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Organogenesis and Functional Revelation of Alimentary Tract and Kidney of Chum Salmon

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Summary

Organogenesis of chum salmon, *Oncorhynchus keta* (WALBAUM), was studied histologically for the purpose of understanding the metabolism of the egg and alevin. The intestine, liver and kidney of the egg embryo were observed to accumulate much glycogen just before hatching at 46 days after fertilization. An accumulation of glycogen was detected during the alevin stage, but not detected in the fry at about 10 days after emerging. At this point the yolk was absorbed perfectly and not accepted in the abdominal cavity, under a nonfeeding condition. The pancreas was first observed in the egg embryo 46 days after fertilization, and furthermore zymogen granules in the exocrine cells and an islet of Langerhans were found in the alevin 21 days after hatching. The stomach and pyloric caeca were distinguishable in the egg embryo 33 days after fertilization and in the alevin 21 days after hatching, respectively. This alevin with a yolk sac did not take food even though his digestive tract had already appeared.

A large nephron was found in the pronephros of the egg 33 days after fertilization and the nephron in the mesonephros appeared in the alevin after hatching. Hence the exhaustional system of waste nitrogen, along with the osmoregulation system, is thought to be established at the hatching stage.

From these results it was concluded that the basic structure of the organs for gluconeogenesis, i.e. liver, kidney and intestine, of chum salmon had been constructed in the egg embryo before hatching.

The energy of egg and alevin of chum salmon seems to come from inner nutriment instead of from out side nutriment, but the mechanism of energy metabolism during development of egg and alevin was not clarified satisfactorily. Study on metabolism of the egg and alevin of chum salmon is basically important for developing techniques of salmon hatchery operations (1).

It is well known that the liver develops in an earlier stage than other parts of the alimentary tract in fish (2). This seems to be related to the energy metabolism

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in these early stages. But the function of early liver of fish has not been reported. Therefore the histological development of the liver of the embryo and alevin of chum salmon were basically studied in relation with the alimentary tract and kidney of chum salmon in this study. The clarification of the organogenesis and functional revelation of the liver, kidney and other alimentary organs will contribute to understanding the energy metabolism of salmon egg and alevin.

Materials and Methods

Fertilized eggs of chum salmon, *Oncorhynchus keta* (WALBAUM), were obtained from the Tsugaruishi River Salmon Hatchery, Iwate Prefecture, Japan on January, 1977. About 2,000 eggs were carefully brought to the Laboratory of Fisheries, Tohoku University, Sendai. Incubation of fertilized eggs was carried out in three plastic tanks (60 liters), shielded from light, in a constant temperature maintained at 10.0°C, for about three months. The water in the tanks was aerated and circulated through wool and gravel, and then replenished with 30 liters of fresh water every fifth days.

About every 10th day 5 undivided eggs or alevins were fixed in Bouin's fixative. In the process of dehydration with etanol, the yolk matter was cut away from the embryo tissues and embedded in paraffin. Serial sections were made to 6 μ in thickness and stained with Mayer's acid hemalum-eosin. PAS reaction with salivary digestion test were carried out with the sections filmed by 1 per cent celloidin after removal from the paraffin for glycogen detection.

Results

As shown in Table 1., the intestine, the liver and the kidney already appear in the egg embryo 22 days after fertilization. The stomach and the pyloric caeca were evident at 33 days after fertilization and at 21 days after hatching, respectively. The first pancreas and primary nephron of the kidney were observed in the egg embryos at 46 days and 33 days after fertilization, respectively.

Liver

In the egg embryo 22 days after fertilization, the liver was observed to exist adjacently to the intestine (Fig. 1). The liver was mainly composed of two layered cords of liver cells. Between the cell cords, in a sinusoid, many primary blood cells were found. Nuclei of the liver cells were comparatively large and have one or two large eosinophilic nucleoli. The cytoplasm of the liver cell displayed a mesh structure composed of basophilic filament, and the PAS reaction was negative. The liver of egg embryo 33 days after fertilization showed the same morphological appearance as at 22 days after fertilization, but glycogen was found in some of the liver cells (Fig. 2).

TABLE 1. *Degree of Differentiation in Characters of Alimentary Tracts and Kidney of the Eggs Embryo and Alevin of Chum Salmon after Fertilization*

Characters	Days after fertilization (Days after hatching)						
	22	33	46 (−2)	57 (9)	69 (21)	84 (36)	106* (58)
Liver							
Liver cells	+	+	+	+	+	+	+
Glycogen	−	−**	+	+	+	+	−
Intestine							
Columnar cells	+	+	+	+	+	+	+
Longitudinal folds	−	−	+	+	+	+	+
Goblet cells	−	−	−	−	+	+	+
Stomach							
Cuboidal cells	−	+	+	+	+	+	+
Gastric glands	−	−	−	−	+	+	+
Pancreas							
Exocrine cells	−	−	+	+	+	+	+
Zymogen granules	−	−	−	+	+	+	+
Islet of Langerhans	−	−	−	+	+	+	+
Kidney							
Renal tubules without brush-border	+	+	+	+	+	+	+
Renal tubules with brush-border	−	−	+	+	+	+	+
Renal corpuscle (pronephros)	−	+	+	+	+	+	+
Renal corpuscle (mesonephros)	−	−	−	−	+	+	+

\+ indicates character already differentiated
− indicates character under differentiated
* starved fry
** Glycogen was partially accumulated

At 46 days after fertilization, i.e. two days before hatching, it was revealed by PAS reaction (Fig. 3) and salivary digestion test (Fig. 4) with sections filmed with celloidin solution that much glycogen had accumulated in the liver cells. But the glycogen was not detected in the nonfilmed sections of liver cells. Furthermore as shown in Fig. 5., the section of liver of alevin at 33 days after hatching shows a stronger PAS positive than the liver of egg embryo.

The liver cells of the fry which emerged from the bottom to feed continued to accumulate much glycogen. In the macroscopic observation, the emerging fry were found to have a small yolk in the abdominal cavity. The remaining yolk disappeared from the abdominal cavity of the fry about 10 days after emerging. During this time glycogen in the liver cells could not be detected under nonfeeding conditions (Fig. 6).

Digestive tract

The digestive canal of chum salmon includes esophagus, stomach, intestine and pyloric caeca. Firstly the intestine of the egg embryo was already distinguishable at 22 days after fertilization. Columnar epithelial cells with elongated nuclei occupied the inner surface of the intestine and thin layered connective tissue wrapped them. But the goblet cells could not be found among the epithelial cells (Fig. 7). The stomach was first observed in egg embryo at 33 days after fertilization. It had a folded epithelium and thick connective tissue around the epithelium (Fig. 8). The digestive canal in alevin 21 days after hatching had basically been formed, hence the goblet cell in the folded epithelium and the gastric glands in the stomach appeared and furthermore the pyloric caeca was observable (Figs. 9 and 10). On the other hand the pancreas was first observed in egg embryo 46 days after fertilization. The pancreas existed in the neighborhood of the liver and assumed a gross of many parenchymal cells, with basophilic and PAS negative cytoplasm (Figs. 11 and 12). In the alevin 21 days after hatching, zymogen was observed as eosinophilic granules in the pancreas exocrine cells (Fig. 13), and a few small isolated groups of endocrine islet of Langerhans occurred as separate structures from the exocrine pancreas (Fig. 14).

Kidney

The kidney of chum salmon is the elongated structures lying above the swim-bladder along a vertebra from the esophagus to the anus. In egg embryo 22 days after fertilization, a simple kidney was observed. It was composed of a pair of long tubule having no brush-border on the surface of the epithelial cells (Figs. 15 and 16).

The functional kidney with a nephron appeared in the egg embryo 33 days after fertilization. So only one nephron could be accepted in the pronephros and it was not accepted in the mesonephros. It was composed of a large Bowman's capsule, a large glomerulus and several large renal tubules (Fig. 17). The renal tubules had a large open lumen and no brush-border (Fig. 18).

The number of haematopoietic cells in the kidney of the egg embryo 46 days after fertilization though no difference in other structures of kidney tissues was noticed at 33 days and 46 days after fertilization (Fig. 19).

According to the development of alevin after hatching, the nephron of the pronephros degenerated, and in it's place a few nephrons were observed in the mesonephros. In alevin at 21 days after hatching a renal corpuscle was found in the mesonephros while on the other hand the pronephros exhibited capillary glomerulosclerosis and necrosis of the epithelial cells of the renal tubules (Fig. 20). The kidney of alevin 36 days after hatching had several nephrons with a small Bowman's capsule, glomerulus and renal tubules (Fig. 21). The blood capillary of the glomerulus was connected to the renal artery. The renal tubules were divided into the neck segment, the proximal segment, the distal segment and the collecting segment (Fig. 22).

Discussion

The intestine, liver and kidney of chum salmon were distinguishable in the earliest stage of development. The intestine is the stem organ of the other digetive tract, i.e. liver, pancreas and so on. The kidney is thought to be needed for osmoregulation in the early stage. But the significance of the earlier genesis of the liver have not been known though it was well known that the liver developed at an earlier stage than in other fishes. Hayes (3) mentioned that glycogen had been stored in the liver near the end of the stage of alevin with the yolk sac and Tanaka (4) said that glycogen could not be detected in liver cells of the fry of rainbow trout before feeding. However in this study much glycogen was observed before hatching. The results of the present study was probably caused by protecting against a loss of the glycogen with a celloidin film. This means that the liver of egg embryo revealed functionally at the stage just before hatching. The accumulation of much glycogen in the liver cells of egg embryo just before hatching is especially interesting with regard to the fact that the changes in chemical composition is conspicuous after hatching in rainbow trout (5) and chum salmon.* The glycogen accumulation in the liver cells continued during the yolk sac stage and was not detected after emerging when the yolk in the abdominal cavity disappeared. This phenomenon shows that the precursor of liver glycogen is derived from the yolk material. Sugar in egg was found to be so little (6, 7) that the liver of alevin will contribute to the glyconeogenesis. The liver glycogen of salmon alevin will usually play the role of energy source. But under the condition of a sudden stimulation or of oxygen insufficiency, a large quantity of glycogen may be consumed by the anaerobic glycolysis.

The intestine was the distinct stem organ from the first. In mammal the intestine is known to contribute to the gluconeogenesis (8). On the other hand islets of Langerhans appeared in the pancreas in the alevin. Gluconeogenesis of chum salmon alevin may be regulated by glucoregulatory hormones. But no more can be said about the regulation of gluconeogenesis, until a histochemical study of the functional revelation of the islets of Langerhans and adrenal cortical tissue is completed.

Other organs, for example exocrine pancreas, stomach, gastric gland and so on, were formed before feeding. This fact can be understood as an adaptation to the severe environment faced by salmonidae, as stated by Tanaka (4).

The kidney had obtained a nephron in the pronephros at the egg and in the mesonephros at the alevin stage 21 days after hatching. Osmoregulation has an important role in the nephron at the alevin stage, as stated by Kashiwagi (9), but in addition to this, the exhaustion of the waste nitrogen (10) is also made possible by the nephron. Furthermore the gill, accepted as a cell gross in the alevin just after hatching and having a lamellae in the alevin of 10 days after hatching in chum salmon (11), will contribute to nitrogen excretion. Namely morphological evidences show that later eggs and alevins will have the ability of the nitrogen metabolism. Therefore it is possible to postulate that they can excrete the waste nitrogen produced by the deaminization in the process of gluconeogenesis.

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* unpublished data

In this sutdy the observations about liver development suggested the existence of gluconeogenesis in the later egg and alevin. The kidney development, along with gill development, also shows the existence of an exhaust system of waste nitrogen produced by the deaminization in the process of gluconeogenesis. Therefore the basic structures for gluconeogenesis have been formed at hatching stage. However to produce evidence of gluconeogenesis in egg and alevin, it is necessary to do a tracer experiment with a marking precursor. Furthermore if the mechanism of gluconeogensis is clarified in egg and alevin, the energy metabolism in egg and alevin of salmonidae can be understood in detail.

Acknowledgement

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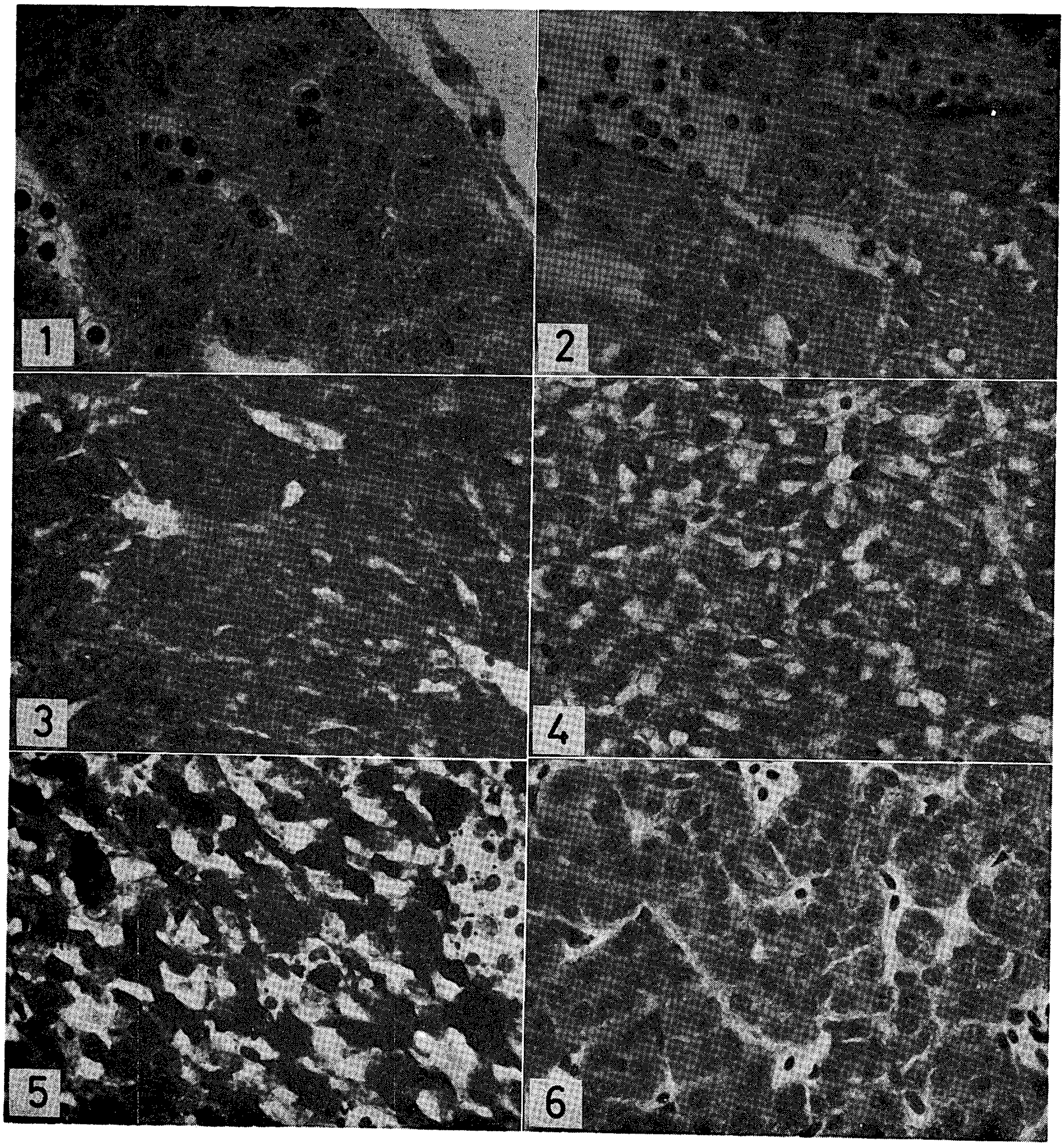
Plate I

Figures showing development of liver (×880)

1. Liver cells of egg 22 days after fertilization, containing basophilic fiber structure in cytoplasm (H. and E.).
2. Liver cells of egg 33 days after fertilization, showing partial accumulation of glycogen in cytoplasm on the right upper corner (PAS reaction).
3. Liver cells of egg 46 days after fertilization, showing moderate glycogen accumulation in the cytoplasm (PAS reaction).
4. Liver cells of egg 46 days after fertilization: portion occupied with PAS material is observed as empty space by H. and E. stain.
5. Liver cells of alevin 33 days after hatching: strongly PAS positive material shows large accumulation of glycogen (PAS reaction).
6. Liver cells of starved fry 54 days after hatching: glycogen is not detected in the cytoplasm (PAS reaction).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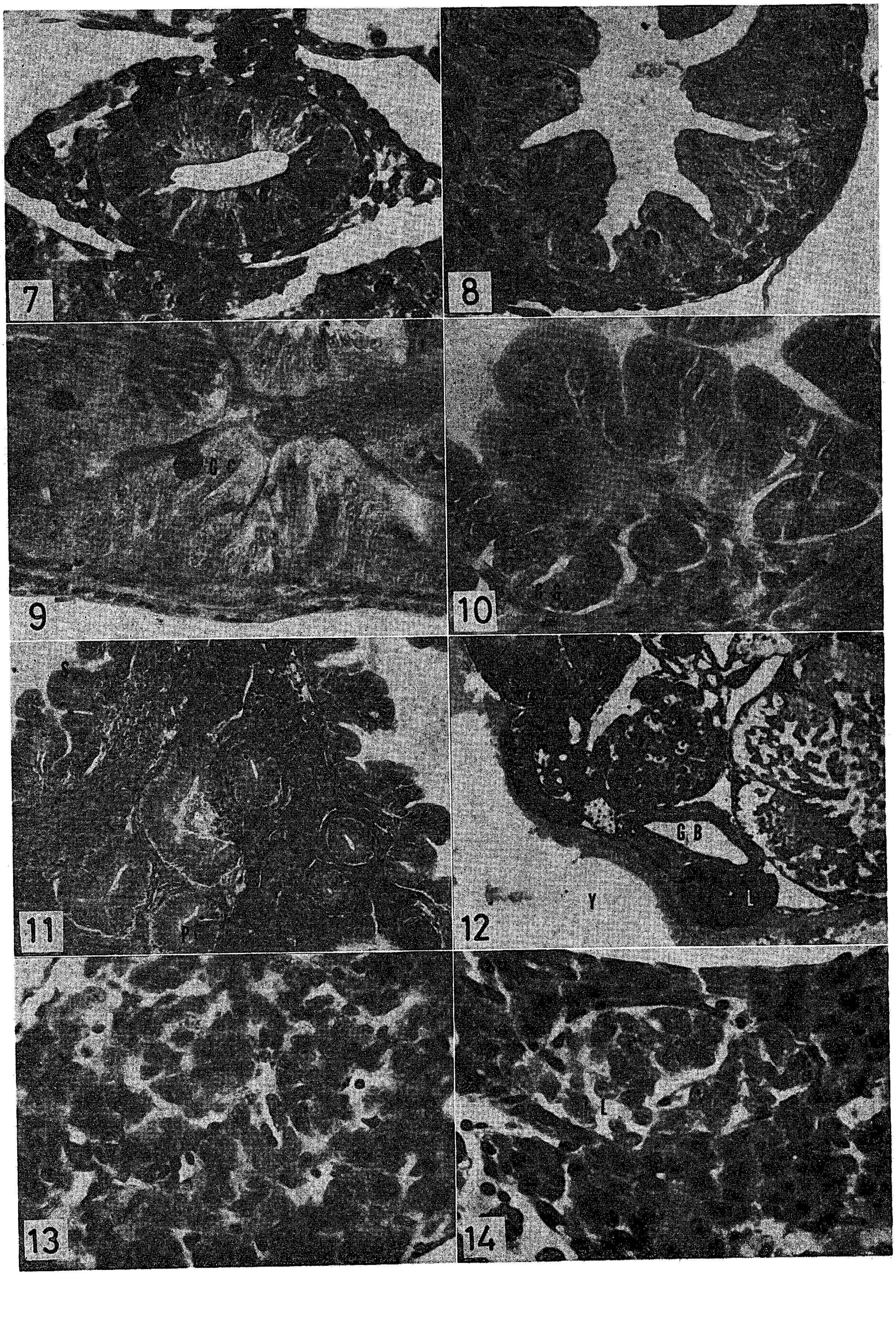
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PLATE II

Figures showing development of digestive tract

7. Intestine of egg 22 days after fertilization (H. and E. ×880).
8. Stomach of egg 33 days after fertilization (H. and E. ×220).
9. Intestine of alevin 21 days after hatching, showing existence of folded mucosa and PAS positive goblet cells (GC) between epithelial cells (PAS reaction ×880).
10. Stomach of alevin 21 days after hatching, showing development of gastric glands (GG) (H. and E. ×880).
11. Pyloric caeca (PC) of alevin 21 days after hatching (H. and E. ×220). P; pancreas, S; stomach
12. Pancreas (P) of egg 46 days after fertilization, adjacent to intestine (I) and not containing PAS positive material (PAS reaction ×220). L; liver, GB; gall bladder, Y; yolk sac
13. Pancreas cells of egg 46 days after fertilization, showing basophilic material but no zymogen granules in cytoplasm (H. and E. ×880).
14. Pancreas of alevin 9 days after hatching: exocrine cells containing eosinophilic zymogen granules and islets of Langerhans (IL) is found (H. and E. ×880).

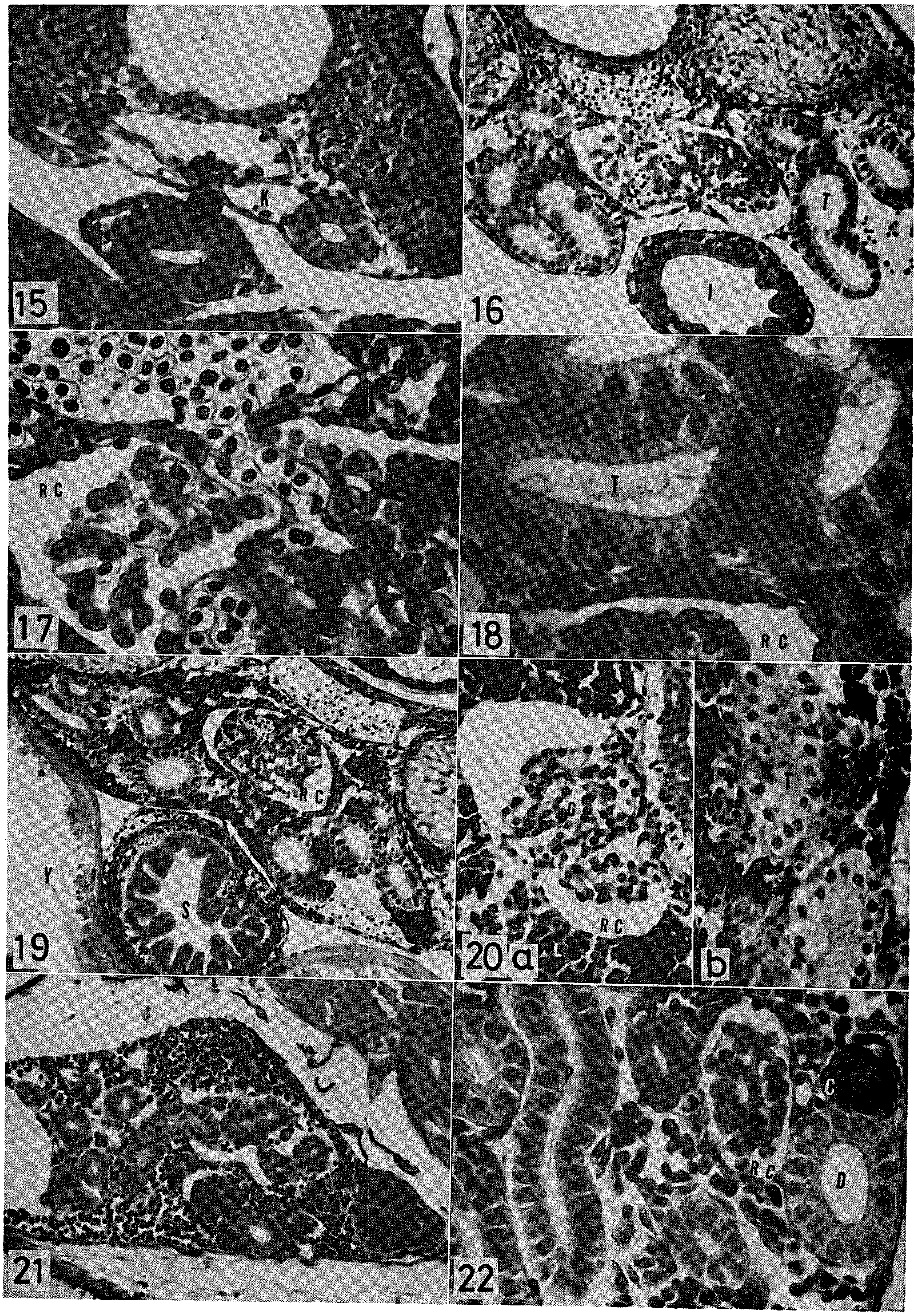
PLATE III

Figures showing development of kidney

15. Kidney (K) of egg 22 days after fertilization: a pair of pronephric tubules are distinguishable and the liver is also observed (H. and E. ×400).
16. Kidney of egg 46 days after fertilization, showing the appearance of a nephron composed of large renal corpuscle (RC) and pronephric tubules (T) (H. and E. ×300). I; intestine

17., 18. Magnified micrographs of Pl. II-8 (H. and E. ×880).

17. Large renal corpuscle (RC), showing entrance of blood cells along glomerular capillary into large Bowman's capsule from dorsal aorta.
18. Large pronephric tubules (T), showing large lumen and epithelium without brush-border. RC; renal corpuscle
19. Kidney of egg 46 days after fertilization, showing increase of number of haematopoietic cells among the renal corpuscles (RC) and pronephric tubules (H. and E. ×250). S; stomach
20. Two micrographs of pronephros of alevin 21 days after hatching, showing degenerative change: 20-a; glomerulus (G) showing shrinkage and sclerosis, 20-b; epithelial cells of renal tubules (T) showing necrosis (H. and E. ×600).
21. One side of mesonephros of alevin 33 days after hatching, showing the existence of small renal corpuscles and tubules (H. and E. ×300).
22. Nephron of alevin 33 days after hatching: renal tubules divide into the neck segment (N), proximal segment (P) having brush-border on epithelial cells, distal segment (D) having no brush-border, and collecting tubule (C) showing basophilia (H. and E. ×900).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