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## MORPHOLOGY OF BURSA OF FABRICIUS IN BURSECTOMIZED AND THYMECTOMIZED DUCKS\*<sup>1</sup>

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The morphology of the bursa of Fabricius was described in hormonally bursectomized, surgically thymectomized and control ducks, aged 5 to 7 weeks.

In the control ducks, the bursa showed two prominent longitudinal folds packed with numerous lymphoid follicles. It was clearly demonstrated that the follicle-associated epithelium showed ability to absorb India ink. In the bursectomized ducks, the bursa significantly diminished in weight and size. The histology of the bursa clearly showed an atrophy of folds and a disappearance or diminution of the lymphoid follicles, although this did not always completely inhibit the formation of lymphoid follicles. It was noted that each of the remnant follicles was perfectly normal in structure. In 5 surgically thymectomized ducks, 4 bursae were normal in structure, but in a bursa, lymphoid follicles have almost completely disappeared.

### INTRODUCTION

Bursectomy and thymectomy in chickens have shown several important facts necessary in immunobiology; the bursa of Fabricius is a central lymphoid organ essential to the development of humoral antibody-producing capability, whereas the thymus relates intimately to cell-mediated immunity or delayed hypersensitivity (COOPER et al., '66; WEBER, '72). In the results of tests conducted by numerous investigators, it was established that the avian peripheral lymphatic organs, such as the spleen and caecal tonsil in chickens, have two different independent areas, bursa- and thymus-dependent areas.

On the other hand, the lymph node of some avian species seems to be the most primitive one in the course of the evolution of the lymph node, except for that of some toads (EVANS et al., '66). As the ducks have two pairs of lymph nodes (LINDNER, '61; SUGIMURA, '73), the lymphatic tissues of the ducks

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may be interesting material for phylogenetic studies with special reference to the bursa- and thymus-dependent areas. However, there are only a few reports concerning the bursectomy of ducks (GLICK, '63) and the normal morphology of the bursa in this species (UYEMATSU, '40; WARD & MIDDLETON, '71). In this report, the fundamental morphology and the changes of the organ are described in hormonally bursectomized, surgically thymectomized and control ducks, and the effectiveness of hormonal bursectomy in this species is discussed.

#### MATERIALS AND METHODS

White Peking ducks aged 1 day to 2 months after hatching were used as materials, but observations were focused on the bursae of 5 to 7 week-old ducks.

**Hormonal bursectomy:** Forty-one fertilized eggs were dipped in 2% testosterone propionate ethanol solution for 5 seconds at the 5th incubation-day by means of Glick's method ('63). In consequence, only ten ducks hatched and out of them two ducks died within 4 weeks; only eight ducks aged 5 to 7 weeks were used as materials for the bursectomized group.

**Surgical thymectomy:** Five ducks were surgically thymectomized at the first day of hatching by application of Yamaguchi's method for chickens ('68).

As a control group, 5 ducks, whose eggs were dipped in ethanol at the 5th day of incubation, and 9 untreated ducks were used to compare with the thymectomized and bursectomized ducks.

Natt & Herrick stain ('52) was used for the blood cell count.

The bursa and other lymphatic organs were weighed and then fixed in 2.5% glutaraldehyde, 10% formaldehyde or Bouin's solution, embedded in paraffin, sectioned 5 to 10  $\mu$  in thickness, and stained with hematoxylin-eosin, toluidine blue, Mallory's trichrome, PAS and Gomori's silver impregnation for reticular fibers. Some pieces of the bursa were fixed in 2.5% glutaraldehyde and 1% osmium tetroxide, embedded in the epoxy resin and observed under a JEM-7 transmission electron microscope in the usual way. The other pieces were fixed in 2.5% glutaraldehyde, dehydrated with graded ethanol followed by isoamyl acetate, and then dried in a critical point drying apparatus (HCP-1). The dried specimens were coated with carbon and gold in a vacuum evaporator and observed under a JSM-S1 scanning electron microscope (SEM) using a beam accelerating voltage of 10 kV.

#### RESULTS

##### 1 Body weight and weights of the thymus, bursa and spleen

Body weight and weights of lymphatic organs were estimated in the bursectomized, thymectomized and control ducks aged 7 weeks, as shown in table 1.

TABLE 1 *Body weight and weights of thymus, bursa and spleen and white blood cell count in 7 week-old ducks*

|  | CONTROL (9)                 | THYMECTOMY (5)              | BURSECTOMY (4)              |
|--|-----------------------------|-----------------------------|-----------------------------|
| Body weight  | 1252.2 ± 197.9 <sup>g</sup> | 1096.0 ± 293.5 <sup>g</sup> | 1242.0 ± 230.0 <sup>g</sup> |
| Thymus   | 4.9 ± 0.5                   | —                           | 5.8 ± 2.2                   |
| Bursa  | 1.4 ± 0.2                   | 1.0 ± 0.8                   | 0.4 ± 0.1*                  |
| Spleen   | 1.2 ± 0.1                   | 1.0 ± 0.4                   | 1.0 ± 0.2                   |
| White blood cells<br>per 1 mm <sup>3</sup> (× 1,000) | 19.9 ± 6.7                  | 9.8 ± 1.8*                  | 14.0 ± 2.6                  |

( ): Number of ducks

\*: Mean is significantly smaller than control mean ( $P < 0.05$ ).

No difference was significantly found among the body weights and weights of the thymuses or spleens in the bursectomized, thymectomized and control ducks. The weight of the bursa in the bursectomized ducks was significantly diminished in comparison with that of the control and thymectomized ducks, although out of 5 thymectomized ducks, two ducks each had an atrophic bursa. The white blood cells decreased in number only in the thymectomized ducks as shown in table 1.

## 2 Structural changes in the bursa of Fabricius

In the control ducks, the bursa is long and cylindrical in shape, about 1 cm in diameter and 4 cm in length at age 5 to 7 weeks. In the cross sections of the middle part of the organ, there are two large longitudinal folds in the ventral wall and 6 to 10 small ones at the lateral and dorsal sides (fig. 1). There are numerous lymphoid follicles consisting of the medulla and the cortex in the folds. Cellular elements in the follicles are comprised mainly a series of lymphocytes, epithelial cells and macrophages. In the medulla, which is lined with a sheet of epithelial cells, however, the small lymphocytes are more dominant than in the cortex, which consists mainly of large and medium-sized lymphocytes.

The epithelium coating the inner surface of the bursa seems to be divided into two parts, the interfollicular and follicle-associated epithelium. The interfollicular epithelium is pseudo-stratified columnar, about 40  $\mu$  in height. The epithelial cells are mucous cells with secretory material, which is PAS-positive and stained metachromatically with toluidine blue. On the other hand, the follicle-associated epithelium is a stratified cuboidal or columnal shape and is extended within the medulla. The epithelial cells are acidophil and faintly PAS-positive but not metachromatical (figs. 3, 5 & 20). Under the SEM, circumvallate papilla-like domes are arranged in almost regular intervals on the surface of the

bursal cavity. The dome corresponds to a group of follicle-associated epithelial cells and is 160 to 200  $\mu$  in diameter at age 7 weeks (fig. 19). In the surface view of the dome, the follicle-associated epithelial cells are larger in size than the interfollicular ones (figs. 4 & 9). The former have numerous irregular microvilli, whereas the latter have long, regularly arranged microvilli (figs. 7~11). It was clearly demonstrated that the follicle-associated epithelium had an absorptive ability to ink in the bursa of a 5 week-old duck 12 hours after injection of India ink into the bursal cavity (fig. 12).

In the bursectomized ducks, the bursa significantly diminished in weight and size. The histology of the bursae clearly showed an atrophy of folds and a disappearance of lymphoid follicles, although this did not always completely inhibit the formation of lymphoid follicles; out of 8 testosterone-treated ducks, three bursae had no follicles in the section, but the remainder had some follicles (figs. 13~18). The average number of remnant follicles was 22.9, ranging from 0 to 90 per section, as shown in table 2.

TABLE 2 *Number of lymphoid follicles per section of bursa in 5, 6 and 7 week-old ducks*

|                                 | CONTROL (12)     | THYMECTOMY (5)   | BURSECTOMY (8)   |
|---------------------------------|------------------|------------------|------------------|
| Number of follicles per section | 138.1 $\pm$ 22.1 | 116.6 $\pm$ 63.1 | 22.9 $\pm$ 31.6* |

( ): Number of ducks

\*: Mean is significantly fewer than control and thymectomized means (P<0.01).

It was noted that no structural changes were found in each of the remnant lymphoid follicles either in the histological sections or under the SEM (figs. 17, 18, 21 & 22). As a result of the disappearance of the lymphoid follicles, there were many irregular crypts on the atrophic folds (fig. 13). The ordinary follicle-associated epithelium was replaced by mucous epithelium (fig. 16) and especially in the bursa having no follicles, the epithelium was found to change to a stratified columnar or cuboidal one (fig. 23).

In the thymectomized ducks, no histological changes were basically found in their bursae (figs. 2 & 6). Out of five surgically thymectomized ducks, however, the bursa of one duck showed a decrease in size and an almost perfect disappearance of lymphoid follicles was detected (fig. 24). The detected histological changes were similar to those of the hormonally bursectomized ducks, but a more conspicuous formation of crypts and villus-like protrusions was noted.

## DISCUSSION

The present study has concentrated on the efficiency of testosterone to bursectomy and the morphological changes in the bursa of Fabricius in the ducks.

The bursa of this species is basically similar to that of chickens (HOFFMANN-FEZER & LADE, '72; YAMADA et al., '73) with the following differences. The bursa of ducks has two prominent folds packed with numerous lymphoid follicles and is coated with a pseudostratified columnar epithelium, as reported by UYEMATSU ('40) and WARD & MIDDLETON ('71). The epithelial layer of the bursa is clearly distinguished into two, follicle-associated and interfollicular, with different morphology and function (BOCKMAN & COOPER, '73; BOSCH, '68). It was also clearly demonstrated in the ducks that the follicle-associated epithelium had an absorptive ability in this experiment, although this fact was already reported in the study on chickens (BOSCH, '68).

The morphology of the bursa in testosterone-treated chickens is described by HOFFMANN-FEZER & LADE ('73), but that of ducks is treated only in GLICK's report ('63), in which the absence of lymphocytes is briefly described. In testosterone-treated chickens, varying structures from no bursa to a bursa, which was morphologically normal, were detected; observed structural changes showed a marked reduction in size, an indistinct formation of bursal folds, and a reduction of number of lymphoid follicles (HOFFMANN-FEZER & LADE, '73).

An atrophy of the folds in testosterone-treated ducks was one of the most noteworthy changes, because the duck bursa has two prominent folds on the ventral side. The other changes were similar to those described in testosterone-treated chickens. Out of 8 treated ducks, there was no follicle in the bursae of 3 ducks, whereas other bursae had more or less typical lymphoid follicles. It was noteworthy that no change was found in each of the remnant lymphoid follicles in either the cellular components in the cortex and medulla or in the structures of the follicle-associated epithelium under the SEM.

WARNER & BURNET ('61) pointed out that with early treatment of testosterone the development of the epithelial sac is inhibited by the lympholytic action of the hormone, whereas at a later stage of incubation the bursa shows complete replacement of the follicles by crypts with a polypoid growth of the epithelium, or regenerates into normal lymphoid follicles. In the present observation of ducks, the development of the bursa was not completely inhibited even if it was treated in an early stage.

MOORE & OWEN ('66) suggested that testosterone treatment does not limit the supply of progenitor lymphoid cells but probably alters the environment in which they proliferate and mature. BOCKMAN & COOPER ('73) pointed out that

lymphoid cells are seen earlier than the specialized follicle-associated epithelium in the embryonal period of chickens.

The present writers paid attention to preserving normal structures in the remnant lymphoid follicles and the intact follicle-associated epithelium in testosterone-treated ducks in spite of replacement of the specialized epithelial cells by mucous epithelial cells. From the obtained and reported facts, it is suggested that early testosterone treatment may be effective in inhibiting the invading of precursor lymphoid cells into the bursal epithelial primordium, although it may change the epithelial environment and inhibit the proliferation of lymphoid cells, as suggested by MOORE & OWEN ('66). It is also clear that the formation of the specialized follicle-associated epithelium is intimately related with the presence of lymphoid cells in the epithelium, because the follicle-associated epithelium is replaced by a mucous one when the lymphoid cells of the bursa were destroyed by cyclophosphamide treatment of posthatching ducklings (SUGIMURA et al., '74). The epithelial primordium may differentiate into the follicle-associated epithelium because of the presence of lymphoid cells in the primordium and then may form a specialized environment for the maturation of B cells.

Testosterone treatment in the ducks did not always completely inhibit the formation of lymphoid follicles, as mentioned above, and there was still an abnormally high mortality rate. The present writers were not concerned with the immunological significance of the bursa in this experiment, but they believe from the obtained results that another method, such as cyclophosphamide treatment, is better for this kind of experiment in ducks (SUGIMURA et al., '74).

Finally, out of 5 surgically thymectomized ducks, the bursa of one duck showed an almost perfect disappearance of lymphoid follicles. This may be due to an accidental atrophy of the lymphoid follicles by other unknown factors. However, HOSHI & MORI ('73) pointed out that germinal centers in the spleen disappeared in x-ray irradiation of the thymus in chickens. It remains a possibility that the bursa is also controlled by the thymus if some factors are added. However, further investigations will need to clarify this suggestion.

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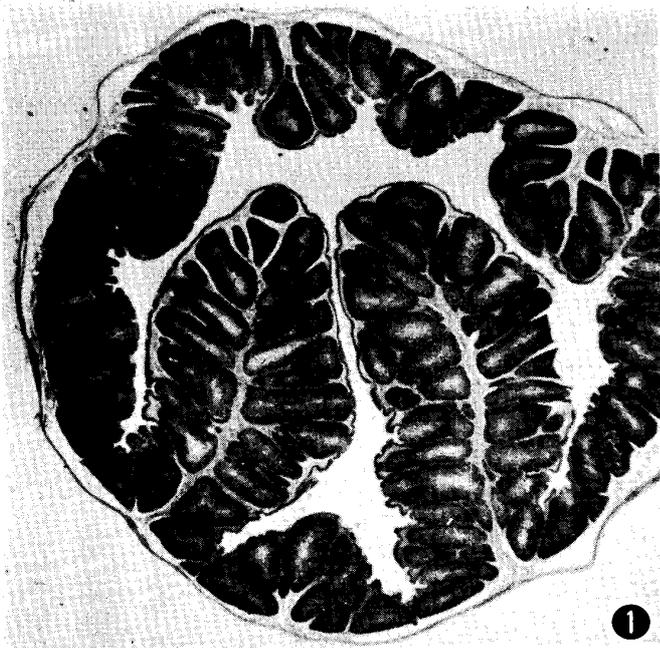
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## EXPLANATION OF PLATES

### PLATE I

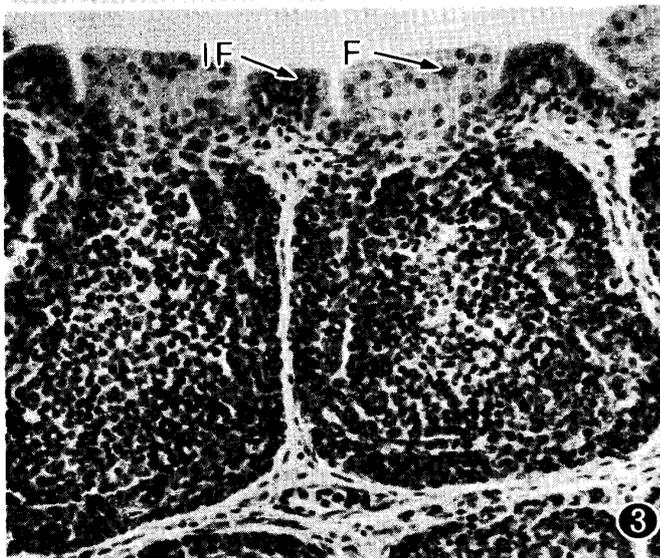
- Fig. 1 The bursa of a control duck, age 7 weeks, has two prominent folds packed with numerous lymphoid follicles. Hematoxylin-eosin  $\times 11$
- Fig. 2 The bursa of a thymectomized 7 week-old duck  
This bursa is somewhat diminished in size, but is normal in structure. Hematoxylin-eosin  $\times 11$
- Fig. 3 The bursa of a day-old control duck  
The cortex and medulla in the lymphoid follicles are clearly distinguishable. Note the difference between follicle-associated (F) and interfollicular epithelial cells (IF). Hematoxylin-eosin  $\times 210$
- Fig. 4 The bursa of a day-old control duck under the SEM  
There are numerous circumvallate papilla-like domes which correspond to follicle-associated epithelium (F). Follicle-associated epithelial cells are larger in size than interfollicular ones (IF).  $\times 270$
- Fig. 5 Lymphoid follicles in the bursa of a control duck, age 7 weeks  
The histological findings are basically similar to figure 3, but the follicles are 3 times larger. Hematoxylin-eosin  $\times 83$
- Fig. 6 The inner surface of the bursa of a thymectomized 7 week-old duck under the SEM  
Papilla-like domes of follicle-associated epithelium (F) are surrounded by another groove. The structure does not differ from that of the control.  $\times 90$



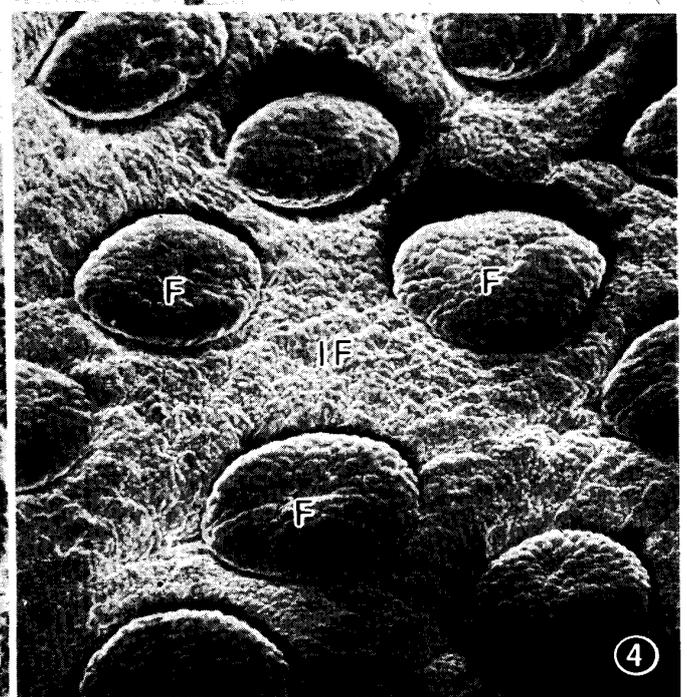
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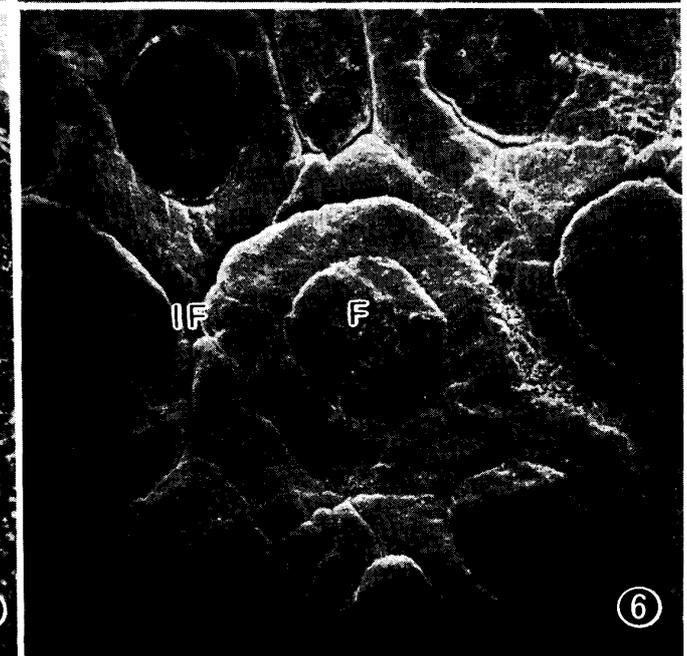
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PLATE II

- Fig. 7 The bursa of a control 5 week-old duck  
Interfollicular epithelial cells have numerous vacuoles with a mucous substance. Note the regular, long microvilli on its apical cell membrane.  $\times 15,000$
- Fig. 8 The bursa of a control 5 week-old duck  
Follicle-associated epithelial cells have numerous irregular microvilli. Note the numerous small vesicles and mitochondria in its apical cytoplasm.  $\times 15,000$
- Fig. 9 Follicle-associated and interfollicular epithelium in the bursa of a day-old duck under the SEM  
The former has numerous irregular microvilli, whereas the latter has regular microvilli (arrow).  $\times 2,700$
- Fig. 10 The bursa of a day-old duck under the SEM  
Note numerous irregular microvilli on the follicle-associated epithelium.  $\times 2,700$
- Fig. 11 Follicle-associated epithelium in the bursa of a 7 week-old control duck under the SEM  
A few mucous cells (M) with regular microvilli are found among the follicle-associated epithelial cells.  $\times 2,700$
- Fig. 12 A lymphoid follicle at 12 hours after injection of India ink into the bursal cavity of a control 5 week-old duck  
Ink particles (arrows) are found in the epithelial cells and medulla. Note a cyst in the follicle-associated epithelium.  $\times 420$

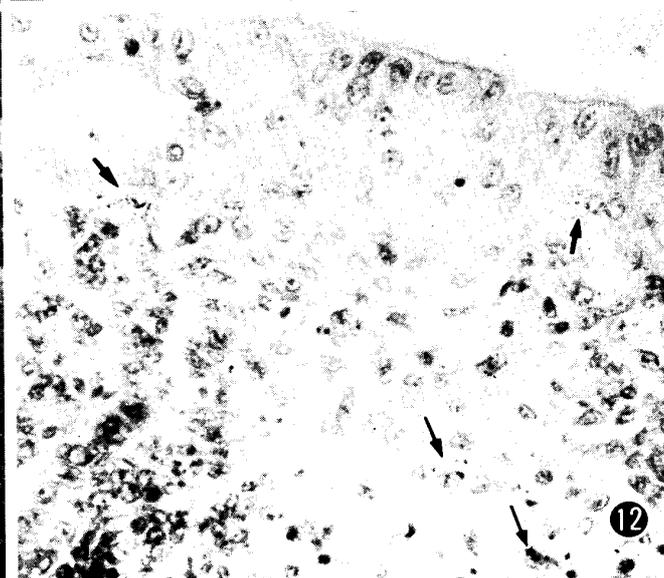
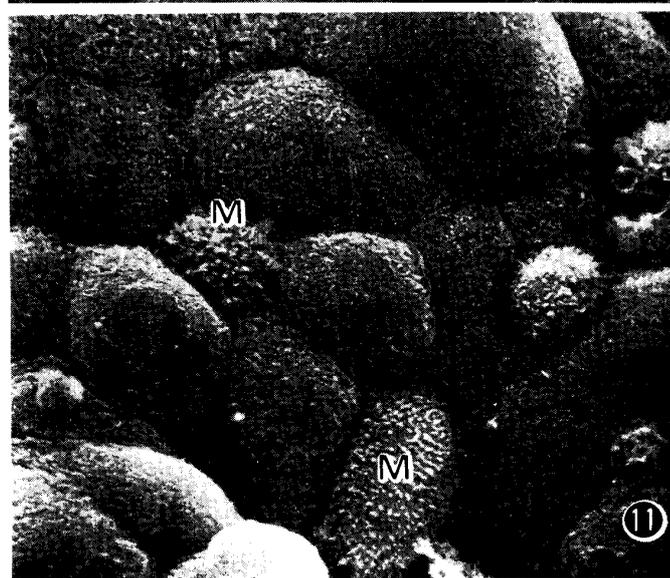
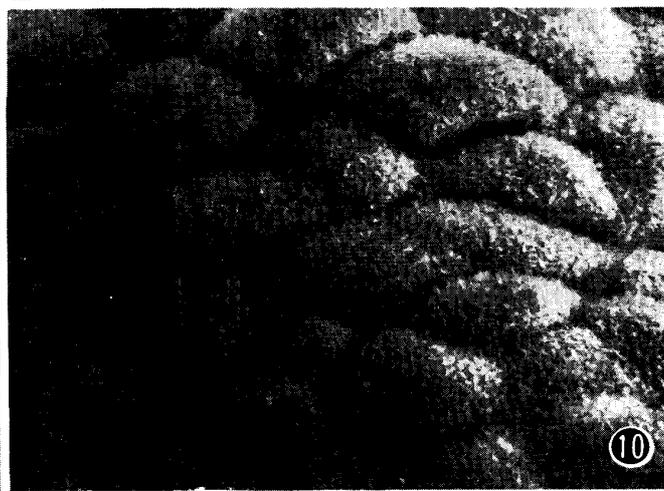
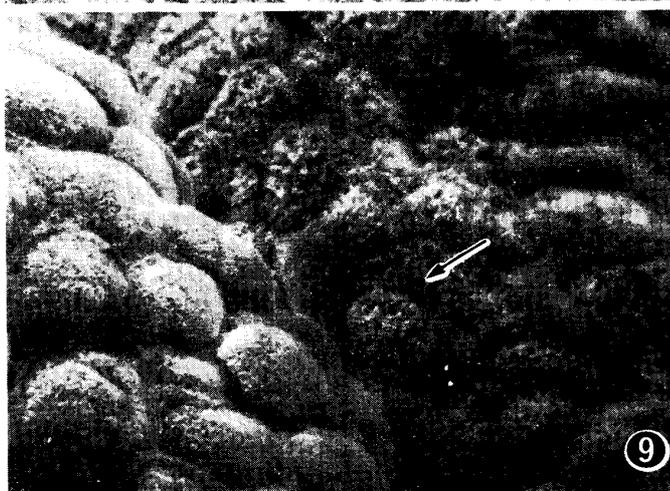
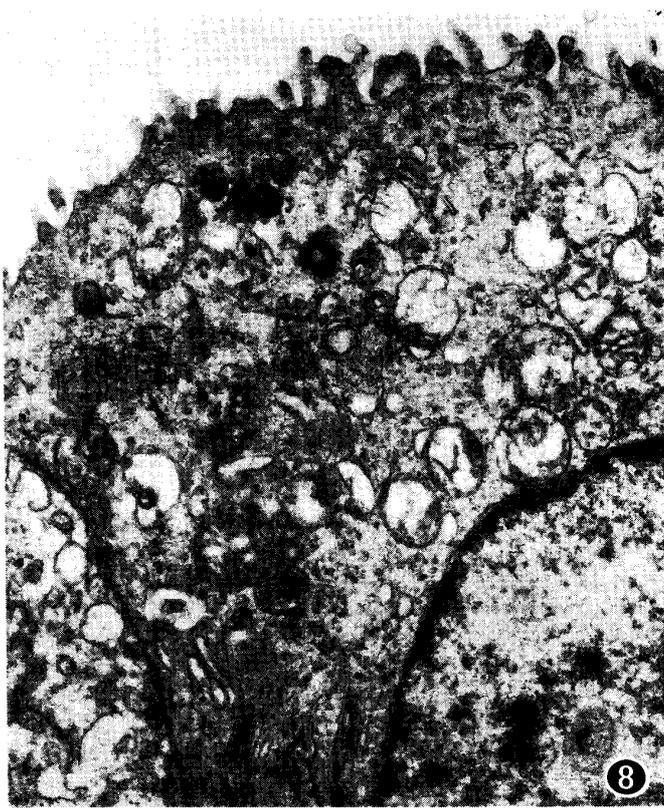
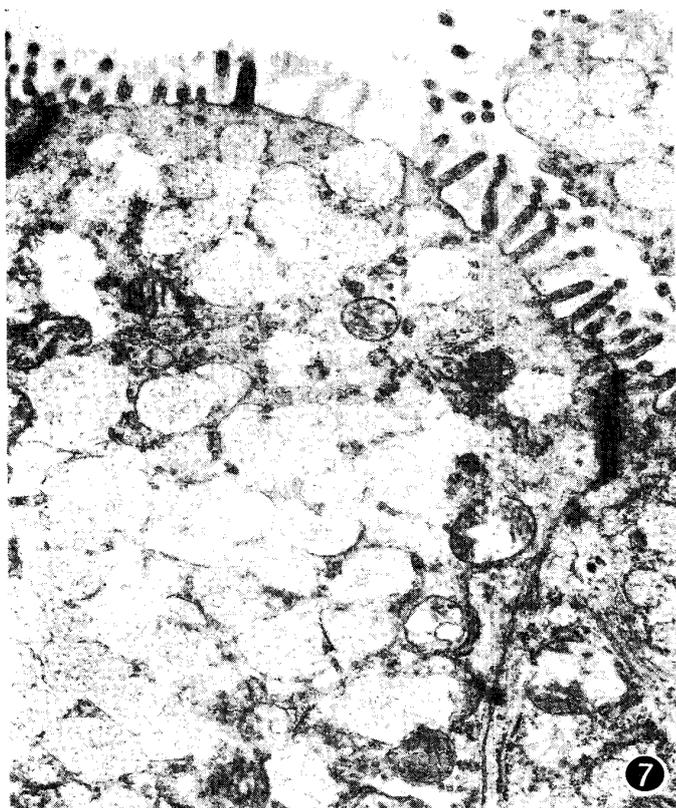


PLATE III

- Fig. 13 The bursa of a hormonally bursectomized 5 week-old duck  
Formation of lymphoid follicles and folds is perfectly inhibited.  
Hematoxylin-eosin  $\times 11$
- Fig. 14 The bursa of a testosterone-treated 7 week-old duck  
In this case, numerous remnants of follicles are found, although  
the number of follicles clearly diminishes.  
Hematoxylin-eosin  $\times 11$
- Fig. 15 The bursa without remnant follicles under the SEM  
No circumvallate papilla-like dome is found.  $\times 27$
- Fig. 16 A part of figure 13  
Replacement of follicles by epithelial crypts is observed.  
Hematoxylin-eosin  $\times 33$
- Fig. 17 The bursa with remnant follicles in a 6 week-old hormone-treated  
duck under the SEM  
Two follicles (F) with papilla-like dome are observed.  $\times 90$
- Fig. 18 A part of figure 14  
Note the small number of follicles and crypts.  
Hematoxylin-eosin  $\times 33$

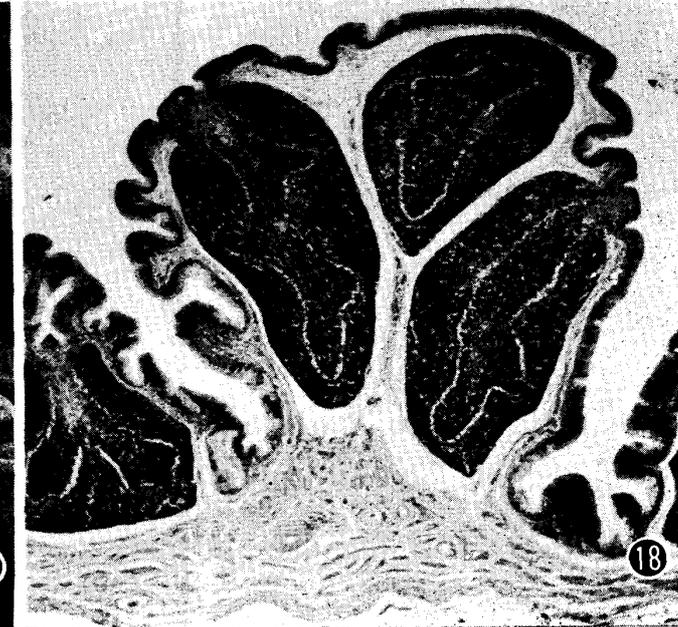
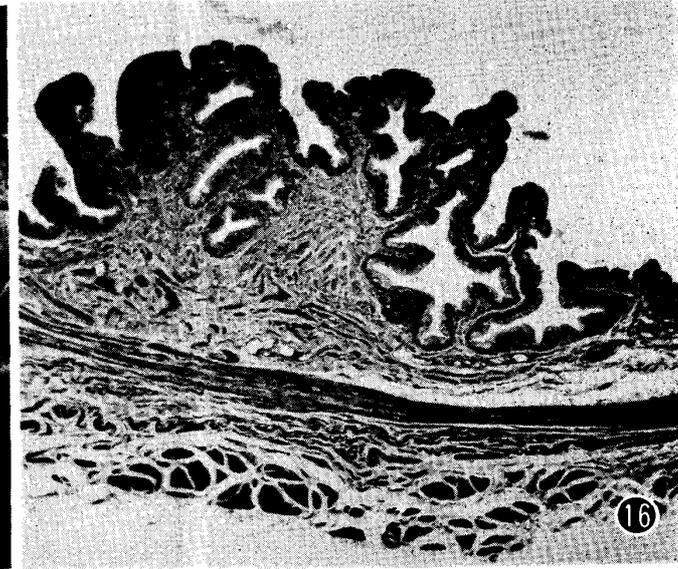
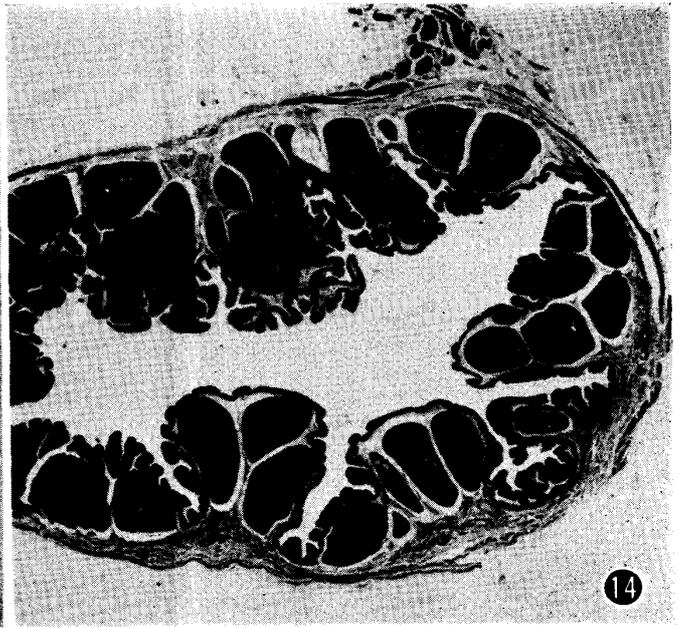
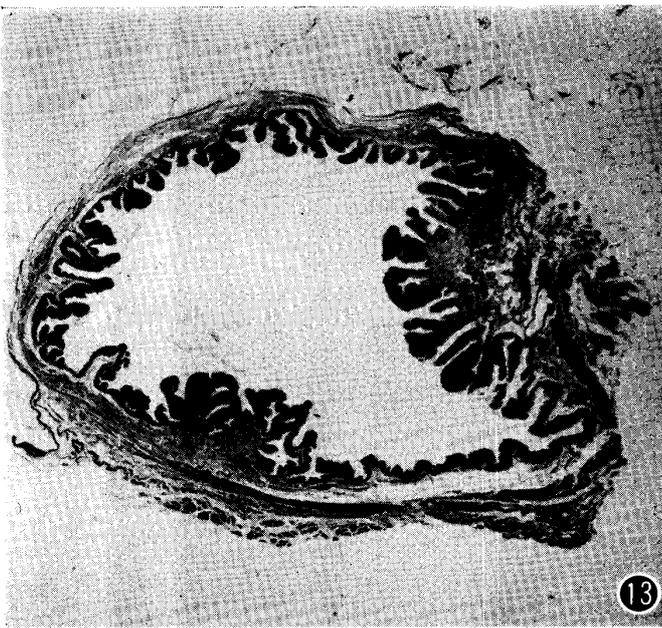


PLATE IV

- Fig. 19 A follicle (F) in the bursa of a 7 week-old control duck under the SEM  
Follicle-associated epithelial cells are larger than interfollicular ones.  $\times 270$
- Fig. 20 Histological section corresponding to figure 19  
Follicle-associated epithelial cells are acidophil.  
Hematoxylin-eosin  $\times 330$
- Fig. 21 A remnant follicle in the bursa of a 6 week-old hormone-treated duck under the SEM  
The surface structure of the remnant follicle is basically similar to that of the control.  $\times 270$
- Fig. 22 A follicle in the bursa of a 7 week-old hormone-treated duck  
Histological structure seems to be normal.  
Hematoxylin-eosin  $\times 83$
- Fig. 23 The bursa of a hormone-treated duck at age 6 weeks  
Note a stratified columnar epithelium and a thickening of muscular sheet. Hematoxylin-eosin  $\times 83$
- Fig. 24 The bursa of a surgically thymectomized 7 week-old duck  
Formation of lymphoid follicles is inhibited. Note polypoid formation of epithelium and a remnant follicle (arrow).  
Hematoxylin-eosin  $\times 16$

