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Histogenesis of Hemopoietic Tissues and Immunoglobulin-forming Cells in the Carp, *Cyprinus carpio*

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Abstract The anlagen with a few hemopoietic cells was first detected in carp at 2 days post-hatch in the thymus and the pronephros. Hemopoietic tissues in both organs developed progressively, and at 9 days post-hatch they appeared to be nearly matured lymphoid organs. The kidney,the mesonephros, and the spleen also developed as hemopoietic tissues after 4 days post-hatch. A large number of immunoglobulin-forming cells were demonstrated immunocytochemically in the pronephros and also a small number in the mesonephros and the spleen.

Key Words : Hemopoietic tissue, Immunoglobulin-forming cell, Carp, Teleost

Introduction

The development of lymphoid tissues in teleosts has recently been reported by Manning and her colleagues[1,2]. However, little is known about correlation between ontogenic development of lymphoid tissues and localization of immunoglobulin-forming cells. On the other hand, extensive investigations of immunoglobulins and immunoglobulin-forming cell in the elasmobranchs, phylogenetically thought to be more primitive than teleosts, have been carried out in our research group. Unexpectedly, the presence of differentiated immune system, that is the presence of well developed lymphoid tissues and of immunoglobulin diversity, has been defined in the elasmobranchs[3-8]. The aim of this study is to analyze ontogenic development of hemopoietic tissues and the localization of immunoglobulin-forming cells in a teleost, the carp, *Cyprinus carpio*.

Materials and Methods

Animals :

Fertilized eggs of the carp, *Cyprinus carpio* were reared to the adult stages in the Ono Limnological Station, Shimonoseki University of Fisheries, Ono, Ube City. Fertilized eggs were kept in an aerated bath at 24℃ until they hatched and grew up to 21 days post-hatch. Then the fry were transferred to ponds of the station. The water temperature of the ponds was 19.2 to 24.0 while the fry were 21-48 days post-hatch. Feeding was started at two days post-hatch with animal planktons *Ploima* sp. and *Branchiopoda* sp., and supplemented at 10 days post-hatch with crumble. When they were more than one-year old, they were further transferred to concrete tanks which have been well aerated with running fresh water. From the time of fertilization to 7 days post-hactch, animals were sacrificed at intervals of 6 hours and afterward they were sacrificed every day until 48 days post-hatch. Seventy two days, 1, 2 and 3 years old young fish were also examined.

Histology :

At each of the intervals described above at least 10 fish were sacrificed and fixed. Fixatives used were 10% formalin, 4% paraformaldehyde or Carnoy's fluid. Frontal, sagittal or horizontal serial sections of the whole egg or the whole body of fry were made in animals smaller than 23 days post-hatch. In the cases of animals older than 72 days post-hatch, animals were sacrificed and various organs were dissected out then fixed. The tissue sections were stained with hematoxylin and eosin and observed under the light microscope.

Immunocytochemistry of immunoglobulin-forming cells :

Immunoglobulin-forming cells were detected by the direct immunoperoxidase or the direct immunofluorescence techniques using horseradish peroxidase (HRP)- or fluorescein isothiocyanate (FITC)- labeled $F(ab')_2$ of rabbit anti-carp immunoglobulin (Ig). Labeled anti-carp Ig was made by Dr. Kunihiko Kobayashi, Department of Pediatrics, Yamaguchi University School of Medicine. Four percent paraformaldehyde-fixed tissues including the pronephros, the kidney, the spleen, the intestine, the liver and the thymus were cut on a cryostat (Bright, U. K.) about 15-20μm in thickness. The tissue slices were incubated with either HRP- or FITC-labeled anti-carp Ig and observed under the ordinary or the fluorescence microscope. In the case of immunoperoxidase method the tissue slices were preincubated in 0.1% phenylhydrazine-HCl solution for 1 hour at 37℃ in the dark to remove the endogenous peroxidase activity[4]. Then the incubation was followed with a substrate solution containing 0.05% 3,3'-diaminobenzidine-4-HCl and 0.01% H_2O_2 in 0.05M Tris-HCl buffer, pH7.6.

Results

1) *Histogenesis of hemopoietic tissues.*

Development of hemopoietic tissues observed in carp was summarized in Table 1. In embryos 30 hours after the fertilization neither blood vessels nor blood cells could be detected, while in embryos 38 hours after the fertilization blood vessels developed in the wall of yolk sac and also in the embryonic body. Developing central nervous system, notochord, endoderm and primordium of pronephric tube were easily distinguishable in the 38 hours embryos. Just beneath the notochord two blood vessels, possibly a dorsal aorta and a postcardinal vein, were observed and in their lumens a few immature blood cells were found.

Embryonic development and histogenesis

Table 1. Histogenesis of carp hemopoietic tissues

Organs / Ages	Thymus	Pronephros	Kidney (Mesonephros)	Spleen	Others
Pre−hatch 1 day	−	−	−	−	Immature blood cells were first found in blood vessels of a 38 hours embryo
Post−hatch 1 day	−	−	−	−	
2 days	+ (Anlage)	+* (Anlage)	−	−	
4 days	+	+	+* (Anlage)	+ (Anlage)	
9 days	++	++*	++*	+	

− : No hemopiesis
+ : Hemopoiesis
++ : Active hemopoiesis
* : Hemopoiesis was observed in interstitial spaces between nephric elements.

in carp at about 24℃ were rapid. It took only two days for hatching after the fertilization. Hatched fish did not have any developed organs yet, but most organs are formed as a primitive form in several days after hatch.

At 2 days post-hatch the thymus anlage was observed in the antero-dorso-medial area of branchial arches(Figs. 1, 2). The anlage was seen as a cell mass of several layers of epithelial cells containing only a few lymphoid cells, which have basophilic round cytoplasm (Fig. 2 arrows). At this stage the pronephros had three components, a pronephric glomerulus, pronephric tubules and a pair of pronephric ducts. Aggregated hemopoietic cells were found in the interstitial spaces around the components (Figs. 5, 6).

By 9 days post-hatch, lymphoid cells in the thymus increased remarkably (Figs. 3, 4), and hemopoietic cells, including lymphoid cells in the pronephros also increased progressively (Fig. 7). At this stage, the pronephric elements, the glomerulus and the tubules became atrophic. At 23 days post-hatch thymus and pronephros developed fully as hematopoietic organs (Fig. 8).

The kidney, the mesonephros, developed in the dorsal wall of the body as a pair of longitudinally oriented mesonephric tubules at the time of hatching. Hemopoietic cells were first detected at 4 days post-hatch in the kidney, and increased gradually accompanying differentiation of nephric tubules (Figs. 9, 10). The major cellular constituents of the kidney, the pronephros and the mesonephros, were lymphoid cells and granulocytes. In the adult pronephros, nephric tubular components became extremely small in number, and the organ was filled with a large number of hemopoietic cells (Fig. 8).

The spleen was first detected on day 4 post- hatch as a small organ attached to the mesogastrium. Immature blood cells and reticular cells were observed as cellular components in the early stage of the development. Figures 11 and 12 show that the spleen of 7 days post-hatch is packed with immature blood cells. Differentiation of so-called white pulp and red pulp did not occur even in the adult carp spleen.

2) *Distribution of immunoglobulin-forimng cells in carp.*

The localization of immunoglobulin-forming cells was analysed immunocytochemically using direct immunoperoxidase and direct immunofluorescence methods in adult fish of 1 to 3 years old. The immunoglobulin-forming cells having positive immunocytochemical staining were observed in numerous number in the pronephros (Figs. 13, 14). A small number of immunoglobulin-forming cells were also demonstrated in the kidney (mesonephros) and the spleen (Figs. 15, 16). However, no positive cells were detected in the liver, the gut mucosa or the thymus.

Discussion

The first part of this paper describes the histogenesis of hemopoietic tissues in the carp, *Cyprinus carpio*. A similar observation in the carp was recently made by Botham and Manning[2]. However, there are at least two major differences between two observations. The first is that Botham and Manning did not distinguish clearly the difference of pronephros and mesonephros and simply described as the kidney. Since the pronephros and the mesonephros of the teleost appear to be quite different structurally and functionally, we described them separately. The pronephros was the major hemopoietic organ containing many lymphoid cells in the carp. Hemopietic cells were already observed in tissue spaces between pronephric elements at 2 days post-hatch, when the lymphoid cells were found in the thymus anlage. On the other hand, mesonephric tubules started to develop around the pronephric duct on day 4 post-hatch accompanying the proliferation of intertubular hemopoietic cells. In addition, the amount of lymphoid elements in the mesonephros was much less than in the pronephros.

The seconed major difference is that the spleen was first identified on day 4 post-hatch, one day earlier than the result of Botham and Manning[2]. This may be simply due to the difference of water temperature in the bath where the fry were grown up (24℃

vs. 22℃), although other factors, such as the genetic difference, can not be ruled out. It is generally known that the temperature is the most influential factor for the early development of fish embryos.

The most important finding on the histogenesis of the carp hemopoietic tissues may be that both the thymus and the pronephros are the first hemopoietic organs to develop as shown in Table 1, and the pronephros contains more lymphoid cells than the mesonephros and the spleen do.

To examine the correlation of histogenesis of hemopoietic tissues with the localization of immunoglobulin-forming cells, an immunocytochemical study was carried out using direct immunocytochemical techniques. As a result, it was found that the pronephros is the major immunoglobulin-forming organ in carp as described in other species of teleosts detected by other methods[9]. It is important to note that although a small number of immunoglobulin-forming cells were also detected in the mesonephros and the spleen, none was observed in the thymus and the gut mucosa.

The teleost is generally considered as a more developed vertebrate than the cartilaginous fish[10]. However, as far as immune system is concerned the teleost apperars to be less developed.

In the classic studies, Yoffey[11] and Kanesada[12] reported that cartilaginous fish have well developed lymphoid tissues in the spleen. Tomonaga and his colleagues[4,6,7] described recently that in addition to the spleen the gut mucosa, Leydig organ of the esophageal mucosa, and the epigonal organ also contribute as immunoglobulin-producing organs in some extents.

In the spleen of cartilaginous fish, especially of elasmobranchs, the white pulp is well developed and numerous antibody-forming cells, some of which are typical plasma cells, are present therein[4,6,7]. On the other hand, as described in the present paper and others [1,2] the white pulp, the organized splenic lymphoid tissue, is generally not detectable in the teleost spleen, and the number of immunoglobulin-forming cells is also scarce.

The presence of developed lymphoid tissues and immunoglobulin-containing cells in the gut mucosa of elasmobranchs indicates that the local immune system, the gut-associated lymphoid tissue, has already evolved during phylogenetic history of lower vertebrates[8]. However, unexpectedly, the carp, a teleost has no lymphoid tissues in the gut mucosa. This suggests that the teleost immune system is much more primitive than that of elasmobranchs.

The pronephros is an excretory organ during early life of most vertebrates, and degenerates after the development of mesonephros and / or metanephros. However, the Cyclostomes and the Teleostei have the pronephros throughout their lives in the abdominal cavity close to the pericardial cavity. According to Fänge[13] the hagfish pronephros contains lymphoid tissues, and Zapata et al[14] recently reported the presence of plasma cells in addition to lymphid cells in the pronephros. Since it is well known that the hagfish, the most primitive vertebrate extant, has no thymus, the pronephros and/or the primitive spleen in the gut mucosa may be the most primitive lymphoid organ in the vertebrate evolutionary history. From morphological view points the carp pronephros in adult life seems to be not functional as an excretory system at all but serves as an important hemopoietic organ and also an immunoglobulin-producing organ.

Much attention has been payed to the biological functions of thymus lymphocytes after the successful demonstration of the basic principles of cellular immunity[15]. Nowadays molecular structures of recognition molecules, T cell receptor and its associates, and their gene structures in mouse and human have been extensively investigated[16,17], although exact reaction mechanism is still obscure[18,19].

T and B cell differentiations in mammals may represent evolutionary background. Analysis of a simple and primitive system such as in fish may help to know exact details of reaction mechanisms involved in recognition, which is most important in the immunobiological system in the living creature.

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Explanation of Figures

Fig. 1. A horizontal section of a carp fry 2 days post-hatch. Note the thymus anlage (arrow) in the antero-dorso-medial area of branchial arches. x75.

Fig. 2. An enlarged view of the thymus anlage in Fig. 1 . Two days post-hatch. The anlage consists of several layers of epithelial cells with a few invading lymphoid cells (arrows). x750.

Fig. 3. A low power view of sagittal section of a carp fry 9 days post-hatch. Two thymus lobes (arrows) in the dorsomedial part of the branchial area. x30.

Fig. 4. An enlarged view of the thymus in Fig. 3. 9 days post-hatch. Small and darkly stained lymphocytes are the predominant cell population. x300.

Fig. 5. A horizontal section of a carp fry 2 days post-hatch. Note primordium of the pronephros in the lower part of photograph. x75.

Fig. 6. An enlarged view of the pronephros in Fig. 5. Two days post-hatch. Immature hemopoietic cells (arrows) are seen in the interspaces between the pronephric tubules. x300.

Fig. 7. Pronephros with numerous hemopoietic cells. Nine days post-hatch. Two tubules are seen in this section. The organ is surrounded by the capsule with melanocytes. x300.

Fig. 8. The pronephros at 23 days post-hatch. Note remarkable increase of hemopoietic cells as compared with the pronephros of a fry 9 days post-hatch. Note rather small number of pronephric tubulers in this stage. x150.

Fig. 9. A sagittal section of a carp fry 7 days post-hatch. The mesonephros having mesonephric tubules and small number of hemopoietic cells are seen in the center to the right. The intestinal wall is on the left side. x300.

Fig. 10. The mesonephros at 23 days post-hatch. The hemopoietic cells increased significantly in the interepithelial spaces. At this stage glomerulus and nephric tubules are well differentiated. x150.

Fig. 11. A sagittal section of a carp fry 7 days post-hatch. Note the presence of the spleen (an arrow) in the abdominal cavity, caudal to the liver and between the intestine and the air sac. x15.

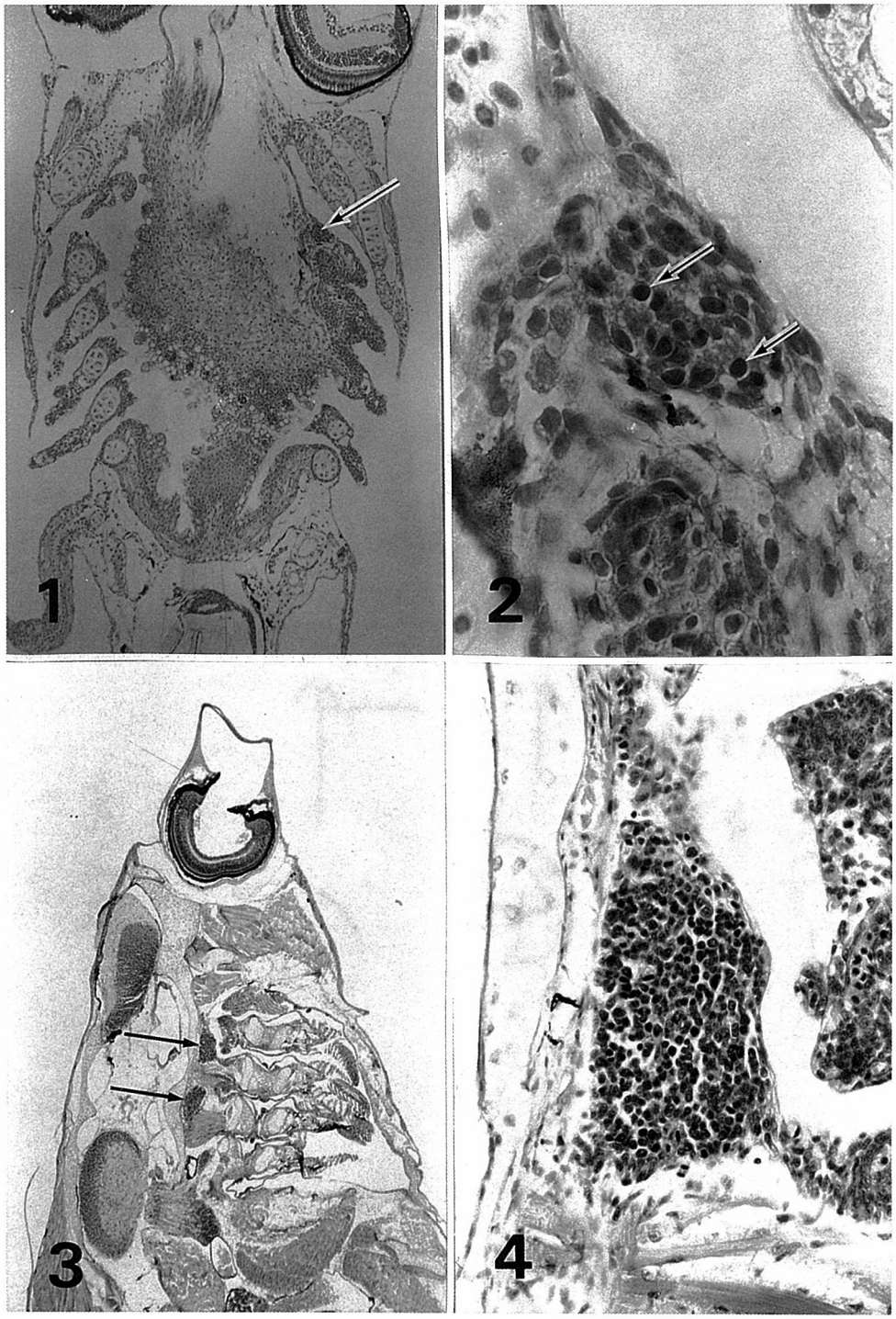
Fig. 12. An enlarged view of the spleen in Fig. 11. Seven days post-hatch. The spleen is filled with many immature blood cells and reticulum cells. x150.

Fig. 13. Numerous immunoglobulin-forming cells in the pronephros of three year-old carp. Dark staining shows presence of a positive reaction product for horseradish peroxidase-labeled anti-carp immunoglobulin. x200.

Fig. 14. A higher power view of Fig. 13. Note some of the positive cells have a rather large cytoplasm. x400.

Fig. 15. A small number of immunoglobulin-forming cells in the mesonephros. The direct immunoperoxidase staining. Three year-old carp. x200.

Fig. 16. An immunoglobulin-forming cell (an arrow) in the carp spleen demonstrated by the direct immunofluorescence technique. Three year-old carp. x320.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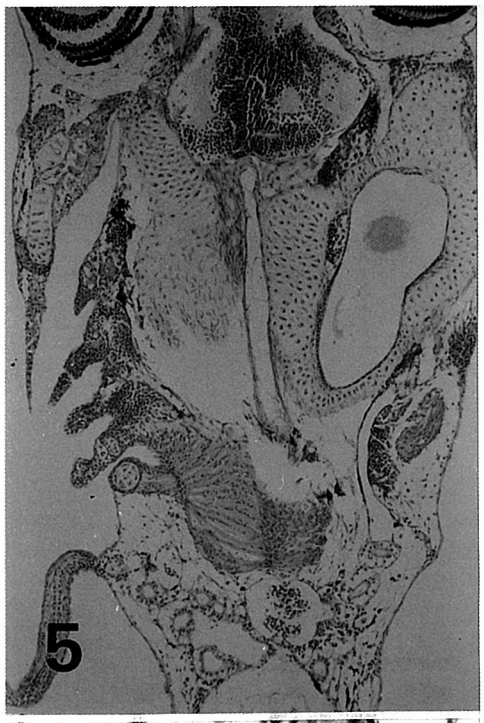
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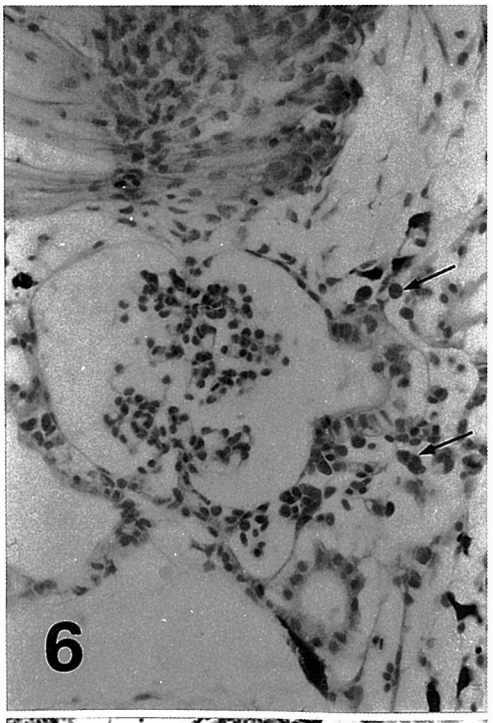
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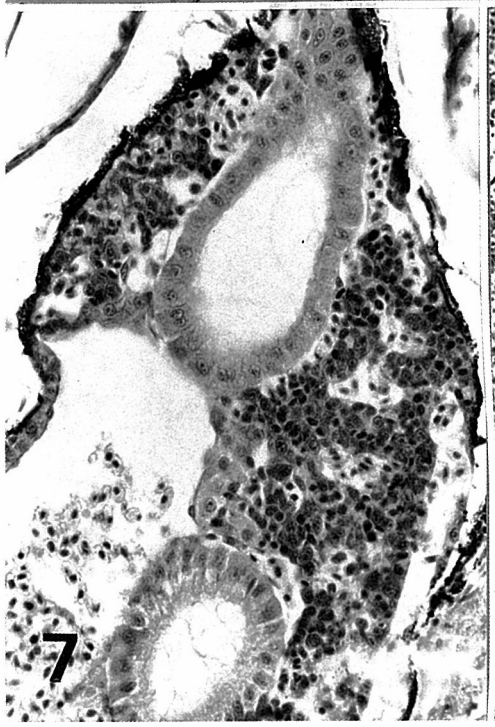
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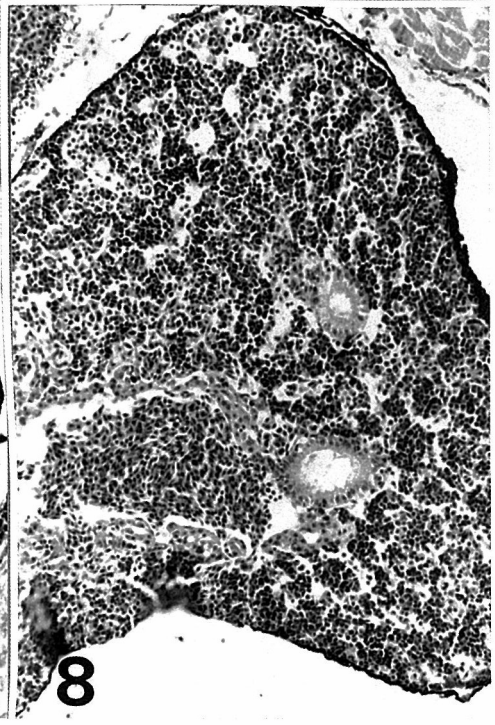
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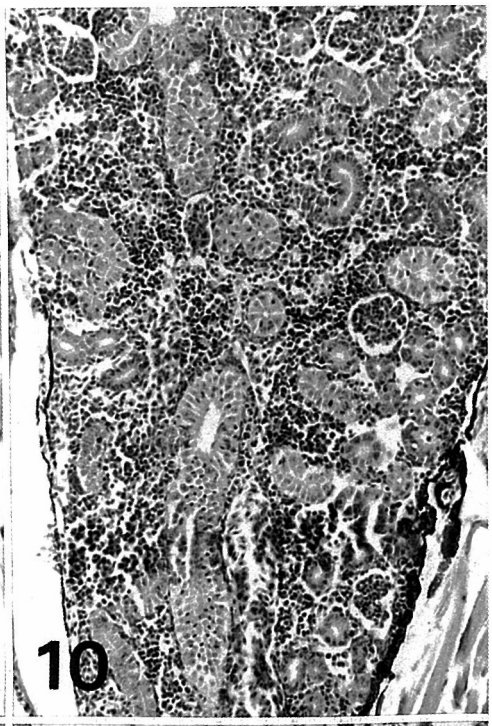
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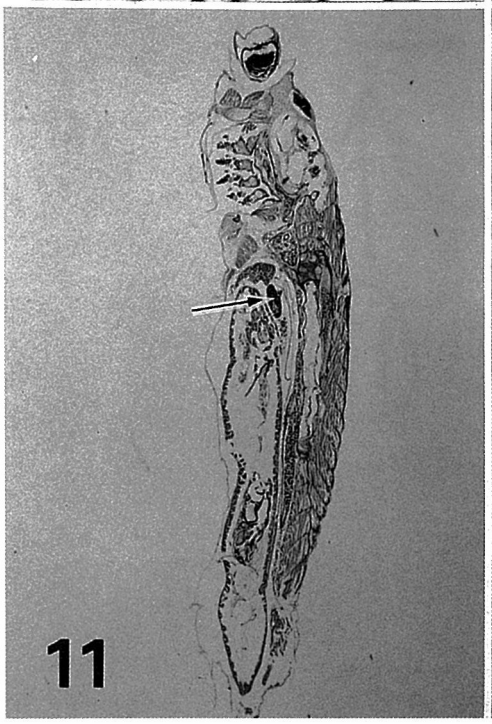
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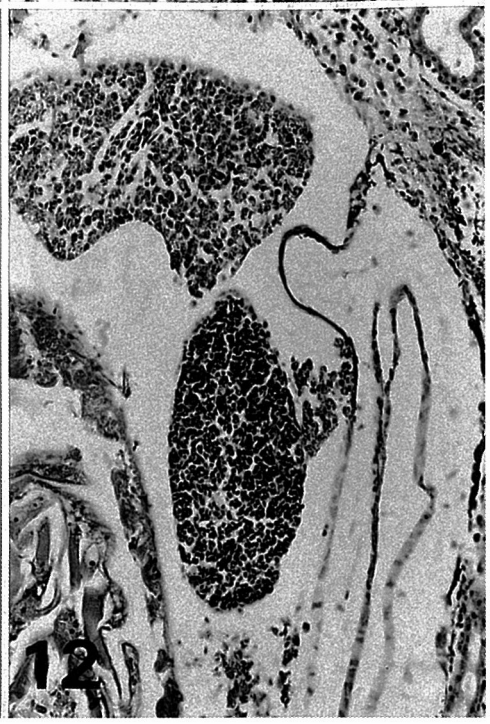
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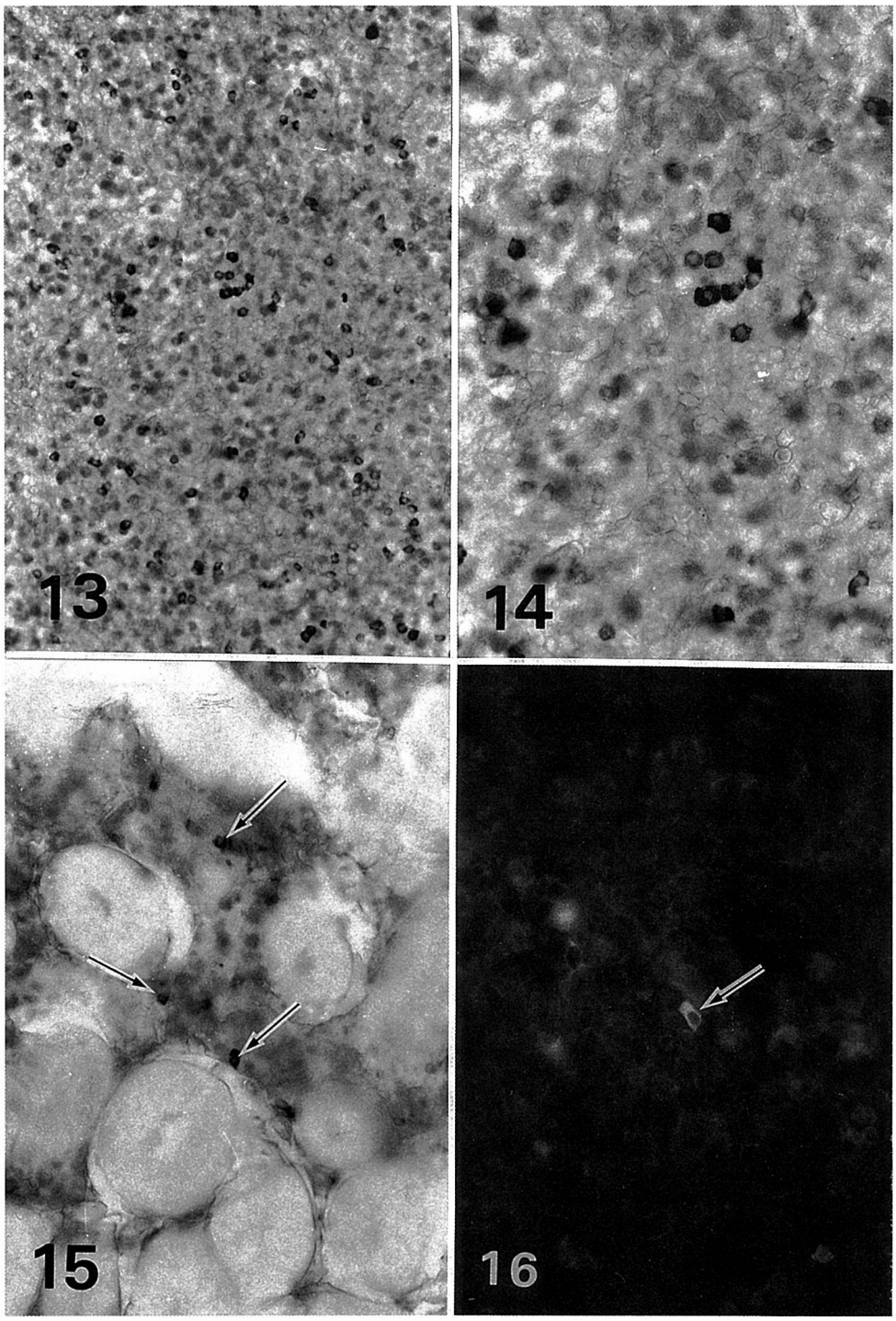
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