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THE DISTRIBUTION AND SIGNIFICANCE OF ORGANICALLY BOUND IODINE IN THE ASCIDIAN *CIONA INTESTINALIS* LINNAEUS

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(Plate I and Text-fig. 1)

INTRODUCTION AND PREVIOUS WORK

Attention has already been drawn (Barrington & Franchi, 1956*a*) to the presence of organically bound iodine in the endostyle of *Ciona*.[1] The purpose of the present work is to provide a fuller account of the distribution of this iodine, to examine how far the binding process is inhibited by the action of the goitrogen thiouracil, and to provide some cytological data for comparison with conditions in the endostyle of the ammocoete larva of the lamprey (Barrington & Franchi, 1956*b*). The importance of this latter aspect arises from recent demonstrations that significant amounts of thyroxin may be formed in various invertebrates (see, for example, Gorbman, Clements & O'Brien, 1954), possibly as the result of the iodination of skeletal scleroproteins. It has been plausibly suggested (Gorbman, 1955) that thyroid hormone may initially have arisen in this way as a biological accident; the thyroidal biosynthesis which is found in vertebrates, and which depends upon the secretion of thyroglobulin (Roche & Michel, 1955), would thus be a later evolutionary development consequent upon the iodination product having become metabolically important in the vertebrate line. From this point of view such protochordates as the ascidians occupy a position of key importance, and the crucial question to be answered is whether their iodine binding also is to be regarded, like that of invertebrates, as a chance by-product, or whether it shows any signs of being, like that of vertebrates, organized as a biochemical specialization.

MATERIAL AND METHODS

The animals were sent to Nottingham from the Laboratory of the Marine Biological Association, Plymouth. Some were fixed on arrival, while others were first immersed for 2 days in sea water containing 200 μc. of ^{131}I per litre. For investigation of the action of thiouracil the animals were immersed for

[1] Some evidence for this has also been obtained in unpublished work of B. C. Abbott and D. A. McGill (see Report of the Council of the Marine Biological Association for 1953–4, this Journal, Vol. 33, p. 775).

1 day in sea water containing 0·03 g. of the goitrogen per litre, after which the radio-iodine was added and they were left for a further 2 days. Constant aeration was provided, and temperatures ranged from 9 to 15° C.

Material was fixed in Susa, mercuric-formol, Champy's fluid in sea water, Bouin's fluid in sea water, and Hollande's modification of Bouin's fluid without acetic acid. For staining and histo-chemistry use was made of the Azan technique, mucicarmine, Kull's technique, the periodic-acid-Schiff (PAS) procedure, the pyronin-methyl-green test for ribonucleic acid (RNA), the ferric ferricyanide test (Fisher, 1953), and fluorescence microscopy; some further reference to these methods will be found in Barrington & Franchi (1956*b*). Autoradiographs were prepared by the stripping-film technique of Pelc, as outlined by Pearse (1954), and were counterstained in Harris's haematoxylin and eosin.

OBSERVATIONS

THE ENDOSTYLE

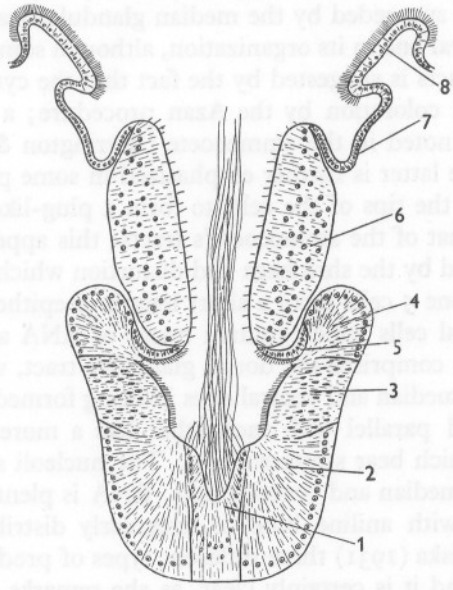
A comparative survey of the endostyle in seven species of ascidians has been reported by Sokólska (1931; see also Hûus, 1937), but the present study has disclosed some new points, both of fact and interpretation, which are of importance for the analysis of the functions and homology of the organ. In *Ciona* (Millar, 1953) it has the form of a deep groove, the epithelial wall of which is composed of a number of distinctive cell types; these will be referred to here for convenience as numbered zones (Text-fig. 1).

Zones 1–6

In the mid-ventral line, at the base of the groove, there is a group of cells (zone 1) which bear exceptionally long cilia. Near their nuclei there are occasional large vacuoles containing material which may be brownish in colour; the significance of these is obscure, but distally there is clear evidence of secretory function, for the apical part of the cell body contains material (diffuse or granular according to the mode of fixation) which stains intensely with aniline blue and with the PAS procedure, and which is the only product in the endostylar epithelium to react positively with mucicarmine. These cells differ, however, from the more typical secretory cells of the three pairs of glandular tracts (see below) in showing neither a large nucleolus nor a significant amount of RNA. Their cell bodies are slender and elongated, and their nuclei are crowded into two rather irregular layers, but the zone extends on each side as a layer of much shallower cells in which both the secretory contents and the ciliation are progressively reduced, the tips of these extensions becoming continuous with the ventral glandular tracts.

The latter are formed of elongated columnar cells (zone 2); each of these has a large spherical nucleus, a very conspicuous nucleolus, and cytoplasm rich in RNA, but it is difficult to judge whether they bear any cilia. In

Sokólska's figures these cells are shown lying more or less parallel with each other, and covered over (and therefore separated from the endostylar lumen) by an entirely separate layer of cubical cells. It is difficult to understand what could be the significance of such an arrangement, which would result in the separation of the glandular cells from the lumen into which they discharge, and in *Ciona* the actual situation proves to be a little different from this. The cells of the glandular tract are in fact arranged in a fan-shaped pattern (Text-fig. 1), with their apices crowded together in a narrow zone at which the discharge of their secretion must take place, and it is the lateral extension of the zone 1 cells over the main body of the fan which gives the misleading impression that the secretory cells are completely cut off from the lumen.



Text-fig. 1. Transverse section of the endostyle of *Ciona* (diagrammatic). The eight zones of the epithelium are indicated by corresponding numbers; for further explanation see text.

This detail is of some importance, not only because it makes the organization of the ventral tract functionally intelligible, but even more because it emphasizes the close resemblance between the endostyle of *Ciona* and that of the ammocoete larva. The cells of the glandular tracts in the latter animal are very like these zone 2 cells, and are arranged in a similar fan-shaped pattern, with discharge taking place through a narrow plug formed by the fusion of the tips of the cells.

The secretory functions of the endostyle of *Ciona* appear to be complex, and an analysis of the activity of the glandular tracts is outside the scope of the present work. It must suffice to say that a characteristic feature of the cells of

the ventral and median tracts is the accumulation above the nucleus of a material, presumably a secretory product, which stains pale grey-blue with the Azan technique, gives a weak PAS-positive reaction, and is negative to mucicarmine. This is sometimes seen as a compact mass lying within a large vacuole, but its actual form is probably much influenced by artifact, and in some preparations, notably after Champy or Bouin-Hollande (without acetic) fixation, it is not sharply demarcated from the surrounding cytoplasm.

The ventral glandular tract is abruptly succeeded by zone 3, composed of slender ciliated cells which are closely crowded and which have their granular nuclei irregularly arranged in a number of layers. RNA is negligible in these cells, but the presence of granular inclusions, PAS-positive after fixation in Bouin-Hollande (without acetic), suggests that they may have some secretory function. They are succeeded by the median glandular tract (zone 4), which resembles the ventral one in its organization, although some differentiation in the secretory products is suggested by the fact that the cytoplasm of the two tracts differs in its coloration by the Azan procedure; a somewhat similar situation has been noted in the ammocoete (Barrington & Franchi, 1956*b*). Resemblance to the latter is further emphasized in some preparations by the apparent fusion of the tips of the cells to form a plug-like structure exactly comparable with that of the ammocoete's tracts; this appearance may, however, be exaggerated by the shrinkage and distortion which is clearly induced by fixation. The zone 5 cells form a short length of epithelium composed of more or less cubical cells which contain traces of RNA and a few granular inclusions. Zone 6 comprises the dorsal glandular tract, which differs in its structure from the median and ventral ones in being formed of elongated cells which are arranged parallel with each other like a more normal columnar epithelium, and which bear scattered cilia. The nucleoli are large, although less so than in the median and ventral tracts, RNA is plentiful, and secretory material (staining with aniline blue) is irregularly distributed in vacuoles. According to Sokólska (1931) three different types of product are discharged from these cells, and it is certainly clear, as she remarks, that the so-called mucous secretions of the ascidian endostyle must be a product of considerable complexity.

In a recent account of the ammocoete's endostyle (Barrington & Franchi, 1956*b*) a distinction has been made between alimentary and thyroidal functions. From this point of view, which seems also applicable to *Ciona*, the portions of the endostyle so far described, constituting the main bulk of the organ, must be regarded as primarily alimentary; there is probably more to be learned as to the full function of its secretions, but they are well known in general terms to be concerned with the trapping of food particles (Orton, 1913). Over the whole of this region (zones 1–6) bound iodine is inconspicuous in autoradiographs and is possibly absent altogether except in association with the long cilia of zone 1, where a small amount of iodine is commonly to be

found; this, however, has a separate significance which will be discussed below (p. 8). In some areas of the epithelium of some specimens, however, the density of the autograph seems to be slightly greater than that of the background, particularly in zone 1 and in the upper part of zone 6, and it is just possible that this is indicative of a slight capacity for iodine binding; it is certain, however, that this would attract little attention if it were not for the highly significant images obtained from the remainder of the organ (zones 7 and 8).

Zone 7

Dealing first with zone 7 (Text-fig. 1; Pl. I, fig. 5), iodine is here found distributed more or less evenly over the cell bodies throughout the whole extent of this epithelium; the autoradiographic image (Pl. I, figs. 6, 11) is not dense, but it is a very definite and constant feature of this zone, and it seems evident that iodine binding must be occurring in the cells. Its clear definition in this particular zone shows that this binding cannot be merely a chance by-product of the general secretory activity of the endostyle, and a further examination of the properties of zone 7 provides good evidence that a specialized secretory process is, in fact, involved, for its cells contain numerous granules of variable size, and may sometimes show a slight indication of RNA. Often there appears to be one large granule per cell (Pl. I, fig. 12), but in some preparations additional smaller ones can be seen, and it is probable that a fusion of a group of these into larger ones may occur as an artifact of fixation. Mucus-like material, not dissimilar in shape although with less sharply defined contours, is plentiful elsewhere in the pharyngeal epithelium, so that the properties of the zone 7 granules, in comparison with the inclusions of other cells, need careful examination; they may be summarized as follows:

(*a*) Colour. They are pale yellow-brown in colour, a property not found anywhere else in the endostylar or pharyngeal epithelium except in the material seen in vacuoles in some of the zone 1 cells (see above, p. 2). The pigment, both in the latter and in the zone 7 granules, is bleached after 24–48 h treatment with 10% hydrogen peroxide, and is thus possibly melanic, although the initial colour is sometimes so weak that this result may not be of much significance.

(*b*) Fluorescence. After fixation in Susa, mercuric formol, or Bouin in sea water the granules exhibit a yellow-brown fluorescence which becomes very pale yellow after bleaching with hydrogen peroxide. Although fluorescence is a conspicuous feature of cells in the blood sinuses, it is found nowhere else in the endostylar or pharyngeal epithelium with the fixatives mentioned; in particular, the brown material in zone 1 is not fluorescent. After fixation in Bouin-Hollande (without acetic) some finely particulate whitish fluorescence appears in zone 6 and in the mucous region of zone 1, but the fluorescence of zone 7, although less striking than with the other fixatives, remains distinctive.

(*c*) Azan staining. The zone 7 granules commonly stain a characteristic

bright orange; the inclusions elsewhere show a varying degree of affinity for aniline blue, the apical secretion of zone 1 and the secretion of the pharyngeal epithelium staining deeply with this.

(*d*) PAS reaction. The granules give a moderately strong positive response. A noticeably stronger response is given by the secretion of zone 1 and of the pharyngeal epithelium, while the response of the remaining secretory products of the endostyle, together with the brown material in zone 1, is weak and indefinite.

(*e*) Mucicarmine. The granules are negative to mucicarmine, as also are all of the products of the endostyle, with the exception of the zone 1 secretion (see above). An occasional indefinite response may be given by the pharyngeal epithelium, but this also is commonly negative.

(*f*) Ferric ferricyanide test. The granules give a moderate but quite definite positive response. The secretion of the pharyngeal epithelium is also positive, but more strongly so, giving a deep blue colour. A strong response is also obtained from some of the contents of zone 1; the response of the remainder of the endostyle is indefinite, although the cytoplasm of the glandular cells may respond to a moderate extent. After fixation in Bouin-Hollande (without acetic) the response of the zone 7 granules is relatively more intense.

The above tests are not, of course, specific, a positive ferric ferricyanide response, for example, indicating no more than the presence of reducing groups (Adams, 1956), and the results are clearly influenced to some extent by the nature of the fixative. It can, however, be concluded that there is present in zone 7 a particular type of secretory inclusion which displays characteristics not found in the same combination elsewhere in the endostylar or pharyngeal epithelium. No useful inferences can be drawn at this stage as to the distinctive chemical nature of this secretion, but it can be said that the granules are associated with iodine binding, and it is significant that their properties, as outlined above, are virtually identical with those of the secretion of the thyroidal epithelium of the endostyle of the ammocoete (Barrington & Franchi, 1956*b*). It seems reasonable, therefore, to conclude that they are homologous with the latter, and, through them, with thyroid colloid, and that they thus constitute evidence for the existence in *Ciona* of a specialized secretory activity providing the molecular basis for the iodination process. On this interpretation the latter is not, therefore, a chance by-product of endostylar secretion, but seems very likely to represent a truly thyroidal biosynthesis. For this reason the zone 7 granules will be referred to as *thyroidal granules*, the name already applied to the corresponding secretion of the ammocoete.

Zone 8

In certain sections one or more of the thyroidal granules of zone 7 can be seen lying in the endostylar lumen, or else at the tip of a cell as though extrusion were about to take place. Such appearances could, however, very well be

an artifact of fixation or of sectioning, and they are too infrequent to be accepted as reliable evidence of discharge. On the other hand, there is reason for believing that in the ammocoete the iodinated product may be extruded into the endostylar lumen in a more fluid and non-staining form, and it is possible that the same may apply to *Ciona*. The importance of this matter lies in its relevance to the interpretation of the condition of the zone 8 epithelium (Text-fig. 1). This is somewhat thicker than zone 7, and is composed of strongly ciliated cells which lack RNA and which show less sign of secretory activity than any other part of the endostylar epithelium. These cells are known (Orton, 1913) to be responsible for driving the secretion of the endostyle laterally out of the groove and on to the pharyngeal wall, up which it is then swept by the cilia of the latter (see below, p. 8). It follows that any iodinated product which may be discharged from zone 7 would also be swept over these zone 8 cilia and some might well be adsorbed to them; thus would arise autoradiographic images which would, however, indicate a transmission of bound iodine across the cells rather than the existence of it within them.

This appears to be the most likely explanation of the fact that zone 8 actually gives a much more intense image than does zone 7 (Pl. I, figs. 5, 6), for close inspection of the distribution of the iodine shows a marked difference between the two zones, the weaker image of zone 7 being almost uniformly distributed over the cells, while that of zone 8 shows an intense concentration over the cilia and cell borders. Pl. I, figs. 6 and 11, illustrate this feature, although it is much less clearly defined in the photomicrographs than in the original preparations. The iodine image extends also over the cell bodies, but this extension is much less intense than that of the surface concentrations, and could quite well be a result of random scatter from them. There is, too, the possibility that if an iodinated secretion does accumulate over the surface of the epithelium there might be some diffusion from it into the cytoplasm. However, it is clearly impossible to be sure that no binding of iodine takes place within the zone 8 cells, but the absence of any obvious secretory basis for it suggests that its occurrence is highly improbable. Moreover, the view that the iodine image over zone 8 is a consequence of the movement of iodinated secretion by the cilia is strongly supported by consideration of the distribution of bound iodine elsewhere in the pharynx, as will now be explained.

THE PHARYNGEAL CONTENTS

First, there is clear evidence that an iodinated product is mixed with the visible secretion of the endostyle and pharynx, for the food cords in the lumen are found to be strongly iodinated (Pl. I, figs. 1, 2). Here, however, a complication is admittedly introduced by the capacity of marine organisms to accumulate bound iodine (see above, p. 1), for any such organisms included in the food would be expected to show this same property. This was, in fact, strikingly exemplified by one specimen in which a large unidentified arthropod

within the pharynx showed a substantial concentration of bound iodine within its exoskeleton (Pl. I, figs. 1, 2). This property could hardly, however, account for all of the iodination of the food cords, the exogenous contents of which are very variable in amount and miscellaneous in nature, including *Ciona* sperm, diatoms and unidentifiable cellular material and detritus. It is perhaps impossible to establish with absolute certainty that an iodinated secretion is added to the food cords from the endostyle, but the strong probability that this does happen is indicated by the fact that radioactive secretion, with very little exogenous material in it, can be observed in the mouth of the organ (Pl. I, figs. 5, 6). Equally significant is the almost invariable association of a weak but definite iodine image with the long cilia which arise from zone 1, for these are thought to have no function in the transporting of food particles, but are believed to assist in the deflecting of the endostylar secretion on to the cilia of zone 8 (Orton, 1913).

THE PHARYNGEAL EPITHELIUM

A second significant aspect of iodine distribution within the pharynx arises from the complicated structure of the wall of this organ. A current of water is maintained by the cilia of the gill openings or stigmata, but internally to the latter there is a system of longitudinal bars from which papillae project into the pharyngeal cavity (Roule, 1884; Berrill, 1950). Now the long threads of secretion which trap the food particles are swept up the walls mainly by the cilia of these papillae, aided in part by a waving movement of the bars themselves (Orton, 1913), with the ciliated dorsal languets becoming involved in the backward movement of the main food cord. If, therefore, there is any tendency for the iodinated component of the endostylar secretion to accumulate over the surfaces of the ciliated epithelia which propel the latter, as has been suggested above for zone 8, an autoradiographic image should be conspicuous on the ciliated regions of the papillae and languets. This is, in fact, readily seen to be so (Pl. I, figs. 1, 2, 9 and 10), the image, as with that of zone 8, showing a concentration over the surface rather than a uniform distribution over the cell bodies. In sharp contrast to this, the ciliated epithelia of the stigmata, which, by virtue of their position, are largely removed from contact with the endostylar secretion, yield very much lighter and often quite negligible images.

THE INTESTINAL CONTENTS

Within the intestine the food cords are distinguishable into two components, one being material which has entered from the pharynx and which is recognizable by the variety of its contents, and the other a clear mucus-like substance, positive to mucicarmine, which has no food material mixed with it, and which is a secretion of the mucus cells of the intestinal epithelium. Auto-

radiographs demonstrate an important difference between these two components (Pl. I, figs. 7, 8), for the former gives an iodine image while the latter does not; here, then, there is no evidence for any association of iodine binding with the normal process of alimentary secretion.

THE TEST

The presence of iodine in the test of ascidians has long been known from the work of Cameron (1914, 1915), who, without reporting on its chemical form, noted that it was especially abundant in the surface layer. The test is now known to be composed of two parts, the tunic proper and the superficial cuticle. The former is made up of the cellulose-like tunicin, with which is associated some glycoprotein, while the cuticle consists of pure protein and is thus chemically different from the remainder (Pérès, 1948). This difference is emphasized by the mode of development, for while the epidermis is mainly responsible for secreting the basic substance of the test, the cuticle arises from 'Tropfenzelle'; the latter initially contain a glycoprotein complex, but after migrating into the test they appear to give up the carbohydrate component to the tunicin and the remainder of their content is subsequently incorporated into the cuticle.

In view of these facts it is of interest that autoradiographs clearly demonstrate (Pl. I, figs. 3, 4) an accumulation of bound iodine in the cuticle, while none appears to be present in the remainder of the test. Mention has been made above (p. 1) of the way in which bound iodine becomes incorporated into the scleroproteins of the skeletal structures of invertebrates; the nature of the proteins of the test clearly demands further investigation from this point of view, for if the tough outer cuticle could be shown to contain scleroprotein the presence in it of bound iodine would be readily explained as a similar by-product of skeletal secretion. The facts at present available certainly suggest that iodination in the test is a process quite distinct from that in the endostyle, although it is tempting to speculate that the existence of the former might have been the starting point for the evolution in ascidians of a biochemically useful iodinated product.

In the earlier stages of this work there appeared to be indications of the association of bound iodine with the mantle epithelium (Barrington & Franchi, 1956*a*), but further studies have not substantiated this, and at the present time significant amounts of bound iodine have only been found consistently in the regions mentioned above. The iodination of the test merits further investigation, however, for, as has been suggested here, it raises an issue of some evolutionary interest, and it is hoped to report on it further in relation to the mode of secretion and regeneration of this tissue. Moreover, the existence of bound iodine in the stolonic septum of *Perophora* (Gorbman, 1941) shows that the present description does not exhaust the possibilities of iodine binding in ascidians.

THE ACTION OF THIOURACIL

In considering the effects of thiouracil it is necessary to bear in mind its supposed mode of action. Thyroidal biosynthesis is thought to depend upon the iodination and subsequent condensation of the tyrosine residues in thyroglobulin, the specific protein of the thyroid gland (Roche & Michel, 1955), but the means by which chemical goitrogens produce their interference with this process are not fully understood. It seems likely, however, that thiouracil, acting perhaps as a reducing agent, prevents the liberation from iodide of free iodine, the presence of the latter being essential if the iodination of tyrosine is to take place. This requirement has been mainly studied in relation to thyroidal biosynthesis, but it appears to be a necessary condition for any protein iodination, including *in vitro* reactions (Rawson, Rall & Sonenberg, 1955). It would seem to follow from this that the iodination of skeletal scleroproteins by radioactive iodide must depend upon the release of free iodine, and that this in its turn might well depend, at least in part, upon an appropriate oxidizing enzyme system which might, therefore, be inhibited by thiouracil. This means that a demonstration of such inhibitory action in any tissue cannot be held as a proof that it is carrying on thyroidal function in the strict sense of the iodination of a specific thyroid protein.

The effect of thiouracil treatment upon *Ciona* is to eliminate almost completely all traces of bound iodine from the sites mentioned above. None at all can be detected in the endostylar epithelium or on the pharyngeal cilia. It is, however, sometimes possible to detect a very slight but significant amount in the food cords, both in the pharynx and in the intestine, but not, of course, in the purely intestinal mucus in the latter (see above, p. 9). It is not clear whether this iodine is associated solely with the exogenous food material, or whether it results from a small amount of bound iodine continuing to be discharged from the endostyle without the prior storage which would be needed to enable that organ to produce a significant autoradiograph, but it is of interest that a similar situation appears to exist in *Amphioxus* (Thomas, 1956), the association of iodine with food particles being demonstrable after thiouracil treatment has eliminated it from the endostyle of that animal. The amount involved in *Ciona*, however, is very much less than in control specimens and could easily be overlooked if it were not being specially sought, and it is clear that the goitrogen substantially arrests the normal iodination process. This applies also to the cuticle of the tunic, for in thiouracil-treated specimens the bound iodine is either completely lacking or is present in a greatly reduced amount. It is impossible to judge the full significance of these results until there has been further clarification of the biochemical basis of the iodination process in the endostyle and cuticle, and until the mode of action of thiouracil is better understood. For the reasons already stated, however, the behaviour of the cuticle cannot be taken as evidence that this region is concerned with specifically thyroidal biosynthesis.

DISCUSSION

The observations recorded here show a close resemblance between the endostyle of *Ciona* and that of the ammocoete, not only in the organization of certain of the glandular tracts, but more especially in the ability of parts of the epithelium to bind iodine, and in the association of the latter property with a characteristic secretory product, here called the 'thyroidal granule'. So close is this resemblance that there can now be little doubt as to the correctness of the classical view that the protochordate endostyle is homologous with that of the ammocoete and, through the latter organ, with the thyroid gland of the vertebrates. It is necessary to consider further, however, the justification for regarding iodine binding in the ascidian endostyle as a truly thyroidal biosynthesis.

Spaul (1928) was unable to accelerate metamorphosis in frog tadpoles by feeding them with *Ciona* endostyle, but in view of the relative weakness of the autoradiograph of the latter organ as compared with that obtained from mammalian thyroid it may be doubted whether the amount of tissue used by him would have been adequate for securing a positive result. As against this negative finding, Sembrat (1953) was able to bring about metamorphic changes in axolotls by inserting into them as many as sixty-five dried endostyles of *Amphioxus*, in which organ iodine binding is known to occur (Thomas, 1956), and in view of this, and of the known occurrence of thyroxin in the ammocoete endostyle (Leloup & Berg, 1954), there is clearly no *a priori* reason why thyroid hormone should not be present in the endostyle of *Ciona*. It is hoped to report later on the chemical form of the bound iodine of this organ and of the test, but in the meantime it must be said that, for reasons indicated above (p. 1), this particular issue now seems less crucial than the question as to whether or not the iodine binding is organized as a biochemical specialization.

It is for future work to show whether, as seems quite likely, the iodination of the cuticle in *Ciona* is a chance result of the presence there of scleroproteins. The situation in the endostyle, however, seems much better defined, for the present work has shown that iodination in that organ takes place within a narrowly limited zone which is cytologically specialized in a manner directly comparable with the thyroidally active regions of the ammocoete's endostyle. The inference is that the product of iodination is a thyroid hormone which has become metabolically important for the ascidian, and that a particular epithelium is set apart for secreting, as thyroidal granules, the necessary molecular basis for the iodination process. It is not possible as yet to suggest what might be the functional importance of this hormone, but it is clearly essential that this inference should now be tested by examining whether thyroxin can be shown to exert any specific effects upon ascidians. At this stage also it is not clear whether any significance is to be attached to the slight traces of bound iodine in other parts of the endostyle. Such traces might represent a generalized capacity from which the special property of zone 7

evolved, but study of a wider range of species is necessary before any useful opinion can be expressed on this.

The autoradiographs do not disclose the route by which the iodine reaches these cells; presumably iodide might be absorbed by them direct from the water, or it might reach them in the body fluid after absorption elsewhere (by, for example, the pharyngeal wall). In this connexion it seems worth noting that the zone 7 cells are separated from the underlying body fluid by a rather thick layer of connective tissue; no doubt this would not preclude absorption through the latter, but, having regard also to the situation of this zone at the upper edge of the endostylar groove where it must be bathed by moving sea water, circumstances would seem to favour the possibility of direct absorption of iodide from the pharyngeal lumen. This would, of course, partly explain the use of endostylar epithelium for thyroidal biosynthesis, and other advantages are its well-developed secretory capacity, which would provide a basis for the evolution of the thyroidal granules, and the ease with which the hormonal product can be mixed with the remainder of the endostylar secretions and so be conveyed into the intestine. From there it is presumably taken up into the body tissues (although there is at present no proof of this), and it is the development of this particular route of absorption which, as Thomas (1956) has also pointed out in connexion with his study of *Amphioxus*, explains why thyroid hormone can be successfully administered by mouth throughout the vertebrates.

Comparison with the ammocoete is complicated by the very different organization of the pharynx in the vertebrates, but it is clear that the division of the endostyle in the latter animal into two halves, amounting to its virtual duplication, and its posterior elaboration into a spiral coil, provide a very large surface area within the limited space available. The proportion of this area devoted to iodine binding is, moreover, very substantially greater than in the ascidian, while many of the thyroidally active cells are columnar in form and, with their large complement of thyroidal granules, seem much more specialized than the corresponding cells of zone 7 (Barrington & Franchi, 1956*b*). These facts suggest a more intensive development of iodine binding in the ammocoete, and this must surely be correlated with the diminished availability of iodine which would have been an inevitable consequence of the migration into fresh water which marked the evolution of vertebrates from the protochordates. It may be suggested also that the form of the endostyle in the ammocoete, where it appears as a sac with only a narrow opening, is not as well adapted as is an open groove for the uptake of iodide from the water circulating through the pharynx; since, therefore, the endostylar epithelium in this animal has close contact with well-developed blood capillaries it seems probable that uptake from the blood stream may have become established with this advance in specialization, although here again the autoradiographs offer no clear evidence.

It is of great interest that iodine binding has also been shown to occur in the

endostyle of *Amphioxus* (Thomas, 1956), for this establishes the phenomenon as a common property of the organ in all three of the groups (Tunicata, Cephalochordata and Vertebrata) in which the latter is present. The endostyle of *Amphioxus* has only two pairs of glandular tracts, and the iodine binding appears to be located in or near the lateral pair, a fact which has led Thomas to regard the endostylar mucus as the direct evolutionary forerunner of thyroid colloid. The present work shows, however, that there are specialized iodine binding cells in the ascidian endostyle which are distinct from the glandular tracts and which would seem to resemble the thyroidally active cells of the ammocoete much more closely than do the mucus cells of *Amphioxus*, despite the fact that the latter animal, with its well-developed metamerism, is in some respects much closer to the vertebrates than are the ascidians. This situation, at first sight somewhat paradoxical, clearly needs further investigation. Its explanation may, however, be found in some recent arguments of Berrill (1955), who has developed with great cogency the view that *Ciona* represents a truly primitive level of chordate organization from which the vertebrates could have been derived by the process of neoteny on which Garstang (1929) placed such emphasis. Berrill suggests that *Amphioxus* is a specialized offshoot of the main chordate stem, and represents a secondary return to a fully marine existence from a stage at which the early chordates were already ascending estuaries and becoming established in fresh water. He ascribes to this the secondary degeneration of their anterior sensory equipment, and it would seem logical to expect that a comparable degeneration in the iodine-binding mechanism would result from such a return to an iodine-rich habitat. For the present this suggestion must remain a matter for speculation, but in the meantime some support for such an interpretation is possibly to be drawn from Thomas's interesting observation that *Amphioxus* does not take up radio-iodine when it is first removed from its natural habitat, but begins to do so when it is kept in the laboratory in circulating water in which the iodine content is probably lower than in the outside sea water.

I am indebted to Mr T. Berbank for preparing the photomicrographs and to Miss J. M. Plumtree for technical assistance. Comparisons with the ammocoete larva have been rendered possible by work carried out on that animal in collaboration with me by Mr L. L. Franchi.

SUMMARY

Some features of the organization of the endostyle of *Ciona intestinalis* are described. Autoradiographs show that bound iodine is present in a limited area of the epithelium (zone 7), where it is associated with characteristic secretory inclusions which are found only in this zone. These are regarded as providing the molecular basis for the iodination process, and are termed 'thyroidal granules', since they resemble in certain properties the similarly

named granules of the endostyle of the ammocoete; it is concluded that they are homologous with these and, through them, with thyroid colloid. Their existence provides evidence that the iodination process is a product of biochemical specialization, and implies that it results in the formation of an hormonal secretion. It is concluded from the distribution of bound iodine over the pharyngeal epithelium and in the food cords that this iodinated secretion is carried in the latter into the intestine. Bound iodine is also present in the cuticle of the test; it is suggested that this may differ from the situation in the endostyle in being a consequence of the iodination of a skeletal protein secretion such as is known to occur in many invertebrates. Prior immersion of the animals in thiouracil solution largely eliminates bound radio-iodine from all of the sites mentioned, although a little may sometimes be detectable in the food cords and cuticle. The results are discussed in the light of recent work on *Amphioxus* and the ammocoete larva, and attention is drawn to their bearing on current views on the relationship between vertebrates and protochordates.

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EXPLANATION OF PLATE I

Fig. 1. Scene in the pharynx of *Ciona*, showing, in transverse section, part of an unidentified arthropod to the left, a food cord to the right, and dorsal languets above. Azan.

Fig. 2. Autoradiograph of an adjacent section. Iodine is present in the exoskeleton of the arthropod, in the food cord, and over the ciliated lateral borders of the dorsal languets (see also figs. 9 and 10).

Fig. 3. Section through part of a colony of *Botryllus* which is growing upon the surface of the test of a *Ciona*. The cuticle of the former is at the top left and that of the latter at the bottom right. PAS.

Fig. 4. Autoradiograph of an adjacent section. Iodine is conspicuously concentrated in the cuticle of *Ciona* (extending across the bottom of the photograph) and is also present to a less extent in that of *Botryllus* (top left).

Fig. 5. Upper part of the right side of the endostyle of *Ciona* (compare Text-fig. 1). The upper part of zone 6 is at the bottom right, zone 7 is above it, and zone 8 forms a crescent to the left; the pharyngeal epithelium extends from the latter upwards and to the right. A small mass of secretion lies in the mouth of the endostyle towards the bottom left corner. PAS.

Fig. 6. Autoradiograph of an adjacent section. Iodine is present over zones 7 and 8, and in the small mass of secretion at the bottom left-hand corner. At the top of the crescent formed by zone 8 there is some indication of the concentration of iodine at the surface of this epithelium (see text).

Fig. 7. Food cord in the intestine of *Ciona*. The dense pharyngeal component, with exogenous material, extends to the left, and is sharply distinguished from the clear intestinal secretion which lies to the right. Azan.

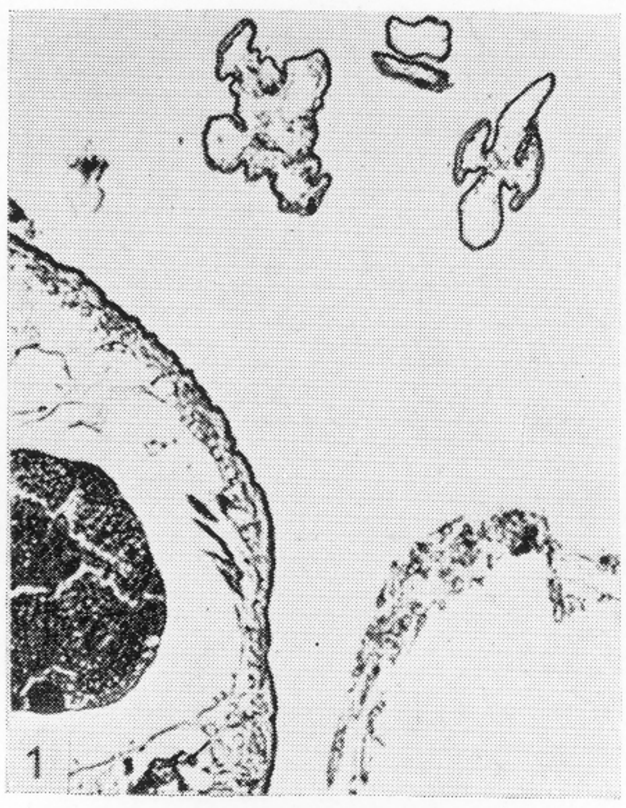
Fig. 8. Autoradiograph of an adjacent section. Iodine is conspicuous in the pharyngeal component and is absent from the intestinal secretion.

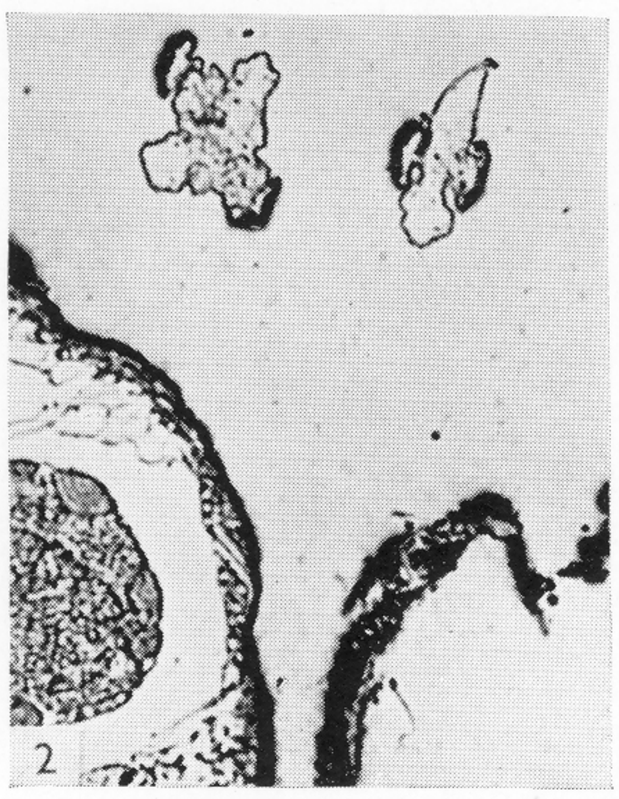
Fig. 9. Lateral border of a dorsal languet; a small amount of secretion overlies the columnar cells, and is associated with their cilia. PAS.

Fig. 10. Autoradiograph of an adjacent section. Iodine is concentrated over the surface of the ciliated columnar epithelium (see text), but is absent from the unciliated cubical epithelium below. The dense image at the top centre is associated with secretion overlying the cells (compare fig. 9, in which some of this secretion is visible).

Fig. 11. Autoradiograph of zone 7 (below) and zone 8 (in the upper third of the photograph). Iodine is equally distributed over the former, but is concentrated at the surface of the latter (see text, and compare figs. 6 and 10).

Fig. 12. Zone 7 epithelium. Thyroidal granules are in focus at intervals; see particularly the large dark granule in the lighter area where the epithelium is bending to the left in the upper part of the photograph. PAS.





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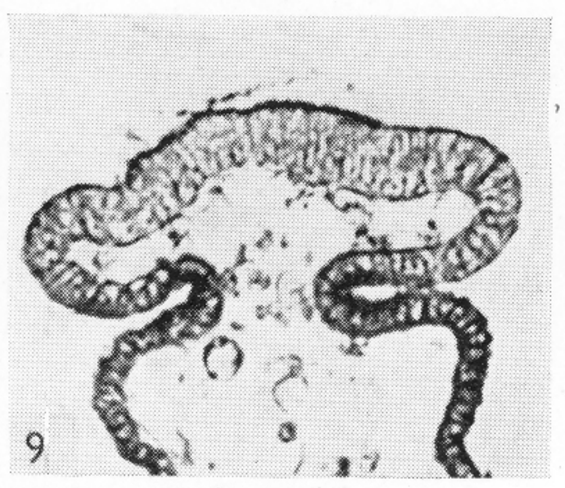
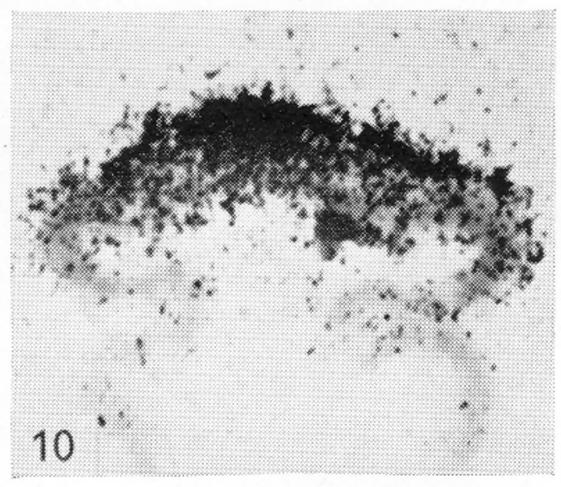
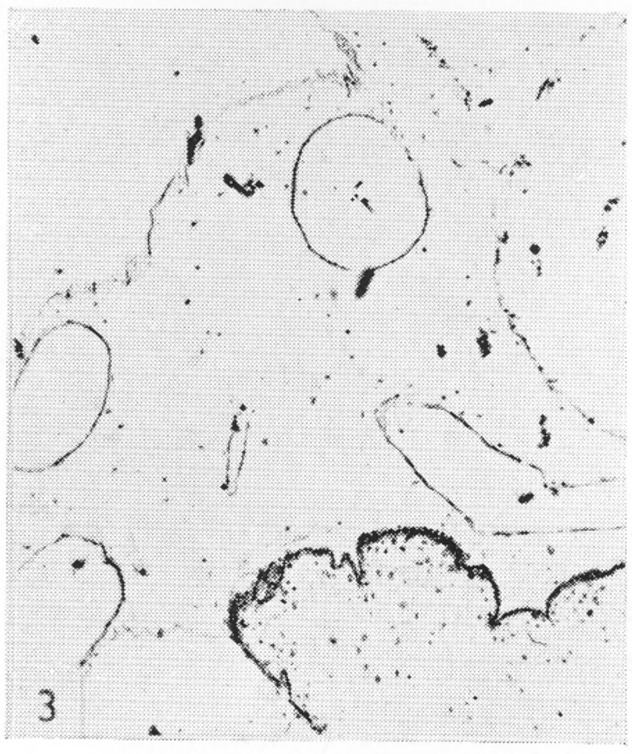
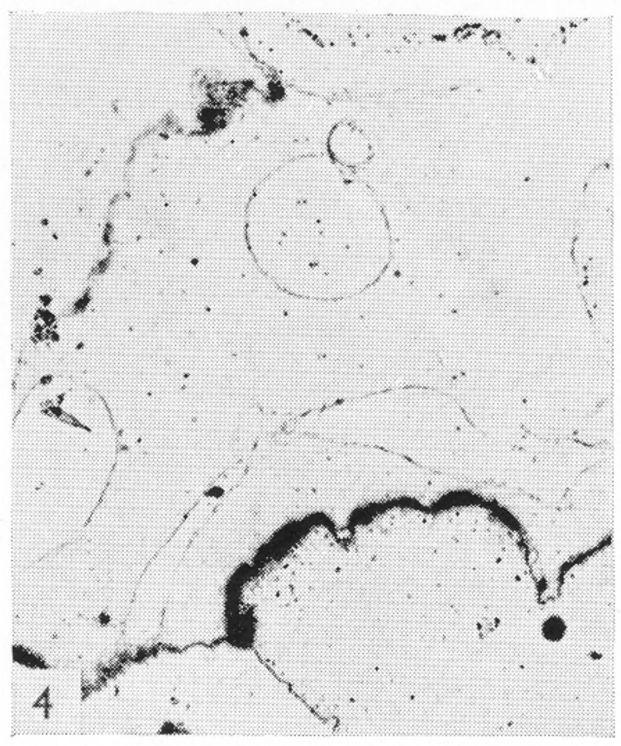
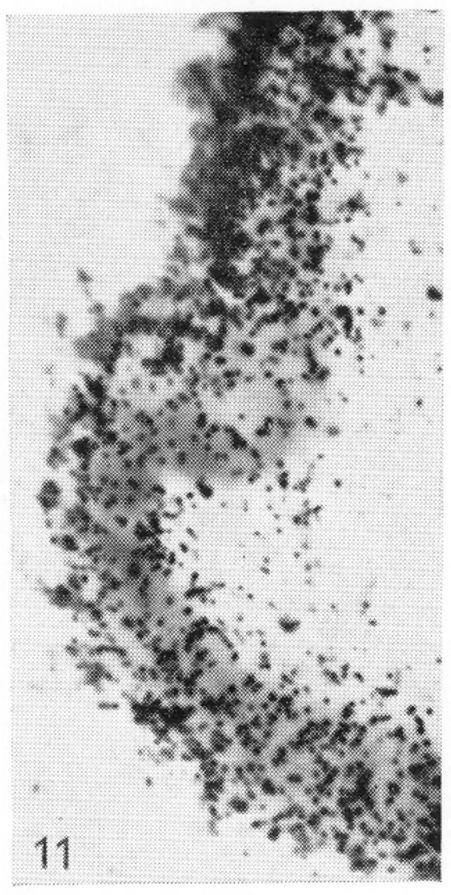


20 μ

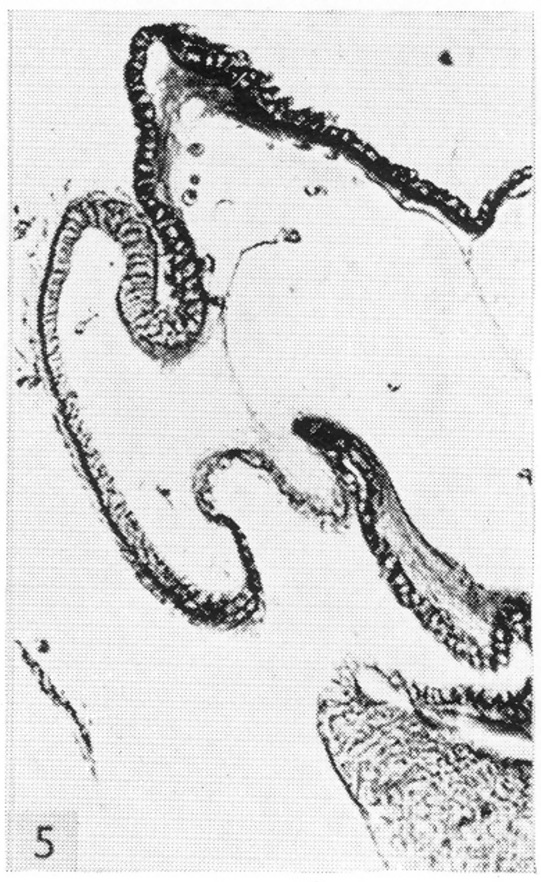


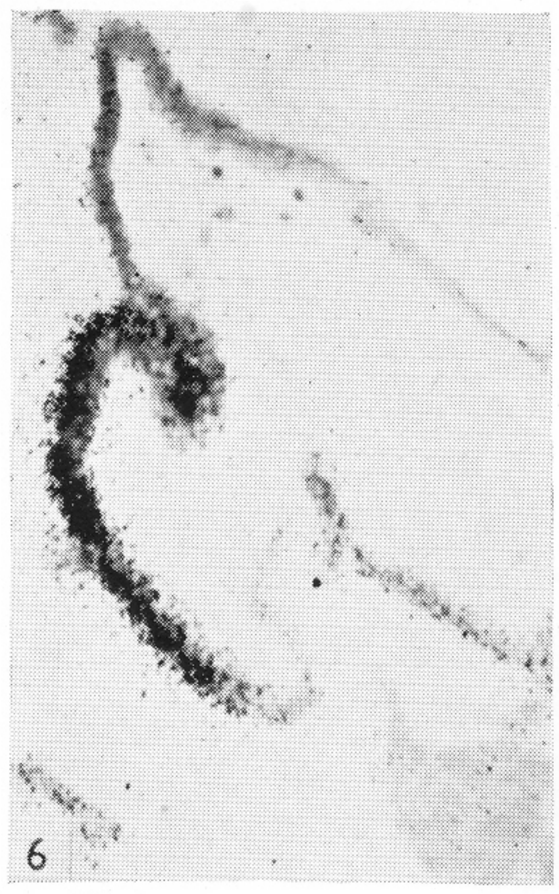


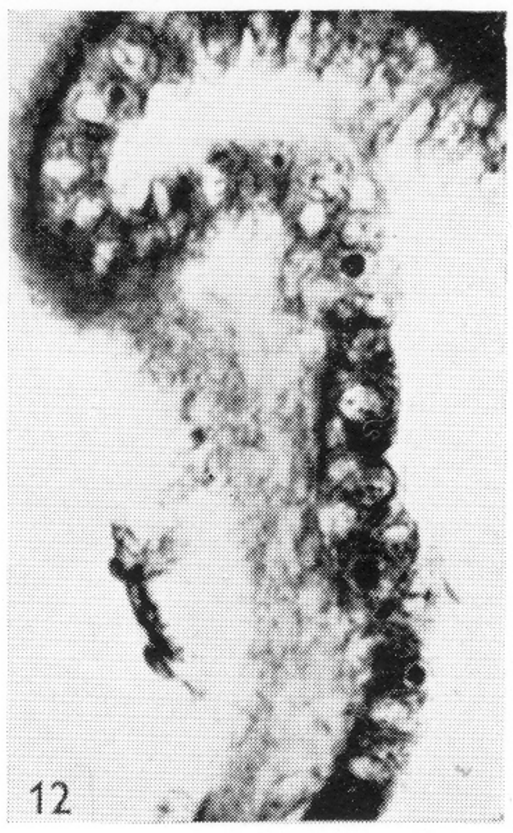




10 μ







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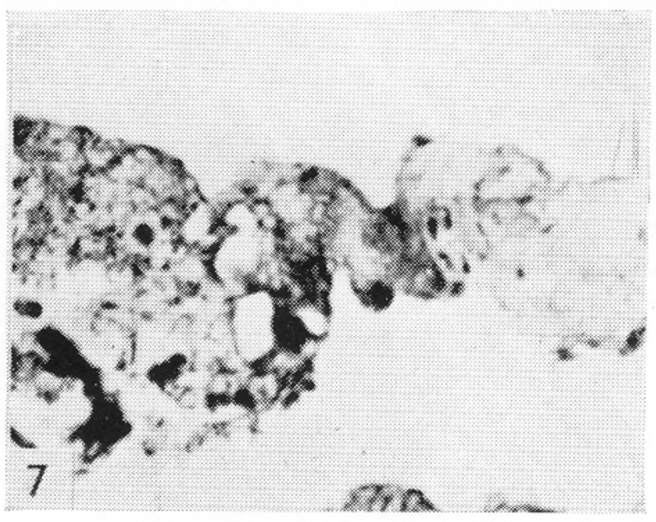

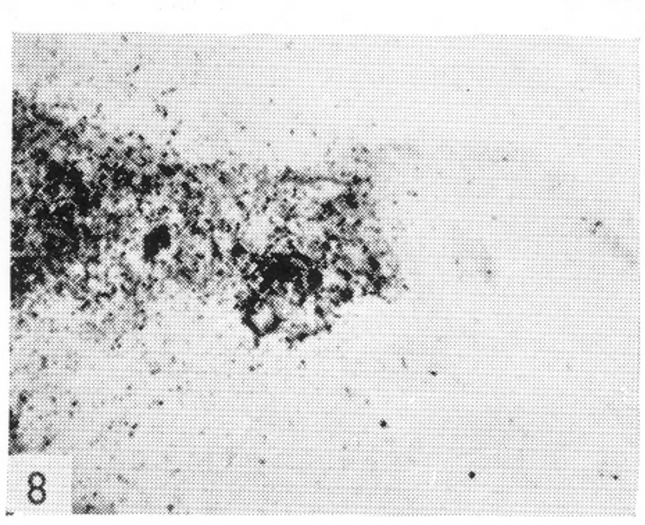

(*Facing p.* 16)