Histological Observation on a Specimen of the Japanese Eel, *Anguilla japonica*, under the Long-term Starvation*

By

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The breeding place of the Japanese eel, *Anguilla japonica*, is located about 1,200 miles far from Honshû, the mainland of Japan[1),2)]. The route from Honshû to that place is surrounded by the complicated oceanographical conditions or environments, and to arrive at that place, there arises a problem in the activity and vital force of no fed eel. In order to know the tolerance of the mature eel to the long-term starvation, this experiment was carried out. Hence, histological examination was performed on this starved specimen to elucidate the changes occurring in various organs and tissues, and the results obtained are described in this paper.

Material and Methods

The eel used in this experiment was caught by the fish-trap in the Misumi River, one of the upper streams of the Chikugo River, at Kuma, Hida City, Oita Prefecture, on October 2, 1969. It was the descending eel measuring about 457 mm in body length and 382 g in body weight. The body color was green-black in dorsal and lateral sides, and green-white in ventral one. After carrying this eel to Shimonoseki University of Fisheries, the animal was kept in the vinyl aquarium (62 x 42 x 20 cm) filled with sea water, which was set up in the constant temperature room regulated at 20°C, and was exposed to the light of about 50 lux in the daytime. During the period from October 5 to December 4, the eel was administered the hormones, such as Gonatropin and Prinogonyl, by six times of injection, although each injection was given intramuscularly every ten days. Since this eel was caught, it has never taken any of food. During the course of experiment, the rearing water was not exchanged but always aerated. The eel died at last on June 17, 1971, being continued to live for 522 days under the

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severe starvation. Thus, the animal was fixed with Bouin's solution for further histologic examination, and observed macroscopically at the outset.

Total length of this specimen looking heavily emaciated is 440.0 (mm), eye diameter is 7.0 in longer axis and 3.5 in shorter, girth at dorsal base 33.0, and weight of body 235 (g) (Fig. 1).

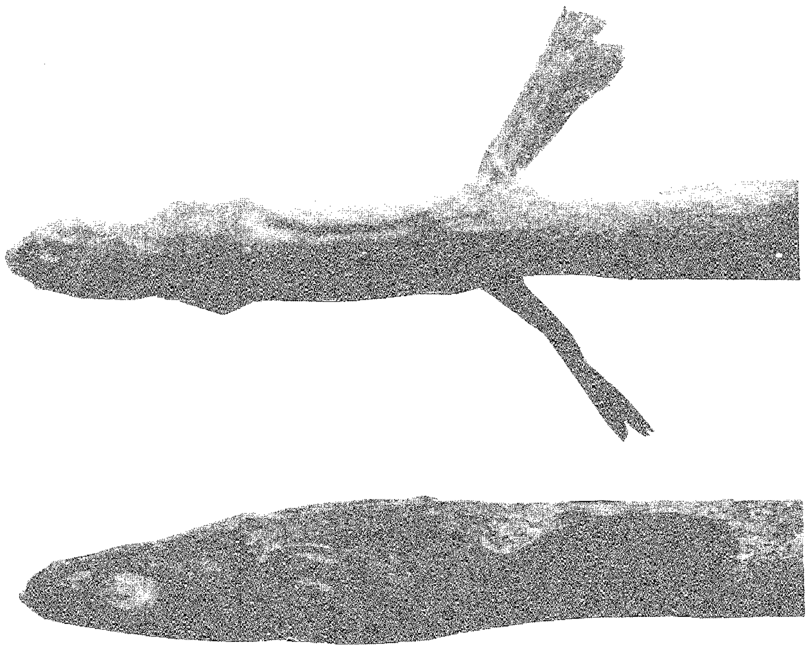

Fig. 1. General appearance of a Japanese eel, *Anguilla japonica*, under starvation for twenty months. 440,0 mm long. A male specimen died on June 17, 1971.

Body is reduced to only skin and bones with comparatively larger eyes. Pectoral fins, black in color, also look considerably large. Although this eel was starved for long time, there is found a small quantity of feces in white tint throughout the digestive canal. Several organs and tissues were then removed, dehydrated, embedded in wax, cut serially at 8 μ thick, and stained with azan trichrome, hematoxylin-eosin and aldehyde fuchsine (AF). Unfortunately, however, on account of fairly long time required to reach the interior part, histologic condition of the hypothalamo-hypophysial complex and other organs revealed considerable postmortem decomposition. Of course, to obtain good fixation, an incision was made to the mid-line of the belly.

Results

Hypothalamo-hypophysial complex

In spite of almost all normal appearance of the brain, a peculially pathological feature was detected in the pituitary structure: as the gland expanded remarkably its volume, a large cyst produced in the rostral part was easily seen by naked eye. By sagittal plane, it was recognized that the cystic space was confined in the rostral pars distalis, although the part swelled in a great degree like that of mature eel caught in the depth of the sea[3] (Fig. 2). The follicles that were the characteristics of the rostral pars distalis of the eel pituitary, and seemed to consist of the prolactin producing cells were still retained in the peripheral region as an exceedingly small number (Fig. 3). Near these follicles, a number of chromophobic cells under the collapse and degeneration were found (Fig. 4). It is likely that this cystic space as a large cavern was produced by a severe necrosis of follicular cells, and the foamy structures were demonstrated in this cyst by AF stain, while achromatic by means of azan stain (Fig. 3).

The proximal pars distalis consisted chiefly of acidophilic cells, many of which were in the course of collapse after expansion of the size. Even in some of the cells that remained in considerably normal appearance, the cytoplasmic granules with dark tint were scarce. A small number of basophils of smaller sizes scattered over the acidophils were also seen, although it was not decided that they correspond to the gonadotropic cells. The pars intermedia was occupied with basophils, the size of which is smaller than that of the acidophils located in the proximal pars distalis (Fig. 5). The pars nervosa contained a large amount of AF positive granules in various sizes. Near the basal region of the gladular cells of pars intermedia, these granules were concentrated heavily, and sometimes the granules of exceedingly larger sizes were encountered. In the dorsal region of proximal pars distalis and just beneath the recessus infundibuli, a number of AF-positive droplets were also seen. There were found the paucity of the capillaries, sinusoids and basophilic fibers surrounding these vascular system.

It was impossible to describe the hypothalamic nuclei, i. e., the nucleus preopticus and nucleus lateralis tuberis, because the pictures were unsuitable to pursue the detail of these neurosecretory cells in the state of deterioration. Further, no secretory material was encountered in the lumen of the saccus vasculosus.

Thyroid

Although the fixation of the thyroid gland was fairly bad, the pathological and regressive figures like those of the radiothyroidectomized gland were seen. A less degree of density of follicles of various sizes was noticed, while a marked increase in the loose connective tissue was recognized. The colloid in the lumen did not show the affinity with dyes. Some of the follicles were in the state of atrophy, but the epithelia were constructed from the cubic cells (Fig. 6).

Liver

The greater part of this tubular compound gland, in particular the peripheral one, was degenerated, while a small amount of tissue still remained active (Fig. 7). The tunica serosa increased its thickness greatly here and there. The tunica fibrosa stained with aniline blue deeply was also thick and undulated. Some of the hepatic cells in healthy condition showed a polyhedral contour with granular cytoplasm, and the nuclei, small in size and round in shape, had the chromatin substances scattered like the axles. The space formed between the cell cords was separated from each other, and the connective tissue (=tunica fibrosa) was noticed. Although the terminal bile duct was indistinct, there were found the large lacunae scattered here and there, containing the fine granular substance. This substance seemed to be derived from the cytoplasm just under degeneration and collapse. The masses of yellow pigment granules considered as lipofuscin were detected (Fig. 7). In some places the development of the interstices between the cell cords were remarkable, while in some other places the substances in fine granules remained between the tunica fibrosa like seminal lobules that contained the mature sperm.

Spleen

A remarkable diminution of the volume of the spleen was noticed: the body indicating the shape of the club was 10 mm long and 2 mm wide. It was difficult to recognize the blood cells, i. e., the contents of parenchymatous splenic pulp, and the architecture consisting of the trabecular net mostly remained (Fig. 8). Accordingly, the collagenous and elastic fibers which made up the trabeculae, the walls of vessels and splenic sinus showed the framework of adipose tissue. There were recognized the debris of degenerated blood cells, in rather larger masses with yellowish tint, scattered in some of the vessels and related portions. The capsule increased its thickness, and the mesothelium in the condition of heavy undulation indicated the feature of fibrous strands (Fig. 9).

Pylorus of stomach

The mucous epithelium without cuticle degenerated heavily, and looked like a string, while the fibrous submucosa and tunica muscularis mucosae in the smooth condition were still thick. It was difficult to see the pyloric gland and lamina propria, although a number of ring-like vesicle were detected, which seemed to be derived from the remnant of faveolae gastricae (Fig. 10). It was also almost impossible to see the cellular elements of blood.

Fundus of stomach

As degeneration of the mucous epithelium and gastric gland were drastic, the gastric mucosa as a whole was coarse and loose, and looked like a network of connective tissue origin (Fig. 11). The epithelium without the striated border was flat, and the nucleus was shifted long along the circular direction. The reduced lamina propria was occupied by an extended lumen derived from the gastric gland. In spite of a marked reduction, only the framework of connective tissue remained well, accompanying a small number of blood cells. The artery containing the paucity of blood cells was seen everywhere without difficulty (Fig. 12).

The circular and longitudinal muscle layers and outer adventitia appeared to be comparatively normal. As a heavy regression of muscle fibers occurred, the contour of nuclei was distinct relatively.

Intestine

Regressive changes in the mucous epithelium of the intestine was also conspicuous. The lumen of the intestinal canal was spacious, because the epithel was reduced heavily in the string-like structure (Fig. 13). As almost all the cells degenerated, the capillaries remained to be the loops or rings showing somewhat similar feature of amphibian lung alveoli. A small amount of the lamina propria was still encountered. The rate of regression of the submucosa and muscle layers, in particular the inner circular one, was heavier than that of catadromous eel, and the vacuoles were seen here and there in the submucosa (Fig. 13).

Gall bladder

The gall bladder had already burst before dissection was made. This seemed to depend largely on a great expansion of the bladder wall. As a result, visceral organs in the vicinity of this bladder were stained with the bile as green tint. The epithelial cells degenerated until the condition of yellow granules, while the lamina propria, smooth muscle layer and adventitia fell into a thin layer as a whole looking like a strand (Fig. 14). This strand was in great undulations with a small amount of bile contents.

Pancreas

Almost all exocrine pancreas degenerated, but the granules of various sizes were retained (Fig. 15). None of acini were detected, while the loose connective tissues which seemed to be derived from the inter lobular septa were seen. On the other hand, the islets of Langerhans increased their number, though the shape of them was rather long ovoid in contrast to the round in normal fish. However, it was impossible to demonstrate the cell types and staining affinities for dyes, because the regressive changes of these endocrine cells was so heavy (Fig. 16). Accordingly, the islet cells were all stained uniformly with acidic dyes, and the vessels and connective tissue elements were barely seen. Due to these degenerative changes, the islet indicated a similar structure of the network.

Testis

The testis as a lobulated and corrugated organ was also atrophied markedly, indicating somewhat the testis of immature fish. The lobule was a fan-shaped or semicircular thin body. Increase in the thickness of the tunica albigenea and its derivatives, i. e., the fibrous septa invading deeply into the interior of the lobule, was recognized (Fig. 17). The fibrous septa considered as previous cyst wall ran zigzag forming a number of labyrinthine cysts. There were found a considerable number of blood corpuscles and their debris in the lumen of the cyst, but none of the germ cells were encountered. On the other hand, one to several number of cells that were thought to be the germ cells were seen in the fibrous membrane (Fig. 17). The cytoplasm of the cell was stained differentially as inner and outer strata.

Corpuscles of Stannius

As described previously, the corpuscles of Stannius of the eel are small encapsulated organs located on the ventral surface of the kidney[3,4]. The corpuscle consisted of aggregates of numerous ovoid alveoli which anastomosed with each other. However, the rate of anastomosis of this specimen was not so marked as in the freshwater eel and the catadromous eel (Fig. 18). The height of the cell in the pyramidal shape that constitutes the alveolus was also lower than that of the other eel reported previously. The cytoplasm of the cell had acidophilia, and the chromatin in the nucleus showed the feature of the axle-tree. It was noticed that the lumen of the alveolus was wide and spacious. However, no prominent degeneration was recognized in this corpuscle (Fig. 18).

Kidney

A marked regression in the hemopoietic tissue, i. e., decrease in the number of lymphocytes, was noticed. The glomerular tuft was atrophied remarkably, condensed, and stained deeply, showing a similar change of sclerosis (Fig. 19). Masses of large, dark granules that were considered to originate in melanin granules were seen here and there (Fig. 20). Although the detail of the striated border was indistinct, the cell of the proximal segment was difficult to stain, having a scanty cytoplasm and a round, deeply stained nucleus. A boundary of each cell was also indistinct. In the distal segment consisting of cubic cell with comparatively rich cytoplasm stained well with eosin, a considerable number of dots were recognized. It is natural to consider that they were the pycnotic nuclei. The nucleus, round in shape, occupied the central part of the cell, and stained deeply with hematoxylin. Further, the free surface of the cells facing the lumen was rugged, and the large vacuoles were occasionally seen in the cell.

Heart

Not so severe, deteriorate change was detected in the heart including both cardiac muscles and blood cells (Fig. 21).

Discussion

Reports dealing with the histologic examination of the fishes under the long, enforced starvation are very scarce, while papers on the physical changes in relation to the chemical constituents are comparatively many. In the case of the eel, the authors could not aware of a written paper on the histologic condition. Apart from this, BOËTIUS and BOËTIUS[5] described that one of the male eels *(Anguilla anguilla)* of different experimental age was able to mature normally after a period of starvation for over three years. According to their unpublished data, the record so far is held by a male silver eel which survived for 1515 days (over four years) at 15°C without food, during which time it lost no less than 76% of its weight. The specimen of *Anguilla japonica* described in this paper was kept at about 20°C. Even in the enforced starved condition, it is no doubt that at a lower temperature some of the species of fishes can survive and hold out for even longer[6-8]. On the other hand SMALLWOOD[9] reported a case of *Amia calva* that could survive for 20 months without food, the period of which is the same in the present case of the Japanese eel.

Drastic changes occurring regulary in the organs and tissues of the species which die soon after spawning have been described on both anadromous and catadromous fishes[3,10-14], and nearly similar changes were induced experimentally by means of androgen injection[15]. The pictures of these maturing fishes are considerably different from those of the present eel of twenty months' starvation. SCHNAKENBECK[16] and BERNDT[17] described the regressive changes in the various parts of the digestive canals accompanying the sexual maturation of the catadromous eel (*Anguilla anguilla*) that ceases eating. However, the rate of regression of the digestive organs in the present eel is more conspicuous and drastic. CREACH and COURNÈDE[18] investigated the weight loss of various organs in the carp (*Cyprinus carpio*) during enforced starvation, and observed that after eight months the liver and kidney are most affected, spleen and intestine less, and heart hardly at all. In the present material, both the brain (except pituitary) and the heart, indicated nearly normal appearance. Therefore, the brain and the heart are allowed to retain virtually all of their chemical and structural constituents and nearly unimpaired function.

A striking deterioration occurring in the endocrine glands and hemopoietic tissue in the present eel is of interest and important to be collated with other pathological condition. As there has not been seen the corresponding case to this exceptional material, the findings described here will deserve well of prospective study.

Summary

Histological study was planned to elucidate the changes occurring in some of the endocrine glands, digestive, urino-genital and hemopoietic organs of a single starved Japanese eel. The animal was reared in the seawater aquarium put in the dark room under the condition of starvation for twenty months. This long-term starvation caused the drastic changes both in the body contour and organs and tissues. Due to the formation of a gigantic cyst, a highly significant expansion was brought about in the hypophysis with a remarkable degeneration of the glandular cells. A marked atrophy was seen in the thyroid, liver, spleen, pylorus and fundus of stomach, intestine, gallbladder accompanied with heavy expansion, exocrine pancreas, testis and kidney, while not so severe features were obtained in the corpuscles of Stannius and heart. Increase in the number of endocrine pancreas was also noticed. These visible changes were discussed in relation to the changes found in sexually mature, starved fishes which die

soon after their first spawning.

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PLATE

PLATE I

Fig. 2. Parasagittal section of the hypothalamo-hypophysial region showing a remarkable expansion of the whole pituitary. Notice a spacious cyst in the region of the rostral pars distalis. ×35

Fig. 3. Follicles remained in the periphery of the rostral pars distalis. In the cyst, there is found a lot of foamy substance stained weakly with AF. ×450

Fig. 4. A number of chromophobic cells near the follicels. Notice a heavy regression in the cell. ×450

Fig. 5. The pars intermedia consisting of small basophil cells. Rootlets of the pars nervosa contain a considerable amount of neurosecretory material. ×450

Fig. 6. The thyroid gland buried in the well-developed connective tissue. Many of follicles have no stainable colloid in their lumen. ×450

Fig. 7. A part of the liver showing a heavy degeneration. Large, lipofuscin-like pigment granules are seen in this picture. ×450

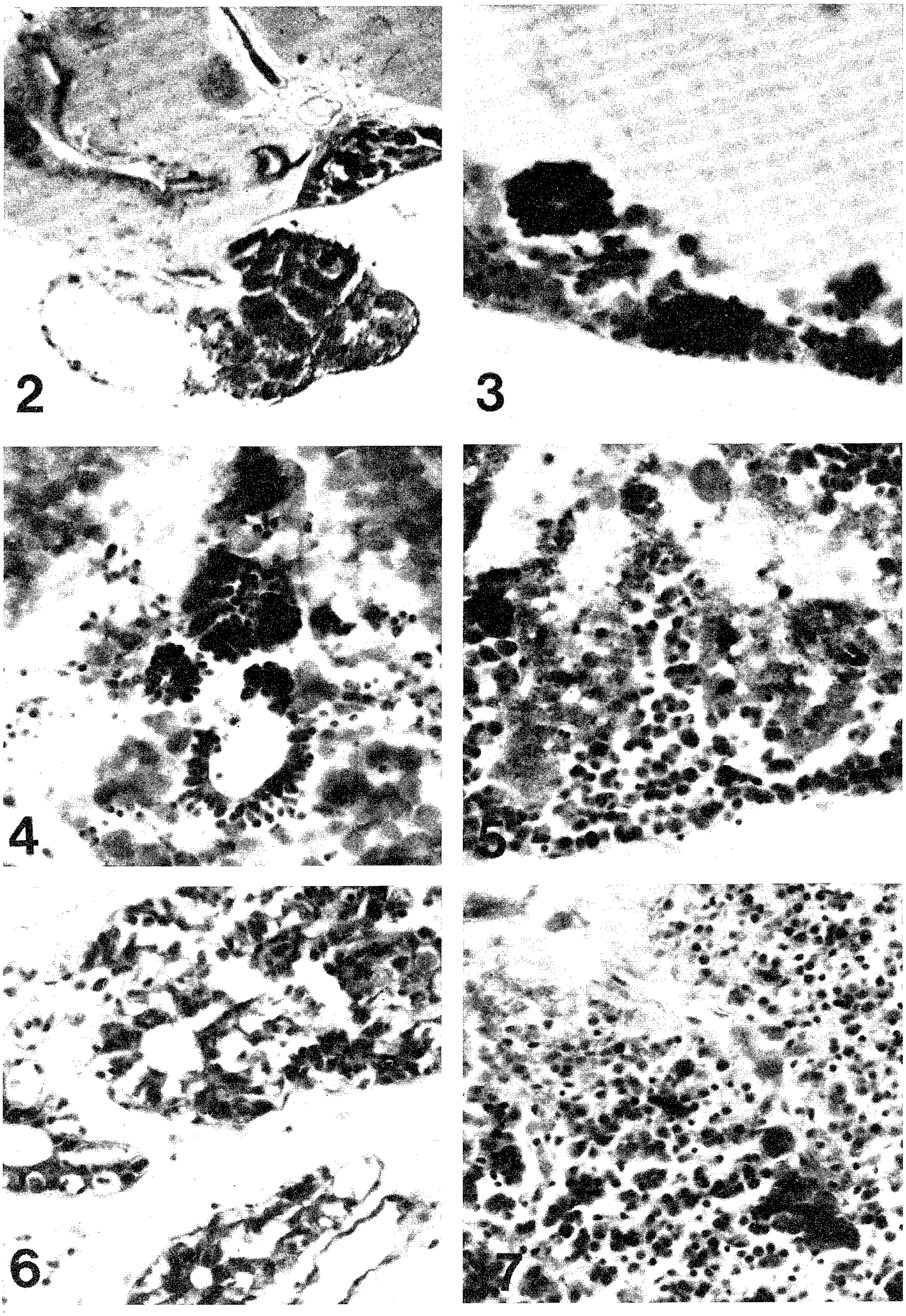
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PLATE Ⅱ

Fig. 8. A part of the regressive spleen showing a sharp decrease in the number of blood cells. ×450

Fig. 9. The thickened capsule of the spleen and nearby structures. ×450

Fig. 10. The pylorus of stomach. Notice the mucous epithelium in a state of marked involution. ×450

Fig. 11. The fundus of stomach consisting of a heavily regressive mucous epithelium and gastric gland. The muscle layer is also atrophied. ×100

Fig. 12. Enlarged view of a part of mucous epithelium of the fundus showing a marked degeneration. ×450

Fig. 13. The intestinal wall under the heavy regression. Notice the mucous epithelium appearing as the string. ×100

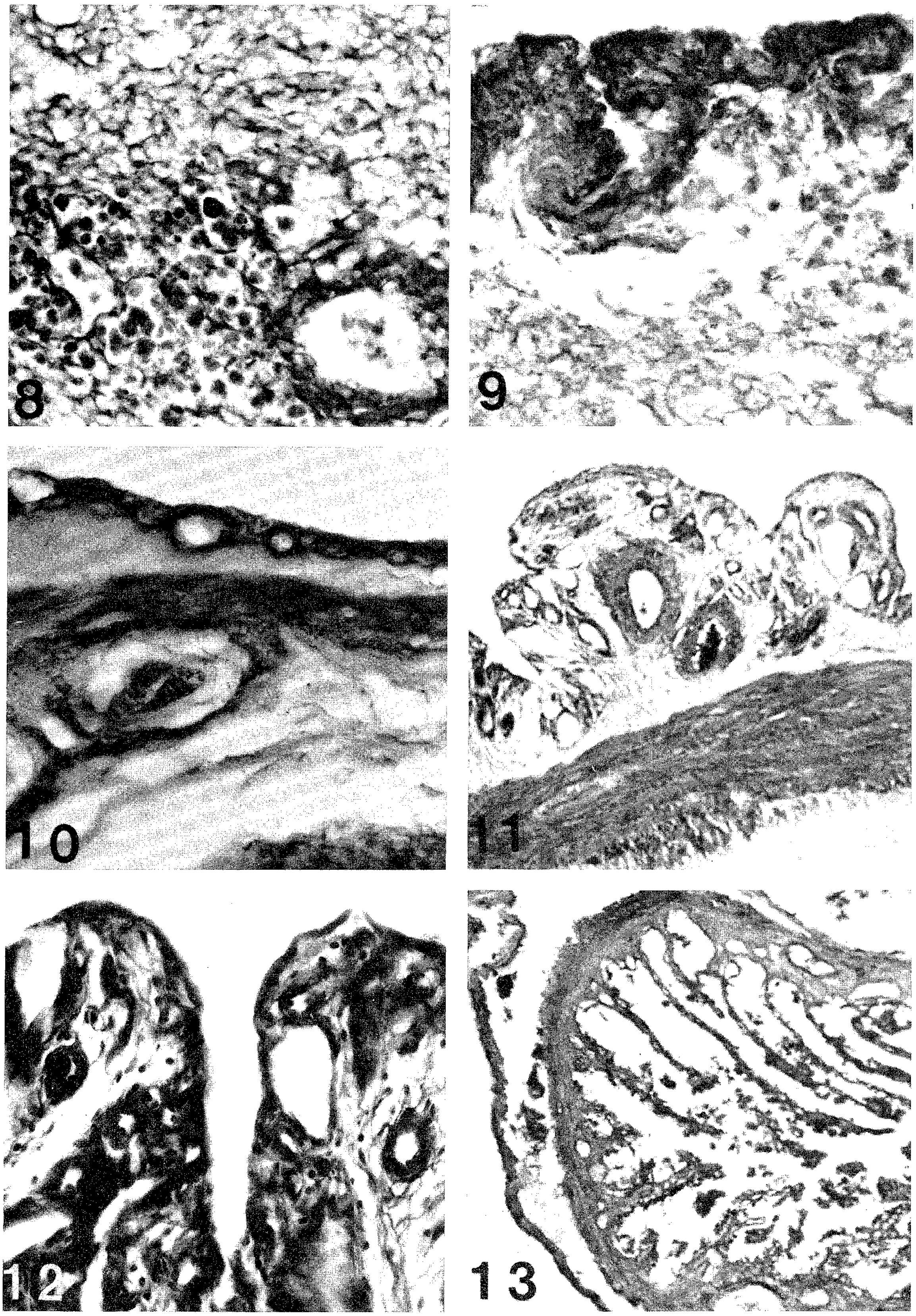
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PLATE Ⅲ

Fig. 14. The wall of the gall bladder in a state of heavy convolution. ×450

Fig. 15. A part of the exocrine pancreas showing a marked regression of acinar cells with a relative increase in the amount of the connective tissue. ×450

Fig. 16. A part of the endocrine pancreas in a state of marked regression. ×450

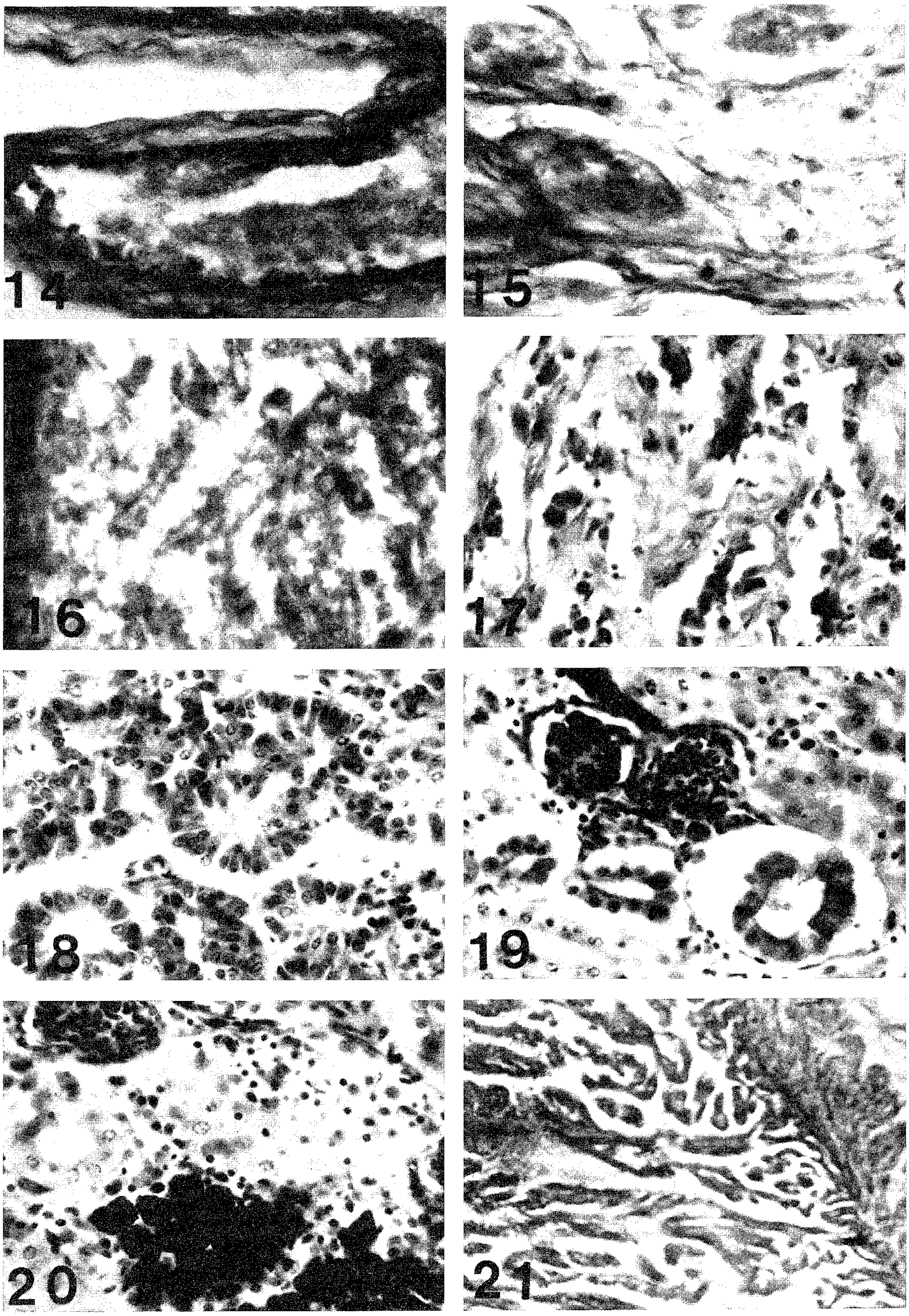
Fig. 17. The degenerative testis consisting of a lot of connective tissue. Several number of blood corpuscles are seen in the lumina of each cyst, and the germ cells in a state of remarkable atrophy are in the cystic wall. ×450

Fig. 18. The corpuscles of Stannius showing a comparatively normal state in appearance. But, the degree of anastomosis is not so marked as in the eel caught in the nature. ×450

Fig. 19. A part of the kidney showing the glomerular tuft in a state of atrophy. A sharp decrease in the number of lymphocytes is noticed. ×450

Fig. 20. A part of the degenerate kidney showing dark granules of melanin origin. ×450

Fig. 21. Section of the heart muscle not showing deteriorate state so much. ×450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