

The Fine Structure of the Adipose Cell of the Leech *Glossiphonia complanata*

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ABSTRACT

The two distinct types of cytoplasm seen with the light microscope in the adipose cell of the leech *Glossiphonia complanata* have been identified in the electron microscope image of this cell.

One of these, the basophil cytoplasm, contains many well oriented, paired membranes which are much more clearly evident when calcium ions are added to the fixative. The membranes sometimes appear as concentric arrays of lamellae and are thought to represent sections through a phospholipide-containing body. The paired membranes and the concentric lamellae have granules attached to them and resemble in size and structure the membranes of the endoplasmic reticulum encountered in many mammalian cells.

Small dense cytoplasmic particles are present throughout the cell; they may be ferritin molecules, derived from the breakdown of haemoglobin taken in as food.

On the basis of a previous histochemical study and the present electron microscope investigation, it is suggested that these paired membranes are similar to the organized type of mammalian ER and the results seem to confirm the belief that these membranes are composed of layers of phospholipoprotein together with attached particles of ribonucleoprotein.

During a recent investigation of the connective tissue of the pond leech, *Glossiphonia complanata*, histochemical studies were made on the adipose cell, which acts as the main centre for storage of reserve food substances (Bradbury (4)). This cell is interesting, as it contains two distinct cytoplasmic regions, one of which colours intensely with basic dyes, and surrounds the fat drops and the nucleus and is sharply marked off from the second, the peripheral cytoplasm of the cell. This basophil cytoplasm has been termed "ergastoplasm" by Bobin (3), but as no evidence about its submicroscopic structure was then available, a new name was suggested—the "surround"—which had no functional implications (Bradbury (4)). When the cell is studied with the light microscope, very little structure is visible in this region of the cytoplasm, though by means of the acid haematein test of Baker (1) phospholipide droplets or bodies are found embedded in it; histochemical tests showed also in the same region of the cell the presence of much ribonucleic acid and protein.

The present study of this material with the electron microscope was made in the hope of discovering whether there was any organized submicroscopic structure in this basophil cytoplasm, and if so, to see whether this could be correlated with the known histochemistry of the cell.

Material and Methods

Specimens of *Glossiphonia complanata*, collected near Oxford, were killed in ice-cold OsO₄ (a 1 per cent solution made up with sucrose and sodium veronal acetate-hydrochloric acid buffer at pH 7.3 as recommended by Caulfield (6)). After about a minute, the leech was transferred to fresh ice-cold fixative and cut into millimetre cubes with a very sharp razor blade. The period of fixation was 1 hour. The tissue was dehydrated through a series of graded alcohols, and then embedded in *n*-butyl methacrylate or in *n*-butyl methacrylate containing 10 per cent methyl methacrylate. Sections were cut on a Porter-Blum microtome; only those sections showing silver or gold interference colours were examined. After being mounted on Smethurst highlight grids covered with a carbon film, the sections were studied in a Siemens Elmiskop I operated at 60 kv.

At the suggestion of Dr. J. R. Baker, a further series of fixations was made, replacing the sucrose in the fixative mixture with calcium chloride to give a final concentration in the fixative of 1 per cent (following the procedure of Chou and Meek (9)).

OBSERVATIONS

The most conspicuous feature of this adipose cell is the large lipide drop (Figs. 1 and 2) which appears from histochemical studies to consist largely of triglycerides. In thin sections, this large lipide drop nearly always cut badly so that in the electron micrographs it appeared to be ridged, but no other structure is visible in it. Its edges appear crenated, but since the drop always appears perfectly spherical when studied with the light microscope in living cells, it is supposed that these crenations are artifacts, possibly a result of shrinkage during dehydration or embedding.

The basophil cytoplasm which forms the "surround" is clearly visible in electron micrographs (Fig. 2). In this figure there is some suggestion of a membranous structure in this region of the cell, but the membranes are not very well defined. Many filamentous mitochondria are embedded in it, but they are also seen to lie free in the peripheral cytoplasm, and there may be a significant concentration of them along the boundary of the basophil cytoplasm. The internal cristae are not here very obvious, but where they are visible, they appear to be plate-like.

When calcium ions were added to the fixative, the results were very striking. All the cytoplasm forming the "surround" then appeared to consist almost entirely of membranes (figs. 3 to 5). Close study of the structure of these membranes showed them to be paired and all were found to have granules adherent to their outer surfaces (Figs. 4 and 5). These granules were very similar in appearance and distribution to those described by Palade (11) as constituting a distinct cytoplasmic component sometimes associated with part of the endoplasmic reticulum. In some adipose cells, the membranes in the basophil cytoplasm were oriented at random (Fig. 5) but in others they seemed to be arranged in parallel layers, resembling the typical arrangement of the endoplasmic reticulum in the pancreatic exocrine cell of the mammal (Palade and Siekewitz (13)) or the Nissl substance of the mammalian nerve cell (Palay and Palade (14)).

The membranes of the adipose cell were found

in places to be arranged concentrically, forming structures about 1 to 3 μ in diameter. From a study of the cell with the light microscope, and histochemical techniques, it seems very probable that these represent sections through the bodies which contain phospholipide and are to be found in this part of the cell. These concentric arrays of paired membranes were first noticed in preparations that had been fixed in the calcium-osmium mixture, but further study of micrographs of material that had been fixed in the standard buffered osmium showed them to be present in the basophil cytoplasm, though they were not very distinct (Fig. 2). The membranes do not appear to compose the whole of the globule; in the centre there appears to be a vesicular "core" (Fig. 4).

It was possible to measure the dimensions of both the membranes and the attached particles. The thickness of each membrane was about 75 A, the separation between the two membranes forming the pair, about 150 to 200 A, and the diameter of the granules 125 A. Membrane pairs were separated by about 750 A.

In all the micrographs of material fixed in the calcium-osmium mixture, the whole of the cytoplasm of the adipose cells was seen to contain numerous very small, dense granules (Fig. 5). At first it was thought that they represented some compound of calcium. When they were seen at higher magnification, however, it became obvious that they were not single granules but each appeared to consist of four sub-units (Fig. 5 a). The whole complex granule was about 60 A in diameter, the subunits about 30 A. It is interesting to note the resemblance between these granules in the adipose cell of this leech, and the pictures of ferritin published by Richter (16). From histochemical work (4) we know that the adipose cells of the leech contain large quantities of haemosiderin derived from the blood which constitutes the food, and (as Richter points out) ferritin may be a component of haemosiderin. Since no specific studies were made on this type of granule, it is not possible to say with absolute certainty that they are ferritin molecules, but it seems that this explanation is the most probable one.

A further interesting feature, which was much more clearly shown when the fixative contained calcium ions, was the complex nature of the cell membrane which in many places was seen to have complicated infoldings. These were usually formed of parallel cytomembranes, but unlike those of the basophil cytoplasm, they did not have any

granules on their surfaces. In this respect they resemble Sjostrand's β -cytomembranes (Sjostrand (18)) and it seems that they may serve to increase the surface area of the cell membrane. As this cell is thought to be a very active centre of metabolism in this animal (4), these infoldings may well be a means of obtaining efficient and rapid transport of substances through the cell membrane.

DISCUSSION

Both parallel lamellae and concentric arrangements of membranes have been described before in the cytoplasm of cells. Weiss (22) observed concentric membranes in the ergastoplasm of active rat pancreas cells. He pointed out that in this cell such cytoplasmic structures are centres of organization of the ergastoplasm. Palay and Palade (14), also noticed similar structures in the cytoplasm of mammalian nerve cells; they considered them to be the Nissl substance or a specialized part of the endoplasmic reticulum. In neither of these studies was there an attempt to correlate the electron microscopy with histochemical studies on the same type of cell.

More recently, Stoeckenius (21) and Policard, Bessis, and Breton-Gorius (15) have described concentric structures in the cytoplasm of phagocytes which have ingested red blood corpuscles. It is suggested that the concentric lamellae represent bimolecular layers of lipide derived from the lipoprotein of the red cell stroma. This accords well with the data from polarized light studies carried out by Schmidt (17) and by Frey-Wyssling (10), who have shown that lipides, especially phospholipides, tend to form layers or lamellae. In the case of a bimolecular layer of phospholipide they calculated a thickness of 63 A, a figure which is very close to the measured value of about 60 to 80 A found by Policard and his coworkers, and of 75 A found in the present study.

In invertebrates, such concentric lamellae have recently been described by Chou and Meek (9) in the neurones of the snail *Helix aspersa*. In this cell, they were able to show by the use of Baker's acid haematein test (Baker (1)) that this appearance was due to inclusions consisting almost entirely of phospholipide. They did not observe any granules attached to the membranes.

In the present study of the adipose cell of *Glossiphonia* similar bodies have been noticed in the basophil cytoplasm of this cell. Histochemical studies of this region of the cytoplasm show that it contains large quantities of ribonucleic acid,

together with phospholipides and proteins. The phospholipides occur not only in well marked bodies, but also in a diffuse form (Fig. 1). These bodies, the so called lipochondria when seen with the light microscope, have a diameter of between 1 and 7 μ and, together with the mitochondria, they are the only conspicuous objects in this region of the cell. The mitochondria may be identified with certainty in the electron micrographs, but the only other structures of comparable dimensions in the basophil cytoplasm are the concentric lamellae; measurements show that they all have over-all diameters which fall within the range of those found for the phospholipide bodies which were seen with the light microscope and it is thus reasonably certain that the concentric lamellae represent sections through such bodies.

Morphologically, the phospholipide bodies in the adipose cell of this leech are not such well defined cytoplasmic inclusions as had been supposed. There is great similarity between the ultrastructure of one of the lipide bodies and that of the rest of the basophil cytoplasm; in places they seem to pass gradually into one another (Fig. 3), and often "strands" of parallel lamellae may be seen in this region of the cell. It may be that these phospholipide bodies represent a means of transporting substances between the cytoplasm and the triglyceride which forms the fat store. An objection to the view that these bodies are not discrete cytoplasmic entities is that in other cells, for instance in the neurone of the snail studied by Chou (7, 8) and later by Chou and Meek (9), no such intergrading is visible. In the living neurone, the phospholipide bodies are quite distinct, and there are no "strands" or special "surrounds" of protoplasm. In the electron microscope studies of this same cell type, though the phospholipide bodies can be shown to have a very definite lamellar structure, the remaining endoplasmic reticulum is not very abundant and not aggregated into such well marked membranes as in the mammalian pancreas or the adipose cell of *Glossiphonia*. It may be that, in the latter type of cell, the high degree of organization of the cytoplasmic membranes will prove, as in mammalian tissues, to be indicative of a very high degree of metabolic activity. This may account for the especial concentration of the cytoplasmic membranes and for the fact that their specialization into bodies or a "surround" is much more pronounced than in a cell which has not such a high metabolic rate.

On purely morphological criteria (structure, size,

and possession of granules) these cytoplasmic membranes in the leech adipose cell (Fig. 5) seem to correspond to the highly organized endoplasmic reticulum described by Palade and Porter (12) in some mammalian cells. This supposition should be considered in the light of the histochemical studies on the same region of the cytoplasm of the adipose cell of the leech; we suggest that here the membranes are principally composed of a protein and phospholipide, in the form of a phospholipoprotein. It may well prove that the phospholipide is serving purely to stabilize the protein into a membrane or sheet but further research will be needed to establish or refute this hypothesis. If the suggestions of Schmidt and Frey-Wyssling are accepted, it may also be justifiable to assert that the membranes are bimolecular, as the correspondence between theoretical and measured dimensions is so close.

It is unwarranted in cytology to generalize too widely from studies on a very specialized type of cell; nevertheless we considered that if similar *in situ* histochemical reactions could be obtained from another cell which was known to contain large amounts of well organized endoplasmic reticulum, there would then be further justification for the belief that those forms of the ER which show the membrane and granule structure are composed of phospholipoprotein with adherent granules of ribonucleoprotein. The possibility of the existence of a large proportion of unreactive lipide in cells was put forward by Bensley and Hoerr (2) and confirmed by the much more recent studies of Smith and his collaborators (20), who showed that in liver cells the residue which remains after repeated extractions of soluble proteins is at least 45 per cent lipoprotein. The fact that this proved so difficult to extract suggests very strongly that it is intimately connected with the structural organization of the cell. *In situ* histochemical studies on the exocrine cell of the mouse pancreas have been made, using a new technique for "unmasking" lipides (Bradbury and Clayton (5)). The basal region has been shown by Palade and Siekewitz (13) and Sjostrand and Hanzon (19) to contain a well organized ER; it can also be shown by standard histochemical methods to contain much protein and RNA. This same part of the cell, however, which was previously thought to be lipide-free, now gives a positive test for phospho-

lipides after the "unmasking" procedure has been applied.

It is now seen that structurally, the ER membranes in both the pancreas cell and the leech adipose cell are essentially the same, and from the histochemical results it seems that there is also some fundamental similarity in chemical composition between these two regions of cytoplasm which contain the organized ER. Since we have shown a correspondence between the presence of membranes and phospholipide in the adipose cell of *Glossiphonia* and also in the mammalian pancreas cell (Bradbury and Clayton (5)), it thus seems that the hypothesis that the membranes forming the ER are predominantly phospholipide in nature receives added support.

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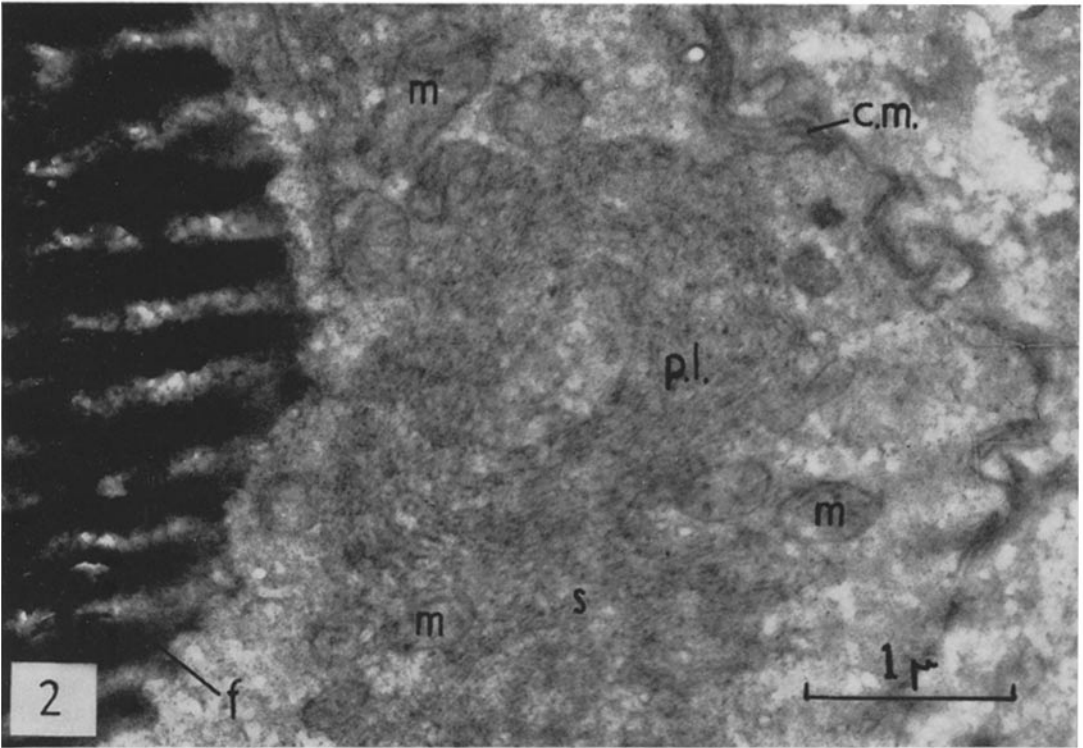
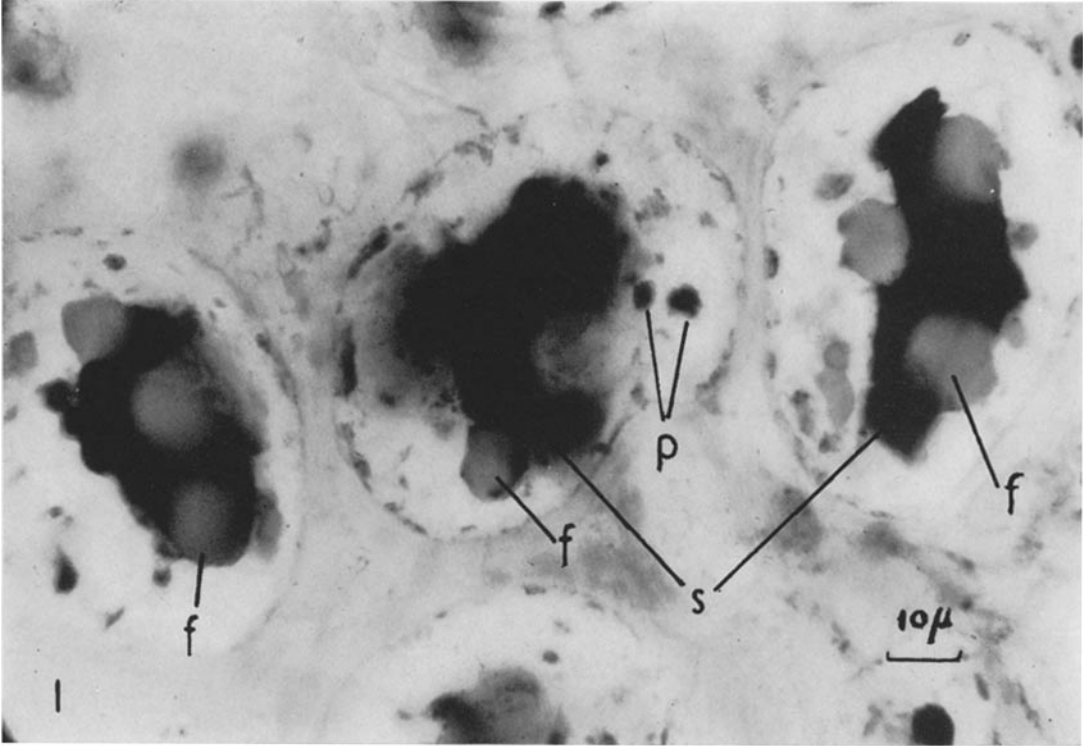
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EXPLANATION OF PLATES

PLATE 286

FIG. 1. A light micrograph of three adipose cells of *Glossiphonia complanata*. The slide was prepared by the acid haematein technique for phospholipides, and the neutral lipides were subsequently coloured with Sudan IV. Note the large droplets of fat (*f*), the phospholipide forming the "surround" (*s*), and two lipochondria (*l*). $\times 1000$.

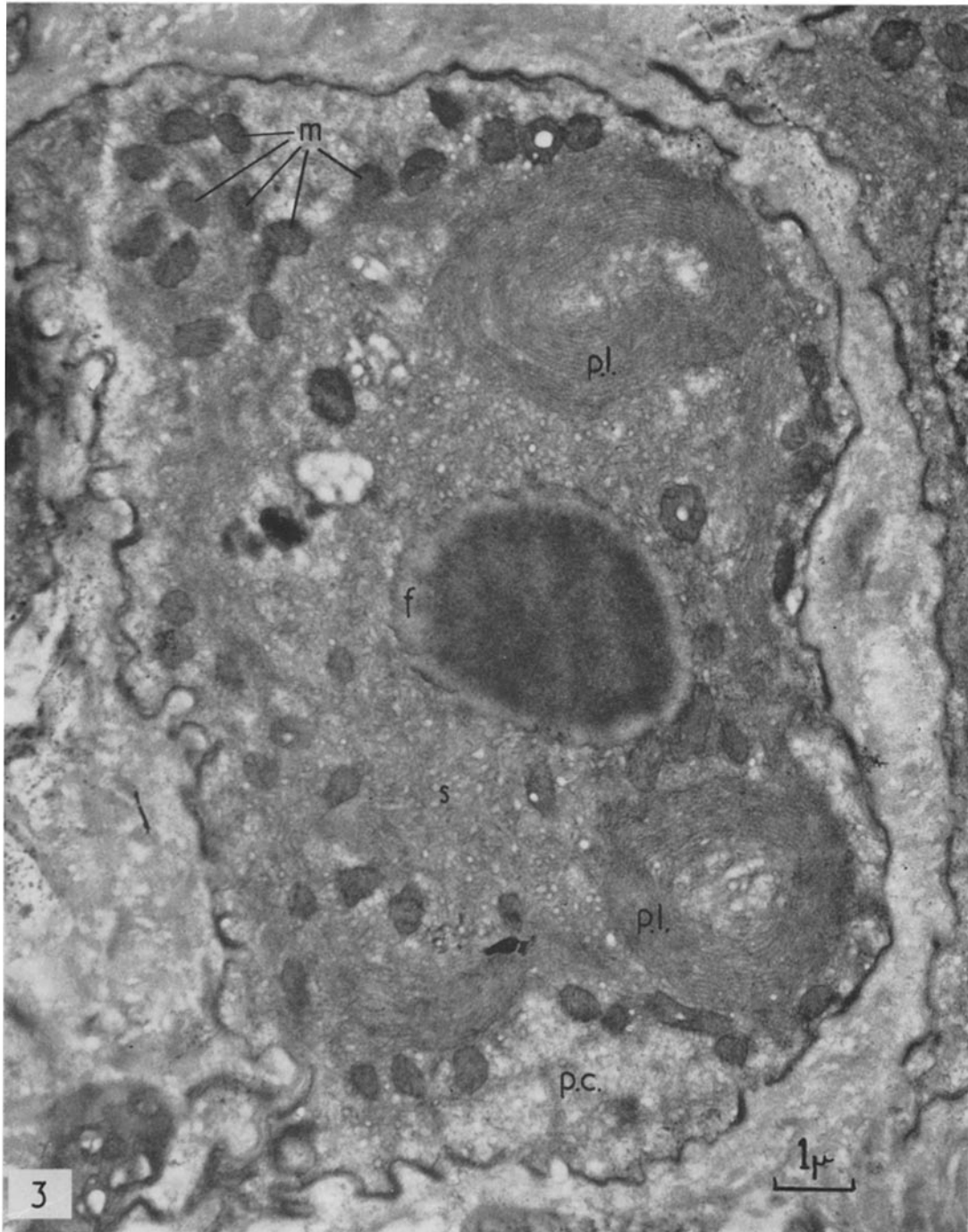
FIG. 2. An electron micrograph of part of an adipose cell fixed in Caulfield's fixative. The "surround" (*s*), mitochondria (*m*), part of the fat drop (*f*), and the multilayered cell membrane (*c.m.*) are clearly visible. The concentric appearance at *p. l.* is thought to represent a section through a phospholipide droplet. $\times 27,000$.



(Bradbury and Meek: Adipose cell of leech)

PLATE 287

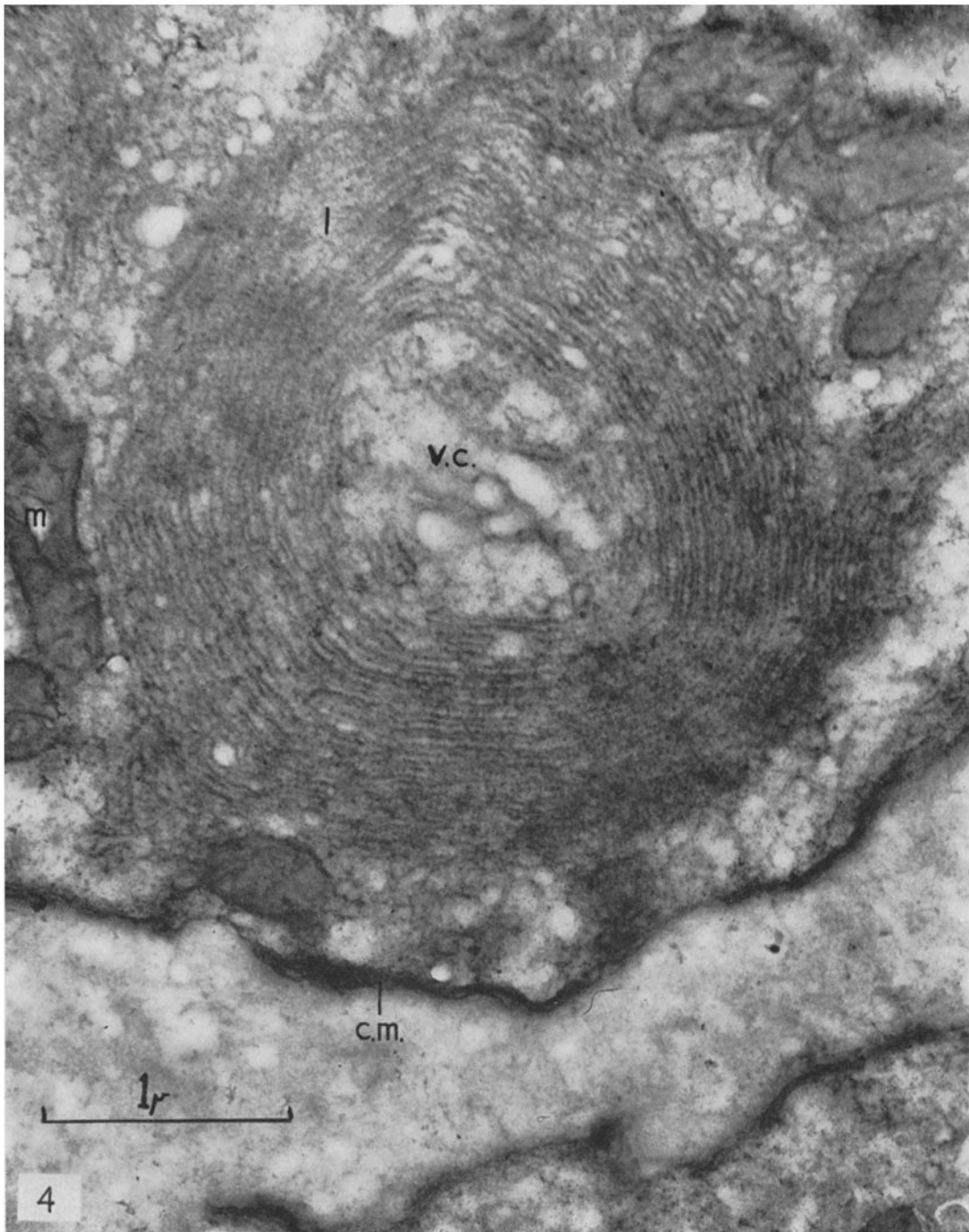
FIG. 3. An electron micrograph of an adipose cell of *Glossiphonia* fixed in calcium-osmium mixture. The distinction between the "surround" cytoplasm (*s*) and the peripheral cytoplasm (*p.c.*) is now very marked. Mitochondria (*m*), part of the fat drop (*f*) and sections of two phospholipide droplets (*p.l.*) are visible. $\times 12,000$.



(Bradbury and Meek: Adipose cell of leech)

PLATE 288

FIG. 4. One of the phospholipide bodies of the cell shown in Fig. 3 at higher power; note the vesicular core (*v.c.*) and the well marked lamellar structure. $\times 38,000$.

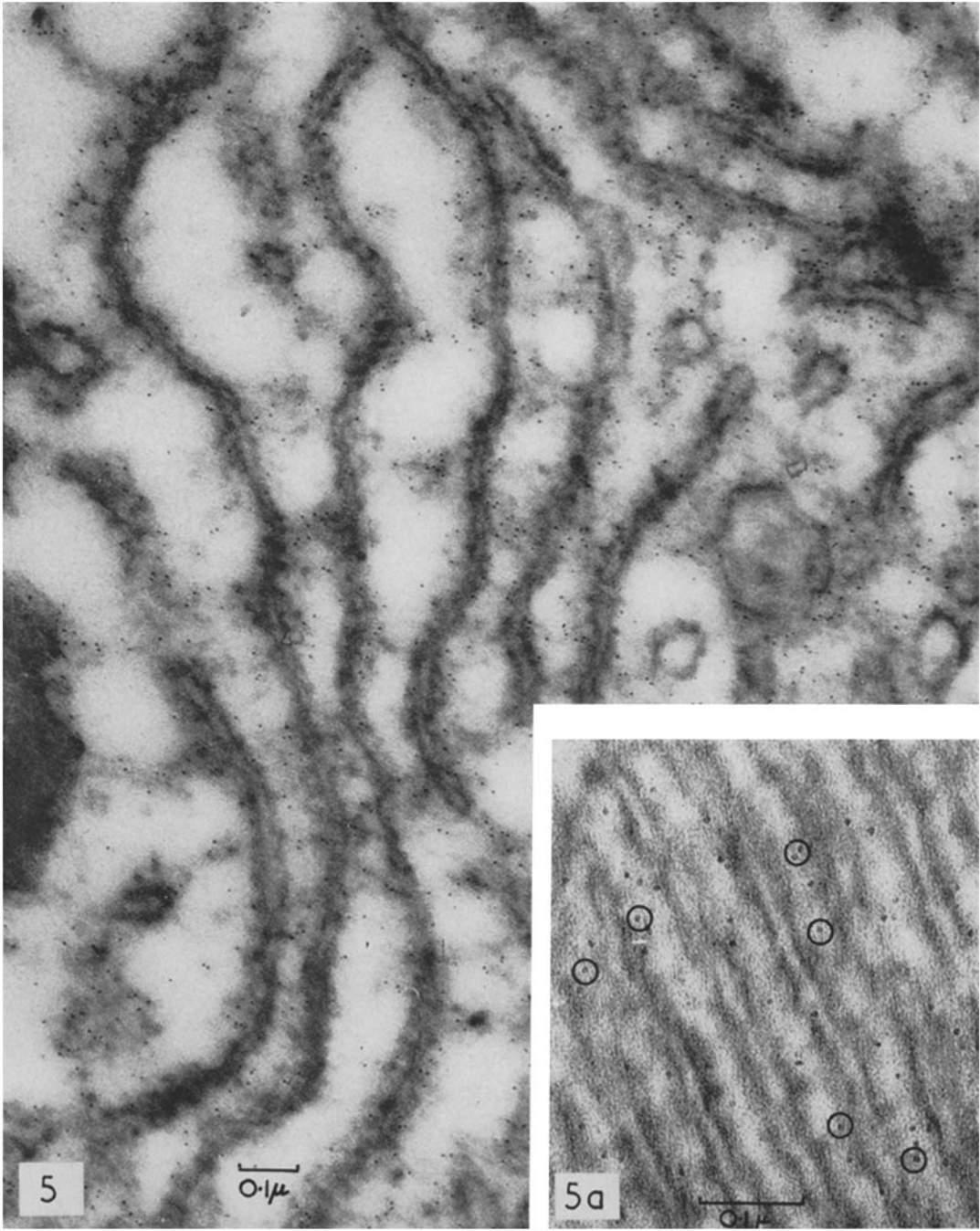


(Bradbury and Meek: Adipose cell of leech)

PLATE 289

FIG. 5. Part of the basophil cytoplasm of an adipose cell of *Glossiphonia* fixed in calcium-osmium mixture. The structure of the paired membranes with their attached granules is well shown. $\times 83,000$.

FIG. 5 *a* (inset). Paired membranes from a similar region to Fig. 5 at higher magnification, showing the small dense particles (encircled) which are thought to represent ferritin. $\times 165,000$.



(Bradbury and Meek: Adipose cell of leech)