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Depigmentation of Extra Eyes in the Planarian, *Dugesia gonocephala*, by Salt of Dithiocarbamide and Recovery of the Pigment

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In our previous paper (Kido and Kishida, 1960), it was found that the depigmentation of planarian eye occurred more rapidly in the solution of thiocardamide containing iodine than in the solution of thiocarbamide alone. Moreover, it was confirmed histologically that the depigmentation was not dissolution of the pigment in the cells but due to preferential disintegration of the pigment cells and, at that time, the retinal rods of the eyes were also damaged. The authour (1960) verified extensively that the effective substance for the depigmentation of planarian eye was a salt of dithiocarbamide. The problem, however, will be taken into consideration whether the chemical exert directly the effect to the pigment cells or the effect firstly given to the brain and the optic nerve would not indirectly come to the disintegration of the retinal rods and the pigment cells. On the other hand, Jähnichen (1896) described with *Planaria gonocephala* that some of the extra eyes accepted incompletely nerve supply and others completely. Furthermore, it has been established that the depigmented eye by the salt of dithiocarbamide could restored the pigment by transferring the worms into fresh water.

From these facts, valuable information about the role of the nerve to the depigmentation and its recovery of planarian eye may be given by the administration of the salt of dithiocarbamide to the extra eye. The present stydy was carried out with such purpose as above.

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Material and Methods

The material used was an asexual form of *Dugesia gonocephala* collected in the suburbs of Kanazawa City. The worms were reared in decalcified tap water for a month in the laboratory in order to keep them in identical conditions. Then the individuals with extra eyes were selected and the location of the extra eye was recorded. Each worm was transferred to a finger bowl receiving 20 cc of a 2×10^{-4} M solution of dithiocarbamide diiodide and kept at 20-22°C. All the tested worms were kept without food throughout the experiment.

The stages of depigmentation of the planarian eyes treated with the solution are denoted by the six letters, N, A, B, C, D, and E in the order of severity, the unaffected, normal state being called N, and the completely disappeared state, E, as in our previous paper (Kido and Kishida, 1960).

Each of the worms was transferred, after its eye pigment had completely disappeared, to a finger bowl containing tap water and kept for 50 days for observing the recovery of eye pigment.

For the histological observation the worms were fixed in Helly's solution and transverse section in 8 μ thickness were prepared by the ordinary technique and stained with heamatoxylin and eosin or Mallory's triple stain.

Experimental results

Types of extra eye and their histological appearance

Out of 5,236 individuals collected from a stream, 329 possessed one or two extra eyes. From the observation by binocular microscope, two types among those extra eyes could be divided. One type, designated type I, has unpigmented area of epithelium as clear space over the eye pigment mass, and the other, designated type II, has no such space (Fig. 1). Among the 176 worms with extra eyes of type I, three had two extra eyes, that is, each of them had four eyes, and each of the other 173 had three eyes, one extra and two normal. Of the 153 worms with extra eyes of type II, 12 had two extra eyes and the remaining 141 a single extra eye each.

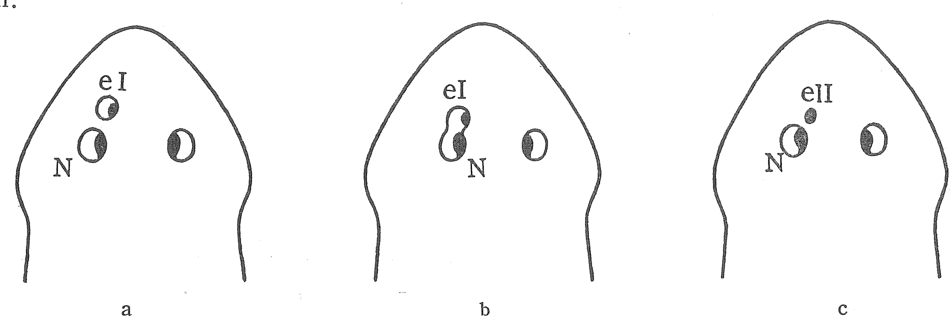

Fig 1. Planarian heads with extra eyes of different types. a and b have extra eyes of type I (eI), and c has extra eye of type II (eII). N is normal eye.

The histological observation revealed in general that the structure of the extra eye of type I was the same as the normal eye, in which the pigment mass formed a cup-shape and the retinal rods were packed in it. Thus the pigment mass was connected with the brain via the optic nerve. But the extra eyes were rather reduced as for largeness of the pigment mass and number of pigment cells and of retinal rods than normal eye (Plate I, A and B). Some of the extra eyes of type II were compressed on the dorsal side of the intestinal tract (Plate I, C), and some others closed conspicously to the brain as the subsequence of deep falling in the body (Plate I, D). The pigment mass of extra eye of type II was not cup-shaped but irregularly vesicular without any opening. Its interior was empty, and no structure such as retinal rods could be observed in paraffin sections.

From above observations, it can be concluded that the extra eye of type I has normal nerve supply and its pigment mass communicated with the brain as the normal pigment mass, but that the extra eye of type II is no such.

Treatment with the solution of dithiocarbamide diiodide.

Seventy-six worms with extra eyes of type I were treated with a 2×10^{-4} *M* solution of dithiocarbamide diiodide. The observation of the depigmentation was made separately on the normal eyes and the extra eyes in the same individuals, and the results are respectively summarized in table 1 -b and 1 -c.

Table 1. Depigmentation of planarian eye treated with 2×10^{-4}M solution of dithiocarbamide diiodide.

a. Depigmentation of normal eye without any extra eye

Stages of depigmented eye	Days during treatment			
	1	2	3	4
	%			
N	0.0	0.0	0.0	0.0
A	2.5	0.0	0.0	0.0
B	5.0	0.0	0.0	0.0
C	35.0	5.0	0.0	0.0
D	52.5	32.5	10.0	0.0
E	5.0	62.5	90.0	100.0

b. Depigmentation of normal eye with extra eye of type I

Stages of depigmented eye	Days during tratment			
	1	2	3	4
	%			
N	0.0	0.0	0.0	0.0
A	2.0	0.0	0.0	0.0
B	4.6	0.0	0.0	0.0
C	38.2	2.0	0.0	0.0
D	52.0	42.8	7.2	0.0
E	3.3	55.3	92.8	100.0

c. Depigmentation of extra eye of type I

Stages of depigmented eye	Days during treatment			
	1	2	3	4
N	0.0 %	0.0	0.0	0.0
A	1.3	0.0	0.0	0.0
B	2.5	0.0	0.0	0.0
C	15.2	1.3	0.0	0.0
D	54.4	13.9	1.3	0.0
E	26.6	84.8	98.7	100.0

d. Depigmentation of extra eye of type II

Deys during treatment	1	2	3	4
Percentage of completely depigmented eye	28.0 %	85.3	98.7	100.0

Since the normal eye and extra eye are usually situated closely one another, there arose the question whether the one influenced the other in respective depigmentation. Accordingly the speed of depigmentation was first compared with the normal eyes with and without extra eyes. As is shown in table 1-a and 1-b, stage N were already absent on the first day after the treatment, and stage D accounted for more than a half, i. e., 52.5% in table 1-a and 52.0% in table 1-b. On the second day, stage A and B also missed, and most of the treated eyes were in stage D or E. On the third day none was in stage C and stage E exceeded 90%. The time required for complete disappearance of eye pigment in all the worms was four days in both cases. These results indicated decidedly that existence of adjucent extra eyes did not affect the speed of depigmentation of normal eye.

The result on the depigmentation of the extra eye of type I is summarized in table 1-c. By the first day after the treatment, the depigmentation had begun in all the eyes of this type, and the eyes of stage D came to 54.4%of the whole. This result is almost the same as that of the normal eyes in table 1-b, except for the difference of the percentage of stage E in both cases, being 26.6 in 1-c, and 3.3 in 1-b. Stage E increased to 84.8% on the second day, to 98.7% on the third, and 100% on the fourth.

These results show that the depigmentation was slightly quicker in the early stage in the extra eyes of type I than in the normal eyes, but that the time required for complete disappearance of eye pigment is almost similar in both cases.

As for the depigmentation of the extra eyes of type II, only the percentage of completely depigmented eyes (stage E) was recorded, because the irregularlly vesicular shape of the pigment mass and the absence of clear space made it difficult to record the sequence of the depigmentation. As is shown in table 1-d, the result is almost the same as that the extra eyes of type I.

Reappearance of the destroyed eye pigment

The worms were kept in the test solution of dithiocarbamide diiodide for one more day after their eye pigments disappeared completely, and each of them was then transferred to tap water. The eye pigment began to reappear in the clear space of normal eyes as small brown spots near the median three or four days after the transfer in tap water. The eye pigment was clearly seen as black spots in all the normal eyes in about ten days. Of the 79 extra eyes of type I, the eye pigment reappeared in 15 (19.0%) as brown spots on the fourth day and in 19 (24.1%) in the first two weeks. In 50 days 40 (50.6%) regained the pigment as black masses. In these extra eyes the repigmentation occurred in the original positions. In 30 worms of which they could not recover the pigment masses of the extra eyes of type I, the clear spaces of extra eyes entirely disappeared and the normal eyes alone recovered the pigment masses, and consequently their heads became normal. Although 9 of the extra eyes which did not recover the pigment masses retained the clear spaces, those space were reduced in size and no retinal rod was observable histologically.

When the worms which had lost extra eyes of type II by the reagent were transferred to tap water, eight extra eyes reappeared in the original positions. Five of them, however, were of type II and remaining three appeared as type I which had clear space (Plate II, E). Three of above five eyes had exceptionally retinal rods in spite of having no clear spaces and deeply fell down in the mesenchyme near the brain as in the cases of some extra eyes of type II (Plate II, F). Accordingly, only two eyes of all 75 reappeared as the eye of type II from the original extra eye of type II (Plate II, G and H).

Beside, there were six cases in which the extra eyes appeared in some other positions than the original. But this phenomenon cannot be called as recovery of eye pigment.

Discussion

In the present experiment, it was found that there were two types of the extra eye in the planarian *Dugesia gonocephala*. One of them has unpigmented area of epithelium called as clear space and consists of same microscopical components as the normal eye (type I). The other has no clear space, and the eye pigment mass was not cup-shaped but irregularly vesicular and devoid of retinal rods (type II). Jähnichen (1896) described an abnormal pigment mass near the normal eye, and pointed out that the interior of this pigment mass was empty. This pigment mass, therefore, is probably the extra eye of type II in the present observation. He also stated that this pigment mass was connected with the plexus of nerve fibers on the way where the optic nerve of the normal eye ran to the brain. But, in the present observation, any extra eye of type II was not found to have such nerve supply. In fact, most of the pigment masses of type II are situated on the dorsal side of the intestinal tract or in front of the pigment mass of normal eye, being far from the way of optic nerve connecting the brain with the pigment mass of the normal eye. Although a few nerve fibers were sometimes observed near the pigment mass of type II, it is doubtful whether they communicate with the pigment mass at least physiologically. Flickinger and Coward (1962) reported that pigment spots appeared in the tail tissue when incomplete reversal of polarity was brought about in planarian pieces. In addition, they claimed that such pigment masses appeared even when the brain was absent. If these pigment spots correspond to the extra eye of type II from the present experiment, such pigment spots may sometimes appear even under a condition of brainless.

What role the brain play in the depigmentation of the planarian eye by the salt of dithiocarbamide? Since depigmentation occurred in both extra eyes of type I and type II in spite of presence or absence of retinal rod and the time required for depigmentation was similar in both cases, it is likely seemed that the depigmentation of the treated eye is ascribed to the disintegration of the pigment cells involved in the direct action of the chemical and that it has nothing to do with the damage of the retinal rod via the brain or nerve affected by the chemical. On the other hand, Lender (1956) assumed with *Polycelis nigra* that formation of the eye was induced by the secretive substance from the brain. If the brain of *Dugesia gonocephala* contributes also to the eye formation, it may be considered that inhibition of the cerebral secretion by the reagent causes disintegration of the eye pigment cells and retinal rods. Further experiments on this point are being carried out.

Next, in the experiment on the recovery of pigment lost in the eye treated with the chemical, only 2.7% (two cases) of the extra eyes of type II regained the pigment, while 50.6% of those of type I did so. Furthermore, since there were six extra eyes appearing in places different from the original location, the two cases which appeared as extra eye of type II may not be cases of recovery in the true sence of the term, but new extra eyes may have appeared in the original situation by pure coincidence. This result, therefore, is seemed to indicate that the extra eyes of type I having retinal rods connected with optic nerve can recover the pigment mass but that those of type II cannot. That is to say, we likely assumed that when the pigment cells of extra eyes of type I are disrupted by the chemical, their pigment masses and retinal rods are also destroyed, but the optic nerve survives in the original position and contributes to reproduce the pigment mass, while, when extra eyes of type II are treated with the same chemical, the pigment masses are irrevocably destroyed so far as they are supplied no optic nerve. To sum up, existence of optic nerve running to the eye pigment mass has no influence on the depigmentation of planarian eyes by the salt of dithiocarbamide, but is necessory for the recovery of the lost pigment.

Since the depigmentation of a normal eye having an extra eye of type I took place with the same speed as that of a normal eye without any extra eye, it is considered that there is no interaction between the normal eye and the extra eye accompanying it in the depigmentation by the salt. Lender (1960) reported that the brain of the planarian inhibited appearance of another brain in the same individual, and Penz and Seilern-Aspang (1961) pointed out that the eye in *Polycelis nigra* inhibited formation of another eye near itself. It is significant for the inhibiting effect of normal eye on the extra eye that in this experiment the pigment of the extra eyes of type I lost by the chemical could not be recovered in 50.6%, while the normal eyes could do in all. The inhibiting effect of normal eye may be more intense for the recovery of extra eyes of type II which have no optic nerve than for the recovery of those of type I which have it.

Summary

1. The depigmentation of the extra eyes of the planarian by dithiocarbamide diiodide and the recovery of the eye pigment thus lost were investigated in the relation with the nerves.

2. From the histological observation of extra eyes, it was revealed that there were two types of the extra eyes, that is, type I had an unpigmented area of epithelium called as a clear space and a pigment cup packed with retinal rods, and type II had no clear space and retinal rods but had a pigment mass of vesicular shape.

3. There was no difference in the speed of the depigmentation between type I and type II.

4. In extra eyes of type I the pigment mass lost in the test solution recovered it with a considerable percentage by transferring the worm to tap water, but in the extra eyes of type II thus treated no recovery occurred.

5. It is likely seemed that the nerve supply to the pigment mass is necessary for the recovery of depigmented eyes but has nothing to do with the depigmentation of the eyes by the reagent.

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Abbreviations

b, brain ganglia	e, extra eye	i, intestinal tract
n, normal eye	p, pigment mass	r, retinal rod

PLATE I

Explanation of figures

Photomicrographic representation of various types of the eye in *Dugesia gonocephala*.

A: The normal eye, having the cup-shaped pigment mass and retinal rods.

B: The extra eye of type I, showing the same constituent with normal eye. Note the smaller size than normal eye in figure A.

C: The extra eye of type II, showing that pigment mass is compresses in the dorsal site of intestinal tract

D: The extra eye of type II, having the vesicular shaped pigment mass without any retinal rod.

PLATE II

Explanation of figures

Photomicrographic representation of reappeared eyes after complete disintegration by treatment with the solution of dithiocarbamide diiodide.

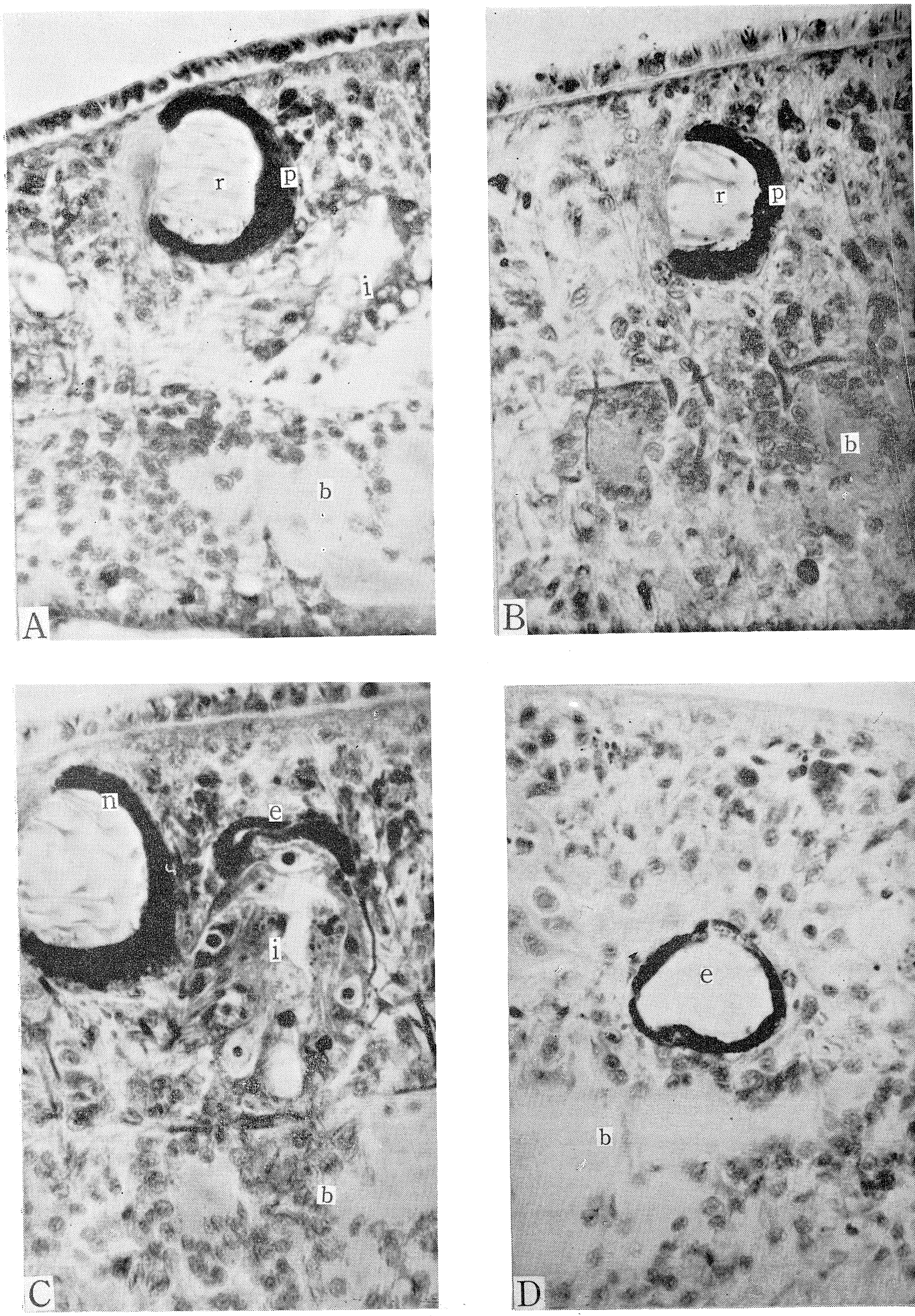
E: The eye reappeared as type I after that of type II disintegrated. Feature of pigment cup and retinal rodsare almost the same with normal eye.

F, G, and H: The eyes reappeared as type II having not clear spaces

F: Incomplete cup-shaped pigment mass and a few retinal rods.

G: Cup-shaped pigment mass without any retinal rod.

H: Vesicle-shaped pigment mass without retinal rod.



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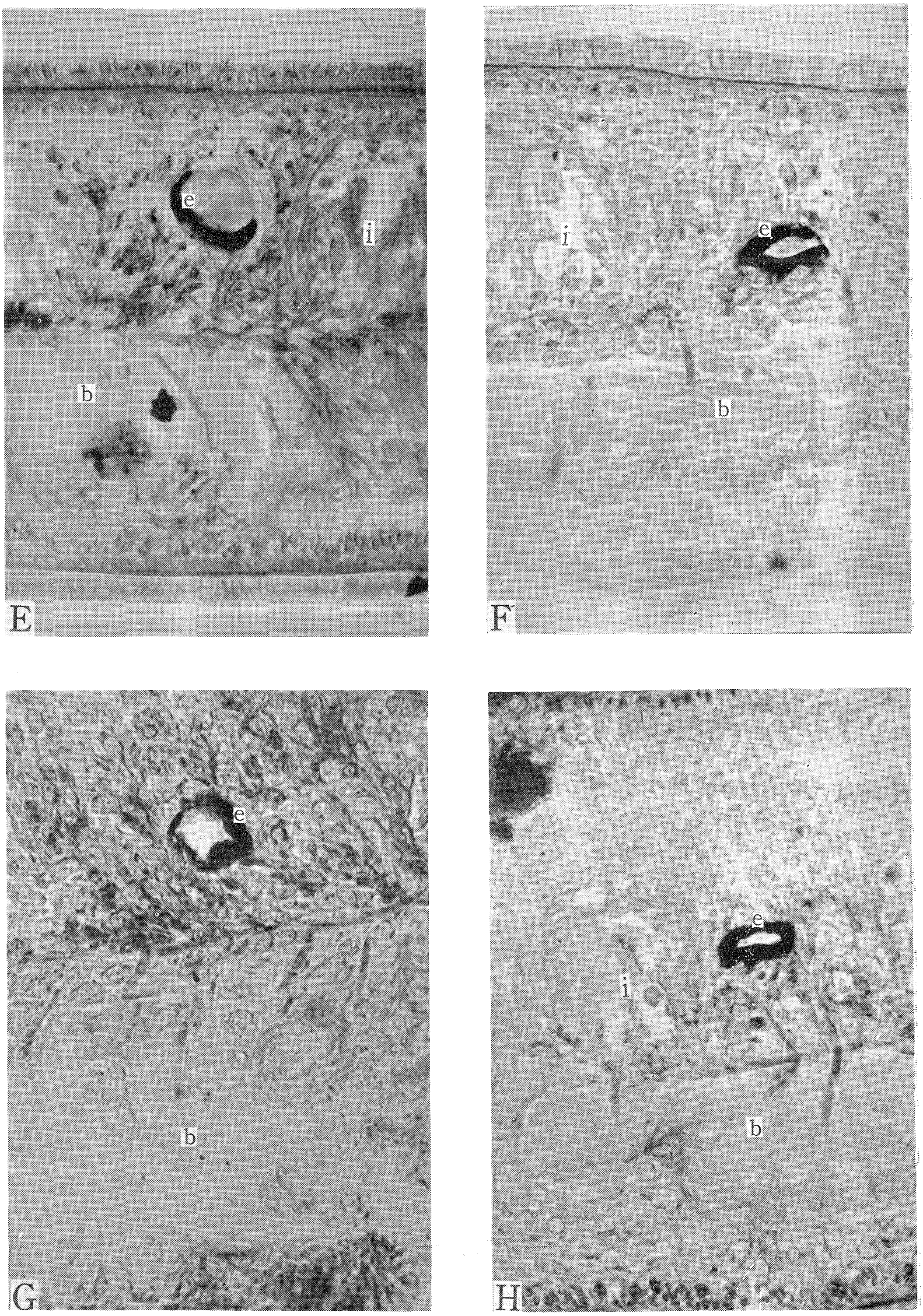

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