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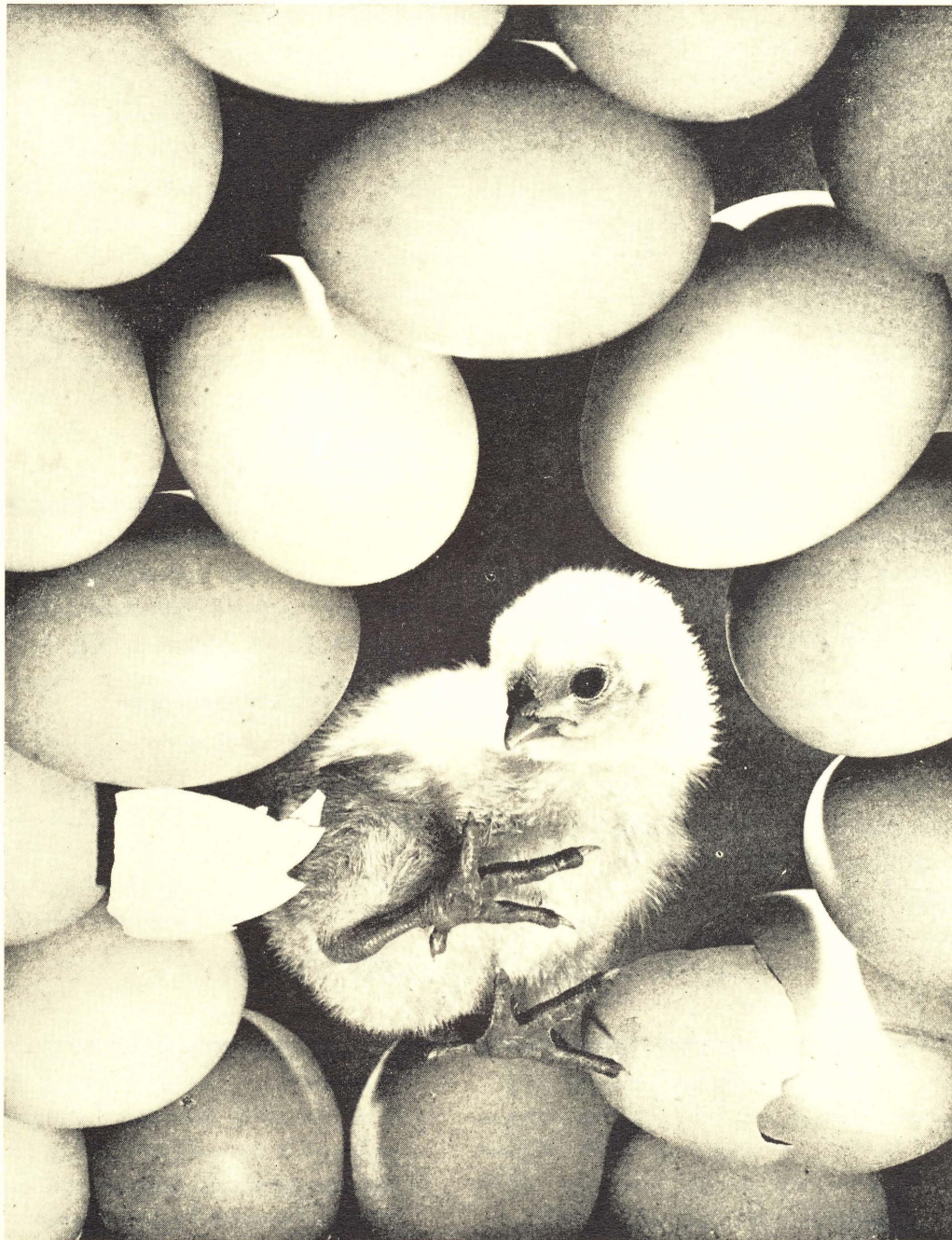
From Egg To Chick – A Guide to the Study of Incubation and Embryonic Development  
Michigan State University Cooperative Extension Service  
4-H Club Bulletin  
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4-H — YOUTH PROGRAMS    COOPERATIVE EXTENSION SERVICE    MICHIGAN STATE UNIVERSITY



# FROM EGG TO CHICK

A GUIDE TO THE STUDY OF INCUBATION AND EMBRYONIC DEVELOPMENT

COOPERATIVE EXTENSION SERVICE

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Community Building

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### A Note to Teachers and Leaders

*The information in this circular has been prepared to help the individual student or 4-H member investigate incubation and embryonic development. The student wishing to pursue these subjects in detail may find it profitable to consult one or more of the publications listed on page 16. For class or group study, you may obtain films, charts, models, and slides from the following sources:*

#### FILMS

Chick Embryo Explanation. Thorne Films, Inc., 1707 Hillside Road, Boulder, Colorado.

The Development of a Bird Embryo. No. 1156, Encyclopaedia Britannica Films, Inc., Wilmette, Illinois.

Embryology of the Chick. Calvin Productions Inc., Kansas City, Missouri.

Where Chick Life Begins. Film Library, New York State College of Agriculture, Cornell University, Ithaca, N. Y.

#### CHARTS, MODELS, AND SLIDES

Carolina Biological Supply Co., Elon College, North Carolina.

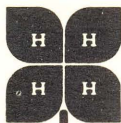
Turtox General Biological Supply House, Inc., 8200 South Hoyne Avenue, Chicago, Ill. 60620.

Ward's Natural Science Establishment, Inc., P. O. Box 24, Beechwood Station, Rochester, N.Y. 14609.

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Cover Photograph: J. H. Vendell

This circular was prepared by S. F. Ridlen and H. S. Johnson, Extension Poultry Specialists of the University of Illinois.



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**T**HE BIRD EGG IS A MARVEL OF NATURE. WHAT IS more fascinating than to see a fully-developed baby chick emerge from an egg? Just three weeks of incubation transforms a seemingly lifeless chicken egg into an active, living being.

If you are interested in a science project for school or 4-H, none can be more interesting and informative than the study of the chicken egg and its development from the unincubated stage to the emergence of the chick from the shell. The developing chick in an egg is called an embryo, and a careful study of the different stages of embryonic development will uncover many interesting facts.

This circular has been designed to help you study incubation and embryonic development. Plans are included for two small, inexpensive incubators which you can make at home or school. Many small commercially built incubators can be purchased at reasonable prices.

Incubation of eggs will show you the effects of heat, air, and moisture control on hatchability. But don't stop your study with the incubation of eggs. Many other interesting aspects of the egg and the chick may be selected for study. For example you may want to find out how an egg is formed, what its different parts and their functions are, and how a chick embryo develops. And there are many other interesting topics you can investigate.

## CONSTRUCTING THE INCUBATOR

An incubator for a science project need not be as elaborately constructed as a commercial incubator. Plans for two still-air incubators are given in this circular. One can be made from cardboard boxes and glass and the other from plywood (or similar material) and glass.

Don't expect 100 percent success in hatching eggs in these or any other incubators. Commercial hatcheries with all their highly automatic and specialized equipment do not average much more than an 80 percent hatch of all the eggs they incubate. You probably should not count on hatching more than 50 percent, and you may not even succeed in hatching 50 percent.

Eggs have been incubated by artificial means for thousands of years. Both the Chinese and the Egyptians are credited with originating artificial incubation procedures. The Chinese developed a method in which they burned charcoal to supply the heat. They also used the hot-bed method in which decomposing manure furnished the heat. The Egyptians constructed large brick incubators which they heated with fires right in the rooms where the eggs were incubated.

Over the years incubators have been refined and developed to the point where they are almost completely automatic. The greatest development has occurred in this century. Modern commercial incubators are heated by electricity, have automatic egg-turning devices, and are equipped with automatic controls to maintain the proper levels of heat, humidity, and air exchange. Present-day commercial incubators vary in capacity from a few thousand to many thousands of eggs, and they have made possible the development of modern hatcheries which produce almost 100 percent of all the chickens grown in this country.

Both still-air and forced-draft incubators are used in hatcheries. However, all the new ones are forced-draft; that is, they have fans to circulate the air. They are capable of maintaining more even temperature, humidity, and oxygen levels than still-air incubators. Many modern commercial hatcheries also use separate setter and hatcher machines.

### A CARDBOARD BOX INCUBATOR<sup>1</sup>

The following supplies are needed to construct an inexpensive cardboard box incubator which will hold three dozen or more eggs:

Two cardboard boxes, one 16" wide x 20" long x 12½" high, the other 14" wide x 18" long x 13" (or more) high

Single-strength pane of glass 16" x 20"

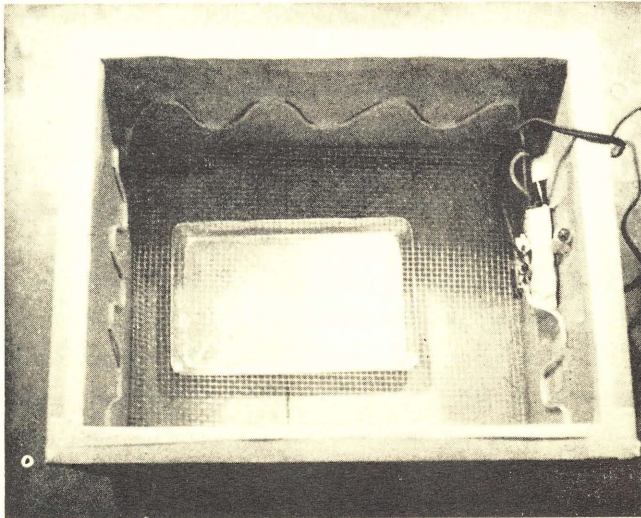
¼"-mesh welded hardware cloth 18" x 22"

Commercial heating unit or porcelain socket and light bulb

Cake tin (water pan) 1½" deep x approximately 9" x 14"

<sup>1</sup> Courtesy of E. A. Schano, Cornell University.



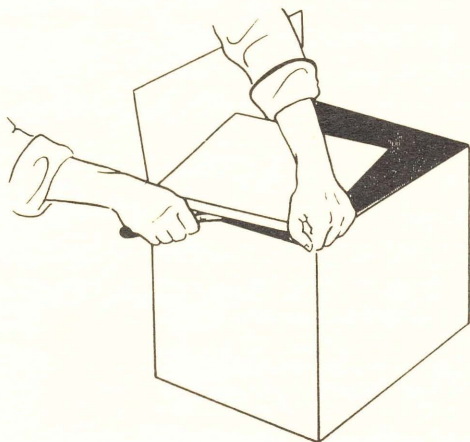


Top view of a cardboard box incubator. The drawings on this and the following page will show you how to construct the box. (Courtesy of E. A. Schano, Cornell University.) (Fig. 1)

- Glue
- Masking or scotch tape
- Newspapers
- Two brooding or incubator thermometers

The incubator is made in the following manner:

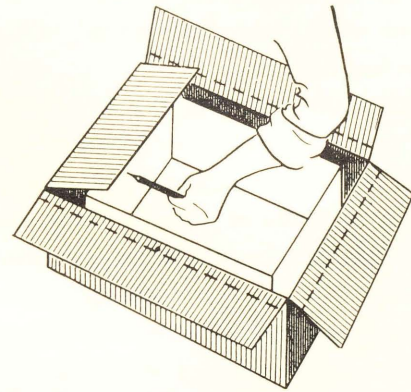
1. Place the smaller box inside the larger one. The inner box should be higher than the outer box and approximately 2 inches smaller in both length and width.
2. Mark a line on the inside box approximately  $\frac{1}{4}$  inch below the level of the outside box. Use a yardstick to make a straight line on the inner box after it is removed from the outer box.
3. Cut off the top of the inside box along the line made in step number 2.



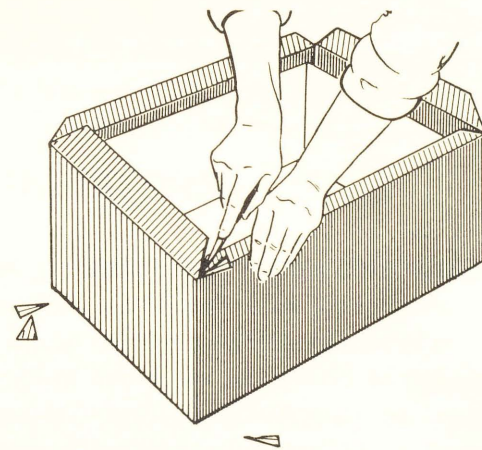
4. Use cut-away pieces of the inside box to line the bottoms of both the inner and outer boxes where the flaps do not meet. If there are no cut-away pieces, use pieces from a third box.

5. Put glue on the bottom of the inner box and then center the inner box in the outer one. There should be a 1-inch space between the sides of the boxes. Secure the inner box until the glue dries.

6. Mark a line on the flaps of the outside box where they come in contact with the inner edge of the inside box.



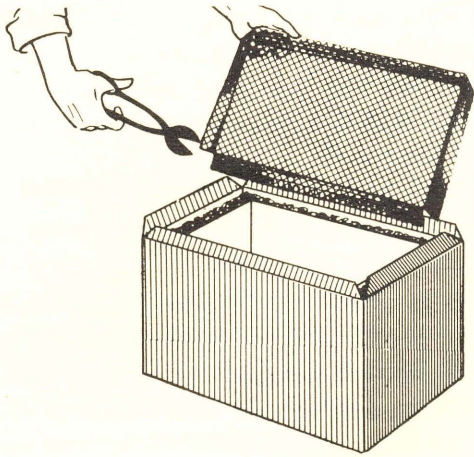
7. Cut off the flaps of the outside box along the lines drawn in step number 6. Cut the corner pieces on a diagonal so that they will make a neat, flat corner.



8. Stuff strips of newspaper lightly into the space between the boxes. Do not bulge the sides of the incubator. Wood shavings, excelsior, or styrofoam can be used in place of the newspaper strips.

9. Use tin snips to cut a 2-inch square from each corner of the  $\frac{1}{4}$ -inch mesh hardware cloth, then bend the projecting pieces of the screen down so that they form legs to support the screen.

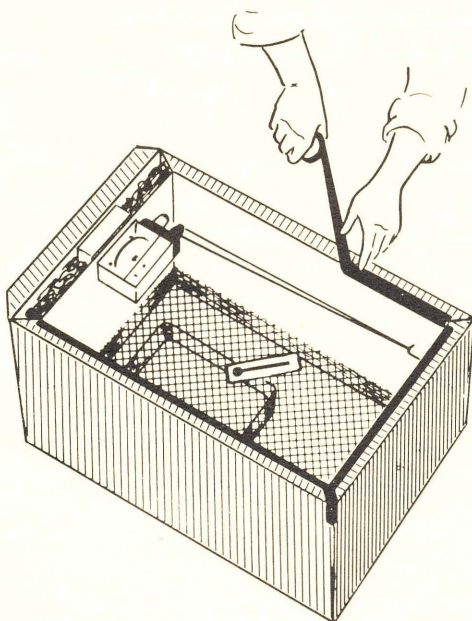




10. Place the cake tin, which will cover about one-half of the surface area of the inside box, under the hardware cloth screen.

11. Install the commercial heating element as directed in the instructions sent with the unit. If you use an electric light for heat, mount the porcelain socket on a board 6 inches square, then place the mounting board on the screen. Next, place a tube of cardboard around the light. Position the tube so that it surrounds the light and stands like a chimney, but to reduce the fire hazard do not let it come in contact with either the light bulb or the covering glass. An oatmeal box makes a good tube.

12. Tape the flaps of the outer box to the sides of the inner box. This seals the area in which the insulating material was placed.

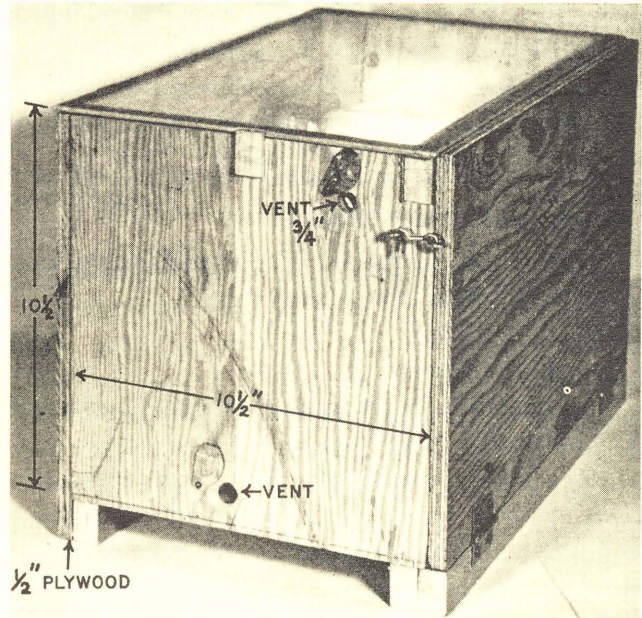


### A PLYWOOD INCUBATOR<sup>1</sup>

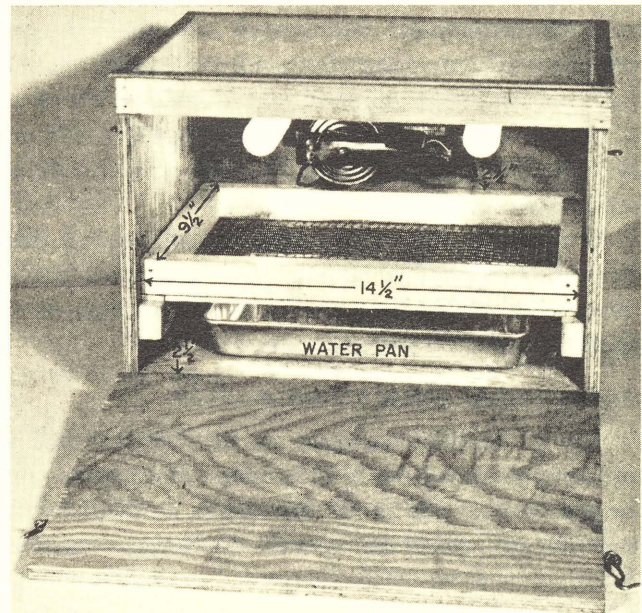
The following materials are needed:

- Plywood
- Glass
- Cake tin
- ¼"-mesh welded hardware cloth

<sup>1</sup> Courtesy of John Bezpa, Rutgers University.



End view of a plywood incubator. Note the tin vent covers for control of humidity and temperature. Glass viewing top is sealed at the sides with masking tape. (Courtesy of John Bezpa, Rutgers University.) (Fig. 2)



Side view of the plywood box incubator shown in Fig. 2. (Courtesy of John Bezpa, Rutgers University.) (Fig. 3)



Heating unit: either a commercial unit or porcelain socket and light bulb

Masking tape

You may construct the incubator according to the size desired. It can be a small one for only a few eggs, or it can be a somewhat larger one which will hold several dozen eggs. The larger the incubator, the more difficult it will be to maintain a uniform temperature in it. In fact, you may find it important in the larger incubator to put in a small fan (3- or 4-inch blade or smaller) with a low revolving rate. For details concerning the construction of a small incubator for about 24 eggs, see Figs. 2 and 3.

#### **OTHER SOURCES OF INCUBATORS**

Some of the companies which manufacture small incubators and incubator parts are:

Brower Mfg. Co.

Quincy, Illinois

(Incubators, incubator thermometers, and circuit breakers)

Fleet Mfg. Co.

P.O. Box 649

Glendale, California

(Small incubators)

Lyon Rural Electric Co.

P.O. Box 30

2075 Moore Street

San Diego, California 92112

(Incubators, incubator thermometers, thermostats, and midget incubator kits)

Montgomery Ward & Co.

Chicago, Illinois 60607

(Farm catalog: Small incubators, incubator thermometers, and thermostats)

National Agricultural Supply Co.

Ft. Atkinson, Wisconsin

(Small incubators)

Sears Roebuck & Co.

Chicago, Illinois 60607

(Suburban-Farm Supplies and Fencing Catalog: Small incubators, thermometers, and thermostats)

## **INCUBATION PROCEDURES**

#### **OBTAINING HATCHING EGGS**

Obtaining fertile eggs may present a problem, especially if you live in an urban area. Most of the eggs sold in grocery stores are not fertile and cannot be used for incubation. Fertile eggs can usually be obtained from hatcheries or poultry breeding farms. Look in the yellow pages of your telephone directory for names of hatcheries and poultry breeders. Or contact the farm adviser in your county agricultural extension service office for suggestions.

If possible, pick up the eggs yourself rather than have them shipped or mailed. It is difficult for hatcheries, post offices, and transportation companies to handle small orders of eggs properly.

#### **CARE OF EGGS PRIOR TO INCUBATION**

The hatchability of eggs can be severely reduced by improper care prior to incubation. Since it may not be practical for you to put the eggs in an incubator as soon as you get them, protect them from extreme

variations in temperature. Ideally, eggs should not be more than 7 days old when they are set (placed in the incubator). Beyond that point, hatchability declines.

If it is necessary to hold the eggs before you set them, turn them daily and keep them in a room where the temperature is around 50° F. and the relative humidity is 70 to 80 percent. The vegetable section of your refrigerator could be used for holding the eggs until it is time to place them in the incubator. Temperatures below 40° F. reduce hatchability. Under no circumstances should the eggs be held at room temperature, because temperatures of this level are detrimental to hatchability. Embryos will begin to develop at subnormal rates when the temperature reaches about 80° F.

#### **LOCATION OF INCUBATOR**

Locate your incubator in a room in which the temperature is between 70° and 75° F., and which is free from drafts and excessive variations in temper-



ature. Do not place the incubator near windows where it will be exposed to the direct rays of the sun. The sun's rays may raise the temperature so much that all of the embryos will be destroyed.

#### READYING THE INCUBATOR FOR OPERATION

Before you set the eggs, be sure that the incubator is in good working order. Put some warm water in the water pan, place each thermometer so that the bulb of the thermometer is 1 inch above the screen, cover the incubator with the pane of glass, and then operate the incubator until the temperature inside it holds at 99° to 103° F.

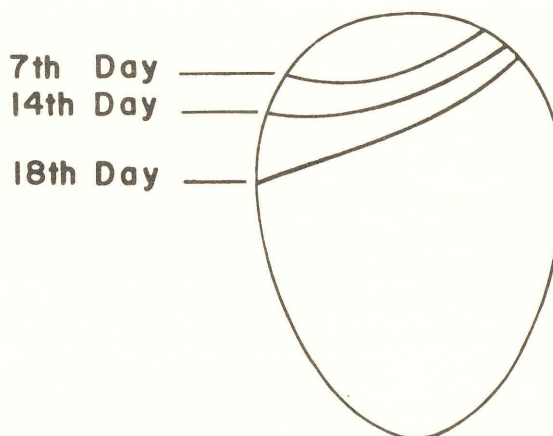
Adjust the thermostat to control temperature in an incubator with a commercial heating unit. In an incubator with a light-bulb unit, control the temperature by adjusting the size of the opening at the top by moving the pane of glass back from the edge of the incubator, unless a thermostat has been installed. If you are using a plywood incubator, adjust the openings as necessary. Or in either incubator you can adjust the heat by using bulbs of different sizes. Forty-watt bulbs will be about the right size for most incubators. Make certain that the sides and top of the incubator fit closely so that no heat is lost. You may need to make many adjustments to reach a proper setting.

#### PREPARING THE EGGS FOR INCUBATION

Eggs must be turned while in the incubator, so before you put in the eggs mark them with a pencil so you can tell when they have been properly turned. An excellent method is to put an "X" on one side of the egg and an "O" on the opposite side. Then you can always tell when the eggs have been turned, because either all "O" 's or all "X" 's are turned up at the same time.

#### HUMIDITY

Nature has provided that the eggs shall dry out to some extent during incubation (Fig. 4). This loss should be about 11 percent of the original weight, but any more than this is detrimental. Water must be placed in the incubator to avoid excessive moisture loss. Keep a pan of water inside at all times. The surface area of the pan should be about as large as the tray of eggs.



Size of the air cell in the egg on the 7th, 14th, and 18th days of incubation. (Fig. 4)

The amount of opening in the incubator also influences the level of humidity. When the humidity is too high, open the vents all the way on the plywood incubator or slide the glass further back on the cardboard incubator. When humidity is too low, the openings should be more nearly closed, but never completely so. Weather conditions will affect the relative humidity in the incubator.

The ideal moisture level is about 50 to 55 percent relative humidity (83°-87° F on a wet bulb thermometer) for the first 18 days and about 65 percent (89°-90° F wet bulb) for the last 3 days. Excessive drying because of low humidity will cause the chick to stick to the shell and fail to survive. Some variation above or below the ideal level usually will not affect hatchability drastically. Frequently, school incubators have too much ventilation and, therefore, too little moisture. This results in delayed or reduced hatches.

When you refill the water pan, use warm water. Hot or cold water will affect the temperature of the incubator too much. To increase the humidity level the last three days, set an extra pan of water in the incubator. Or you can put a wet sponge in the incubator to raise the humidity. A word of caution: Do not let the eggs come into direct contact with the water at any time.

#### TEMPERATURE

Temperature in the still-air incubator can vary from 99° to 103° F. with no harmful effects if the temperature varies between these limits rather than staying at either extreme. If it stays at either extreme for several



days, the hatch may be reduced somewhat. Overheating is much more critical than underheating. Overheating will result in abnormal embryos, speed up development, and lower hatchability. A thermometer should be in the center of the incubator if possible, and the bulb of the thermometer should be level with, but not touching, the tops of the eggs. If light bulbs are used to supply the heat they should be distributed as evenly as possible in the incubator.

### VENTILATION

Proper ventilation is very important during the incubation process. While the embryo is developing, oxygen enters the egg through the shell, and carbon dioxide escapes in the same manner. As the chicks begin to hatch, it is essential that they receive an increasing supply of oxygen. This means that the air openings need to be opened gradually to increase the flow of air.

### LENGTH OF INCUBATION

Chicken eggs require 21 days to hatch, but the incubation period for the eggs of other species of poultry varies. The approximate periods of incubation required for various species of poultry and game birds are:

	<i>Days</i>
Chicken . . . . .	21
Turkey . . . . .	28
Duck . . . . .	28
Muscovy duck . . . . .	33-35
Goose . . . . .	29-31
Guinea . . . . .	26-28
Pigeon . . . . .	16-18
Ring-neck pheasant . . . . .	23-24
Mongolian pheasant . . . . .	24-25
Bobwhite quail . . . . .	23
Japanese quail . . . . .	17-18
Chukar partridge . . . . .	22-23
Peafowl . . . . .	28

### TURNING THE EGGS

When the eggs are put in the incubator, lay them on their sides and turn them at least three times a day. Turning prevents the embryo from sticking to the shell membranes as it will do if it is left in one position too long. Good results can be obtained by turning the eggs the first thing in the morning, again at noon, and the last thing at night. But it is better to turn the eggs more than three times a day. In any

case they should be turned an odd number of times so that the side that is up longest will be staggered from day to day. Otherwise the egg will be in the same position every night, which is the longest stretch of time between turns.

When you turn the eggs, move them to a different part of the tray to offset variations in temperature in the different parts of the incubator. Continue to turn the eggs from the first through the 17th days but do not turn them after the 17th day.

### TESTING THE EGGS

Although it is not necessary to test eggs for fertility, you can eliminate the eggs which are not going to hatch by doing so. It is also an interesting phase of the project, since it is possible to see clearly the developing embryo.

Get a shoe box or a box of similar dimension made from wood or tin and cut a hole in one end about one inch in diameter. Use an extension cord and mount a 60-watt bulb in the box. Darken the room and hold the large end of the egg to the light. What you will see depends mostly on the age of the embryo. It is difficult to see much development until the 4th or 5th day of incubation.

The first parts of the embryo which you will be able to see by candling will be the head and eye, and they will appear as a dark object. If the embryo is alive and circulation is established, the contents of the egg will have a pinkish color or cast. But if the embryo is dead the contents will appear muddy or brownish. The live and growing embryo will eventually occupy all of the interior of the egg and will not transmit light; thus, it will be impossible to see anything but the air cell at the end of the incubation period. Infertile eggs and early dead embryos can be detected readily because they appear clear.

Removing the eggs from the incubator for candling does little harm if you handle them gently. It may slow up development of the chick, though, depending upon how much the egg is cooled. Generally, if the eggs are removed from the incubator two or three times for a period of no more than 15 minutes each, such cooling will make little difference in the total incubation time required for hatching. On the other hand, if the eggs are cooled for several hours because of power failure or some other reason, hatching time may be delayed. It is as important not to cool the eggs too long as it is to avoid overheating.



## FINAL STAGES OF INCUBATION

After the 17th day, eggs should not be turned, and the incubator should not be opened unless it becomes necessary to add water, replace a light bulb, or make some other necessary adjustment. Chicks will start to pip the shell around the 19th day. All chicks which are going to hatch should be out of their shells by the 21st day. If the eggs were chilled or you ran into operational difficulties during the incubation period, the hatch may be delayed. Chicks that hatch beyond the 22nd day are usually not healthy, vigorous ones.

When most of the eggs are hatched, lower the temperature to approximately 95° F. This permits the newly-hatched chicks to dry off. At this time, all the air vents in the plywood incubator should be opened, and the glass top on the cardboard incubator should be opened wider.

## HOW THE CHICK EMERGES FROM THE SHELL

The head of the chick develops at the large end of the egg. Between the 15th and 16th days, the chick orients itself so that its head is near the air cell at the large end of the egg. Not long before the chick is ready to attempt to make its way out of the shell its neck acquires a double bend so that its beak is under its right wing and pointed toward the air cell. About the 19th day the chick thrusts its head forward. Its beak quickly breaks through the inner shell membrane,

and the chick's lungs begin to function. Complete breathing by the lungs usually does not occur until the 20th day of incubation.

Using its egg tooth (a tiny, sharp, horny projection on the end of its beak), the chick pecks at the shell thousands of times. Finally, the young bird pips its way through the shell and begins to breathe air directly from the outside. After the chick has made a hole in the shell, it stops pipping for three to eight hours and rests. During this time, it is acclimating its lungs to the outside atmosphere. After the resting stage is completed, the second stage of pipping begins.

The chick begins to turn slowly inside the egg. As it turns, usually counter-clockwise, the cutting edge of the chick tooth continues to chip away. In two to five hours, the chick has made about three quarters of a turn inside the egg. As the chick progresses in its movement around the shell, it begins pushing on the egg cap (large end). Squirming and struggling, the chick works feverishly for about 40 minutes pushing at the cap. Finally with a vigorous shove, the chick breaks free from the shell, still wet and panting.

When the chick is freed completely from the shell, it lies still. Its energy has been virtually exhausted, and it is extremely tired. After a rest of some few minutes, the chick begins to rise to its feet and gain coordination of its muscles. Within a few days the egg tooth, its usefulness over, will disappear.

### REASONS FOR POOR HATCHES

1. Infertile eggs.
2. Eggs too old when set.
3. Parent stock weak, unhealthy, or fed a nutritionally deficient diet.
4. Improper care of eggs prior to incubation.
5. Shell contamination.
6. Eggs not turned often enough.
7. Temperatures too high, too low, or too variable during incubation.
8. Too little humidity in the incubator or occasionally too much.
9. Improper ventilation.
10. Oxygen starvation.



## WHAT TO DO AFTER THE CHICKS HATCH

As soon as the chicks have dried and fluffed up completely, remove them from the incubator and place them in holding quarters where the temperature is approximately 95° F. Then give them fresh water and feed. Rearing the chicks as a project has certain limitations, but if they are to be kept for a few days, they should be given a chick-starting mash obtainable at any feed or farm supply store. Fresh water is also important.

Since the disposal of day-old or started chicks may be difficult, have the solution to this problem worked out before you undertake this project. If you are

going to rear the chicks at home, secure your parents' permission and cooperation in advance.

**Cleaning the incubator.** When the hatch is completed, disconnect the incubator. Remove all shells and unhatched eggs and wipe the interior clean with a soapy sponge. Permit the incubator to air dry for several days by leaving the door open.

Cleaning can be made easier if you place a layer or two of cheesecloth or crinoline on the rack on the 17th or 18th day of incubation to catch the egg shells and other debris. This will also help to prevent injury to the chicks' navels. After the chicks are removed the cheesecloth can be discarded.

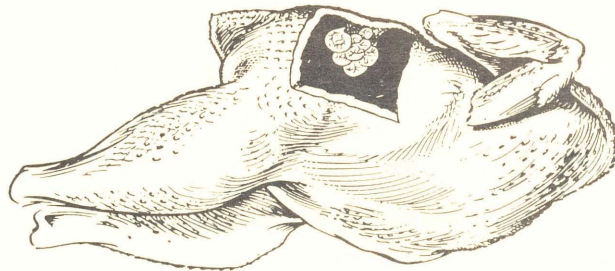
## FORMATION AND PARTS OF THE EGG

Incubation is not the only project a student may undertake with eggs, and in the remainder of this circular we shall look into some of the more complicated and technical projects that are of interest. Let us start by paying some close attention to the egg itself.

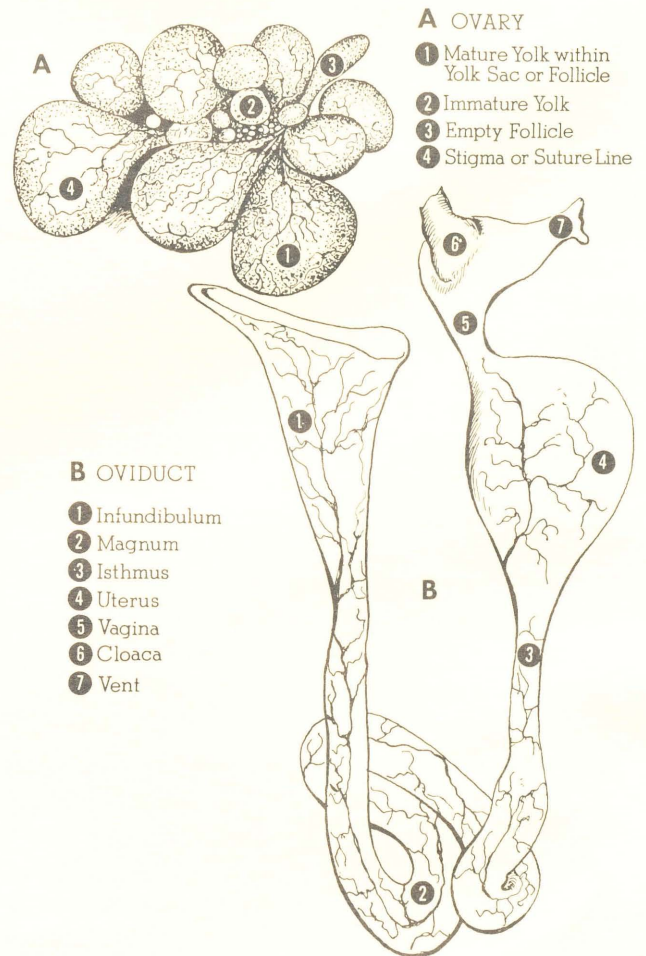
The avian egg is one of nature's finest foods. The yolk, white, and shell of the egg contain all the proteins, fats, carbohydrates, minerals, and vitamins necessary to nourish the embryo during the 21-day incubation period.

Since the embryo actually begins with the formation of the egg within the hen's body, some understanding of the formation of the egg will lead to a better comprehension of embryonic development. The major reproductive organs of the hen are the ovary and the oviduct (Figs. 5 and 6).

The females of most animals have two functional



Position of the ovary in a hen. (From Egg Grading Manual, USDA Agricultural Handbook No. 75, July, 1961.) (Fig. 5)



A hen's reproductive system. The position of the ovary in the hen's body is shown in Fig. 5. (From Egg Grading Manual, USDA Agricultural Handbook No. 75, July, 1961.) (Fig. 6)



ovaries, but the hen has only one. The right ovary stops developing when the female chick is hatched, but the left one continues to develop. The left ovary will contain up to about 4,000 tiny ova from which the full-sized yolks will develop when the hen matures.

Each yolk or ovum is contained in a thin membranous follicle. Blood vessels in the follicle carry nutrients to the developing yolks. When a yolk matures, the follicle ruptures along a line relatively free from blood vessels known as the stigma, and the yolk is released. The yolk is kept intact by the vitelline membrane surrounding it.

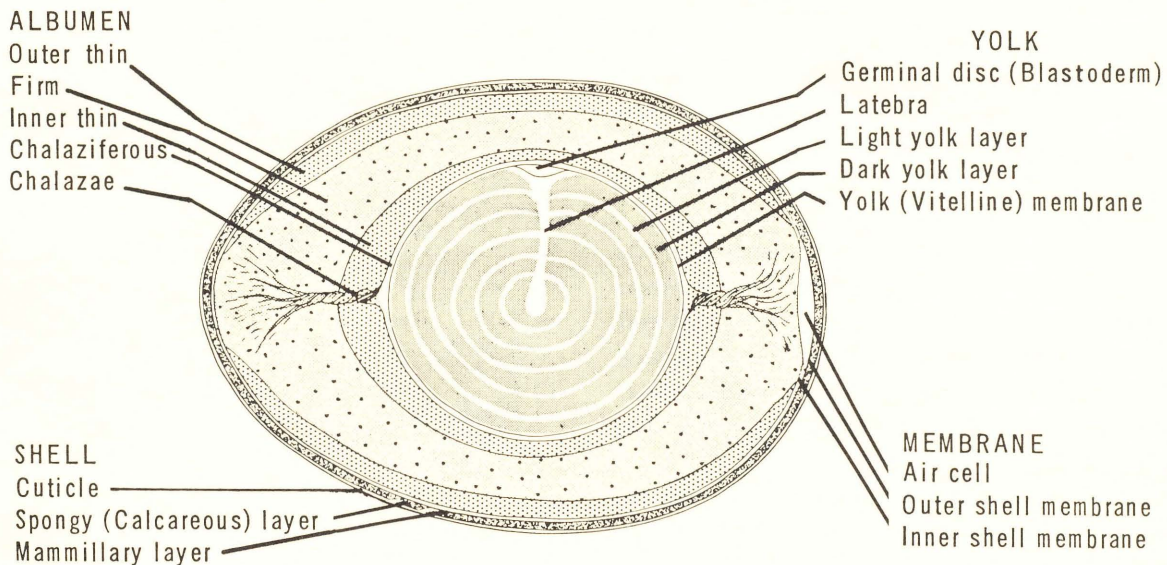
Upon release from the follicle, the yolk drops into the body cavity. There the infundibulum, or funnel, engulfs the yolk and starts it on its way down the oviduct. The oviduct is more than two feet long and is lined with glands which secrete the materials for the albumen, shell membranes, and shell. Twenty-four hours or more are required from the time the yolk is released until the completed egg is laid.

The fertilized egg is a highly complex reproductive cell and is potentially a small center of new life (Fig.

7). The germinal disc from which new life develops is attached to the yolk of the egg. Surrounding and protecting the germ cell and yolk is the white or albumen consisting of several layers.

The albumen is somewhat elastic, and it is a shock-absorbing, semi-solid material with a high water content. The albumen and the yolk of the egg serve as food for the growing embryo during the incubation period. On opposite sides of the yolk are two twisted, whitish cord-like objects known as chalazae. They anchor the yolk and keep it from rising and touching the shell. Nature provided them also to serve as a rotating axis to keep the germ cell on the top side of the yolk and, therefore, next to the heat of the hen's body. So, the yolk and albumen work together to protect and sustain the life of the growing embryo.

Around the albumen and yolk are two shell membranes and the shell itself. These give protection to both the yolk and albumen, and the shell provides for an exchange of gases and serves as a method of conserving the food and water supply incased within the shell for the benefit of the developing embryo.



The parts of an egg. (From Egg Grading Manual, USDA Agricultural Handbook No. 75, July, 1961.) (Fig. 7)



# DEVELOPMENT AND PRESERVATION OF EMBRYOS

## STAGES OF EMBRYONIC DEVELOPMENT

Shortly after the ovum has been released from the hen's ovary, it is picked up by the funnel or infundibulum. Sperm from the male are found in the folds of the infundibulum. Soon after the ovum is picked up by the infundibulum, many sperm contact the germinal disc, but only one unites with the germ. Thus fertilization occurs about 24 hours before the egg is laid.

Since the fertilized germinal disc, or blastoderm, spends about 24 hours in the warmth of the hen's body (about 107° F.) while the egg is being completed, certain stages of embryonic development occur during that time. About three hours after fertilization the newly formed single cell divides and makes two cells. Then there are four, eight, sixteen, and more. Cell division continues until there are many cells grouped in a small, whitish spot visible on the upper surface of the egg yolk.

When the egg is laid and its temperature drops below about 80° F., cell development ceases. Cooling at ordinary temperatures will not kill the embryo, and it will begin to develop again when the egg is placed in the incubator. Keeping eggs at temperatures above about 80° F. prior to incubation will cause a slow growth which leads to a weakening and eventual death of the embryo.

During incubation various processes occur. They are mainly respiration, excretion, nutrition, and protection. Extraembryonic membranes are membranes outside the embryo's body which make these functions possible (Fig. 8). The extraembryonic membranes are the yolk sac, amnion, allantois, and chorion.

The yolk sac is a layer of tissue growing over the surface of the yolk. Its walls are lined with a special tissue which digests and absorbs the yolk material. The amnion is a transparent sac filled with a colorless fluid. The amnion and amniotic fluid provide protection from mechanical shock and permit the developing embryo to exercise.

Respiration by the embryo is made possible by the allantois. Blood vessels in the allantois bring oxygen to the embryo and take carbon dioxide away. The allantois also stores excretions, absorbs albumen used as food by the embryo, and absorbs calcium from the shell for the structural needs of the embryo. The

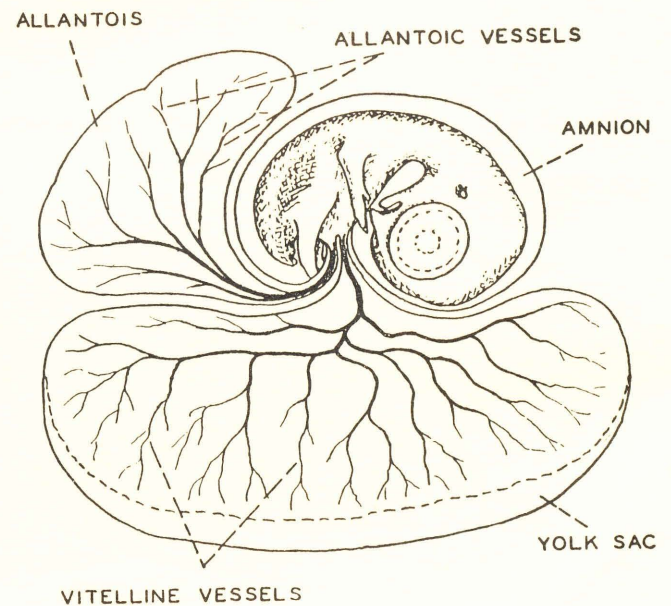
allantois ceases to function when the chick punctures the air cell and starts to breathe on its own.

A fourth membrane (not shown in Fig. 8), the chorion, surrounds both the amnion and yolk sac. Initially the chorion has no apparent function, but later the allantois fuses with it to form the chorio-allantois membrane. None of these extraembryonic membranes become a part of the chick.

## STUDYING EMBRYONIC DEVELOPMENT

You can study the external form of chicken embryos at various stages of development. Beginning with the 3rd or 4th day of incubation you can, by carefully breaking open an egg each day, observe many events in the development of embryos. And it is easy to preserve the embryos for display or additional study.

To study the early embryonic development from the beginning to the 4th day of incubation, you will need to prepare a set of whole mounts and observe them under a microscope. This is a rather exacting procedure which requires considerable time. It should not be attempted by the younger student or 4-H member.



Seven-day old embryo with its embryonic membranes and embryonic blood vessels. (Slightly modified from Duval, in A. L. Romanoff, Cornell Rural School Leaflet, September, 1939.) (Fig. 8)

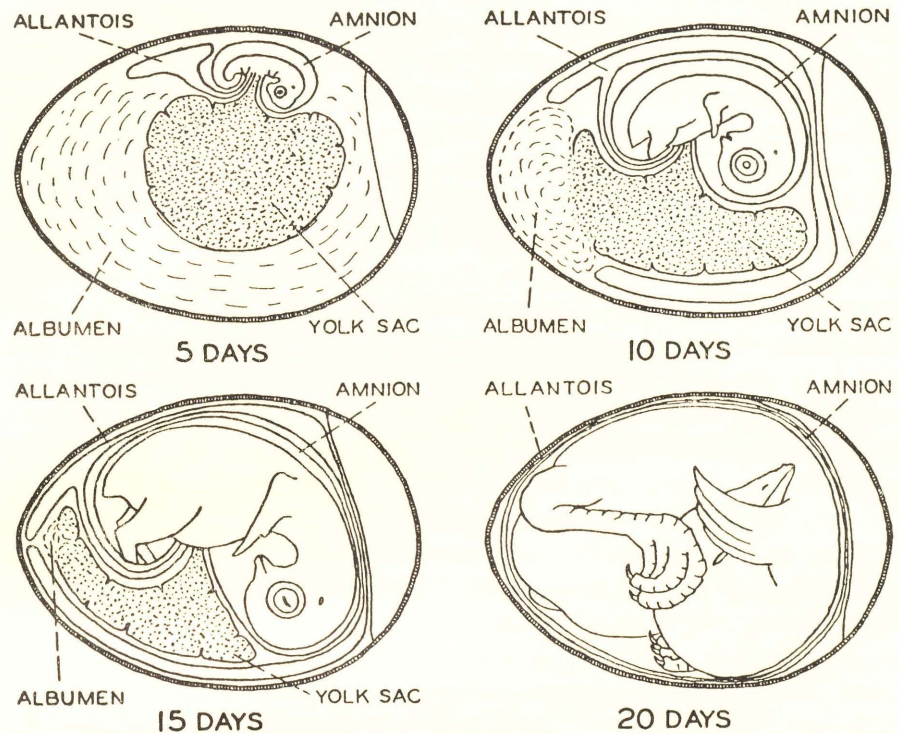


Bone formation and growth can be studied by clearing and staining the embryo. This process requires advanced and exacting techniques, considerable time and equipment, and some financial outlay. Therefore, clearing and staining of embryos should be at-

tempted only by the advanced student under the supervision of an experienced person.

If you plan to study the different stages of embryonic development by one or more of the above methods, be sure to incubate enough eggs for this pur-

Successive changes in the position of the chick embryo and its embryonic membranes. (From A. L. Romanoff, Cornell Rural School Leaflet, September, 1939.) (Fig. 9)



**IMPORTANT EVENTS IN EMBRYONIC DEVELOPMENT**  
(From A. L. Romanoff — Cornell Rural School Leaflet, September 1939.)

**BEFORE EGG LAYING**

- Fertilization
- Division and growth of living cells
- Segregation of cells into groups of special function

**BETWEEN LAYING AND INCUBATION**

- No growth; stage of inactive embryonic life

**DURING INCUBATION**

**FIRST DAY:**

- 16 hours — First sign of resemblance to a chick embryo
- 18 hours — Appearance of alimentary tract
- 20 hours — Appearance of vertebral column
- 21 hours — Beginning of formation of nervous system
- 22 hours — Beginning of formation of head
- 23 hours — Appearance of blood islands — vitelline circulation
- 24 hours — Beginning of formation of eye

**SECOND DAY:**

- 25 hours — Beginning of formation of heart
- 35 hours — Beginning of formation of ear
- 42 hours — Heart begins to beat

**THIRD DAY:**

- 50 hours — Beginning of formation of amnion

- 60 hours — Beginning of formation of nose
- 62 hours — Beginning of formation of legs
- 64 hours — Beginning of formation of wings
- 70 hours — Beginning of formation of allantois

**FOURTH DAY:** Beginning of formation of tongue

**FIFTH DAY:** Beginning of formation of reproductive organs and differentiation of sex

**SIXTH DAY:** Beginning of formation of beak and egg-tooth

**EIGHTH DAY:** Beginning of formation of feathers

**TENTH DAY:** Beginning of hardening of beak

**THIRTEENTH DAY:** Appearance of scales and claws

**FOURTEENTH DAY:** Embryo turns its head toward the blunt end of egg

**SIXTEENTH DAY:** Scales, claws, and beak becoming firm and horny

**SEVENTEENTH DAY:** Beak turns toward air cell

**NINETEENTH DAY:** Yolk sac begins to enter body cavity

**TWENTIETH DAY:** Yolk sac completely drawn into body cavity; embryo occupies practically all the space within the egg except the air cell

**TWENTY-FIRST DAY:** Hatching of chick



pose. The stages of embryonic development are listed in the Table on page 13 (see also Figs. 9 and 10). You will not be able to see all of these with the naked eye. But just by breaking and observing an egg each day you can identify many of the stages.

**Preserving embryos.** You can either break open an egg and preserve the embryo each day, or you can set eggs on succeeding days and do them all at one time. Most students prefer to do one egg each day, because doing all stages at one time will require several hours.

Break the egg gently in the region of the air cell, which is at the large end of the egg. Use blunt tweezers to peel away the upper portion of the shell and shell membranes and to separate the embryo from the remainder of the egg. Remove all extraembryonic membranes, and with small scissors sever the umbilical stalk near the body wall. Wash the embryo gently and thoroughly in running tap water to insure a nice, clean appearance. Be careful not to lose the embryo, especially those in the early stages of development.

If you want to make close-up pictures or slides, place the embryo in a small petri dish or similar container. Then to preserve the embryo, place it in a 10

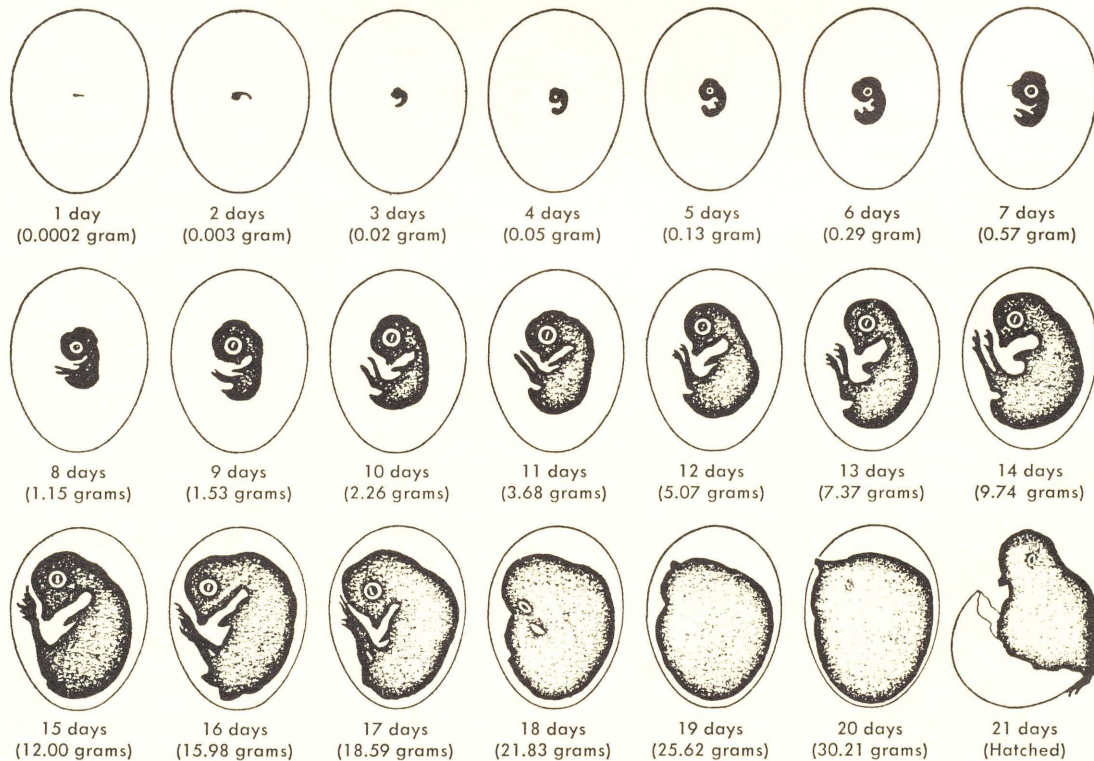
percent solution of formalin (1 part of 37 percent formaldehyde with 9 parts of water) in glass jars with screw caps. Small baby food jars are excellent for this purpose. Use a gallon jug or similar container to prepare the formalin — never use a metal container.

Label each jar with pertinent information. This might include species, variety, age of embryo, date, and similar information.

**Preparing whole mounts.** Early embryonic development between zero and four days of incubation can be studied by preparing whole mounts of embryos and examining them under a microscope. This is a difficult procedure, so you will need to arrange with an experienced person for guidance and counsel.

The first step in preparing whole mounts is fixing the embryo, that is, preserving the actual form of the embryo and producing optical differences in its structure or making it possible to produce these differences in subsequent treatment.

The chick embryo, albumen, and yolk must be removed from the shell and the embryo then separated from the albumen and yolk before the embryo can be fixed. Since the early stages of embryonic develop-



Daily changes in the weight and form of the developing chick embryo — in this case White Leghorn. (From A. L. Romanoff, Cornell Rural School Leaflet, September, 1939.) (Fig. 10)



ment are the most difficult to work with, you should start with a 4- or 5-day-old embryo and work back to the 1-day embryo.

Advanced students who want to prepare whole mounts of embryos can find the procedures in such works as *Animal Micrology*, *Introduction to Vertebrate Embryology*, and *Handbook of Microscopical Technique* (see the reference list on page 16).

**Clearing and staining embryos.** Bone formation and growth in chick embryos can be studied by clearing and staining the embryos. First the embryo must be fixed. Then it must be cleared, that is, made more transparent. Finally it is stained with a color such as

alizarin red S, so that the skeletal structure can be readily seen.

As is true of preparing whole mounts, clearing and staining requires advanced techniques. So it should not be attempted except by the advanced student under the supervision of a qualified person. Procedures for clearing and staining embryos can be found in such references as *Introduction to Vertebrate Embryology*, *Staining the Skeleton of Cleared Embryos with Alizarin Red S*, *Alizarin Red S and Toluidine Blue for Differentiating Adult or Embryonic Bone and Cartilage*, and *Bone Development in the Chick Embryo*. (See the reference list.)

## COLORING CHICK EMBRYOS

Coloring embryos by injecting dye into eggs before they hatch has been practiced for a number of years. It is done to identify the young of certain hatches or groups. And it makes it easier to observe movements of wild birds (especially water fowl) after they leave the nests.

The process of coloring chicks by injecting dye into the eggs also provides an opportunity to study early feather growth. Juvenile plumage will replace the colored down in about two weeks. As this happens, the dyed background amid new growing feathers provides a constantly changing pattern.

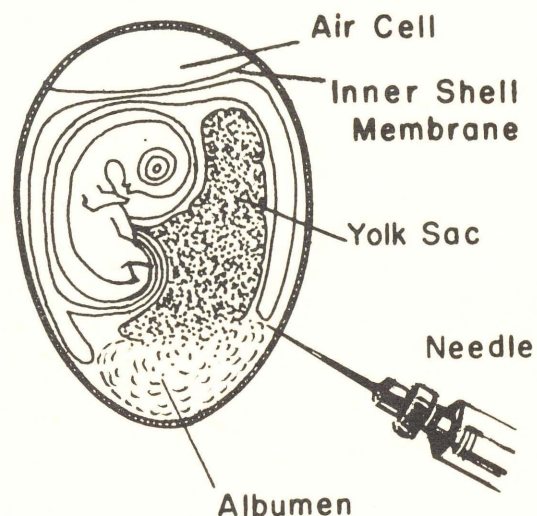
While it is possible to inject eggs from about the 10th to 19th days of incubation, the period from the 11th to 14th days appears to be ideal. Only one treatment is necessary if the injection is done at this time. When injections are made after the 14th day the color usually remains localized because the embryo occupies most of the egg; so it may be necessary to inject the egg in more than one place.

Harmless vegetable dyes, such as food coloring dyes sold in grocery stores, work very satisfactorily. Most of these dyes are sold in concentrations of 2 percent and 3 percent solution and are suitable for direct injection into the egg. No further dilution or sterilization is necessary. Dyeing by injection of the egg will not affect the chick's health, appetite, or growth.

Different colors of dyes can be used. Red, green, and blue usually give the clearest and most distinct results. Yellow and orange vary only in intensity and

do not give sharp differences. Different colors can be blended from the primary colors. Best results can be obtained by using naturally white chicks.

When you are ready to make the injections, remove the eggs from the incubator, candle them, and discard any infertile ones or those which contain dead embryos. Work with only as many eggs as you can complete in one-half hour or less. This will avoid excessive chilling. Wipe the small end of each egg with a 95 percent alcohol solution to sterilize the area where the shell will be punctured. Tincture of iodine (2 percent) or merthiolate can be used in place of alcohol. Allow the eggs to dry.



Cross section of an 11-day old embryo showing the site of injection. (Fig. 11)



At a point about one-half inch from the small end of the egg (see Fig. 11), make a small hole in the shell with a sharp probe, such as a dissecting needle or large hypodermic needle. The hole should be just large enough for the hypodermic needle you use to inject the dye.

Inject about 0.2 to 0.5 cc of dye into the egg with a sterile hypodermic needle and syringe. Use a 22 to 27 gauge needle,  $\frac{3}{8}$  to 1 inch long. The amount of dye injected will affect the degree of coloring somewhat. Release the dye gently and slowly (to prevent overflow) just beneath the inner shell membrane. To avoid injury to the embryo, be careful not to penetrate too deeply.

Close the hole in the shell with a drop of melted paraffin, fast-drying cement, collodion, or a piece of plastic tape cut from a Band Aid. Return the eggs to the incubator. If you handle the eggs carefully and use clean techniques, they should hatch in the normal time and rate for the conditions under which you incubate them.

If you wish to do additional reading on coloring embryos, a good reference is "A Method of Color Marking Young Waterfowl," by C. D. Evans, *Journal of Wildlife Management*, Volume 15, No. 1 (pages 101-103), 1951.

## ADDITIONAL REFERENCES

If you wish to go deeper into the study of incubation and embryonic development, the publications listed below provide both technical and non-technical information. Some of these references should be available in your local public, school, or college libraries.

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Evans, C. D. A Method of Color Marking Young Waterfowl. *Journal of Wildlife*, Vol. 15, No. 1, 1951. (Highly recommended.)

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Williams, T. W., Jr. Alizarin Red S and Toluidine Blue for Differentiating Adult or Embryonic Bone and Cartilage. *Stain Technology*, Vol. 16, No. 1, 1941. (Recommended for advanced students.)

Winter, A. R., and Funk, E. M. *Poultry Science and Practice*. J. B. Lippincott Co., New York, 1960. (A general poultry book with information on fertility, hatchability, and principles of incubation.)