ニワトリの消化管におけるmonoamine 含有線維と細胞の分布について

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著者	池田, 衡 犬飼, 初男 五島, 治郎
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LOCALIZATION OF MONOAMINE-CONTAINING FIBERS AND CELLS IN THE ALIMENTARY CANAL OF CHICKENS

Hitoshi IKEDA, Hatuo INUGAI and Jiro GOTOH

Department of Animal Physiology, Faculty of Agriculture, Nagoya University, Chikusa-ku, Nagoya

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Since a fluorescent-histochemical procedure was established by the Scandinavian school of investigators (FALCK, 1962; CARLSON et al., 1962), the accurate localization of monoamines in various tissues of different animal species could be demonstrated by this procedure. A number of investigations were carried out in mammals (DAHLSTRÖM and FUXE, 1964; HOLLANDS and VANOV, 1965; JACOBOWITZ, 1965; EHINGER, 1966). In the alimentary canal, specific green fluorescent structures showing the adrenergic innervation were observed in the wall of blood vessls, muscle layers, and Meissner's and Auerbach's plexuses (HOLLANDS and VANOV, 1965; JACOBOWITZ, 1965; MURYOBAYASHI et al., 1968). In addition, yellow fluorescent structures containing 5-hydroxytryptamine (5-HT) were demonstrated in the mucosal, submucosal, muscular, and serosal layers of the alimentary canal (TOBE et al., 1966; PENTTILÄ, 1966; TOBE et al., 1967).

No investigation has been carried out as yet on birds by the fluorescent-histochemical procedure. Therefore, the present study was undertaken to determine the distribution of monoamine-containing fibers and cells in the alimentary canal of chickens.

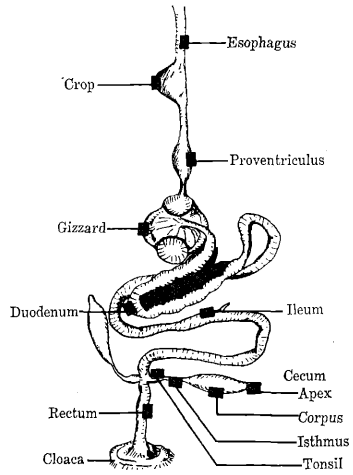
MATERIALS AND METHODS

Experiments were carried out by using 30 male White Leghorn chickens 8 to 15 weeks of age, weighing from 600 to 1500 g. Of them, twelve were injected with reserpine (1–3 mg/kg, intramuscular, 4–96 hr), three with nialamide (500 mg/kg, intraperitoneal, 5 hr), and two with nialamide and l-dopa (100 mg/kg, intraperitoneal, 20 min).

The birds were killed by decapitation. Several parts of the alimentary canal, including the esophagus, crop, proventriculus, gizzard, small intestine (duodenum, upper and lower ileum), large intestine (rectum), and ceca, were rapidly removed and dipped into isopentane cooled by liquid nitrogen. Specimens from each organ investigated are shown with a solid square in Chart 1. They were freeze-dried for 3 to 5 days at −35°C in vacuo. Then, they were treated with formaldehyde gas derived from paraformaldehyde in the oven at 80°C for one hour by the method of Falck (1962). Thereafter, they were put into soft paraffin (Nakarai product, m.p. 52–54°C) in vacuo and embedded in hard paraffin (Nakarai product, m.p. 56–58°C). Sections 10 μ in thickness were cut every 100 μ of each specimen, and stretched on a slide-glass while heating at 60–65°C. Then, they were mounted with an Entellan-xylene mixture (1:1). Histological examination and photographical recording were done on each section by using a Nikon fluorescence-microscope (Nikon, Japan). Kodak Tri X film was used with an exposure time ranging from 50 to 100 sec.

The numbers of 5-HT cells per mm in length of the tissue and per mm^2 in area of the mucosal layer were counted under a magnification of 100 ×. The mean number of

Chart 1. Alimentary canal of the chicken. The site of each organ investigated is shown with a solid square.



5-HT cells was calculated from 10 sections. After recording of fluorescence, some of the sections were stained by the argentaffin method of Masson-Hamperl. The stained sections were used to determine the anatomical relationship between the fluorescence-emitting structures and stained structures.

RESULTS

I. Catecholamine-containing fibers (CA fibers)

Green fluorescence indicating the presence of catecholamine was observed on the wall of blood vessels, the submucous and myenteric plexuses, and the musculature in the tissues examined. A more detailed description for each part of the canal is presented below.

Esophagus: Specific green fluorescences were divided into three types. One was a spot-like emitting structure on the wall of blood vessels in the epithelium and submucosa. Another was a small fiber with very fine varicosities seen in the muscle layer (Fig. 1). The third was a small, weakly fluorescent material surrounding the ganglion cells of the myenteric plexus in the longitudinal muscle layer (Fig. 2). There were, however, no fluorescent structures in the whole area of the submucous plexus.

Crop: The crop showed histologically the same structures as the esophagus. Weakly green fluorescences were observed on the wall of blood vessels and in the myenteric plexus, but not in the muscle layer.

Proventriculus and gizzard: Essentially the same distribution of fluorescent structures was observed in the proventriculus and gizzard as in the esophagus (Figs. 3, 4, and 5). In the *gll. gastricae propriae profundi* of the proventriculus, specific fluorescent fibers with varicose structures ran toward the inside (Fig. 6). In the *pars pylorica*, specific green fluorescences increased considerably in the muscle layer and appeared also in the submucous plexus.

Small intestine (duodenum and ileum): In the duodenum and ileum, green fluorescences were recognized on the wall of blood vessels, in the muscle layer, and in areas around the submucous and myenteric plexuses (Figs. 7 and 8). In the submucous plexus, an intense, green fluorescence, in the form of bead or small fiber, was associated with

groups of ganglion cells of the plexus. Some of the cells wer surrounded by fluorescent structures, but showed no fluorescence themselves. This finding indicates that the submucous plexus is innervated by adrenergic nerves in the chicken as in the mammal (HOLLANDS and VANOV, 1965; JACOBOWITZ, 1965). Plexuses were connected with one another by fasciculi containing adrenergic fibers. The myenteric plexus of chickens was different in distribution from that of mammals. The former was located mainly within the longitudinal muscle layer, while the latter was reported to be between the circular and longitudinal muscle layers. Many of green fluorescent materials were also observed in the myenteric plexus. The fluorescent fibers were arranged in two ways; some of them followed the direction of the circular muscle layer, while the others penetrated the muscle layer towards the submucous plexus or the outer serous layes. The fibers were connected with one another, and many of them were accompanied by blood vessels.

Rectum: The fluorescent materials in the rectum exhibited essentially the same distribution as those in the small intestine, although the rectum was richer in green fluorescent materials. One characteristic finding in the rectum was an abundance of bright, green fluorescent varicose fibers following the direction of the muscle layer (Fig. 9). Moreover, the fluorescences of th submucous and myenteric plexuses were not only greater in number but also larger in size in the rectum than in any other organ examined (Figs. 10 and 11). The structures showing specific fluorescences were compared between one section and essentially the same section as this stained by the argentaffin method of Masson-Hamperl. A close histological relationship was seen between the fluorescent materials and the ganglion cells.

Cecum: The ceca are situated at the junction between the small and large intestines (Chart 1). In the chicken, they are large and prominent (about 6 inches long) and in pairs (STURKIE, 1965). The cecum was divided into four portions (i.e., tonsil, isthmus, corpus, and apex; see Chart 1), all of which were examined. The fluorescent structures observed in the cecum presented essentially the same distribution as those in the small intestine (Fig. 12). There was no differences at all among the four portions of the cecum.

II. 5-Hydroxytryptamine-containing cells (5-HT cells)

5-HT cells exhibiting a strong yellow fluorescence in the cytoplasm were present in the mucosal layer, but not in the submucosal, muscle, or serosal layer, of the proventriculus, duodenum, ileum, rectum, and cecum. The fluorescence was abundant in the lamina propria mucosae of the alimentary canal, as described above. It was composed of spindle-shaped cells, but contained no fluorescent nuclei. Moreover, some of these cells were identified as enterochromaffin cells (argentaffin cells) in Lieberkuehn's crypts (Fig. 13).

The number of 5-HT cells varied considerably in different parts. Chart 2 shows the number of these cells per mm in length (solid circle) and per mm^2 in area (blank circle) of the mucosal layer. The number of 5-HT cells per mm in length was the largest in the duodenum, moderate in the ileum, rectum, cecal tonsil, and cecal isthmus, and the smallest in the corpus and apex of the cecum. The number of 5-HT cells per mm^2 in area was the largest in the rectum. 5-HT cells were the same in amount in the cecum as in the ileum. There were no 5-HT cells in the esophagus, crop, or gizzard, but a few in the glandular epithelium of the proventriculus. Autofluorescent materials, which were small in size and bright-orange in color, were observed in the mucosal layer of the esophagus, proventriculus, rectum, and cecum.

III. Effects of drugs administered

Reserpine: An almost complete disappearance of the specific green fluorescence was noted in all the organs treated with a single injection of reserpine (3 mg/kg, 4 hours).

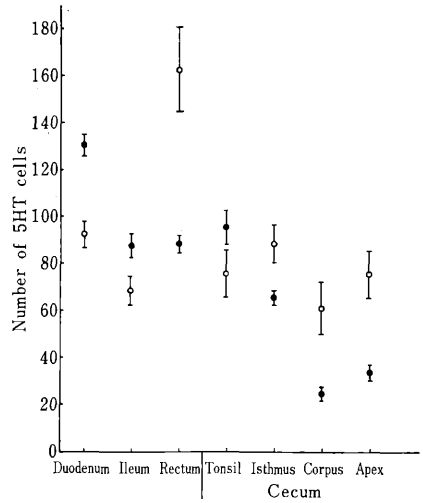


Chart 2. Distribution of 5-HT cells in various organs of the alimentary canal.

This phenomenon continued for about 48 hours. On the other hand, 5-HT cells still showed a specific yellow fluorescence, but decreased greatly in number after the treatment (Fig. 14).

Nialamide alone or in combination with l-dopa: Sections from all the organs were examined in chickens treated with a single dose of nialamide or a combined dose of nialamide and l-dopa. None of them showed a marked alteration in either specific fluorescence.

DISCUSSION

The present studies have revealed that fluorescent materials are contained abundantly in the alimentary canal of the chicken. Specific green fluorescences due to the presence of catecholamine, probably noradrenaline, were generally observed on the wall of blood vessels and in the muscle layer of the alimentary canal investigated. Moreover, the submucous and myenteric plexuses were intensely fluorescent in the small intestine (duodenum and ileum), rectum, and cecum. The myenteric plexuses were weakly fluorescent and the submucous plexuses non-fluorescent in the esophagus, crop, proventriculus, and gizzard. These fluorescences were presented by numerous fluorescent fibers with very fine varicosities, these fibers were in contact not only with non-fluorescent ganglion cells but probably also with non-fluorescent nerve fibers in these plexuses. Most of CA fibers in the smooth musculature may have been derived from the fluorescent fibers surrounding the submucous and myenteric plexuses. The rest of those fibers may have been originated from sympathetic postganglionic nerves directly innervating the smooth musculature. These observations are in agreement with those made earlier on the alimentary canal of mammals (HOLLANDS and VANOV, 1965; JACOBOWITZ, 1965; MURYOBAYASHI et al., 1968). This agreement strongly suggests that both plexuses of the chicken may also be innervated by adrenergic nerves.

It should be noted that the myenteric plexus of the chicken was present within the longitudinal muscle layer. This fact has been discovered by SHIMADA (1969). It was distinctly recognized that the myenteric plexus was linked to the submucous plexus with

fluorescent fibers, which were accompanied mainly by blood vessels. Thus, such a linkage seems to be related to the complicated reflex-arch responsible for the intestinal motility (FUKUHARA et al., 1960; SHIMADA, 1969).

Specific yellow fluorescent materials were contained in the mucosal layer, but not in the submucosal, muscle, or serosal layer, of the proventriculus, small intestine, rectum, and cecum. No yellow 5-HT cells were shown in the esophagus, crop, or gizzard. Some of these cells were identified as enterochromaffin cells (argentaffin cells) in Lieberkuehn's crypts, and the other seemed to be argyrophile cells. These observations are in agreement with those reported by MONESI (1960). Mast cells, which have been found in the gastrointestinal canal of mammals (HOLLANDS and VANOV, 1965; MURYOBAYASHI et al., 1968), were not detected from any part of the alimentary canal of chickens. The number of 5-HT cells per mm in length was the largest in the duodenum. It was comparatively large in the ileum, rectum, cecal tonsil, and cecal isthmus, but distinctly small in the corpus and apex of the cecum. On the other hand, the density of 5-HT cells was the highest in the rectum. It differed significantly in value between the rectum and any other part of the alimentary canal ($P<0.001$). It was approximately at the same level in the other parts of the canal than the rectum. In addition to this fact, 5-HT cells in the rectum seemed to be slightly different from those in any other part of the canal, for the former had a greater resistance to the action of reserpine than the latter (unpublished data). Further investigation is required to clarify the role of 5-HT cells in various parts of the alimentary canal of the chicken.

SUMMARY

Investigation was undertaken to study the distribution of monoamine-containing fibers and cells in the alimentary canal of the chicken by using a fluorescence histochemical method.

Catecholamine-containing fibers, characterized by a green fluorescence, were seen as chains of beads or as fibers themselves. Specific green fluorescent fibers were observed on the wall of blood vessels and in the muscle layers at all levels of the canal investigated. The most conspicuous feature was the presence of intense or weak catecholamine-fluorescent fibers in the myenteric plexuses. The submucous plexuses were also intensely fluorescent in the small intestine (duodenum and ileum), rectum, and cecum, but were not fluorescent in the esophagus, crop, proventriculus, or gizzard. These facts suggest that the structures, especially the myenteric and submucous plexuses, of the alimentary canal of chickens may be innervated by adrenergic nerves.

5-Hydroxytryptamine-containing cells, characterized by a yellow fluorescence, were observed in the mucosal layer of the proventriculus, small intestine, rectum, and cecum. There were no yellow cells in the mucosal layer of the esophagus, crop, or gizzard. The density of 5-HT cells was the highest in the rectum.

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ニワトリの消化管における monoamine 含有線維と細胞の分布について

池田　衡・犬飼初男・五島治郎
名古屋大学農学部家畜生理学教室

（昭和45年8月28日受付）

FALCK (1962) および CARLSO 等 (1962) によって確立された螢光組織化学法を用いて，ニワトリの消化管（食道・嗉のう・腺胃・筋胃・十二指腸・回腸・直腸・盲腸）における monoamines の分布を追究し，次の結果を得た．

catecholamine 含有線維（緑色螢光線維）は，観察したすべての消化管の各部で，小動脈壁・筋層および壁内神経叢に見られた．もっとも顕著な特徴は，消化管のすべての部位で，縦走筋層内の壁内神経叢に強または弱発色の CA 線維が存在することであった．更に，十二指腸・回腸・直腸および盲腸の粘膜下組織の壁内神経叢においても，強い緑色螢光が見られた．これらの事実は，従来 choline 作動性と考えられていた壁内神経叢が，adrenaline 作動性調節を受けることを示唆する．

5-hydroxytryptamine 含有細胞（黄色螢光細胞）は，腺胃・十二指腸・回腸・直腸および盲腸の粘膜層の Lieberkuehn 腺に分布し，enterochrom 親和細胞と一致した．粘膜面 1 mm² 当りの 5-HT 細胞の数は，直腸が最も多かった．一方，食道・嗉のうおよび筋胃では，5-HT 細胞は認められなかった．

reserpine (3 mg/kg) 処理後 4 時間と 18 時間の標本では，catecholamine の緑色螢光は，全て完全に消失したが，5-hydroxytryptamine の黄色螢光は，一部消失せず残存した．

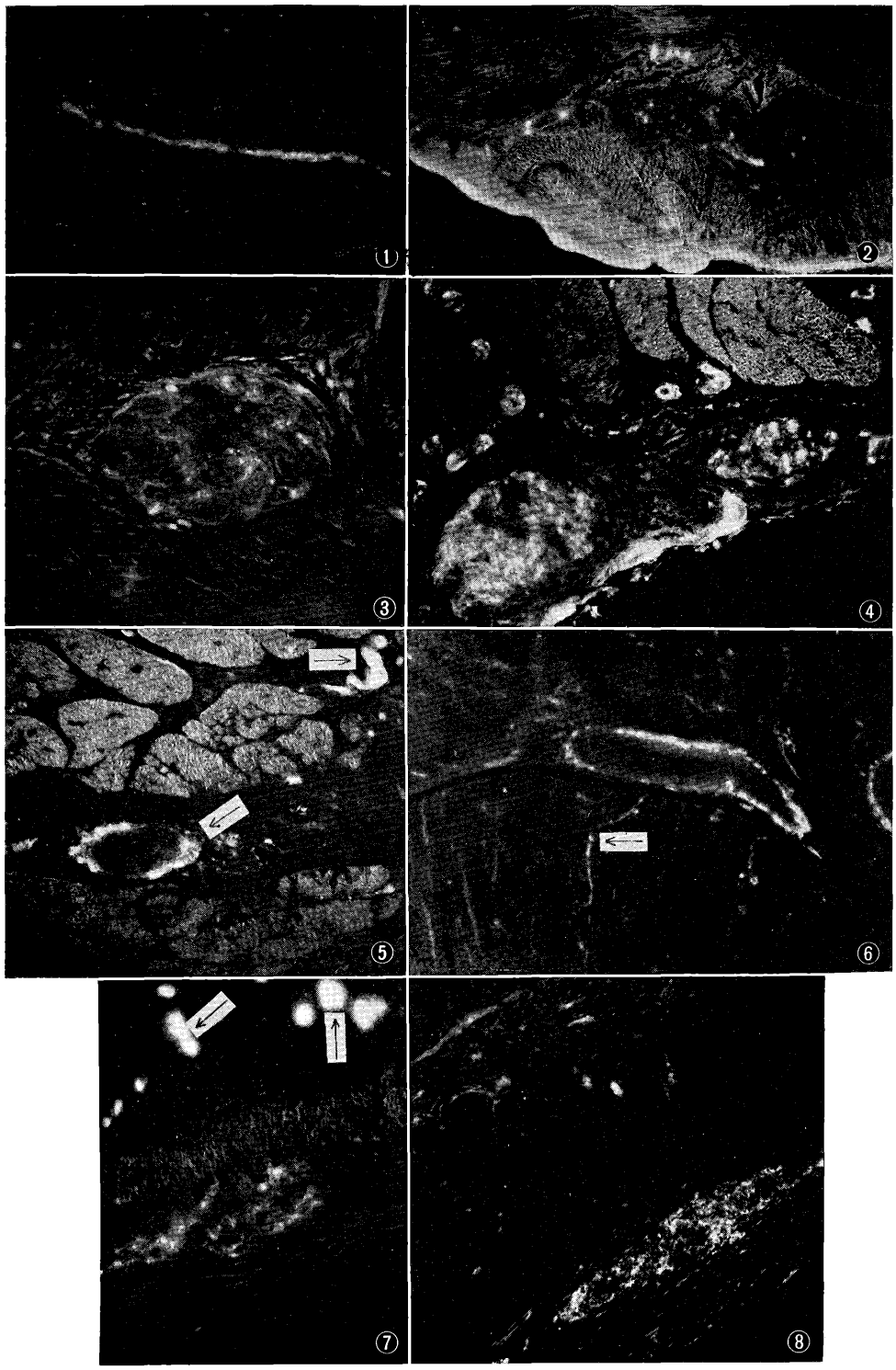
EXPLANATION OF PLATES

PLATE I

Fig. 1. Fluorescent nerve fiber in the circular muscle of the esophagus. ×400.
Fig. 2. Distribution of fluorescence in the myenteric plexus of the esophagus. ×200.
Fig. 3. Distribution of fluorescence in the myenteric plexus of the proventriculus. ×100.
Fig. 4. Distribution of fluorescence in the myenteric plexus of the gizzard. ×100.
Fig. 5. A small artery with fluorescent structures observed in the circular muscle of the gizzard. ×100.
Fig. 6. A small artery and nerve fiber (←) with fluorescent varicosities observed in *gll. gastricae propriae profundi* of the proventriculus. ×100.
Fig. 7. Distribution of fluorescence in the submucous plexus of the duodenum. ×200. Arrows indicate 5-HT cells in the mucosal layer.
Fig. 8. Distribution of fluorescence in the myenteri plexus of the ileum. ×100.

PLATE II

Fig. 9. Fluorescent nerve fiber with varicose structures seen in the circular muscle of the rectum. ×400.
Fig. 10. Distribution of fluorescence in the submucous plexus of the rectum. ×100. An arrow indicates a fluorescent nerve fiber in the circular muscle. 5-HT cells were seen in the mucosal layer.
Fig. 11. Distribution of fluorescence in the myenteric plexus of the rectum. ×100.
Fig. 12. Distribution of fluorescence in the myenteric plexus of the cecum. ×200.
Fig. 13. 5-HT cells seen in Lieberkuehn's crypts of the rectum. ×100. Some of these cells were identified as enterochromaffin cells (argentaffin cells). An arrow indicates the submucous plexus with fluorescent structures.
Fig. 14. Distribution of fluorescence in the rectum treated with a single injection of reserpine (3 mg/kg, 4 hr). ×100. Note that no fluorescence is present in the blood vessel, submucous plexus, or muscle layer. However, 5-HT cells can still be found.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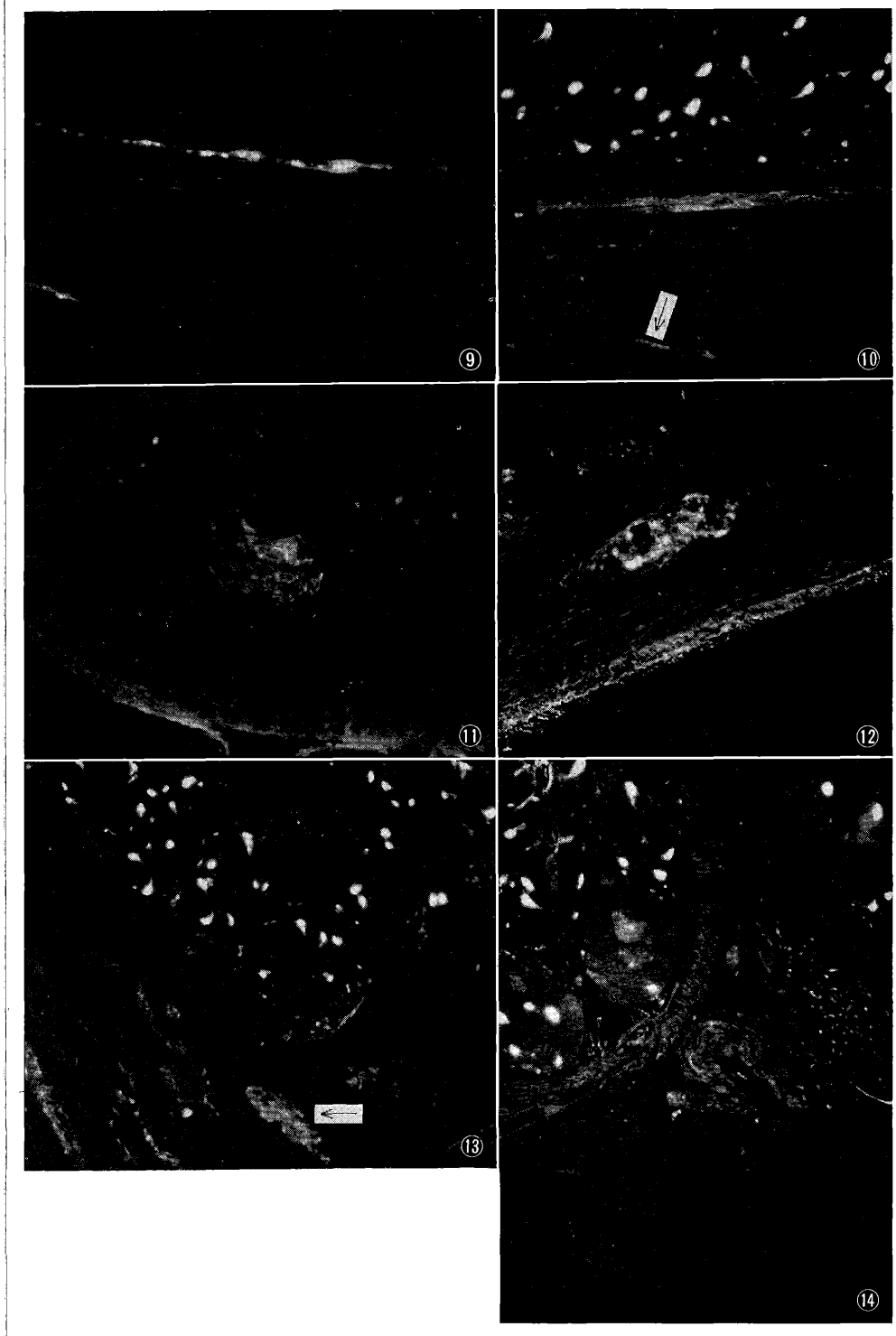
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