Demonstration of Lipine in the Golgi Apparatus in Gut Cells of *Glossiphonia*

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With two Text-figures

INTRODUCTION

THE Golgi apparatus has long been thought to consist in part at least of lipoids. Baker (1944) has summarized and discussed the evidence. The fact that it can be coloured by lipoid colorants (sudan black and sudan III) is a proof that a lipoid or lipoids are present in it. Baker has given very strong evidence, not amounting to histochemical proof, that lecithin or cephalin (or both) are present. The opinions of other workers on the nature of the lipoid contained in the Golgi apparatus are not based on reliable tests.

The purpose of this paper is to present histochemical proof that the Golgi apparatus in the epithelial cells of the alimentary canal of the leech, *Glossiphonia complanata*, contains lipine.

In this paper, the word 'lipoid' is used to include fats and all other substances that occur in plants and animals and resemble fats in solubility. The word 'lipine' refers to lipoids that yield fatty acids, phosphoric acid *or* galactose, and a basic nitrogen compound.

MATERIAL

This study was made on the gut epithelium of the Rhynchobdellid leech *Glossiphonia complanata* (L.). This animal was originally chosen for the suitability of its fat-cells for work on lipines, and because it is common and occurs throughout the year in the adult stage. The Golgi apparatus is visible in the gut after formaldehyde-calcium fixation in unstained preparations mounted in Farrants's medium, and is very easy to demonstrate by a variety of methods. It appears to have the same structure in both stomach and intestine, but although it is easily shown in both by sudan black, the standard silver and osmium techniques give good preparations far more often with the intestine than with the stomach. On the other hand, the acid haematein test rarely gives good results with the intestine, partly because of interference by a diffuse lipine in the cytoplasm.

METHODS

For definitive demonstration of the Golgi apparatus, the following standard techniques were used:

Silver: Da Fano
Ramon y Cajal
Aoyama

Osmium: Mann-Kopsch-Weigl (Ludford)

These were supplemented by Kull's method, after Helly fixation, for mitochondria.

For investigating composition, Baker's acid haematein test for lipines (Baker, 1946) was used, and the following method for sudan black:

(1) Fix, postchrome, wash, embed in gelatine, and cut frozen sections exactly as for the acid haematein test.
(2) Leave for a few minutes in 50 and 70 per cent. alcohol.
(3) Transfer to a saturated solution of sudan black in 70 per cent. alcohol for 7 minutes or longer (the exact time does not matter; see Lison, 1936, Baker, 1944).
(4) Pass through three lots of 50 per cent. alcohol, 30 seconds in each.
(5) Rinse in distilled water and mount in Farrants's medium, or counterstain first if desired. One counterstained preparation is useful if the tissue is not familiar.

This method is a modification of Baker's. It was used in preference to Lison's because Dr. Baker informed me that the use of potassium dichromate helps to make the Golgi apparatus colourable with sudan black in certain cases in which it is not shown by Lison's technique.

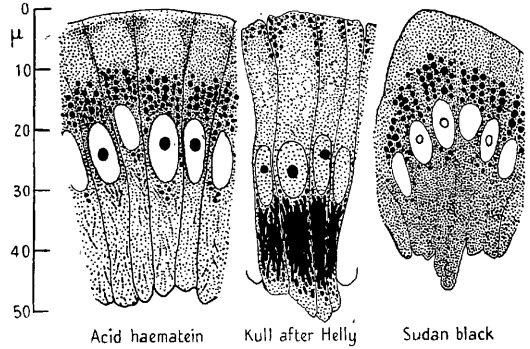
It was found that no difference could be seen between preparations made in this way and those fixed in formal-calcium with no postchroming, so the same block was used for acid haematein and sudan black.

The method for nile blue given by Lison (1936) was used, except that again, as there was no difference between postchromed and not-postchromed sections, postchromed ones were used. Also, a variety of tissues (sections of *Glossiphonia complanata*, *Lumbricus castaneus*, *Dendrocoelum arboreum*, and liver, lung, and kidney of mouse) were stained in a saturated aqueous solution boiled with ½ per cent. sulphuric acid (as advised by J. L. Smith, 1908, and recommended by Lison, 1935*b* and 1936), and compared with others stained in the untreated solution. Again, no difference could be seen, so the untreated solution was used, as it appears to keep indefinitely. In addition, the following method was used.

(1) Prepare a section as for acid haematein.
(2) Stain in saturated aqueous solution of nile blue, 10 minutes.
(3) Leave in 1 per cent. acetic acid for about 18 hours, until the Golgi apparatus is pale pink and the cytoplasm colourless.
(4) Wash in distilled water, and mount in Farrants's medium.

Lison (1935, *a* and *b*) concludes, after a very detailed study of nile blue, that the red coloration is due to nile red, an oxazone occurring as an impurity in nile blue, and that this behaves like any other lipoid-colouring agent,

e.g. sudan black. It cannot distinguish between the various classes of lipoid. (This conclusion, as Kay and Whitehead (1937) point out, supersedes that given in his book (1936).) He states also that the blue dye is simply a basic dye, and no histochemical conclusion whatever can be drawn from its uses, and (1935*a*) that it has weak powers of metachromatic staining with ordinary chromotropic elements, the metachromatic colour being *violet bleuâtre*. The method with prolonged differentiation given above does enable one to distinguish between the metachromatic and allochromatic colorations in some cases. It is believed, from some unpublished work, that Lison's conclusions



TEXT-FIG. 1. Parts of transverse sections of the stomach of *Glossiphonia*.

are a little over-pessimistic, and that under certain well-defined conditions distinctions can be drawn between certain classes of lipoid; this will be the subject of a separate paper. In the meanwhile, it must be emphasized that no more specificity is claimed for this method than that a red coloration with an aqueous solution of nile blue does show a lipoid. Its value here is purely morphological, as an adjunct to the sudan-black method.

These methods are further discussed below.

RESULTS

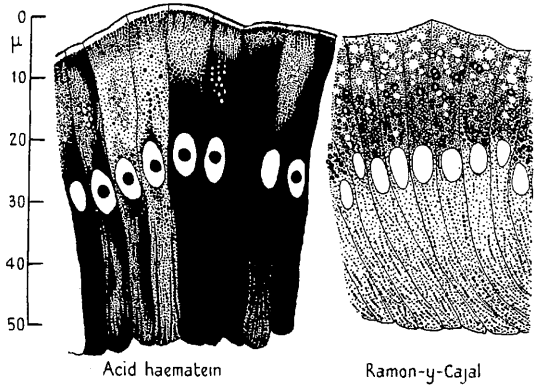
(I) *Description of the Golgi apparatus in the alimentary canal of* Glossiphonia

The stomach and intestine of *Glossiphonia* are both very distensible, and the shape of the epithelial cells varies greatly, so that the differences figured by Brumpt (1900) are not always as clear as one might wish. The surest distinction is the position of the lateral diverticula relative to the testes. Those of the stomach lie between or outside the testes, those of the intestine above them.

When the alimentary canal is not distended, both epithelia may be described as columnar (Text-figs. 1 and 2). The nucleus, with a conspicuous

plasmosome, lies in the middle region of the cell. The mitochondria are massed at the base of the cell (most proximal part) and may occupy almost the whole of that region up to the nucleus, but a few are scattered in the distal area, chiefly away from the Golgi region, and close to the sides of the cell. The main mass of the Golgi apparatus, in both epithelia, lies immediately distal to the nucleus (that is, on the side towards the lumen of the gut), but parts may extend down its sides, or even surround it.

The constituents of the Golgi apparatus, as shown in undistended epithelia by the standard methods, appear as numerous uncoloured globules, nearly



TEXT-FIG. 2. Parts of transverse sections of the intestine of *Glossiphonia*.

every one of them surrounded, at least in part and usually completely, by a shell of strongly osmiophil and argentophil substance. Almost all are arranged in lines parallel with the long sides of the cell. If the globules in one cell are more or less uniform in size, then that size is at the lower end of the range of variation seen. If they are not uniform, then usually a wide range of size is seen, the largest having no shell and being in the most distal part of the cell. The rest of the cytoplasm in osmium preparations is a general grey, usually rather darker in the region of the mitochondrial mass. In toned silver preparations this area is definitely darker than the rest of the cytoplasm. There is no definite evidence for a diffuse osmiophil or argentophil substance specially concentrated in the Golgi region.

The standard techniques usually fail to show anything in the stomach epithelium, but are quite reliable for the intestine. A good Da Fano stomach preparation was obtained, but the Mann-Kopsch technique usually failed completely; and in the only good preparation obtained, the stomach was in the distended state, and the cells so reduced in height that interpretation was very difficult.

(II) *Composition of the parts of the Golgi apparatus*

In both epithelia, after coloration with sudan black, the Golgi apparatus is seen as a crowd of black, usually nearly spherical bodies, arranged in rows as before; occasionally some are apparently coalescing. There is no special indication of a diffuse lipoid between them. A colourless internum is not to be seen.

After acid haematein, it is seen that in the stomach the Golgi apparatus is stained blue quite deeply, the mitochondrial mass much less so. The cytoplasm is greyish from the Golgi area to the free edge, colourless or faintly yellowish elsewhere. The nucleus is almost colourless, except for the plasmosome, which varies from brown to black. The pyridine extraction test gives a completely negative result (except for the nucleus and plasmosome) in both stomach and intestine. As before, the apparatus takes the form of spherules or nearly spherical bodies arranged in rows, more or less, and again, it is difficult to see in any a definite internum. In the intestine, an altogether different appearance is seen. Staining with acid haematein is by far the best method of distinguishing between these two parts of the alimentary canal if there is any doubt. Almost every intestinal cell is full of the blue stain, sometimes so much so that it seems black rather than blue. The plasmosome is again stained, more usually black than brown, and the rest of the nucleus is the only transparent part of the cell. It is usually pale-yellow or yellowish-brown. Nearly always, the whole of the intestine and its diverticula that can be seen in the section is stained like this (and, on a few occasions, the most posterior part of the posterior pair of stomachic diverticula may appear the same), but sometimes a cell here and there, or a small group of cells, is seen to be comparatively clear. In that case the mitochondrial mass is still very darkly stained, and there is a blue patch just inside the free border; but the Golgi region is almost unstained. Often spherules can be seen, but by refraction; a few grains are usually stained, but are very small, and extremely like the small mitochondria in the most distal region. Sometimes, when the whole cell is stained blue, small clear bodies can be seen clustered in the Golgi region. They resemble, quite closely, those seen in that region in unstained sections.

Staining with nile blue by Lison's method shows the Golgi apparatus in both stomach and intestine as spherules (or nearly spherical bodies), darker blue than the surrounding cytoplasm and slightly redder in tone. (As globules of fat in the cell, if present, are coloured red, so that confusion is very unlikely, this is a good, simple method for demonstrating the Golgi apparatus.) Their disposition in the cell is as already described. After prolonged differentiation, quite another picture is seen, but unfortunately a rather faint one. The cytoplasm is now colourless or slightly yellow, the nucleus a very pale blue. The spherules of the Golgi apparatus are seen as colourless or faintly pink globules with definitely pink shells. The internum can now be distinguished clearly from the externum, and it appears to be the latter which is coloured. Under the low powers only, a general diffuse

pinkness is seen in the Golgi region; this is probably due to the colour in the shells of spherules that are out of focus.

Discussion

Lison (1936), speaking of the use of the lipoid colorants, says: 'La spécificité de ces méthodes est parfaite; seuls les lipides donnent des réactions positives.' Nevertheless, if a piece of paper be coloured with sudan black by either Baker's or Lison's methods, it will be seen to retain a faint coloration. And the same depth of colouring will be produced on paper that has first been soaked in pyridine for 24 hours at 60° C.—a procedure that, after suitable fixation, in Baker's pyridine extraction control test results in removing all lipoid except the faintest trace of lipine in the fat cells from a piece of *Glossiphonia*, as may easily be seen by taking a section prepared by the pyridine extraction test method and colouring it with sudan black, instead of staining with acid haematein in the regular way. With such a section, sudan IV, used in the same way as sudan black, gives a quite definite general pink tinge, as it does with paper. It seems reasonable to conclude that if a tissue is not coloured by the very powerful sudan black; a coloration by sudan IV does not show a lipoid. The general pale-pink tinge given by sudan IV has no significance. It is possible, I suppose, that such a powerful colorant as sudan black may actually be colouring an absorbed lipoid, not removable by pyridine, on the paper. However, for very faint colouring by sudan black there remains a slight doubt, unless it disappears after pyridine extraction. As the gut cells of *Glossiphonia* are completely uncolourable by sudan black after pyridine extraction, the point does not arise in this case.

The coloration of paper by sudan black was first noticed by Dr. J. F. A. McManus, who pointed it out to me in conversation.

Nile blue preparations made by the method quoted by Lison show the Golgi apparatus quite clearly, in a darker and slightly redder blue than the surrounding cytoplasm. Tarao (1939) noticed that it was 'blue with red tone' in the liver cell of the mouse. The result with prolonged differentiation makes it clear that this is principally, if not entirely, a case of allochromasy, not metachromasy; but this method is chiefly valuable for showing that the lipoid substance is greatly concentrated in, or confined to, the shell or externum. This makes it very probable that the only reason why the internum was not visible in the sudan black preparations was that the shell was so strongly coloured as to be opaque. That at least the principal lipoid in the Golgi apparatus of the stomach cells is lipine is shown by the positive result with Baker's test. It is suggested, therefore, that the Golgi apparatus in the stomach is composed of rows of (comparatively) fat-free globules with lipoid shells containing lipine.

In the intestine, as exactly the same picture is obtained with sudan black and the standard techniques, the structure appears to be the same as in the stomach, but with this important difference, that where the Golgi apparatus is visible after acid haematein, it is seen to contain little or no lipine as shown by

that test. That it contains much the same amount of lipoid as the stomach Golgi is seen from the sudan black preparations; and as the largest globules, nearest the free border, are not osmiophil or argentophil, it seems likely again that the opacity of the spherules with sudan black is due to intensity of coloration, as in the stomach. It is worth noting that the amount of lipine in the rest of the cell seems to vary inversely with that in the Golgi, there being very little in the stomach cells, and usually a great deal in the intestine. Nevertheless, the bulk of mitochondrial substance appears to be much the same in both. As lipine appears to be particularly plentiful in the actively metabolizing nephridial cells (where it is confined to the very long and numerous mitochondria), and in the fat-cells, where it occurs throughout the cytoplasm, it might be suggested that the abundance of lipine in the intestinal cells is correlated with the active role of the intestine as the food-absorbing region (see Brumpt, 1900).

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SUMMARY

(1) The standard silver and osmium techniques show a typical Golgi apparatus in the epithelial cells of the stomach and intestine of the leech, *Glossiphonia complanata*.

(2) Histochemical studies reveal the presence of lipoids in the Golgi region of these cells. In the stomach, part, at least, is lipine. (In the intestinal cells, the rest of the cytoplasm contains much lipine, but the Golgi apparatus little or none that can be shown by the method used.)

(3) The Golgi apparatus in these cells appears to consist of rows of non-lipoidal spheres, each with a lipoid coat. In the stomach, this coat contains lipine.

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