STUDIES ON EMBRYOGENESIS AND MORPHOGENESIS OF BLACK SEA BREAM, AND DISEASES OF SPARID FISHES IN LARVAL PRODUCTION

By

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クロダイの種苗生産時における発生・形態学的変化とタイ類の種苗の疾病について

Studies on Embryogenesis and Morphogenesis of Black Sea Bream, and Diseases of Sparid Fishes in Larval Production

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CONTENTS

本研究ではタイ類の種苗生産に関する知見を集積するため、クロダイ Acanthopagrus schlegeli の発生と仔稚魚の形態変化を経時的に観察し、あわせて、クロダイとマダイ Pagrus major の仔稚魚の疾病について病理組織学的に検索した。クロダイの親魚養生、受精卵の採取と孵化、仔稚魚の飼育は高知県水産試験場内の水槽で行なった。

受精と孵化：クロダイの受精卵は直径0.8～0.85mmで、正常卵は1個の油球を、異常卵は2個以上の油球をもっていた。水温約20℃の孵化水槽で、受精後、15～30分で卵割が始まり、38～50時間で孵化が起こった。

仔魚の発育：孵化仔魚については孵化後50日まで、形態学的変化の観察、器官の発生と発達の組織学的観察を経時的に行なった。孵化仔魚は体長約2mmで、4時間後に遊泳を始めた。水温約20℃で、前期仔魚期は孵化後6日までであり、卵黄の吸収、鰓原基の形成、眼球・口器・膵細胞・胆のう・腎実質・造血

組織などの分化、腸管の発達がみられた。また生態面では4日目に摂餌が始まり、遊泳力の向上と集団形成がみられた。中期仔魚期は7～20日までで、各鰭が発育して仔魚の形ができ上った。また、摂餌物の消化・吸収、肝臓・膵臓・ランゲルハンス氏島・鰓弁・鰓薄板・歯・味蕾などの分化と発達、造血が特徴であった。後期仔魚期は21～35日までで、全身の血管系の発達、メラニン細胞の増加、親魚の類型の完成、胃と幽門垂の分化と発達、消化・吸収の向上と肝細胞内貯蔵物質の増加が特徴であった。

仔稚魚の疾病：クロダイとマダイの種苗生産時に、適時病魚を採取し、病理組織学的に観察した。その結果、消化管内での細菌の異常増殖、鞭毛虫のIchthyobodo necatorの皮膚寄生、および前記の細菌と寄生虫の二重感染、滑走細菌によるロぐされと尾ぐされがそれぞれ認められた。

1. INTRODUCTION

The Greek government wants to develop culture of marine fish especially that of gilt-head bream Sparus aurata in the coastal regions of Ionian and Aegean seas. Advancement and accumulation were needed on fundamental informations about fish mariculture. Black sea bream Acanthopagrus schlegeli and red sea bream Pagrus major are close relatives to gilt-head bream Sparus aurata. In Japan culture of red and black sea bream were totally established due to their high market value, and improvement of their culture technics during the early life stages has mainly contributed to this establishement.

The acquisition of eggs and mass culture of juvenile red sea bream have been formulated by Kitajima (1978). Tanaka (1973) histologically studied on the structure and function of the digestive tract in larval teleosts including black and red sea bream. Shelbourne (1955) and Umeda (1979) have given evidence of a utilization of yolk through direct transfusion into the subdermal space in plaice and yellowtail larvae respectively. Yamada (1959) has demonstrated a yolk utilization process through the early circulatory system in larvae of salmonids. In spite of all these studies above there is no detailed publication on the embryology and early life history of black sea bream.

In this study embryogeny and morphological development of larval black sea bream were described in detail. The acquisition, rearing morphological observations, collection and preservation of larval fish were done at Kochi Fisheries Experimental Station from May 1st of 1983 to June 6th of the same year. The formalin fixed samples were then transferred at Fisheries Department, Laboratory of Fish Pathology of Mie University for histological examinations.

Loss of larvae usually occurred in considerable amounts in artificial larval production of black sea bream and red sea bream, but this fact has been neglected to be investigated on.

In this study histopathological observations were also done on diseased larval fish and revealed causes of their diseases.

2. MATERIALS and METHODS

2.1 Acquisition of eggs.

In Kochi Fisheries Experimental Station, parental fish of black sea bream were outside reared in a round metalic tank as big as 120 tons which was covered with a black net for avoiding light stress on the fish. Running sea water was continually supplied into the tank. Overflowing water was collected with a 10 cm in diameter plastic pipe located at the top margin of the tank to be brought into another 1 ton plastic tank with a net of 0.4 mm mesh-work inside it. Spawned fish eggs were brought through the pipe into the small tank and kept floated on the surface of the water. The fertilized eggs were boyant and the unfertilized or damaged eggs sank after some interval of time into the bottom of the water.

The eggs used in this experiment were taken on May 1st. Rapidly an enough high quantity of eggs were accumulated, the collection net was emerged the eggs washed off gently into a container, and then poured into several volumetric tubes filled with sea water to make a preliminary determination of fertilized eggs. Fertilized eggs tend to afloat occupying the upper part of the container, while unfertilized eggs remain suspended. Apart from this, estimation of percentage of fertilized eggs was done by microscopic analyses too.

2.2 Incubation and handling of fertilized eggs.

Fertilized eggs of black sea bream were transferred to indoor

500 liter plastic tanks in which they were contained in 500-600 cm^3 suspended incubating nets for the whole incubation period. Aeration was supplied and the water temperature was between 19.0-19.8°C. Continually flow of sea water of an average 1.023 sp.gr. salinity was maintained. Continuous observations of the embryonic development were done on properly designated time intervals of sampling, using a light stereo microscope. The significant stages of embryo development were recorded and photographed. Sinking dead eggs were regularly removed using a siphon for maintaining the possible best conditions of healthy eggs.

2.3 Larval rearing

After hatching, larvae of black sea bream were transferred from the incubation tanks to outside concrete-lined tanks as big as 1.6 ton. Aeration was supplied and the water temperature was 20°C. Chlorella was added to the tanks two times daily at a density of $100x10^5$/ml as much as to maintain the green water condition. Four days after hatching live food consisting of rotifers Brachionus plicatilis at a density 4-8 rot./ml was supplied to the larvae. The introduced rotifers were of a 150-200μ size strain. As the larvae grew older this strain was changed with another strain 200-300μ in size, combined with copepods Tigriopus japonicus, at around the 20-day old larval stage. Later on at the metalarval stage the living food was gradually decreased in amount as artificial diet (finely granulated commercial formulated diet), was supplementing it. Sizes of artificial diets were graded from 0.3 to 0.6 mm in diameter in accordance with the growth of fish. The food was given three times daily. Finally during the early prejuvenile stage the food was composed of fish (sandlance) and shrimp made in a minced

form. Through the whole observation period one third of the tank water was changed daily. The water temperature fluctuated between 20.0-21.5°C and salinity between 1.025-1.045 sp.gr.

2.4 Morpho-histological preparations

Samples of eggs of black sea bream during the incubation period and of larvae during the period from hatching to prejuvenile stage were taken periodically, to record morphological and histological characteristics at significant stages in the early life history of this fish. Photographs were taken on every significant stage, observations and sketching were done using a light stereo microscope. Samples were taken every day at a fixed hour and after measurement of total body length were preserved with 10% formalin solution for histological study. These fixed samples after dehydration were embedded into paraffin wax and were serially sectioned 3 to 4 μ in thickness. Sections were stained with hematoxylin-eosin (H-E) and periodic acid Schiff (PAS) reaction. Observations and taking of photographs were carried out using a light microscope.

2.5 Preserving diseased fish

During the larval rearing, sickened or moribund larvae of black sea bream were collected. Diseased juvenile fish of black sea bream and red sea bream were also collected at rearing tanks in Kochi Fisheries Experimental Station. These fish were preserved with 10% formalin solution and then sectioned with routine histological procedures. Sections were stained with H-E stain, PAS reaction and Giemsa stain.

3. RESULTS

3.1 Fertilization and hatching

Spawning occurs in the late day-time around sunset, when the fishes swimm near the surface of water, and exhibit sudden bursts of sexual activity with an evident agitation of surface water. The eggs of black sea bream are sticky and boyant, floating along the water surface, and although semi-transparent can only barely seen with the naked eye. They often made masses in condition of no aeration at some corners of the containers in which net was set for collection.

The fertilization rate of eggs was estimated to be 70 %. The newly spawned eggs are spherical in shape and measure 0.80 to 0.85 mm in diameter. Several minutes after fertilization the egg increases slightly in diameter, from 0.80-0.85 mm to 0.90 mm approximately,due to the formation of perivitelline membrane that creates a marginal space in between the outermost chorionic membrane.

The normal fertilized eggs have a coarsly granulated cytoplasm with a distinct centrally located oil globule of a size about 0.19 mm. Abnormal eggs usually have two or more oil globules and the percentage of the abnormal eggs was less than 5% in the current study. The distinct micropyle along the animal pole disappears gradually as the egg develops into a one cell zygote. The size and spherical shape of the egg and oil globule do not change until hatching.

A general description of ontogenic development of the egg is summarized in figure 1.

3.2 Embryonic development

Embryonic characters of all developmental stages were clearly distinguishable. First cleavage was observed 15-30 minutes after fertilization followed by 2, 4, 8, 16 and succeding cleavages which occurred within 4 hours after fertilization. In the last cleavage phase an uneven meridional division of cells was observed, some appearing bigger in size and irregularly shaped until the blastoderm formation.

The morula occurrence timed four hours before reaching the blastula stage, which in turn gives way to the blastoderm invasion over the entire yolk. The gastrulation processes lasted for about 2 hours until the yolk plug stage with closure of blastopore. At this stage the neural keel and the anlage of the early embryo were already visible. Formation of embryonic shield further developed as it reaches the neurula stage where the expansion of the cephalic region was evident.

The late phase of organogeny to the pre-hathing stage was the longest stage in embryogeny, taking more than 10 hours as an average time to be completed.

Several eggs had different speed for each of these developmental processes. The Kupffer's vesicle is the distinguishing character of the late phase of organogeny, while melanin pigmentation begins to appear on the oil globule and the dorsal margin of the embryo. In the pre-hatching stage the fully functioning heart and the twiching motions of the embryo are very obvious. Optic cup and auditory placode are also more obvious at this stage. The observed first hatching occurred at about 38:50 hours after fertilization where jerking of the embryo in almost all eggs eventually breaks-off the egg capsule releasing the larvae.

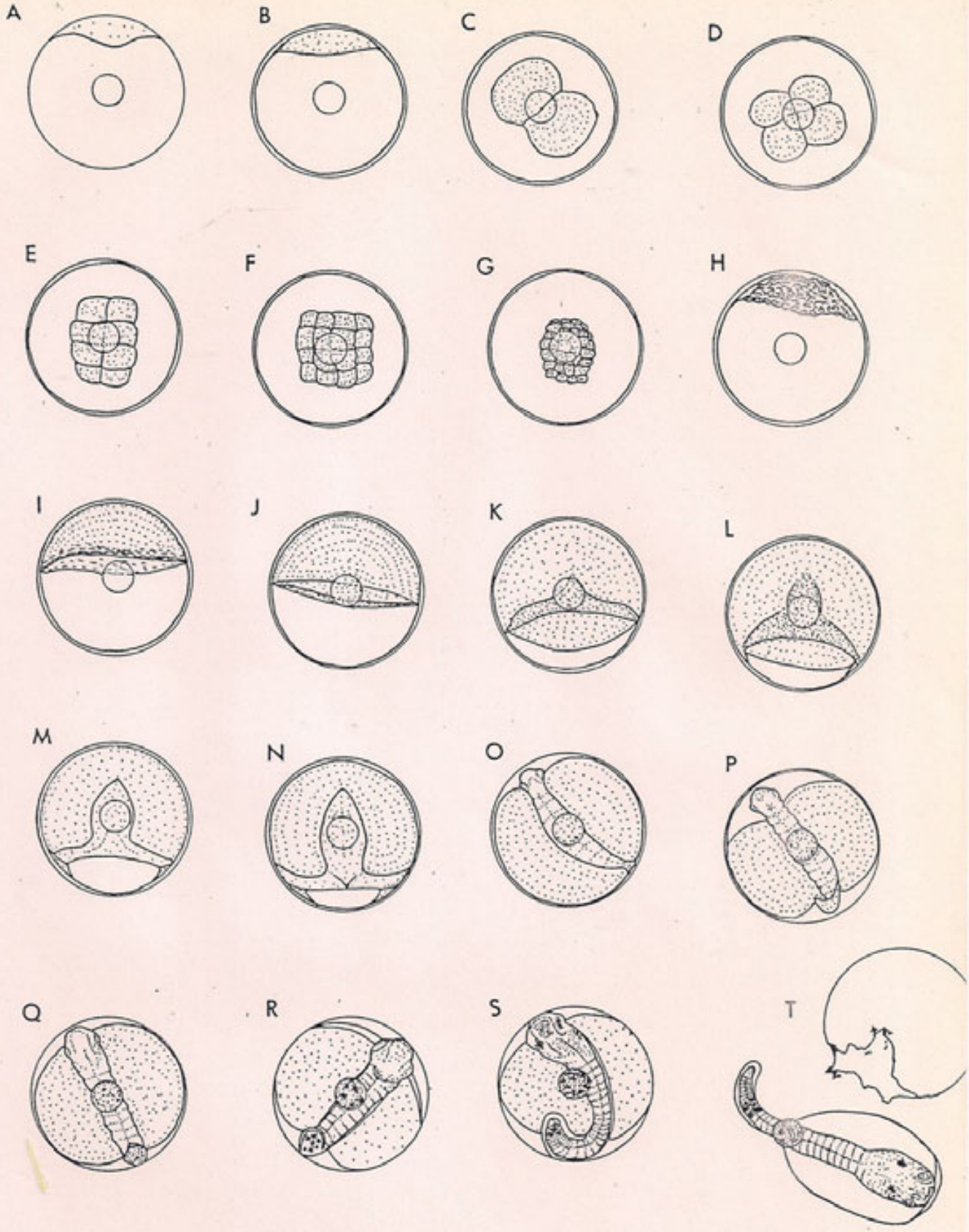

Figure 1. Schematic illustration of embryonic development of black sea bream.

Explanations of schematic illustrations in figure 1.

A: Unfertilized zygote; Egg is spherical in shape, 0.80-0.85 mm in diameter. Transparent. Single oil globule is centrally located, 0.20mm in diameter. Presence of cytoplasmic granules is in less density. Abnormal occurence of two oil globules is observed in some eggs. Micropyle is evident on the animal pole.

B: Fertilized egg; 0:10-0:15 hours post-fertilization; Thin perivitelline layer is evident in the outer egg margin increases in size to 0.90 mm. Cortical granules are evident in all the cytoplasmic area. Cytoplasmic accumulation process in progress along the animal pole. Micropyle has disappeared.

C: Two cells stage; 0:40-1:00 h. Polar cap division is in two dome-shaped cells.

D: Four cells stage; 1:20-1:30 h. Perpendicular division of the first cleavage to the second results to 4 blastomeres.

E: Eight cells stage; 1:55-2:20 h. Third cleavage results to 8 blastomeres.

F: Sixteen cells stage; 2:25-2:30 h. Fourth cleavage results to 16 blastomeres. Future longitudinal axis is elongated.

G: Thirty two cells stage; 2:40-3:00 h.
Sixty four cells stage; 3:05-3:10 h.
Many cells stage to morula 3:30-3:40 h, 4:00-8:20 h.
Smaller dividing cells to several cells results in thick blastoderm formation. The small and irregularly shaped cells of this stage obscure the complexity of cleavage on this fifth plane division. Cleavage is not simultanously completed in all eggs. Precise number of dividing cells is difficult to be distinguished. Mass minute blastoderm starts to spread on the animal pole.

H: Early high blastula;11:10-11:20 h. Blastomeres mass on the upper region. Many minute celled-formations are at the dome-shaped blastoderm.

I: Late high blastula; 15:00-15:15 h. Smaller blastomeres develop and spread. Dome-shaped blastoderm is considerably elevated. Blastoderm invasion commenced over the yolk.

J: Flat expanding blastula (Mid-yolk invasion); 16:10-16:25 h. Yolk is half invaded as germinal ring formation invades the yolk. Blastoderm becomes more flattened. Periblast cells are spread.

K: Early gastrula (late yolk invasion); 17:40-17:46 h. More than half of the yolk is covered by the periblast. Primitive endoderm moves forward beneath presumptive ectoderm. Germ ring is formed as embryonic shield develops. Marginal ridge is visible within the inner layer of the germ ring.

L: Mid gastrula; 18:00-18:10 h. Upper half of the yolk mass is occupied by the embryo. Neural keel appears in mid-line embryonic shield.

M: Late gastrula; 18:30-18:42 h. Within the membrane embryo rotates. About $\frac{3}{4}$th of the yolk is took up by the periblast. At the ectodermal area the anlage of the central nervous system is thickened.

N: Yolk plug (closure of blastopore); 19:50-20:00 h. The periblast covers almost all the part of yolk.

O: Neurula; 20:05-20:12 h. Embryonic shield elongates and becomes a raised streak from the surface of the yolk sac indicating the formation of the anterior axis of embryo. Solid neural keel invagination indicates the formation of central nervous system. Head region expands to form the optic bud. Embryo-like form can be observed.

P: Early phase organogeny; 20:50-21:05 h. Optic bud is differentiated. Neural keel is prominent. 3-4 somites and Kupffer's vesicle appear.

Q: Mid phase organogeny; 22:05-22:16 h. 7-9 somites are visible. Differentiation of head and tail region is evident. Eye cup starts to be formed.

R: Late phase organogeny; 25:15-29:05 h. Somites develop to 10-20 in number. Heart tubules can be seen at ventral cephalic region. Pigmentation starts along the dorsal body area and along the outer pole of the oil globule. Kupffer's vesicle is more prominent.

S: Pre-hatching stage; 32:10-37:50 h. Heart starts to beat. Egg yolk has been depressed as head and tail further develop. The tail bud curves along the yolk. Body twiching is observed. Optic cup is very distinct. 20-25 somites developed. Auditory placode appears posterior to the head region. Finfold membrane formed along the tail region. Pigmentation increases especially at head and peduncular region.

T: Hatching stage; 38:50-40:10 h. At hatching time somites have developed to 26-28 in number. Urinary vesicle is prominent along the ventral margin of the finfold. Oil globule is sited along the posterior base of the yolk mass. Melanophore pigmentation is intensive in the peduncular region.

3.3 Larval morpho-ecological development

The newly hatched larvae measured 2 mm in average body length and 0.6 mm in body depth. They had about 26 somites and a prominent pigmentation was massed between the 17th and 19th caudal myomeres. The oil globule was positioned posterior to the yolk mass in front of the urinary vesicle. The fin bud was found between the optic cup and the otolith. The larvae floated with the oval shaped yolk sac belly up and head down, and made sudden movements of the posterior half region of the body. These movements became more and more intense 10 minutes after hatching. Touching with a pinset slightly the head region did not show any sign of sensitivity by the larvae. About 4 hours after hatching the larvae tried to swimm but with no success.

The 1-day old larvae possesed a slightly decreased yolk sac to about 0.5 mm in diameter. The average body length increased to 2.5 mm and the oil globule at the position of the 5th and 6th myomere slightly decreased in diameter. The urinary bladder was enlarged and the optic lobe pigmentation was more prominent. The pectoral fin bud was more elongated but remained unfunctional. Alimentary tube was almost invisible along the ventral body margin.

The 2-day old larvae measured 2.85 mm in average body length. The yolk bulk has been decreased more and the oil globule could be seen somewhere in the middle between snout and anal region. The anal region differentiated and the alimentary canal was more evident, with the urinary bladder joining behind the anal region. The auditory placode moved more close to the eye lobe. No blood vessel is still evident. Larvae at this stage drifted on a fully vertical position but a slight gain of control was evident in comparison with the 1-day old larvae.

The 3-day old larvae gained swimming locomotion with subsequent effect that the larvae distributed from subsurface down to the upper bottom surface of the tank. The first appearance of the eye pigmentation made the larvae more visible to the naked eye. In the pectoral fin the first segment can be observed. The upper and lower jaws were clearly separated but the mouth appeared to be not yet functional. The posterior gut expanded but the anal pore was still closed. The mid-gut showed early sign of convolution in the region just behind the yolk sac which was continuously shrunken.

The 4-day old larvae had the completely separated jaws, the mouth being continuously functioning. The eyes were fully pigmented. Between the presumptive fore and mid-gut a loop curvature demarkated the magor gut subdivisions, and the post-constriction of the digestive tube was prominent. The anal pore was likely to open. The yolk and oil globule still remained between the loop structure and the heart. The larvae well controlled body motion balance with the locomotion of pectoral fins. The ability to avoid approaching objects was also noted.

The 5-day old larvae fed on rotifers and grew to a size of 3.6 mm.

The 6-day old larvae had circulation of unpigmented blood cells to the ventral aorta and post -cardinal vein. Nasal pores start to be formed at the antero-orbital region. The yolk sac was almost completely absorbed and the liver is formed at the site of absorption. Air bladder was also detected in many fish. The larvae at this stage exhibited schooling behaviour concentrated along the tank corners in patches. Feeding rate increased showing visible food in the digestive tract with occassional peristalsis.

The 9-day old larvae displayed the size of 3.8 mm in total body length and pigmentation of the ventral side of each myotome.

The 10-day old larvae attained a size of 4.5 mm. The vertebral spines are well distinguished and the general demarkation of the future finfolds is evident. The hypural rays initiated to be produced along the ventral side of the caudal tip. Primordial rays were also formed along the ventral and dorsal margin of the body, along the bases of finfolds.

The 15-day old larvae grew to 6 mm and showed segregational tendencies of individuals. The development of caudal and dorsoventral fin rays and the frequently inflated air bladder were very prominent.

In the following 16, 17, 18, 19th days the caudal ossification progressed rapidly, and ossification of hypural, epural and urostyle bones were almost completed on the 20-day old larvae, which attained a size of 7.9 mm in average total body length. Segmentation of two caudal rays was evident at this stage and the whole appearance of the larvae, with clearly visible dorsal and ventral fin rays, verified the fact that the larvae of this stage being resistant to stress and transportation.

The metalarval stage at about 20 to 24-day old larvae showed the following changes. The myomeres were more reddish and semi-transparent. The whole blood vessel system was functionally completed and the gill arches became reddish. Mass lateral pigmentations were concentrated along the caudal bone margins, and melanophores increase in number with development of larvae. The abdominal peritonium appeared silver in color. The fish at this stage attained an average size of 9mm but wide variation in growth was noticeable.

At ages over 25th day elongation of the snout to an adult-like form, and pigmentations along the dorsal and ventral fin bases and

the dorsal side of spinal cord were clearly evident. When the metalarvae grew older than 32 days in age, body pigmentations formed bands extending vertically across the body axis from nape to caudal region. The nasal pores at this stage and the teeth on both jaws were very evident, indicating a clue to the feeding capabilities of larvae. The fish exhibited rheotaxic and phototaxic tendencies and is very tolerable to water currents and handling.

During the larval development the three major stages termed protolarval, mesolarval, and metalarval could be distinguished as follows.

1. Protolarval (or pro-larval stage). From hatching through the yolk sac stage and feeding commencement, until the yolk is fully absorbed around the 6th day. Duration 6 days.

2. Mesolarval (or mid-larval stage). From the stage that all yolk has been utilized, until the whole marginal and caudal fins have been fully formed and functional, around the 20th day. Duration 14 days.

3. Metalarval (or post-larval stage). From the 20th day until the pyloric caecae have been fully formed, around the 35th day. Duration 15 days.

In account to the process of pigmentation the following were noted. Stellar pigmentation predominated the oil globule from the late phase organogeny, and retained until the oil globule being fully absorbed at the end of protolarval stage. In the pre-peduncular region pigmentation was also present, even before hatching, and it was maintained through the first 3 days of protolarval stage disappearing thereafter. As the larvae grew older melanin pigmentation,

varying slightly with individuals, became progressively increased in distribution along the base of dorsal and ventral fin, peritonium, dorsal area of spinal cord and head region. Finally from the 35th day and on, the heavy pigmentation on body surface of the larvae made them intransparent for morphological observations of internal structures.

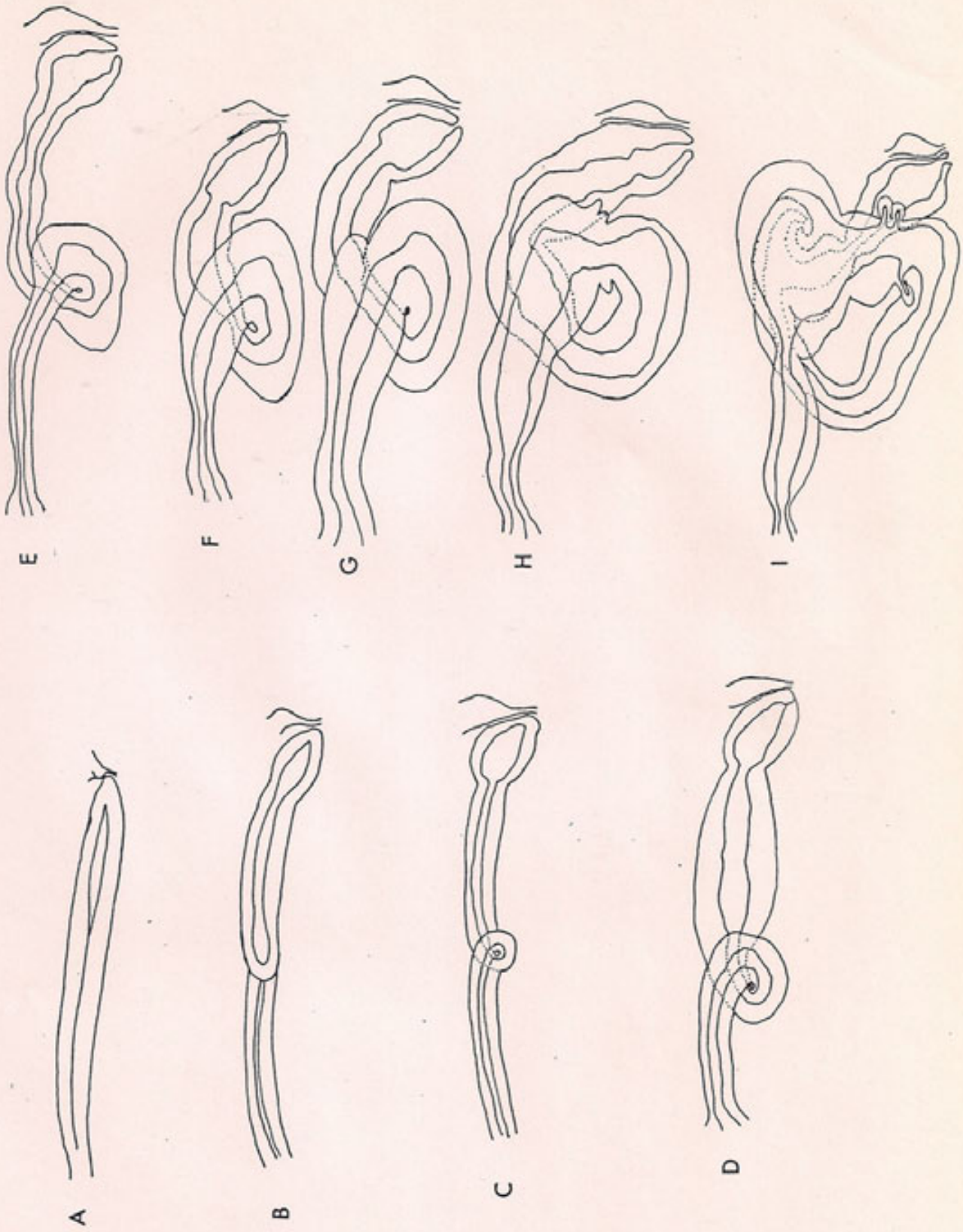

Figure 2. Schematic illustration of the digestive tract of larval black sea bream.

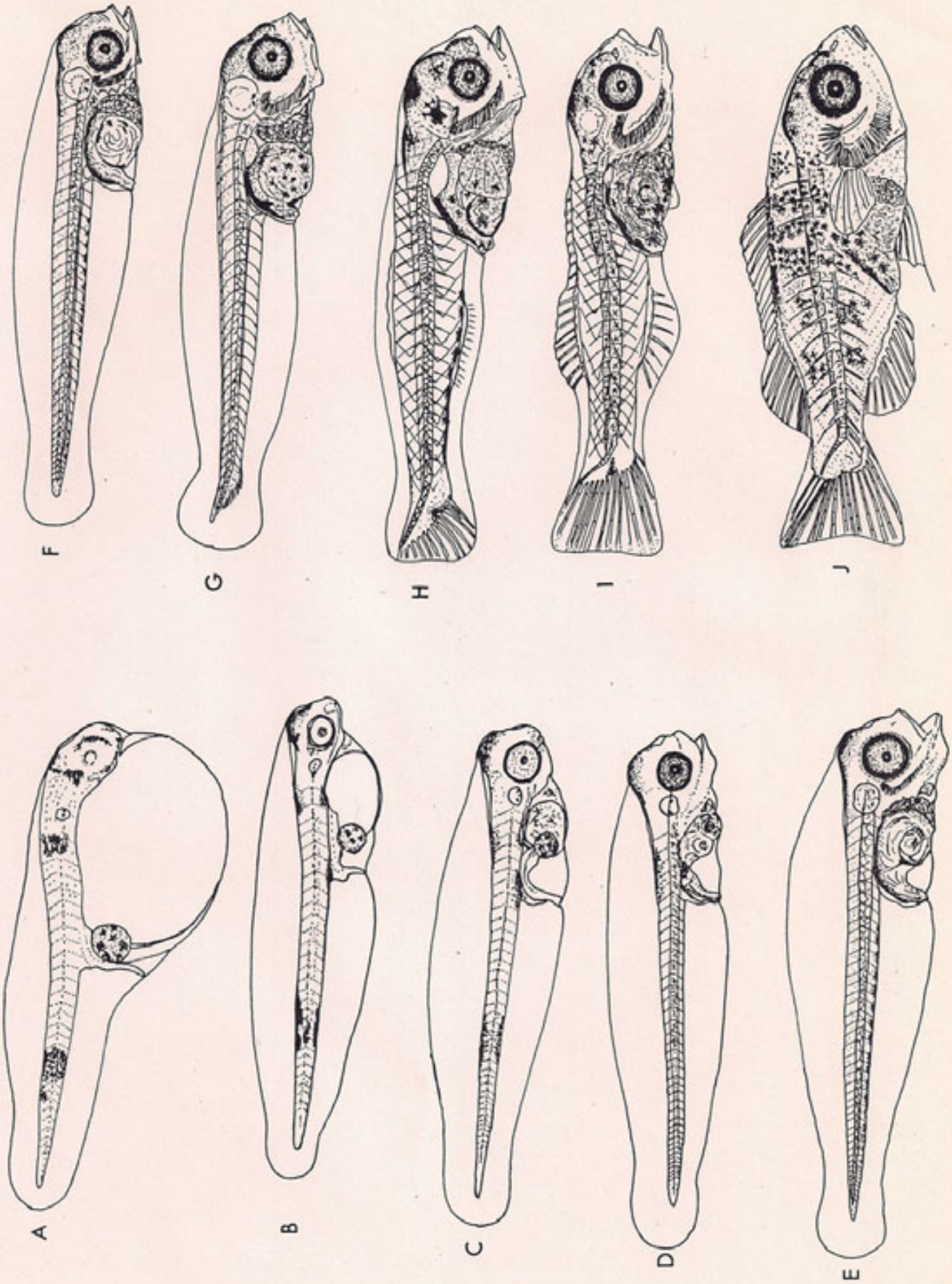

Figure 3. Schematic illustration of larval black sea bream.

Explanations of figure 2.

A: Digestive tract of a newly hatched larva.

B: Digestive tract of a 2-day larva.

C: Digestive tract of a 4-day larva.

D: Digestive tract of a 7-day larva.

E: Digestive tract of a 12-day larva.

F: Digestive tract of a 18-day larva.

G: Digestive tract of a 26-day larva.

H: Digestive tract of a 34-day larva.

I: Digestive tract of a 40-day larva.

Explanations of figure 3.

A: Newly hatched larva. 2 mm in average total body length.

B: 2 days old larva. 2.8 mm in average T.B.L

C: 3-days old larva. 3.3 mm in average T.B.L

D: 4 days old larva. 3.5 mm in average T.B.L

E: 6 days old larva. 3.6 mm in average T.B.L

F: 8 days old larva. 3.7 mm in average T.B.L

G: 13 days old larva. 5.4 mm in average T.B.L

H: 18 days old larva. 7.0 mm in average T.B.L

I: 24 days old larva. 9.3 mm in average T.B.L

J: 40 days old larva. 15.0 mm in average T.B.L

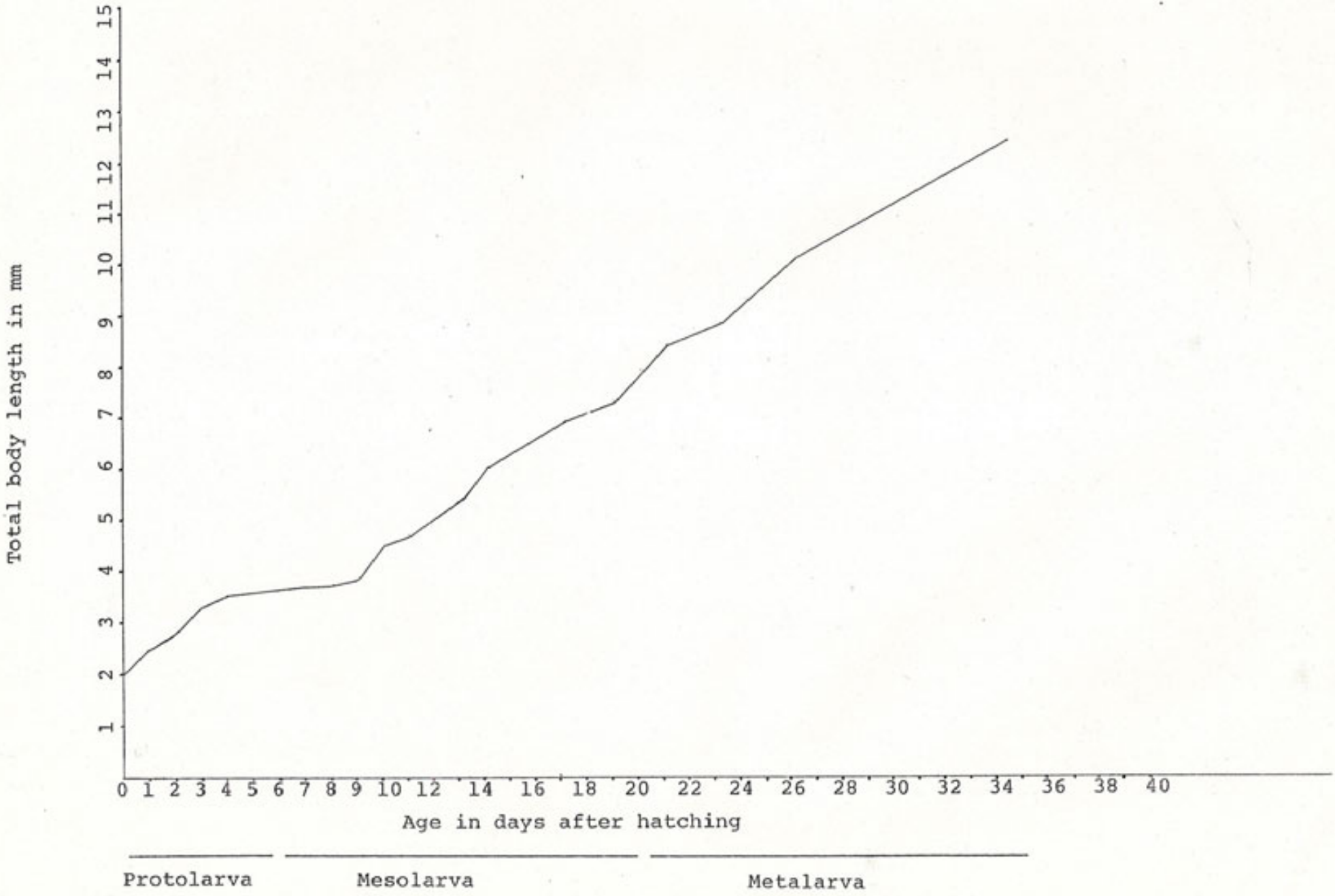

Fig. 4. Growth curve of black sea bream

Table 1. Record of larval rearing of the black sea bream from hatching to the early juvenile stage.

Age from hatching (in days)	Number of fish examined	Total length (mm) Mean	SD	Range	Temperature (°C)	Salinity (sp.gr.)	Food*	Culture accommodation **
0	10	2.0	0.187	1.6-2.2	20.0	1.022	-	F T
1	10	2.5	0.141	2.2-2.6	20.0	1.028	-	F T
2	10	2.8	0.141	2.6-3.0	20.1	1.031	-	F T
3	10	3.3	0.173	3.1-3.6	2o.o	1.025	-	F T
4	10	3.5	0.154	3.3-3.7	20.4	1.026	R	C T
5	10	3.5	0.174	3.2-3.7	20.2	1.039	R	C T
6	10	3.6	0.161	3.4-3.9	20.4	1.035	R	C T
7	10	3.7	0.194	3.4-3.9	20.9	1.030	R	C T
8	10	3.7	0.184	3.4-4.1	21.2	1.025	R	C T
9	10	3.8	0.173	3.5-4.1	20.9	1.029	R	C T
10	10	4.5	0.214	4.0-4.7	20.9	1.032	R	C T
11	10	4.7	0.100	4.5-4.8	21.1	1.040	R	C T
12	10	5.0	0.282	4.4-5.3	21.2	1.035	R	C T
13	10	5.4	0.279	4.9-5.8	21.3	1.035	R	C T
14	10	6.0	0.794	4.8-7.0	21.0	1.036	R	C T
15	10	6.0	0.728	4.9-7.2	21.2	1.029	R	C T
16	10	6.6	0.507	5.7-7.4	21.4	1.032	R	C T
17	10	6.9	0.800	5.6-7.6	21.4	1.035	R	C T
18	10	7.0	0.828	5.7-8.0	21.5	1.036	R	C T
19	10	7.3	0.809	5.9-8.1	21.4	1.042	R	C T
20	10	7.9	1.165	6.0-10.0	21.5	1.040	R T	C T
21	10	8.4	1.168	6.7-9.7	21.6	1.039	R T	C T
22	10	8.7	1.368	6.9-10.8	21.5	1.045	R T	C T
23	10	8.8	1.322	7.0-11.1	21.4	1.043	R T	C T
24	10	9.3	1.525	7.0-11.4	21.3	1.024	R T	C T
25	10	9.5	1.663	7.0-11.8	21.3	1.035	R T	C T
26	10	10.1	1.719	7.2-12.4	21.0	1.030	R T	C T
30	10	10.9	2.074	8.0-13.9	21.4	1.032	C F AD	C T
34	10	12.4	2.636	8.2-15.6	21.4	1.030	F AD	C T
40	10	15.0	3.825	10.0-20.1	21.5	1.033	F AD	C T

* R= rotifers (Brachionus plicatilis), T= copepods (Tigriopus japonicus), C= naturally collected copepods, F = powderized fish meal, AD= artificial diet.

** F T= 500 liter fiberglass tank, C T= 1600 liter concrete tank.

Table 2. Morphological records on the differentiation of major organs and body structures of black sea bream, from hatching to the early juvenile stage.

Age (in days)	Head region	External and internal body region	Fins
0	av, h, oc	uv	pfb
1			
2		ac	
3	lj,m,sf,uj	ic,ig,pc	pfs
4	ep,ga	ag,ao	
5		bc,fm	
6	np	bf,bv,ls,oga,p,ya	
7	gf	ab,vs	
8		my	
10	ob		
13	ov,rgs		
14	rha	rbc	
15		rsp	cb,dmf,vmf
16			cfr,hy
18			cfs
19			afr
20	tj		
24			ffa
26			hcf
30		ll	
32			plfs
40		sc	

Legends:

Head region-av,auditory vesicle; ep,eye pigmentation; ga,gill arches; gf, gill filaments; h, heart; lj, lower jaw; m, mouth; ob, opercular blades; oc, optic cup; ov, oral valve; rha, reddish heart; rgs, reddish gill structure; sf, snout formation; tj, teeth on jaws; up, upper jaw; np, nasal pores.

External and internal body region- ab, air bladder; ac, alimentary canal; ag, anal groove; ao, anal opening; bc, blood cells; bf, blood flow; bv, blood vessels; fm, food materials; ic, intestinal convolution; ig, intestinal groove; ll, lateral line; ls, liver structure; my, myomere; oga, oil globule absorption; p, peristalsis; pc, post constriction; rbc, red blood cells; rsp, reddish spleen; sc, scales; uv, urinary vesicle; vs, vertebral spines; ya, yolk absorption.

Fins-afr, anal fin rays; cb, caudal bending; cfr, caudal fin rays; cfs, caudal fin segments; dfr, dorsal fin rays; dmf, dorsal marginal fin; ffa, finfold absorption; hcf, homocercal caudal formation; hy, hypural; pfb, pectoral fin bud; pfs, pectoral fin segments; plf, pelvic fin; plfs, pelvic fin segments; vmf, ventral marginal fin.

Table 3. Histological records on the differentiation of the major digestive organs and accessory parts of black sea bream from hatching to the early juvenile stage.

Age (in days)	Buccupharyngeal	Esophagus	Stom.	Int.	Rect.	Panc.	Liv.	Others
0								h,ub
1	gic							
2							hc	kt*
3	ma			ic		pc,zg	bd	gb
4	ga,lj,uj,mo,			cc,m	ao,rv	pd	v	tf,ab
5			cuc					bc,bv*
7		lf			vc	Lc*		s
8	mc	mc*		vc			lv	gcc
9							gl	
10			lf					gl
15				gc	gc			
16	pt*,tb*							
17			bs					
21	tj		pv		as			
22			gg					
30				pc				
47								at

* denotes structures that might have been differentiated earlier.

Legends: Buccupharyngeal- ga, gill arches; lj, lower jaw; ma,mouth anlage; mc, mucous cells; mo, mouth opening;pt, pharyngeal teeth; tb, taste buds; tj, teeth on jaws; uj, upper jaw; gic, gill initiating cells.

Esophagus- lf, longitudinal folds; mc, mucous cells.

Stomach - bs, blind sac; cc, columnar cells; gg, gastric glands; lf, longitudinal folds; pv, pyloric valve; cuc, cuboidal cells.

Intestine- cc, columnar cells; gc, goblet cells; ic,intestinal convolution; m, microvilli; pc, pyloric caeca; vc, vacuole cells.

Rectum- ao, anal opening; as, anal sphincter; gc, goblet cells; vc, vacuole cells; rv, rectal valve.

Pancreas- Lc, Langerhans cells; pc, pancreatic cells; pd, pancreatic duct; zg, zymogen granules.

Liver-bd, bile duct; gl, glycogen; hc, hepatic cells; lv; liver vacuoles; v, vascularization.

Others- ab, air bladder; bc, blood cells; bv, blood vessele; gb, gall bladder; gcc, gill chloride cells; gl, gill lamellae; h, heart; kt, kidney tubules; s, spleen; tf, thyroid follicles; at, adipose tissue.

3.4 Histological observations

One day old larvae possessed a furrow in the site of the still closed mouth anlage between the optic lobes in the head region. Gills are recognized as a basophilic mass of cells, but no structure is still evident. The eye cortical layers and lens developed unpigmented. Renal tubules are evident close to the urinary bladder and a perivitelline syncytium of enthothelial origin covers the entire oil globule and the yolk sac. The digestive gut is recognized as a straight tube lined with cuboidal cells (fig. 19).

In 2-day larvae the eye cortical layers and lens are well formed but still unpigmented (fig. 21). The digestive tube bends toward anal pore (fig. 22). Liver cells differentiated on ventral side of anterior part of digestive tract, but they do not comprise as yet a distinct organ. The upper jaw cartilage formation is also in progress.

In 3-day larvae a lot of remarkable changes occured. The intestinal convolution forms a loop-like structure behind the partially absorbed oil globule, and the post constriction marks the boundary between intestine and rectum (fig. 23). Pancreatic cells are formed and produce zymogen granules. The gall bladder with a narrow lumen has been formed in ventral part of the liver.

In 4-day larvae the digestive tube is lined with columnar cells with a well developed striated border. The mouth has opened and the eye is fully pigmented (fig. 24), indicating active feeding. The upper and lower jaws are formed. Gill arches are also formed. The rectal valve clearly separates the rectum from intestine (fig. 26). Hematopoietic tissue forming immature erythrocytes has also been observed in the anterior kidney (fig. 25). The air bladder appeared inflated.

In 5-day larvae the yolk particles in absorption are seen to be invaded by liver cells (fig. 27). Food materials are evident in the intestinal lumen and blood erythrocytes are also evident in hepatic sinusoids.

In 7-day larvae longitudinal folds in oesophagus and its enlarged posterior part, judged to be the rudimentary stomach are very evident. The considerably enlarged intestinal lumen fills with food particles (fig. 28). Eosinophilic granules appeared in rectal epithelium indicating albuminous absorption (fig. 29). The spleen is formed (fig. 30). Langerhans islets are also detected in pancreatic cells (fig. 30). Liver cells are storing glycogen.

In 8-day larvae minute fat vacuoles in mid-intestinal apical region and big fat vacuoles in posterior intestine are evident (fig. 31). The oesophageal epithelium possesses mucous cells and chloride cells were detected on gill filaments.

In 9-day larvae the mucous cells of oesophagus have been increased in number. Renal glomerulus was detected with no blood in capillaries. Blood erythrocytes were detected into gill filaments.

In 10-day larvae the intestinal mucosae is thickening and early signs of pyloric sphincter formation can be distinguished. Glycogen accumulation is obvious in liver. Gill lamellae initiate development. Hematopoietic tissue of anterior kidney increases in cell number.

All the above characteristics of the 10-days larvae were carried on in increased developmental intensity through 11, 12, 13, 14-days old larvae.

In the 15-day larvae goblet cells were for the first time detected in intestine and rectum. Gill lamellae are well developed.

In 16-day larvae pharyngeal teeth and taste buds were detected (fig. 32). The kidneys appear fully developed (fig. 33).

In 17-day larvae pharyngeal teeth and taste buds abruptly increase in number. The stomach appears to be considerably enlarged with its future blind sac already formed.

In 18-day larvae the gill filaments have taken the adult form.

In 19-day larvae goblet cells are detected increasing in number in the intestinal epithelium (fig. 34). 20-day larvae did not show any significant changes.

In 21-day larvae canine teeth formation on both jaws and numerous mucous cells on oesophageal epithelium are well prominent features.

In 22-day larvae gastric glands in an early stage of development were first detected (fig. 35), and blind sac of stomach is also well developed.

In 23-day larvae the pyloric sphincter is fully formed (fig. 36). In following 24, 25, 26, 27, 28-day larvae, the gastric glands development continued increasing in number. Glycogen in liver gradually diminished.

In 29-day larvae fat vacuoles detected in the liver (fig. 38).

In 30-day larvae the gastric glands appear to be fully developed (fig. 39). The pyloric caeca start to form and eosinophils were detected in pyloric submucosae.

In 31, 32, 33, 34-day larvae taste buds increase in number in the pharyngeal epithelium (fig. 41).

In 35-day larvae the pyloric caeca are fully formed marking the transitional stage to prejuvenile fish. At this stage fat vacuoles are filling the liver cells. The last significant change in histological characteristics was the development of adipose tissue in the

47-day larvae (fig. 42). It should be noted that the pancreas and liver remain separate for the whole larval and early prejuvenile stage, and they do not fuse to form the hepatopancreas. This is formed around the 50th day.

Four phases could be distinguished in account of utilization of yolk and oil globule, related to the topographical position of the oil globule.

1st phase: The oil globule is lowered from its original in a newly hatched larva, to a point where it reaches the level of the presumptive anal pore in a 1 day old larva.

2nd phase: A loop-like structure of intestinal tube is formed behind the yolk mass and oil globule on a 3-day larva.

3rd phase: Yolk mass remnants still cover the oil globule, and the whole structure is lying behind the cardiac wall and bounded by newly differentiated liver tissue on a 4 to 5-day larva.

4th phase: The liver is totally covering the yolk droplets, and the oil globule is already diminished in volume and obscure in observation. Absorption takes place by diffusion in the liver tissue. This is completed on the 6-day larva.

DISCUSSION

The breeders of black sea bream in this experiment were of variable ages and weight, and were kept altogether in a 120 ton metalic outdoor tank, and were spawning regularly every day around sunset.

The spawning season of black sea bream starts late on March and ends late on May. The eggs used in this experiment were acquired early in May, when it was nearly the end of spawning season. One batch of eggs was taken and treated properly from incubation of eggs through hatching and larval rearing to prejuvenile stage.

The hatching rate, estimated through random microscopical observations to be 46 %, was lower than usual average rate of more than 75 %. This can be due to rather high temperatures of May, and to suspected deteriorating conditions of spawners because the experiment started rather late in the spawning season of this species. However developing larvae did not show any sign of lacking yolk nutrients.

The fertilized eggs can be easily distinguished from unfertilized ones due to the perivitelline space which is created after impregnation. Laale (1980), indicated the existence of a perivitelline-like space in unfertilized eggs of Salmo salar, Salmo gairdneri and Catostomus commersoni among others, but this was not observed on the unfertilized eggs of black sea bream.

The longest phase recorded in embryogeny was about 7.5 hours during the late phase of organogenesis, and about 5.5 hours in the pre-hatching stage. Within the temperature fluctuation range of 19.0-19.8°C in this experiment, hatching occurred at 39-40 hours after fertilization. This significantly differs from that of the red sea bream when it occurs after three days at temperatures 16.0-17.0°C, (Kitajima, 1978).

The density of larvae at this experiment was 50 larvae per liter of water. Fukuhara (1979) reported stocking density of 5-50 larvae per liter to be effective to avoid stress and outbreak of disease.

No apparent mortality was observed during the protolarval and mesolarval stages. This could be probably attributed to the intensive care paid to the larvae, concerning food supply and renewing of water. However during the later period of metalarval stage and early prejuvenile stage, significant loss of larvae was noted due to cannibalism.

First feeding was observed on the 4-day larvae in which mouth was functional, eyes fully pigmented, and digestive organs developed. At that time the yolk sac was two days prior to full absorbance and therefore the larvae were judged to have two sources for acquisition of nutrients. The tiny larvae with a high metabolic demand cannot afford a great stress condition to occur when their internal nutrients are depleted. By starting feeding before the yolk is consumed, they smooth out the transitional period of switching from internal to external source of energy. The so called critical period coinciding with a growth stagnacy at the first days of active feeding, is an indicator of the difficult time they face. The larvae which acquire a surplus of nutrients will survive further and the excess is accumulated in the form of fats in the intestinal epithelium. The growth recovery coincides with the appearance of these fat vacuoles in the intestinal epithelium (Tanaka, 1973). Tanaka (1973) also indicated that exept for nourishement these intestinal fat vacuoles have and an ecological significance, bringing about boyancy in the larvae at a time when they do not yet possess well developed fins.

In account of the yolk utilization process of black sea bream; a direct transfusion of yolk materials into larval tissues and especially subdermal space is not supposed to occur, since no discontinuity was histologically observed in the yolk sac syncytium. Shelbourne

(1955) in plaice and Umeda (1979) in yellowtail, have referred to a direct transfusion of yolk into the subdermal space of larvae. Yamada (1959) in pond smelt, dog salmon and rainbow trout larvae, and Balakrishnan and Devi (1970) in larvae of Ambassis, Mugil, Dorosoma and Thrissocles species have indicated an absorption proccess through the early circulatory system. This latter one seems to be the case in black sea bream larvae. Utilization of yolk takes place through the early circulatory system in the first half of protolarval stage, with the vitelline syncytium acting as the primary site of absorption. This utilization is then completed at the later half of protolarval stage with the direct diffusion in the hepatic cells.

Larvae with digestive organs fed on zooplanctons. However the digestive mechanisms of larvae are absolutely different from those of adult fish since no stomach is yet formed. Gastric glands firstly formed at around the 22nd day but, judging from their appearance and number, they remain unfunctional until the 30th day when they predominate the stomachal mucosa. But even at this stage no zymogen granules were detected casting doubt on the functional role of stomach. It seems thus likely that the pancreas plays the important role of digestive enzymes secretion throughout the larval stages of black sea bream.

The liver played an important role for the storage of nutrients acquired by the larvae after the yolk sac has been consumed. The liver is the main site for glycogen storing in all the mesolarval and the first half of metalarval stage. Fat starts to be stored in the liver only during the later half of this stage. Mid-intestinal fat vacuoles indicate that fat was absorbed by the mid-intestinal mucosa.

Proteins on the other hand, are absorbed from the rectum in the mesolarval and metalarval stages, in which large eosinophilic granules

are detected. This indicates that the rectal epithelium is the site to absorb protein. Same findings were reported in larvae of black and red sea bream (Tanaka, 1973), yellowtail (Umeda and Ochiai 1973), and Tilapia nilotica among others (Watanabe, 1981).

DISEASED LARVAL FISH

1. MATERIALS and METHODS

Diseased larval fish were collected from rearing tanks of Kochi Fisheries Experimental Station. These specimens were fixed in 10 % formalin solution, embedded in paraffin and serially sectioned at 4 to 6 μ in thickness. They were then stained with hematoxylin eosin (H-E) stain, PAS reaction and Giemsa stain. Some larvae were also imprinted on slide. The imprinted specimens were then air-dried, fixed with methanole and stained with Giemsa stain and PAS reaction.

The total number of larvae fixed in formalin was 39, (24 of red sea bream and 15 of black sea bream).(Table 4).

The age range of collected specimens was from 17 days old to 31 days old for black sea bream, and from 17 days old to 52 days old for red sea bream.

2. RESULTS

2.1 Gross symptoms

Diseased larval fish of red and black sea bream usually exhibited abnormal swimming behaviour, loss of appetite and segregetional tendencies. They often appeared dark in coloration or with the abdomen swollen.

A 52 days old red sea bream fish exhibited red mouth and fin erosion.

2.2 Histopathology

1. Enteric bacterial disease

Bacteria with various shapes were excessively multiplied among food particles in the intestinal and rectal lumen (fig. 46). They produced metabolic gases expanding the intestine and rectum. Some

of the expanded intestine and rectum also underwent desquamative catarrh (fig. 53). The hepatocytes were atrophied and vacuolated without bacterial invasions (fig. 48). The spleen was engorged with blood (fig. 49). Bacteria were also seen to migrate into heart chambers (fig. 47). Pancreas, gill filaments, kidney and musculature did not show any sign of bacterial invasion, nor other histological structures.

2. Dermal bacterial disease

One fish showed hemorrhagic ulcerative lesions in the mouth and fin erosion. In the mouth lesion, long rods attack the epithelium (fig. 58), and short rods invade the dermis and lateral musculature (fig. 59). The infected tissue was necrotized. In the caudal region and the dorsal fin region, long rods attack the epithelium necrotizing it. Short rods subsequently invade through the necrotized epithelium and attack the underlying musculature necrotizing it (fig. 60), (fig. 61).

3. Ichtyobodo necator infestation

Larvae of black and red sea bream were infested with Ichtyobodo necator on the skin (fig. 51). Protozoan attacked the topmost layer of the skin causing epithelial necrosis and erosion (fig. 43). The infested skin occassionally showed secondary bacterial invasion. The hepatocytes were atrophied. The air bladder appeared to be considerably inflated. There were also larvae with both of enteric bacterial problem and Ichtyobodo necator infestation.

4. Starvation

The larvae which were speculated to have been in diseased condition because of starvation, exhibited as a main histopathological change atrophied hepatocytes.

2.3 Ichtyobodo necator

In imprided specimens Ichtyobodo necator showed the follows. The protozoans had two flagellae of unequal length, one nucleus, one blepharoplast containing glycogen-like material, one cytostome, few vacuoles and many granules (fig. 57). In the parasitic condition the protozoans showed pyriform or triangular shape. The protozoans attached to single epithelial cells with penetration of the disk or processes of the pointed end of the body.

Table 4. Record of collected diseased fish.

Diseased fish	Red sea bream	Black sea bream
Total number of fish examined	24	15
Fish with bacterial diseases	14 (58.3%)	10 (66.6%)
Fish with Ichtyobodo infestation	1 (4.1%)	2 (13.3%)
Fish with double infection	3 (12.5%)	2 (13.3%)
Starvation suspected	6 (25%)	1 (6.6%)

DISCUSSION

The main cause for mortality among the collected specimens was seen to be enteric bacteria infection. This was characterized by bacterial multiplication in the digestive lumen. Bacteria produced metabolic gases to expand intestine and rectum. These fish must have suffered loss of appetite resulted in starvation, with subsequent atrophy of hepatocytes. Some intestine and rectum were invaded by bacteria undergoing catarrh. These tissue damages were recognized to result in larval mortality.

Some fish were infested with Ichtyobodo necator on skin. This occurred either singly or accompanied by enteric bacterial trouble. This incident indicates that these protozoa are usually opportunist

to attack weakened fish with bacteria, but this also is able to weaken fish with single infestation.

The pathogenicity of Ichtyobodo necator has been investigated mainly on fresh water fish. On salmonids by Fish (1944), Robertson (1979), Robertson, Roberts and Bullock (1981). On catfish by Rogers and Gaines (1975), Rogers (1979) and Miyazaki (in printing).

On marine fish pathogenicity of Ichtyobodo necator has been confirmed on plaice, Pleuronectes platessa by Bullock and Robertson (1982).

Robertson (1979) studied Ichtyobodo infestation on farmed young salmonids, and observed a remarkable decrease in the protozoan number on gills and skin of the fish as they grew older. He then speculated that they may develop a defensive mechanism as they grew in age. The same may hold true for the marine species of black and red sea bream, as there is no literature on mortality of adult fish because of Ichtyobodo infestation. This fact needs further investigation for evaluation of host-parasite interactions between the concerned fish and Ichtyobodo necator.

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SUMMARY

This study was carried out for the clarification of the physiological developmental stages taking place in a young teleost, to advance informations on the early life stage characters in the black sea bream Acanthopagrus schlegeli. The mature spawners were kept in 120 ton metalic tanks covered with black net and fed on minced or chopped fish twice a day. Spawning took place every day and eggs were collected by an overflow system with a proper size net. Eggs were incubated in indoor 500 liter plastic tanks to hatch and subsequently reared to prejuvenile stage through 50 days. Embryogeny of the developing eggs and larvae was observed with a light microscope.

Hatching occurred 38-40 hours after fertilization at temperatures 19.0-19.8°C.

The newly hatched larvae measured 2 mm in total length and possessed a functional heart. This newly hatched larvae had an early formation of mouth furrow and digestive tract.

First feeding was observed on the 4-5th day after hatching. Larval stages were divided to protolarval,mesolarval and metalarval which were characterized by development of the functional organs. In this study average duration of an each stage was 6 days in protolarval stage, from hatching to the 6th day at 20.0-20.4°C. 14 days in mesolarval stage, from the 6th day to the 20th day at 20.9-21.5°C. 15 days in metalarval stage from the 20th day to the 35th day at 21.0-21.6°C.

Larvae of an each stage were also histologically observed and the markers were described.

Diseased larvae of black sea bream (Acanthopagrus schlegeli), and red sea bream (Pagrus major), reared in various outdoor tanks in Kochi Fisheries Experimental Station, were collected and investigated histologically. The diseased larvae exhibited abnormal swimming or floated passivelly on the surface of the water in a moribund condition. Most of diseased larvae showed bacterial multiplication in the digestive tract lumen, with subsequent production of gas causing swelling of the digestive tract and catarrh. Ichtyobodo necator infestation was also observed in the skin, causing necrosis and subsequent bacterial invasion. Double infection by Ichtyobodo necator and bacteria on skin, and bacteria in the digestive tract was also seen. An individual red sea bream exhibited red mouth and caudal fin erosion. The pathogenic agent was Flexibacter that caused necrosis of the epidermis in the infected regions, and secondary infection of short rodded bacteria that caused necrosis in the underlying musculature.

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EXPLANATIONS OF FIGURES IN PLATES

Figure 1. Cleavage, 4 cells stage 1:20 hours post fertilization (x40).

Figure 2. Cleavage, 32 cells stage 2:50 h. post-fertilization (x35).

Figure 3. Early gastrulation 17:40 h. post-ferrtilization (x22).

Figure 4. Early phase organogeny 20:55 h. post-fertilization (x26).

Figure 5. Mid-phase organogeny 22:30 h. post-fertilization (x30).

Figure 6. Pre-hatching stage 36:50 h. post-fertilization (x30).

Figure 7. Black sea bream; newly hatched larva (x45).

Figure 8. 2 days old larva (x38).

Figure 9. 5 days old larva (x32).

Figure 10. 8 days old larva (x30).

Figure 11. 19 days old larva (x12).

Figure 12. 29 days old larva (x11).

Figure 13. Caudal peduncle of a 13 days old larval black sea bream (x43).

Figure 14. Caudal peduncle of a 15 days old larva (x44).

Figure 15. Caudal peduncle of a 16 days old larva (x44).

Figure 16. Caudal peduncle of a 20 days old larva (x40).

Figure 17. Caudal peduncle of a 29 days old larva (x35).

Figure 18. Caudal peduncle of a 36 days old prejuvenile black sea bream (x35).

Figure 19. 1 day old larval black sea bream. Straight digestive tube and perivitelline syncytium of yolk sac and oil globule H-E (x80).

Figure 20. 1 day larva. Perivitelline syncytium covers the oil globule cuboidal cells line the digestive tube H-E (x320).

Figure 21. 2 days larva. Eye; formation of lens and retina (X320).

Figure 22. 2 days larva. The digestive tube bends toward presumptive anal pore, uv: urinary vesicle H-E (x200).

Figure 23. 3 days larva. Mouth furrow, post intestinal constriction and pancreatic cells, mf: mouth furrow; pc: post constriction; H-E (x100).

Figure 24. 4 days larva. The eye is fully formed with the retina pigmented H-E (x320).

Figure 25. 4 days larva. Hematopoietic tissue of anterior kidney produces immature erythrocytes, rt: renal tubules; ie: immature erythrocytes H-E (x600).

Figure 26. 4 days larva. Rectal valve, striated border of columnar intestinal epithelium, air bladder and pancreatic cells containing zymogen granules. rv: rectal valve; zg: zymogen granules; ab: air bladder; sb: striated border H-E (x200).

Figure 27. 5 days larva. Liver tissue absorbs yolk particles yp: yolk particles; H-E (x300).

Figure 28. 7 days larva. The intestinal lumen is enlarged and filled with food materials H-E (x100).

Figure 29. 7 days larva. The rectal epithelial cells possess eosinophilic granules, eg: eosinophilic granules; H-E (x400).

Figure 30. 7 days larva. Islets of Langerhans and spleen Li: Langerhans islets; sp: spleen; H-E (x600).

Figure 31. 8 days larva. Small fat vacuoles in the apical region of intestinal epithelial cells, fv: fat vacuoles; H-E (x320).

Figure 32. 16 days larva. Taste bud and pharyngeal tooth, tb: taste bud; pt: pharyngeal tooth; H-E (x400).

Figure 33. 16 days larva. The nephron is well developed gl: glomerulus; ht: hematopoietic tissue; rt: renal tubule; H-E (x320).

Figure 34. 19 days larva. Goblet cells in intestinal mucosa gc: goblet cell; PAS (x600).

Figure 35. 22 days larva. First appearance of gastric glands gg: gastric gland; H-E (x320).

Figure 36. 23 days larva, pylorus, H-E (x160).

Figure 37. 28 days larva, well developed canine teeth, H-E (x320).

Figure 38. 29 days larva. Fat cells develop in liver, fc: fat cells; H-E (x320).

Figure 39. 30 days larva. The gastric glands are numerous and well developed H-E (x160).

Figure 40. 30 days larva. Eosinophilic granules in rectal epithelium, eg: eosinophilic granules; H-E (x320).

Figure 41. 34 days larva. Numerous taste buds in pharyngeal epithelium, H-E (x160).

Figure 42. 47 days juvenile. Adipose tissue H-E (x80).

Figure 43. 31 days old diseased black sea bream. Ichtyobodo necator infests skin epithelium causing necrosis, Ich: Ichtyobodo necator; Giemsa (x160).

Figure 44. High magnification of fig.43. Bacteria secondary infect necrotic epithelium, ba: bacteria; Giemsa (x320).

Figure 45. 31 days diseased black sea bream. Abnormal multiplication of bacteria in intestinal lumen produces metabolic gases causing swelling of intestine, Giemsa (x50).

Figure 46. High magnification of fig. 45. Bacteria, ba: bacteria; Giemsa (x320).

Figure 47. 31 days diseased black sea bream. Bacteria multiply in the atrium of heart, ba: bacteria; Giemsa (x480).

Figure 48. 31 days diseased black sea bream. Atrophied hepatocytes H-E (x400).

Figure 49. 31 days diseased black sea bream. The spleen is engorged with blood H-E (x160).

Figure 50. 18 days diseased black sea bream. Abnormal multiplication

of bacteria in intestinal lumen expands it, Giemsa (x160).

Figure 51. 45 days diseased red sea bream. Ichtyobodo necator infests skin epithelium. Epithelial cells exhibit atrophy, vacuolization and necrosis, Ich: Ichtyobodo necator; Giemsa (x200).

Figure 52. 45 days diseased red sea bream. Expanded intestine due to metabolic gases produced by abnormal multiplication of bacteria, Giemsa (x40).

Figure 53. 28 days diseased red sea bream. Bacteria cause catarrh in rectum, Giemsa (x200).

Figure 54. High magnification of figure 53. Catarrh in rectum, ba: bacteria; Giemsa (x400).

Figure 55. 17 days diseased black sea bream. Heavy bacterial multiplication in intestinal lumen, Giemsa (x100).

Figure 56. 17 days diseased red sea bream. Hepatocytes are vacuolized and intestinal epithelium undergoes catarrh, Giemsa (x200).

Figure 57. Ichtyobodo necator. Giemsa (x600).

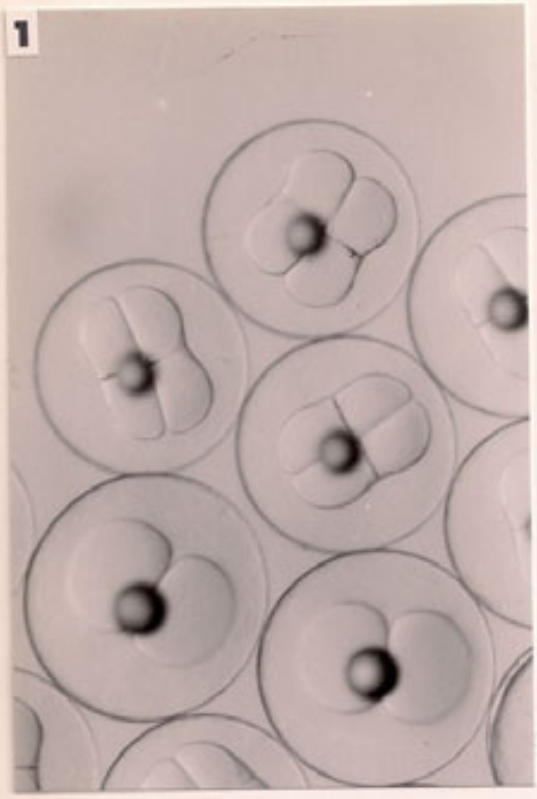
Figure 58. 52 days old diseased red sea bream. Red mouth. Bacterial long rods attack the epidermis and cause necrosis in the mouth region. Giemsa (x460).

Figure 59. 52 days old diseased red sea bream. All the layers of the epidermis have been destroyed by long rodded bacteria and subsequent invasion of short rodded bacteria to the underlying musculature occurs. Giemsa (x320).

Figure 60. 52 days diseased red sea bream. Bacterial long rods excessivelly multiply and destroy the epidermis in the

prependuncular region. Giemsa (x320).

Figure 61. 52 days diseased red sea bream. Short rods have invaded through the necrotized epidermis to attack and necrotize the underlying musculature. Giemsa (x400).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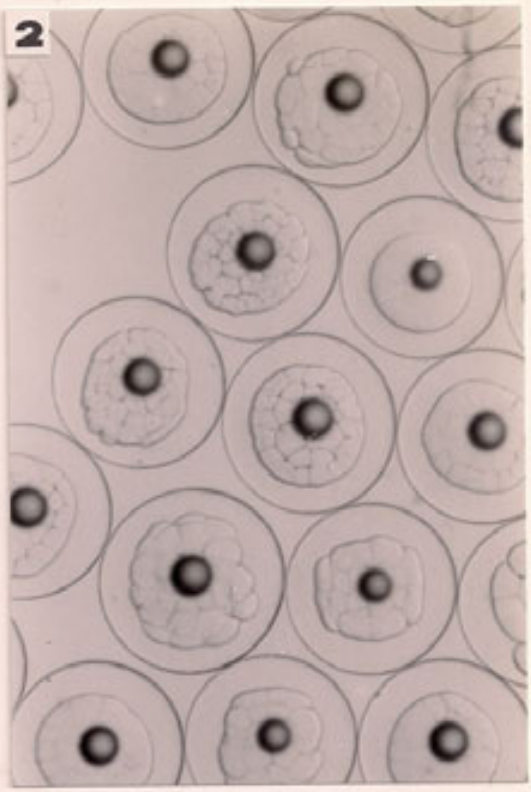
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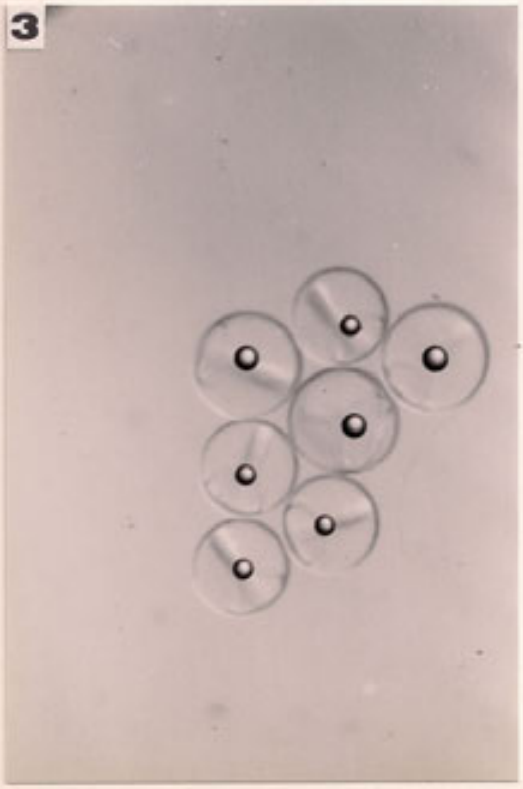
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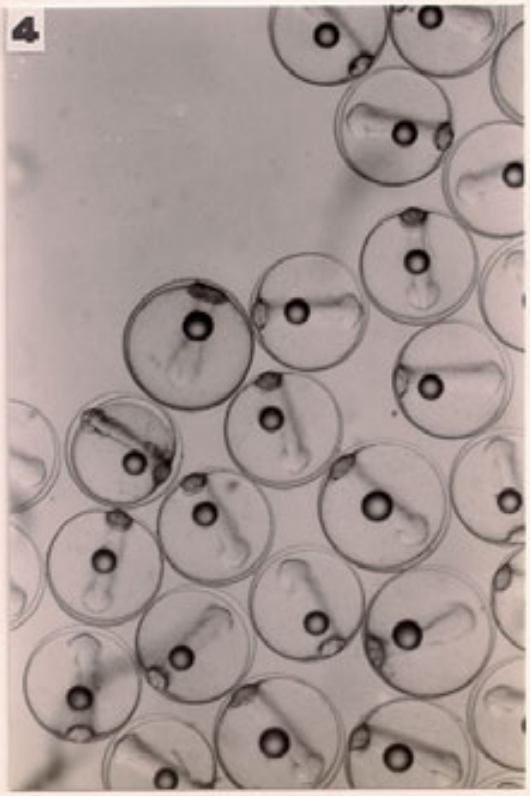
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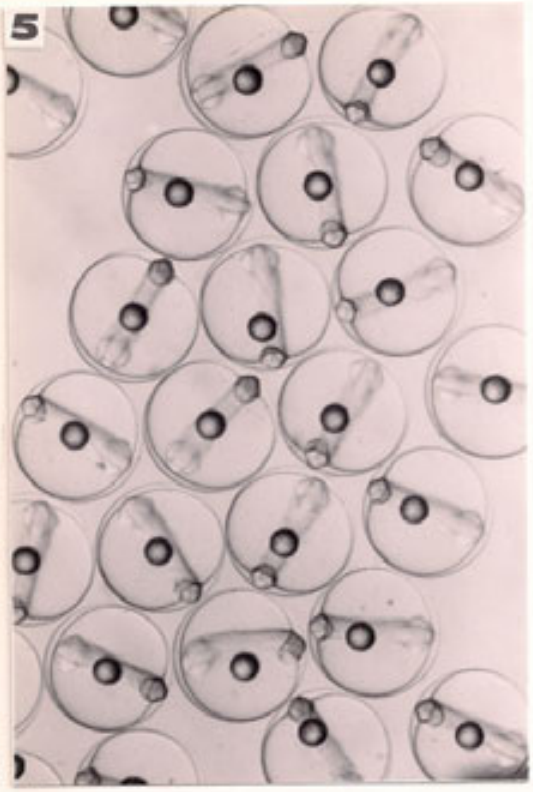
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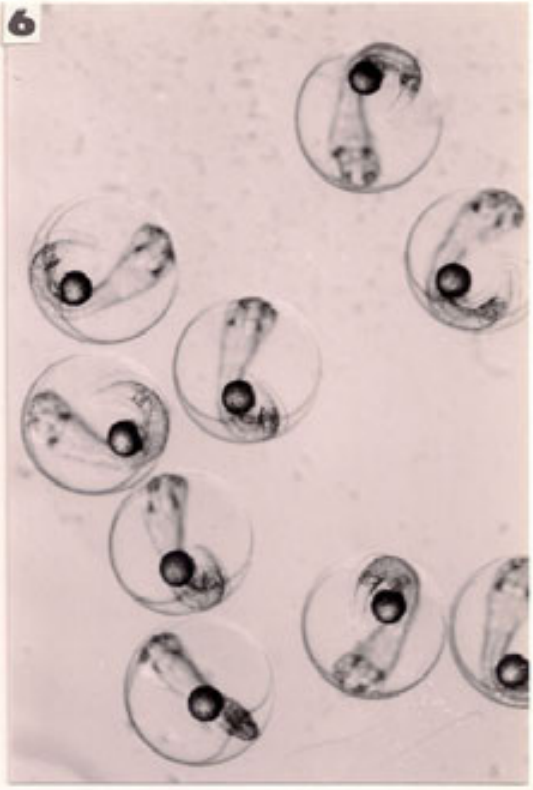
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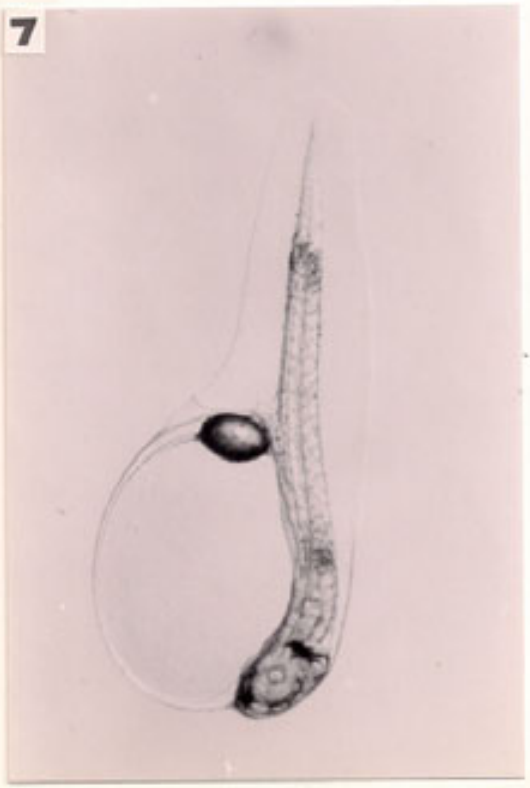
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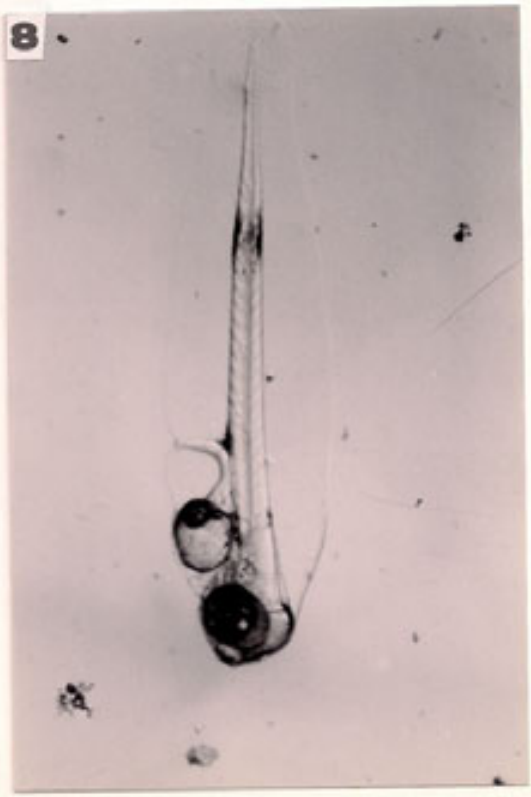
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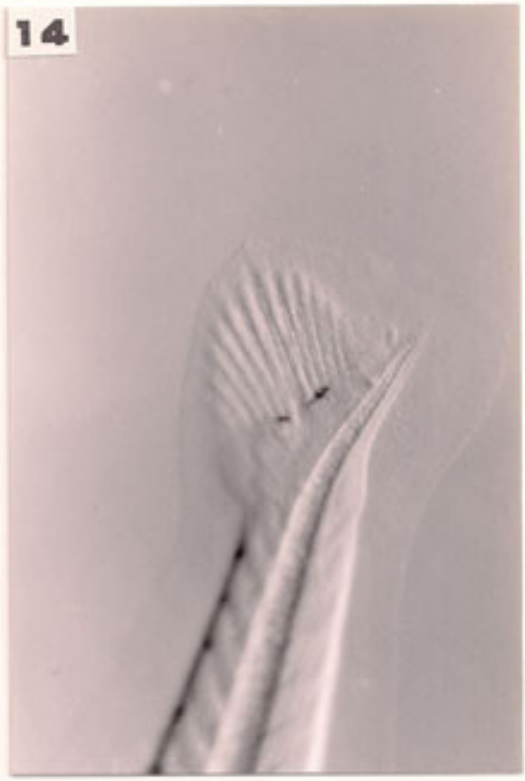
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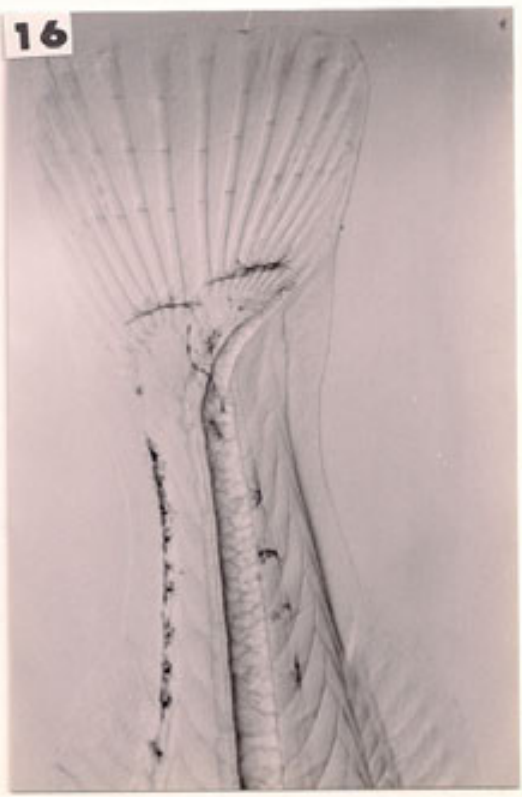
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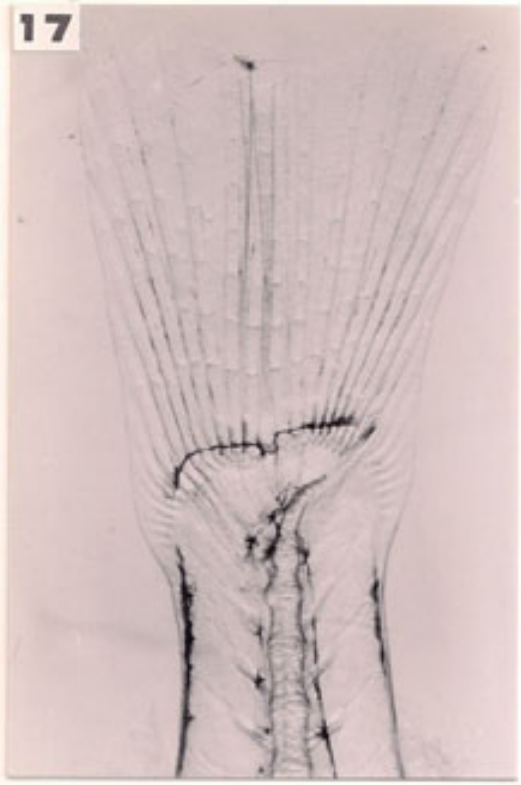
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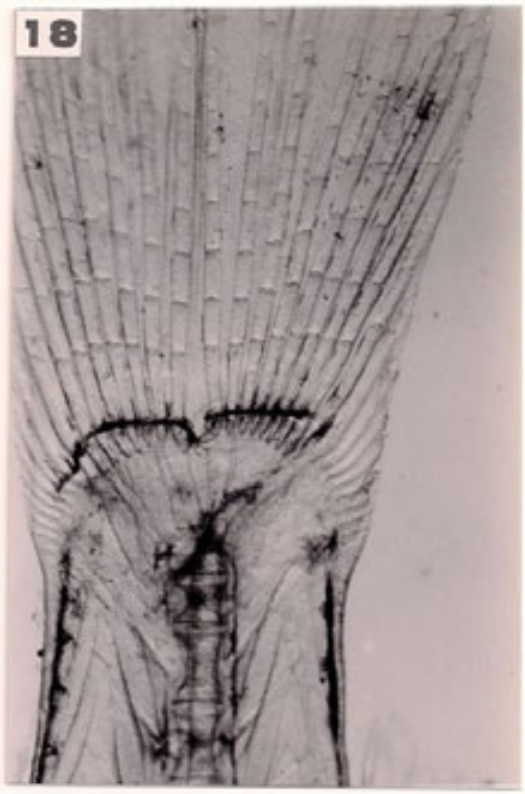
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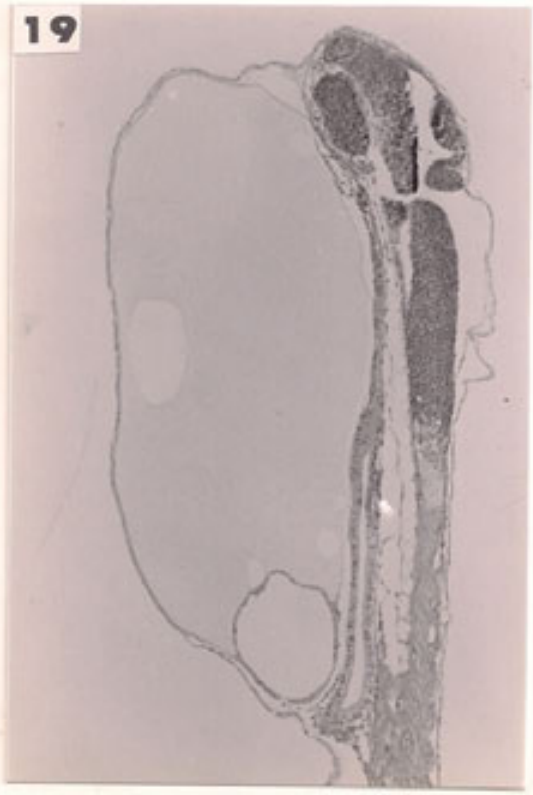
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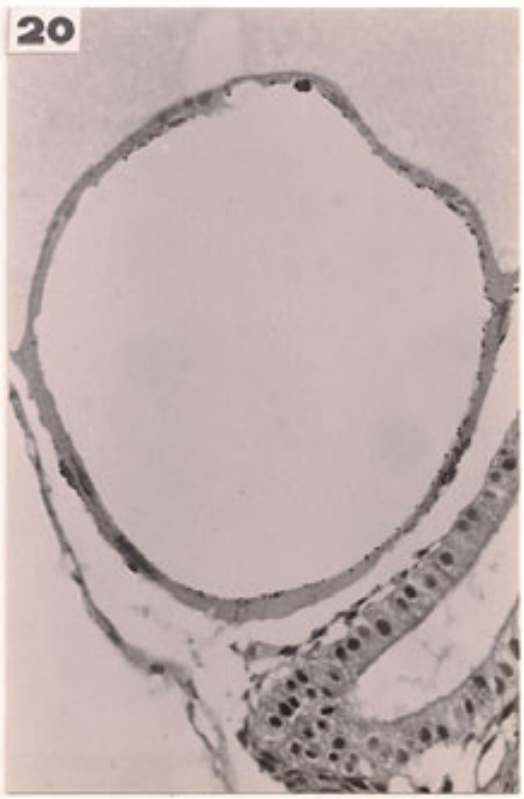
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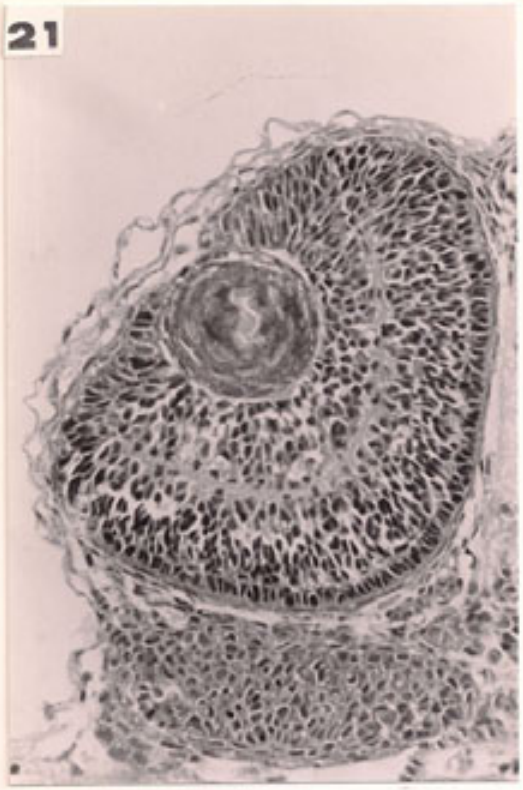
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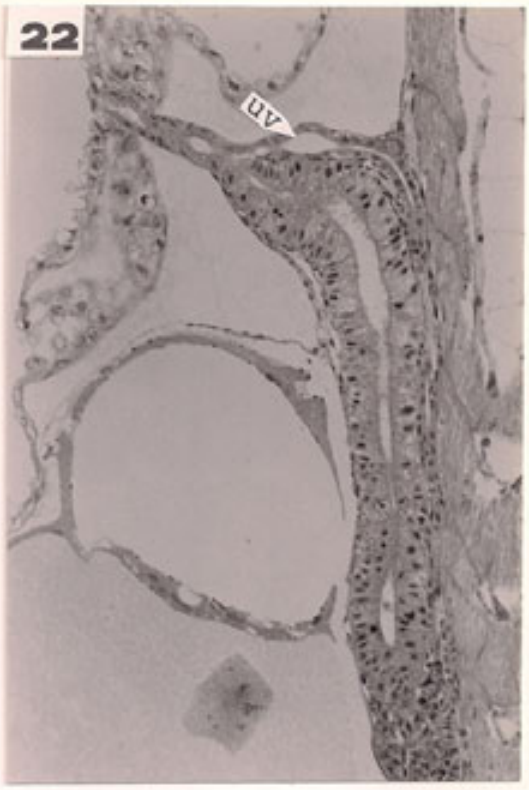
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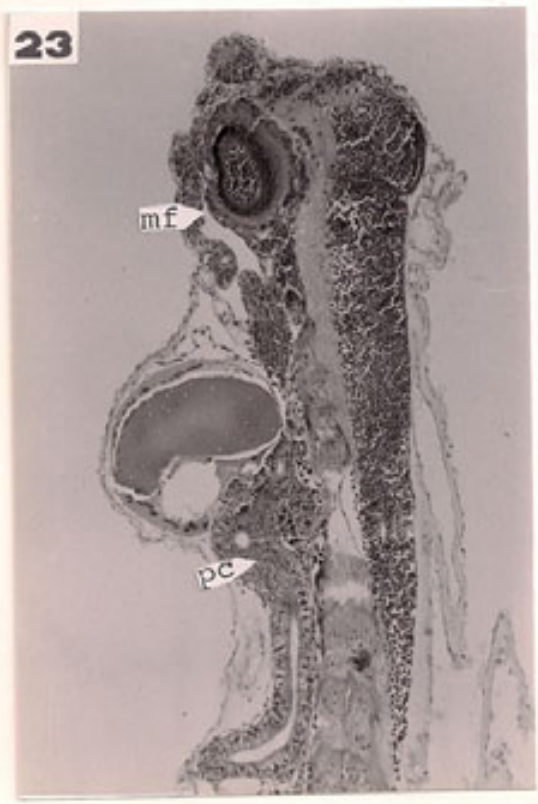
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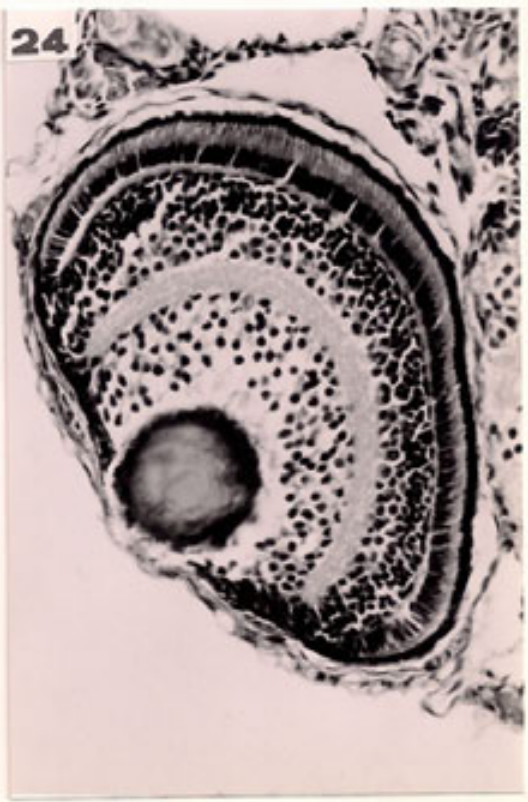
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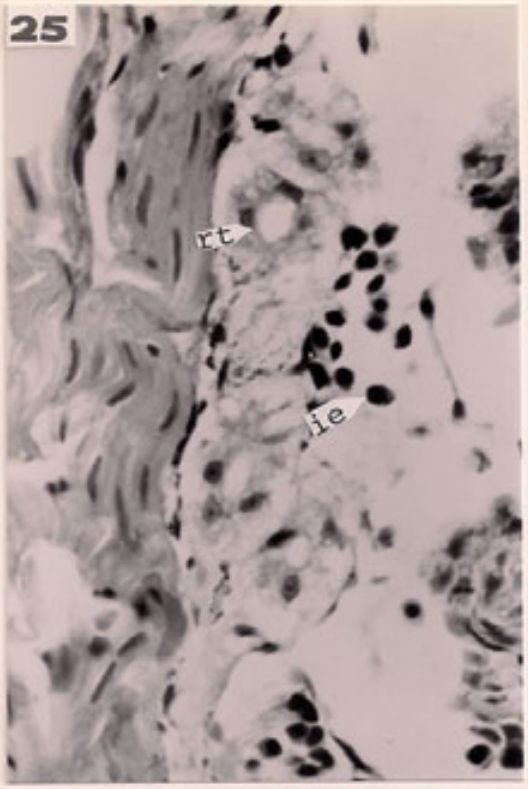
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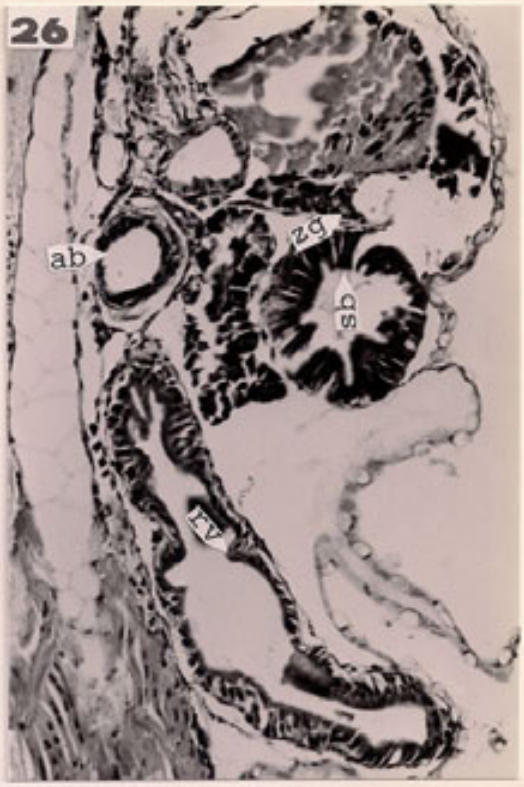
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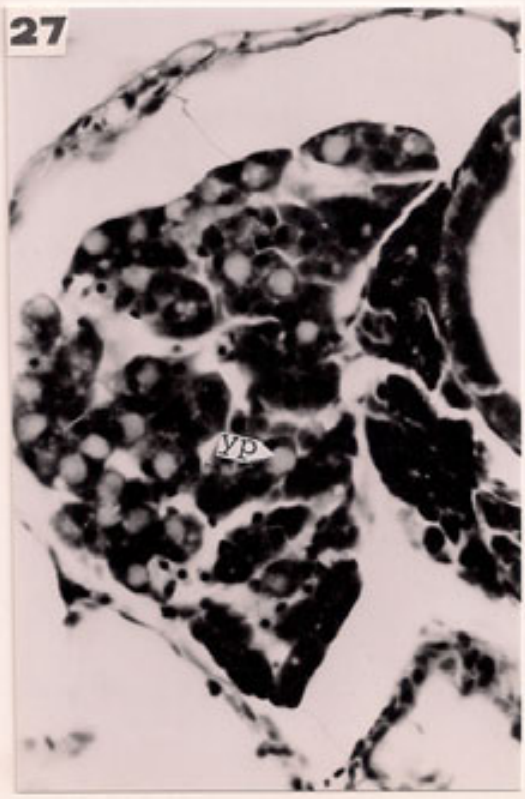
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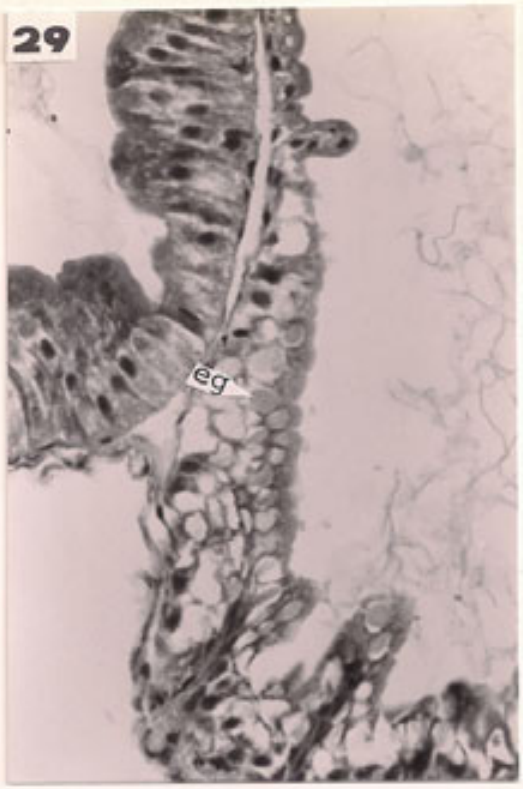
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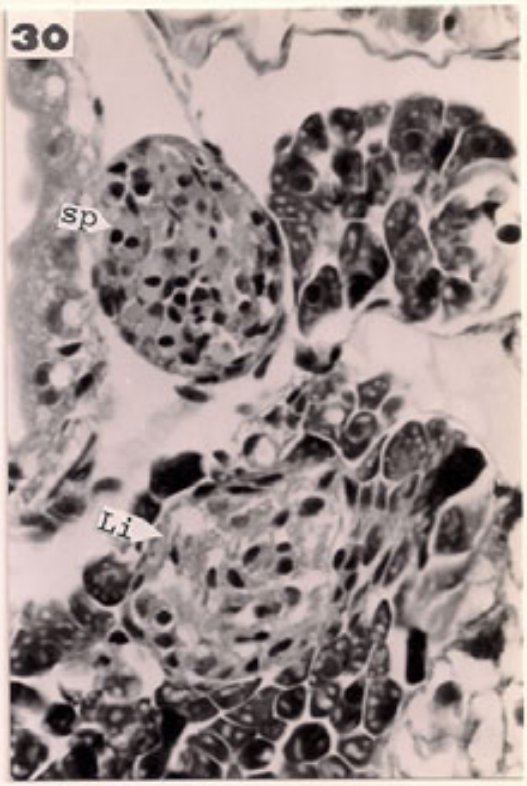
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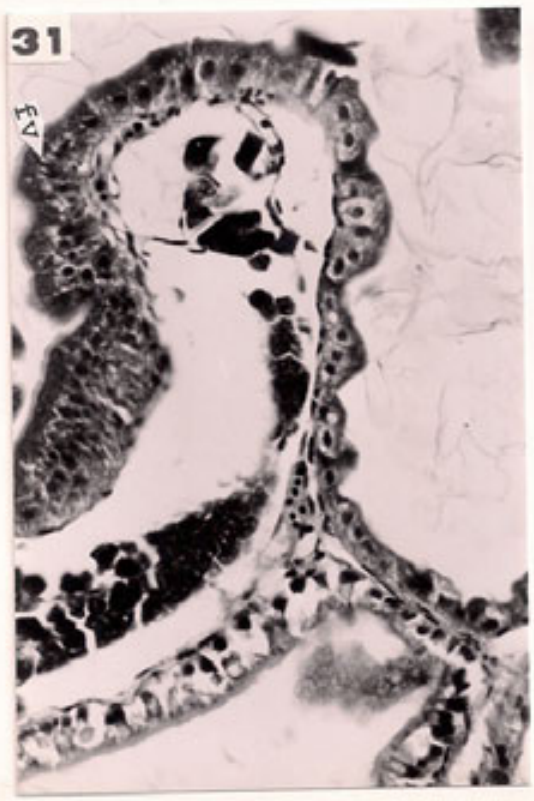
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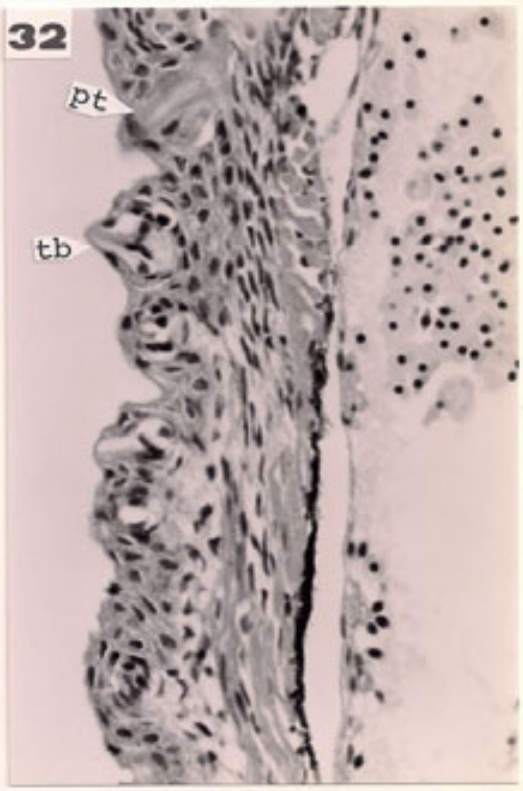
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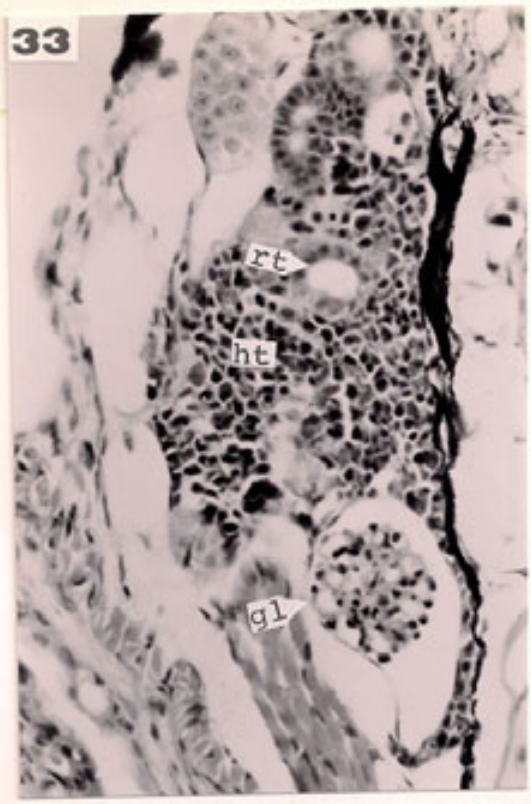
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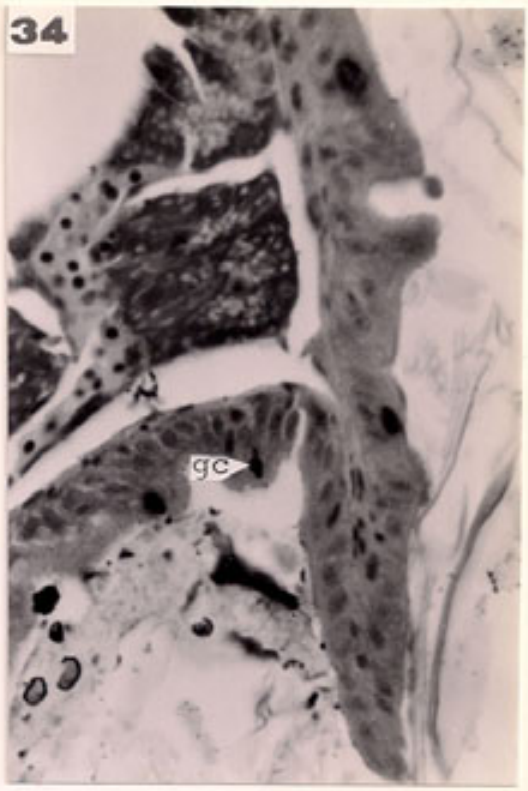
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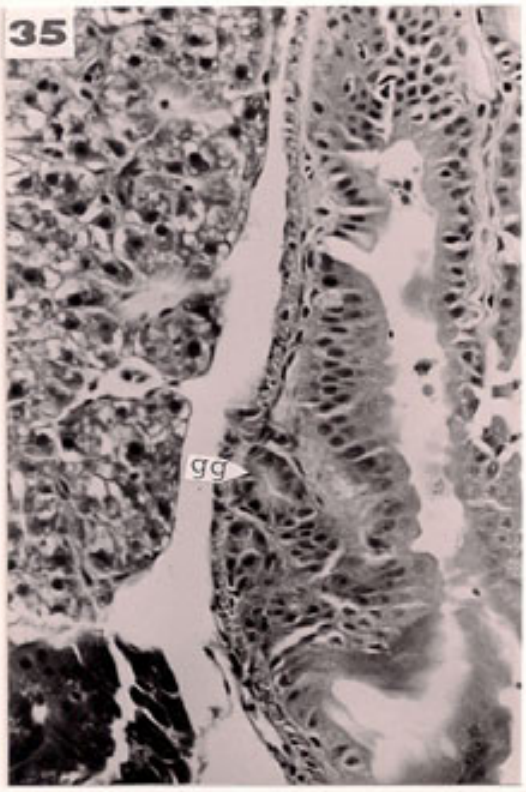
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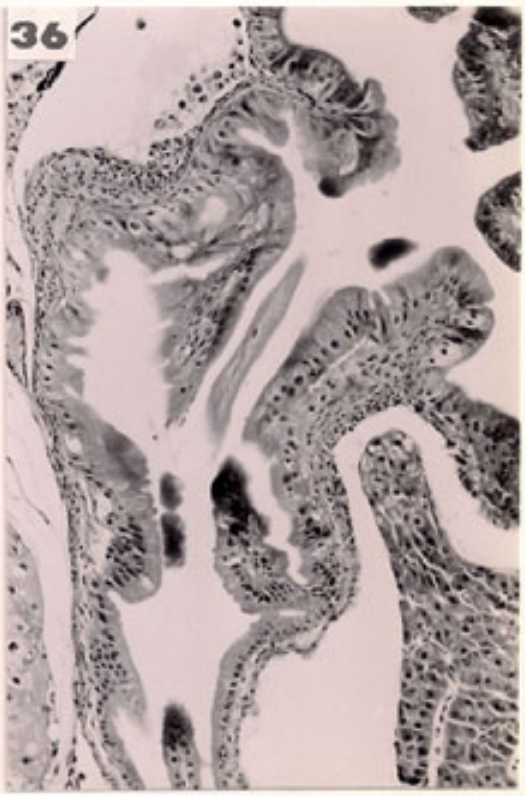
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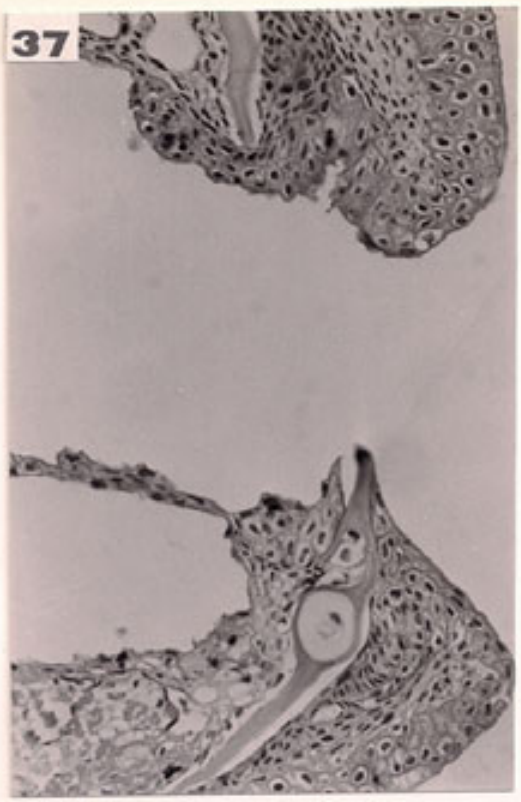
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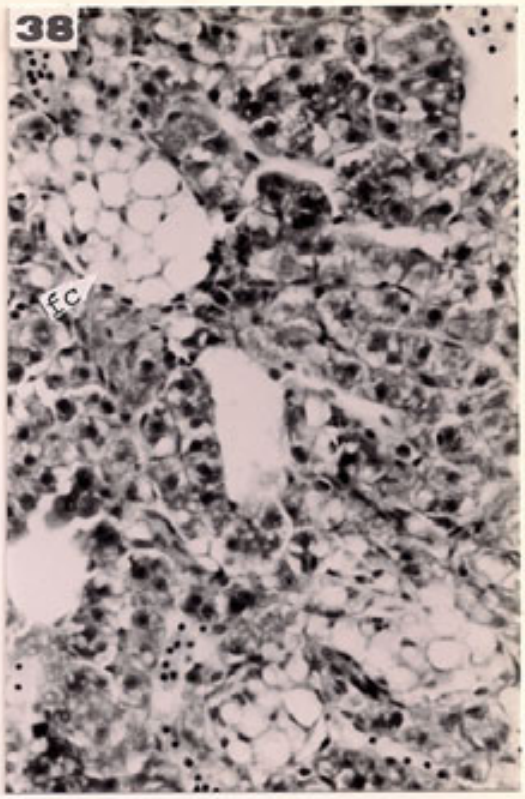
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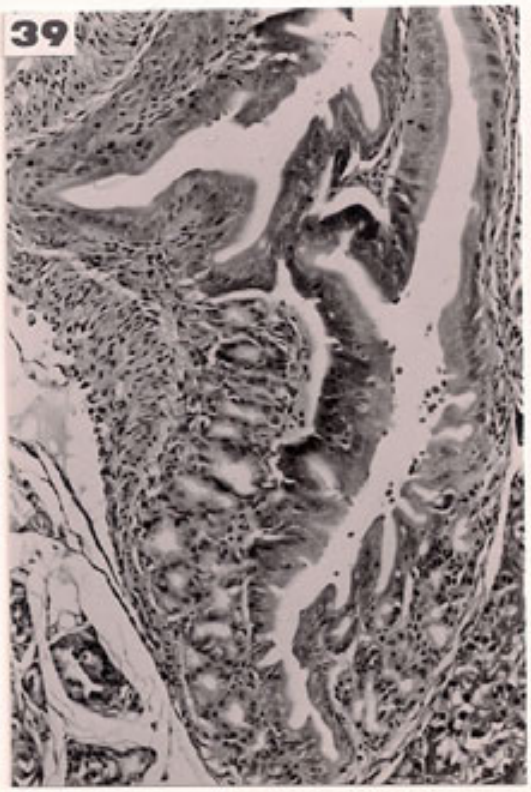
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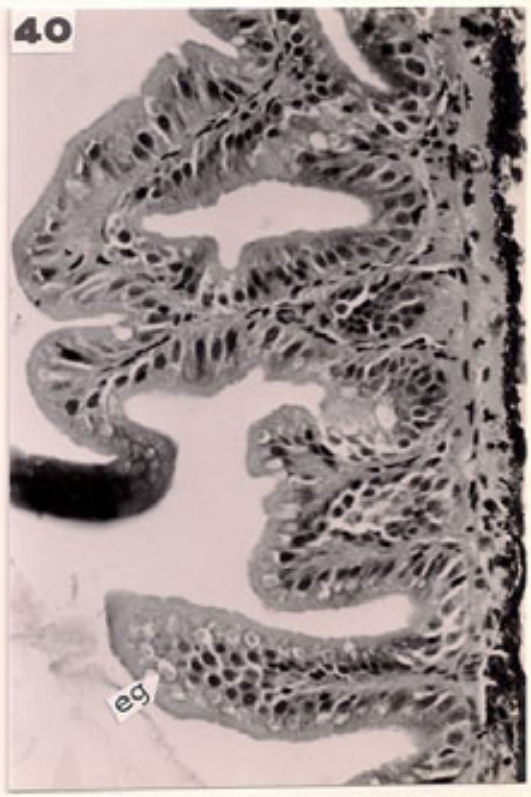
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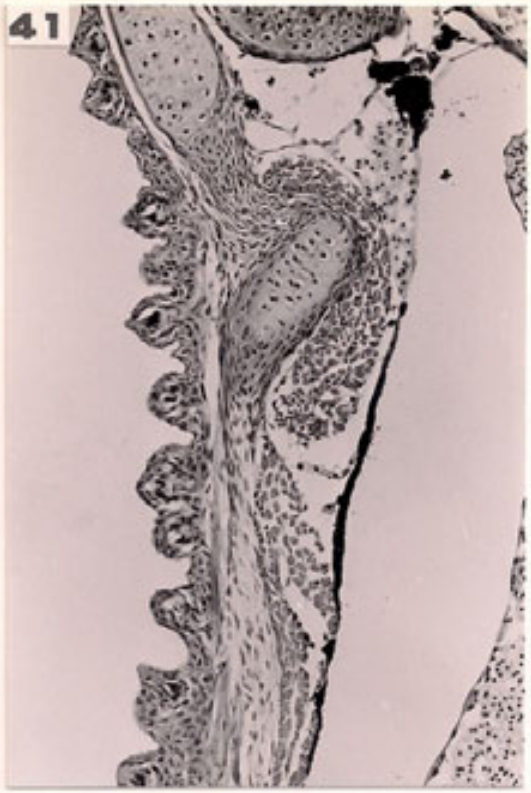
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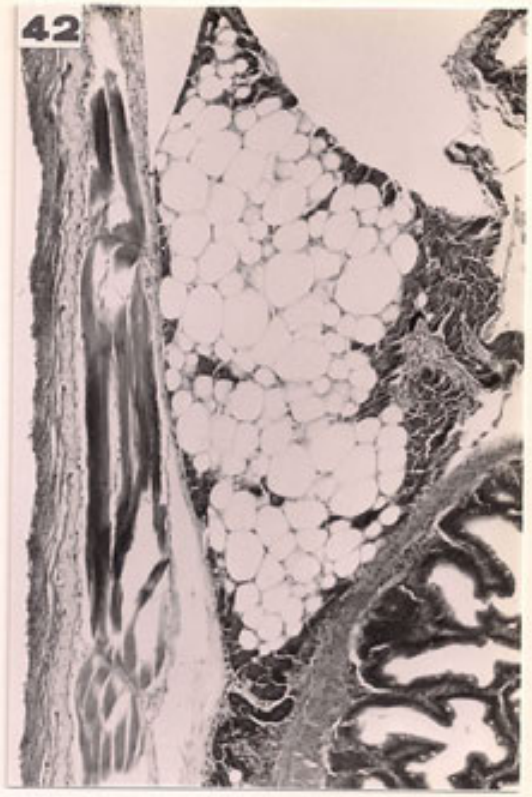
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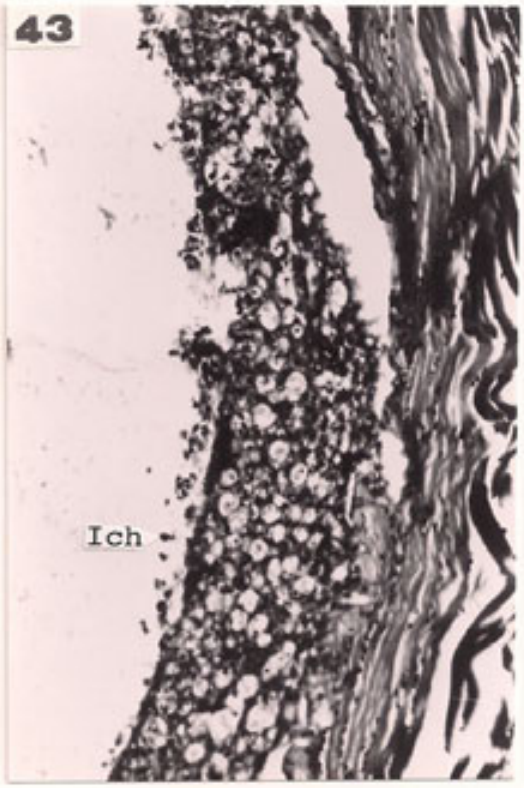
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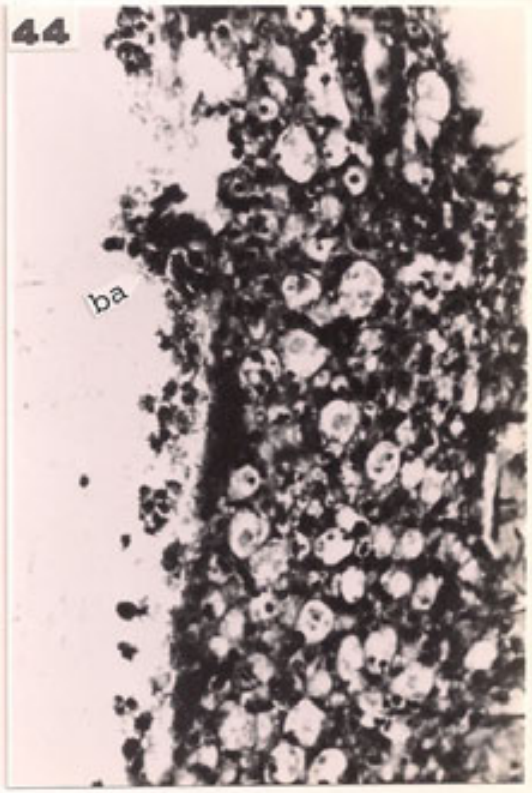
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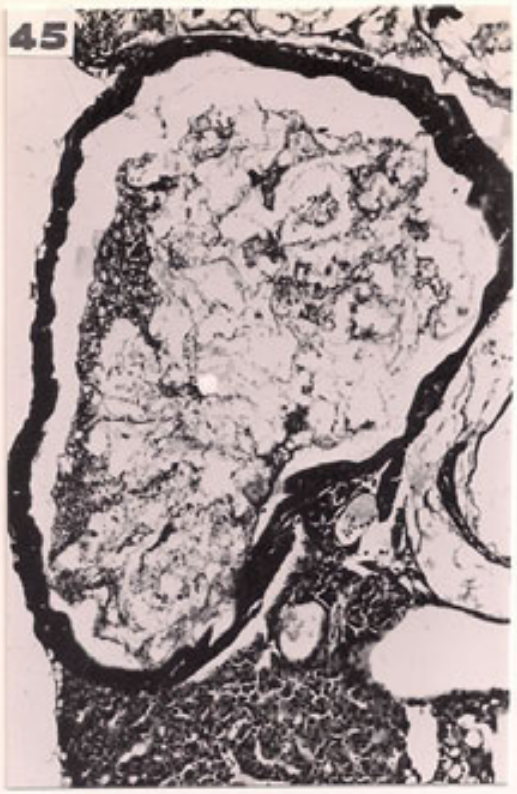
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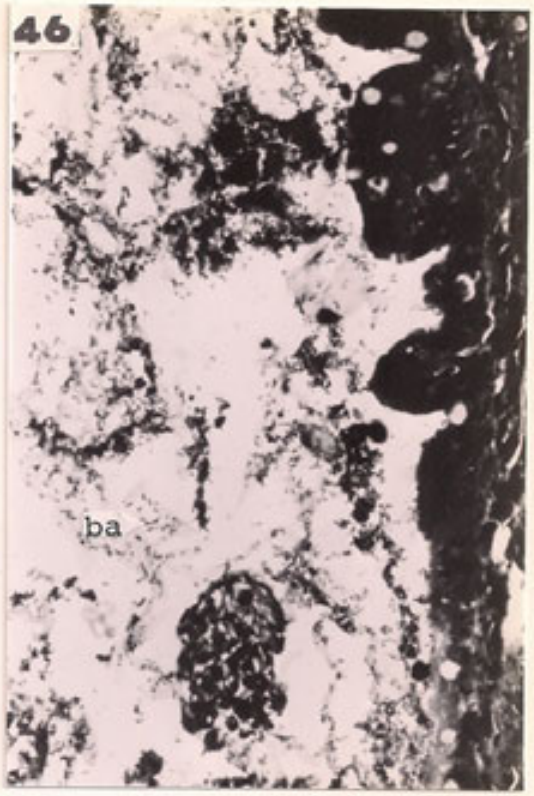
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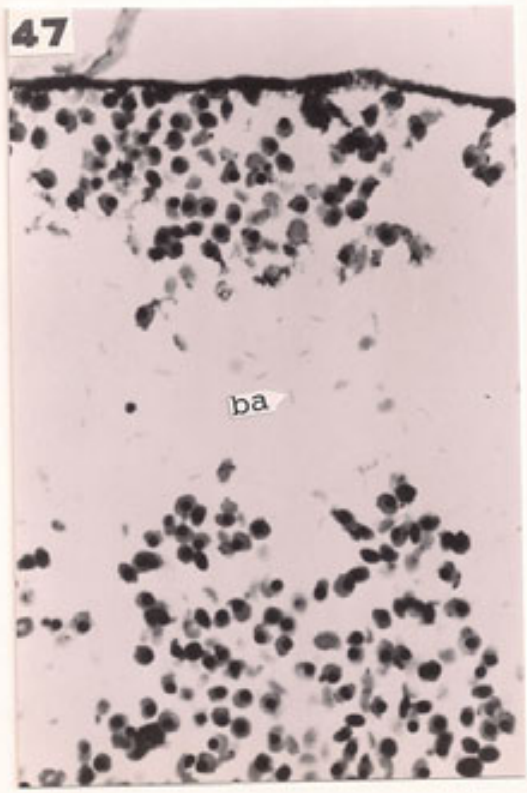
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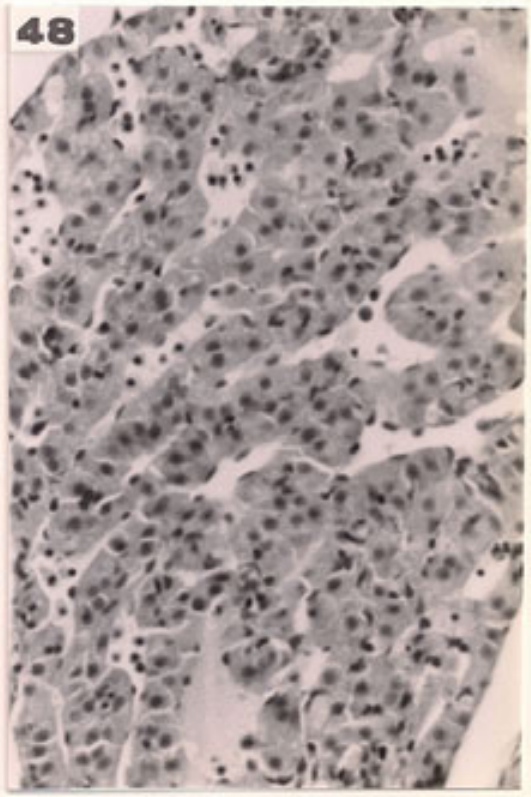
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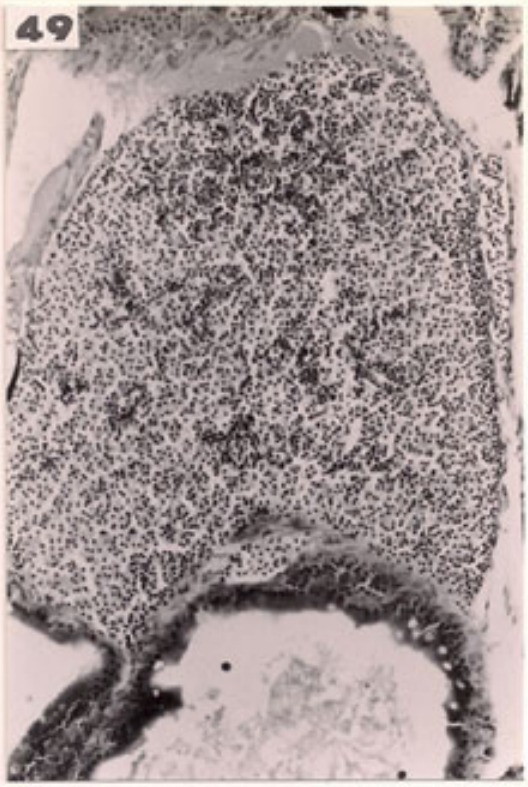
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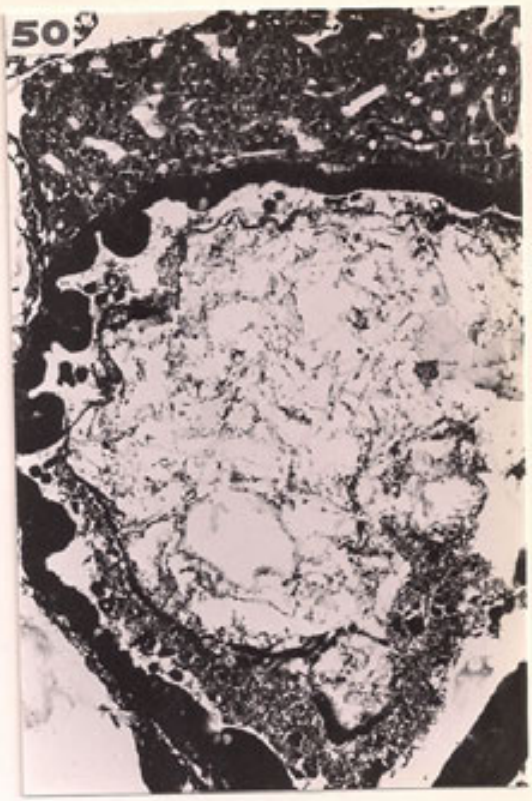
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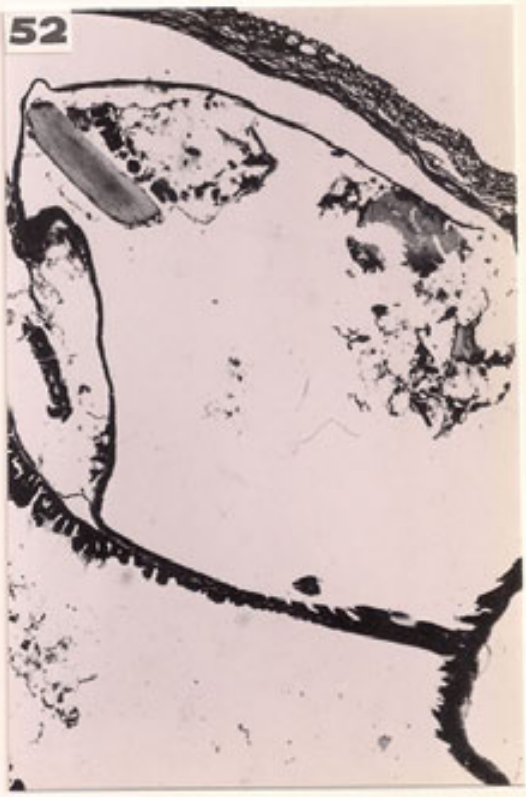
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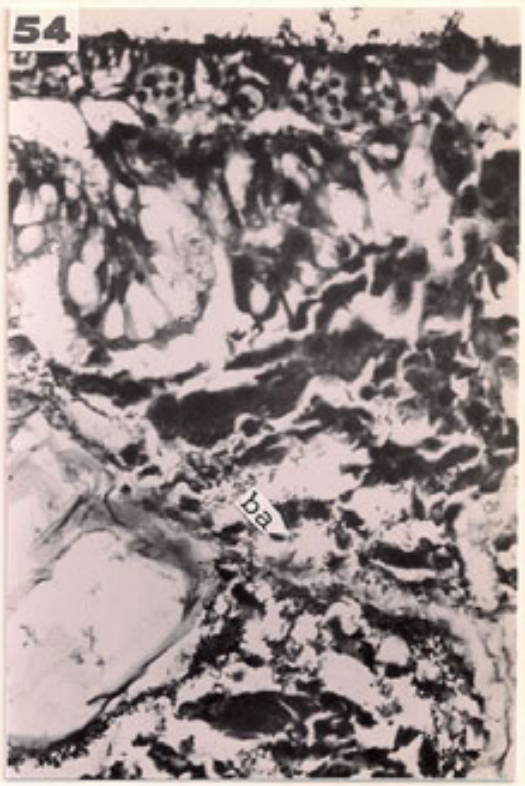
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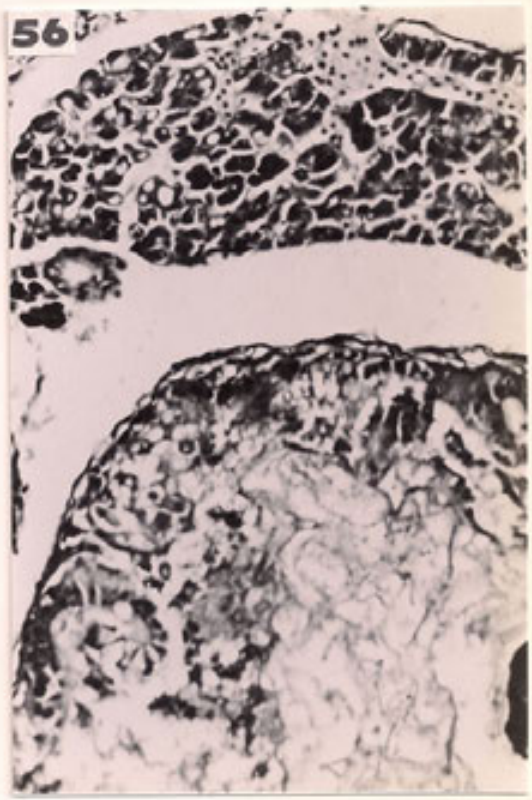
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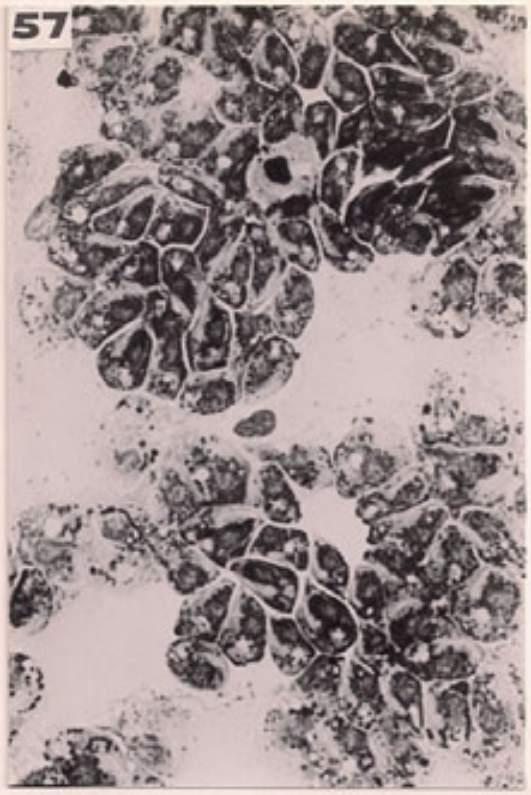
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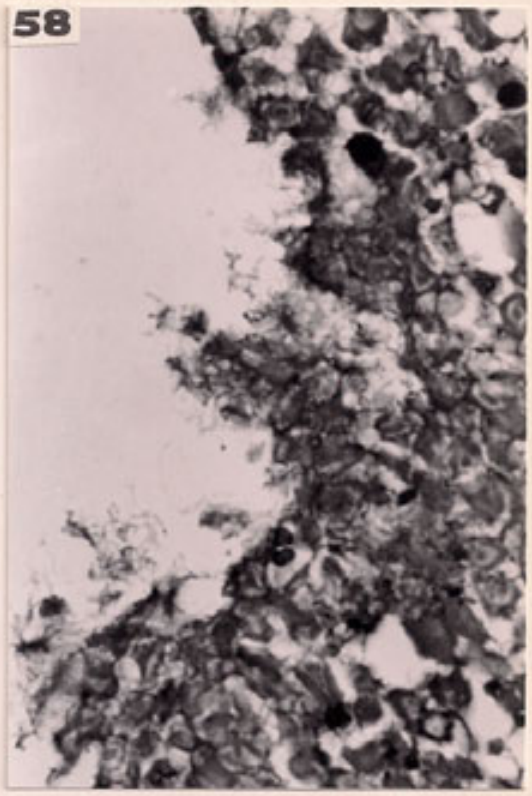
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