STUDIES IN BURSECTOMIZED AND UNBURSECTOMIZED CHICKENS

by

MARY WOODYARD

A. B., Oklahoma City University, 1929

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

KANSAS STATE COLLEGE

OF AGRICULTURE AND APPLIED SCIENCE

1931

TABLE OF CONTENTS

STUDIES IN BURSECTOMIZED AND THYMECTOMIZED CHICKENS

INTRODUCTION

An analysis of the results obtained by Ackert and Morris (1939) indicated that thymectomized pullets were slower in maturing than normal. Riddle and his associates inferred that the bursa is allied to the thymus. Since Ackert and Morris bursectomized some mature pullets it seemed desirable to study the effect of bursectomy and thymectomy on younger chickens, noting especially the effect upon the blood picture, the growth of the chickens, the age of sexual maturity, egg weights, egg shell strength, and the relative weights of the different glands.

REVIEW OF LITERATURE

Literature on the Bursa

Fatavii (1821) "De Formatione Ovi et Pulli" described the bursa as a blind sac lying behind the uterus of the fowl, but emptying into the cloaca close to its external orifice.

The early belief of the function was that this was the place into which the cock injected his semen, and forced it

in so that it was kept there. Harvey and DeGraaf showed its presence in both sexes and so disproved the belief.

Markow (1829) showed while the bursa is developed in young birds, it is absent in the old birds. Huske (1838) described its development, showing that it arises in the superior part of the cloaca. It is differentiated in the embryo of the fowl from the eighth to the ninth day of incubation, acquiring by degrees a more perfect form but that after a time it increases but slowly in comparison with other parts of the embryo.

Beginning with the middle of the nineteenth century the bursa was subjected to microscopic examination and thereafter repeatedly studied, one group of investigators (Leydeg and his successors) holding that it was purely of lymphoid origin and another group (Stieda and his school) maintaining on embryological grounds that it was primarily a glandular organ.

Following Kolliker's description of the epithelial origin of the thymus in 1879, these views were partially reconciled but gave rise to the new discussion as to whether the epithelial primordium is replaced by invading tissue or whether it is itself transformed into a reticulum containing lymphocytes. Most authors since Wenckebach (1906) have

held that the epithelium undergoes transformation without invasion.

Forbes in 1877 investigated the bursa of ninety species of birds to see if there were characteristic structures of this organ to be used in classification of birds. This work was done for Professor Garrod. In this work Forbes concluded the following:

1. That the bursa Fabricii exists in both sexes and probably in all species of birds.

2. That it is most developed in the young birds but becomes atrophied and more or less obliterated in adults, the period, however, of the commencement and the conclusion of this process differing greatly in various birds. In some it persists through a state of functional inactivity throughout life.

3. That the bursa is a glandular organ, of which lymphatic follicles are the essential constituents, but has no exact homologue in other classes of vertebrates.

Jolly (1914-15) found that there were produced in the bursa structures like the Hassels corpuscles in the thymus. In his work Jolly found that the maximum development of the bursa is attained at the time when spermatogenesis is beginning, and that the involution of the bursa corresponds

exactly with the appearance of sexual maturity as measured by the sudden increase of testicular weight and the appearance of the ripe spermatozoa. In writing of the bursa Jolly gives the following: "The bursa Fabricii is a transitory organ which disappears at the time of sexual maturity. Its involution precedes that of the thymus. The phenomena which one observes during the physiological involution show the structure of these follicles. The lymphocytes disappear at first; the cortical substance atrophies progressively. In the medullary substance the lymphocytes die and the epithelial frame-work appears in a homogeneous state. The epithelial cells compact themselves and tend to reconstruct an epithelial bud as compact as in the beginning of histogenesis. This phenomenon shows that the process is not one of a tissular transformation but one of functional adaptation, association, and symbiosis. From this moment, which occurs more or less shortly after the maturation, the follicles cease to be separated from their original base of implantation by the progressive atrophy of the fibers of the organ. In the follicles thus separated and atrophied one recognizes the bordering epithelial zone, which permits one to distinguish these special follicles of the lymphoid type from those nearly purely mesodermic. It

is not rare to see the medullary substance transformed into cystic epithelium. The follicles disappear in that portion which is more atrophied and more fibrous, although one still finds some remaining lymphoid tissue which follows the direction of the vessels."*

Jolly also produced involution of the bursa from the action of X-rays and by starvation. In the use of X-rays Jolly found that the process was similar to that of the physiological involution but was much more rapid. The experimental involution produced by starvation Jolly found was not permanent for upon feeding the chickens the lymphocytes in the follicles gradually came back to normal. Thus this involution of the bursa may be compared to that of the thymus and would lead one to believe that they played a part in the exchange of nutrition.

In writing of physiological involution of the bursa Fabricii Jolly states as follows: "In the physiological involution of the bursa Fabricii, the glandular cavity gradually disappears throughout the entire bursa; at the same time the mucus which lines it loses its glandular aspect and seems to resemble the mucus of the cloaca. Finally in the atrophied condition the bursa exists as a blind sac consisting of fibrous trabeculae which bore the glandular fol-

* Translated from Jolly: "La bourse de Fabricuis et les Organes Lymphoepitheleaur."

licles. In the posterior portion of the cloaca lined by cloacal mucus this blind sac seems to persist for a long time--well after the appearance of sexual maturity. In the adult chicken it is not rare to be able to isolate it. This fact coupled with that which one observes at the beginning of development permits one to suppose that the bursa represents an ancestral glandular cloacal caecum in regression (appendix in mammals) but which by a particular adaptation necessitated by a new function has composed itself entirely of epithelial and mesodermal lymphoid tissue."*

Jolly points out that the thymus and the bursa are closely related in their function and structure. The bursa might be considered as a cloacal thymus. He would class them both under the term "lympho-epithelial organs."

Osawa (1911) believed that the bursa is homologous with the prostate gland, even though the latter is well developed in the males only. This theory would be discarded because of the fact that the bursa degenerates at the time when the prostate gland would be functional.

Gadow (1909) believed it to be homologous to the anal sacs (bursa anales) of the turtle.

* Translated from Jolly: "La bourse de Fabricuis et les Organes Lymphoepitheleaux."

Riddle and Tange (1928) tried the effects of removal of the bursa Fabricii in young doves. In this experiment they found, upon bursectomizing 66 young ring-doves, that bursectomy did not change the rate of growth or the normal adult body weight of those birds that lived to maturity, and had no effect on the age at which the females became sexually mature. Riddle, from these results and from the work of Jolly, suggested that only the removal of both the bursa and the thymus in the birds would adequately demonstrate the functions of either organ.

Riddle (1928) brings out the fact that in the common pigeon the bursa Fabricii attains its maximum size at the age of 2.9 months and in the ring-doves in 3.1 months (age being calculated from the beginning of embryonic development). This period of its growth coincides with the rapid growth of the body and of the thymus, and with very slow growth of the gonads. In this work Riddle also found that the involution of the bursa begins, at least in early maturing birds, immediately after the maximum size is attained. At precisely the same time the thymus begins its partial involution the testes and the ovary begin to show a greatly increased rate of growth. Involution of the bursa is usually, but not invariably, practically complete by

the time sexual maturity is attained. Notable sex differences in size, growth, and involution of the bursa are not present.

An intensive review of the work done on the bursa emphasizes its relationship to the period of extreme rapid body growth and to a period of repressed growth in the gonads as well as to the time at which either of the sexes attains the capacity to reproduce. These data furnish some indication that the organ has an endocrine function. Also that the bursa is closely associated with the thymus and that these two organs may have identical functions.

Literature on the Thymus

The thymus gland has been studied by many workers, not only in birds but other animals. Birds have been used more than other experimental animals because there are fewer complications encountered, that is, there are no bones, muscles, or pleura in the region of the thymus glands.

Tarullo and Lo Monaco (1897) performed total thymectomy on young chickens. The heavy losses, however, were probably due to leg weakness (deficiency of vitamine D) rather than the effect of the removal of the thymus.

Fischl (1907),working on the thymus of chickens, concluded that the removal of the thymus was without signifi-

ment effects. Fischl stated that the thymus probably functioned in fetal life.

Soli (1906) found that the removal of the thymus glands from young cocks delayed the development of the testes. In 1910 Soli carried on further investigations with chickens. He found that the removal of the thymus from pullets had an effect upon the calcium metabolism, thus modifying the deposition of calcium in the egg shell.

Basch (1908) found that the removal of the thymus in dogs created a change in the skeleton and a doubling in the calcium excretion. His work on thymectomy in birds was unsuccessful, due to the regeneration of the thymus. This has been explained as probably due to his failure to remove all of the thymus tissue.

Katsura (1922), working on the results of thymectomy in chickens, concluded that the function of these glands was to aid in growth and to retard maturity. He also investigated the relation between the removal of the thymus and the calcium metabolism and found no significant effect on the shell structure and only one per cent difference in the calcium content.

Riddle (1924), working with thymectomized pigeons, found certain changes in the eggs. These were a deficiency

in the shell and albumen, a frequent reduction of normally paired ovulation to single ovulations, a diminished fertility, and a restricted hatchability of the eggs. In addition, birds that had initially shown quite normal reproduction showed abnormalities after thymectomy. He found that complete thymectomy in pigeons was difficult to obtain and that there were many sources of error in evaluating the completeness and the results of the operation. The whole of the data seemed to indicate the presence in thymus of birds of a substance having a highly specific action on the oviduct. This substance is indispensable to the production of normal egg envelopes. It is apparently of the nature of a true hormone.

Riddle and Frey (1925) found that a true involution occurs in apparently all species and races of pigeons. The relationship of disease and other adverse conditions to the thymus size in various pigeons of all ages was considered. They found that the age at which involution occurred was 3.0 months and that, before age, this involution begins, the thymus of the females was as large as that of the males. During the period immediately succeeding the attainment of sexual maturity, the thymus of male pigeons is notably larger than that of the females.

By feeding thymus to normal birds, Krizenecky and Nevalonney (1927) secured an increase in pigmentation or intensity of color in chickens, which they attributed to hyperthymism. By feeding thyroid and thymus, these authors concluded that there is an antagonism between the thymus and thyroid glands, hyperthyroidism tending toward the lessening of pigmentation and hyperthymism stimulating pigmentation. Krizenecky (1930) found a similar thing to be true regarding the antagonistic effect of thymus on thyroid in the weight of pigeons.

Literature on the Blood

Since the beginning of the twentieth century, various workers in different phases of poultry work have taken up the subject of bird blood from the standpoint of physiology, pathology, and embryology.

Warthin (1907) published work on Leukemia of the common fowl. In his work on infected and normal chickens he reported the following results:

	Infected	Normal
Red blood cells per c. mm.	450,000	2,000,000-3,000,000
White blood cells	380,000	12,000-39,000
Small lymphocytes	1.5%	35.5%
Large lymphocytes	84.5%	14.5%

	Infected	Normal
Granular eosinophiles	none	10.0%
Crystalloid eosinophiles	11.5%	21.5%
Degenerating white cells	3.0%	16.5%
Mast cells	.5%	2.0%

It is to be noticed that in normal chickens there are about 105 to 225 red cells to one white cell, while in the infected chickens the proportion is less than two to one.

In this work, Warthin considered Toisson's solution as satisfactory for the dilution fluid. Haematoxylin and eosin, Ehrlich's triple stain, and Wright's stain were used to make the smears. Warthin considered that Wright's stain gave the best results. The percentages of different white cells were made from the stained smears rather than from the counting chamber.

Schmeisser (1915) worked on Leucosis in fowls. He reported white cells as 30,000 to 80,000 per cubic millimeter. He classified them as follows:

Lymphocytes	42.3%
Polymorphonuclear cells with eosinophilic rods	39.0%
Polymorphonuclear cells with eosinophilic granules	4.3%
Large mononuclears	13.4%

Mast cells	3.2%
Unclassified cells	3.2%

In this research Schmeisser used Wright's stain. He also reports the number of red cells as 3,000,000 to 4,000,000. This made the proportion of red cells to the white as much as one to 50 or one to 150.

Ellerman and Velhelm published work on Leucosis in 1921 under the title, "The Leucosis of Fowls and Leucaemia Problems."

In 1921 Sabin and Simpson introduced the supervital method of blood counts. This was more satisfactory because it caused much less injury to the cells and consequently eliminated the uncertain group previously classified as degenerating cells and allowed the assortment of the cells of that group into their proper classification. It likewise permitted a much finer demarcation between the lymphocytes and the monocytes.

In 1923 Jolly published a work entitled "Traite technique d'hematologue."

Maximow and Aschoff (1924) published separate papers on the origin of the blood cells.

Hedson and Knight (1924), working on anemia in hens, found the results given in Table I.

Table I*

Case	Total Count	Erythrocytes	Abnormal Red Cells	Leucocytes	Polymorphonucleocytes	Monocytes	Thrombocytes
I	1,194,600	872,000	274,000	4,800		100.0%	
Normal	3,096,049	3,000,000		33,777	38.7%	61.3%	55,272
II	634,728	482,832	33,072	145,644	16.0%	84.0%	3,180
III	844,288	697,440	36,240	100,424	34.6%	65.4%	11,984
IV	877,640	587,000	46,484	170,546	14.0%	86.0%	83,600
V	1,272,000	1,139,536	104,304	36,160	16.6%	83.4%	none
VI	1,190,000	1,050,300	57,820	57,820	11.1%	88.9%	14,160

*Results of work of Bedson and Knight. Journal of Pathology and Bacteriology. Vol. 27, 1924. Page 236.

Doan, Cunningham, and Sabin (1925) worked on the origin and maturation of blood. They concluded that there is a system of collapsed capillaries in the bone marrow and that new blood cell formation occurs inside these capillaries.

J. P. McGowan (1926) published a book on pernicious anemia. After the examination of the bone marrow of anemic chickens, he concluded that, under the condition of great stimulation, there is a return to intravascular formation of red blood cells such as occurs in the embryo. In 1928 McGowan published a treatise, "On Rous, Leucotic, and Allied Tumors in Fowl." Chapter IV of this work is devoted to normal and infected blood.

Daniel Blain, of the Department of Anatomy, Vanderbilt University School of Medicine, wrote papers on "A Study of White Blood Cells of the Normal Fowl by the Supervital Technique" (1928). The blood work was carried on in connection with his work on tuberculosis of chickens. In his investigations on normal chickens, Blain found the results as given in Table II. A little later M. Coates (1929) reputed a new dilution fluid.

Three papers of importance to the worker in bird blood were published in 1929: H. P. Bayon, "The Pathology of Anemia in Fowl"; Forkner, of Rockefeller Institute for Medical

Table II*

Number of Chicken	Total White Blood Count	Differential Counts				
		Polymorphonuclear Cells			Lymphocytes	Monocytes
		Eosinophilic Rods	Eosinophilic Granules	Basophilic Granules		
231	20,532	58.5	10.0	4.5	22.7	10.3
232	10,600	53.5	8.1	4.4	29.9	4.1
233	18,400	35.5	13.5	8.3	39.7	3.2
234	10,130	47.3	8.0	1.0	33.6	10.0
235	29,460	43.5	8.3	2.1	44.1	3.0
236	23,660	64.5	4.1	1.5	26.6	3.3

*From Blain.

Research, "Blood and Bone Marrow Cells"; Keyes, "The Normal Leucocyte Content of Bird Blood."

Bayon found that, in healthy fowls, the total white blood cells per cubic millimeter may vary between the limits of 15,000 to 25,000, counting erythrocytes and leucocytes in the same graduated chamber in a one to 200 dilution of Toisson's fluid. Confirmation of this report was obtained by examination of dry films where one leucocyte of lymphocyte type was seen to every 125 to 150 cells counted. Bayon found that in various morbid conditions of blood forming tissues of fowls, this proportion is disturbed, owing to a decrease in the number of erythrocytes and an increase in the number of leucocyte-like cells. Proportions of one to 50 or even less may be observed, corresponding to a total number of leucocytes of at least 100,000 per cubic millimeter. Bayon classified these cells as follows:

	Healthy Fowl	Erythromyelosis
Total Cells	20,000	100,000
Immature erythrocytes,	a few	76,000
Lymphocytes	8,000 to 12,000	12,000
Polynuclear	6,000 to 8,000	10,000
Monocytes	2,500	1,500
Undefined	800	500

Forkner in his work found the following results:

Total number of polymorphonuclear cells and monocytes in 0.9 c. mm.	7200
Total number of polymorphonuclear cells and monocytes in 1 c. mm.	8000
Differential leucocyte count	
Polymorphonuclear eosinophiles	50.0%
" neutrophiles	4.0%
" basophiles	2.0%
Monocytes	10.0%
Total polymorphonuclear cells and monocytes	66.0%
Small lymphocytes	30.0%
Large lymphocytes	4.0%
Total	100.0%

Therefore, 66 per cent of the total count, 8,000 cells

100 " " " " "

12,121 cells

The difficulties in direct method of counting as found by Forkner were that the blood cells were nucleated, the thrombocytes were difficult to distinguish, and the rapidity with which coagulation occurred.

Preston Keyes (1929), in his work on normal leucocyte content of bird blood, summarizes the leucocyte counts of

various workers as follows:

Hagem	36,300 per c. mm.
Moore	30,800 " "
Albitone and Kaszona	33,300 " "
Barthin	12,000-28,000 per c. mm.
Ward	36,186 per c. mm.
Burnett	17,931 " "
Mack	33,777 " "

The number of leucocytes, reported by Keyes, contained in blood of normal pigeon and in that of the domestic fowl is 8,000 to 13,000 per cubic millimeter rather than being double that of mammals (mammal leucocytes, 3,000 to 15,000).

Shaw in 1931 published a paper on a new method of making supervital counts. In this method Shaw combines the methods of previous workers, thus using both the crystal violet and the neutral red.

Little work has been done on the hemoglobin content of the blood of chickens. One of the earliest workers was Preger (1871) who by the use of the iron determination found the following results:

10.1 grams Fe per 1,000

16.75 grams Fe per 1,000

Muller (1886), by the use of the spectroscopic method, found that a very young chick had a hemoglobin content of 8.91 grams per 100 cubic centimeters.

Schmeisser (1913), by using the Sahli method of determining hemoglobin, found that the average for fowl blood to be between 60 and 70 per cent.

Fritsch (1930), by the use of the spectrophotometer, found the following results:

12.3 grams per 100 c. c. in the cocks

9.6 grams per 100 c. c. in the hens

Hart, Kivehyen, Kemmer, and Kelpin (1930) found in the chickens six to seven grams per 100 cubic centimeters.

Dukes and Schwartz (1931) found in White Leghorn pullets 11 .4±0.7 (uncorrected) or 8.9±0.7 in cockerels 16.4 ±0.2 (uncorrected) or 13.4 0.3 (corrected). They also found that the sex differences were slight and inconstant.

METHODS AND ANATOMY

General Methods

The chickens used in this experiment were purebred single-comb White Leghorns. They were bought as day old chicks by the station from a commercial hatchery and were raised under a brooder. They were raised in confinement

on a natural adequate diet.

The diet is similar to the one used by Merrick, Ackert and Dunbine (1923) which they showed to be adequate. The mash was made up on the following formula:

Yellow corn meal	48.24%
Powdered skimmed milk	7.4%
Meat Meal	13.03%
Oatena	13.87%
Cracked wheat	13.87%
Leaf meal	4.62%
Cod liver oil	1.95%

The chickens had the mash available at all times; the same was true of water and grit. When the age of sexual maturity approached, oyster shell was placed in the pens.

The chickens were kept in indoor pens about 12 feet by 20 feet by 10 feet. The floors were cleaned regularly and covered with shavings. The temperature was kept fairly constant, since the rooms were well ventilated and heated.

Anatomy of the Bursa Fabricii

Kingsley in his text (Comparative Anatomy of Vertebrates) writes of the bursa Fabricii as follows: "Many birds have a pocket, the bursa Fabricii, of unknown func-

tions developed from the dorsal part of the cloaca. It arises from the entodermal portion and extends forward, dorsal to the rectum, later connecting with the ectodermal part of the cloaca. In some cases it degenerates in the adult."

Knapp in his text (Anatomy of Domestic Fowls) makes the following statement:

"On the dorsal wall of the cloaca, between it and the spine, is a small sac called the bursa of Fabricius, which has a duct communicating with the cloaca. The mucous membrane of this sac is thrown into folds and is studded with glands. The bursa Fabricius is larger in young than in the adult birds. It apparently atrophies as the birds become older. When the birds are four months old the bursa is best developed and at this age it may be as much as two to three centimeters in diameter."

Jolly (1914) states as follows:

"The bursa of Fabricius is made up of a diverticulum from the back of the cloaca lined by mucus of cylindrical epithelium, containing a number of lymphoid follicles which have been reported to be continuous with this epithelium."

Method of Bursectomy

At the age of about six weeks the chickens were bursectomized. The wings and the feet were tied and the chicken tied to the board. A general anesthetic (ether) was used. Two people were necessary for the operation, one for the anesthetic and one for the operating. A horizontal slit was made through the skin and the body wall just above the anus. The bursa was sufficiently freed from the tissue by the use of blunt dissection so that hemostatic forceps could be set on it. The bursa was then freed, all except where it is united with the posterio-dorsal wall of the cloaca. It was then drawn out of the body cavity, the connection tied and cut. The incision was sewed with absorbable cat gut. The wound was then greased well with vaseline. No infection was noticed after the operation, probably due to the high native resistance of chickens. The chickens were kept in small individual pens for 12 or more hours until the effect of the anesthetic had worn off and the shock of the operation had passed. They were then placed in the large pen, along with the controls.

PLATE I

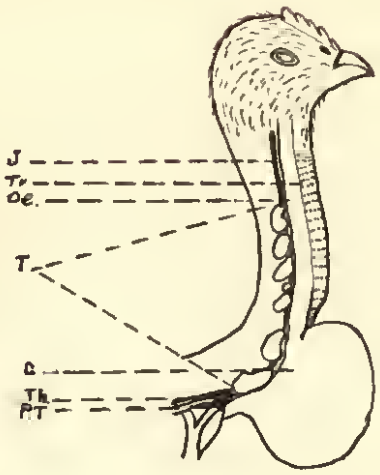

Fig 1

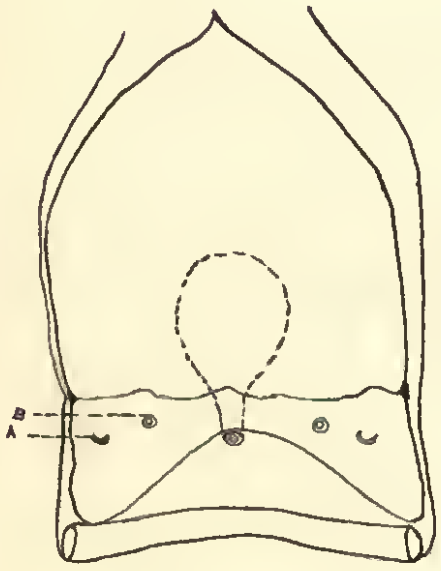

Fig 2

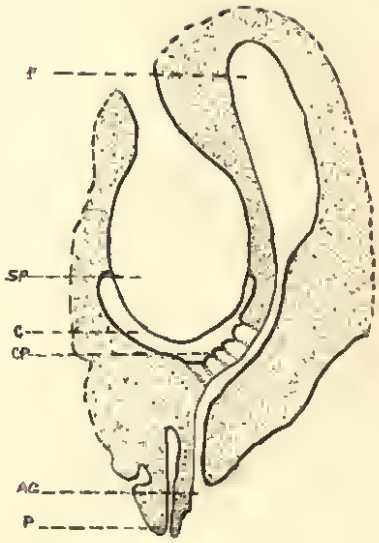

Fig 3

The Anatomy of the Thymus Gland

The thymus gland arises on either side from the mucous membrane to the pharynx, as a proliferation of the epithelium of the gill clefts. It is impossible to state with certainty whether it was originally a glandular, that is, secretory organ, or whether it consists of material that was at one time designed for the formation of gill-filaments.

In post-embryonic development the organ always shows a lymphoid structure, and on account of its richness in white blood corpuscles certainly has important physiological relations to the organ as a whole.

Method of Thymectomy

The chickens were thymectomized at the age of about six weeks. They were anesthetized with ether and operated. A medio-ventral incision was made. This exposed the trachea and the crop and allowed all the lobes of the gland to be easily seen. The lobes of the thymus were removed from the fascia which surrounded them by blunt dissection, a small curved scalpel and toothed forceps being used. Care was taken not to cause hemorrhage when each lobe was sepa-

PLATE II

Plate II

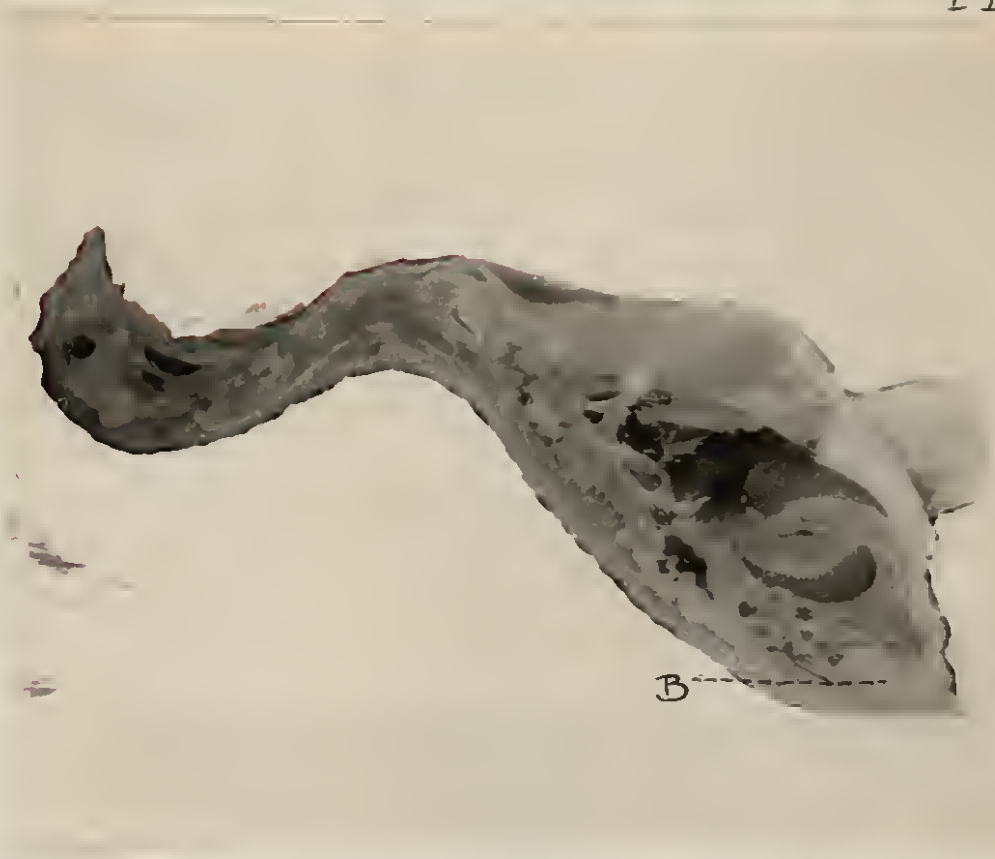

Fig. 1

Fig. 2.

rated from the blood vessels. In removing the fascia, there is danger of injury to the jugular vein. Much of the fat surrounding the glands was taken out to be sure that all of the glandular tissue was removed.

Anatomy of the Blood

The blood cells of the chicken are classified in the following manner:

The red blood corpuscles, which are nucleated, are larger than those of man (chicken 7.5 micra by 13 micra). The nucleus is usually slightly irregular and oval in shape and is smaller than that of the small lymphocytes.

The thrombocytes, which are also nucleated, are the size of small or intermediate lymphocytes. These cells are smaller than erythrocytes.

Polymorphonuclear leucocytes with eosinophilic rods are from 8.to 12 micra in diameter. The lobulated nucleus is filled with a reticular chromatin network. The cytoplasm is filled with rod shaped, specific granules which stain red with eosin.

Polymorphonuclear leucocytes with eosinophilic granules differ from those with eosinophilic rods, in that the rods are in the shape of granules and the nucleus is bilobed.

The basophiles or mast cells have small round granules which are uniform in size and stain a brick-red color in neutral red or deep blue in Wright's. The nuclei are less polymorphic than those of the cells with eosinophilic rods. They often appear as a single mass with slight and irregular indentations like the eosinophilic cells.

The monocytes are 8 to 10 micra in diameter. The nuclei are usually either oval or tend toward a horseshoe shape with but slight indentations.

With the exception of the monocytes all of the mononuclear cells of the chicken blood are characteristic of the lymphocyte group. The diameter of the lymphocytes varies from 3 to 12 micra and are usually classified as large and small lymphocytes.

In comparing chicken blood with that of mammals it was found that the principal differences between them were the absence of a structure which could be identified as corresponding to the platelets of mammals and the presence of a nucleated erythrocytes.

Methods of Blood Counting

In making a total blood count, the standard dilution pipettes and the Levy counting chamber with Neubaur ruling

were used. In most of the work a standard Leitz microscope was used.

The feathers were removed from the under part of the wing, in the region of the joint of the ulna and the humerus. The skin was then washed with 70 per cent alcohol. The ulnar artery was cut by use of a needle with a flattened point. The first drop of blood was wiped away. The dilution pipette, which had been washed in 70 per cent alcohol, then in ether and dried, was placed in the drop of blood, and the blood drawn to the 0.5 mark. The pipette was placed in the dilution fluid and this was drawn in to the 101 mark (or to the 11 mark according to the pipette used). The blood should be drawn into the pipette quickly from a freely flowing drop of blood, as fowl blood clots very rapidly. If the red dilution pipette was used the dilution was one to 200, if the white dilution pipette was used the dilution was one to 20. After shaking according to the directions for the different fluids, the diluted blood was placed in the Levy counting chamber and allowed to settle for several minutes. Fifty of the small squares were counted and this multiplied by eight to obtain the number in a square millimeter. As the column of fluid was 1/10 millimeter deep, to obtain the number in a cubic millimeter the result was multiplied by 10. Since the dilution was one to

200 (or one to 20) the result must also be multiplied by this factor thus:

Number in 80 small squares times 8 times 10 times 200 (or 20) equals the number of cells in one cubic millimeter.

Various methods suggested by different workers were tried. The common mammalian method, which consists of a dilution fluid of one per cent acetic acid, embodies the principle of the destruction of the red blood cells in order that the white cells may be counted without confusion. In avian blood, this is not satisfactory because the circulating red cells are nucleated, and while acetic acid solution hemolyses the red cells it does not destroy the nuclei, and these laked red cells are then confused with the white cells.

Keyes' method, using two per cent osmic acid at 43 degrees centigrade with the pipettes at the same temperature, is capable of rapid cell fixation. At the above temperature, no hemolysis occurs and the complicating stromata are absent.

Forkner (1928) employed the following method for counting the white cells: 25 milligrams of neutral red were dissolved in 100 cubic centimeters of 0.9 per cent sodium chloride solution and the solution filtered and kept at room temperature. The ordinary red blood pipettes were

used, the blood was drawn to the 0.5 mark, and the pipette filled to the 101 mark with the dilution fluid. The pipette was shaken for four or five minutes.

Hayem (1939), in his work, used Toisson's solution, which is made as follows:

Distilled water	160 c. c.
Glycerin neutral	30 c. c.
Sodium sulfate	8 gms.
Sodium chloride	1 gm.
Methyl violet	0.025 gms.

Dr. Ida Gentes (1939) reported the following method for making solutions but the same method was given in McClung as the Wright and Kinnicutt's method. This method involves two solutions:

Solution I

Brilliant cresyl blue	1.0 gms.
Distilled water	300 c. c.

This solution must be kept in the refrigerator to prevent the growth of yeast.

Solution II

Potassium cyanide	1.0 gms.
Distilled water	1,400 c. c.

The solutions are kept separate and are mixed immediately before using. The remainder of the procedure is exactly

that of an ordinary whole count.

Herrick (1933), in his work on blood counts and their relation to parasitism, used Hayem solution, that is

Distilled water	200 c. c.
Sodium chloride	1 gm.
Sodium sulfate	5 gms.
Mercuric chloride	0.5 gms.

In the first experiment, it was found that Klein's method gave the best results. Two solutions were used:

Solution I

Neutral red (one to 5,000) made up in Locke's solution and adjusted to a pH of 7.4.

Solution II

12 per cent formalin, also made up in Locke's solution and adjusted to a pH of 7.4.

Locke's solution is made as follows:

Sodium chloride	0.9%
Calcium chloride	0.025%
Potassium chloride	0.042%
Sodium acid carbonate	0.02%
Dextrose	0.1%
Distilled water to make	100%

The blood was drawn up to the 0.5 mark in a standard red blood pipette. The solution of neutral red (which was kept

at a temperature of 39 degrees centigrade) was drawn into a pipette until the bulb was half filled, and the pipette shaken about 15 minutes. The pipette was then filled to the 101 mark with the formalin solution and the shaking continued for two or three minutes. The counting chamber was then filled.

In the counts made during 1930-31 in the second and third experiments, the method reported by Shaw (1930) was used. The dilution fluid was made up of two solutions:

A.

Neutral red (Grubler)	25.0 mgms.
Sodium chloride	9 gm.
Distilled water	100 c. c.

B

Crystal violet (Grubler)	12.0 mgms.
Sodium citrate	3.8 gms.
Formaldehyde	0.4 c. c.
Distilled water	100 c. c.

Shaw directed to make these up fresh each time but instead the following modification was found to give the best results:

A_1

Neutral red	25 mgms.
Distilled water	50 c. c.

A_2

Sodium chloride	0.9 gm.
Distilled water	50 c. c.

B_1

Crystal violet	12 mgms.
Distilled water	25 c. c.

B_2

Sodium citrate	3.8 gms.
Distilled water	50 c. c.

B_3

Formaldehyde	4 c. c.
Distilled water	35 c. c.

These were mixed in proportion to the amount of water, that is, equal parts in the first, and in the second one part B_1, two parts B_2, and one part B_3. Both solutions were filtered through No. 42 Whatman filter paper immediately before using and then heated to about 10 degrees F. (clinical temperature). The warmed counting tube was filled to the 0.5 mark with blood. Solution A was drawn up to fill about half of the mixing chamber and was immediately followed by solution B. The pipette was shaken for five minutes.

When the chicken blood was mixed with these solutions under the above conditions, differential staining took place as follows:

"Erythrocytes, cytoplasm unstained, that is, pale yellow; nucleus a faint blue; in a small number of erythrocytes deficient in hemoglobin the nucleus stains more deeply and the cytoplasm is hyaline and colorless; granular leucocytes (polymorphs, eosinophiles, basophiles) round or ovoid, coarse, and refractile, with a sharp dark border; stain a deep red; lymphocytes round or ovoid, less refractile than the granular cells; border sharp and dark; nucleus purple; cytoplasm sky blue; monocytes similar to lymphocytes but larger and more irregular in shape; thrombocytes elliptical, translucent, and hyline; stain a pale bluish green without sharp differentiation of the nucleus and the cytoplasm; at one or both poles are one or more granules staining bright red; the colorless polar vacuole characteristic of these cells in fixed films may also be seen; some of the thrombocytes are refractile, owing to injury when the blood is shed, but apart from some shrinking in transverse diameter they retain their characters sufficiently to permit differentiation from the lymphocytes."*

* From Shaw (1930).

EXPERIMENTS

Experiment I

The chickens in this experiment were secured from a commercial hatchery November 16, 1929. They were single-comb White Leghorns and Rhode Island Reds.

At the age of six weeks the chickens were operated: 11 White Leghorns and three Rhode Island Reds were bursectomized and thymectomized; 11 Leghorns were bursectomized. Seven White Leghorns and three Rhode Island Reds were used as controls. The chickens were kept in a common pen, thus insuring like care for all chickens.

The weights were taken every week by means of a balance animal scale. The first weight was taken at the age of six weeks--a short time before the operations were performed. The last weight was taken at the age of six months. At the age of six weeks the average of the females (operated) was 312 grams while the controls averaged 333 grams. Throughout the experiment the controls weighed more than the operated, except that of the 13th week after operations, then they were about the same. At maturity the controls were still about 50 grams heavier. (Figure 2.)

PLATE III

Plate III.

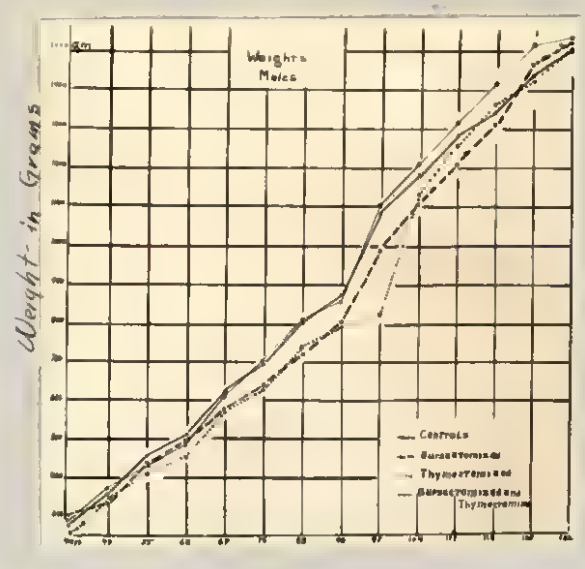

Fig. 1

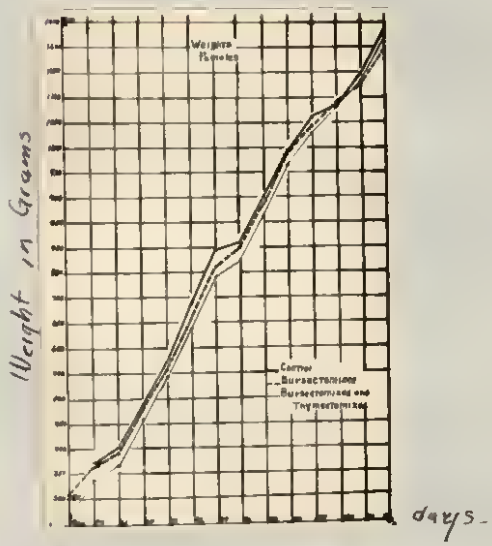

Fig 2.

The cockerels were killed at the age of 17 weeks.

At the time sexual maturity was reached the pullets were separated into small pens, because they would not go into the trap nests. They started to lay when they were 22 weeks old. A bursectomized pullet (No. 4220) was the first to lay, but within three days all of the pullets, both controls and operated, were laying.

Each egg was marked with the number of hen or with the group number; the date and the weight of the egg in grams were also indicated. The eggs were then tested as to shell appearance. This test was made with an ordinary candling apparatus and the eggs recorded as smooth, slightly pitted, pitted, or badly pitted.

The shell strength was tested by placing the egg, small end up, in an apparatus which exerted pressure upon the small end by means of a wooden rod, which supported a receptacle for sand. The sand was poured into this receptacle very slowly (so as to have the time element as nearly constant as possible) until the egg shell was broken by the weight of the sand. The quantity of sand was weighed and the weight in grams was taken as a measure of shell strength. The results of this work are shown in tabular form

	Ave. Egg Wt.	Ave. Shell Strength
Thym- and bursectomized	40	2,740
Bursectomized	41.4	3,178.6
Control	42.7	3,616

The chickens were autopsied at different times. One group of two bursectomized and one control was autopsied about five weeks after sexual maturity. Their conditions were found to be as follows:

In No. 4335 (bursectomized) there was one egg in oviduct. This egg was normal in appearance. The thymus glands were medium in size. The bursa was not regenerated but there was an abundance of scar tissue.

In No. 4381 (bursectomized) the oviduct was poorly developed and the ovary seemed to be degenerated. This condition might have been due to injury during operation. The thymus was very large in size but seemed to be of a normal texture. The bursa was partially regenerated but was of abnormal texture.

A control (No. 4255) was killed and examined. There were many eggs in the oviduct of this chicken and the oviduct seemed to be healthy and normal. The ovaries were well developed. The bursa and the thymus were both degenerated to a great extent.

PLATE IV

Plate IV.

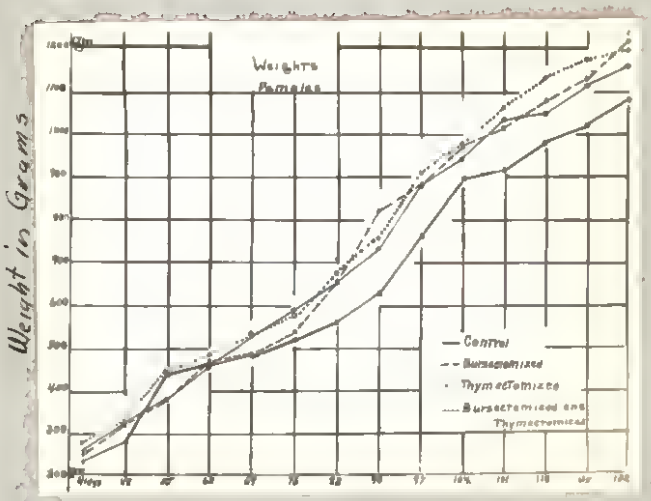

Fig. 1.

The conditions of the other pullets that were killed later are found in the following chart:

Date of Autopsy	Chicken Number	Group	Bursa	Thymus	Other Conditions
6-7-30	4375 RR	control	large	rather small	excellent
6-7-30	4543	thym-bursect.	no trace	no trace	good
6-14-30	4547 RR	control		very large	good
6-14-30	623	bursect.	no trace	very small	good
6-28-30	4313	thym-bursect.	no trace	no trace	good
6-27-30	4302	"	"	"	"
6-27-30	4333	control	degenerated	small	excellent
7-3-30	4304	bursect.	no trace	no trace	very fat
7-5-30	4273	"	"	very small	good
7-25-30	4219	control	"	no trace	good
7-26-30	4230	bursect.	"	"	"

*RR; Rhode Island Red. All others White Leghorns.

Experiment II

The chickens in this experiment were secured from a commercial hatchery on September 19, 1931. They were raised under an electric brooder and given care as described

PLATE V

Plate V.

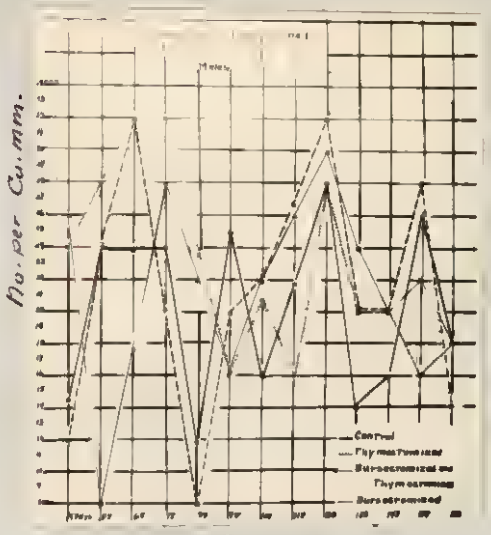

Fig. 1

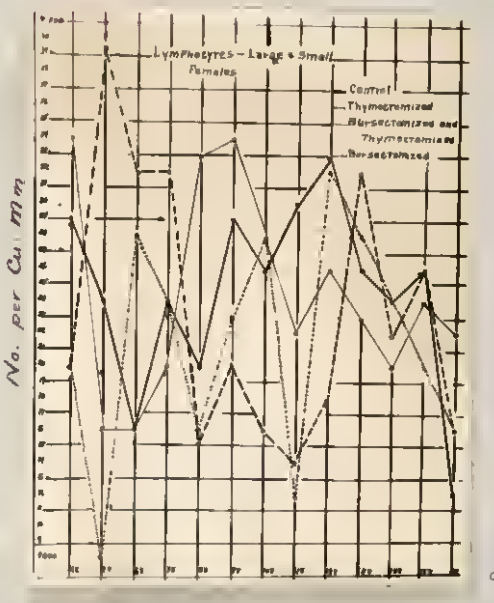

Fig. 2.

PLATE VI

Plate VI.

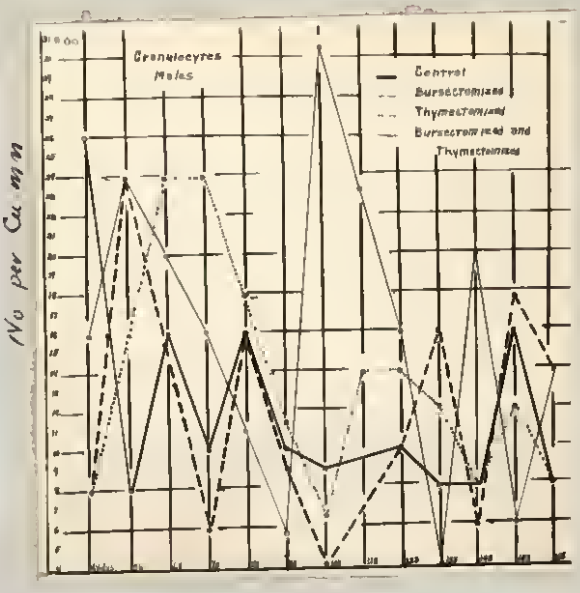

Fig. 1

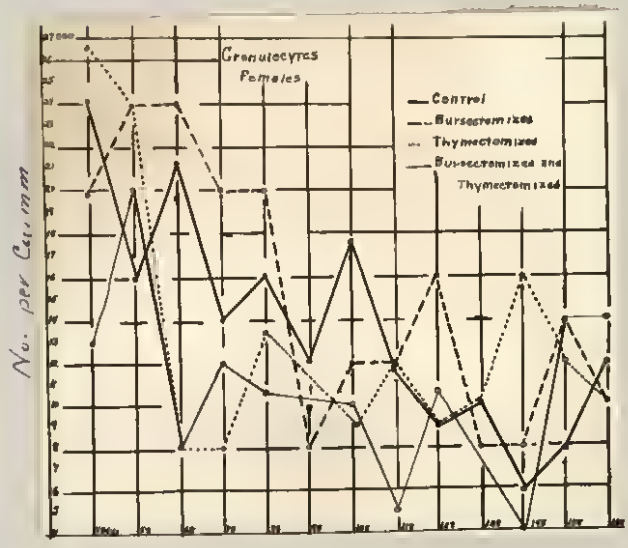

Fig. 2.

PLATE VII

Plate VII.

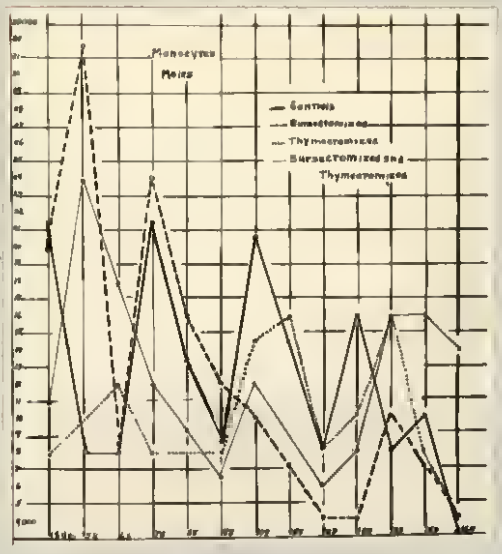

Fig. 1.

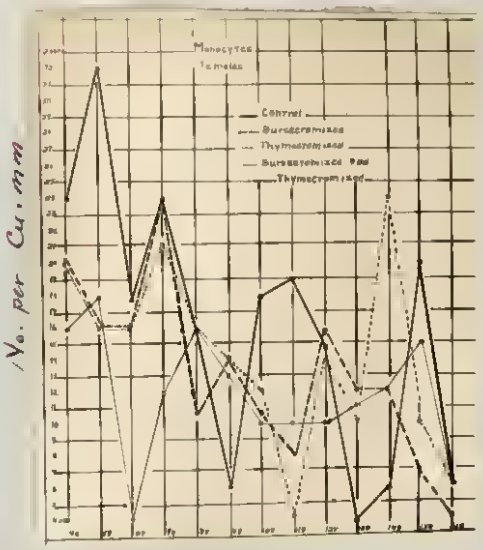

Fig. 2

PLATE VIII

Plate VIII.

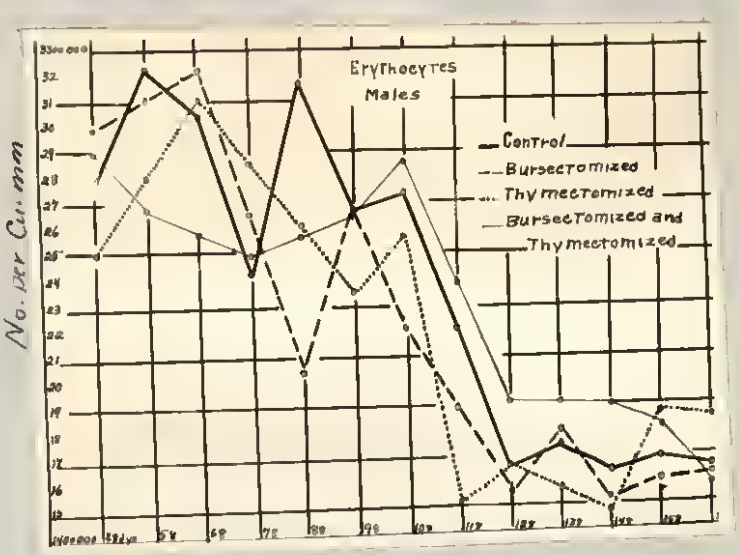

Fig 1.

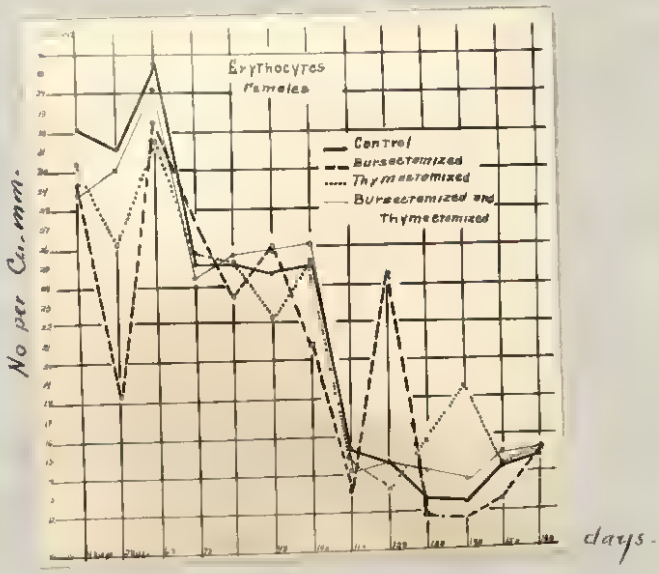

Fig. 2.

in the general methods.

The chickens were operated when they were 60 days of age. Four females and four males were bursectomized. Four females and five males were thym- and bursectomized. Four females and five males were thymectomized. Eight females and seven males were kept for controls. The chickens were confined to small pens for several days after operation to observe if there were any post-operative effects.

The chickens were weighed before operations were performed, thus trying to make the weight as equal as possible in all groups. After the operation the chickens were weighed every week and the weight recorded. The weights are plotted and shown in Figures 1 and 2. The weights of the males and females were plotted on different graphs. In these there was a little more variation than in Experiment I. Still the variations were not enough to be an indication of a relation of the removal of the organs to the growth of the chickens.

After 10 days when all effects of the operation were thought to be worn off, the blood counts were started and continued at intervals of 10 days. The plotted results of these counts are seen in Figures 1 and 2.

At the age of 23 weeks the chickens matured. As they refused to go into the trap nests it was necessary to di-

vide them into separate pens. It was found that the chickens in the different groups matured about the same time. A bursectomized pullet laid the first egg, but the others were laying in three days.

The eggs were not tested for shell strength in this experiment. The hatchability of the eggs was, however, tested. A week's laying from each group was tested. It was found that the per cent of eggs that developed ran about the same in all groups. The number of eggs laid by the different groups also was about the same. In 19 days two thyma- and bursectomized hens laid 26 eggs. During the same time two bursectomized pullets laid 21 eggs and two thymectomized hens laid 29 eggs. Two hens were run in the control group but an autopsy it was found that one of them had a tumor in the oviduct that had also infected the ovary, so that this hen would have been unable to produce eggs; thus the one hen in the control test laid 13 eggs in 19 days.

At the age of 23 weeks, 33 of the chickens were killed and autopsied, thus leaving four chickens in each of the groups. The results of these autopsies are shown in Table This chart also shows the weights of the organs computed as the per cent of dressed weight of the chicken.

Three weeks later the rest of the chickens were autopsied. In these chickens almost all of the bursa and the

thymus had involuted. The results of these autopsies are recorded in Table One should note especially the comb "area" and the wattle "area." These are purely arbitrary figures and represent the multiplicand of the greatest horizontal distance in millimeters and the greatest vertical distance.

At the time of the autopsies the hemoglobin was determined. This determination was made with a Dare's hemaglobenometer. The result of this is given in the chart.

Experiment III

Since operating at six to eight weeks showed no conclusive effect, it was thought desirable to start another experiment in which the chickens were operated at an earlier age.

The chickens were secured as day old chicks from a commercial hatchery on January 10. When the chickens were 10 days old they were bursectomized and thym- and bursectomized. Another group was added to these. This was a group in which bursal tissue was implanted. The bursa was removed from five chickens and transplanted into three other chickens. Two chickens were anesthetized at the same time, and after the bursa was removed from one it was at once

PLATE IX

Plate IX.

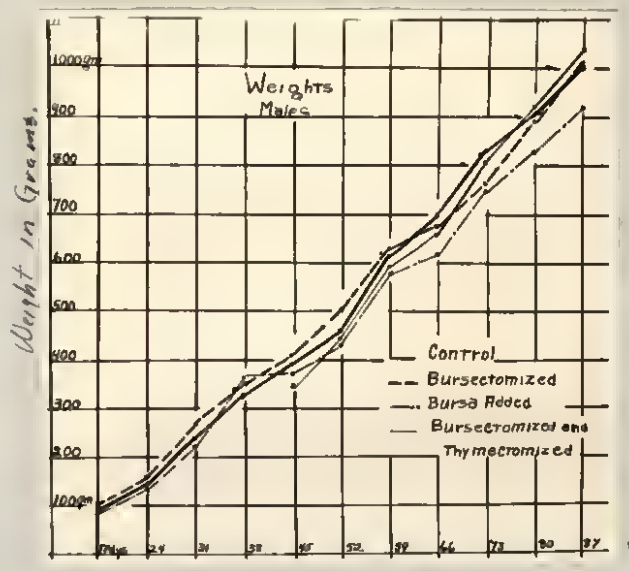

Fig. 1

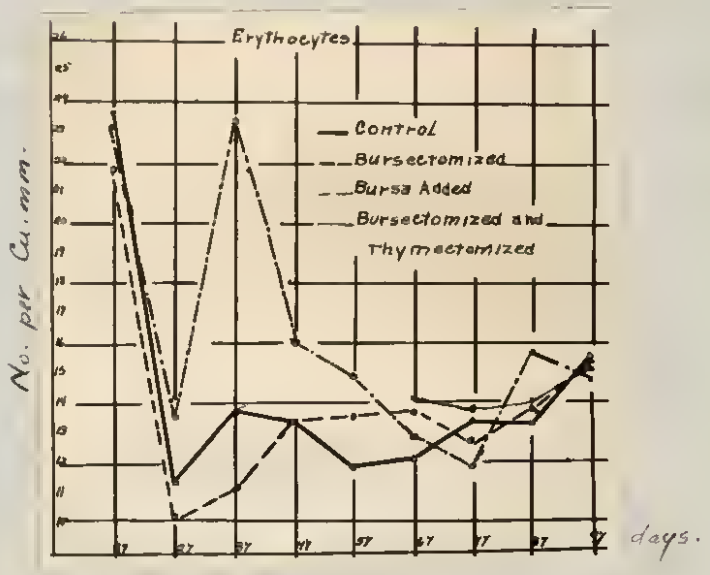

Fig. 2

PLATE X

Plate X.

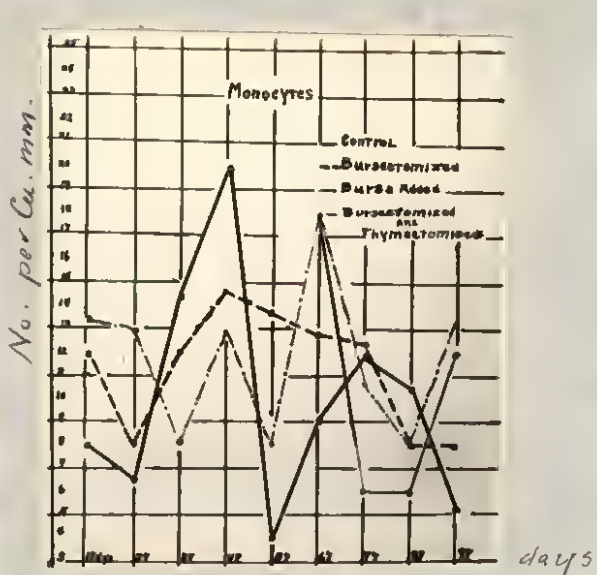

Fig 1

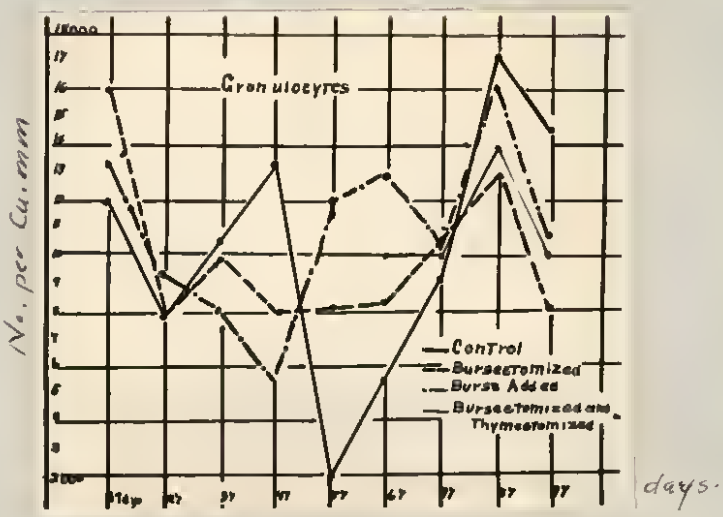

Fig 2.

TABLE XI

Plate XI.

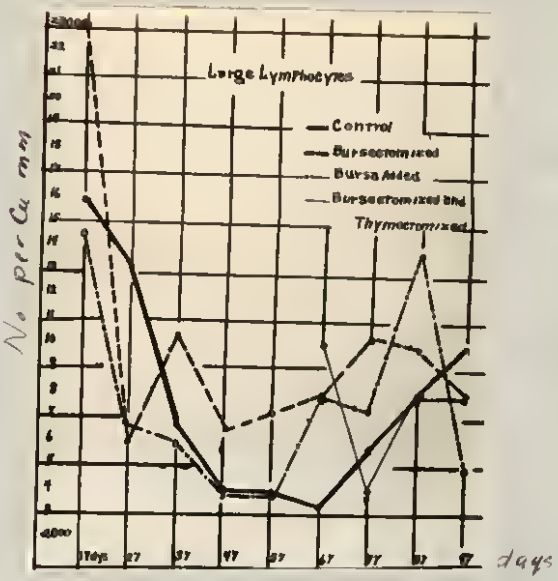

Fig. 1

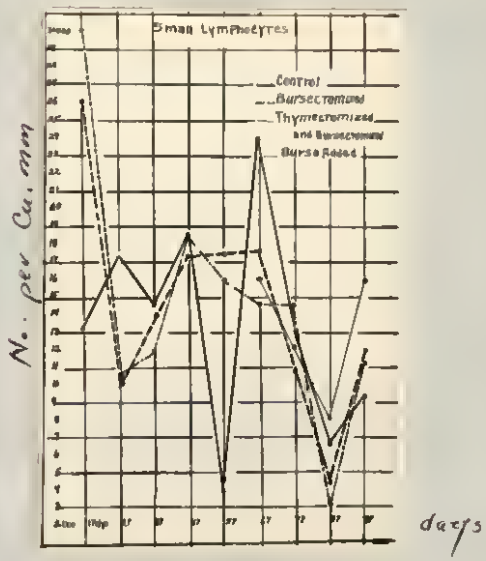

Fig. 2.

placed under the skin of the other chicken immediately under the wing, thus in a position where there would be little effect of exercise of the muscles. It was found on observation from time to time that the tissue was absorbed very rapidly. After three weeks these chickens were again implanted with more bursal tissue.

The weights of these chickens were recorded weekly, and the blood counts were made every 10 days for a period of about 80 days. The graphs of the results of the weights and the blood counts are found in Figures 1 and 2, Pl IX, X, XI.

The chickens in this experiment were autopsied at the age of about 16 weeks. The results of these autopsies are shown in Chart No.

DISCUSSION

The growth of the chickens in the different groups was found to be much the same. The variation from week to week was similar to that found by other workers in chickens and by Riddle in pigeons. The growth of the chickens seemed normal, thus it would indicate that these organs had no great functional relation to the metabolism of the chickens. If the calcium metabolism were affected it would seem that there would be an effect that would show in the growth and

CHART I

Results of the Autopsies in Experiment II and III

Weight	Exp. IIa	Exp. IIb	Exp. III	Exp. IIa	Exp. IIb	Exp.III
C	1082.5	1297	858.7	723.5	948	551
T	1258	1266		884.3	1003	
B	1060	1354	834	937	866	597
TB	1299	1248	975	932.5	876	694
BA			807			662
BI			777.5			531
Length in millimeter of tibia-tarsus						
C	92.5	97	91.7	77	82	73
T	90	87.5		77.5	87	
B	82.5	95	90	76	80	76
TB	98.0	93.5	100	80	83	87
BA			87			86
BI			88			77
Comb Area						
C	7920	11400	4832	1897	4950	375
T	9585	11100		1696	3550	
B	7971	12600	5367	3755	3831	397
TB	10847	12375	8024	2200	5529	465
BA			6900			740
BI			5030			355
Wattle area						
C	2313	4037	1408	516	1050	105
T	2632	2480		585	755	
B	2426	3638	1336	900	787	885
TB	3920	4200	1806	525	1068	75
BA			1130			200
BI			1130			107

	Males						Females					
	Exp.IIa		Exp.IIb		Exp.III		Exp.IIa		Exp.IIb		Exp.III	
Thyroid:	Gms.	%	Gms.	%	Gms.	%	Gms.	%	Gms.	%	Gms.	%
Control:	1.0	.09	.29	.022	.48	.054	.35	.049	.4	.043	.15	.027
T	1.1	.01	.05	.004			.38	.038	.1	.012		
B	.32	.09			3	.033	.175	.019	.2	.025	.21	.036
TB	.42	.032	.18	.014	.12	.012					.3	.047
BA											.3	.045
BI					.15	.016					.42	.091
Thymus												
C	2.84	.265	.75	.058	2.28	.52	6.09	.81	.4	.043	2.9	.52
T	1.05	.09					.87	.103	.07	.007		
B	1.06	.104	.91	.089	4.2	.47	2.32	.25	.35	.041	2.9	.49
TB	1.21	.096	.035	.009	1.2	.12	1.05	.095			0.4	.06
BA					3.8	.57					2.5	.3
BI					4.25	.54					3.95	.75
Gonads												
C	18.25	1.68	20.7	1.4	7.77	.9						
T	14.71	1.15	21.2	1.67								
B	16.3	1.58	21.7	1.61	6.6	.79						
TB	18.5	1.50	18.8	1.53	8.1	.83	No weights taken					
BA					6.8	.84						
BI					5.6	.79						
Pancreas												
C	2.61	.24	2.45	.189	2.2	.26	2.3	.319	2.7	.365	1.5	.27
T	2.45	.18	2.05	.161			3.11	.368	2.45	.241		
B	2.45	.24	1.7	.124	2.5	.28	2.4	.262	2.5	.280	1.62	.28
TB	2.67	.20	2.67	.233	2.3	.36	2.67	.307	2.5	.286	2.7	.27
BA					2	.21					3	.3
BI					2.26	.285					1.97	.35
Spleen:												
C	1.91	.177	1.55	.119	1.95	.22	1.425	.198	1.3	.138	1.2	.21
T	2.2	.175	2.4	.18			2.13	.278	1.5	.15		
B	2.4	.238	1.45	.104	2.7	.33	1.1	.110	.78	.092	1.	.16
TB	2.05	.155	1.5	.121	3.05	.21	1.56	.166	1.2	.137	1.1	.17
BA					1.4	.17					2.2	.33
BI					1.7	.215					1.6	.32
C	3.0	.285	none		3.22	.38	2.6	.37	none		3.8	.68
T	1.05	.083	"				3.17	.357	"			
B					0	0					0	0
TB					0	0					0	0
BA					1.8	.22					3.	.45
BI					3.2	.405					2.5	.42
Heart												
C	7.6	.704	11.2	.86	6.5	.75	5.07	.705	6.4	.67	3.5	.63
T	9.14	.725	9.4	.743			5.68	.846	6.05	.615		
B	9.55	.89	9.1	.67	7.7	.87	5.75	.61	6.15	.71	3.75	.64

TB	11.66	.9	10.27	.826	8.	.82	6.5	.636	5.47	.636	4.5	.7
BA					4.8	.59					5.4	.81
BI					6.5	.80					3.4	.48
Liver												
C	19.33	1.69	24.15	1.86	22.3	2.31	22.65	3.12	25.5	2.7	14.	2.57
T	22.22	1.7	26.9	2.15			31	2.5	33.3	3.27		
B	23.65	2.34	27.2	2.02	24.3	2.75	28.95	3.	33.6	3.88	15.1	2.63
TB	15.66	1.89	19.7	1.6	22.2	2.7	23.75	2.5	31.3	3.45	16.5	2.60
BA					20.5	2.54					17.1	2.5
BI					17.3	3.93					16.9	3.1
Blood Clotting time (sec.)												
C	31.5	95			17.5	25						
	999.				999.							
T	40	98			15.6	86.3						
B	38	101			17.6	86						
TB	14	101			12.5	63						

C - Controls

T - Thymectomized

B - Bursectomized

TB - Thym- and Bursectomized

BA - Bursa Implanted

BI - Bursal Tissue Injected

in the structure of the bones, thus causing variation in weight of body, also in the length and structure of the bones.

There seemed to be periodic plateaus in the growth curves--one coming about the age of 54 days to the 61 day, the second coming from the 82 day to the 96 day, and a third from the 117 day to the 124 day. These were found in all groups. These plateaus were more apparent in the females than in the males. This fact was also found in the work of Latimer (1928), and was accounted for as a period in which there was a beginning of sexual development. The variation in the male and female growth curves was similar to the findings of Latimer, except that the variation began before the chickens reached a body weight of 900 grams as reported by him. It would seem that age was a greater factor in the sexual variation than weight. A general weight for sexual variation could not be given because of the variation of weight in the different breeds of chickens. The periodic plateaus were also found in the work of Ackert and Morris (1929).

If the activity of the bursa inhibits sexual maturity, and if its involution is a factor in initiating sexual maturity, then its removal in the young chick should bring

on the plateaus at an earlier date. In these experiments, however, no such acceleration in the appearance of the plateaus occurred.

The pullets were considered sexually mature when they produced their first egg. The time of sexual maturity was not determined on the cockerels, thus there would be some variation from the result of Jolly and Riddle. In the groups run in this experiment little variation was found in the time of sexual maturity. These results did not coincide with the findings of Ackert and Morris (1939), who found that the thymectomized were slower to mature. Riddle and other workers suggested that the bursa and the thymus were glands functioning during growth that seemed to prevent the development of the sexual characteristics and the attainment of sexual maturity. This would suggest that the bursectomized chickens might mature earlier than the normal chickens. Jolly thought it not unreasonable to suppose that the bursa prepared a hormone-like substance somehow related to the sexual crisis. Workers have found that the growth of the gonads is repressed during the growth of the bursa and the thymus. This experiment showed none of these suggested relations of the thymus, the bursa, or the thymus and the bursa as a unit, to the time of sexual maturity.

There was some regeneration of the thymus tissue in the chickens used in these experiments. This was also found by Ackert and Morris (1929) who performed a second operation. The operations of this group may have been the cause of the chickens being slower to mature.

Soli in his work on thymectomy in chickens concluded: that thymectomy caused a notable modification of the calcium laid down in the shell of the fowl's egg; that this probably resulted from a decreased absorption of the calcium by the intestine; and a decreased utilization of the blood calcium by the tissues; that these effects are never produced immediately, but 15 to 20 days after operation; and that still later a complete recovery gradually ensues; that neither the trauma involved in the operation nor the partial thymus extirpation causes any of the disturbances.

A test of the shell strength would indirectly indicate the amount of calcium in the eggs. As all groups showed some variation in the shell strength no conclusive effect due to operation seems apparent. The results of these tests are shown in column I. These would indicate that there was an effect upon the weight and upon the shell strength, but some tests were made on the eggs of a similarly operated group of chickens and the results were as shown

in column II. In another test on this group the results were as shown in column III.

	Ave. Egg Wt. I	II	III	Ave. Wt.	Ave. Shell Strength I	II	III	Ave. Shell Strength
Thym- and bursectomized,	40	44.9	47.3	44.08	2740	3175	2727.6	2880.8
Bursectomized	41.4	47.6	53.6	47.53	3176.5	2363	3082	2873.8
Thymectomized		41.5		(41.58)		4013		(4013)
Control	42.7	41.3	45.1	43.03	3616	2804	3052	3157.3

The variation within groups is well shown. This variation is also brought out with the individual chicken's records, as pullet No. 701, thym- and bursectomized, varied from 1,940 grams shell strength to 5,242 grams shell strength; another 3,623, same as above, varied from 1,035 grams to 3,130 grams shell strength. The variation in the thym- and bursectomized in Experiment I was from 1,078 to 5,035 grams, that of the bursectomized was from 1,279 to 4,895 grams shell strength. This variation was not a gradual increase or decrease but was a variation from one egg to the next egg laid by the pullet.

Soli and others concluded that the thymus and the bursa have to do with the formation of the egg envelope, especially the shell. These facts were not found in this experiment. The shell strength results were similar to those

of Ackert and Morris (1929). If there were a deficiency in calcium due to the removal of these organs, it seemed that it would show up in the formation of the egg if the deficiency were small, and if large it would be noted in the coagulation time of the blood.

The tests on hatchability showed that there was no outstanding difference between the groups. Riddle in his work fed thymus and found an increase in hatchability. Thus it would suggest that the removal of the thymus might cause a decrease in hatchability. Since he suggested a like function for the bursa it might be said that the bursa had a relation to hatchability of eggs. The records show that there was no such relation to hatchability. These results are similar to those of Ackert and Morris (1929).

The results of the work on blood likewise show no distinct effect of the activity of either of the glands. There were fluctuations in the numbers from period to period. These fluctuations of the blood count are also found to accord with the data of other workers.

The erythrocytes in the chickens varied more or less throughout the experiment. In Experiment I the control females started with the highest count, 3,300,000, and then lowered only a little, and at the age of 92 days reached

the peak of all counts, 3,500,000. They then dropped down to 3,500,000 to run along with the other groups, ending with a count of 1,500,000. The bursectomized for the majority of the time ran the lowest but this was not enough to say that their count was uniformly below the others. The thymectomized and the thym- and bursectomized were found to vary, sometimes above and sometimes below the others.

In comparing the males with the females it was found that the female count was higher during the early part of the experiment, but at the time of sexual maturity and after, the females on the whole showed lower erythrocyte counts. The erythrocytes in Experiment III showed similar variations. The first count was very soon after operation and was the highest count of the entire experiment. The Burse Added group showed the highest general average count throughout the experiment, with only one low count that was at the age of 77 days.

There was a gradual lowering of the erythrocyte count at the time of sexual maturity. This decrease in the blood count as the individual grows toward maturity is also found in other animals, especially in mammals.

The granulocytes in the males and females in general were found to be about the same, though there was more vari-

ation among the groups. The females in the thym- and bursectomized group in general had the lowest count. This was not observed in the males. In experiment III no conclusion could be drawn because of the variation of the granulocyte count, but it was found that in general the counts varied from six to 18,000 per cubic millimeter. The controls in general ran highest except for the 57th day, when they went down to 2,000 per cubic millimeter.

The lymphocyte counts in Experiment II indicated that the females had a higher count than the males. In Experiment III the lymphocytes were counted apparately, that is, the small and the large lymphocytes. There was a gradual decrease in the counts in all groups. The large lymphocyte counts were lower than the small.

In considering the autopsies one again finds few significant results. The results showed that there was a larger comb "area" and wattle "area" in the thym- and bursectomized group and also in the bursectomized group, thus showing that the bursa may have, as suggested by other workers, an influence upon the growth of the sexual characteristics, which would indicate that the removal of the bursa allowed the secondary characteristics to manifest themselves during the growth period of the body.

There seemed to be no interrelation in the growth of the bursa and the thymus. When one was removed it would seem that if the function of the other were the same, there would be a hypertrophy of the remaining gland, which did not seem to be the case in the results of the autopsies.

In the autopsies of the chickens at different ages it was found, as was also found by Riddle and Jolly, that the involution of the bursa started before that of the thymus, thus there remains thymus after the bursa has become so involuted that it could not be functional. It was also found, contrary to the indications of Riddle, that the involution of the thymus and the bursa was not complete at sexual maturity.

The weights of the other organs compare well with those reported by Latimer ( ). The time of involution of the thymus depends upon the age of the chicken rather than the body weight.

SUMMARY

1. Three experiments were carried out to ascertain the possible effects of bursectomy, thym- and bursectomy, and thymectomy upon chickens. These experiments covered a period of two years and involved the use of about 110 single-comb White Leghorn chickens and several Rhode Island Red chickens.

2. The following tests were made upon the above mentioned groups and a group of controls without securing a significant different among the groups in any test.

(a) weight of eggs

(b) strength of egg shell

(c) appearance of egg shell

(d) fertility and hatchability of eggs

3. A record of the weights was taken and there was found to be no significant variation in the rate or amount of growth. There was no change in the time of maturity. There was a variation between the males and females, beginning when the chickens were about 80 days of age.

4. The blood counts were made on the chickens of each group.. The different types of cell and the number of cells were recorded and graphs of the averages of the groups made. These graphs showed no significant variation of any type of cell in the different groups. A modification of Shaw's method of blood counting was found to give the best results.

5. The autopsies showed no significant changes in the organs and glands, except in the comb "area" and the wattle "area" of the thym- and bursectomized and the bursectomized groups, in which it was found that these organs were larger.

ACKNOWLEDGMENTS

The writer wishes to thank Dr. J. E. Ackert, who suggested the problem and directed and assisted with the last part of the experiment, the research assistants in parasitology, and others who helped make this experiment possible.

Literature Cited

Blood

Aschoff
1924. Lectures on Pathology. New York. Chapter I, page 33.

Bayon, H. P.
1929. The Pathology of Transmissible Anemia in the Fowls. Parasitology. Volume 21, pages 339-372.

Blain, Daniel
1938. A Direct Method for Making Total White Blood Counts on Avian Blood. Proceedings Soc. for Exp. Bio. and Med. April, pages 594-596.

Blain, Daniel
1938. A Study of Blood Cells of the Normal Fowl by the Supervital Technique. Anatomy Rec. 33: 285-291.

Bedson, Phillips S. and Keith
1924. Anemia in Hens. Jour. of Path. and Bact. 27: 239.

Coates, Ida
1928. A Method of Counting White Cells in the Blood of Fowl. Report of the Ontario Veterinary College Sessional Paper No. 39. 1929, page 63.

Doan, C. A., R. S. Cunningham, and F. R. Sabin
1926. Experimental Studies on the Origin and Maturation of Avian and Mammalian Red Blood Cells. Contributions to Embryology Nos. 78-84. Publication No. 361.

Duke, H. N. and L. H. Schwarte
1931. The Hemoglobin Content of the Blood of Fowls. Am. Jour. of Physiol. Volume 96, pages 89-93.

Ellerman, Vilhelm
1921. The Leucosese of Fowls and Leucaemia Problems. English Edition.

Forkner, Claude E.
1929. Blood and Bone Marrow Cells of the Domestic Fowl. Jour. of Exp. Med. Volume 50, pages 131-141.

Fritsch, G.
1920. Pflueger's Arch. CLXXXI 78.

Hart, [illegible] B., C. A. Elvehjem, A. R. Kemmer, and J. G. Halpin
1930. Poultry Sci. IX [illegible]

Herrick, C. A.
1923. Some Physical Effects of Ascaridia perspicillum on Growing Chickens. Master Thesis, 1923.

Jolly, J.
1903. Traite technique d'hematologue.

Keyes, Preston
1929. Normal Leucocyte Content of Birds' Blood. Anatomical Record. Volume 43.

Maximow, A. A.
Relation of Blood Cells to Connective Tissues and Endothelium. Physiological Rev. 4; 533-563.

McGowan, J. P.
1926. On Rous, Leucotic, and Allied Tumors in the Fowl. New York. The Macmillan Company.

McGowan, J. P.
1926. Pernicious Anaemia Leucaemia and Aplastic Anaemia. London. H. K. Lewis and Company.

Muller, C. A.
1886. Inaugural Dissertation. Erlangen.

Preger, W.
1871. Die Blutkrystalle. Jena.

Sabin, F. R.
1921. Studies on Blood. Bull. Johns Hopkins Hosp. 32; 314.

Schmeisser, H. C.
1915. Leukemia of the Fowl. Jour. of Exp. Med. 22: 820-838.

Simpson, Marion E.
1921. On the Reaction of the Living Blood Cells to Dyes. Proc. Amer. Assoc. Anat. Anat. Rec. Volume 21. page 82.

Shaw, A. F. B.
1930. Direct Method of Counting Leucocytes, Thrombocytes, and Erythrocytes of Birds' Blood. Jour. of Path. and Bact. 33: 935-935.

Warthin, A. S.
1907. Leukemia of the Common Fowl. Jour. Infect. Dis. Volume 4. Pages 388-391.

Thymus and Bursa

Ackert, James E.
1924. Effect of Parasitism on Fowl Thymus. Anat. Rec. 29:120.

Ackert, James E., and M. H. Morris
1929. Studies of the Effect of Thymectomy on Growing Chickens. Master's Thesis. 1929.

Basch, K.
1908. Zur Thymusexstirpution beim Jungen Huhn. Monatschr. f. Kinderd. 7:41.

Barkow, S.
1929. On the Cloaca of Birds. Meckel's Archiv. 1929, page 443.

Boyden, E. A.
1918. Vestigial Gill-filaments in Chick Embryos with a Note on Similar Structures in Reptiles. Am. Jour. Anat. Volume 23, pages 205-36.

Fischl, R.
1907. Über die Folgen der Thymus Ausschaltung bei jungen Hühern Monatschr. Kinderd. 5:329.

Forbes, W. A.
1877. On the Bursa of Fabricius in Birds. Proc. Zool. Soc. London. Pages 304-319.

Muscke, Emil.
1938. Die Bursa Fabricii. Origine Jenne.

Jolly, J.
1915. La Bourse De Fabricius et Les Organes Lympho-Epitheleaux. Archives D'antomie Microscopique 16- 363-647.

Kaupp, B. F.
1918. The Anatomy of Domestic Fowl. W. B, Saunders Company.

Katsura, K.
(Influence of Thymus on Growth) Mitteil. a. d. med. Fakult d. k. univ. zu. Tokyo: 30:1-208.

Kingsley
Comparative Anatomy of Vertebrates.

Krizenecky, J.
1928. Uber den Einfluss der Hypert hyroreoidiectim und der Hyperthymusatim auf des gewuktdeir. Ausgewachsene Vogel.

(Zeitschrift fur vergleichende Physiologie) 9 bd 1 heft.

Latimer, H. B.
1924. Postnatal Growth of the Body Systems and Organs of Single-comb White Leghorn Chickens. Jour. Agri. Research. Volume 29. Pages 363-397.

Patavii
1621. De Formatione Ovi et Pulli.

Riddle, Oscar
1934. Studies on the Physiology of Reproduction in Birds. A Hitherto Unknown Function of the Thymus. Am. Jour. of Physiol. 68:557-580.

Riddle, Oscar
1925. Studies on the Reproduction in Birds. Am. Jour. of Physiol. Volume 86, No. 2.

Riddle, Oscar, and Paul Frey
1924. The Growth and Involution of the Thymus in Male and Female Pigeons. Am. Jour. of Physiol. 71: 413-429.

Riddle, Oscar, and Masaharu Tange
1928. Studies on the Reproduction in Birds. On the Extirpation of the Bursa Fabricii in Young Doves Am. Jour. of Physiol. 86: 366-373.

Soli, U.
1906. Contribution a la connaissance de la function du thymus le Poulet et chez Quelques Mammiferes (abstr.) Arch ital debiol, turin 52: 353-370.

Tarulli, L., and Lo Monaco, D.
1897. Richrche Aperimentali sul Timo. Bull. d. r. Acad. med. di Roma. 23: 311.

Wenckebach, K. F.
1896. Die Follikel der Bursa Fabricii. Anat. Anz Bd. 11e 159.