STUDIES ON THE EFFECT OF PARASITISM UPON THE TISSUES. I. WITH SPECIAL REFERENCE TO CERTAIN GASTEROPOD MOLLUSKS

By

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Studies on the effect of Parasitism upon the Tissues. I. With special reference to certain Gasteropod Molluscs.[1]

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With Plates 12–21.

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[1] Contribution from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 235.

Introduction.

The cytological changes which take place in parasitized tissue is one of the details of parasitology that now may be undertaken without any suffering from lack of a proper foundation; for many admirable works on parasitology have long since appeared and are steadily being added to this important branch of zoology. Histological and anatomical changes in parasitized organisms have been studied in various ways and by a number of workers. And yet detailed cytological studies of the effect of parasitism upon the host is nearly a virgin field. Perhaps this may be demonstrated, although necessarily only briefly, by referring to the labours of certain workers in parasitology and pathology, to wit:

Schaper (1889) in his study on ' Die Leberegel-Krankheit der Haussäugethiere ' made a distinct addition to the development of pathology of the subject. His pathological anatomical descriptions of the host are perfectly clear, but one may be justified in stating that he did not give an adequate treatment of the subject since the cytological aspect was of significance. This is true both as regards the text and illustrations.

Naumann (1892), commenting on the pathological anatomy of the sheep, says: ' The first effect of the penetration of the flukes into the bile-ducts is inflammation (period of traumatic hepatisis, according to Gerlach). The liver is enlarged, and contains more blood than usual; it is friable in texture, and its surface is smooth, or marked in places by openings the size of a pin's head to that of a millet-seed, from which exudes a sanious fluid on pressure. There are traces of local peritonitis, or exudations, which cover the young flukes; and small haemorrhagic centres exist in the parenchyma. The bile is slightly reddened and the peritoneal serosity is more abundant, and often contains small flukes. The faeces do not yet contain ova ' (p. 535). ' The hepatic parenchyma is soft, and the thickened connective tissue slightly grates on section; the surface of the latter is of a dirty-gray, yellowish-red, or blood colour, and perforated by spaces the size of a pea, which

contain one or more young flukes in a blood-clot, or in a sanious fluid formed of white and red corpuscles, hepatic cells which have undergone fatty degeneration, and a finely granular detritus. . . . Over the entire liver the cells are granular, and infiltrated with fat; the connective tissue is in process of proliferation' (p. 536). Neumann's description indicates that he made cytological studies of the livers of hosts suffering from fluke-infection. Unfortunately, however, his methods are not mentioned, nor are his statements quoted above supported by illustrations.

Acland and Dugeon (1902: 1313), in their study on Primary carcinoma of the liver, made a general histological examination to show the relative amount of connective tissue, and the normal and abnormal liver cells of that infected organ. Their purpose of study would certainly have been advantaged by cytological studies. In such an event, however, a different fixing agent than was used should have been employed. The fixing agent used was alcohol and the stain was Mayer's haematoxylin.

Leiper (1915: 177), in his study on Bilharzia, pointed out the avenue of infection of the Bilharzian trematode larva of its vertebrate host, and stated that the cercaria 'are able to pierce the skin very rapidly'. He also referred to the intermediate hosts by saying: 'The glandular tissue of an infected organ disappears apparently through pressure atrophy (fig. 44).'

Fantham, Stephens, and Theobold (1916), in describing the pathological anatomy of the sheep, give graphic pictures of the general condition: 'The bile ducts are conspicuous on the surface of the liver. They are thickened and much dilated and in part saccular, and considerable atrophy of the liver cells accompanies the condition. Histologically there is immense proliferation of the epithelium of the bile ducts leading to "adenomata"' (p. 241). 'Anaemia through loss of blood to worm; enlarged spleen, toxic in origin (?); phlebitis, thrombosis, due to portal stasis; the eggs, however, cause the greatest mischief. They are carried by the circulation to various

organs where they produce inflammation, granulation tissue, and later connective tissue ' (pp. 280–1).

Cawston (1918), Manson-Bahr, and Fairley (1920) may also be mentioned, although they do not give any pathological facts about the tissues of either the primary, intermediary, or secondary hosts. Walton (1918), on ' Liver Rot in Sheep ', points out certain facts briefly and says : ' The liver of many are found to be atrophied and hardened to the consistency of leather ' (p. 237). And again : ' . . . her liver was then found to be " like a stone " ' (p. 243). In his study on the effect of cercariae on the snails he says : ' The only conclusion arrived at was that, while differences were observable in structure of the liver of infected snails, yet no rediae or cercariae were recognizable ' (p. 264).

Noguchi (1918, 1918 *a*, 1918 *b*, 1918 *c*, 1919, 1919 *a*, 1919 *b*) demonstrated the yellow fever organism through his study of the pathological changes of the tissues of the human host. Up to 1918 the causative agent of yellow fever outside the mosquitoes Aedes aegypti (Linnaeus) was thought to be an ultra-microscopic virus. By painstaking and resourceful methods and skilful technique he demonstrated definitely the mysterious organism which, in more than one way, had been an object of much speculation for a long time. Whether the swamp fever virus may not be demonstrated also is a question of some interest (Van Es, 1911 ; Swingle, 1913 ; Van Es and Schalk, 1917 ; Schalk, 1920).

The work of Faust (1920) is significant. To my knowledge it is the first scientific treatise which contains a careful analysis of the liver of gasteropods suffering from trematode infection. Unfortunately the original coloured illustrations of this paper were redrawn in order to reduce the cost. The author did not see the paper after the figures were redrawn until the contribution appeared in print. The published article lacks some of the original merit of the contribution. This explains why the illustrations of Faust's paper do not quite support the text. This contribution nevertheless is by far the most comprehensive work on this subject up to the present time. It concerns

itself, however, with the hepatic organ only. Further comments will be made on this later.

Pirie (1921), in his important discourse on Carcinoma and Cirrhosis of African natives, points out Schistosomiasis as the causal factor. This author's comment on the cytological condition of the organs involved is much to his credit. Since, however, he does not describe his technique one cannot tell whether the 3,900 specimens sent him for ' general histological examination ' were favourable for cytological study. Unfortunately so many doctors who have an excellent opportunity to secure important material of immense biological importance do not realize the significance of the fixing of the tissues for microscopic examination. A twenty-four hours' post-mortem examination cannot possibly reveal the same cytological structures as existed ante mortem or in articulo mortis (vide Hance, 1917, 1917 *a*). Every cytologist will agree with this statement. In point of fact Pirie states : ' I have also been strongly impressed by the variety of structure to be met with in a single case, so that where only a single piece of tissue is examined a false idea of the variety of tumour might easily be given, and it is possible that in some of the cases received a different opinion might have been given, at all events of the type of structure, had a large number of pieces been examined.' Indeed, this variety of structure may be due in part to the methods of fixing the tissue. And the variety may be exaggerated greatly by the lapse of time between death and the preserving of the specimens in question. A great deal of credit is due to Pirie for his care in analysing the cytological structure of the tissues with which he dealt.

The labours of many other authors might have been cited. Suffice it, however, to add : It is clear from the works reviewed above, their meritorious nature notwithstanding, that the pathological changes of the tissues of almost any organism suffering from parasitic attack have not been studied by many authors, neither in very much detail by any one. Of course, in order to understand the pathological condition of any organ,

it is necessary first to know its normal condition. The writer, therefore, has attempted to raise Physa gyrina (Say), the most common species studied in this work, in the laboratory, and studied a number of young while in different stages of growth and age and before they became infected. This was quite necessary since all the specimens collected in nature were infected with flukes in various stages of development.

The works of human pathologists have hardly touched on the cytological phase of the problem. Leading men in this field, Cohnheim (1882), Sternberg (1893), Adami and Nichols (1919), and MacCallum (1920), indeed, might have increased the value of their published works considerably had they considered their subject-matter from a cytological point of view also and not only from a histological one. The work of Faust (1920) deals with the liver only, and no consideration is paid to any of the other organs. All the other students of vertebrate and invertebrate hosts suffering from trematode and other infections have not studied the tissues from a cytological point of view. Their technique, except in a few cases (Noguchi, Faust, Ward, and Calkins), has not lead to new discoveries. Merely some of the gross histo-morphological changes of the host have been studied, and many of these are recorded only briefly. As we shall see, then, ample excuse exists for approaching to this subject seriously from a cytological standpoint.

The purpose, then, if this investigation has been to study the cyto-physiological changes which take place in the hepatic organ and all the tissues of certain organism affected by parasites. This has not been attempted earlier. The aim of this paper is to present a general survey of the subject and give some of the data obtained. It is hoped that subsequent papers dealing with further and more detailed phases of this topic may be published.

For this investigation fresh-water snails were used; partly because several species (Physa gyrina (Say) and Planorbis trivolvis (Say)) were easily obtainable in nature all the year, and partly because of the direct application which

may be readily made of fresh-water gasteropod material of this nature both from an economic and medical standpoint. Some material was collected each month from September 1921 to May 1922, and also during August 1922, from the Drainage Ditch of Crystal Lake at Urbana, Illinois. Some material of Physa was raised in the laboratory from January to August. Some of these and their progeny were kept alive until September 1922, when most of them were killed and preserved for later study. This Drainage Ditch has been a collecting ground for several previous investigators (vide Cort, 1914; Faust, 1919). It is remarkably suitable for this purpose because of its considerably diversified fauna. The Drainage Ditch is artificial, and was built to carry off the surplus storm and seepage water. At times the streamlet may nearly overflow its banks. At other times it decreases very much in size, and filamentous algae, Elodea, et al., flourish abundantly. This offers a suitable breeding-place for animals. In these aquatic plants are found representations of several animal phyla: Amphibians—adult and larvae; Pisces—Boleosoma nigrum (Raf.), Notropis anogenus (Forbes), et al.; Pelecypoda and Gasteropoda; Crustacea and Insecta, the latter both in adult and larval stages; Polychaeta. Among the higher forms may be mentioned the kingfisher, wading birds that feed on the lower forms. I have not attempted to give a full list of the fauna, but to show that the opportunity is good for parasites to find primary and secondary hosts.

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Material and Technique.

The material used for this study was three different species of fresh-water snails: Lymnaea obrussa (Say), Physa gyrina (Say), and Planorbis trivolvis (Say). Some specimens were studied alive. The shell was first removed and the animal placed on a slide with a few drops of water. Very slight injury to the surface of the snail made it apparently easy for the cercaria, if present, to come out of the host, for they frequently came out in considerable number. For killing purposes specimens were partly dissected and immersed in the fixing mixture. The smaller specimens (3 to 7 mm.) were placed whole in the killing fluid.

A peculiar difficulty developed in connexion with fixing the specimens. A great deal of free gas developed in the body of the specimens which were immersed in acid mixtures, so that small bubbles of this gas covered the surface of these specimens and prevented penetration of the fixatives. A modification of Carnoy's fixing agent caused a steady stream of gas to flow from the fixing object for several seconds. The regular standard acid-mixture-fixatives, which penetrate more slowly, proved satisfactory, provided the fluids were constantly and gently stirred. Because the gas liberated tended to be caught in the mucus exuded by the snail. Hence good fixations were often impaired. Hammersten (1885: 393) found grains of $CaCO_3$ present in the mucus of the mantle of Helix pomatia. Substances of this nature may be the cause of some of the gas formed. Owing to some bad fixation, caused by too slow a penetration of the fixative, it was necessary to prepare a large number of duplicates of fixed specimens of both infected and apparently uninfected individuals.

The fixing agents used were (1) F.W.A., a formula intro-

duced by Gatenby for Flemming's mixture without acetic acid. (2) Flemming's strong mixture. (3) Lams' fixative, which consists of equal parts of the following: (*a*) saturated aqueous solution of picric acid; (*b*) 2 per cent. osmis acid aqueous solution; (*c*) 2 per cent. chromic acid aqueous solution; (*d*) glacial acetic acid; (*e*) 40 per cent. formaldehyde; (*f*) saturated aqueous solution of corrosive sublimate; and (*g*) absolute alcohol. This fixing agent was used by Dr. Honoré Lams (1910) on the ovotestis of Arion empiricorum (Fér.) with very fine results. (4) Saturated aqueous solution of corrosive sublimate with 3 per cent. to 5 per cent. glacial acetic acid. (5) Corrosive sublimate saturated in normal saline solution with 3 per cent. glacial acetic acid. (6) Bouin's picro-aceto-formol fluid. (7) Zenker's solution. (8) Müller's solution. (9) Hetherington's modification of Carnoy's fixing agent; this is made as follows: (*a*) absolute alcohol, 20 parts; (*b*) chloroform, 15 parts; glacial acetic acid, 5 parts; and phenol crystals to raise the volume by 10 parts. The material is handled thus: fix for half an hour to twenty-four hours or until clear; add oil of wintergreen, little by little, until transparent (the object becomes hard); rinse in wintergreen, chloroform, cedar-wood oil, or clove oil, to get rid of the acid; imbed. (10) Saturated aqueous solution of corrosive sublimate with glacial acetic acid, and from a few drops to equal parts of normal urine at 37° C. (11) 10 per cent. formaldehyde. (12) Alcohol. (13) Bowen's modification of Weigl's sublimate-osmic fixative; fix in glass-stoppered bottles for twenty-four to thirty-six hours in Mann's sublimate-osmic made up of equal parts of 1 per cent. osmic acid solution and corrosive sublimate saturated in normal salt solution. (14) Kopsch's method for mitochondria and Golgi apparatus. (15) Mann-Kopsch's method for cell-organs. (16) Champy's method for mitochondria, &c. (17) Champy-Kull's method for mitochondria, and Golgi apparatus and cytoplasmic granules.

A number of stains were used. (1) Tests for glycogen: (*a*) the iodine method, (*b*) Best's carmine stain. (2) Heidenhain's iron haematoxylin. (3) Weigert's iron haematoxylin.

(4) Delafield's haematoxylin. (5) Mallory's phosphotungstic acid haematoxylin, and (6) safranin. For counter-stains were used : (*a*) light green, (*b*) orange G, and (*c*) eosin.

Hetherington's Carnoy-phenol penetrates very rapidly, and the fixing is quite uniform throughout. The sublimate-aceto-normal-urine-mixture is good as a cytoplasmic fixative. It preserves a large number of cytoplasmic granules which cannot be stained otherwise, e.g. if fixed in Flemming's or Bouin's fixing agents. The results obtained by the last four methods cannot be discussed here. Fine results were obtained with material fixed in Flemming's solution and stained with safranin and light green. Most sections were made less than 10 micra in thickness. The thickness of the sections ranges from 4 to 15 micra. All specimens sectioned were mounted serially.

Observations.

I. Condition of Normal Cytoplasm.

The classical works of Altmann (1889, 1894, 1896, 1896 *a*), Wilson (1895, 1899, 1904), and later of Schreiner (1916, 1918), show that the cytoplasm is primarily granular in structure. The ultra-microscope reveals objects ca. 2 to 5$\mu\mu$—far below the possible limit of vision. There are numerous kinds of recognized cytoplasmic granules specified both in structure and in origin : (1) microcomes, (2) mitochondria, (3) chromidia, (4) metachromatic granules, (5) secretory granules (vide Schreiner 1916, 1918), (6) pigment granules, (7) metaplasmic and paraplasmic granules, such as fat, yolk, starch, leucocytes' granules and many others. In addition to these may be mentioned the Golgi bodies. Gatenby's treatises on ' The Cytoplasmic Inclusions of the Germ-cells ' (1917, 1917 *a*, 1917 *b*, 1918, 1919, 1919 *a*, 1920, 1920 *a*, 1922), and Gatenby and Woodger (1921) are in support of the workers who claim that protoplasm is fundamentally granular in structure. But the works of Gatenby, &c., need not be considered in detail in this connexion as these treatises are concerned with the germ-cells only, and the germ-cells will not be discussed in the present

paper. It is, however, the opinion of the foremost cytologists of to-day that the protoplasm is fundamentally granular in nature. This is in agreement with Altmann's contention that the cytoplasmic granules exist in a constantly regressive size, from larger granules to smaller ones, which finally become ultra-microscopic. In my own studies at Wood's Hole in 1920 (not yet published) I was able to demonstrate in the growing egg of nudibranchs an apparent constant gradation in size of the cytoplasmic granules from fairly large ones to the ultra range of the microscope. These granules had in most cases affinity for three different stains.

Schreiner (1916, 1918) builds on Altmann's work. He carries this phase of cytology to a climax. He shows better than any other cytologist up to the present time the inter-nucleo-cytoplasmic relationship. He found that cytoplasmic granules of different nature had their origin in the nucleolus and the nuclear ' Netzknoten '. From the works of Schreiner it is seen that the nucleus plays an important rôle in the various activities of the cell. In my study on Physa and Planorbis I have come to the conclusion that the large amount of black granules (which will be discussed later) among the tissues of individuals newly infected by trematodes have their origin in the nucleus also. A more detailed report on this phase of this problem will follow later.

Müller (1896 : 321) also agrees with Altmann that the saliva ' aus typischen Granula stammt '. Indeed, Altmann claimed that the granules originated from the homogeneous inter-granular substance. In his opinion both the granules and the intergranular substance are living (1894 : 51). Nussbaum (1882), in his study on vertebrate glands, saw a special cytological activity in the vicinity of the nucleus. Lange (1902) elucidated this point better than Nussbaum in his work on the structure and function of the salivary glands of gasteropods. He showed that the nucleus takes part in the secretory activity of the salivary glands, in that the nuclear membrane dissolves and the nuclear contents mixes with the cytoplasm, so that the first part of the secretory activity of the gland is noticeable

on the nucleus. And Korschelt (1891), in his ' Morphologie und Physiologie des Zellkernes ', brings out this point by showing that the nucleus and cytoplasm are dependent on each other in the life of the cell, and yet, ' im Allgemeinen erscheint der Kern als ein Theil der Zelle, der sich vom Zellplasma scharf sondert '. My findings (Agersborg, 1923) for the nuclear activity of the nidomental gland of Melibe, Lange's for that of the salivary glands of certain gasteropod molluscs, and Korschelt's for a variety of cells, agree with Schreiner's observations on Myxine. Dahlgren and Kepner's (1908 : 8–9) secretion substances, secretion fibrils, and secretion material which arise in the nuclear environs of the gland-cells resemble very much the paranuclear bodies (Pl. 18, fig. 34, *pnb*) of the liver of Planorbis (vide infra). Fuchs (1902) demonstrated some very interesting facts in regard to the secretion activity of the epididymal epithelium of the mouse. A certain ' Fadenknäuel ' arises at the distal region of the nucleus, and from it ' Zellfäden ' pass to the periphery or free border of the cell. The argument brought forth by Fuchs substantiates the works of Altmann and others. His illustrations are strikingly similar to points found in my study of the intestinal epithelium of Physa gyrina (Say) (Pl. 21, figs. 53, 54), in which case excretory accumulations on the luminal side of the nucleus appear as a nuclear cap (*nuc*), and from it may be seen passing similar delicate strands as in Fuchs's figures to the border and beyond it into the lumen. In the liver of Planorbis trivolvis (Say) paranuclear bodies are sometimes seen (Pl. 18, fig. 34, *pnb*). These resemble very closely the nuclear caps (Pl. 21, figs. 53, 54, *nuc*) and the ' Fadenknäuel ' of Fuchs. In this case, however, as may be noticed in Pl. 21, figs. 53 and 54, no fibrillar stands (*st*) are seen. A fact which seems to be peculiar to the entire hepatic organ. The paranuclear body (Pl. 18, fig. 34, *pnb*) represents apparently some nuclear products preparatory for secretory material, which of course sooner or later leaves the cell. That this is most probable is gathered from the vacuolar condition of the cell in the immediate vicinity of the paranuclear body. This is also the opinion

of Fuchs. Schneider (1902 : 570) recognized two kinds of granules in liver cells of Helix pomatia. Both kinds may be jointly voided into the lumen frequently in the process of forming into spheres. The excretory cells of the liver have bubbles which contain a large excretory ball of similar consistency as the excretory granules of the liver cells. It appears thus that leading cytologists agree upon a very essential point, viz. the cytoplasm is fundamentally granular in structure, Wilson (1899 : 23) ; even the astral rays in the dividing sea-urchin egg are formed by linear arrangements and fusion or close union of granules or microsomes of the reticulum (Wilson, 1895 : 467). It may be fair to say, however, that Wilson is cautious in his statements, but he is not far from supporting Altmann's view altogether. Altmann even thought that the secreted extracellular substance was granular also. On this point his claim agrees with my observations on the pathological tissues of the aquatic pulmonates: Physa and Planorbis. For, in this case, it is very evident that there are in the intercellular (extracellular) substance an abundance of granules which had their origin in the cells. The presence of an extra amount of certain granules among the tissues, located generally intercellularly, is the main secondary feature of the tissues of the parasitized snails. This condition, as will be pointed out presently, is most striking in the newly infected individuals. After some time following an infection the granules seem to decrease quantitatively. That is, the black intercellular granules of parasitized Physa and Planorbis are a temporary element which under a certain physiological condition appears in the homogenous intercellular matrix, and which origin is intracellular.

II. Normal Tissue.

The anatomy and morphology of pulmonates in general have been worked out by several investigators : Swammerdamm (1737), Leydig (1850), Semper (1857), Leydig (1876), Simroth (1885), Bronn (1896), and others. Leydig (1850) points out that there are present black and yellow pigments

in the tissues, and in 1876 he calls them chromatophore granules. But otherwise he does not record anything about intercellular granules. In this second work this author worked on four different genera of pulmonates: eight species of Limax, two of Helix, one Physa fontinalis, and one Arion. But he does not record anything about granules other than those mentioned. Semper (1857) also pictures pigment, but that is as far as he went on this point, although he dealt with seven genera of pulmonates, containing nine different species, one of which was Planorbis marginatus.

Since all the specimens I examined were infected at the time of examination, it was impossible to ascertain from them the exact nature of the normal tissues. But judging from many cases with an early or mild infection, I believe that upon infection the tissues become less responsive to ordinary stains. Infected specimens are difficult to fix properly. It is difficult to make good sections. The tissues have a tendency to crumble before the edge of the knife. None of these difficulties were met with in the uninfected material which was raised in the laboratory. The normal tissue has less tendency to shrink during the process of preparation. And the black intercellular granules are absent.

III. Parasitized Tissue.

Since serial sections were made of all the specimens sectioned, it was relatively easy to make a survey of the entire animal from the anterior to the posterior and in this way ascertain the degree of infection. At first a general survey of ca. 40,000 sections were made and notes recorded on points observed. Then additional material was prepared, both from fresh specimens collected in nature and from some reared in the laboratory. After this mode of procedure a more intensive study ensued. This was coupled with the preparation of drawings made to scale as indicated below. Every specimen collected in the Drainage Ditch was infected with trematode larvae. The infection consisted of miracidia, rediae, and cercariae in various stages of development. All of these stages were present every-

where in the body of the host (Pl. 12, figs. 1, 2 ; Pl. 13, 3–14 ; Pl. 16, figs. 20–3 ; Pl. 17, figs. 24–30 ; Pl. 18, figs. 31, 35 ; Pl. 20, fig. 48 ; Pl. 21, fig. 55). In Pl. 12, fig. 1 (*par*), a parasite (probably a cercaria) may be seen just below the ectoderm of the foot.

1. Avenue of Infection.

Infection is apparently established by way of the blood system. (1) Because the earliest stages of the parasite are found in the sinuses all through the body. This is most manifest as regards the finer connective tissue of the respiratory organ. (2) Because later stages are found in the loose connective tissue of any organ. By the time the cercaria stage is reached the parasite has arrived in the hepatic sinuses. In fact the hepatic sinuses seem to be a general collecting-place in so much that they may be completely filled even to the extent that the hepatic wall is distended and stretched until the epithelium is practically obliterated (Pl. 13, figs. 3–14 ; Pl. 18, fig. 34). On this point my observations agree with Leiper's findings, for this author claims that the epithelium disappears apparently through pressure atrophy. And Faust (1920 : 81) verifies this when he says : ' The food which the parthenita takes in first of all is from the lymph.' That is, the infection takes place by way of the blood system. Faust also says : ' The parasite is always found in the connective tissues and the interstices between tubules, while the portion of the epithelial cell bordering the lumina are always intact except in the most necrotic tissues.' According to Leiper (1915 : 177), if an infected Planorbis boissyi is kept in tap-water which is renewed daily, it may discharge large numbers of cercariae daily for weeks. But the cercariae will die within thirty-six hours if they do not find a definite host. Cort (1914 : 74) found specimens of Planorbis trivolvis infected with encysted stages of Cercaria trivolvis (Cort), showing, as this author claims, that Planorbis trivolvis (Say) is able to serve both as intermediate and secondary-intermediate hosts for this trematode. In this connexion it may be

noted that such a condition seems to be common for Physa gyrina (Say) as well. Does this mean that the bilharzian cercaria of Planorbis boissyi is more specialized than Cercaria trivolvis?

2. Effect of Infection.

The first and immediate effect of infection as represented by the changes in tissues of the host is that of a distorted and disintegrated condition (Pl. 12, fig. 1). As the parasite passes from one stage to another it migrates into all the parts of the host's body. Cercariae or rediae may be found in the tentacles (Pl. 12, fig. 2), the foot (Pl. 12, fig. 1), and the pharyngeal wall.

The second noticeable change in the tissues of the secondary-intermediate host infected with trematode larvae is an attempt apparently by the tissues of the host to react to the presence of the parasite. This is shown first by an increase of black granules throughout the host. According to Schreiner (1916, 1918) the origin of certain cytoplasmic granules is in the nucleus. Evidence as to the origin of the black granules so abundantly present (Pl. 14, figs. 15–17 ; Pl. 15, figs. 18, 19 ; Pl. 16, figs. 20–23 ; Pl. 17, figs. 24–30, *gr*) in all the tissues of newly infected Planorbis trivolvis and Physa gyrina, seems also in this case to point toward the nucleus as a source. A detailed discussion of this will be given in another paper. Heavily infected specimens seem to have less of these granules, reverting, as it seems, toward the normal even while parasites in all stages are abundantly present. It does not seem that the parasite is the source of this excessive pigmentation. But, as pointed out by Ward (1920 : 51–2), it is the product of the tissues of the host. It is well, however, to note that Ward, in the case of the cyst of the myxisporidian parasite Myxobolus aureatus (Ward), finds that the cyst itself has a pigmentation of its own. This pigmentation of the cyst may be the product of Myxobolus. The later phase of this stage of infection is noted by an apparent readjustment (in some cases) ; this readjustment does not take place in every

specimen. When it does not the organism dies. After the readjustment of the tissues of the host is accomplished there is a diminution of pigmentation or granules, and the tissues approach the normal condition. This is easily comparable to the tissues of young uninfected individuals and adults with only a few cercariae in the foot. The response of the tissues of the host to the parasite may be partly illustrated by figs. 20–30 (Pls. 16, 17). The black granules (*gr*) are shown definitely in the tissues, and there are no transitional stages of granules passing from the parasite to the host. An acute case of maximum pigmentation is demonstrated in figs. 15 and 16 (Pl. 14). This case represents a condition of a newly infected young Physa (Pl. 14, fig. 16) in which miracidia could be found. Fig. 15 (Pl. 14) represents a condition in Planorbis generally infected. During the second or readjusting period the tissues may proliferate considerably also, as in certain cases, e.g. in the walls of the hepatic sinuses (Pl. 18, figs. 31, 33, 35, *mfct*, *mct*). This is accompanied by a rapid growth of the parasite (Pl. 18, fig. 35, *par*).

The third stage of the parasitized tissues is a much distorted, disintegrated, and shrunken condition. During this time the host ' hangs between life and death '. In the liver the epithelium may be nearly destroyed. Only small nuclei remain, and these are in a highly concentrated condition giving the effect of squamous epithelium instead of columnar (Pl. 18, figs. 32, 33).

The fourth period is marked by definite changes toward the normal condition ; this may be called a period of regeneration. During this time the parasite is less abundant and the tissues are slowly recovering.

3. Glycogen.

Nearly forty years ago Barfurth demonstrated the presence of glycogen in the various parts of the body of gasteropods (Limax variegatus and Helix pomatia). He writes (1885 : 342) : ' Bei der mikrochemischen Untersuchung fand sich in fast allen Organen reichlich Glycogen.' That is, he

found glycogen in all the organs save the tentacular retractor. In the case of Physa gyrina I found that glycogen was present in the muscles. But as a granular substance it is quite different from the black granules mentioned above. Granules, then, may be present everywhere in, and between, the tissues. Shun Ichi (1920) finds that osmicated mitochondrial fixatives preserve glycogen which can be stained in Best's carmine and iron haematoxylin. Hammersten (1885) substantiates the findings of Blundstone and Barfurth. The works of Barfurth, Blundstone, Hammersten, and Pflüger deal with normal tissues. It is necessary, however, in such a work as the present one, to have in mind the condition of the normal tissue as a comparative basis for pathological tissue. Parasitized Physa has glycogen in its muscles. In this connexion it may be well to recall that Faust demonstrated the presence of glycogen in the parasite. And, in his opinion, this glycogen was obtained from the host. That is, the parasite which may be anywhere in the body of the snail may not only obtain food from the blood-stream but also from stored-up food of the body in general.

IV. The Liver.

1. The Anatomy of the Liver.

The liver offers an interesting topic of study because of its important physiological activity in the life of the organism. Several morphological regional differences of this organ are noticeable. These differences may be exaggerated in parasitized specimens. The anatomy of this organ has been worked out by several workers as stated above (Swammerdamm, Leydig, Semper, Simroth, Bronn), and also Faust (1920) described it partly. It will therefore not be necessary to go into any description of the anatomy here, although the anatomy of Physa gyrina (Say) has not been described before. It is not so very different, however, from other related aquatic pulmonates. This also is true as regards the liver. The molluscan digestive gland has also been the subject of the attention of a number of other authors, who have studied

it both from the standpoint of its finer structure and from the standpoint of physiology.

2. The Physiology of the Liver.

Before considering the livers of parasitized Physa and Planorbis it is necessary, although briefly, first to point out the normal condition in other gasteropods. De Quatrefages (1842) maintained that the liver in nudibranchs was of a threefold function; hence his term 'plebenterism' to designate that species of gradation which consists in the union of different function in one system of vessels. One unquestionable function of the hepatic tubules, as far as Aeolidia is concerned, is as an exit of harmful indigestible parts taken in with food (Alder and Hancock, 1845; Glaser, 1903; Hertwig, 1912). Frenzel (1886: 278) believed with Max Weber (1880) and Barfurth (1883) that the liver of molluscs has a double function: (1) as in Crustacea it is a digestive gland; (2) in addition this gland, according to Weber for the Crustacea and Barfurth for the Mollusca, is of 'excretorische Funktion'. They think that the liver of these forms is analogous to that of vertebrates. Frenzel described three kinds of epithelial cells of the liver of the nudibranchiate mollusc Tethys leporina (Linnaeus): (1) 'Körnerzellen', (2) 'Keulenzellen', and (3) 'Kalkzellen'. These are also described by Hecht (1895: 671). Eliot and Evans (1908) think that some of the liver cells in a doridiform cladohepatic nudibranch are excretory in function and are dropped into the follicle as they become extended with excreted material. Eliot (1910: 39) attributes to the liver the function which in the case of the nudibranchiate mollusc Melibe leonina (Gould) I have shown (Agersborg, 1923) to be that of the epithelium of the posterior chamber of the stomach, e. g. the gizzard. The epithelium of the liver of Melibe leonina (vide Agersborg, 1923) shows a similarity to the 'Keulenzellen' of Frenzel, or 'Cellules vacuolaires excrétrices' of Hecht. The nucleus as a rule is basal in position and contains one or two nucleoli. That part of the function of the liver of Melibe is secretory and digestive may be

judged from the fact that some of its product passes into the stomach. This is readily shown by the fact that the surface of the stomachal contents gives the same staining reaction as the wall of the hepatic ducts which pass through the walls of the gizzard. In fact, these are stained differently from any other part of the organism treated chemically in the same way. In looking at the hepatic epithelium one is impressed with the nucleo-cytoplasmic relationship. There is a strong indication that the nucleus takes an active part in the secretory activity of this gland (Agersborg, 1923). Lange (1902) recorded a similar phenomenon for the salivary glands among gasteropods, viz. the nucleus seems to take an active part during the secretory activity of the cells. This he could observe easily after feeding and starving experiments on the snails with which he worked. Boas (1916 : 389) states that the liver of gasteropods is a large compound acinous gland which secretion among certain snails has been shown to have a strong dissolving effect on cellulose. But besides being a secretory organ the liver also acts as an absorption organ in that it takes up finely parted solid particles of food (for example, starch granules), dissolves and absorbs them. Further, it serves as a storage chamber partly for nutrition material (glycogen, fat), partly for calcium salts which are stored in different cells of the liver. This is also the opinion of Barfurth (1883 : 332–4) and Bierdermann and Moritz (1899 : 61). But the liver is not the only organ in which glycogen may be stored, for Barfurth (1885) and Blundstone (1885) demonstrated independently the presence of glycogen in practically all parts of the body of Limax variegatus and Helix pomatia. Using Best's carmin test, I found glycogen in the muscle-tissue of Physa gyrina (Say). The liver in gasteropods, according to Barfurth (1883), performs several activities which in higher animals may be divided between several organs.

3. The Livers of Parasitized Physa and Planorbis.

It was pointed out that the tissues of the host try to adjust themselves to the presence of the parasite. In the liver this

may be manifested in various ways. The fluke, being first of all in nearest contact with the interstitial cells of the sinus, affects them first. The earliest change seems to be a quantitative increase in cell material in the lumina of the sinuses (Pl. 18, fig. 31, *mfct*). The nucleus of the hepatic epithelium is uniformly remarkably nearer the free luminar border than is usually the case. Presumably the parasite uses up available food quite quickly, which source of supply both for the hepatic wall and the parasite is the blood-stream. The nearest supply for the hepatic wall is in the hepatic epithelium itself. The nucleus must migrate to the source of supply—the free luminar border—a fact also observed by Korschelt (1891). I have reason to think that the hepatic epithelium takes a more active part in digestion than is generally accepted (Pl. 21, figs. 49, 53, 54). If such be the case then digestive fluid may be available in the hepatic lumen. Hence the position of the nucleus of the hepatic epithelium. Be this as it may, the hepatic gland seems to be an organ of secretion, which is also contended, among other things, by several investigators. The position of the nucleus near the free border, as seen in fig. 31 (Pl. 18), may be initial to disintegration, since in this position—at this level within the sinus—may be found a parasite (*par*). The striking condition of the basement membrane (*bm*) should be noted. The basement membrane is not discernible in fig. 33 (Pl. 18) (taken from Planorbis). But this is an exception to the rule, as will be seen presently.

The nucleus of the atrophied hepatic epithelium does not drop out of the cell altogether, as may be inferred from fig. 31 (Pl. 18). Because, in another follicle adjacent to it, the nucleus is still present although the cytoplasmic reduction is exceedingly great, the cytoplasm having practically disappeared (Pl. 18, fig. 32). In other young Physa (5 mm. long) killed in strong Flemming's and F.W.A. fixing reagents (Pl. 20, figs. 46 and 47) respectively, and in which the liver was not infected, the nucleus was basally situated. And on the luminar border are seen in many cells prominent secretion vacuoles (*vac*) and granules (Pl. 20, fig. 47, *sg*). The secretory activity

is beautifully demonstrable in the liver of Planorbis (Pl. 18, figs. 33–6; Pl. 19, figs. 37–8, 40–2). In fig. 33 (Pl. 18) nearly all the cells on this plane of the organ have large secretion vacuoles (*vac*) with a secretion ball (paranuclear body) in it. The free border in some of them is characterized by fine granules. This vacuolization (*vac*) is much more easily demonstrable in fig. 34 (Pl. 18). The paranuclear body (*pnb*) is in closer proximity to the nucleus in this case than in the previous figure, and the border is even more oxyphil (*oxb*). The specimen was quite heavily infected. The sinuses (*s*) are in many places crowded with developing flukes in various stages (Pl. 18, fig. 35, *par*). In spite of such high infection the hepatic epithelium (*he*) presents a normal picture. This specimen was killed in strong Flemming's fixing fluid, and the figs. 33–5 (Pl. 18) were drawn from three different places of the same individual in order to show the variable condition of the liver. The nucleus in all these cases is located basally, and shows quite a contrast to the condition found in a heavily parasitized two-months old Physa (Pl. 18, fig. 31) also killed in Flemming's mixture. In the case of Planorbis (Pl. 18, fig. 35) the interstitial cells (*mct*) of the sinus (*s*) are greatly modified. This is no doubt a direct response on the part of the tissue of the host to the presence of the parasite (*par*). The globules (*mct*) are nuclei located in a cyncitium. Black granules as shown in the connective tissue (*ct*) immediately below the basement membrane (*bm*) and in the epithelium near the basal border are stained black by the osmic acid. Quite a contrast to the condition as represented in the last three figures (33–5) may be seen in fig. 36 (Pl. 18). This drawing was made of a section of the liver of another individual of Planorbis killed in Bouin's fixative. The organism was generally infected and showed the same general features as demonstrated in figs. 18 and 19 (Pl. 15). In fig. 36 (Pl. 18) the epithelium may be seen in the process of eliminating secretion products into the lumen. The cells show vacuolization and fibrillar formation, a condition common to normal glandular activity.

As pointed out already by Barfurth, Boas, Faust, and others, the hepatic gland has several functional properties, among others that of absorption. Planorbis killed in F.W.A. shows absorption substances (Pl. 19, fig. 38, *sgr*) in great abundance in the epithelium, some of which seem to have passed into the sinus (*cts*). In this connexion it is imperative to note the simple condition of the basement membrane (*bm*) and compare this fact with that shown in fig. 31 (Pl. 18). The specimen upon which fig. 38 (Pl. 19) is based was very heavily infected, also the specimen represented by fig. 31 (Pl. 18). Whether the condition of the basement membrane is affected by the parasitic action on the host or by the absorption action on the part of the epithelium of the hepatic organ of the host remains a question. This same condition is manifested in fig. 41 (Pl. 19), taken from the same specimen as fig. 38 (Pl. 19). But here the epithelium, besides having the absorption substances well distributed within it, also exhibits the sloughing off or discharging of products into the lumen (*sp*).

The nucleus, indeed, may not be situated basally as shown in fig. 42 (Pl. 19), in which case it is located at various positions within the cell. Previous to this I have stated that it has held a basal position (Pl. 18, figs. 33–6 ; Pl. 21, fig. 49). There is an exception to this, as has already been pointed out and demonstrated in fig. 31. The basement membrane even in the same individual may fluctuate considerably (Pl. 19, figs. 38, 41 ; Pl. 20, fig. 43). Such a condition as represented in certain parts of the gland (Pl. 20, fig. 43) is quite extraordinary. But it shows that the liver of the same individual is capable of extensive variation (Pl. 18, figs. 33–5 ; Pl. 19, figs. 37, 38, 41 ; Pl. 20, fig. 43). Planorbis killed in Bouin's fluid may show the basement membrane quite prominently (Pl. 20, fig. 45) and the secretory activities of the epithelium as well. The vacuolated and fibrillated condition of the hepatic organ may be demonstrated also in the epithelium of the genital duct (Pl. 19, fig. 39). An apparently non-infected Physa (except with a few miracidia (?) in the foot) shows a highly vacuolated hepatic epithelium (Pl. 20, fig. 44) with practically no basement membrane.

Discussion.

I. General Discussion.

Metabolism of Host and Parasite.

As pointed out by Ward (1907), the effects of parasites on the host may be mechanical, morphological, and physiological. Mechanical changes are recognized as the local structural disturbances; morphological changes such as abnormal proliferation of the tissues and cyst-formation are common phenomena; and physiological effects are expressed in the limitation or modification of the normal physiological processes of the host. The body of an organism, in order to live, must carry on the necessary metabolic activities. It is evident that the metabolism of an individual whose body is invaded by other organisms becomes very greatly augmented. In addition to the normal metabolic activities of the host, if it is to continue its own existence, it must provide for itself and its guests. This is done in various ways according to the nature of the parasitism. Metabolism being a process of taking in food, assimilating it, building up new material, and voiding material of no longer use—a process alike in host and parasite—it is evident that the host has to work harder if it is to continue its existence successfully. Endoparasites do not only feed upon the host, but their metabolic wastes must be taken care of by the host as well as its own. This is often a greater difficulty than the providing of board for the parasites. The parasitic excretions become frequently poisonous to the host. That is, the metabolism of the host may be impaired in various ways: either by lack of sufficient food, owing to the drainage on the food-supply of the parasite; or by the impaired function on the part of certain organs of the host, owing to the particular specificity of the parasite which is adapted to certain host-organs only and the consequent overwork of these organs in the attempt of the host to maintain metabolic equilibrium, or by actual general or local weakening of the host by the toxic parasitic excretions. The life of the host may be absolutely

shortened. This, of course, is a disadvantage for the parasite inasmuch as the death of the host, indeed, may result in the death of the parasite also. On the other hand, it is of advantage to the parasite that a tolerable condition, or balance in relationship between host and parasite, be reached. The continued existence of the host may ensure the life and reproduction of the parasite. Therefore it becomes of mutual importance both to host and parasite whether an adjustment in the physiological relationship between the two can be established quickly. Since this may save the life of the host it may also secure the propagation of the parasite and of the host without which neither may long endure.

II. Is an Antidote[1] formed?

Antidote for Parasitic Toxin.

As stated above, the first reaction of the tissues of Physa and Planorbis infected with trematodes is to provide an antidote against the parasitic excretions. In the case of the monkey and of man the bilharzial excretions are very toxic (Fairley, 1919 : 299). This author states that pigmented cells were not uncommonly found in the vicinity of bilharzial lesion. He was not able to tell, however, whether the pigment originated in the haemolytic action of the bilharzial toxin or was derived from blood metabolized in the process of digestion by the adult parasite. This pigment seems to resemble closely malarial pigment, and it actually exists in the intestinal coeca of the worm. Brown (1911 : 299) found that melanin is the product of the action of a proteolytic enzyme of the malarial parasite upon the haemoglobin of the erythrocytes. The condition of the liver in Schistosomiosis in man, as shown by Phalen and Nichols (1908), is an increase of connective tissue of the liver at the expense of the hepatic epithelium. This, of course, leads to serious metabolic disturbances of the

[1] I purposely avoid the term antibody, which has a very precise significance, and which, so far as I know, no one has ever seen. I prefer also not to coin a new term.

host, which ultimately is fatal. There is not the proper adjustment between host and parasite. Fairley (1919 : 312) showed that there is a definite relationship between the cellulo-humeral response in experimentally infected monkeys (infected with Bilharzia haematobia and Bilharzia mansoni), and the prognosis. In hyperinfected monkeys dying within few weeks he found that there was a constant leucopenia, absence of eosinophilia, and a negative complement fixation reaction. In monkeys surviving the sixth week of infection there was constantly present an eosinophil leucocytosis associated with a positive serological reaction. Finally, the death of the hyperinfected monkeys prior to the deposition of the ova, and the constant presence of positive serological reactions in monkeys recovering from the initial stages of infection, go far to prove the action of some toxic body elaborated by the metabolic activities of these parasites, and the protective immunization of the definite host by antibody production. From this excellent work of Fairley, one may well conjecture that the molluscan host suffering from trematode infection also has to solve the problem of immunization.

Faust (1920) states that melanin deposition in the connective tissue is a matter of record in mollusc and vertebrate liver, as a result of trematode infection. I am not able to agree yet on this point as far as the gasteropods which I have studied are concerned. That ' the flukes work their way to the liver through the blood sinuses (in molluscs) coming to reside in the interstices between the liver tubules ' is substantiated by my studies. Faust proved that a great deal of absorption of the digested foodstuffs on the part of the fluke took place in the hepatic sinus of the host. More than that, it may be stated that the hepatic epithelium in some cases is actually greatly decreased quantitatively by the presence of the parasite (compare Pl. 18, figs. 32 and 35, *he*) ; also that flukes ' empty a burdensome amount of excretory wastes into the tissues and cause serious cytological changes both in the epithelial cells of the (hepatic) tubules and in the intertubular connective tissues ' (Pl. 18, figs. 31, *mfct* ; 32, *ct* ; 35, *mct*). In other words, Faust then

anticipated me in my opinion relative to the problem which the infected snail has to solve. That is, molluscan hosts suffering from trematode infections have to solve the problem of immunization.

That the parasites produce and liberate a toxin or toxins into the system of the snail may not be doubted. That a visible difference between non-infected, newly infected, and long-infected snails exists is very apparent. Part of this difference consists in the presence of a large amount of an intercellular granular substance which may be blackened by osmic acid. It seems to be of a lipoid nature. This granular substance does not originate in the parasite. It originates in the cells of the tissues of the host, e.g. in the muscles. Is the granular substance something produced to counteract the parasitic toxin? Or is it the result of the parasitic waste upon the tissues of the host? Why is it more abundant during an early infection stage than at a later and even more heavily infected stage? Has the host-metabolism adapted itself to the extra task of caring for the elimination of the parasitic wastes? Are even necrotic tissues of heavily infected snails better able to take care of the parasitic toxins than normal tissues of lightly and newly infected snails? These are some questions that must be answered. It does not seem reasonable that tissues which are highly abnormal morphologically can function better than normal tissues. Therefore to conjecture that the gradual decrease in intercellular granular substance proportionally with the duration of the infection, irrespective of the continuous increase of the infection and the consequent destruction of the tissues, is an indication of a balance in reaction between a tissue-reaction substance and the parasitic waste, does not seem logical although it may be a helpful suggestion. However, if this supposition is correct, do antibodies counteract the parasitic toxins? What is the nature of the intercellular granules so abundantly present during an early infection? Are they metabolic wastes of the host? What becomes of them later? Are they voided by the host and are less and less formed even during progressively increased infection? Without

designating any rôle to the intercellular granular substances at present, I must call the attention to their presence. If they represent metabolic waste of the host in its combat with the parasitic toxins, then their consequent decrease may suggest that the metabolic activities of the host becomes more effective because of the antibodies which counteract the toxins, the necrotic condition of the tissues notwithstanding.

Summary and Conclusions.

1. This work shows that the fresh-water snails Physa gyrina (Say) and Planorbis trivolvis (Say), infected with trematode larvae, respond to the infection by a cytological secretion on the part of all the tissues.

2. This secretion probably originates in the nucleus and is further developed in the cytoplasm before it is discharged into the intercellular spaces.

3. The secretion remains intercellularly all the time during an infection. It seems to be most abundant during the early infection stage, and decreases toward the normal after the infection has lasted for some time.

4. This cell-product, which possibly may have the function of an antidote for the parasitic excretions, is represented in the prepared specimens and in my drawings by fine black granules situated everywhere in the tissues.

5. These granules may be blackened by osmic acid (OsO_4). They can be demonstrated also, but less easily, in material fixed in Bouin's fixative.

6. Physa gyrina (Say) raised in the laboratory became infected in the aquarium containing several minnows[1] (Notropis anogenus (Forbes)) collected in the same

[1] Mr. R. E. Richardson, who kindly identified this species for me, was quite unwilling to believe that it had been collected at Urbana, Illinois, because he had hunted for it all through the state for twenty years without any success of finding it. (See also S. A. Forbes and R. E. Richardson, ' The Fishes of Illinois ', 1920.) However, this fish was present in large numbers during the fall and winter of 1921–2 in the Drainage Ditch of Crystal Lake, Urbana, Illinois.

place as the parents of the snail. The aquarium was balanced with filamentous algae (Spirogyra, Cladophora) and Elodea canadensis. They were very heavily infected when three weeks old. At this time cercariae filled the hepatic sinuses.

7. The liver may present a normal physiological aspect although the organism may be heavily infected.

8. Several striking morphological differences in the liver of the same individual may be demonstrable at one and the same time.

9. The basement membrane seems to wax and wane according to the physiological state of the liver (Pl. 18, figs. 31–6; Pl. 19, figs. 37–8, 41–2; Pl. 20, figs. 43–5). This is in strong contrast to the condition found in the ectoderm of the foot in which the basement membrane is never present. The ectoderm passes imperceptibly on to the underlying connective tissue (Pl. 21, figs. 50–2). This condition is common both for Physa and Planorbis in young as well as adult states.

10. Absorption and secretion are demonstrable functions of the liver of Physa and Planorbis. Conspicuous bodies, which I have called paranuclear bodies, are present in the cells of the hepatic epithelium of Planorbis trivolvis (Say). They are usually situated between the nucleus and lumen; but sometimes this position may be altered. They are granular in structure and resemble the Golgi bodies. Their variable position seems to indicate that they are related to the different functional states of the cell (Pl. 18, fig. 34, *pmb*). In the intestinal epithelium of Physa gyrina (Say) there are definite nuclear caps which break up and pass out of the cell and into the lumen (Pl. 21, figs. 53, 54, *nuc*).

11. The adult cercaria having left the host (Planorbis trivolvis and Physa gyrina) may re-enter and encyst.

12. Trematode larvae in various stages of development may enter any part of the host.

13. A macerated condition of the tissues caused by the invasion of larval flukes may be overcome by subsequent

reaction on the part of the host. This reaction is of two kinds : (1) the building up of an antibody ; (2) the regeneration of the macerated tissues.

14. Four infection stages may be recognized. (1) The parasite invades the host. The tissues shrink and become more and more difficult to handle for cytological purposes. The tissues become friable. (2) The tissues secrete an intercellular granular substance which becomes present everywhere in the host. It is not present in the parasite. (3) During prolonged heavy infection the tissues disintegrate. The epithelium of the liver may be reduced from tall columnar to squamous. The host may die. (4) If the host does not die, then there follows after the third stage a gradual return to the normal. The parasites also decrease in number within the host.

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EXPLANATION OF PLATES 12–21.

N.B.—The drawings were made by the use of the camera lucida and Leitz microscope. Tube length 117 mm. and 334 mm. total length from the ocular to the drawing. Ocular no. 3; objectives nos. 3, 5, 8, and $\frac{1}{12}$ ($\frac{1}{12}$ = the oil-immersion). The magnification is indicated in each case. Also the fixatives and stains employed are mentioned. F.W.A. = Flemming's solution without acetic acid.

Plate 12.

Fig. 1.—Section through the foot of Planorbis trivolvis (Say), killed in Bouin's picroformol-acetic solution; stained with Heidenhain's haematoxylin and light green. Sections 8 micra thick. *ct*, connective tissue; *ect*, ectoderm; *ics*, intercellular secretions; *mus*, muscle-fibres; *par*, parasites. In this specimen, though it was highly infected, pigment granules are relatively scarce. Magnification: 50 mm. = 250 micra.

Fig. 2.—Longitudinal section of a tentacle of Planorbis trivolvis (Say), to show encysted parasites (*par*). *n*, optic nerve; eye. In this instance, too, note the scarcity of intercellular granules. The section is from the same specimen as fig. 1. Magnification also the same.

Plate 13.

Figs. 3–14.—Serial sections of a hepatic sinus from two-months-old Physa gyrina (Say), to show: (1) the presence of two cercariae, *a* and *b*, in the sinus, and (2) the distended condition of the hepatic wall. *t*, tail; *vs*, ventral sucker; *wlf*, hepatic wall. Killed in Flemming's strong solution; stained with safranin, light green. Sections 8 micra thick. Miracidia are found in the foot; pigmentation less abundant; hepatic follicles filled with cercariae. Magnification: 50 mm. = 100 micra.

Plate 14.

Fig. 15.—Section of part of the body-wall covering the connective-tissue capsule (tunica propria) of the liver, to show the intercellular secretion granules among the muscle-fibres of Planorbis trivolvis (Say). Killed in Flemming's solution without acetic acid. Stained with Heidenhain's haematoxylin. Infection general; intercellular secretion granules abundant. Trematode larvae were noticeable during dissection. Sections 8 micra thick. *mf*, muscle-fibres; *nf*, nerve-fibres; *sgex*, intercellular secretion granules. Magnification: 50 mm. = 50 micra.

Fig. 16.—A section through the base of a tentacle of Physa gyrina (Say), to show the intercellular secretion granules (*sgex*). Killed in

Hetherington's carnoy-phenol; stained with Mallory's phosphotungstic acid haematoxylin. Sections 5 micra thick. *mf*, muscle-fibres. The specimen was partly infected with rediae; pigmentation was quite prominent throughout. The liver of this specimen shows a striking pathological picture: many cells are sluffed off in part into the lumina. The magnification is the same as in the previous figure.

Fig. 17.—A few muscle-fibres from Physa gyrina (Say), to show the presence of black granules within the muscle-fibres; killed in Weigl's corrosive sublimate acetic mixture: stained with Heidenhain's haematoxylin. Sections 10 micra thick. *mf*, muscle-fibres; *nu*, nucleus; *sgint*, intracellular granules (mitochondria). Magnifications the same as the last figure.

PLATE 15.

Fig. 18.—Horizontal section of the right jaw of Planorbis trivolvis (Say), killed in Flemming's solution; stained with safranin and light green. Sections 10 micra thick. *csmc*, cross-sections of muscular columns; *ct*, connective tissue; *gr*, intracellular cytoplasmic granules; *mf*, muscle-fibres. The specimen showed a general infection throughout its body. Magnification: 45 mm. = 30 micra.

Fig. 19.—Longitudinal vertical section of the jaw of Planorbis trivolvis (Say), showing the muscle-columns (*lsmc*) cut longitudinally. *epc*, epithelial cover of the organ; *gr*, intercellular granules; *mf*, muscle-fibres; *nu*, nucleus. Magnification the same as in fig. 18.

PLATE 16.

Figs. 20–3.—Serial sections of a parasite in the foot of Planorbis trivolvis (Say), killed in Flemming's strong mixture; stained with safranin, light green. These sections show: (1) a scanty amount of secretion granules (*gr*), and (2) these granules are not present in the immediate proximity of the parasite, (3) the dark spherules in the parasite are the nuclei which are all stained uniformly red by the safranin. *cap*, cyst-wall; *gr*, secretion granules; *nu*, nuclei; *par*, parasite; *su*, sucker. Sections 10 micra thick. Magnification: 50 mm. = 50 micra.

PLATE 17.

Figs. 24–9.—Serial sections from the foot of Planorbis trivolvis (Say), to show the parasites imbedded in the tissues. Killed in Flemming's mixture. Stained with safranin, light green. Sections 10 micra thick. *cap*, cyst-wall; *gr*, secretion granules; *nu*, nuclei; *par*, parasite; *pg*, pedal gland; *spar*, small parasite (micracidia ?). Magnification: 50 mm. = 100 micra.

Fig. 30.—Section through the foot of Planorbis trivolvis (Say), killed in Flemming's fixative. (Same specimen as in figs. 24–9.) To show the intercellular secretion granules (*gr*) of the host in relation to the

parasite (*spar*). The muscle-fibres have been omitted from the drawing. Six encysted parasites are seen in the field. All the parasites stain faintly oxyphil (green). Stained with safranin, light green. The granules are black; muscles red; parasites green. These parasites are probably developmental stages of rediae. Magnification: 50 mm. = 50 micra.

PLATE 18.

Fig. 31.—Cross-section of a hepatic follicle of Physa gyrina (Say), about two months old. Killed in Flemming's solution. Stained with safranin, light green. Micracidia found in the foot; pigmentation less abundant; liver filled with cercaria. The cercariae are not in the lumen, but in the sinus. Sections 8 micra thick. *he*, hepatic epithelium; *lu*, hepatic lumen; *mfct*, modified connective tissue; *par*, parasite within sinus; *s*, sinus. Magnifications: 50 mm. = 50 micra.

Fig. 32.—Cross-section of a hepatic follicular wall adjacent to the one represented in fig. 31, to show the flattening of the hepatic epithelium (*he*) and the secondary change of the connective tissue (*ct*) which borders on the sinus (*s*); *bhm*, basement membrane of hepatic epithelium; *bms*, basement membrane of cells lining the sinus; *ibmr*, inter-basement-membranal substance; *lu*, hepatic lumen.

Fig. 33.—Cross-section of a hepatic villus showing a central sinus (*s*) of Planorbis trivolvis (Say). Killed in Flemming's solution without acetic acid (F.W.A.); stained with Heidenhain's haematoxylin and light green. *he*, hepatic epithelium; *lu*, lumen; *s*, hepatic sinus; *vac*, vacuole. Sections 8 micra thick. Magnification the same as in fig. 31.

Fig. 34.—Section of a liver tubule from the same specimen as in fig. 33. The epithelium is highly vacuolated, and oxyphil in staining reaction. Note the paranuclear bodies which are acid or oxyphil in staining reaction. *bm*, basement membrane; *he*, hepatic epithelium; *lu*, lumen; *oxb*. oxyphil border; *pnb*, paranuclear body; *vac*, vacuoles; *s*, sinus. Sections 8 micra thick. Stained with Heidenhain's haematoxylin. Magnification as above.

Fig. 35.—Section of a liver tubule from lumen to lumen of the same specimen as shown in fig. 33. The columnar hepatic epithelium shows a striking condition of an actively functioning epithelium at the time of death. Immediately below the interstitial connective tissue (*ct*) are a large number of densely basic (red with safranin) bodies (*mct*) which in general resemble nuclei of mesenchymous tissue. The granules of the epithelium are black (from the osmic acid). *bm*, basement membrane; *ct*, connective-tissue cells of the interstitial cells; *he*, hepatic epithelium; *mct*, nuclei of modified connective-tissue cells; *nu*, nucleus; *par*, parasite within the hepatic lacuna; *s*, lacuna or sinus. Sections 8 micra thick. Magnification as above.

Fig. 36.—Section through the hepatic wall of Planorbis trivolvis (Say), killed in Bouin's fixative. The specimen at the time of killing showed

the same general condition as the specimen represented in fig. 19. Neither was apparently infected, but microscopic investigation revealed that both were infected. *bm*, hepatic epithelium. Stained with Heidenhain's haematoxylin and light green ; 10 micra in thickness. Magnification same as above.

PLATE 19.

Fig. 37.—Cross-section of the hepatic follicle of Planorbis trivolvis (Say), killed with F.W.A. ; stained with Heidenhain's haematoxylin, light green. *chl*, interhepatic lacunae ; *ilt*, interstitial hepatic tissue ; *lu*, lumen ; *x* (vide fig. 38). Magnification : 50 mm. = 250 micra.

Fig. 38.—*x* from fig. 37 ; magnified 50 mm. = 50 micra. *bm*, basement membrane ; *cts*, hepatic sinus ; *he*, hepatic epithelium loaded with fat granules blackened with osmic acid ; *sgr*, absorption granules.

Fig. 39.—Section of oviduct of Planorbis trivolvis killed in Flemming's fixing agent ; stained with safranin and light green. Sections 8 micra thick. *ct*, connective-tissue capsule ; *e*, epithelium ; *nu*, nucleus ; *vac*, semi-vacuolar space. The lumen had considerable mucilaginous substance which had stained with safranin. Magnification the same as in fig. 38.

Fig. 40.—Section through the tunica propria of Planorbis trivolvis (Say), fixed in Bouin's fixative. *ct*, connective tissue of tunica propria ; *hc*, ectoderm ; *bm*, basement membrane ; *he*, hepatic epithelium ; *lu*, lumen hepaticum. Stained with safranin and light green. Magnification : 50 mm. = 100 micra.

Fig. 41.—Section of a liver-tubule of Planorbis trivolvis (Say), fixed in F.W.A., and stained with safranin, light green. *bm*, basement membrane ; *ct*, interstitial connective tissue ; *cts*, lacuna ; *he*, hepatic epithelium ; *sp*, cell-particles sluffed off into the lumen. Note the irregular condition of the hepatic border ; the marked basement membrane as in fig. 38 ; also the granules of the epithelium. Sections 8 micra in thickness. Magnification : 50 mm. = 50 micra.

Fig. 42.—Section of a liver tubule of Planorbis trivolvis (not the same specimen as in fig. 41), fixed and stained in the same way as the previous one (fig. 41). *bm*, basement membrane ; *he*, hepatic epithelium. The liver shows a very variable condition of its epithelial lining. Sections 10 micra thick. Magnification as in fig. 41.

PLATE 20.

Fig. 43.—Section of the liver of Planorbis trivolvis (Say), fixed in F.W.A. and stained with safranin, light green. Sections 8 micra thick. *bm*, basement membrane ; *ctc*, connective-tissue capsule ; *he*, hepatic epithelium ; *lu*, lumen ; *vac*, vacuoles of the epithelium ; *sp*, secretion particles sluffed off from the epithelium into the lumen of the organ.

The finely stippled border is oxyphil ; the remainder is basiphil. Magnification the same as the foregoing.

Fig. 44.—Section of the liver of Physa gyrina (Say), fixed in four parts absolute alcohol, one part glacial acetic acid ; stained in Best's carmin stain for glycogen. A few rediae were found in the posterior end of the foot ; pigmentation of the mantle quite general, also in the anterior region of the body. The liver was highly vacuolated. Sections 8 micra thick. *bm*, basement membrane ; *lu*, lumen ; *sgr*, secretion granules ; *vac*, vacuoles. Magnification as above.

Fig. 45.—Section of a liver tubule of Planorbis trivolvis (Say) (the same specimen as in fig. 36), killed in Bouin's fixative ; stained with Heidenhain's iron haematoxylin, light green. Note the general feature as seen in fig. 36. This section was taken from the inner portion of the hepatic organ. Sections 10 micra thick. Magnification as above.

Fig. 46.—Cross-section of the liver of Physa gyrina (Say), ca. 5 mm. long, killed in Flemming's solution and stained with Delafield's haematoxylin. The organism was a little infected with miracidia (?). Note the vacuoles between the nuclei and the hepatic lumen. *nu*, nucleus ; *s*, hepatic sinus. Section 7 micra in thickness. Magnification : 45 mm. = 30 micra.

Fig. 47.—Section of the hepatic tubule of Physa gyrina (Say), ca. 5 mm. long, killed in F.W.A. and stained with safranin, light green. The organism was found to be a little infected with miracidia (?). Sections 7 micra thick. *bm*, basement membrane ; *lu*, lumen ; *nu*, nucleus ; *s*, hepatic sinus ; *sg*, secretion granules ; *vac*, vacuoles. Magnification : the same as in fig. 46.

Fig. 48.—Section of the genital tubule of Planorbis trivolvis (Say), killed in Bouin's fixative and stained safranin, light green. Sections 10 micra thick. *alc*, alimentary canal ; *gd*, genital duct ; *gh*, hepatic gland ; *icto*, inter-organ connective tissue ; *par*, rediae ; *spar*, miracidia. Magnification : 50 mm. = 250 micra.

Plate 21.

Fig. 49.—Section of the stomach of Physa gyrina (Say), killed in F.W.A., and stained with Heidenhain's iron haematoxylin. Sections 9 micra thick. *bgr*, basal granules ; *bm*, basement membrane ; *cil*, cilia ; *ctc*, nucleus of connective-tissue capsule ; *nu*, nucleus of entoderm. Magnification : 45 mm. = 30 micra.

Fig. 50.—Cross-section of the foot of Planorbis trivolvis (Say), showing ciliated ectoderm with pedal glands, both of which are interspersed with black granules. The basement membrane is absent ; the ectoderm rests imperceptibly upon the connective tissue. Section 10 micra in thickness. *bgr*, basal granules ; *cil*, cilia ; *ect*, ectoderm ; *ct*, connective-tissue cells ; *sgr*, secretion granules ; *pg*, pedal glands. Magnifica-

tion : 50 mm. = 50 micra. (Specimen killed in strong Flemming's fixative and stained with safranin and light green.)

Fig. 51.—Cross-section of the foot of Physa gyrina (Say), ca. 5 mm. long, raised in the laboratory ; killed in Flemming's fixative ; stained with safranin, light green. Section 7 micra thick. The organism was infected with a few miracidia in the foot. A basement membrane is absent. *bgr*, basal granules ; *cil*, cilia ; *ect*, ectoderm of the foot ; *nu*, nucleus. Magnification : 45 mm. = 30 micra.

Fig. 52.—Cross-section of the foot of Physa gyrina (Say), one day old. Killed in Bouin's fluid ; stained with Delafield's haematoxylin. This demonstration stands in striking contrast with those represented by figs. 50 and 51. The absence of the basement membrane in this part of the body is apparently a common characteristic of both the young and adult of Physa and Planorbis. Sections 8 micra thick. *bgr*, basal granules ; *cil*, cilia ; *ct*, connective tissue ; *ect*, ectoderm ; *mgl*, mucous gland ; *nu*, connective-tissue nuclei. Magnification as above.

Fig. 53.—Cross-section of the intestine of Physa gyrina (Say), 2 mm. long ; killed in Hetherington's carnoy-phenol fixing agent ; stained with Mallory's phosphotungstic acid-haematoxylin. Sections 5 micra thick. This drawing demonstrates an interesting phenomenon relative to secretion. Note the nuclear caps (*nuc*) and strands (*st*) which extend from them and beyond the ciliated border. *bgr*, basal granules ; *bm*, basement membrane ; *cil*, cilia ; *ctc*, connective-tissue capsule ; *nu*, nucleus ; *nuc*, nuclear cap ; *st*, mucous strands. There are no miracidia in the foot ; pigmentation general. Magnification : 50 mm. = 50 micra.

Fig. 54.—Cross-section of the intestine as in fig. 53, showing the same phenomenon still better ; stained in Delafield's haematoxylin. Section 5 micra thick. *bm*, basement membrane ; *nu*, nucleus ; *nuc*, nuclear cap ; *usbi*, unstained internal border. Magnification : 45 mm. = 30 micra.

Fig. 55.—Section of the foot of Planorbis trivolvis (Say), infected with flukes in various stages. Killed in Bouin's fixing agent ; stained in Delafield's haematoxylin. Section 8 micra thick. This shows an encysted cercaria in the foot ; the tissues around the parasite does not seem to have any of the black secretion granules present. *cap*, capsule of the parasite ; *mf*, muscle-fibres of the host ; *nu*, nuclei of the parasite ; *par*, parasite ; *spar*, smaller parasites (miracidia ?) ; *su*, suckers. Magnification : 50 mm. = 100 micra.

H. P. K. Agersborg, del.


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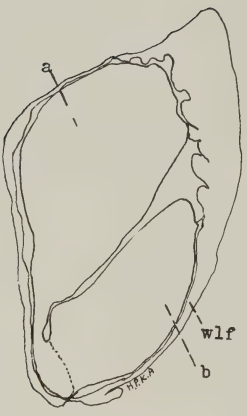

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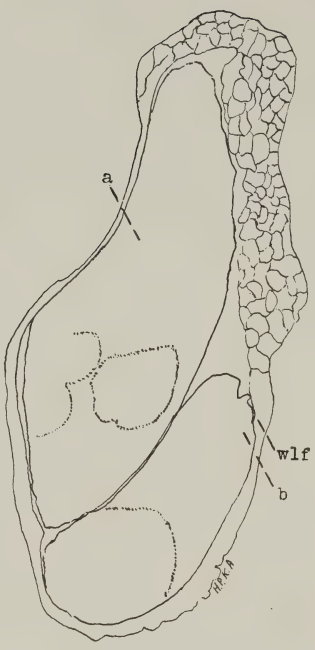

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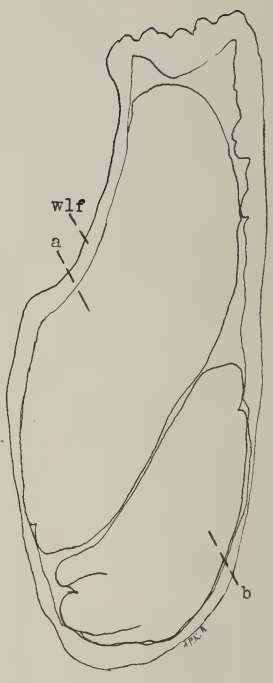

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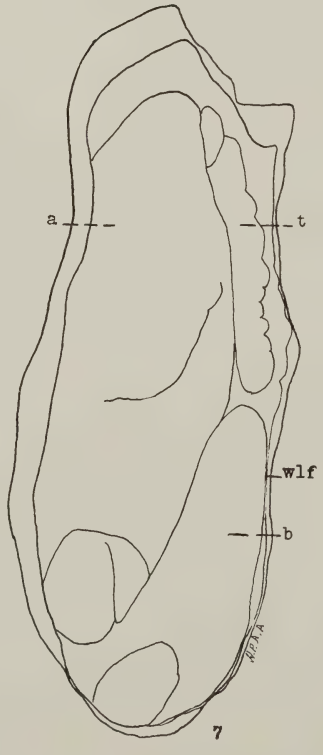

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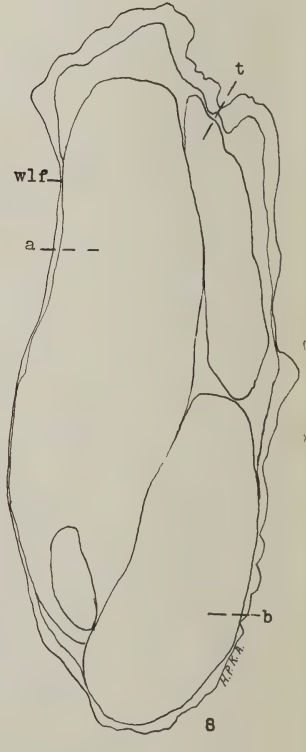

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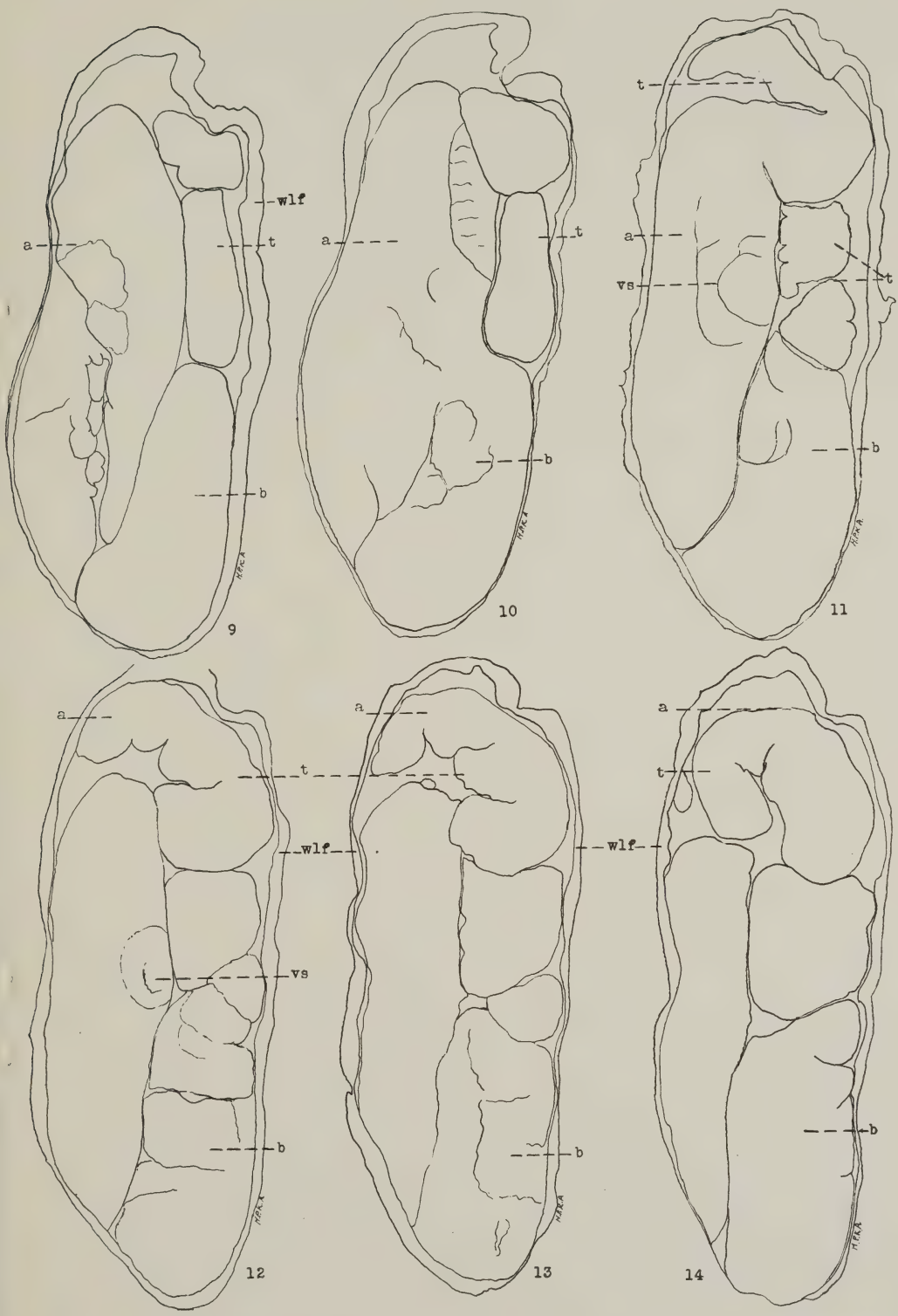
H. P. K. Agersborg, del.



Vaus and Crampton.

15

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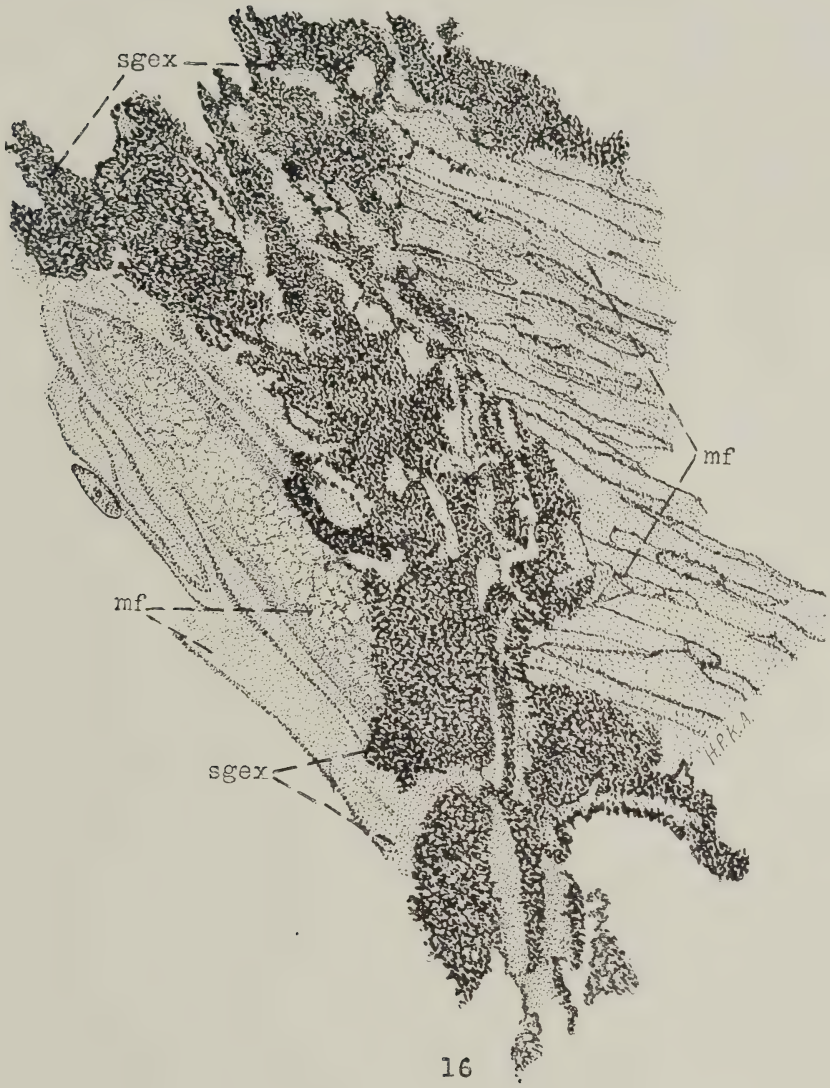

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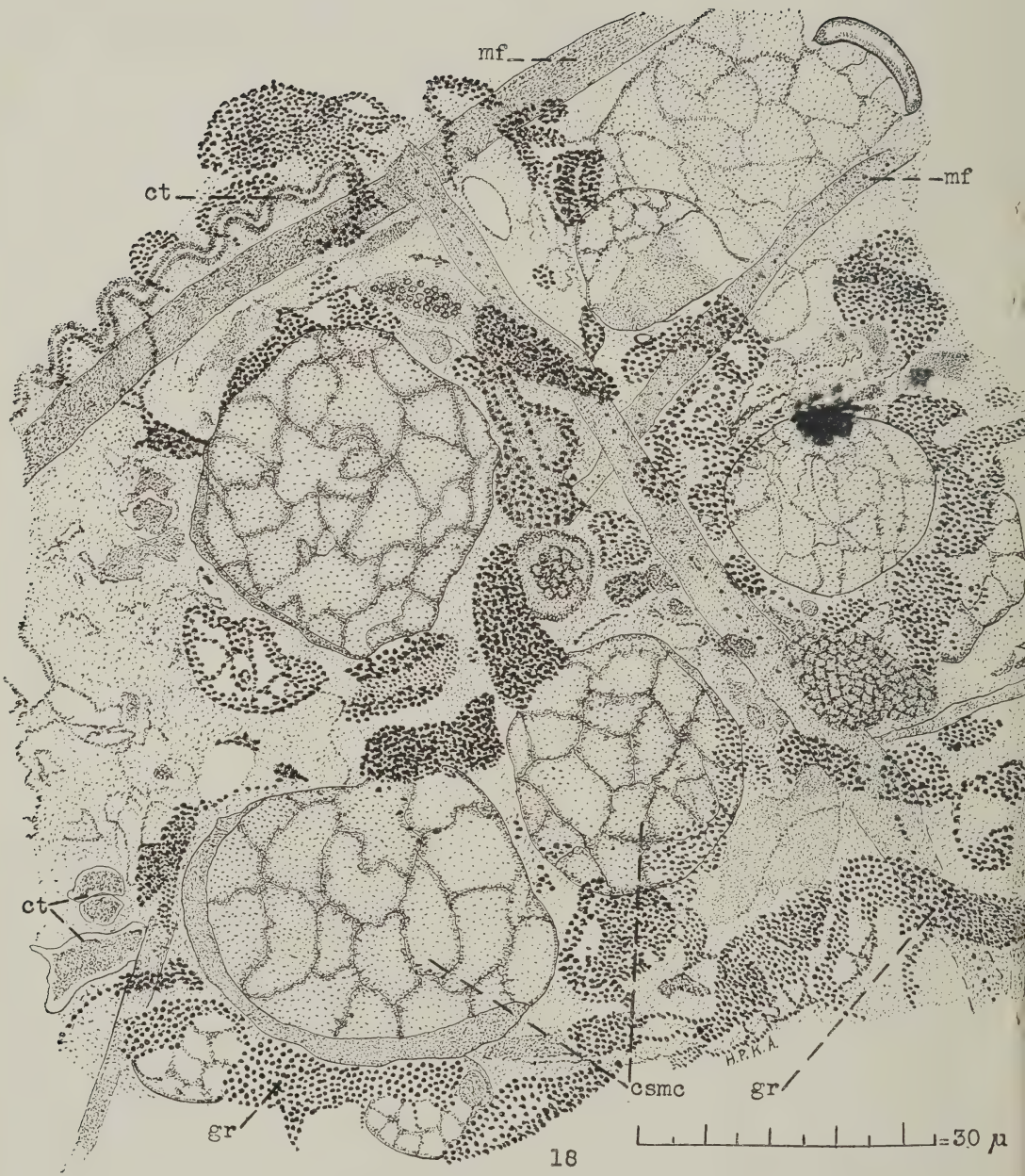

18

H. P. K. Agersborg, del.

Vaus and Crampton.

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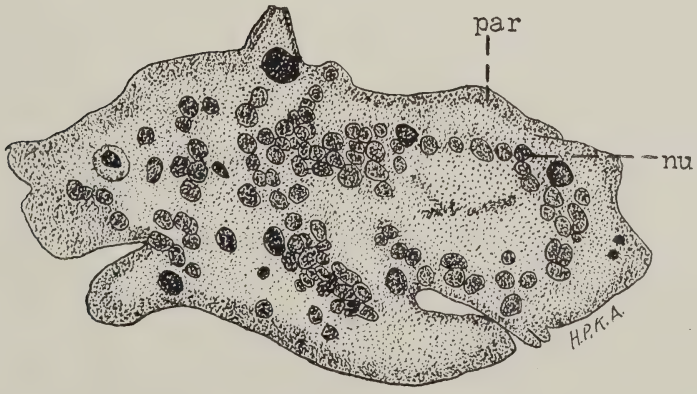

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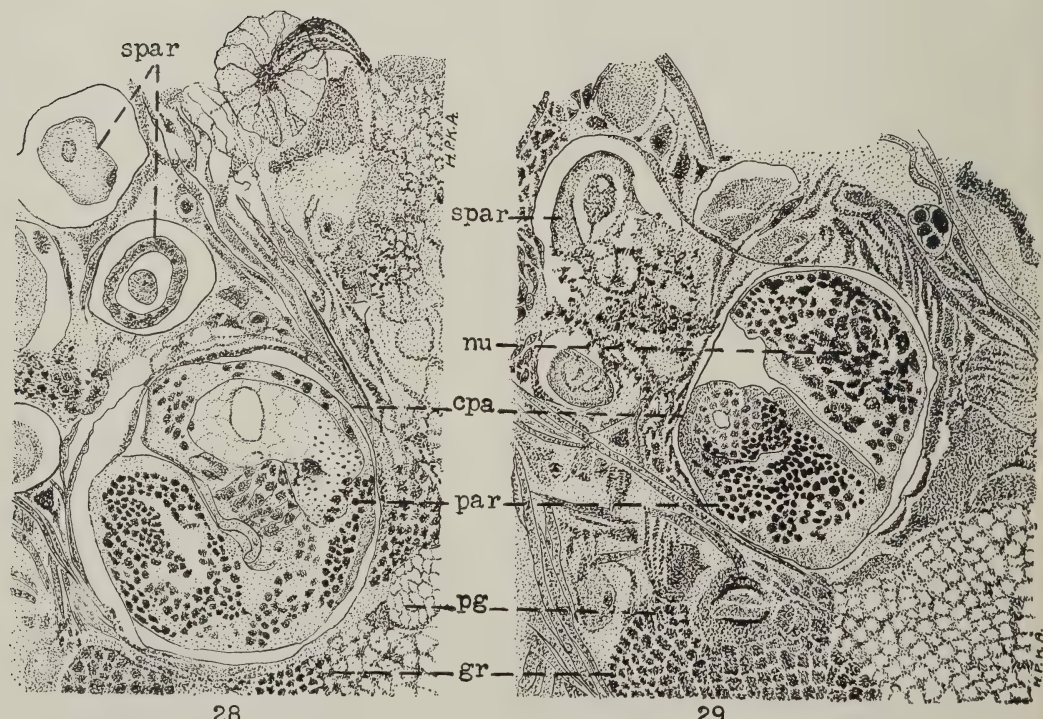

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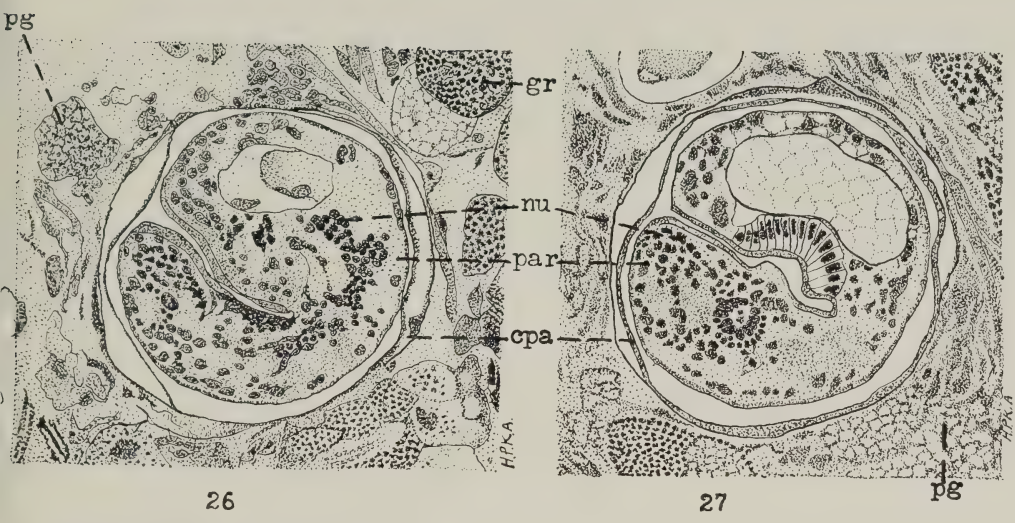

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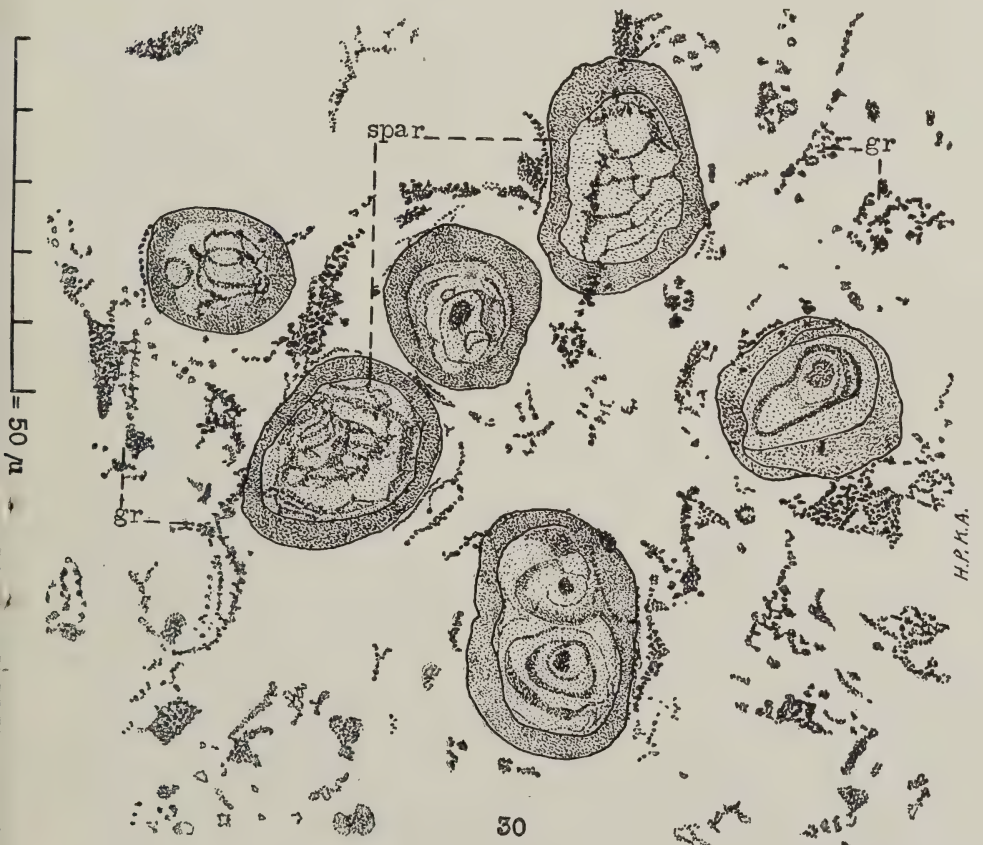

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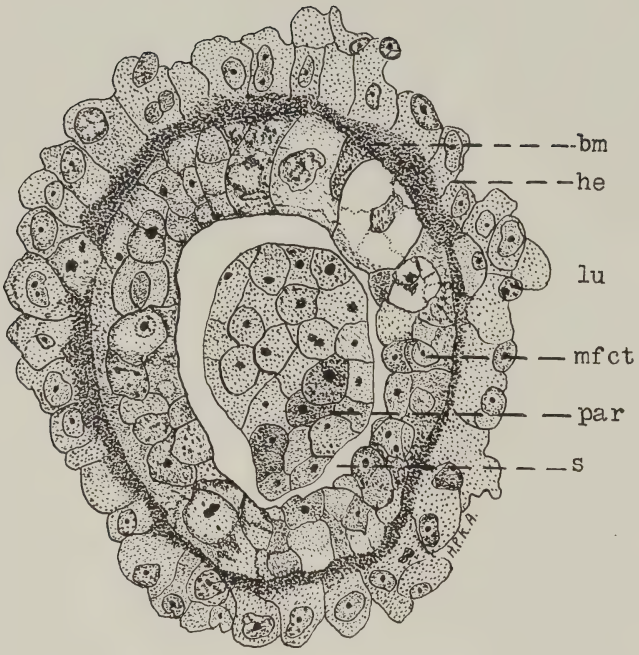
Vaus and Crampton.



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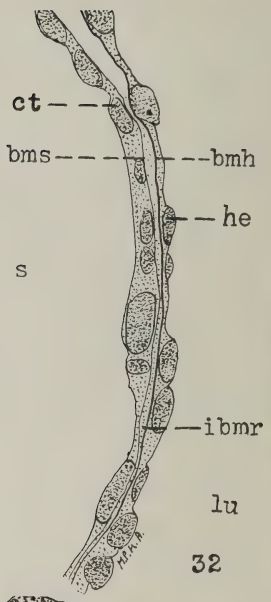

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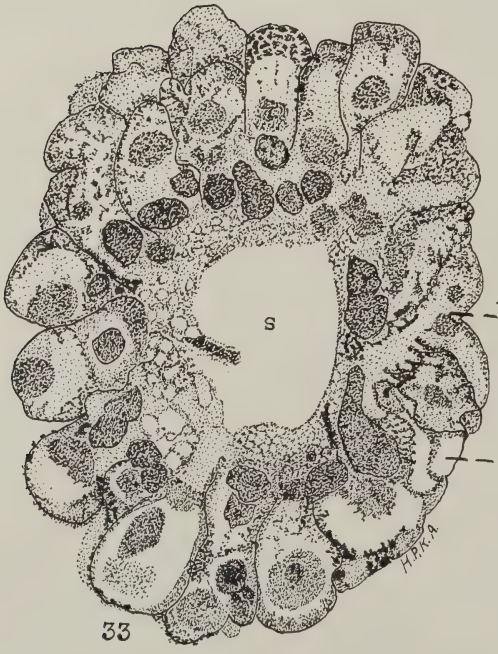

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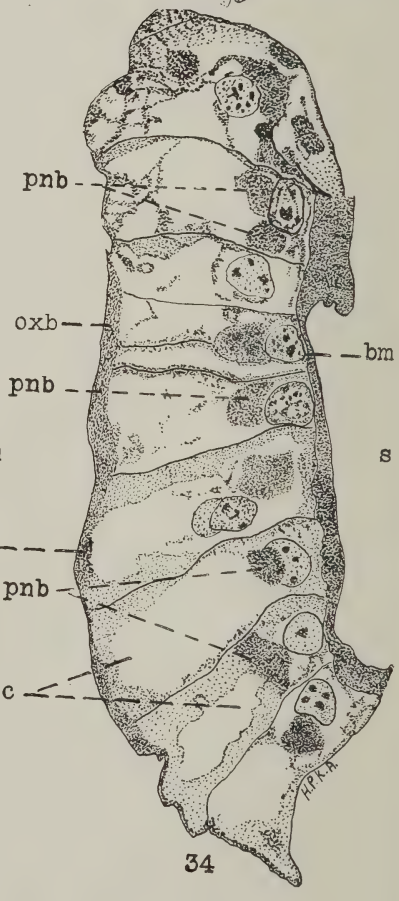

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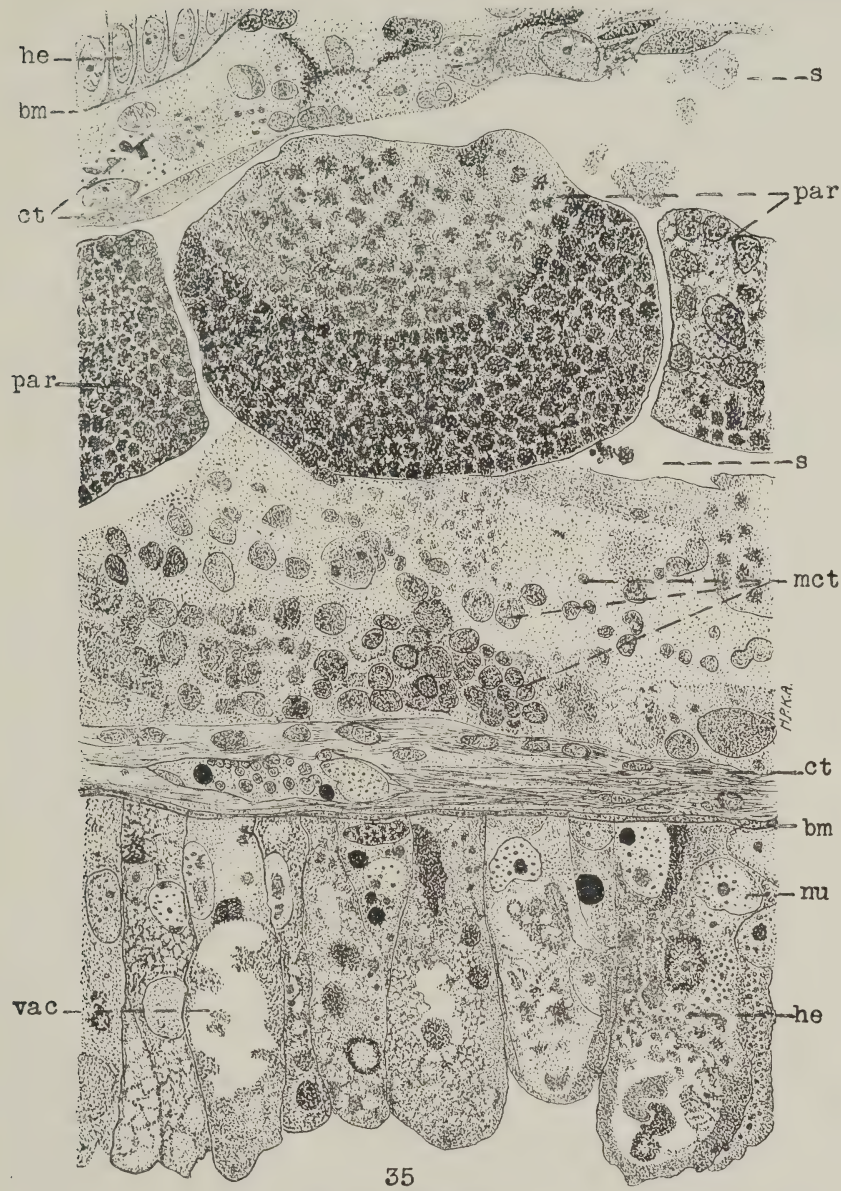

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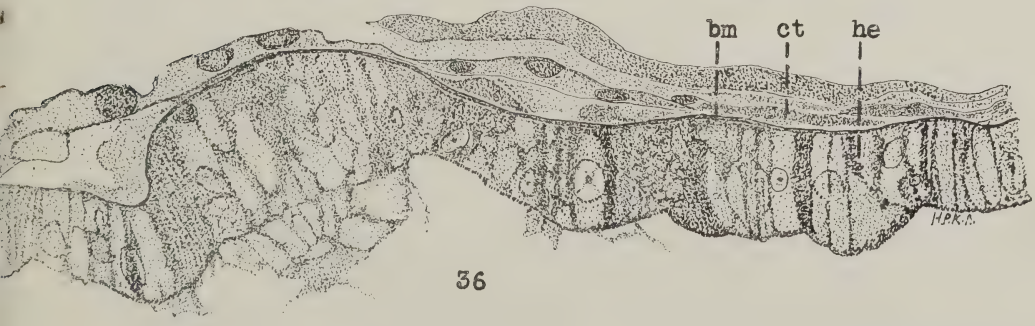

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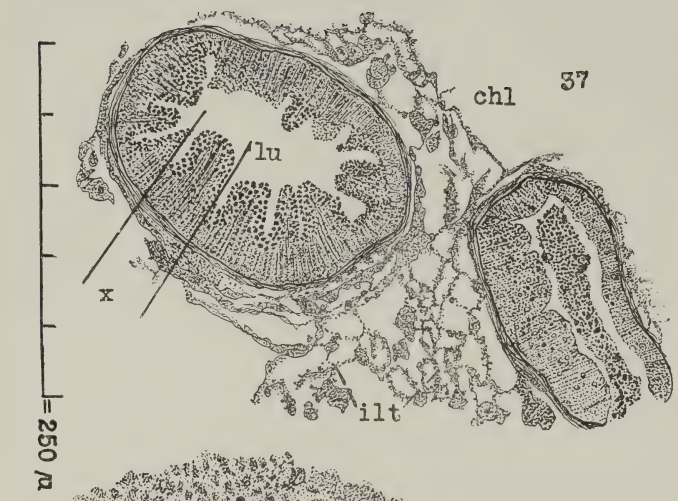

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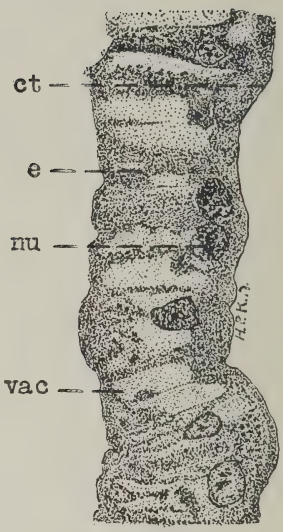

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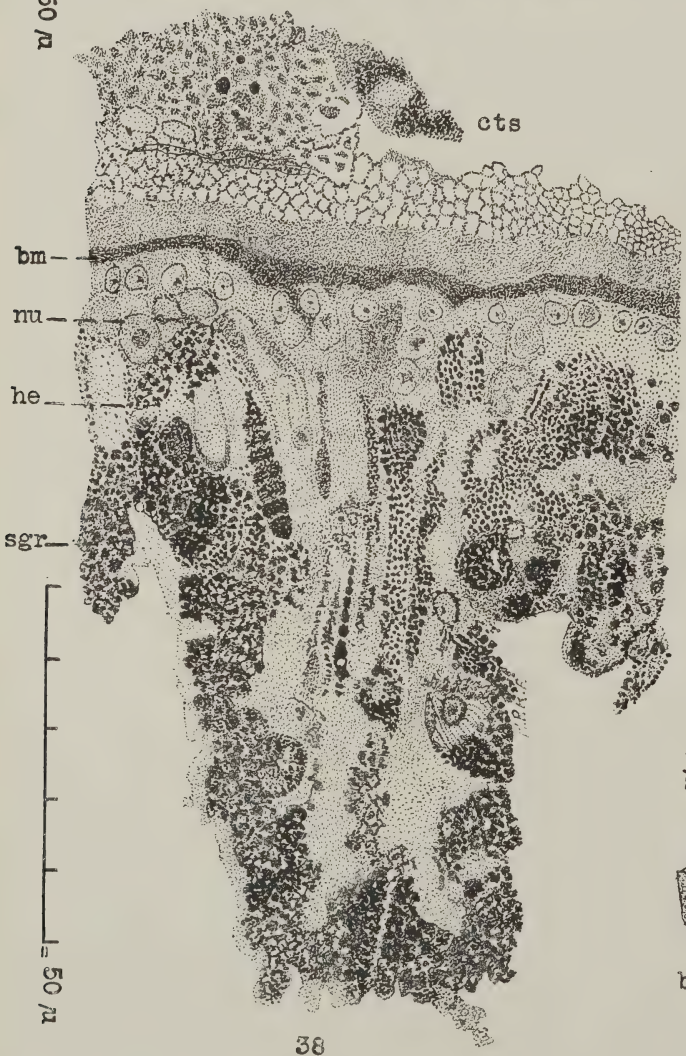

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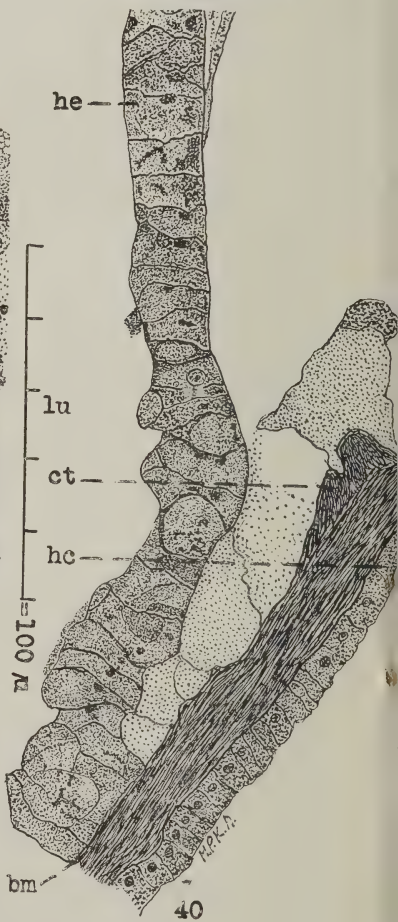

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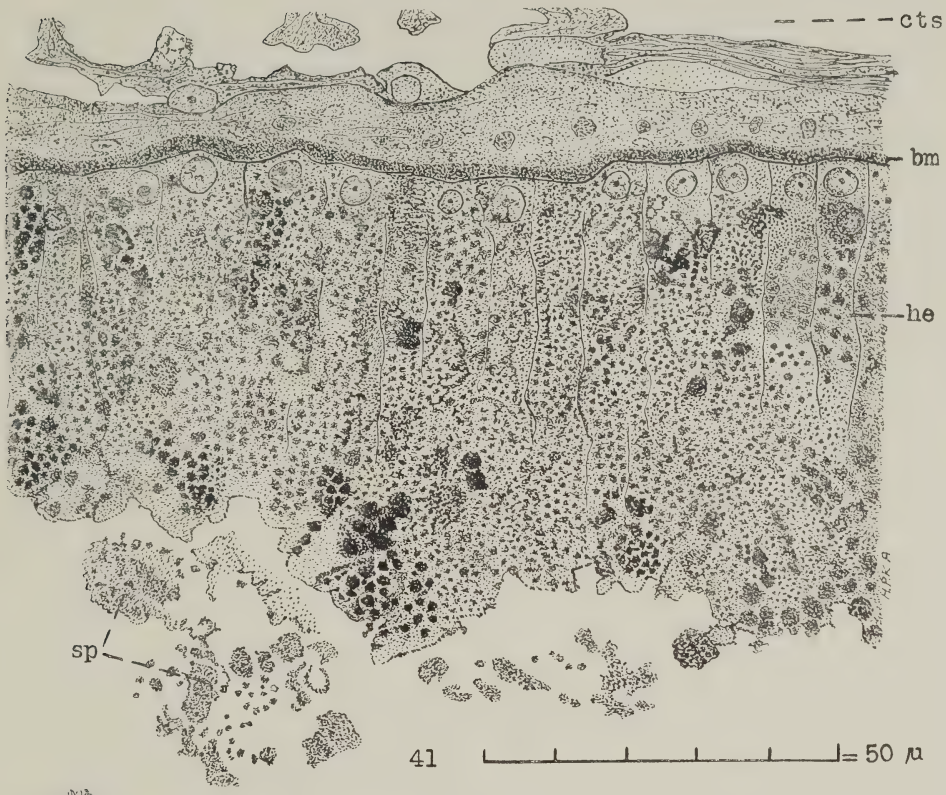
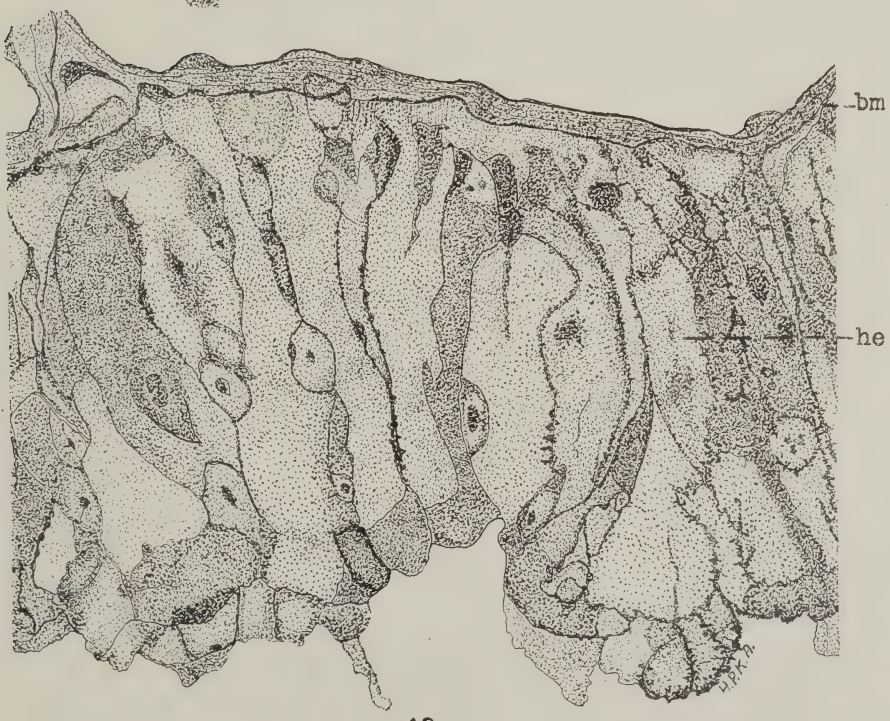
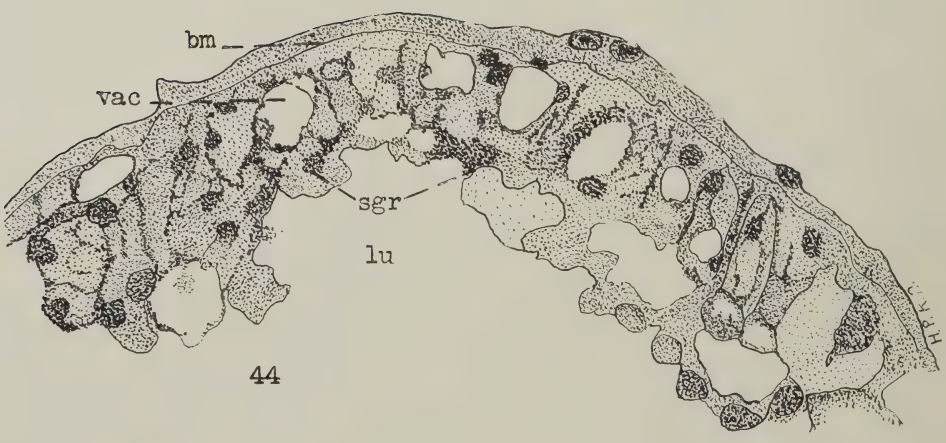




Vaus and Crampton.

43



44

H. P. K. Agersborg, del.


bm
sg
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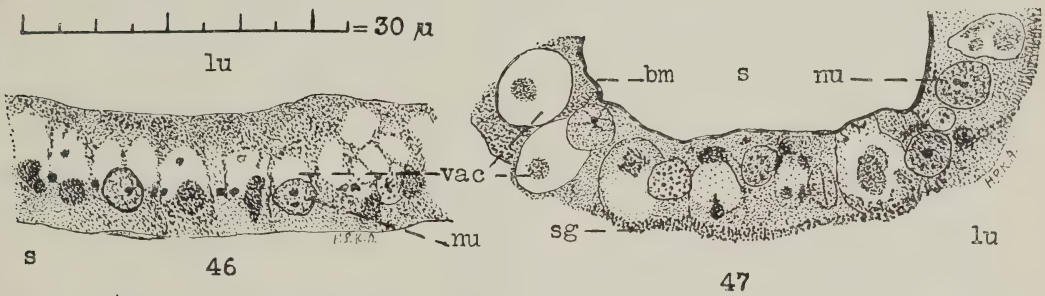
45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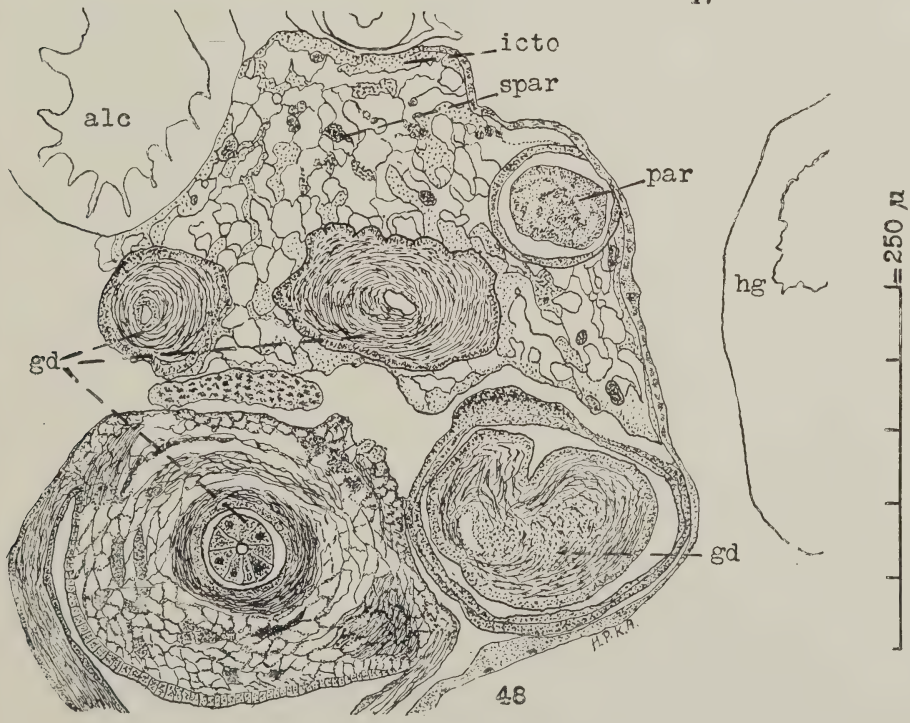
46 47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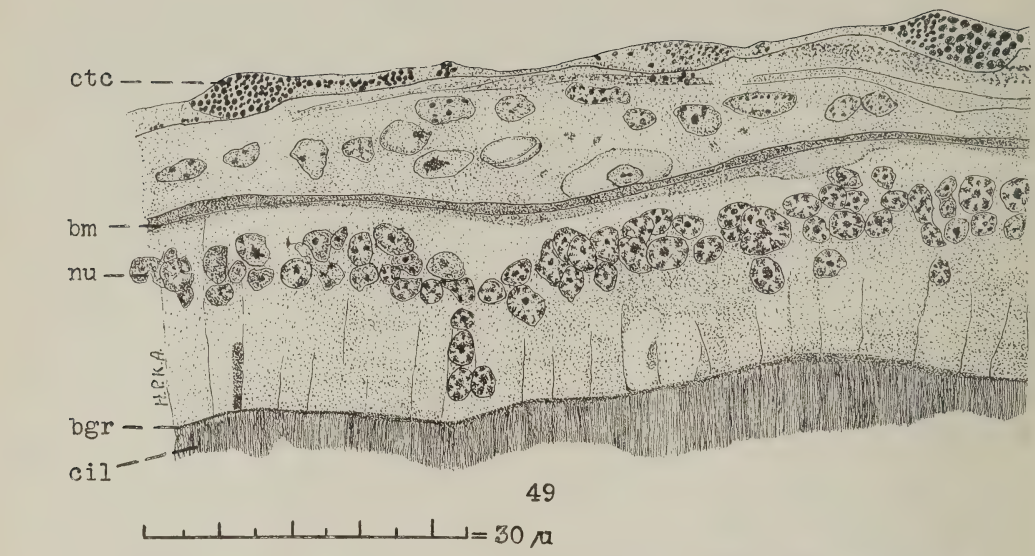
48

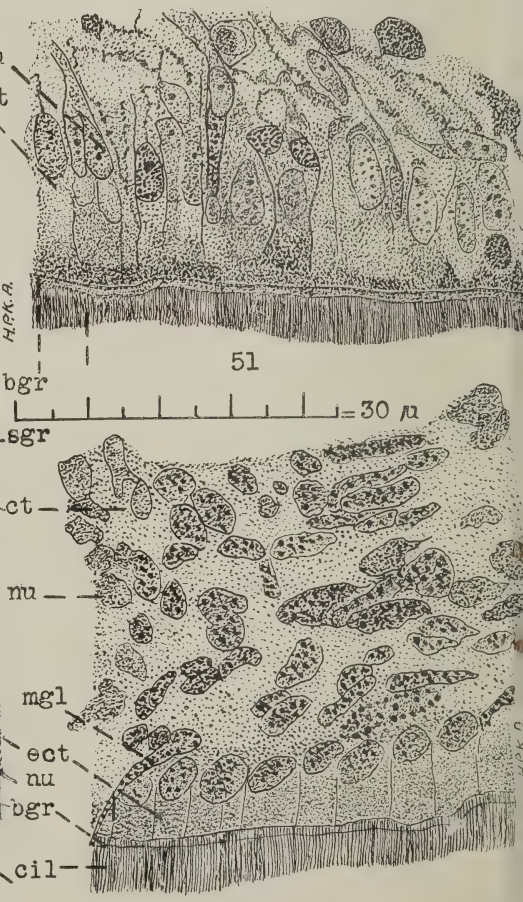


49

= 30 µ

50

= 50 µ



51

= 30 µ

52

H. P. K. Agersborg, del.


ctc
bm
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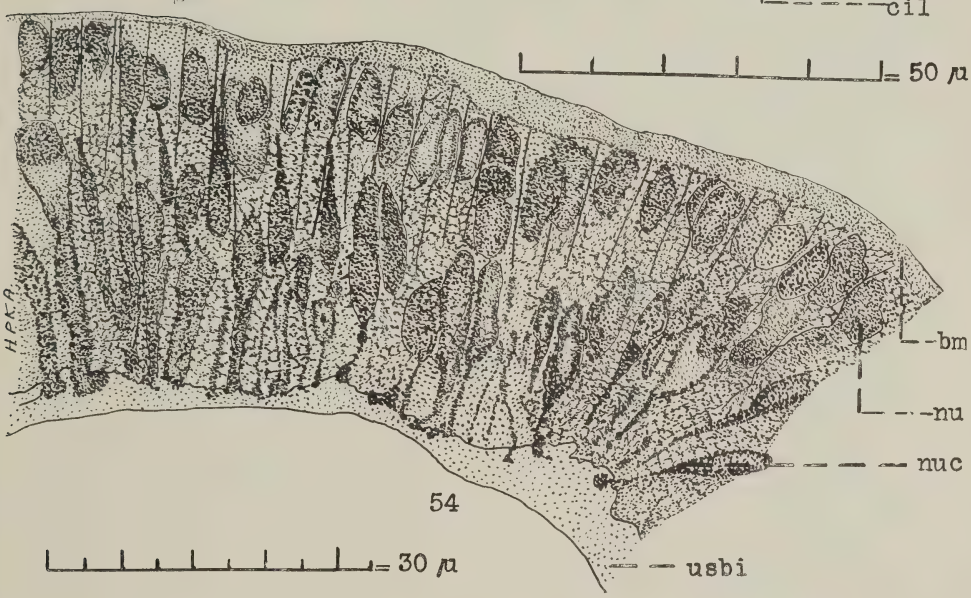
cil
H.P.K.A.

53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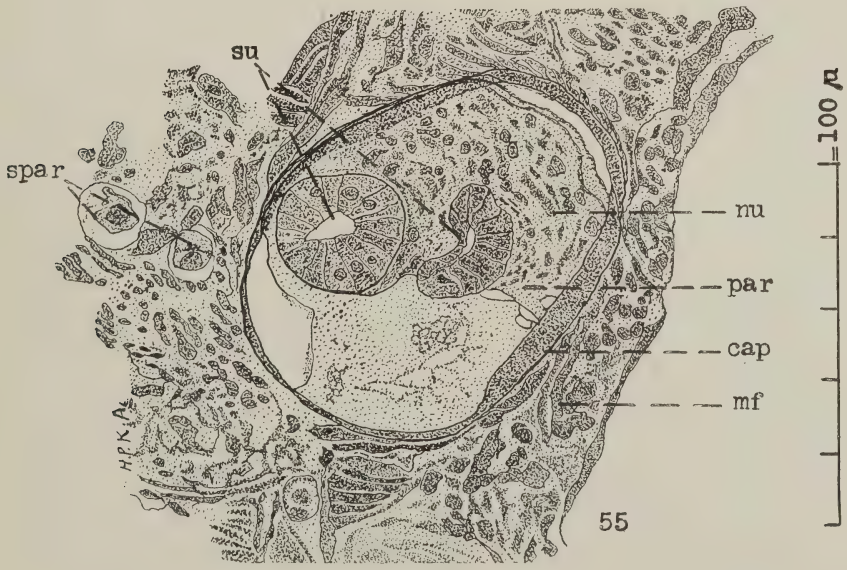
H.P.K.A.
bm
nu
nuc
usbi
= 30 μ

54


su
spar
nu
par
cap
mf
= 100 μ
H.P.K.A.

55

VITA

Helmer Pareli Von Wold Kjerschow Agersborg, born on the 26th of October, 1881, at Gjersvik in Rödöy, Nordre Helgeland, Norway.

Parents

Hr. Albert Martin Petersen Agersborg, and fru Hansine Marie Zahl Agersborg (Née: Hansine Marie Zahl Christensen Mechlenborg).

Education

1887-1900. Attended school in the District, and under private Tutor at home.

1900-1906. Lived on parental estate, and traveled in Norway.

1906-1908. Attended evening schools in Brooklyn, N. Y., and Seattle, Wash.

1908-1912. Attended Seattle Seminary (Seattle Pacific College) Seattle, Wash.

1912-1916. Attended the University of Washington, Seattle, Washington.

1917 Attended the Universitas Regia Fredericiana, Oslo, Norway.

1918-1820. Attended Columbia University, New York, N. Y.

1921-1922. Attended the University of Illinois, Urbana, Illinois, and the Summer Session of the University, 1922.

1913, 1914, 1921, Student and investigator at the Pugent Sound Biological Station, Friday Harbor, Washington (summers).

1917, 1920, Expeditions to the arctics of Norway, to collect littoral mollusks for the purpose of a study of distribution, variation and evolution (summers).

1918, 1919, 1920, 1923, Student and investigator at the Marine Biological Laboratory, Woods Hole, Massachusetts (summers).

Degrees

1916. Bachelor of Science, University of Washington, Seattle, Washington.

1916. Master of Science, University of Washington, Seattle, Washington.

1917. Akademisk Borger, Universitas Regia Fredericiana,, Oslo, Norway.

1920. Master of Arts, Columbia University, New York, N. Y.

1923. Doctor of Philosophy, University of Illinois, Urbana, Illinois.

Positions Held

1913, 1914, Assistant and Acting Curator, respectively, Pugent Sound Biological Station, Friday Harbor, Washington (summers).

1914-1916. Charge of the Biological Supply Department, University of Washington, Seattle, Washington.

1913-1916. Student Assistant in Zoölogy (general invertebrate zoölogy), University of Washington, Seattle, Washington.

1917-1918. Tutor in Biology (botany, general zoölogy, and comparative vertebrate anatomy), and in the Evening College (comparative anatomy, and histology), College of the City of New York, New York, N. Y.

1918-1919. Assistant in Zoölogy (general biology, and vertebrate comparative anatomy), Columbia University, New York, N. Y.

1919-1920. First Assistant in Zoölogy, Columbia University, New York, N. Y.

1917-1919. Demonstrator in Anatomy (histology and embryology), Long Island College Hospital Medical College, Brooklyn, N. Y.

1919-1920. Instructor in Anatomy (histology and embryology), Long Island College Hospital Medical College, Brooklyn, N. Y.

1920-1921. Assistant Professor of Zoölogy (general zoölogy, comparative vertebrate anatomy, histology, and economic zoölogy), University of Wyoming, Laramie, Wyoming.

1920-1921. Assistant Parasitologist, University of Wyoming Agricultural College, Laramie, Wyoming.

1921-1922. Graduate Assistant in Zoölogy (ontogeny and general zoölogy), University of Illinois, Urbana, Illionis.

1922-1923. Instructor in Zoölogy (general zoölogy), University of Nebraska, Lincoln, Nebraska.

1923-1924. Instructor in Biology (general invertebrate zoölogy), Williams College, Williamstown, Massachusetts.

1924- Professor of Biology (and Head of the Department), The James Millikin University, Decatur, Illinois.

Positions Declined

1920. Assistant in Anatomy, College of Physicians and Surgeons, Columbia University, New York, N. Y.

1920. Associate Professor of Anatomy, Emory University, College of Medicine, Atlanta, Georgia.

1921. Associate in Anatomy, University of Illinois College of Medicine, Chicago, Illinois.

1921. Konservator av den zoölogiske Avdeling, Tromsö Museum, Tromsö, Norway.

Member of Scientific- and Other Learned Societies, Since:

1915- Pacific Fisheries Society, Seattle, Washington.

1917- Det kongelige norske Videnskabers Selskab (Trondhjems Museum), Trondhjem, Norway.

1918- The Corporation of the Marine Biological Laboratory, Woods Hole, Massachusetts.

1918a- The American Scandinavian Foundation, Fellow, New York, N. Y.

1919- The American Association for the Advancement of Science, Washington, D. C.

1919a- The Ecological Society of America.

1920- The American Society of Mammalogists.

1921- The International Honorary Scientific Society of the Sigma Xi, (Illinois Chapter).

1922- The National Geographical Society, Washington, D. C.

1922a- The Nebraska Academy of Science, Lincoln, Nebraska.

1922b- The American Society of Zoölogists.

1922c- The American Nature Association.

1923- The American Tree Association.

1924- American Association for Medical Progress, Inc., Boston, Massachusetts.

1924a- The Honorary Scientific Society of the Gamma Epsilon Tau, Alpha Chapter, Decatur, Illinois.

1925- The Illinois State Academy of Science.

1925a- The American Ornithologists' Union.

Contributions to Science

1918. "Nematodes on marketable fishes." Science, N. S., 48: 493-495.

1918a. "Bilateral tendencies and habits in the twenty-rayed starfish, *Pycnopodia helianthoides* (Stimpson)." Biological Bulletin, 35: 232-254, 3 text figures, and 1 plate.

1919. "The teaching of natural science in the Primary and Secondary Schools of Norway." School and Society, 9: 675-678.

1919a. "Notes on the nudibranchiate mollusk, *Melibe leonian* (Gould)." Publications Puget Sound Biological Station, 2: 264-277, 2 plates.

1920. "The utilization of echinoderms and of gasteropod mollusks." American Naturalist, 54: 414-426, 4 text figures.

1921. "Contribution to the knowledge of the nudibranchiate mollusk, *Melibe leonina* (Gold)." American Naturalist, 55: 222-253, 12 text figures.

1921a. "On the status of *Chioraera* (Gould)." Nautilus, 35: 50-57.

1922. "The relation of the madreporite to the physiological anterior end in the twenty-rays starfish, *Pycnopodia helianthoides* (Stimpson)." Biological Bulletin, 42: 202-216, 10 text figures.

1922a. "Notes on the locomotion of the nudibranchiate mollusk, *Dendronotus giganteus* O'Donoghue." Biological Bulletin, 42: 257-266, 4 text figures.

1922 b. "Some observations on qualitative chemical and physical stimulations in nudibranchiate mollusks with special reference to the rôle of the 'rhinophore'." Journal Experimental Zoölogy, 36: 423-444, 4 tables and 2 text figures.

1923. "A critique on Professor Harold Heath's *Chioraera dalli*, with special reference to the use of the foot in the nudibranchiate mollusk, *Melibe leonina* (Gould)." Nautilus, 36: 86-96, 3 plates.

1923a. "Notes on a new cladohepatic nudibranch (*Olea hansineënsis*) from Friday Harbor, Washington." Nautilus, 36: 133-138, 1 plate.

1923b. "Gymnosomatous Pteropoda (*Clione kincaidi* and *Trichocyclus hansineënsis*) from Friday Harbor, Washington." Annales Sciences Naturelles, Zoölogie, (10) 6: 391-402, 5 text figures.

1923c. "The morphology of the nudibranchiate molluse, *Melibe leonina* (Gould)." Quarterly Journal of Microscopical Science. 67: 507-592, 10 double plates.

1923 d. "The sex of the nudibranchiate Mollusca. I. With special reference to Germ-cell secretions in *Melibe leonina* (Gould)." An abstract in Anatomical Records, 25: 346, (Read in part before the American Society of Zoölogists, Cincinnati, December 28, 1923).

1923 e. "The sensory receptors and the structure of the oral tentacles of the nudibranchiate mollusk, *Hermissenda crassicornis* (Eschocholtz 1831), syn. *Hermissenda opalescens* Cooper 1862, 1863." An abstract in Anatomical Records, 25: 347 (Read in part before the American Society of Zoölogists, Cincinnati, December 28, 1923).

1924. "Studies on the effect of parasitism upon the tissues. I. With special reference to certain gasteropod molluscs." Quarterly Journal of Microscopical Science, 68: 361-401, 10 double plates.

1924 a. "The sex of the nudibranchiate Mollusca. I. With special reference to Germ-cell secretions in *Melibe leonina* (Gould)." Archives de Biologie, 34: 215-233, 3 plates.

1924b. "A proposed biagraphical entomological dictionary." Science. 60: 431.

1925. "The sensory receptors and the structure of the oral tentacles of the nudibranchiate mollusk, *Hermissenda crassicornis* (Eschscholtz 1831), syn. *Hermissenda opalescens* Cooper 1862, 1863." Acta Zoölogica. (in press), 23 figures.

1925 a. "Studies on the effect of parasitism upon the tissues. II. With special reference to a new diplostomous trematode found in the minnow, *Notropis anogenus* (Forbes)." (In press) 3 plates. (Read in part before the Illinois State Academy of Science, Springfield, Illinois, February 21, 1925).