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HISTOLOGICAL AND QUANTITATIVE STUDIES ON THE POSTNATAL GROWTH OF THE THYMUS AND THE BURSA OF FABRICIUS OF WHITE PEKIN DUCKS*

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Postnatal growth of the thymus and the bursa of Fabricius of White Pekin ducks was studied with respect to their morphology and gravimetric changes from the day of hatching to 22 weeks of age when they showed sexual maturity. The thymic mean weight reached its maximum at 11 weeks of age, and thereafter, a rapid regression mainly due to the depletion of cortex lymphocytes and cyst-formation by epithelial reticular cells in the medulla was observed. Histologically, myoid cells, large and small types of cystic structures formed by epithelial reticular cells, and Hassall's corpuscles could be distinguished in the medulla. The bursa of Fabricius grew actively during the first few weeks of posthatching life and reached its maximum mean weight at 9 weeks of age. Thereafter, the bursa, as well as the thymus, showed a severe regression, and at 22 weeks of age many of the bursae lost their lymphoid follicles and remained as a fibrous sack. Gravimetric changes of the bursa in posthatching life coincided with the growth of bursal lymphoid follicles. The relative number of plasma cells in the interfollicular spaces of the bursa reached their maximum at 3 weeks of age.

From morphological results, it was ascertained that the duck's lymphoid system functions actively after the 3-5 weeks of posthatching life, and thus, ducks are available for immunobiological experiments from these weeks.

INTRODUCTION

The postnatal growth of the thymus and the bursa of Fabricius has been described in detail by several authors (WOLFE et al., 1962; DIETER & BREITENBACH, 1968, in chickens; YAMADA et al., 1971, in Japanese quails). These data are available for immunobiological experiments of immune responses by some antigenic stimulations. To the authors' knowledge, however, there are only a few reports regarding the postnatal growth of the central lymphoid

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organs of Anatidae (GLICK, 1960; WARD & MIDDLETON, 1971), and there are, significantly, no reports on the thymus of ducks. Ducks have two pairs of true lymph nodes and may be experimental models for considering the phylogenetic immunological relations between mammals and birds.

In the present study, the authors focused their attention on the postnatal changes in weight and histology of both central lymphoid organs in White Pekin ducks from the day of hatching to 22 weeks of age, and the quantitative changes of the cellular components of the bursa were histologically analysed in detail.

MATERIALS AND METHODS

Fertile White Pekin duck eggs were incubated in an automatic incubator. All of the newly hatched ducks were given the "Golden Chick" for newly hatched chickens (Kyodo Feed Company, Japan), and after 5 weeks of age they were fed on "Sakuraboshi" for adult chickens (Kyodo Feed Company, Japan) with water, ad libitum.

Ducks of hatching day age, 5 days of age, 1, 3, 5, 7, 9, 11, 13, 17 and 22 weeks of age were utilized to determine the weight and histological growth of the thymus and the bursa of Fabricius.

The thymus and the bursa were removed from the ducks immediately after death, then weighed to the nearest milligram on a Sartorius-scale before being fixed in Carnoy's or 10% formalin fluid. The lymphoid organs were embedded in paraffin. For histological examinations, sections of 4~5 microns were cut from each organ and stained with Mayer's hematoxylin-eosin, toluidine blue, PAS, Goldberg and Chaikoff's trichrome, and Gomori's silver impregnation for reticular fibers. Samples for electron microscopy were obtained from the thymus of 5 days and 7 week-old ducks and fixed in 3% glutaraldehyde following 1% OsO_4. They were embedded in epoxy resin according to the routine method. Ultrathin sections were cut on a Porter-Blum MT-1 ultramicrotome and photographed under the JEM-7 electron microscope.

In the bursa, the number of lymphoid follicles per one cross section was counted. The relative-follicle size was represented by multiplying the length by width of the follicle, which was selected from the most typical ones sectioned at the mid plane in each bursa. The height of the interfollicular epithelium and the diameter of the bursal cross sections were also recorded.

RESULTS

1. Body weight

The mean body weight of the White Pekin ducks showed a rise in 11 weeks

after hatching, and the greatest increase occurred during the first 5 weeks. On the day of hatching, the body weight showed only 59.6±4.5 g ; it then grew rapidly and reached 2300.0±291.2 g in 11 weeks of age, as shown in table 1. Though a slight decline could be seen at 13 weeks, thereafter, the body weight was maintained throughout the observation period without much variation.

2. Growth of the central lymphoid organs

(1) Thymus: The duck thymus consisted of 10～12 lobes, 5～6 on each side, which lay along the jugular veins. The lobes were irregular in number.

The weight of the thymus of White Pekin ducks during posthatching life is shown in table 1 and text figure 1. On the day of hatching, the thymus weighed 0.13±0.03 g (0.22% of the body weight) and there was a continuous increase in the mean weight of the thymus up to 11 weeks of age. Between 3 and 11 weeks the thymus weight was maintained at 0.31–0.43% of the body weight, as shown in table 1. After 11 weeks of age, however, a sharp decrease occurred from 13 to 22 weeks, and at a latter age the thymus showed only 0.02% of the body weight. The maximum absolute mean of the thymus weight was 9.96±4.90 g at the 11th week, and there was a wide variation in weight at all stages.

(2) Bursa of Fabricius: On the day of hatching the bursa of Fabricius had a mean weight of 0.08±0.02 g (0.13% of the body weight), and it grew most

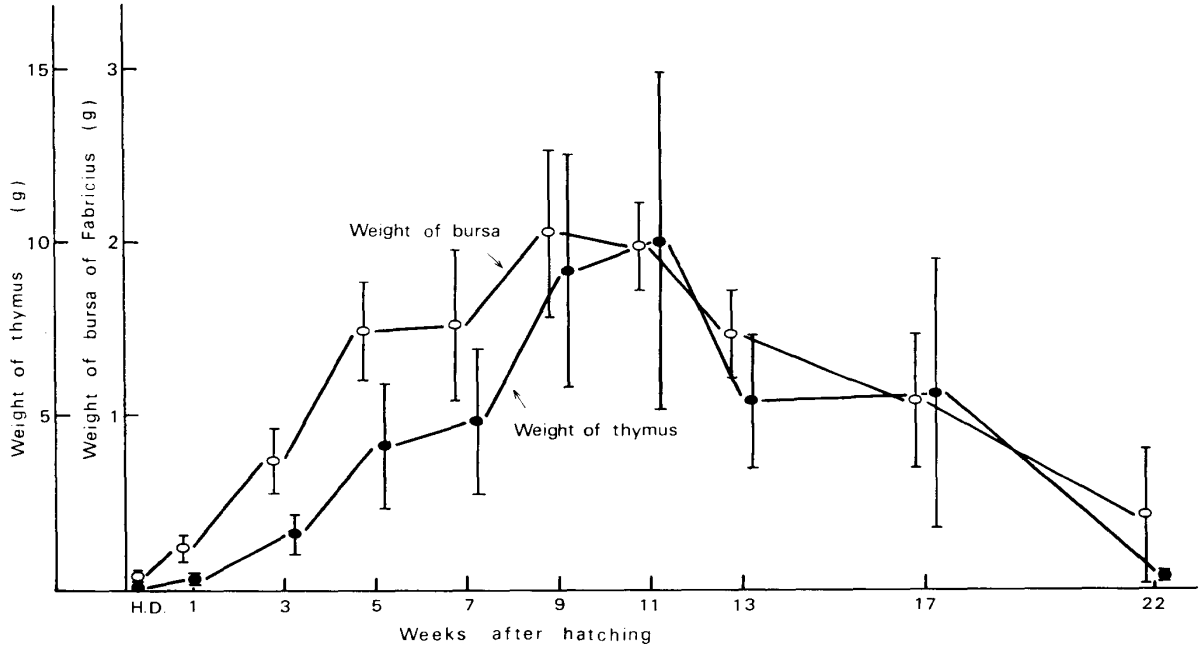
TABLE 1 *Postnatal growth of body weight, weight of thymus and bursa of Fabricius, and organ weight percentage of body weight in ducks*

AGE*	BODY WEIGHT	THYMUS	BURSA	ORGAN WEIGHT PERCENTAGE OF BODY WEIGHT	
				Thymus	Bursa
week	g	g	g		
H.D. (8)	59.6± 4.5	0.13±0.03	0.08±0.02	0.22	0.13
1 (5)	103.8± 26.9	0.30±0.15	0.25±0.08	0.29	0.24
3 (5)	359.0± 64.7	1.56±0.60	0.74±0.20	0.43	0.21
5 (8)	1335.0±117.8	4.13±1.85	1.49±0.28	0.31	0.11
7 (7)	1260.0±238.0	4.79±2.10	1.53±0.44	0.38	0.12
9 (7)	2194.0±263.1	9.16±3.42	2.07±0.48	0.42	0.09
11 (6)	2300.0±291.2	9.96±4.90	1.97±0.25	0.43	0.09
13 (5)	2066.0±366.0	5.36±1.95	1.46±0.27	0.26	0.07
17 (6)	2150.0±273.3	5.63±3.88	1.07±0.39	0.26	0.05
22 (4)	2315.0±179.4	0.39±0.12	0.41±0.43	0.02	0.02

Mean weight (g) ± Standard deviation

* H. D.: Hatching day ( ): Number of ducks

TEXT-FIGURE 1 *Postnatal changes in thymic and bursal weight in duck*



(Mean ± Standard deviation)

rapidly during the first 5 weeks after hatching. The maximum absolute mean of the bursal weight was 2.07±0.48 g (0.09% of the body weight) at 9 weeks of age. From the next week there was a decrease in the mean weight, and this was followed by a steady regression. The initial regression of the bursal weight probably immediately after 9 weeks of age, and then, at 22 weeks the weight reached 0.41±0.43 g (0.02% of the body weight). At this age, three ducks had almost no remnants of the bursa, while in another duck the bursal weight was still 1.03 g.

3. Histological observation

(1) Thymus: The histological appearance of the thymus of ducks was similar to that of the chickens. Each thymic lobe was clearly divided into an outer cortex comprising densely packed small lymphocytes with scattered mesenchymal and epithelial reticular cells, and an inner medulla consisting of less densely packed lymphocytes, myoid cells, and epithelial cyst-forming cells, including Hassall's corpuscles.

On the day of hatching, the thymus seemed to be almost completely established, and the cortex and medulla were clearly identified (fig. 1). In the cortex, the small lymphocytes were packed more densely than the medium- or large-sized lymphocytes, and mesenchymal reticular cells were frequently among them. The epithelial reticular cells were also found in the cortex. In the

medulla, myoid cells, epithelial reticular cells and small or medium-sized lymphocytes, which filled a greater proportion of medulla, could be distinguished (figs. 5–8).

Myoid cells were frequent in the medulla and in the juxta-medullary cortical portion, but were rare in the cortex. The cells from the 5 day-old juvenile ducks showed a very long cytoplasm and contained regularly arranged myofibrils in the longitudinal view (fig. 9). In the more aged ducks, the myoid cells tended to show a round to ovoid appearance (fig. 5).

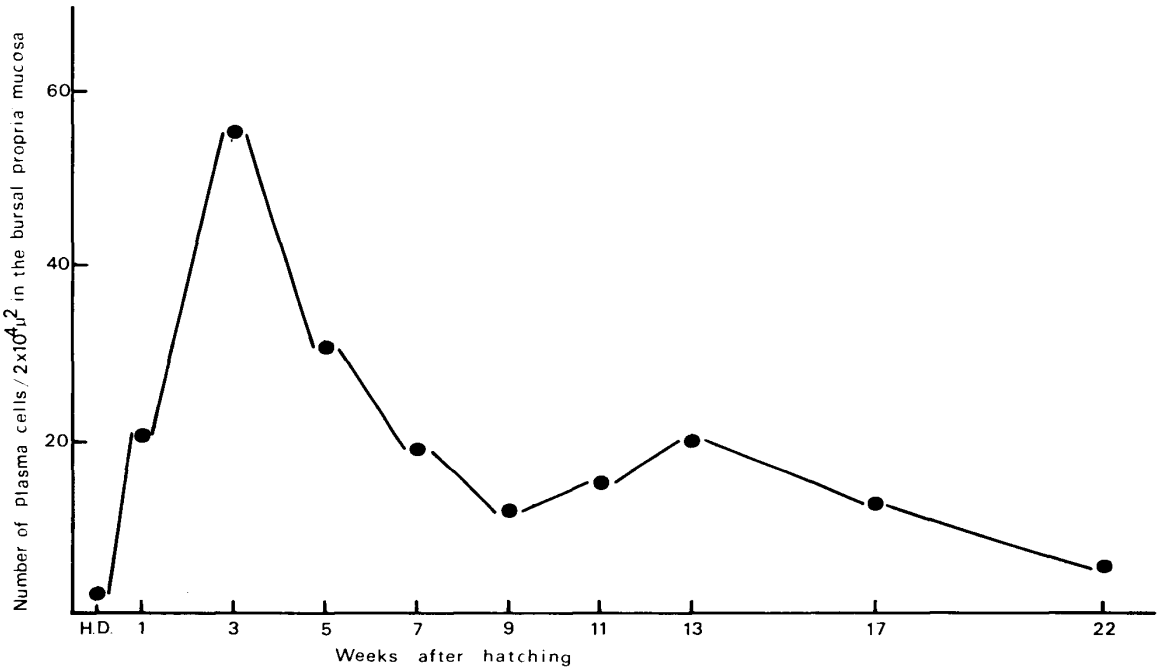
Many epithelial reticular cells in the medulla were involved in the cyst formation. Regular small type cysts were formed by a few epithelial reticular cells. Some of the cysts appeared to be empty, while the others contained some amount of PAS positive materials (fig. 6). The large type cysts were considerably varied in shape and were formed by many epithelial reticular cells. On many occasions, the cysts were filled with substances similar to colloidal or granular materials, including cell debris (figs. 3, 4 & 7). Both structures appeared variable in number and no fixed tendency was observed with age. Hassall's corpuscles could be found frequently in the medulla, and this structure slightly increased with age (fig. 8).

The histology of the thymus in ducks was generally unchanged until the regression time began. At 13 weeks of age there was a slight disappearance of lymphocytes from the cortex, and thereafter, regressive change, which was mainly the depletion of lymphocytes and cyst-formation by epithelial reticular cells, could be observed in the medulla of all cases. In 22 week-old ducks, the thymic lobes were embedded in the adipose tissue, and in the histological sections none of the cortical portion could be found at all (fig. 2).

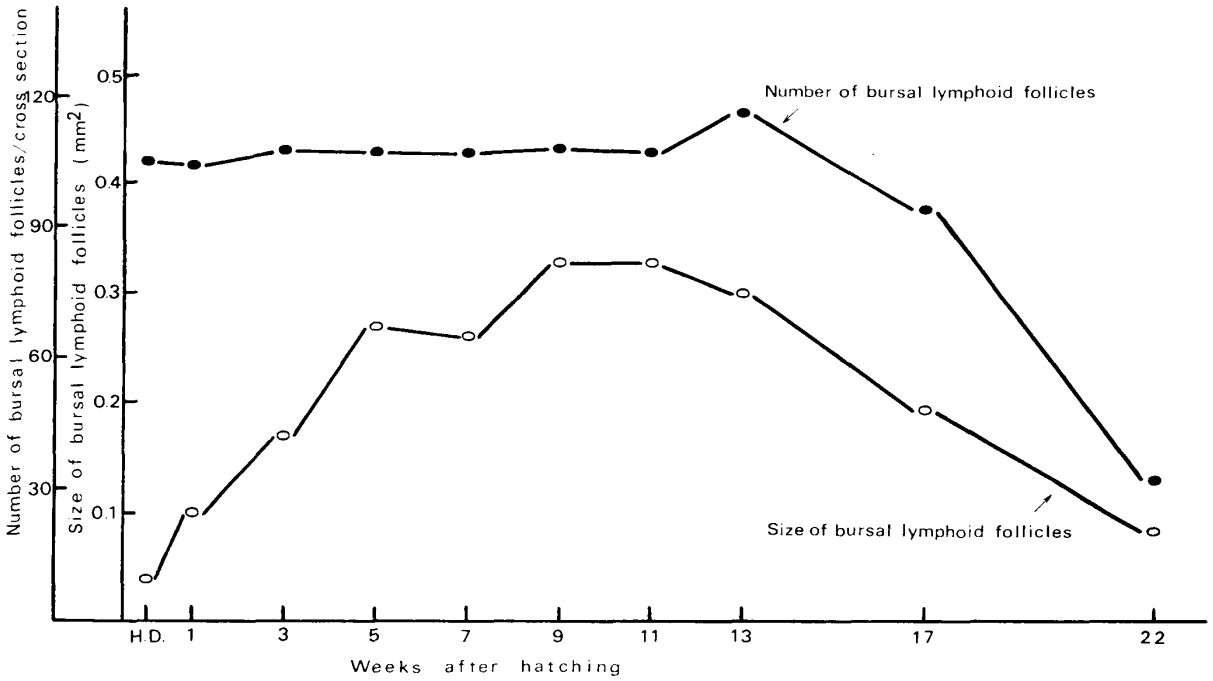
(2) Bursa of Fabricius: The bursa of Fabricius of ducks was long and cylindrical in shape and connected by a short stalk to the dorsal region of the cloaca. On the day of hatching, the lymphoid follicles in each fold consisted clearly of a well-developed outermost cortex with densely packed lymphocytes supported by a mesenchymal reticular networks and capillaries, and the inner medulla comprising epithelial reticular cells and loosely packed lymphocytes separated from the cortex by a thin layer of epithelial cells and basement membrane (figs. 11 & 12).

The epithelium consisted of two kinds of epithelial cells, named follicle-associated and interfollicular epithelium, respectively. Interfollicular space was filled with connective tissue, including the vascular and nervous system, and a small number of lymphocytes, plasma cells, and granular leucocytes could be found. The plasma cells in this area already appeared on the day of hatching, and the number increased markedly to the 3rd week of posthatching life;

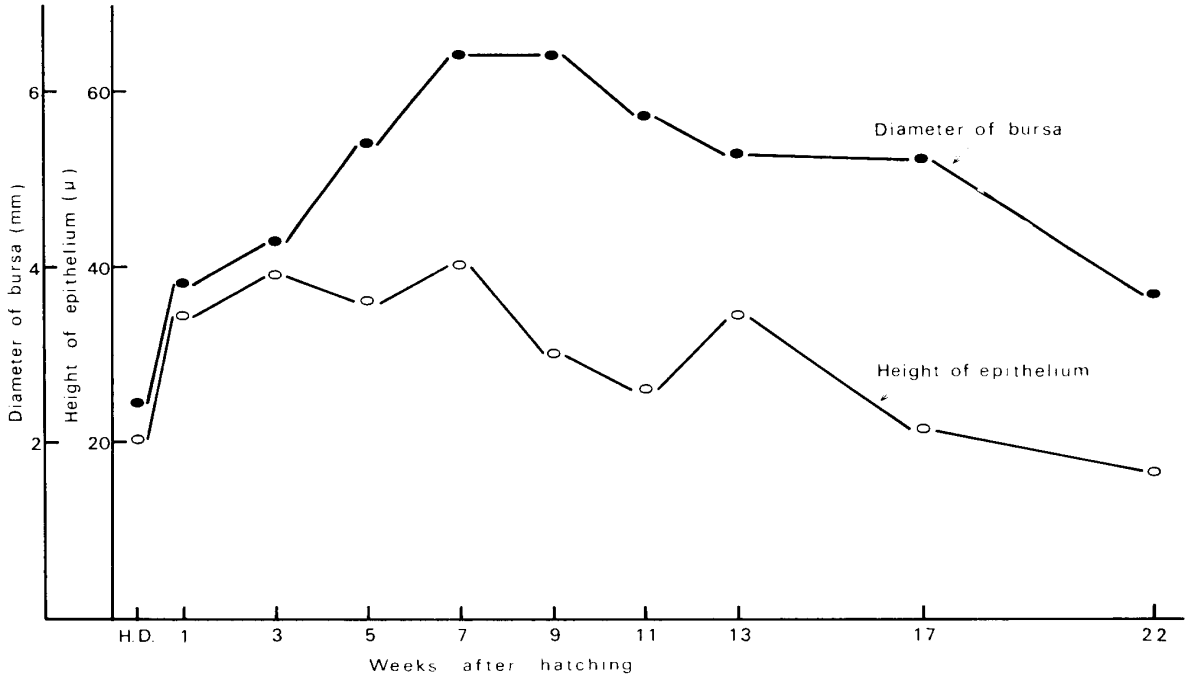
TEXT-FIGURE 2 *Quantitative change of plasma cells in the bursal interfollicular space in the posthatching life*



TEXT-FIGURE 3 *Quantitative changes of duck bursal lymphoid follicles in number and size with age in cross section*



TEXT-FIGURE 4 *Postnatal changes of the height of interfollicular epithelium and the diameter of bursa in cross section*



thereafter, a decreased was observed (text fig. 2).

The outermost part of the bursa in the cross section consisted of a circular strand of smooth muscle and serosa.

The number of bursal lymphoid follicles during postnatal life showed almost no changes up to 13 weeks of age, as shown in text figure 3. Thereafter, however, a sharp decrease could be observed, and at 22 weeks of age, three ducks out of four had only a few lymphoid follicles within the bursa, which consisted mainly of hyperplastic smooth muscle and adipose tissue (fig. 15). Follicle size was about 0.035 mm^2 on the day of hatching and a steady increase was observed up to the 9th to 11th week in the size of 0.33 mm^2, but thereafter, the size, as well as the number of follicles, began to decrease sharply, accompanied by the depletion of cortical lymphocytes. The size finally decreased to about 0.08 mm^2 at 22 weeks (text fig. 3).

The interfollicular epithelium was 20.1 μ in height on the day of hatching, and in the following weeks a sharp increase was observed and it reached approximately 40 μ at 5 to 7 weeks. Thereafter, although a small re-increase could be observed at 13 weeks of age, there was generally a steady decrease and fall into 17.5 μ at 22 weeks of age. There were no change in the follicle-associated epithelium until 13 weeks of age. The epithelium disappeared, accompanied by the cyst-formation in the medulla.

The diameter of the bursa, cross-sectioned almost to its mid portion, showed a maximum size of 6.45 mm at 7 to 9 weeks, and during the following weeks a steady decrease could be observed (text fig. 4).

Initial histological changes in the bursa during its natural regression were found in the 13 week-old ducks due to the loss of lymphocytes, mainly from the cortex, and the interfollicular epithelium became thinner. The bursal lymphoid follicles consequently started to diminish in size (figs. 13 & 14). Thereafter, more prominent regressive changes occurred in the propria mucosa; the thinned follicle-associated epithelium gave rise to cystic structures lined by flattened epithelial cells. The lumen of cysts was connected with the bursal cavity in many cases. A marked increase of connective tissue in the interfollicular spaces could also be found. At 22 weeks of age, almost all of the bursal lymphoid follicles disappeared. The bursa may persist as a very small sack near the cloaca for some period after the onset of sexual maturity.

DISCUSSION

In the present study, the postnatal growth of the central lymphoid organs of the White Pekin ducks was clarified, with special attention given to the change of weight and histology.

The growth of the thymus in the posthatching life was reported by WOLFE et al. (1962), DIETER & BREITENBACH (1968) in chickens, and YAMADA et al. (1971) in Japanese quails. WOLFE et al. (1962) reported that there was a continuous increase in the mean weight of the thymus up to 12 weeks of age and practically no change occurred between the 12th and 14th week. The maximum mean weight of the thymus was 15.76 g at 17 weeks of age in Arbor Acre White Rocks. The data described by DIETER & BREITENBACH (1968) indicated that the maximum weight of the thymus of White Leghorn cockerels was attained at 11 weeks of age; by the 15th week, the age involution had begun, and thereafter, a sharp decrease of the thymic weight occurred from 17 to 23 weeks. As to the Japanese quails, YAMADA et al. (1971) reported that the male thymus reached its maximum weight at the 5th week, and the female at the 4th week, respectively.

In the present study, it was observed that the duck thymus continued its conspicuous growth up to 11 weeks of age and attained 9.96 ± 4.90 g in weight. In the following two weeks the regression began, and then a sharp decrease of the thymic weight could be observed throughout the observation period. In the report of WOLFE et al. (1962), the thymus attained its maximum mean weight 7 weeks later than the bursa in chickens, while our data indicated that the duck thymus attained its maximum mean weight only 2 weeks later than

the bursa.

The regressive changes of the duck thymus seemed to be due to the depletion of the lymphocyte series in parallel with the decrease of the thymic weight. As for the involution mechanism of the thymus, DIETER & BREITENBACH (1968) suggested that the beginning of age involution coincided with a further logarithmic increase in the testicular mass. The gravimetric changes in the adrenals, testes and comb suggested that the endogenous testosterone level increases just before the beginning of age involution. With this in mind, it is theorised that the testosterone may have an effect on the thymus. However, KING et al. (1965) reported that the thymus appeared to be almost unaffected in its morphogenesis by testosterone as opposed to its complete inhibitory effects on the bursa. In this regard, the mechanism of thymus regression in birds needs clarification as to the nature of the influence of this hormone upon the thymus.

Histologically, the fine structures of myoid cells, cyst-forming cells and Hassall's corpuscles in chickens were described by FRAZIER (1973), and GILMORE & BRIDGES (1974). The myoid cells and cyst-forming cells are thought to be established in number on the day of hatching. As for the large type of cysts and the Hassall's corpuscles, their number increased, on the whole, with age; the cysts may also play a role in disposing of exhausted materials, including cell debris.

The presence of myoid cells within the thymus of many species has been acknowledged by previous workers (BOCKMAN & WINBORN, 1968, in lizards; TÖRÖ et al., 1969, in frogs; FRAZIER, 1973, in chickens; SUGIMURA, 1972, in calves; ITO et al., 1969, in man). Therefore, though the cells exist as an integral part of the normal thymus and may play some physiological roles, it is reasonable to consider that the actual function is unknown at the present time.

FRAZIER (1973) referred to the intra- and intercellular cysts in the chicken, and the structures appeared to be fairly common in the thymus of various mammals (HOSHINO, 1962; LUNDIN & SHELIN, 1965; ITO & HOSHINO, 1966). The intracellular cysts in the mammalian thymus are usually small, and several are often contained within a cell, while in the duck thymus the intracellular cyst-like small cyst were present, and they contained a certain amount of PAS positive materials. Some authors are of the opinion that the cyst-forming cells show cytoplasmic features suggestive of a secretory function. The small cysts as well as the intercellular large cysts were frequently observed in the duck thymus, but owing to their morphological diversity, it is difficult to assign definitely a secretory role; its exact role remains to be clarified.

On the other hand, the postnatal growth of the bursa of Fabricius was studied by many investigators. WOLFE et al. (1962) described that the mean maximum weight of bursa occurred at 10 weeks of age in the Arbor Acre White Rock. GLICK's data (1956), using three different breeds of chickens, showed a mean peak bursal weight at 8 weeks for male Rhode Island Reds, 4 to 4 1/2 weeks for male White Leghorns, and 8 to 14 weeks for males hatched from a cross between Barred Plymouth Rocks and Dominant White Rocks. GLICK (1960) reported that in White Pekin ducks, the bursal weight attained its maximum at 8 weeks of age, and the mean weight was significantly larger than the mean bursal weight of chickens at all ages except 3 and 4 weeks. In wild mallards, the mean bursal weight reached its maximum at 8 weeks in males and 6 weeks in females, and decreased steadily after 12 weeks in males and 16 weeks in females (WARD & MIDDLETON, 1971). In Japanese quails, as reported by YAMADA et al. (1971), the bursa attained its mean maximum weight at 7 weeks in the males and at 6 weeks in the females, and reached its mean maximum weight earlier than chickens, with the exception of GLICK's data for male White Leghorns. In the present study, the ducks' bursa reached its maximum weight at 9 weeks of age, and thereafter a rapid decrease in weight could be observed. As compared with the chick's data by WOLFE et al. (1962), DIETER & BREITENBACH, (1968) and YAMADA et al. (1973), the duck's data showed little weight gain, with the exception of the first few weeks after hatching, and it opposed GLICK's data (1960) of White Pekin duck, in which the bursal weight predominated over the chick's level. The difference may be attributed to differences in breeding conditions.

The bursa of ducks contains a lot of lymphoid follicles in each fold, and the cortex of the follicles is already formed on the day of hatching, while there is no formation of the cortex in the chicken at this time (YAMADA et al., 1973). The number of bursal lymphoid follicles of duck is almost fixed from the day of hatching up to 13 weeks, while the size is apparently changeable with age and coincides with the weight of the bursa. This supports the fact that the gravimetric changes of bursa with age is mainly influenced by the quantitative changes of the lymphoid follicles.

Histological changes in the chick bursa during its natural regression were described by YAMADA et al. (1973). The change which appeared firstly in the duck's bursa was a decrease of epithelial height and hyperplasia of the connective tissue around the follicles accompanied by the pits formation at the epithelium. The follicles then grew smaller as they lost cortex lymphocytes. The cysts formed in the medullary portion were lined by flattened epithelial cells and may have originated from the follicle-associated epithelium. The appearance of these

structures is one of the most apparent signs of bursal regression.

In the present study, the growth of the central lymphoid organs of duck was the most active from 3 weeks of age, and the splenic elements of ducks, mainly the periellipsoidal lymphoid sheaths, were almost completely established at this time (HASHIMOTO & SUGIMURA, read the paper at the meeting of the Japanese Association of Anatomists, 1975).

As for the plasma cells, the existence of the greater number of cells in the interfollicular space of the bursa at 3 weeks of age may be suggestive of the beginning stage of active antibody production in ducks, and the ability may start from the 2nd or 3rd of the posthatching week when the serum gammaglobulin coming from the maternal bird has fallen off, as reported by ISHIHARA & SUGANUMA (1966) in chickens.

From these results, it was suggested that the immunobiological function might be demonstrated from the 3rd or 4th week in the postnatal life, and that the most active function might be after 9 or 11 weeks of age in ducks. Ducks may be effectively used for immunomorphological experiments, however, further studies on the spleens and lymph nodes are necessary to confirm this hypothesis.

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Explanation of plates

Plate I

Fig. 1 Thymus from a day-old duck
Outer zone of densely stained cortex and inner zone of medulla are clearly identified at this stage.
PAS hematoxylin stain × 50

Fig. 2 Thymus from 22 week-old duck
No cortical zone can be seen and many islets of epithelial reticular cells are scattered in the medulla.
HE stain × 50

Fig. 3 Thymus from 22 week-old duck
Numerous granulocytes can be seen along the septa.
Trichrome stain × 80

Fig. 4 Thymus from 17 week-old duck
Granulocytes can be found around the cyst.
Trichrome stain × 120


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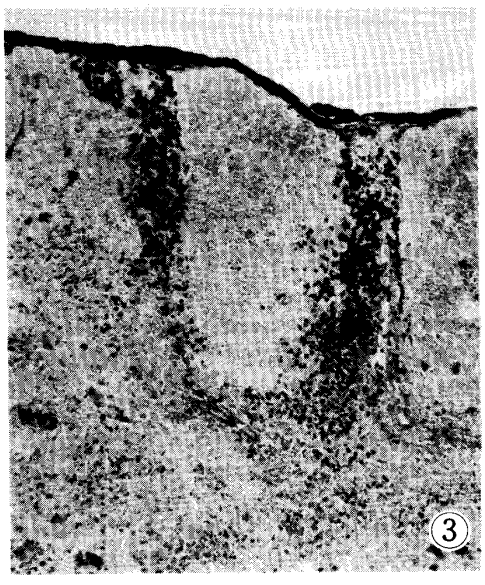
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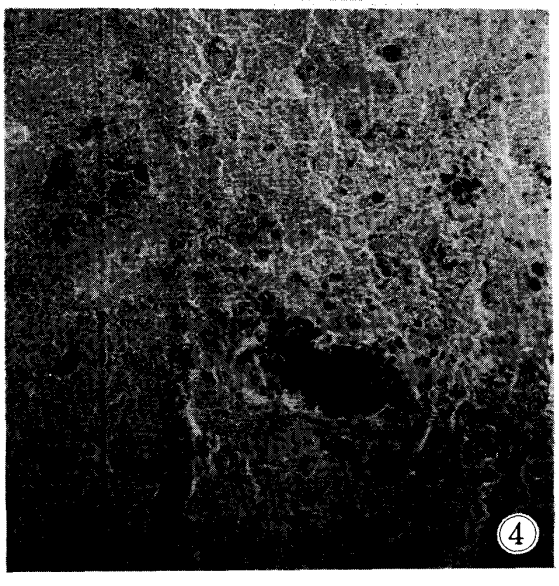
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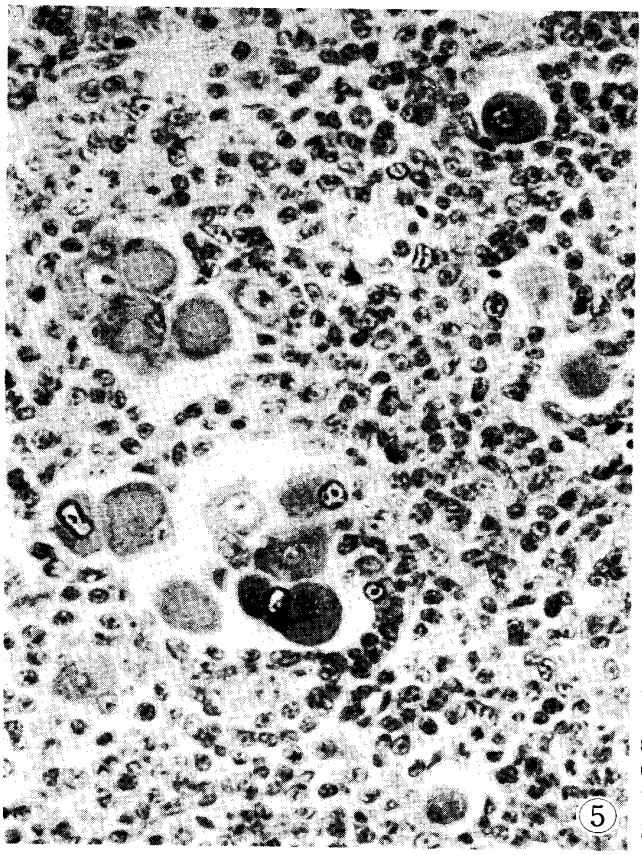
PLATE II

Fig. 5 Myoid cells in the medulla of thymus from 22 week-old duck
They are round to ovoid in shape and usually contain a large nucleus. HE stain × 590

Fig. 6 Small type of cysts in the thymus from a day-old duck
The cysts usually contain a certain amount of PAS positive materials. PAS hematoxylin stain × 590

Fig. 7 Large type of cyst in the thymus from a day-old duck
It consists of a number of epithelial reticular cells and includes plenty of cell debris. Many mast cells are scattered around the cyst. Toluidine blue stain × 300

Fig. 8 Hassall's corpuscle and small cysts in the medulla of 7 week-old duck thymus
The concentric circular architecture of Hassall's corpuscle can be seen; a few small cysts exist nearby.
PAS hematoxylin stain × 590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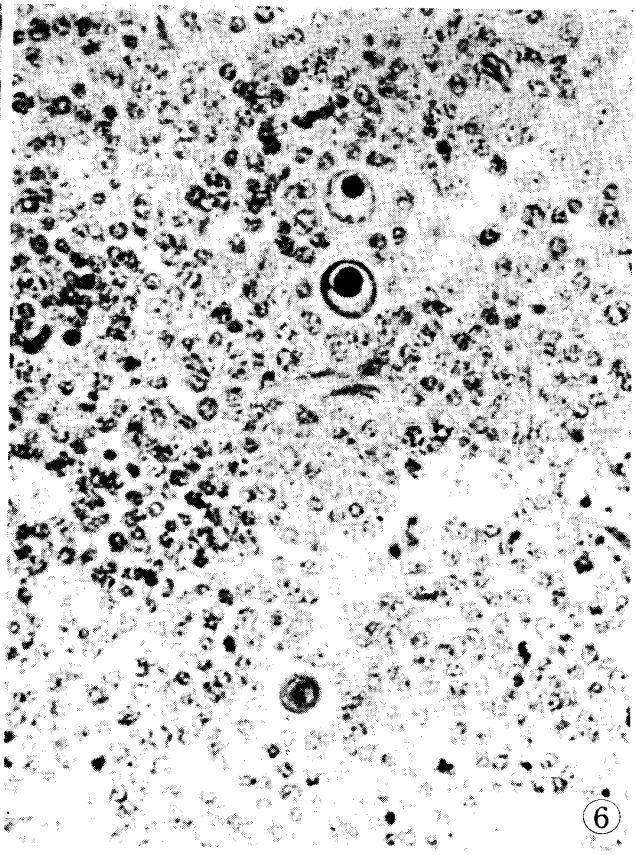
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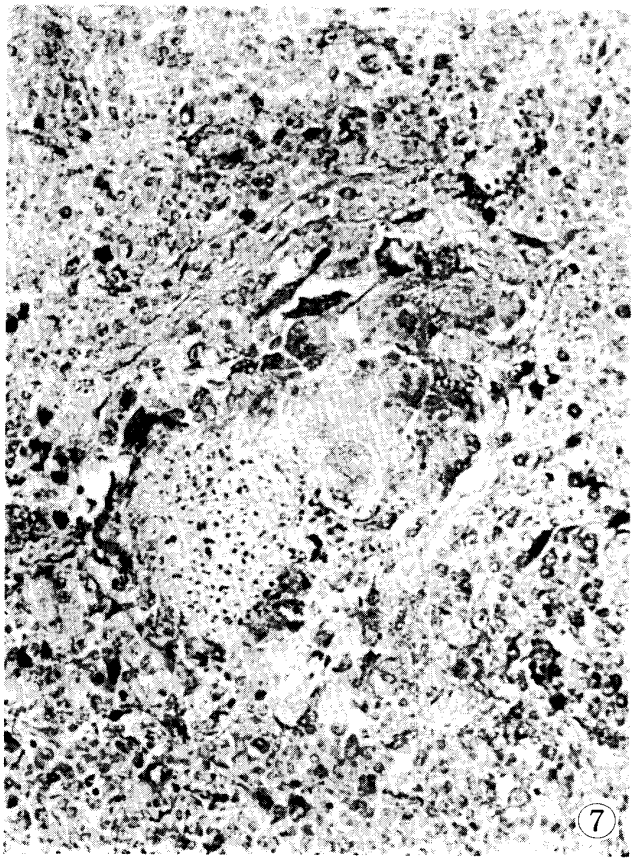
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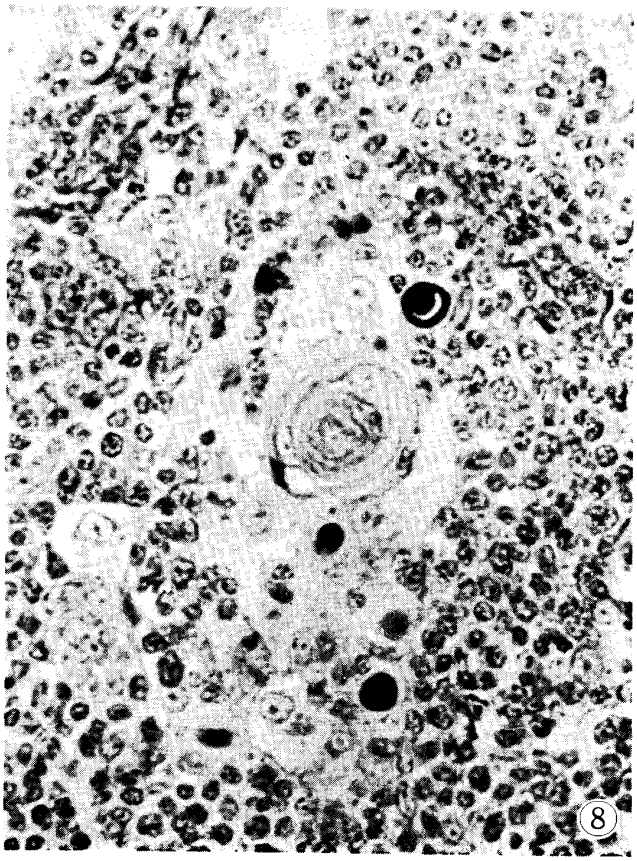
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PLATE III

Fig. 9 Electron microscopic survey of myoid cell from 5 day-old duck
Myoid cell of juvenile ducks have an elongated appearance and contain regularly arranged myofibrils crossed by dense plaques, which correspond to the Z lines in the cytoplasm. × 5000

Fig. 10 Electron micrograph of cyst-forming cell in the duck thymus
A small cyst furnishing with numerous microvilli can be seen and the cytoplasm is filled with a number of mitochondria and dense bodies. × 5000

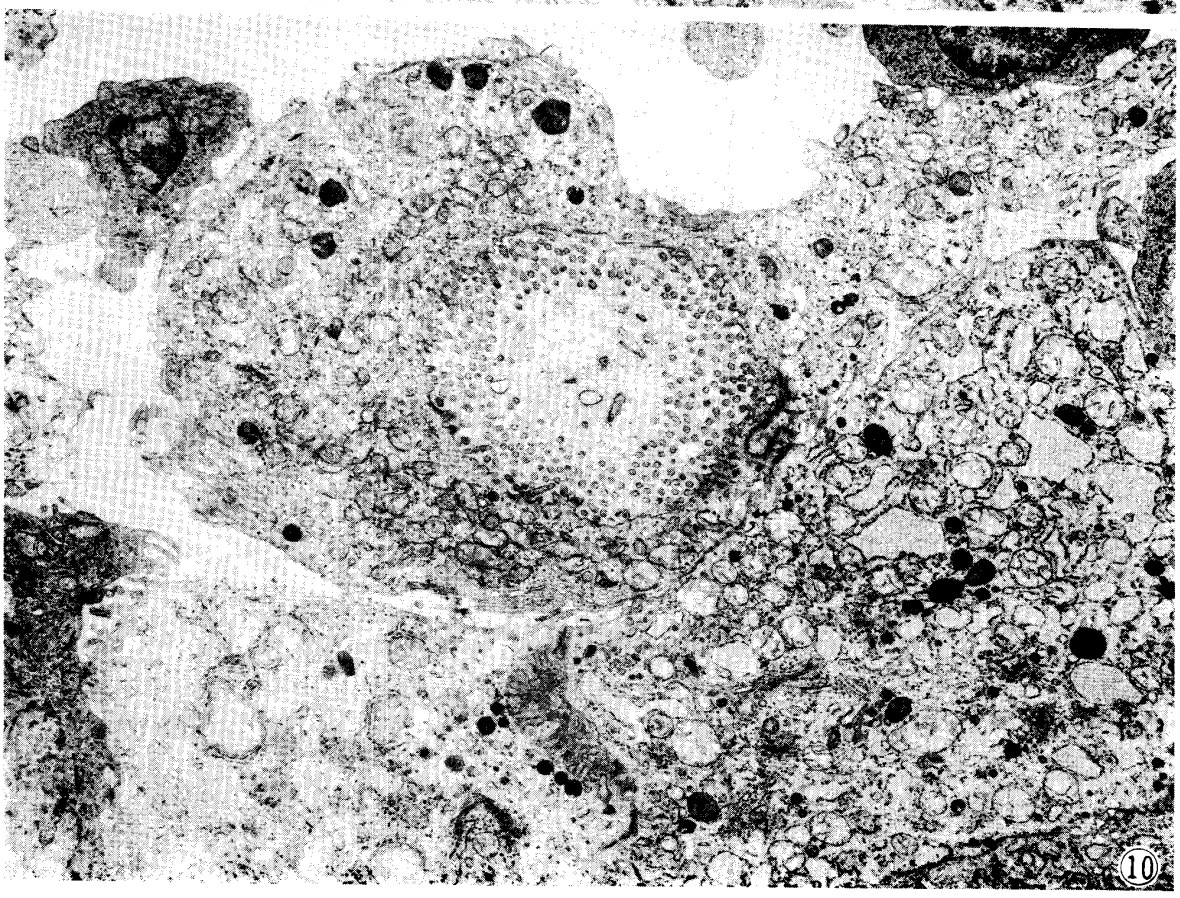


PLATE IV

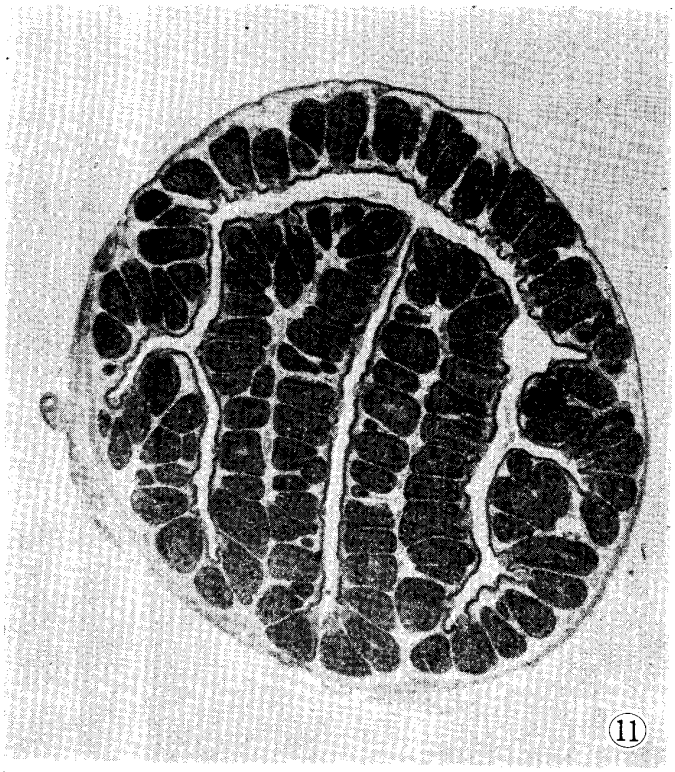
Fig. 11 The bursa from a day-old duck
It has a pair of prominent folds packed with numerous lymphoid follicles. HE stain × 30

Fig. 12 Bursal lymphoid follicles of a day-old duck
The cortex and medulla, and the follicle-associated and interfollicular epithelium are apparently distinguished. Note the marked invasion of reticular fibers in the cortical area.
Silver impregnation × 150

Fig. 13 Bursa in regression stage from 22 week-old duck
A marked hyperplasia of smooth muscle can be seen in the propria mucosa, and the bursal lymphoid follicles are consequently constricted, forming the cyst-like structures in the medullary portion. HE stain × 60

Fig. 14 Cross sectioned view of the regressing bursa from 17 week-old duck Many bursal lymphoid follicles still remain but seem to be pushed away around the outermost muscle sheath as a result of hyperplasia of smooth muscle and connective tissue in the propria.
Note the flattened epithelium and thickened smooth muscle layer. Trichrome stain × 20

Fig. 15 Almost completely regressed bursa from 22 week-old duck
A few irregularly arranged remnants of lymphoid follicles can still be seen, and a part of the propria is replaced by adipose tissue. HE stain × 60



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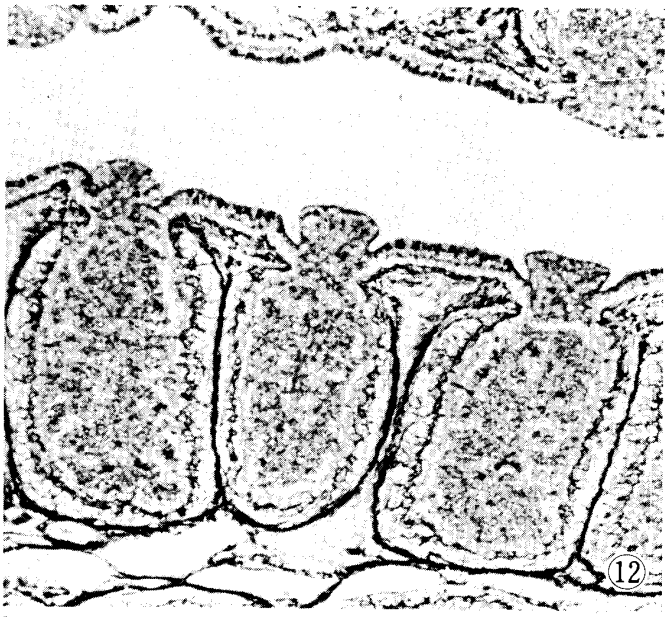

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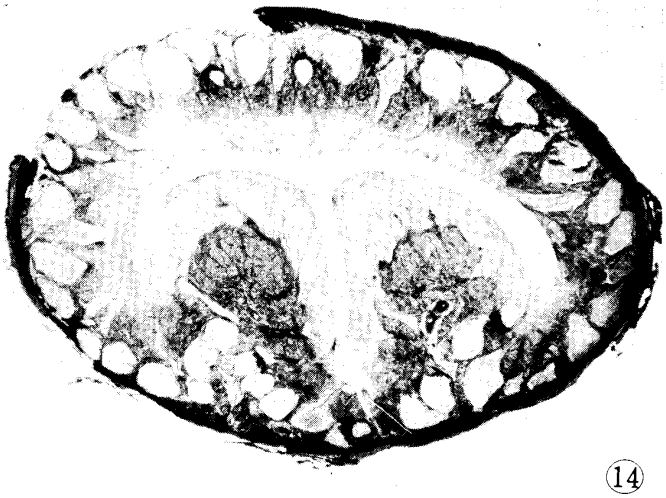

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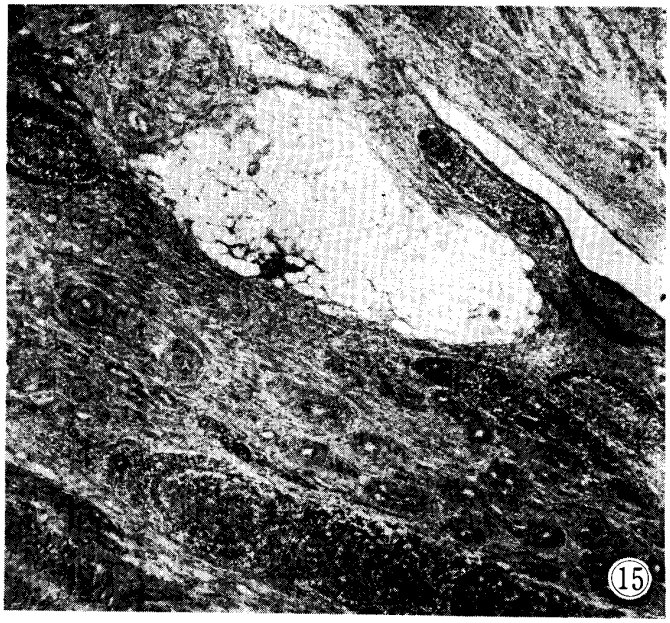

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