, deeply basophilic mass of chromatin resembling the intermitotic mass in female somatic cells has also been described in polymorphonuclear cells by Murthy and Emmerich (1958). However, they never observed this mass in combination with the accessory nuclear lobule. Romatowski et al. (1958) suggested that counting both the accessory nuclear lobule and the intranuclear chromatin mass would give results comparable with the percentages reported in the tissues.

It is gratifying to note that in no case of a positive chromatin individual has there been functioning testicular tissue and likewise there is no ovigenesis in chromatin negative subjects (Briggs and Kupperman 1958).

MATERIALS AND METHODS

This study includes blood smears obtained from five normal male and five normal female specimens from each of the following species: Bos, Canis, Capra, Equus, Felis, Ovis and Sus. Fresh smears were made on new pre-cleaned microscopic slides (MacGregor et al. 1940). Smears were dried immediately to prevent osmotic loss of water (Christopher 1954). All smears were stained with Wright's stain using the modification of Osgood and Ashworth (1937). Coverslips were not used.

At least six smears were prepared from each animal. A differential white blood cell count including four hundred cells was made on these smears to check for cell distribution and technical perfection (Sturgis and Bethell 1943). Animals whose differential counts did not comply with the normals as given by Albritten (1953) were replaced.

Three smears from each animal were selected for nuclear study. The oil immersion objective was used with 15X oculars. No area of any smear was re-examined.

Five hundred polymorphonuclear neutrophil leukocytes with entire cell membranes were counted unless six accessory nuclear lobules were observed first. Although many nuclear appendages were considered, only those having a round chromatin-rich head measuring 1.5 micra and attached to one lobe of the nucleus by a fine chromatin strand were counted. The number of the cell on which each accessory nuclear lobule was seen was recorded.

The true accessory nuclear lobule must be differentiated from several other projections (Plate I - 1). The chromatin-rich "sessile nodule" measuring one to two micra, may cause confusion (Plate I - 2). Morphologically this appendage is not well defined and therefore not considered in actual counts. "Small clubs" are also seen which resemble the sex influenced appendage but are less than one micron in diameter (Plate I - 4). The "racket" has a pale clear central area and is attached to a lobe of the nucleus by a fine filament (Plate I - 6). Actual nuclear lobules small enough to cause confusion are always found to be attached by two filamentous strands to adjacent lobes (Plate I - 5). Small terminal nuclear lobes are generally not as perfectly formed as the accessory nuclear lobule (Plate I - 3).

PLATE I

Figure 1 Accessory nuclear lobule

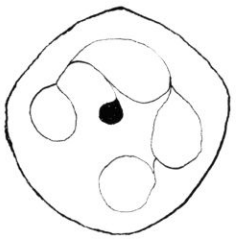
2 Sessile nodule

3 Terminal nodule

4 Small club

5 Intermediate lobe

6 Racket



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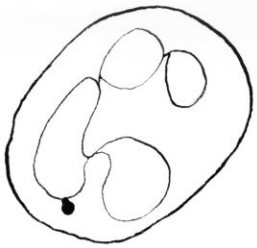

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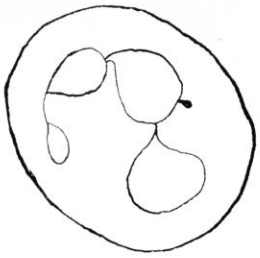

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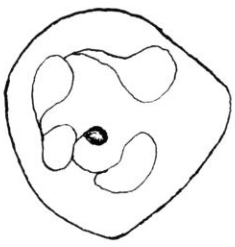


6

RESULTS AND DISCUSSION

The data from this study tend to agree with previous research. Six accessory nuclear lobules were observed in all female animals except Bovis, before five hundred polymorphonuclear neutrophil leukocytes were counted (Table I). It is difficult to correlate this work with many of the former investigations because there were variations in the cells studied. In many cases neutrophils in all maturing stages were used; in others, only mature granulocytes were counted, and some workers have included both these groups. Because of the variation in eosinophilic granulation and the differences in nuclear lobulation in domestic animals, a more precise evaluation was obtained in this study by counting only polymorphonuclear neutrophil leukocytes.

Statistical studies done on these data show all counts to be within the 95 percent confidence limits of random sampling. The standard deviations were calculated on the actual counts rather than on the averages. This, plus a discussion of the results by species, will aid other investigators when considering an individual case.

Bovis

This was the only animal in which six accessory nuclear lobules were not observed when five hundred polymorphonuclear neutrophil leukocytes were counted. In the entire 2,500 cells from females examined, only two such lobules were seen (Plate II - 1). In smears from males there were none. The granulocytic nuclei in cattle are extremely dense and compact, containing heavy clumps

TABLE I

Polymorphonuclear Neutrophil Leucocytes Counted*

	Bovis	Canis	Capra	Equus	Felis	Ovis	Suis
All males	500	500	500	500	500	500	500
Females							
1	500	166	434	446	429	457	381
2	500	321	449	281	342	502	468
3	500	416	310	261	227	434	222
4	500	263	339	230	314	356	294
5	500	330	239	306	286	438	440
Averages	500	229	354	305	330	437	361
Standard Deviation**		±92	±87	±84	±61	±53	±102

* Counting was discontinued at 500 or when six accessory nuclear lobules were observed.

** Standard deviations of the actual counts.

PLATE II

Polymorphonuclear Neutrophil Leukocytes from Female Animals Showing Typical Accessory Lobules

Wright's Stain. Magnification 1900X.

Figure 1 Bovis

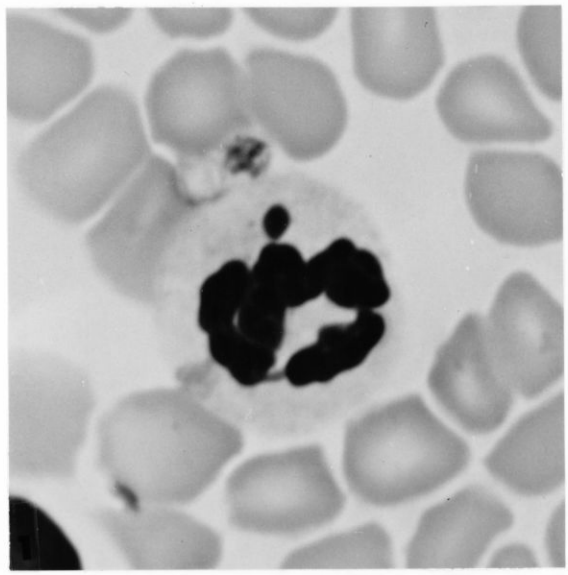
2 Canis

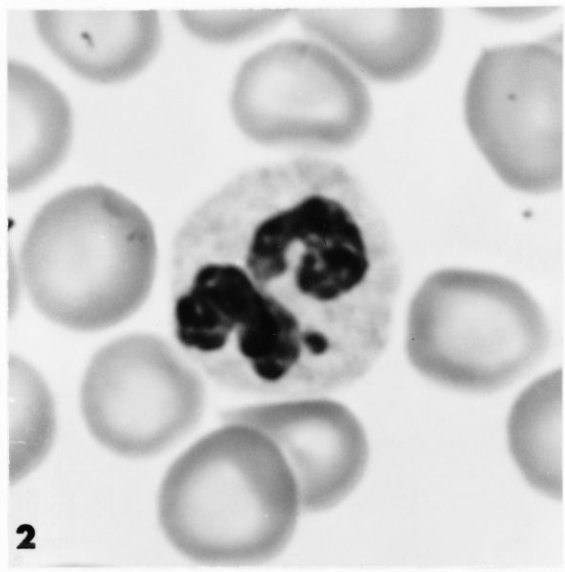
3 Capra

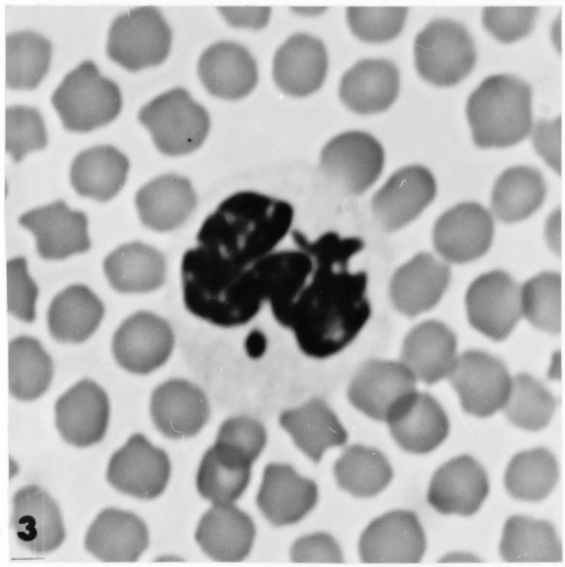
4 Equus

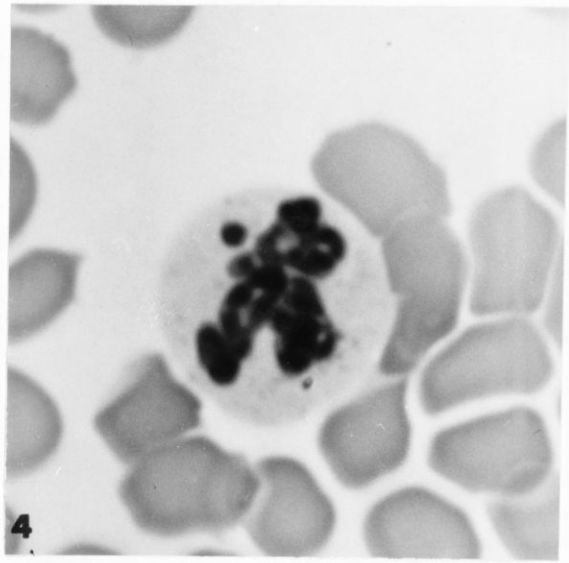
5 Felis

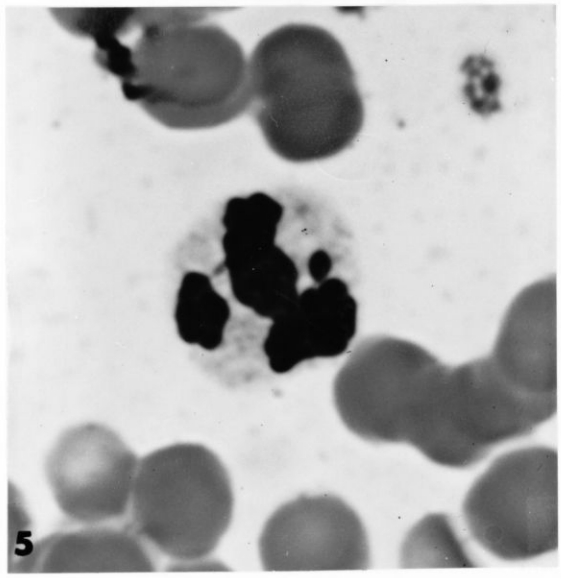
6 Ovis

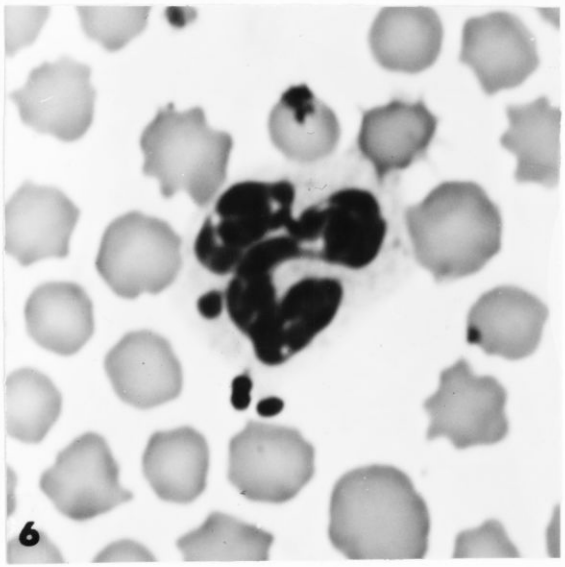












of chromatin (Plate III - 6). No other studies of this nature have been done on Bovis; however, Moore et al. (1955, 1957) have studied other bovine tissue. They observed a sex chromatin mass only in the neurons and stated that coarse chromatin clumps obscured it in other tissues.

Since cattle do exhibit a sex influenced chromatin mass in the blood, further investigation is needed to determine its frequency. The results of this study suggest that the frequency is too small to make this procedure an applicable method for sexing cattle.

Canis

The accessory nuclear lobule occurred in every 60 ± 9.14 polymorphonuclear neutrophil leukocytes in female dogs and was not observed in male dogs (Plate II - 2). The lowest number of cells counted to obtain six accessory nuclear lobules was 166; the highest was 416. This accounts for the higher standard deviation (Table I). The differential leukocyte counts done on these animals showed no shifts which might account for this variation in frequency.

Although accessory nuclear lobules observed on band forms (Plate III - 5) and eosinophil leukocytes were not counted, they were noted in these smears. Sessile nodules were seen more frequently in the female dog than in other species. A dense mass of chromatin measuring 1.5 micra was often seen as a terminal lobe in the female (Plate III - 4). These were never observed in the male, but under the conditions of this problem such appendages were not counted. This may explain the frequency lower than that cited by Porter (1957).

PLATE III

Polymorphonuclear Neutrophil Leukocytes
Wright's Stain. Magnification 1900X.

Figure 1 Accessory nuclear lobule, female Suis

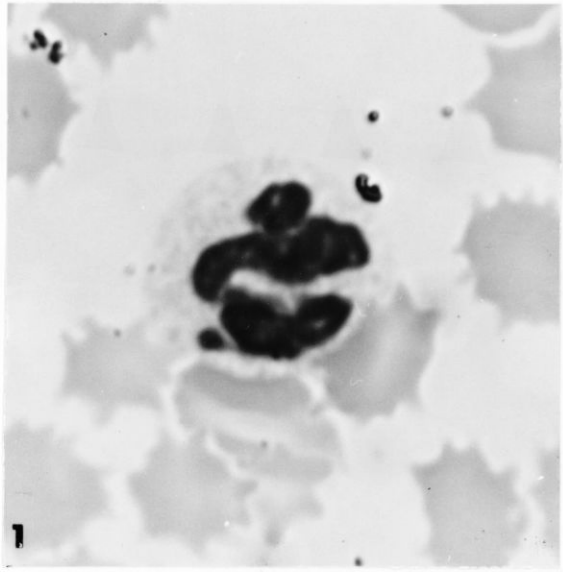
2 Terminal lobe, male Equus

3 Racket, female Felis

4 Terminal lobe, female Canis

5 Band cell with accessory nuclear lobule, female Canis

6 Typical dense chromatin, female Bovis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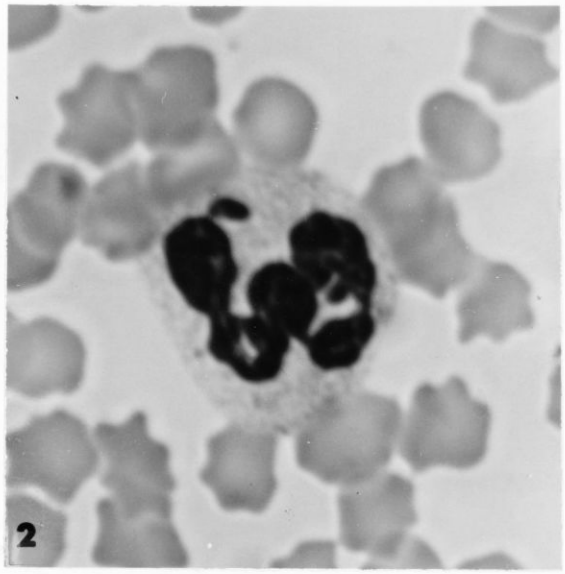
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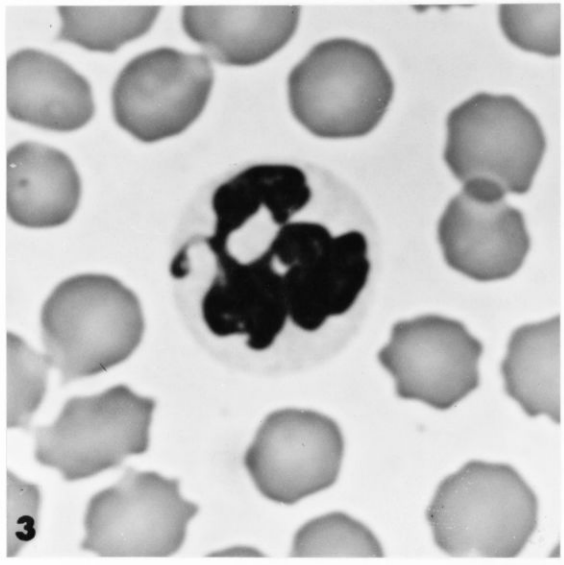
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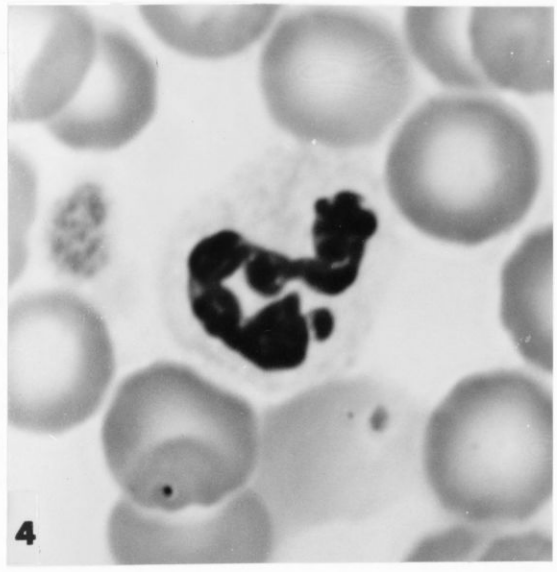
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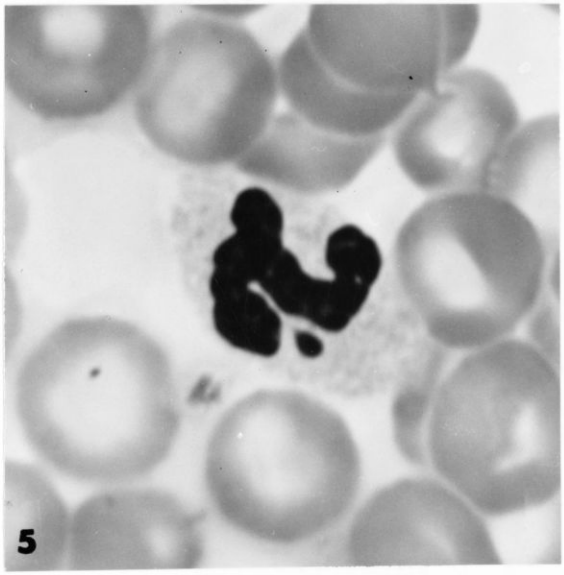
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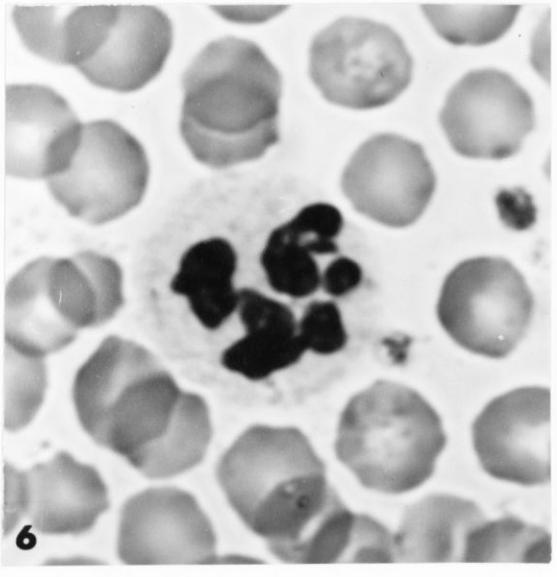
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Capra

Accessory nuclear lobules were observed in the female goat (Plate II - 3). The average frequency was 60 $\pm$ 7.98. A similar structure was never seen in smears from male goats. There was a range of only 110 cells between the highest and lowest counts obtained in females (Table I). Unless a worker is experienced, these appendages should be very carefully measured, for the smaller size of the goat red blood cell (4.1 micra) may cause confusion. There were very few appendages in either sex that could be mistaken for the accessory nuclear lobule.

Equus

The female horse exhibited a sex influenced chromatin mass on the polymorphonuclear neutrophil leukocytes in about the same proportions as other domestic animals (Plate II - 4). The accessory nuclear lobule was seen in every instance before five hundred cells were counted, the average being 305 (Table I). A typical chromatin mass was observed on an average of every 51 $\pm$ 8.82 cells in female smears, but none were seen in the smears from male horses.

In general, these appendages were more perfectly rounded and did not taper toward the filamentous attachment. In many cases, this attachment was extremely short. The granulation of the horse eosinophil completely obscured any appendages on these cells. The beginning of lobulation in the horse granulocyte appears to start toward one end of the band cell in both sexes and these small lobes may cause confusion (Plate III - 2). Sessile nodules were frequently observed in smears from both male and female horses.

Felis

In female cats, an average of every 55 $\pm$ 6.52 polymorphonuclear neutrophil leukocytes exhibited an accessory nuclear lobule (Plate I - 4). These were not seen in the male. The fewest cells counted to obtain six such lobules was 227 cells. The average was 330 (Table I).

No sessile nodules were observed in the males. Smaller lobes were seen with two attachments to the rest of the nucleus (Plate I - 5). Many small terminal lobes were noted but a larger more densely staining chromatin mass was always present elsewhere in the cell.

In one instance, a structure similar to the accessory nuclear lobule, but having a clear central area, was observed in a female (Plate III - 3). This is the racket described by Riis (1955, Plate I - 6). However, he attributed this structure exclusively to the male.

Ovis

The frequency of accessory nuclear lobules was the lowest in the sheep (Plate II - 6). In one instance, due to an oversight in counting, the sixth lobule was observed on the five hundred and second cell. The average was 73 $\pm$ 9.63 cells counted to obtain one appendage. The average number of cells counted to obtain six lobules was also highest in the sheep -- 437 (Table I). However, a statistical study using the standard deviation of this average shows that in counting five hundred polymorphonuclear neutrophil leukocytes all but .001 percent of similar subjects will exhibit six accessory nuclear lobules.

Suis

The average number of polymorphonuclear neutrophil leukocytes counted to obtain an accessory nuclear lobule in the female pig was 51 $\pm$ 8.36 (Plate III - 1). There was no chromatin mass in the male resembling these lobules. Six accessory nuclear lobules were seen in 222 cells in one case, and the highest number of cells counted before six were observed was 468 (Table I). The average number was 361 and although this is smaller than the average number obtained in sheep the standard deviation of the individual counts was higher. A standard deviation of this average shows that by counting five hundred cells only 78 percent of similar subjects would be included. Therefore, any studies using this method of sexing pigs should include a larger number of polymorphonuclear leukocytes. At present, this procedure would be most useful in non-terminal experiments as Cantwell et al. (1958) found only the nervous tissue to exhibit a definite nuclear morphology according to sex.

SUMMARY AND CONCLUSIONS

This investigation demonstrated the accessory nuclear lobule in domestic animals. Blood smears were collected from five males and five females of the following species: Bos, Canis, Capra, Equus, Felis, Ovis and Sus. Differential leucocyte counts were made and five hundred polymorphonuclear neutrophil leucocytes were examined, unless six accessory nuclear lobules were noted. In females, with the exception of cattle, it was possible to demonstrate six such lobules before five hundred cells were counted.

Cattle exhibits a sex influenced chromatin mass but the frequency is too low to make this procedure practical. Although the pigs used in this study exhibited six accessory nuclear lobules per five hundred polymorphonuclear neutrophil leucocytes, the frequency and standard deviations indicate that this method does not include the entire population.

All the domestic animals studied exhibited an accessory nuclear lobule. By examining five hundred polymorphonuclear neutrophil leucocytes it is possible to determine the sex of Canis, Capra, Equus, Felis and Ovis.

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