IX. "On the Occurrence of Glycogen as a Constituent of the Vesicular Cells of the Connective Tissue of Molluscs." By EDWIN RICHARDSON BLUNDSTONE, Scholar of Christ's College, Cambridge. Communicated by E. RAY LANKESTER, M.A., LL.D., F.R.S. Received June 2, 1885.

The following results were obtained in connexion with a research "On the Connective Tissue and Vascular System of Mollusca," on which I acted as assistant to Professor Lankester, according to the terms of a grant from the Government Grant Committee of the Royal Society: other results will be published subsequently.

The connective tissues of Molluscs, as presented by Helix, Planorbis, Anodon, Cyclas, and Solen, may be divided into two main groups. In one of these the constituent cells are little advanced from their original mesoblastic condition; they have an irregular stellate form, and they are joined together by the tips of their processes. In the other variety, which will be spoken of as lamellar connective tissue, the cells are more irregular in form and their processes more attenuated, but, by the deposition of an intercellular ectoplasm in certain planes, the cells come to lie in plates or films. These films form the walls of the sinuses and lacunæ (occasionally vein-like in appearance) of Molluscs. All the cells, however, of the lamellar connective tissue do not lie embedded in the films, for some of enormous size project into the blood, being only attached by a small portion of their superficies to the film.

These cells are the "vesicular cells" (Lankester) of the lamellar connective tissue: they contain *glycogen*.

1. *Tissues operated upon.*

In extracting glycogen from Anodon I have made use of the mantle, thus avoiding all contamination and complications of results on the part of the liver, and, by rejecting the mantle edge, ventral to the pallial muscles, have been able to work upon a region of the simplest composition, for with the exception of a very few muscular fibres which pass from side to side of the mantle, the region operated upon comprises only the two epidermes and the lamellar films with their glycogenous cells. The blood offers no difficulties, as it readily drains out of the mantle.

2. *Processes of Extractions, &c.*

In extraction I have made use of the process and tests detailed for investigating the liver of the rabbit for glycogen in Foster and Langley's "Elementary Physiology," and also of those in the admirable essay of Dr. Errera, to which I am much indebted.

To observe most of the following results, however, the more complicated processes alluded to above are not really essential. I therefore give a simpler process, whose results are *sufficiently* accurate.

The mantles of twenty Anodons (previously preserved in strong spirit) are treated, after removing the adherent spirit with blotting paper, with 3 oz. of boiling distilled water. While in the water, the mantles are disintegrated as far as possible with a glass rod. The mixture is freely shaken, and in a few minutes filtered. The filtrate is rapidly cooled, and then twice its bulk of absolute alcohol is added to it, and allowed to stand. The precipitate so obtained is taken up, after washing with 90 per cent. spirit, in distilled water, and again precipitated by absolute alcohol as before.

Upon dissolving this precipitate in distilled water a bluish opalescent solution is obtained which—

1. Gives mahogany colour with iodine solution—the colour disappearing upon warming, and reappearing upon cooling.
2. Gives no reaction with Fehling's solution upon warming.
3. After digestion with saliva at 30—35° for about 10 minutes, the solution gives Fehling's reaction.
4. Is precipitated by 60 per cent. alcohol.

The above is briefly the evidence of the occurrence of glycogen in the mantle of Anodon.

3. *The Localisation of the Glycogen in certain Cells.*

By a little care the mantle may be split in half (this is performed more easily with a spirit specimen). Placing one of the halves so obtained upon a glass slip, epidermis downwards, and treating the preparation with solution of iodine, a remarkable appearance is observed.

The tissues generally are hardly stained at all, but with the naked eye it is seen that the connective tissue is copiously sprinkled with dark brown dots. By the microscope these dots are found to be very large vesicular cells, some of whose contents have been deeply stained by the iodine.

4. *Some Reactions and Particulars of these Glycogenous Vesicles.*

For the study of these cells, thick sections of the frozen mantle of Anodon or preparations of the "mesentery" of Helix are best.

The vesicles are then seen to be very large round or oval cells, with very brilliant (though not doubly refracting) contents.

By treatment with water, the cells are emptied of contents except the nucleus and the cell protoplasm, which is very small in amount. By crushing, it is seen that the metaplasm ("endoplastic

product" Lankester) is fluid, and dissolves to an opalescent fluid in the surrounding water.

With strong spirit, the metaplasm undergoes a very remarkable and quite characteristic clotting or pseudo-crystallisation (also noticed by Errera), which takes place equally well either within or (in crushed specimens) outside the cell. Osmic acid yields no reaction.

It is the metaplasm or endoplastic product of the vesicle which is *deeply* stained by iodine, and also by borax carmine, and not the nucleus and cell protoplasm. I do not propose here to further describe the glycogenous vesicles, for they are described and figured, both fresh and after the action of various reagents, at great length by Professor Lankester and myself in the forthcoming paper to which allusion has before been made, but I will take this opportunity of pointing out—

1. That these "vesicles" are the same as the "plasma cells" of Brock and others, the "Langer's bladders" of very many writers, and are equivalent to many of the "lacunæ" of Kollmann, Griesbach, &c. For many years the existence of these vesicles has been denied and affirmed by two schools of observers; we shall bring forward indubitable proof in favour of Fleming and his adherents.

2. That these cells are trustworthily figured and described by Fleming ("Archiv für Mikros. Anat., vol. xiii), and may be readily seen in Anodon by the method described above, or in Helix by merely spreading out a portion of the "mesentery" on a glass slip.

5. *Distribution of the Vesicles.*

These vesicles occur in Anodon wherever there is lamellar connective tissue, except in the very muscular *tip* and *edge* of the foot, labial palpi and gills, Keber's organ, organ of Bojanus, mantle edge.

In Helix, they are found especially on the lining of the great lacunar spaces, and on the "mesenteries."

They are especially associated with the arteries in all Molluscs I have examined. The brilliant whiteness of the slug's arterial system is due to their presence in the connective tissue, *outside* the arteries.

I have reasons for believing that these cells, or slight modifications of them, are very widely distributed throughout the Invertebrata.

6. *General Remarks and Conclusion.*

Although glycogen has frequently been stated to occur in Invertebrates (*e.g.*, by Professor Foster for Ascaris, and by Fredericq for Mya), yet I believe that hitherto it *has never been definitely localised in certain cells*, and far from being associated with connective tissue, has been thought to be for the most part a liver product. I hope to be able to publish further results in the above directions at a future time.

In connexion with the Mollusca certain points seem worthy of special notice.

In the first place, it is held by many comparative anatomists that the lacunar system of Molluscs has a partly enterocoelous origin, or at least has enterocoelous elements in its nature. If this be so, it is interesting to note that some cells of the lacunar walls may be glycogenous, for glandular surfaces seem to be specially characteristic of the ectoderm and endoderm. Moreover, these cells are also to be found on the mesenteries of Holothurians, which are undoubtedly enterocoelous.

In the second place, one of the greatest objections which can be urged against the feasibility of water inception by Molluscs is removed, if (1) the specific gravity and (2) the nutritive quality of the blood can be maintained in spite of the process. It is supposed that this would be accomplished by the discharge of the contents of the glycogenous vesicles.

Finally it is interesting to note, that one of the functions of the vertebrate liver seems in Molluscs with ease to be performed outside its domain, and this, moreover, in animals whose liver is essentially a digestive gland.

In conclusion I have to thank Professor Lankester and Professor Foster, to whom, as also to Mr. Langley, Mr. Lea, and Mr. Gardiner, I am very greatly indebted.

X. "On the Development and Morphology of *Phylloglossum Drummondii*. Part I. Vegetative Organs." By F. O. Bower, M.A., F.L.S., Regius Professor of Botany in the University of Glasgow. Communicated by W. T. Thiselton Dyer, M.A., C.M.G., F.R.S. Received June 18, 1885.

(Abstract.)

The morphological history of *Phylloglossum* has up to the present time rested on a very slender basis. The following brief summary given by Sachs ("Textbook," 2nd English Edition, p. 463) practically comprises the whole of it:—"A small Australian plant, only a few centimetres high. It consists of a stem arising from a small tuber, and bearing at its lower part a rosette of a few long leaves, and one or more lateral roots; it is prolonged above this as a thin scape, and terminates in a spike of small leaves bearing the sporangia. The plant is propagated by means of adventitious shoots, consisting of a tuber with a rudimentary leafless bud; in this respect it resembles our native *Ophrydeæ*."

The study of so reduced a form of a group, usually so remarkable