2. Observations on the microscopic Anatomy of the Medicinal Leech (Hirudo medicinalis).

By Professor Ray Lankester, University College, London.

Whilst we have from the pen and pencil of Leydig a very detailed account of the structure of the nervous system and sense organs of the common medicinal Leech — which has been supplemented by the researches of Hermann — the histology generally of the integument, of the connective tissue, blood-vessels, alimentary canal and nephridia (excretory organs, Schleifencanäle), has never been satisfactorily investigated. In Leuckart's excellent »Menschlichen Parasiten« we find a description of the anatomy of the medicinal leech in which the earlier researches of Gratiolet, Brandt and Leydig are cited and supplemented by the author's own observations made by means of transverse transparent sections.

During the past twelve months a certain amount of attention has been given in my laboratory to the minute anatomy of the medicinal Leech, by myself and by my pupils MM. J. E. Blomfield and A. G. Bourne. Some of the results which we have obtained are new and some in contradiction with the conclusions arrived at by Leuckart. They may be briefly summarized as follows.

1) Epidermis. The cells forming the epidermis are in a single row as in the Earthworm but smaller and more completely cylindrical than those constituting the epidermis of that animal. They do not present a branched base as do the latter, nor are there goblet-cells scattered at definite intervals amongst them. They are all, with the exception of the very large unicellular glands which sink far into the body-wall, of the same cylindrical form and appearance and produce on their free surface a thick cuticle which is readily separable. As Leuckart has observed, in many places the branches of coloured connective-tissue cells pass in amongst the single layer of epidermic cells. This also occurs in the Lumbricidae, — notably in *L. olidus*. — An important fact which has not hitherto been recorded, is that in all parts of the epidermis of the Leech, excepting the extreme ends of the body, fine blood-vessels penetrate the epidermic layer and take a horizontal course amongst the cylindrical epidermic cells. The respiratory function of the body-surface is no doubt effected through this arrangement.

The clitellus of the Earthworm exhibits a similar interpenetration of blood-vessels and epidermic cells — but in this case the interpenetration is in an exceptionally thickened and hypertrophied layer of the

epidermis — and not as in the Leech in a single layer of small columnar cells.

2) Epidermic gland-cells. These were first described and figured by Leydig, — but as Leuckart has remarked, their great abundance and the very deep position which they may occupy, was not insisted upon by him. They are seen in transverse sections as transparent globular bodies with nucleus and either clear or granular contents — often in close proximity to the wall of the alimentary cavity — whilst their narrow ducts reach forward in a radial direction to open between the epidermic cells on the body-surface. The salivary glands are aggregations of closely similar cells each with its own fine duct — and their identity with the unicellular glands of the body-surface is correlated with the fact that the pharynx is an invagination of the ectoderm (stomodaeum).

Leuckart appears to me to have made a mistake in confusing the true unicellular glands of the epidermis which sink so deeply into the body — with certain deep-yellow granular cells which form a well marked layer nearly surrounding the alimentary canal. The epidermic gland-cells are pale and translucent and quite distinct from the yellow or brown botryoidal tissue which Leuckart identifies with them. This brown tissue as Leuckart rightly states is what Brandt indicated as liver, whilst Leydig considers it to be a kind of fatty body like the fatty body of Insects (Leuckart, Menschl. Par. Vol. I. p. 641). Whilst the brown perienteric tissue or botryoidal tissue is quite distinct from the epidermic glands — its true nature has been overlooked by all previous observers, as will be shewn directly.

It must be distinctly understood that we are able to confirm Leuckart's distinction of the epidermic unicellular glands into those which are more superficial and those which sink deeply into the body so as to abutt upon the wall of the alimentary canal. But the brown botryoidal tissue (»Zellen von gelblichem Aussehen und gewöhnlich zu unregelmäßigen Strängen und Trauben an einandergereiht« Leuckart) has nothing to do with them.

Connective tissue. The connective tissues of the Leech include three chief varieties which run into one another and further are not to be sharply distinguished from the tissue forming the wall of the smaller blood-vessels. The most massive tracts of connective tissue are formed by a slightly fibrillated but otherwise homogeneous ground-substance in which are very numerous oblong corpuscles with exceedingly fine processes. The younger of these corpuscles are finely granular, the older contain abundant coarse straw-coloured granules of larger size and high refractive index. Silver staining gives the appea-

rance of colourless branched spaces on a dark-brown ground. The second variety is one in which the ground-substance is thrown into the form of ramifying fibres, the protoplasmic corpuscles being placed at intervals on the fibre. These fibres are usually beset with fine brown-coloured granules. They form an investment or tunica propria to nearly all the more important organs — e. g. to the blood-sinus of the nerve cord, to the lateral blood-vessels, to the nephridia (excretory glands), and to the separate muscular bundles of the body wall. It is to this variety that the pigment bodies of the integument belong. There appears to be no sharp distinction between these branching pigmented fibres and some of the blood-vessels. Blood-vessels occur which are nothing more than such fibres hollowed out. This is not surprising when we compare the facts which have been ascertained as to the development of blood-vessels in the omentum of the Rat. Nevertheless there are in the Leech abundant minute blood-vessels which have exceedingly delicate walls devoid of granules and a distended appearance and regular diameter — which makes them resemble the finer blood-vessels of the Earthworm. The third variety of connective tissue in the medicinal Leech might perhaps be better described as a variety of blood-vessel — but as a matter of fact the walls of blood-vessels are nothing more nor less than connective tissue. This third variety is the brown botryoidal tissue surrounding the alimentary canal. It consists of moniliform tubes which are highly tortuous and branched, — the branches ultimately loosing their moniliform character and becoming ordinary blood-vessels. The cells which form these tubes are closely set around the lumen which they enclose and are oval and of great relative thickness; their protoplasm is entirely obscured by an abundance of dark yellow or brownish granules. The granules stain deeply with osmic acid, also with goldchloride.

The lumen of the tubes forming the botryoidal tissue is occupied by the red blood of the Leech: the fat-body of Leydig, the liver of Brandt, the epidermal glands of Leuckart, is in reality a vascular plexus. This I have most amply proved again and again both in fresh preparations and in transverse sections. For the examination of the latter I can strongly recommend the use of Ranvier's Picrocarmine, as a colouring agent. The vascular plexus of the botryoidal brown tissue must not be confounded with that which occurs on the wall itself of the intestine.

A vascular plexus adjoining the alimentary canal and having upon its walls large cells highly charged with deep-yellow granules is well-known to occur in the Chaetopodous worms and more especially

in the Oligochaeta. The botryoidal tissue of *Hirudo* has I can not doubt precisely the same signification as the yellow-coloured tunic of the intestine and large vessels of *Lumbricus*. More especially I would compare it to the coecal vascular processes covered with yellow granular cells which project from the walls of the alimentary canal of *Lumbriculus*.

I am inclined to think that both Leydig and Brandt may be right as to its function to a certain extent. Leydig has already pointed out that the liver in Paludina may have a function as a fatty body — a store-house of combustible material. At the same time there is evidence for the opinion that not only in Vertebrates but in Mollusca also the cells of the liver are active in the production of colouring matters similar to Haemoglobin. Sorby has found such a colouring matter in the stomach of *Helix* in the winter (Quart. Journ. Microsc. Science. Vol. XVI. p. 78). Now though the yellow tunic of the Chaetopods intestine and the brown botryoidal tissue of the Leech are morphologically quite distinct from hepatic-cells, being products of the mesoblast and not cells contained within diverticula of the enteron, — yet in function these cells may have some analogies. The yellow tunic and the botryoidal tissue certainly do not secrete any substance which aids in digestion or which can directly reach the alimentary cavity, but so far as their fatty nature is concerned they may represent the hepatic cells and also, it seems very possible, they may be the seat of the production of the haemoglobin which is diffused in the liquid of the blood-vessels related to them.

It is to be noted in comparing the yellow tunic of Chaetopods with the brown botryoidal tissue of the Leech, that the cells of the former are freely exposed to the body-cavity and separated from the closed vascular system by the proper walls of the blood-vessels; whilst in the latter, in which there is no differentiation of closed vessels from body cavity, the brown cells form the walls of fine channels containing the haemoglobin-coloured fluid which is common to all spaces in the Leech's body (excepting of course the alimentary canal).

Blood-vessels and blood-corpuscles. There is still much to be made out with regard to the relationships between the Leech's system of blood-spaces and the double system of body-cavity and red vascular system obtaining in Chaetopods. It may be definitely stated that in the medicinal Leech there is no system of spaces occupied by a colourless liquid. At the same time the large vessels with definite muscular walls can be distinguished from the large sinus-like spaces, and the small regular vessels with clear walls from shrunken vessels with pigment granules (mentioned above as related to connective tissue

fibre) and the moniliform brown vessels constituting the botryoidal tissue. In the small vessels with irregular walls I have observed minute pale corpuscles sometimes aggregated in quantity — but I have not seen them in the clear walled vessels.

The exceedingly fine capillaries which take a horizontal course among the epidermic cells (noted above) furnish an example of the completeness of the Leech's vascular system: this is still further exemplified in the very complete set of minute capillaries which are distributed to the nephridia in such a way that each constituent cell of the gland is encircled by at least one loop of the vascular network and in section may be seen to have as many as five separate capillaries interposed between it and neighbouring gland-cells. In the completeness of its vascular supply the nephridium of the Leech strongly recalls the similarly complete vascular supply of the Mammalian liver.

The nephridia or excretory glands (Schleifencanäle). Leydig has figured a bit of the nephridium of Haemopis, showing the large cells of which it is composed, each cell being actually perforated or traversed by the duct. Claparède has figured the same structure for the nephridium of the Earthworm. The actual disposition of the perforated cells in the nephridium of the medicinal Leech and the mode in which they are set together so as to form a solid gland has never been described. It is impossible without figures to give an idea of the true relations of the lobes of the nephridium which has been carefully worked out by my assistant Mr. A. G. Bourne. In the apical region of the gland the cells are not perforated through and through by the duct, but the duct has a finely arborescent origin in each cell; in another part the cells are perforated through and through by a branching duct which joins the branching ducts which perforate neighbouring cells; again in the basal region of the gland the ducts become very large and simply traverse successive gland-cells which are little more than hollow cylinders. Everywhere the duct has a fine cuticular lining which resists maceration and can be isolated. In addition to the traversing or intra-cellular ducts, the lobes of the nephridium present each a large axial or inter-cellular duct. The wall of the nephridial vesicle or bladder is finely ciliated. The bladder contains a number of excessively fine needle like particles — not merely granules as stated by Leuckart. Micro-chemical examination renders it probable that these needles are of a nature allied to that of the Uric acid.

Silver-staining or maceration in Müller's serve to render apparent in the nephridial gland-cells a very remarkable fibrillar structure. After long maceration the cells will break up into a number of exceedingly delicate rods — which are all set so as

to radiate from the surface of the cell towards the duct. They appear to be the same structures which were detected by Heidenhain in the epithelial cells of the convoluted tubules of the Mammalian kidney.

The cells constituting the various lobes of the nephridium are bound together by fibrillar connective tissue beneath which one blood-vessel (possibly a second also) derived from the great lateral trunk penetrates and breakes up to form a marvellously complete plexus, its branches running between the contiguous nephridial gland-cells.

I have not been able to detect any stroma-tissue in the nephridia nor to determine the cellular structure of the walls of the blood-vessels. The walls in question are distinct enough, and the natural injection of the plexus and dilatation of the vessels varies in different specimens.

A prolonged examination of the nephridia of *Hirudo* by various methods, has served to confirm the usually accepted opinion viz. that the duct has no communication with the body-cavity.

Epithelium and walls of the alimentary tract. The cells which line the coeca of the alimentary tract of the medicinal Leech are remarkable. They are not ciliated, nor are they columnar, but are short hemispherical cells with pale nucleus around which are numerous coarse granules. During digestion (as shewn in transverse sections) they one and all give origin to a secretion which stands forward on the top of each cell as a hyaline, homogeneous drop of viscid consistence, compressed and elongated by the viscid drops adjoining it. It will be interesting to compare these cells with those in the corresponding region of *Aulostomum* in which the material to be digested differs very considerably from blood.

The muscular fibres in the wall of the alimentary canal are band-like and arborescent — often terminating in a stellate group of processes.

Clear-walled blood-vessels (quite independent of the botryoidal tissue) form a plexus in the wall of the alimentary canal.

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3. Beiträge zur Kenntnis des anatomischen Baues der Geschlechtsorgane bei den Phalangiden.

Aus dem Zoologischen Institute in Amsterdam.

Von J. C. C. Loman, Cand. Phil. in Amsterdam.

Auf Veranlassung der »Beiträge zur Kenntnis des anatomischen Baues der Geschlechtsorgane bei den Phalangiden« von H. W. de Graaf in No. 47 des Zoolog. Anzeigers, theile ich die Hauptresultate mit, welche ich bei der Beantwortung derselben von der pilosoph. Facultät der Universität zu Leiden gestellten Preisfrage erhalten habe.