

THE STRUCTURE AND FORMATION OF PROTEIN GRANULES IN THE FAT BODY OF AN INSECT

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ABSTRACT

In the larva of the butterfly *Calpodés ethlius*, the fat body begins to store protein in the form of granules at about 30 to 35 hours before pupation, at a time when the endocuticle is being resorbed. At least two sorts of granule can be distinguished. The first granules to arise are those within vesicles of the Golgi complex. These may increase in size by incorporating material from microvesicles at their surface and by coalescence with one another. Later, at about 10 hours before pupation, another sort of granule arises by the isolation of regions of the endoplasmic reticulum (ER) within paired membranes derived from Golgi vesicles. Several of these ER isolation bodies coalesce, with fusion of their outer isolating membranes. The ribosomes and membranes may then disappear and the granules become indistinguishable from the protein granules formed from Golgi vesicles, or the ribosomes may remain and be embedded in dense crystalline protein, forming a storage body for both protein and RNA. Mitochondria are isolated within paired membranes in the same way as regions of the ER. The isolated mitochondria also coalesce in a similar manner. When the inner membranes are lost, the structure of a group of isolation bodies is indistinguishable from that of a cytolysome. Isolation within paired membranes, as described here, may be of general importance in segregating regions of massive lysis or massive sequestration.

INTRODUCTION

The mechanism by which reactions may be localized within a cell, each with its appropriate environment, poses a general problem. There is a need for the physical separation of processes, which becomes most obvious in cells undergoing gross changes, both massive synthesis and massive lysis. The fat body of insects provides useful material for a study of this problem, for it commonly contains a single cell type which is functionally diverse. At various times it stores and mobilizes fat, protein, and glycogen which it may have synthesized or sequestered (10, 11). It may also play a part in excretion by synthesizing and storing uric acid (12). Many of these events depend upon both the hormonal and nutritional milieu, so that there

are sequential changes during the cycles of molting and feeding in larval life, and during metamorphosis from the larva to the pupa and from the pupa to the adult. The fat body cell is thus ideal for studying patterns of synthesis that are changing in space and time.

In this study we have been concerned particularly with the morphology and origin of several sorts of protein granule and the membranes which surround them in the fat body of the butterfly, *Calpodés ethlius*. We have also tried to relate the formation of these structures to other physiological events taking place at metamorphosis. The structural changes in the fat body have turned out to be extraordinarily complex. To simplify the presentation, this report is restricted to the origin of

the granules. The source of the protein and the fate of the granules will be discussed separately.

MATERIAL AND METHODS

Changes in the fat body of *Calpodex ethlius* Stoll (Lepidoptera, Hesperidae) have been followed through the 4th, 5th, and pupal stadia into the adult. Larvae in the stadium before they were needed were transferred from a greenhouse to an incubator at 22°C in the dark. Under these conditions the 4th stadium lasts 87 hours (SD 9 hours), the 5th, 168 hours (SD 18 hours), and the pupa 10 days (SD 1 day). Most events in the molting cycle can be timed from the moment of eclosion from the exuvium. This system of timing has disadvantages in that changes are most rapid and extensive shortly before ecdysis when the method of dating is least accurate. To make the timing more precise before molting, two other markers were used. In the 4th stadium the thoracic segments enlarge at about 27 to 25 hours before ecdysis. In the 5th stadium the rectum empties slightly after the rest of the gut at about 28 hours before pupation.

Glutaraldehyde (5 per cent with 0.1 M phosphate buffer at pH 7) at 0 to 4°C for 1½ to 12 hours was used as a fixative for all material, for both the light and electron microscopes. For the electron microscope the material was postfixated for 1 to 3 hours in 1 per cent osmium tetroxide in 0.1 M phosphate buffer, at pH 7, containing 4 per cent sucrose. Rapid initial fixation of all the body organs was obtained in the following way. The larvae were decapitated and the gut contents and some blood allowed to escape; the thorax was then ligated and the hind part inflated by the injection of cold fixative, after which the whole larva was immersed in iced water for 2 minutes. Small pieces were cut from the 7th, 8th, and 9th segments for further fixation.

For electron microscopy best results were obtained with silver or gray sections mounted unsupported and coated with carbon. Before coating, the sections were stained for 5 to 20 minutes in saturated uranyl acetate in 70 per cent ethanol or absolute methanol (7) followed by 5 minutes in lead citrate (5).¹

¹ A very simple device has been used for the routine handling of large numbers of sections to give identical staining conditions with no contamination. 1-mm rings were cut from a polyethylene tube or vial with an outside diameter of about 8 mm. The inside of the ring was scored halfway through, with a thin razor blade, into 4 quadrants. When the ring is compressed in line with one pair of cuts by squeezing gently with coarse forceps, the cuts open and a grid can be inserted into each. A ring can carry 4 grids. The whole ring floats on aqueous stains or sinks in alcohol. A stream of reagent from a wash bottle is passed through the ring, drop by drop, to wash the

For histochemistry the material was embedded in ester wax and sectioned at 4 to 8 μ. Sections were prepared from a series timed at all stages from the early 4th instar larva to the adult. Stains used routinely, according to the methods mentioned by Pearse (4), included: for ribonucleic acid (RNA), methylene blue at pH 2-10 and methyl green-pyronin, both with and without ribonuclease; for proteins, especially tyrosine-rich proteins, Millon reaction, bromophenol blue, the Morel-Sisley diazotization with 1-amino-8-naphthol-4-sulphonic acid (S-Acid), the dinitrofluorobenzene (DNFB) method followed by diazotization and treatment with 1-amino-8-naphthol-3:6-disulphonic acid (H-Acid); for glycogen, the periodic acid-Schiff reaction. Changes were also followed using the natural fluorescence of unstained sections excited by ultraviolet (UV) light. The osmium-ethyl gallate staining method (Wigglesworth, 13, 14) was used for comparison with electron micrographs and to show lipid droplets.

Changes in the proportion of cell components were estimated using an ocular micrometer disc with 50 randomly positioned dots. The component under each dot was recorded in a series of samples over the field.

RESULTS

Light Microscopy

In the 4th stadium the fat body stores mainly lipid. In the 5th and pupal stadia, glycogen and protein appear and the pattern of lipid storage changes markedly. These differences between the 4th-5th molt and the 5th-pupal metamorphosis reflect the marked changes in pattern of syntheses. Protein granules appear towards the end of the 5th stadium, 30 to 35 hours before pupation. They have a natural yellow fluorescence when excited in the ultraviolet, as would be expected for proteins containing aromatic amino acids. They stain clearly with the protein stains mentioned in Materials and Methods. Fig. 1 *a* shows the granules in a new pupa stained with bromophenol blue, and Fig. 1 *b* shows the granules after treatment with DNFB, diazotization, and treatment with H-acid. The former stain is a general protein stain, and the latter procedure indicates tyrosine and NH₂ and SH groups. With these methods the granules stain in varying shades which are not correlated with size. From these results it was not possible to correlate the size of the granules with the size of the grids. The grids drain instantly when the ring is placed on filter paper. This simple technique is time saving and fool-proof; it has consistently given the cleanest, best stained grids we have obtained.

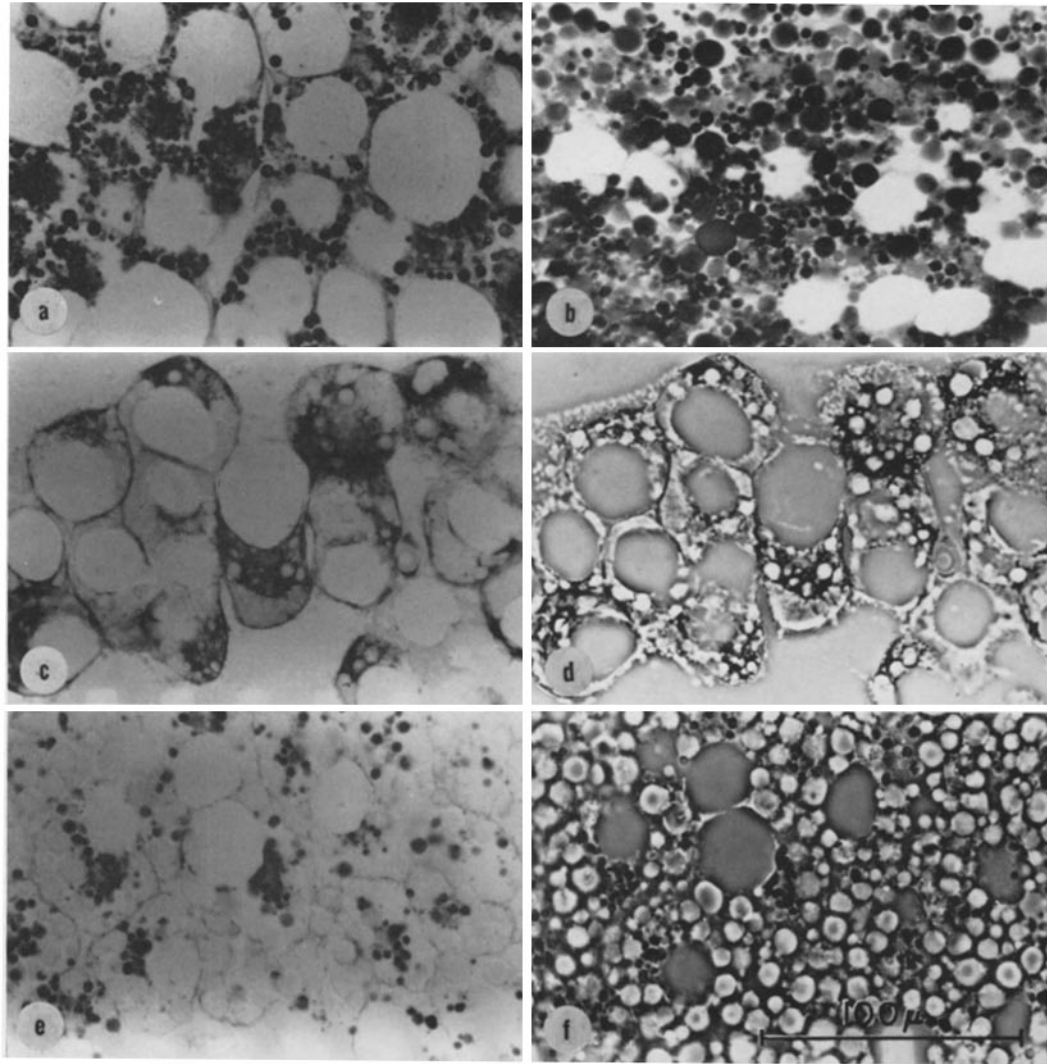


FIGURE 1 Sections of the fat body in *Calpodes ethlius*. The clear circular areas are lipid. $\times 350$.

Fig. 1 a: Pupa 6 hours old, bromophenol blue. Several kinds of granule are present but they cannot be distinguished by this stain.

Fig. 1 b: Pupa 18 hours old, DNFB, diazotization and treatment with H-acid. All the granules stain for protein.

Fig. 1 c: 5th instar larva 27 hours before pupation, methyl green-pyronin. Part of each cell contains RNA which outlines the protein granules just appearing.

Fig. 1 d: The same field as Fig. 1 c viewed with phase-contrast microscope. The protein granules stand out clearly.

Fig. 1 e: Pupa 18 hours old, methyl green-pyronin. All the RNA is now in granules.

Fig. 1 f: The same field as Fig. 1 e under phase-contrast microscope, showing that the cells are now almost filled with the two sorts of granule.

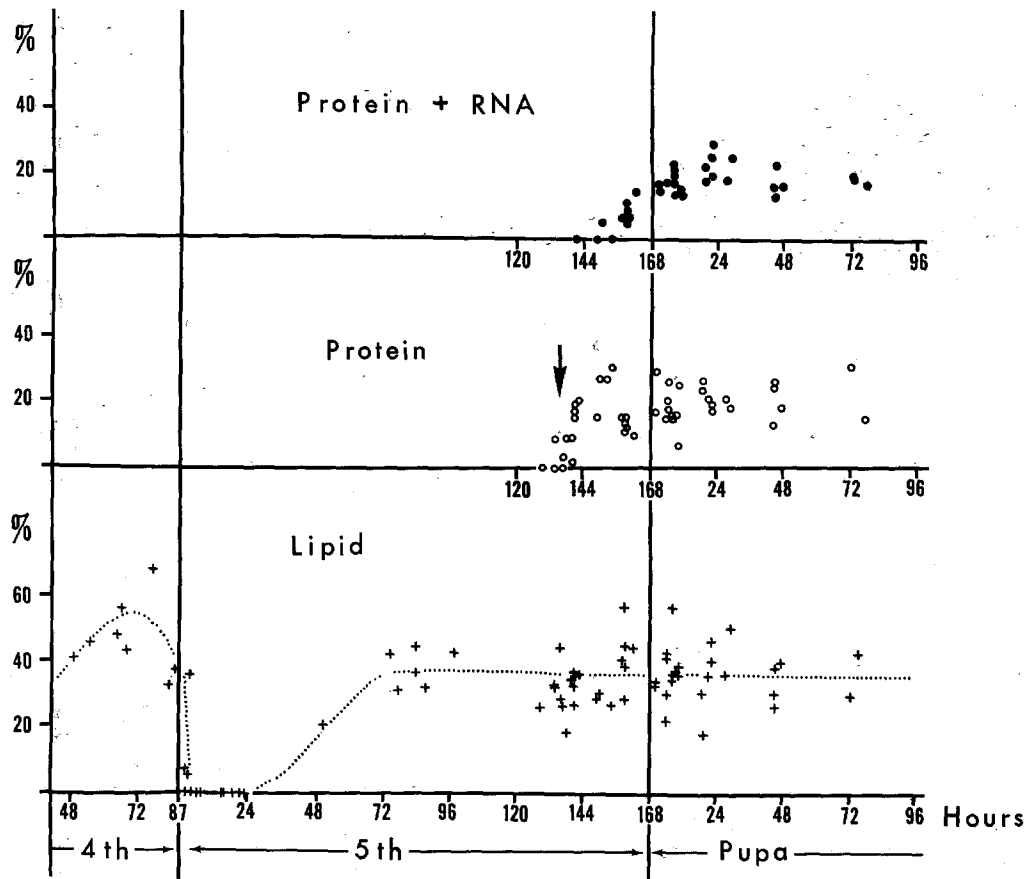


FIGURE 2 Changes in the composition of the fat body at various stages. Ordinate: percentage of each component, sampled with a randomly dotted eyepiece. Abscissa: age in hours during the latter part of the 4th stadium, the 5th stadium, and the first half of pupal life. The arrow marks the time at which the 5th instar endocuticle begins to be resorbed by the epidermis, coinciding with the beginning of deposition of protein granules in the fat body.

sible to distinguish different sorts of granule or different stages in their formation, but there is little doubt that all the granules are composed mainly of protein.

A completely different picture is obtained after staining for RNA. This staining shows that there are two different sorts of protein granule, one containing protein alone and one containing protein and RNA. The first formed granules contain no RNA. Until about 10 hours before pupation all the RNA is distributed in strands of cytoplasm around the protein granules and lipid droplets, and through regions of glycogen (Figs. 1 *c* and 1 *d*). A little later nearly all this RNA becomes gathered in granules which also stain for protein, until

in the new pupa the cell is almost devoid of RNA not in granules (Figs. 1 *e* and 1 *f*).

In the pupal fat body, parts of some granules of both sorts are birefringent even when in mounting media, suggesting that the protein sometimes forms crystals. In the pupa the granules are fewer and larger: many granules of both sorts appear to have increased in size by coalescence.

In order to correlate changes in the fat body with other physiological events, the results just outlined were quantified. The amount of different components was estimated from sections, using the random dot sampling technique. Fig. 2 shows how the percentage composition of the fat body alters at different times. Some of the scatter may be due

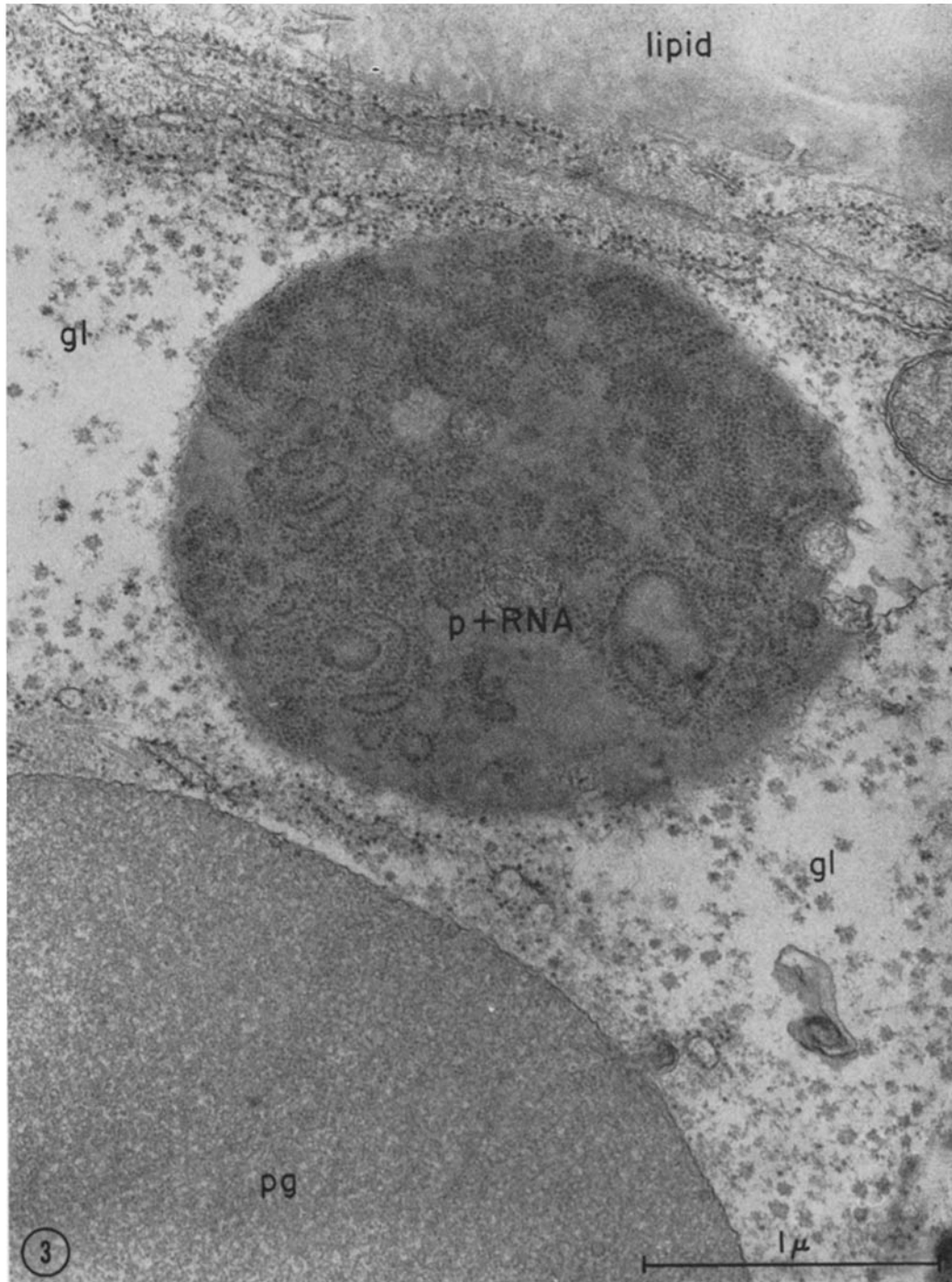


FIGURE 3 The fat body in a pupa 38 hours old. The four main components stored by the fat body:—lipid, glycogen (*gl*), protein + RNA granules (*P + RNA*), and protein granules (*pg*). The lipid spheres are very large with a smooth texture. The protein + RNA granules are so dense that it is sometimes difficult to make out the closely packed ribosomes. The protein granules have a granular texture. Parts of both sorts of protein granule may be crystalline. $\times 43,000$.

to variation in nutrition, for the composition of the fat body in some insects (10) varies with the type of food ingested. The first appearance of the protein granules coincides exactly with the time when the cuticle is being digested by the molting fluid for resorption by the epidermis. This suggests that the fat body protein granules may be derived from the protein of the cuticle. The time when the protein + RNA granules first appear coincides approximately with the formation of the pupal epicuticle.

From the histology we may conclude that the protein granules are of two sorts, differing in their composition and time of formation. Those which appear first we shall refer to as protein granules. Those which appear later and which retain RNA we shall call protein + RNA granules.

Electron Microscopy: Introduction

The main components stored in the late larval or pupal fat body are all easily recognized in electron micrographs (Fig. 3). The lipid droplets are very large, with smooth contours outlined by profiles of the endoplasmic reticulum (ER). The edge of the lipid may be an interface or a thicker, denser layer, but there is no unit membrane. The protein granules are enclosed in a unit membrane and have a granular texture, tending to be fibrous in parts. The center of some granules is crystalline, as predicted from their birefringence. The protein + RNA granules are also surrounded by a unit membrane, but their texture is smooth or crystalline. Many ribosomes are embedded in this much denser protein, but still are arranged for the most part as if they were on membranes of the ER. Rosettes of glycogen fill up most of the space between the lipid and the protein granules.

The picture in the pupal fat body is very clear, the cells containing little else than the structures described above. There are only two complica-

tions. Some of the protein granules are being resorbed, and there are also stages in the formation of an intermediate type of protein granule which contains RNA when first formed. The latter are considered in a subsequent section.

In the late 5th instar larva, however, the picture is complicated. There appear to be a bewildering number of granules differing in their form. This complexity can now be seen as part of a much simpler pattern involving a small number of processes. These are:

1. The formation of protein granules in Golgi vesicles.
2. The isolation of cell components for lysis or sequestration by paired membranes derived from the Golgi complex.
3. Growth by microvesiculation of the unit membrane surrounding the granules.
4. Growth by fusion of granules at various stages of their development.

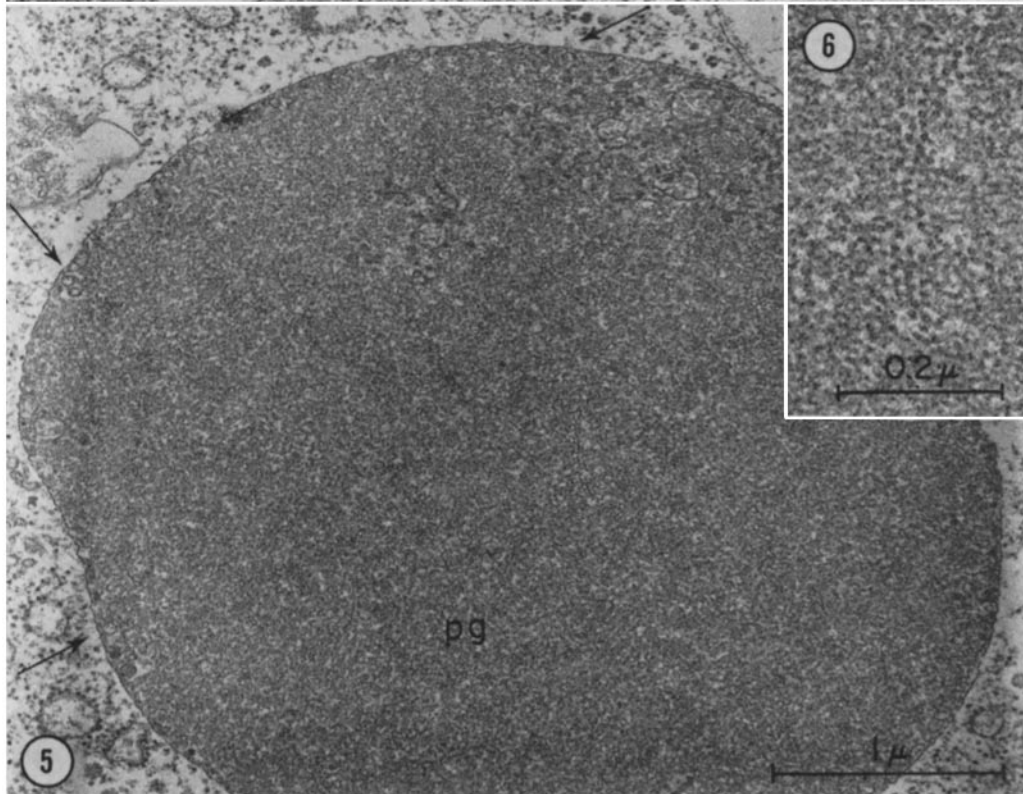
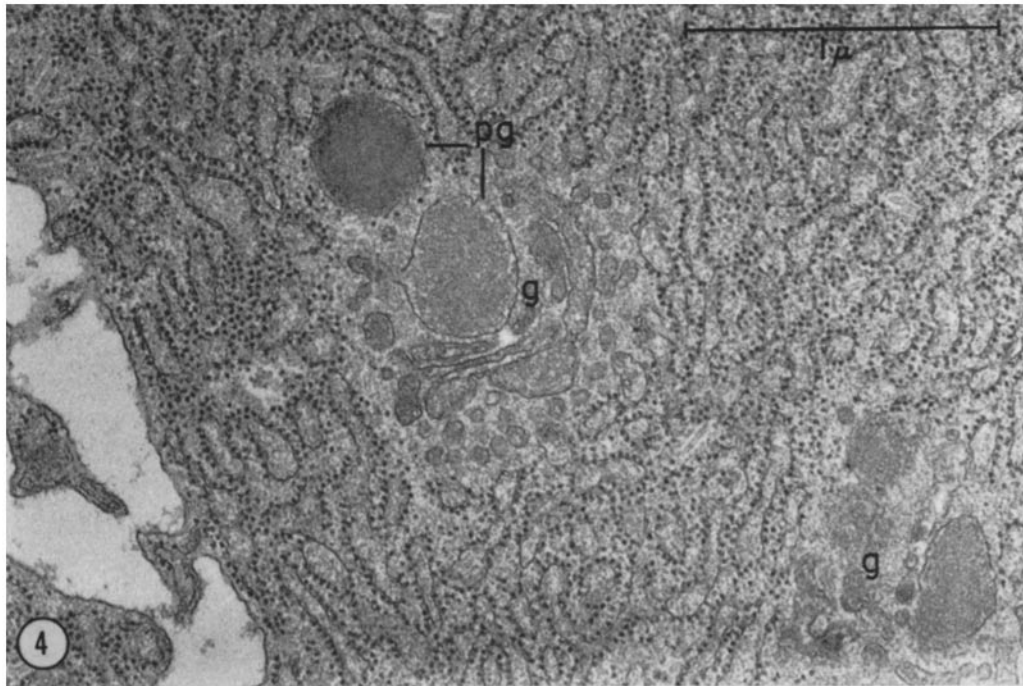
The Structure of the Protein Granules

There are 10 to 15 Golgi complexes in a typical profile through the middle of a fat body cell about 35 hours before molting. Although small, they are actively giving rise to protein-containing vesicles which enlarge to about $\frac{1}{2}$ to 1 μ in diameter before separating from the parent Golgi complex (Fig. 4). The vesicles may already have the granular texture characteristic of fully formed granules (Figs. 5, 6). The membrane surrounding the granule may form microvesicles about 400 to 800 A in diameter at any time after separation from the parent Golgi complex (Figs. 5, 7, 8, 21, 24, 27). Since the microvesicles accumulate inside the emergent granules, and since these granules are known to be growing very rapidly at this time, it is reasonable to assume that the vesicles are transporting material into the granule from the cytoplasm. In agreement with this hypothesis, the

FIGURE 4 5th instar larva about 34 hours before pupation, showing the origin of protein granules from the Golgi complex. *g*, Golgi complex; *pg*, protein granule. $\times 42,000$.

FIGURE 5 Pupa 1 hour old, showing a large protein granule *pg*. There are still some microvesicles (arrows). $\times 32,000$.

FIGURE 6 Enlargement from the center of the protein granule in Fig. 5 to show the granular texture. $\times 87,000$.



cytoplasm becomes less dense around such granules; there is some glycogen but no ER, ribosomes, or dense ground substance. In larger granules many of the microvesicles appear broken as though they are being transformed into the contents of the protein granule.

This interpretation of the origin of the protein granules is summarized in Fig. 9.

The Structure of the Protein + RNA Granules

By the time the protein + RNA granules begin to form at about 10 hours before pupation, the ER is restricted to regions between groups of protein granules and lipid droplets. It is these regions of the rough ER that are transformed into granules. Vesicles from the Golgi complex can be traced between membranes of the ER. The vesicles flatten and fold back to invest a more or less spherical mass of the ER in a two-layered shell. These ER bodies, as we have called them, are thus isolated from the rest of the cell by a space derived from the lumen of the Golgi vesicles (Figs. 10 and 12). For this reason we have referred to the paired membranes derived from the vesicles as isolation membranes. From the time when the isolation membranes meet, the ER body is topologically external to the cell. Any communication between it and the cell would have to take place across two isolation membranes and the lumen they enclose. In the next stage several of these ER bodies come together with confluence of their outer isolation membranes (Fig. 13). The inner isolation membranes break down, so that the ER is set free within the surviving isolation membrane (Fig. 14). Within this membrane, derived from those of several ER bodies, the granule forms. The membranes of the ER become progressively less distinct

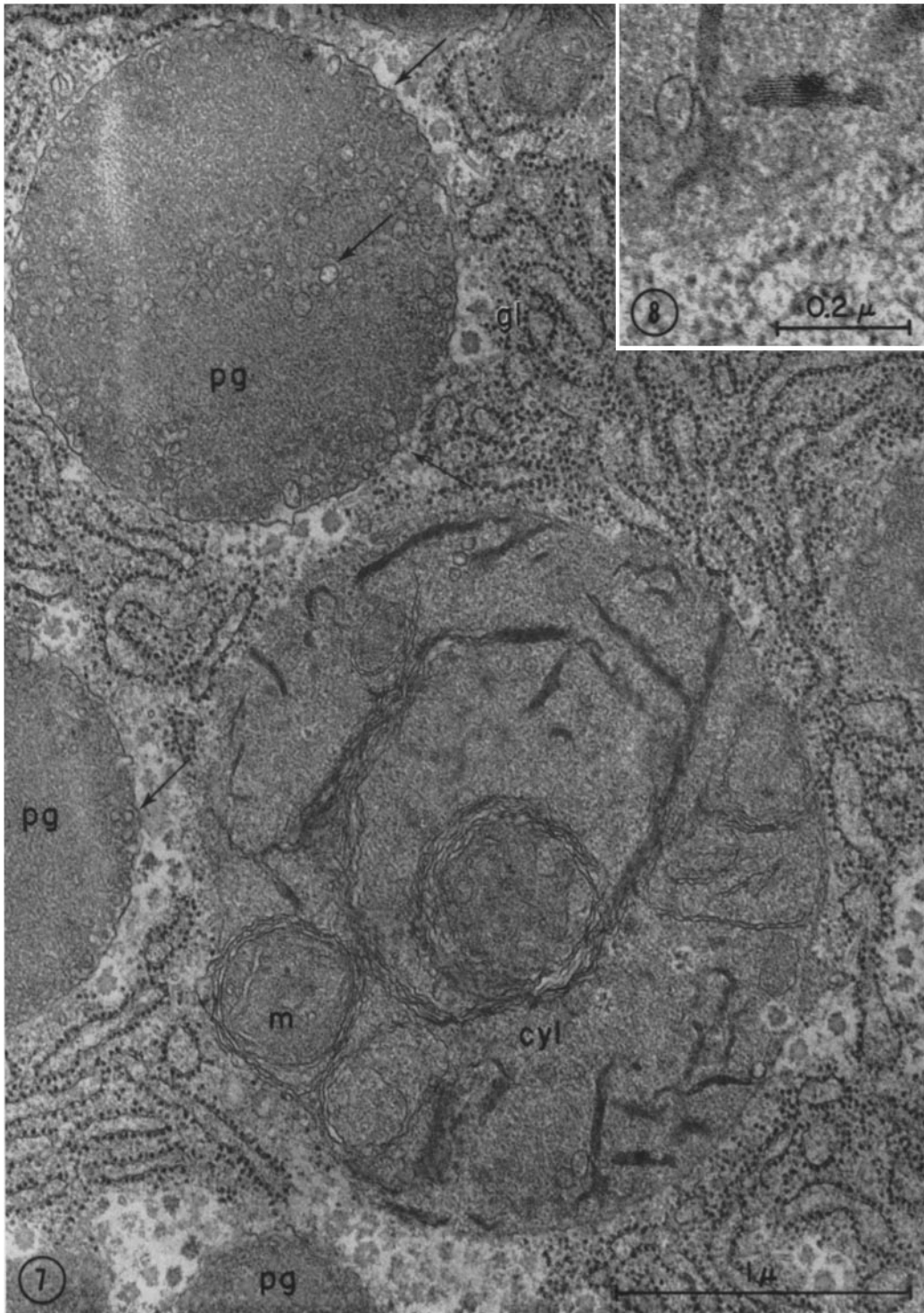
and the whole space between the ribosomes becomes filled with dense, finely granular material (Figs. 15 and 16). These ER membranes are probably not lysed suddenly and completely, but gradually decrease in area. The profiles become rounded and the ribosomes become more evenly spaced and closely packed together. Sections tangential to an area of ribosomes show that the ribosomes are now no longer randomly scattered, but are packed tightly, in a hexagonal pattern (Figs. 17 and 18), with only 230 to 240 Å from center-to-center of each ribosome. This is the pattern we should expect when membrane substance is progressively removed, if the ribosomes somewhat repel one another and are free to move at the membrane surface as it decreases in area. A very simple model can be used to demonstrate this effect. A rubber balloon is inflated and smeared with vaseline or sticky oil. Mustard seeds or similar small, more or less symmetrical particles take up a random pattern when sprinkled on the balloon surface. If the balloon is now deflated the particles become progressively more ordered until a closely packed hexagonal pattern is formed.

In the final stages of their formation the granules become very dense, and the ribosomes, although they maintain their positions as though they are still on membranes, can be seen only with difficulty. This may be caused by a contraction of the granules, since the dense granules are smaller than most granules in the stage shown in Fig. 14. In many cases the protein component becomes crystalline with a repeat spacing varying from 90 to 160 Å. The outline of the granules itself may become angular, perhaps caused by the crystal orientation.

The origin and formation of the protein + RNA granules is summarized in Fig. 19.

FIGURE 7 5th instar larva about 34 hours before pupation, showing protein granules (*pg*) with microvesicles (arrows) and a cytolysome (*cyl*). Glycogen (*gl*) is beginning to appear in clear regions around the protein granules which have a characteristic granular texture. The cytolysome originates from isolation bodies containing mitochondria (see Figs. 24, 25 to 29). Traces of a mitochondrion (*m*) can still be seen in this cytolysome. $\times 45,000$.

FIGURE 8 Enlargement from the cytolysome in Fig. 7 to show what are perhaps phospholipids in a lamellar phase. They have a repeat distance of 60 Å. $\times 101,000$.



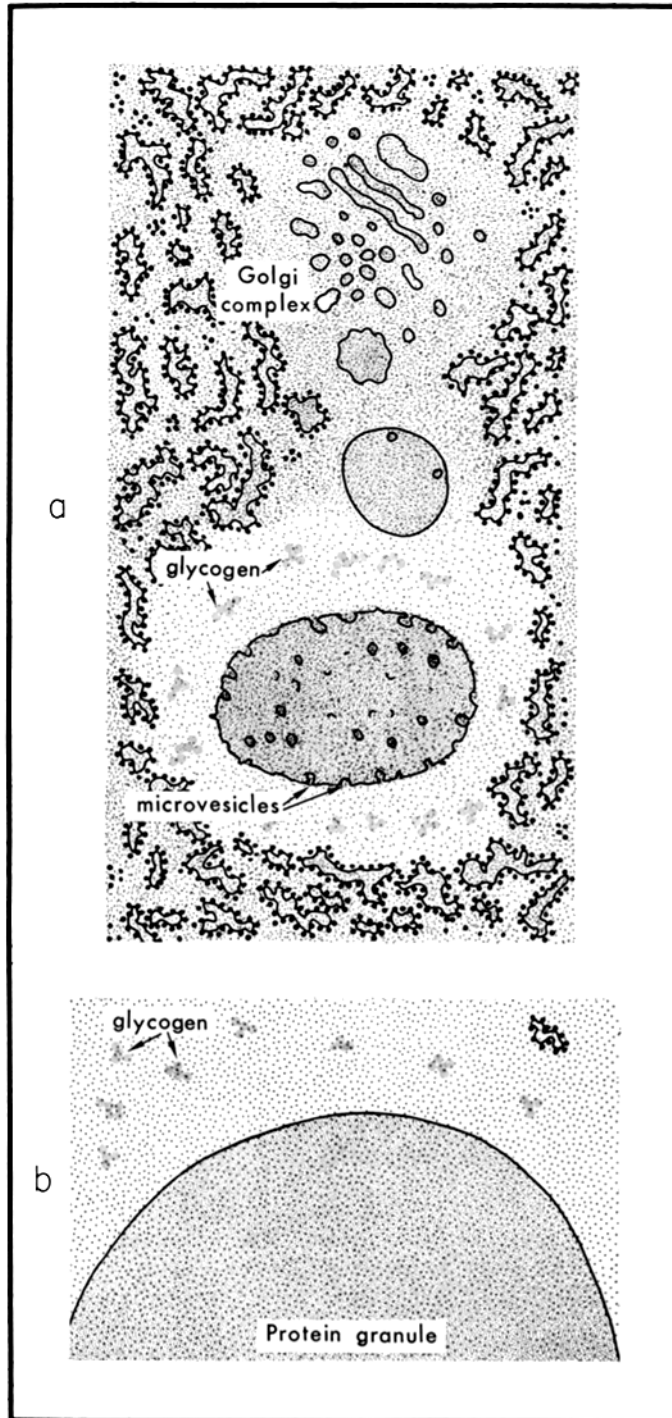


FIGURE 9 Diagram summarizing the events leading to the formation of a protein granule. Fig. 9 *a*, a vesicle from the Golgi complex enlarges and grows further by the incorporation of microvesicles. In Fig. 9 *b*, microvesiculation has ceased in a granule from the pupa. Compare with Figs. 3 to 7, 12, 24, and 27.

The Structure of Protein Granules Derived from the ER Bodies

The light microscope gave evidence for only two sorts of granule, but the electron microscope showed the situation to be more complicated. Not all the aggregations of the ER bodies go on to become protein + RNA granules. In some, the ribosomes are lost and the result becomes indistinguishable from a protein granule. The fate of the ER bodies is often distinguishable from very early on. Fig. 20 shows an early stage of a granule in which the ribosomes are destined to be lost. The membranes of the ER are perfectly clear and sharp but the ribosomes have puffed up to about four times their usual size and have become diffuse at their edges. Fig. 21 shows a later stage in which the ribosomes have almost disappeared but membranes are still present. At this time the isolation membrane often forms microvesicles similar to those formed by the membranes round the protein granules arising directly from the Golgi vesicles. Still later the protein takes on a granular texture (Fig. 22), and finally only the remains of a few membranes may distinguish this sort of granule from a protein granule, although their early states are quite distinct. The origin of this sort of granule is summarized in Fig. 23.

The Fate of Mitochondria

It has been claimed that in the fat body of *Drosophila* the mitochondria give rise to the protein granules (2). This seemed plausible in view of Ward's (9) observation that yolk proteins form within mitochondria in the oocytes of *Rana pipiens*. The fate of the mitochondria in *Calpodes* was therefore of particular interest.

At about the time the protein granules are being formed from the Golgi complex, some other Golgi vesicles are already in the process of forming isolation membranes. Although there is abundant ER at this stage, the membranes isolate mitochondria. A mitochondrion may appear normal and completely surrounded by ER except for a few Golgi vesicles. Closer examination shows that the mitochondrion is very closely invested by paired membranes in the same way as the ER bodies. Mitochondria invested in a somewhat similar way have been described in *Tetrahymena* during the destruction of cell components which takes place during the transition from the log to the stationary phase of growth (1). All stages of investment can be made out (Figs. 25 and 26).

The invested mitochondria aggregate with fusion of their outer isolation membranes in the same way as the ER bodies (Fig. 24). The inner isolation membranes break down and the mitochondria progressively degenerate. This stage (Fig. 7) is identical with the structure described as a cytolysome by Novikoff (reference 3, p. 54, Fig. 11). Later stages are less dense, containing only a few membranes which finally disappear altogether. Thus, although the isolated ER may result in a sequestered mass of protein, or protein + RNA, isolated mitochondria may disappear quickly and completely. The important point is not whether we should call one or all of these structures cytolysomes, but that we now have a description of the way in which membranes can isolate organelles for lysis or any other process requiring separation from the rest of the cell. The fate of the mitochondria is summarized in Fig. 29.

Occasionally, but rarely, both the ER and a mitochondrion may be isolated together (Fig. 27). These mixed isolation bodies will account for some of the mixture of components occasionally seen in the early stages of granule formation. Another source of mixed components is the aggregation of isolation bodies of different origin. Fig. 28 shows a small aggregation of five isolation bodies, 3 containing the ER and two containing mitochondria. The body in Fig. 7 may be a later stage of such a mixed aggregation, for it is much more dense than others believed to be derived from mitochondria alone. It also contains a few microvesicles, so that it may be destined to form a protein granule.

DISCUSSION

A corollary of the unit membrane hypothesis (6) is the topological continuity of cellular membranes, if not spatially then temporally, *e.g.* the cisternal space of the ER, the space in the Golgi vesicles, and the space between inner and outer mitochondrial membranes can all be considered continuous with the environmental space outside a cell. All membranes in a cell are polarized, with one face adjacent to cytoplasm and the other face either external or limiting a space in continuity with the outside. There are no membranes which have cytoplasm on each side. The only exceptions to this rule are the membranes enclosing cytolysomes which pose a topological problem not previously explored. This work shows that the cytolysomes are only apparent exceptions. When cytolysomes are first formed there are two isolation membranes

separated by a space topologically continuous with the exterior of the cell. As the cytolysome matures this space and the inner isolation membrane are obliterated, leaving the outer isolation membrane with its internal face adjacent to the cytoplasm and its external face in contact with the contents of the cytolysome. We may suppose that the two isolation membranes are similar, but the inner one has not survived exposure to lytic enzymes upon its cytoplasmic face, whereas the outer membrane is not affected since only its external face has been exposed.

The ribosomes in the protein + RNA granules can be considered as "stored" in some sense, whether they are used again as whole ribosomes or not. In some of the aggregates of isolation bodies the ribosomes are the first component to be lysed, whereas in the protein + RNA granules they are the only components to retain their identity. The major task of cellular construction and reconstruction begins soon after pupation. By the end of this period, in the imago, all the granules may have been resorbed. It would seem reasonable that some of the ribosomes should have been set aside as an RNA pool to be used in adult development.

Growth by the formation and incorporation of microvesicles occurs in both the protein granules derived directly from Golgi vesicles and those derived from the isolated ER bodies. If the formation of microvesicles is a characteristic property of membranes derived from Golgi complexes, we

may ask what induces their formation in the membrane around these two structures but not in that around two others (protein + RNA and mitochondria). Further study may reveal the environment within the granules that stimulates this reversed form of micropinocytosis.

Isolation membranes derived from the Golgi complex are probably of general occurrence. We have seen them in other insect tissues (epidermis and oenocytes) and around other cellular components. Many of the structures described in papers on lysosomes and cytolysomes may also be stages in a sequence similar to the ones we have outlined (see, for example, reference 8, Fig. 3, and reference 1, Fig. 12). The structures we have described undoubtedly serve to remove and condense cellular machinery no longer needed. The granules which result also appear to function as reserves. Whether we emphasize the lytic or the storage functions of these bodies will depend upon the type of granule and the time of observation.

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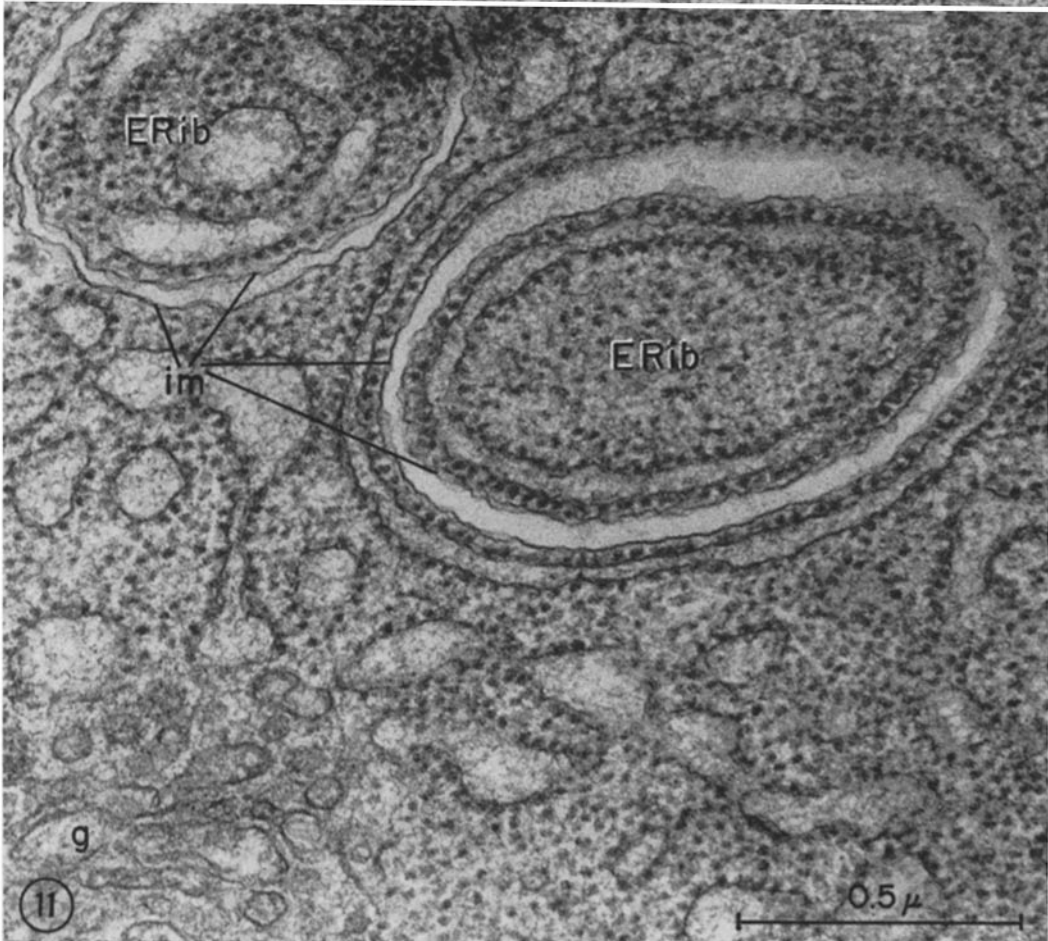
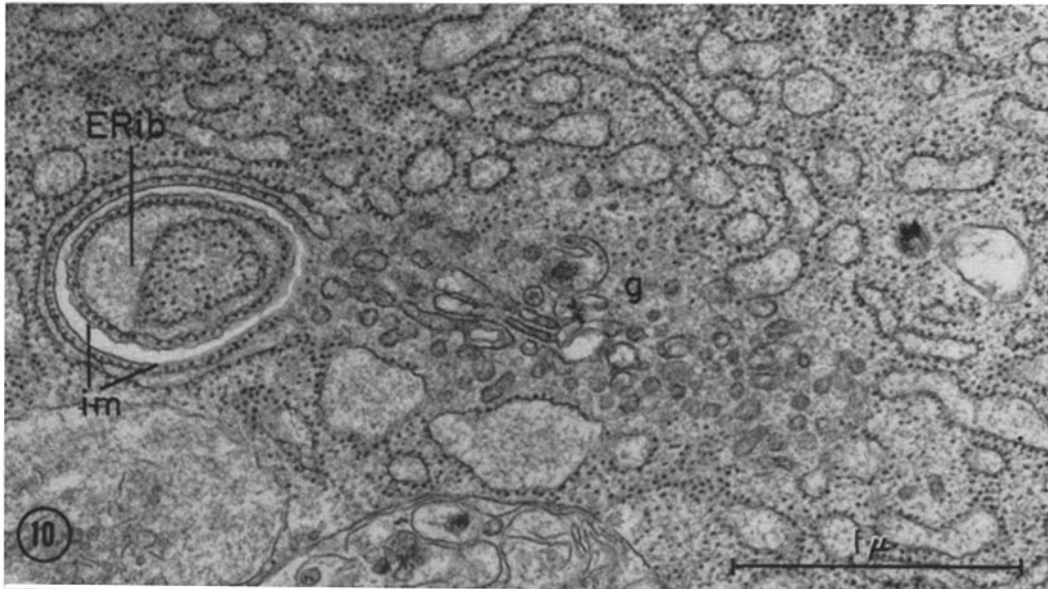
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FIGURE 10 5th instar larva 12 hours before pupation, showing the formation of an ER isolation body. Small vesicles of the Golgi complex (*g*) join up to isolate a region of the ER. *ERib*, ER isolation body; *im*, isolation membranes; *g*, Golgi complex. $\times 39,000$.

FIGURE 11 5th instar larva 12 hours before pupation, showing two ER isolation bodies. The isolation membranes are distinguished from membranes of the ER by their lack of ribosomes. Abbreviations as in Fig. 10. $\times 77,000$.



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FIGURE 12 5th instar larva about 10 hours before pupation, showing the formation of isolation membranes from Golgi vesicles. *pg*, protein granule; arrow, microvesicles; *im*, isolation membranes; *ERib*, ER isolation body; *g*, Golgi complex; *m*, mitochondrion. $\times 31,000$.

FIGURE 13 5th instar larva about 10 hours before pupation, showing several ER bodies aggregating with fusion of their outer isolation membranes in the first stage of formation of a protein + RNA granule. In the bottom left corner there is a slightly later stage in which the inner isolation membranes have been lost. Abbreviations as in Fig. 12. $\times 43,000$.

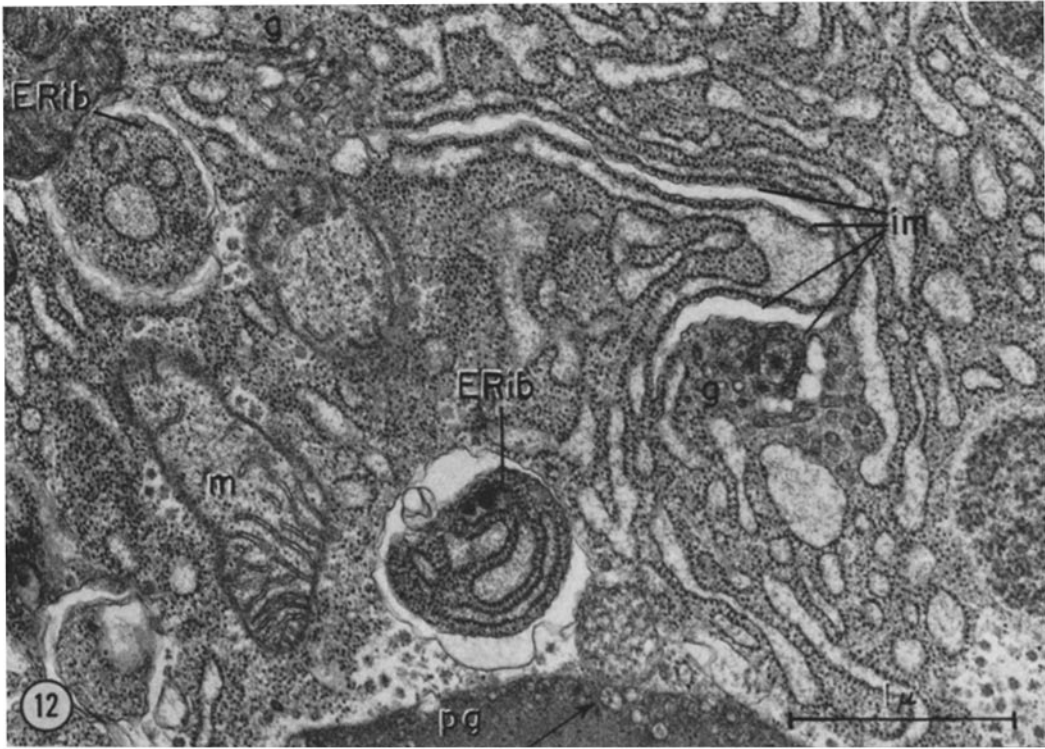
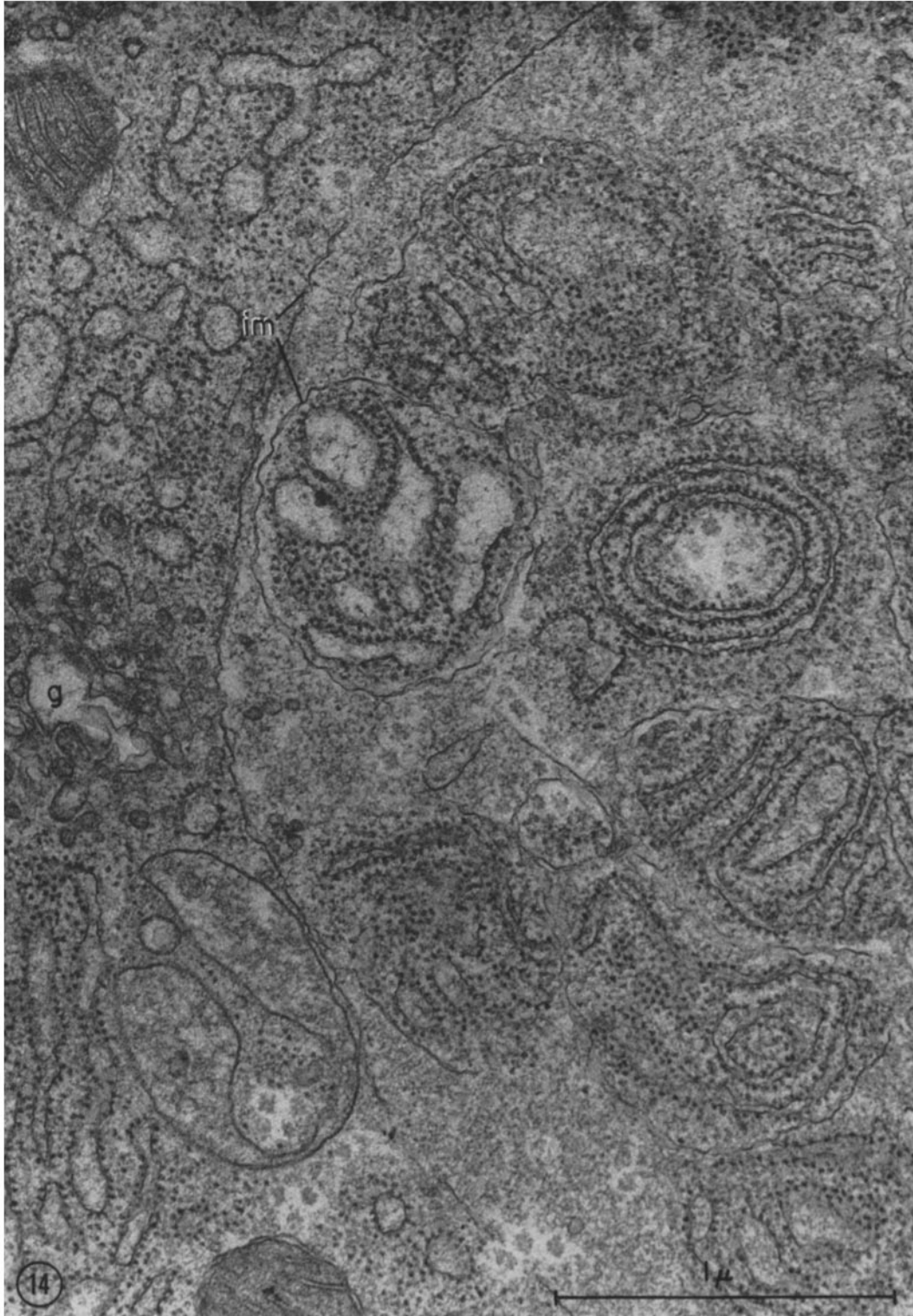
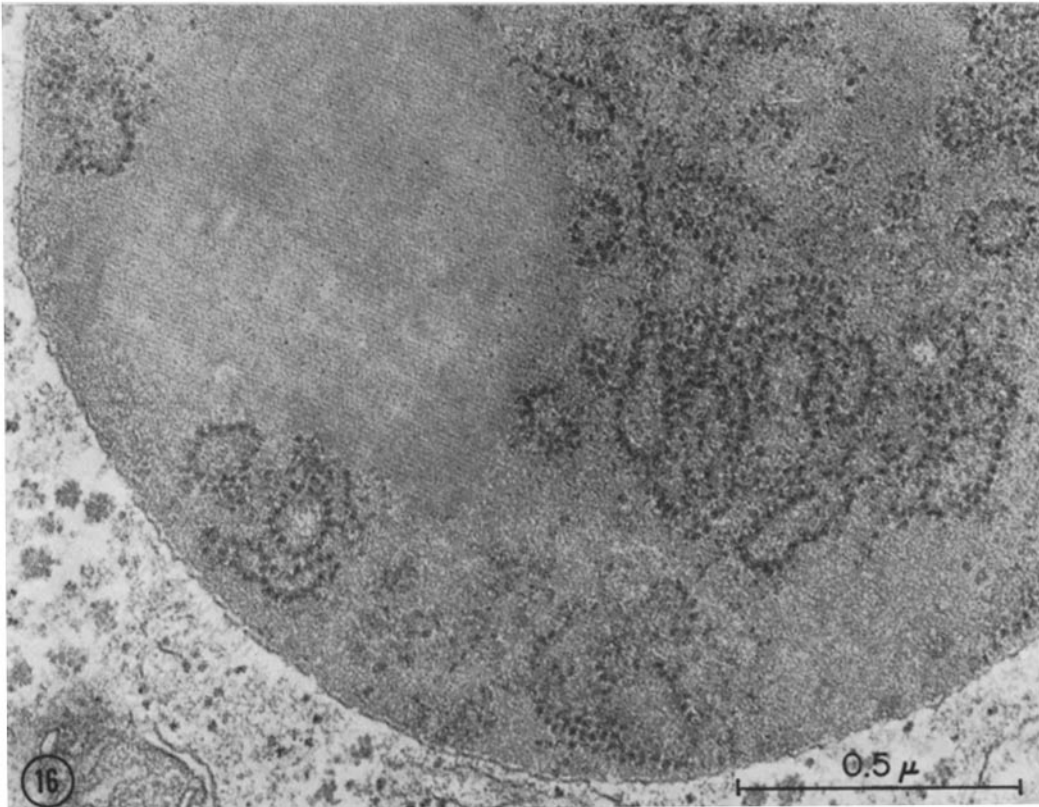
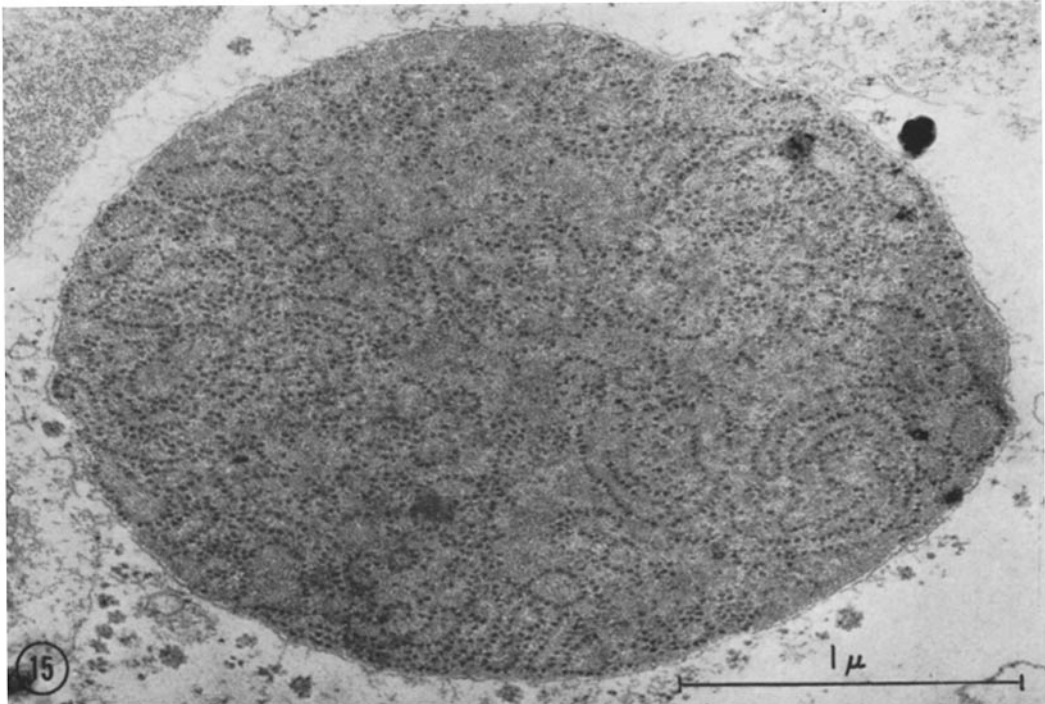
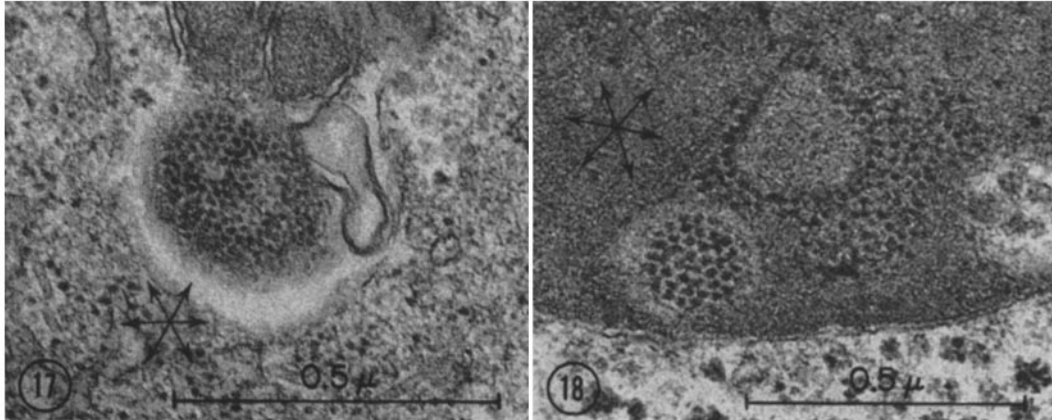


FIGURE 14 5th instar larva about 10 hours before pupation, showing an early stage in the formation of a protein + RNA granule. The outer isolation membrane runs the length of the micrograph. Some of the ER bodies have lost their inner isolation membranes, others are still invested. Some Golgi vesicles seem to have made their way into the future granule. *im*, isolation membrane; *g*, Golgi complex; $\times 52,000$.







FIGURES 17 and 18 Enlargements of protein + RNA granules, showing the ribosomes close packed in hexagonal array. The 3 directions of the array are shown by arrows. Fig. 17, $\times 87,000$; Fig. 18, $\times 75,000$.

FIGURES 15 and 16 Protein + RNA granules from a pupa 38 hours old. The granule is very dense but the ribosomes are still spaced as if they are on membranes. There is a single bounding membrane. Part of the protein is crystalline. Fig. 15, $\times 46,000$; Fig. 16, $\times 74,000$.

FIGURE 19 Diagram summarizing the events leading to the formation of the protein + RNA granules. Fig. 19 *a*, regions of the ER are separated by isolation membranes to become the ER bodies. Fig. 19 *b*, several ER bodies coalesce with fusion of their outer isolation membranes. Fig. 19 *c*, the inner membranes are lost and the ribosomes and proteins are condensed into a dense, partly crystalline, granule. See Figs. 3, and 12 to 18.

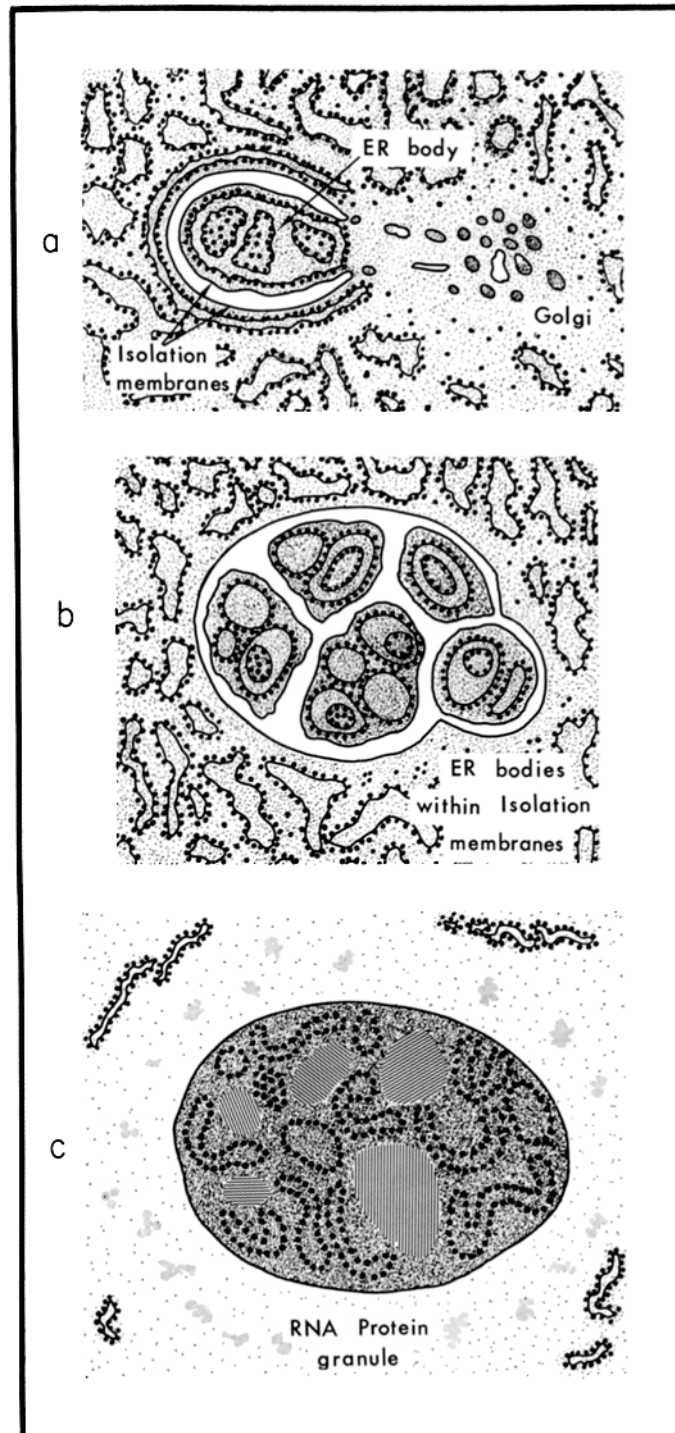
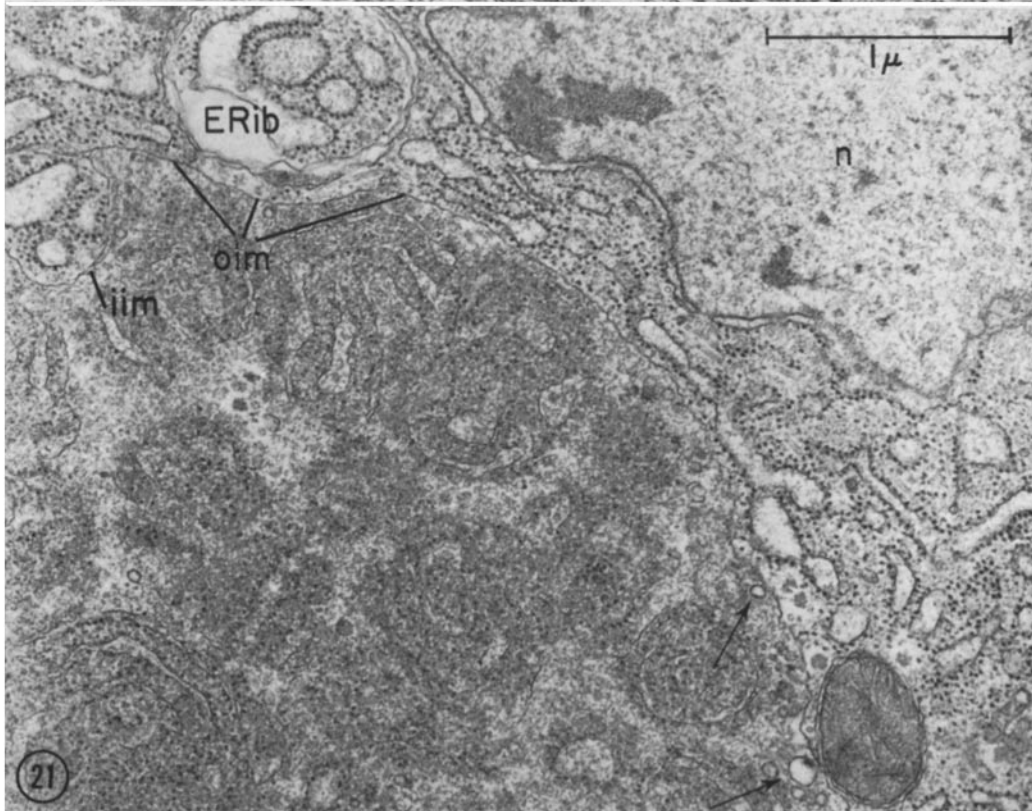
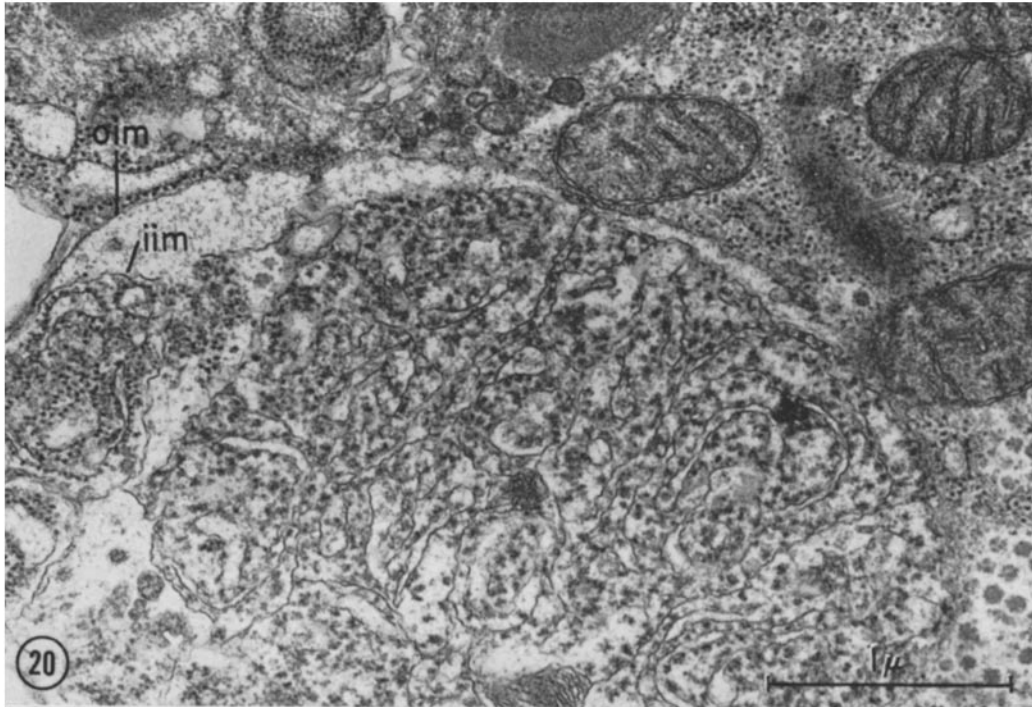


FIGURE 19

FIGURE 20 The lysis of ribosomes within an isolation membrane in a larva about 10 hours before pupation. Most of the ribosomes have puffed up in the first stage of disintegration, but there is one ER body with normal-looking ribosomes. This is the only one still invested by the inner isolation membrane; *im*, inner isolation membrane; *om*, outer isolation membrane. $\times 33,000$.

FIGURE 21 The lysis of the ER at a slightly later stage than Fig. 20. Very few ribosomes on membranes are now distinct. One ER body still has an inner isolation membrane and clear ribosomes. Another ER body may be about to join the main mass. The outer isolation membrane is beginning to form microvesicles. *n*, nucleus; *ERib*, ER isolation body; arrows, microvesicles; other abbreviations as in Fig. 20. $\times 33,000$.



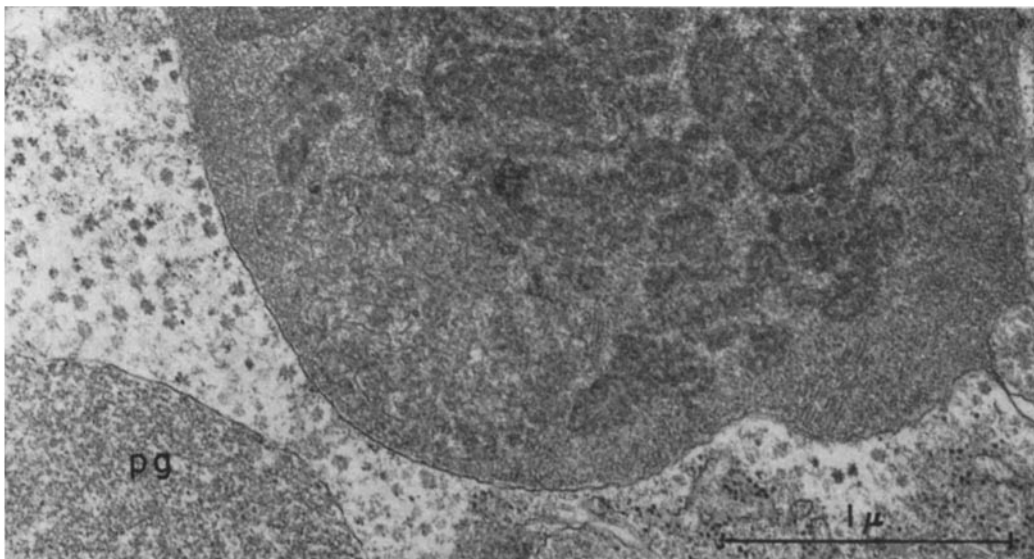


FIGURE 22 A granule of mixed origin. The center still contains the remains of lysed ER while the riphery has the texture of a protein granule. *pg*, protein granule. $\times 38,000$.

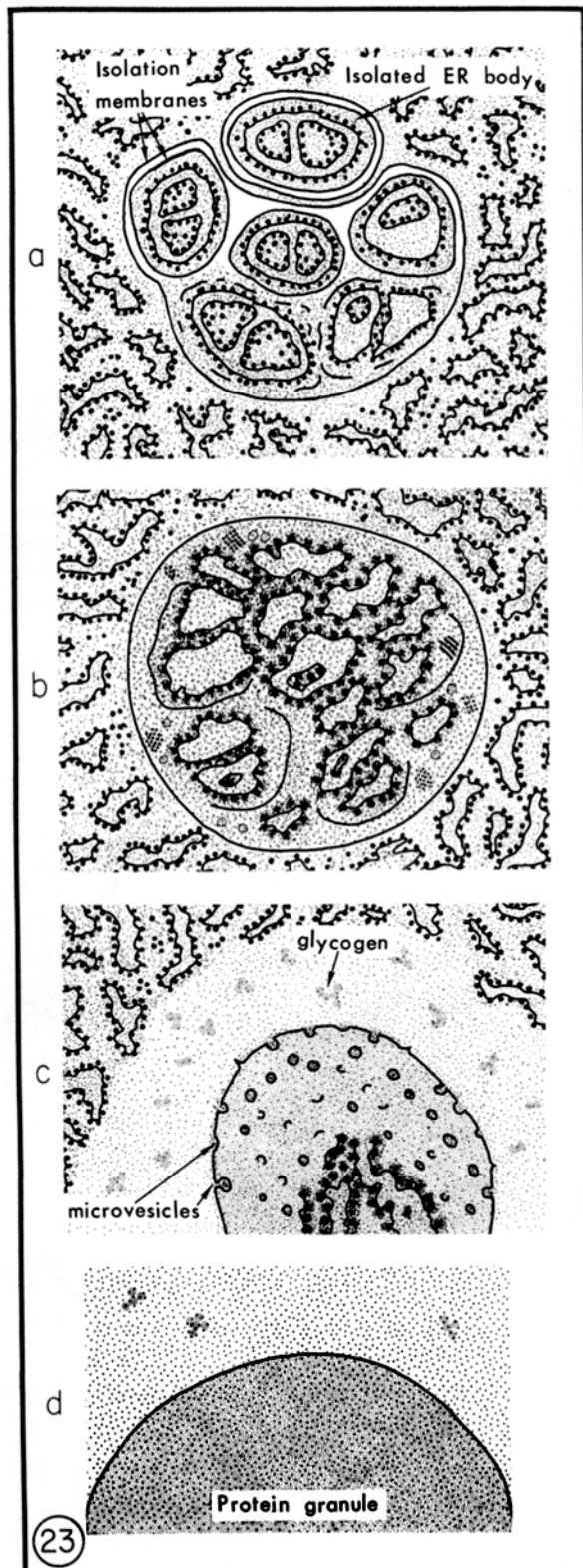


FIGURE 23 Diagram summarizing the events leading to the formation of a protein granule from isolated ER bodies. Fig. 23 a, the aggregation of isolated ER bodies within a common membrane derived from the outer isolation membrane. Fig. 23 b, the lysis of the ribosomes and later the membranes. Fig. 23 c, the addition of material by microvesiculation. Fig. 23 d, the mature protein granule. This may be partly crystalline and retain traces of the ER. See Figs. 20 to 22.

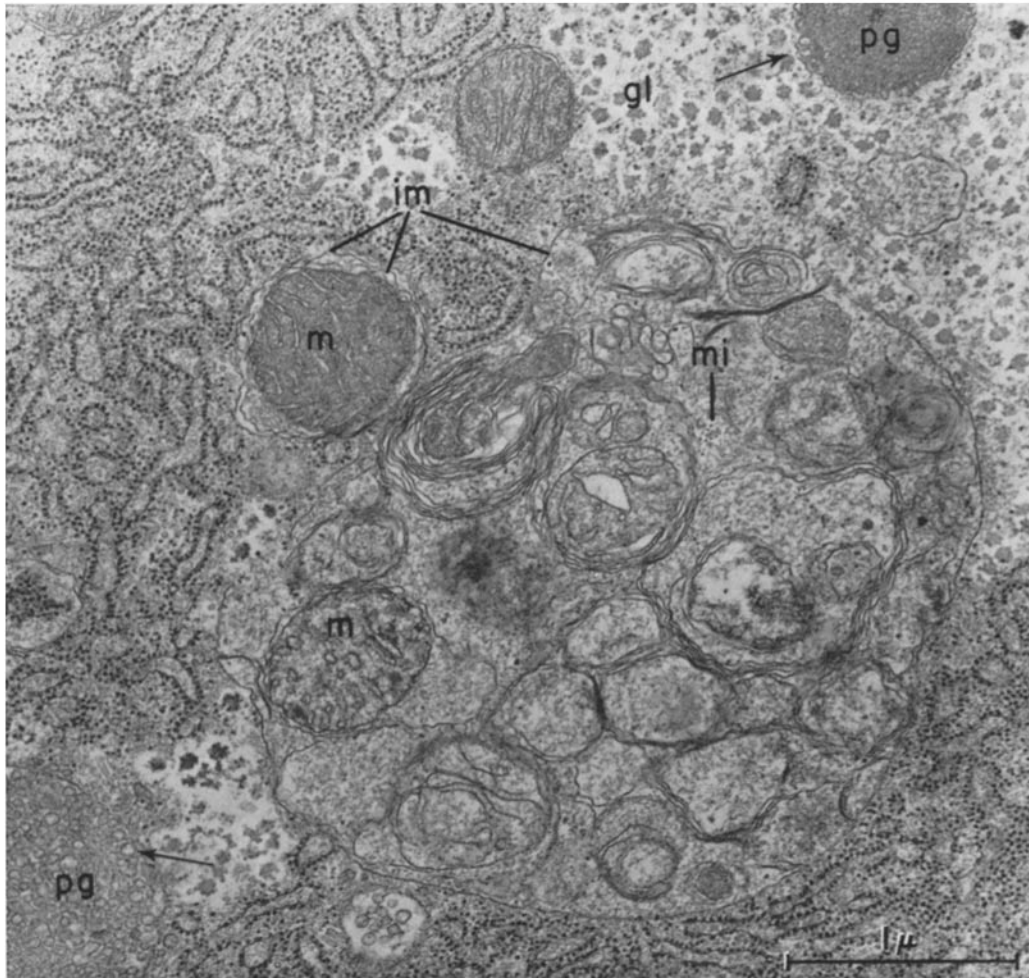


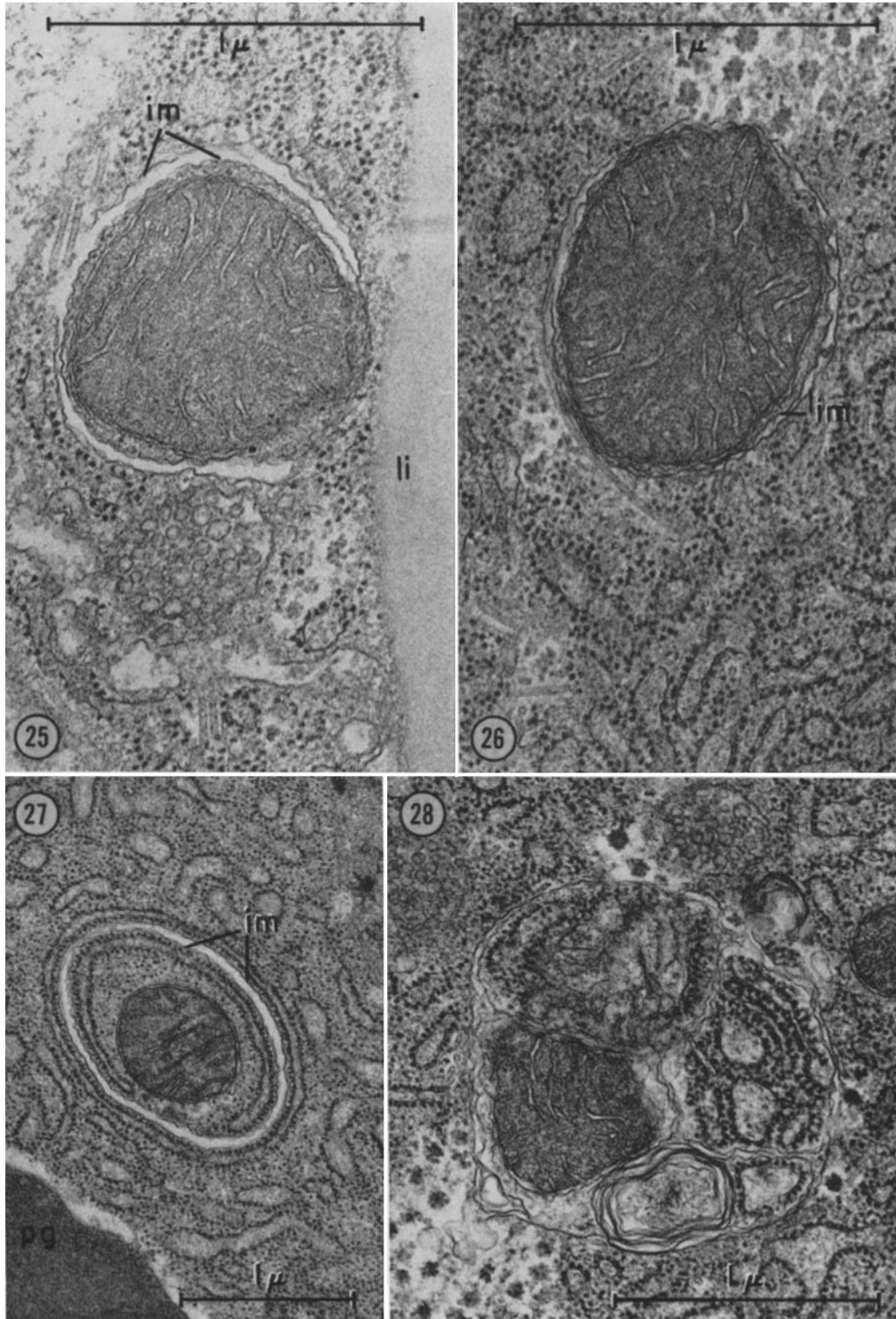
FIGURE 24 The formation of a cytolysome from mitochondria enclosed in isolation membranes; 5th instar larva about 34 hours before pupation. An enclosed mitochondrion is about to join a larger group of mitochondrial isolation bodies, many of which are in an advanced state of lysis. This stage is probably a little earlier than the cytolysome in Fig. 7. *m*, mitochondrion; *im*, isolation membrane; arrow, microvesicles; *mi*, microtubules; *pg*, protein granule; *gl*, glycogen. $\times 31,000$.

FIGURE 25 A mitochondrion partly sheathed by isolation membranes. *im*, isolation membranes; *li*, lipid. $\times 57,000$.

FIGURE 26 A mitochondrion completely surrounded by isolation membranes. Abbreviations as in Fig. 25. $\times 55,000$.

FIGURE 27 A mixed isolation body containing both ER and a mitochondrion. *pg*, protein granule; other abbreviations as in Fig. 25. $\times 27,000$.

FIGURE 28 An aggregate of different isolation bodies, 3 containing ER, one containing a mitochondrion, and one containing possibly a lysed mitochondrion. $\times 41,000$.



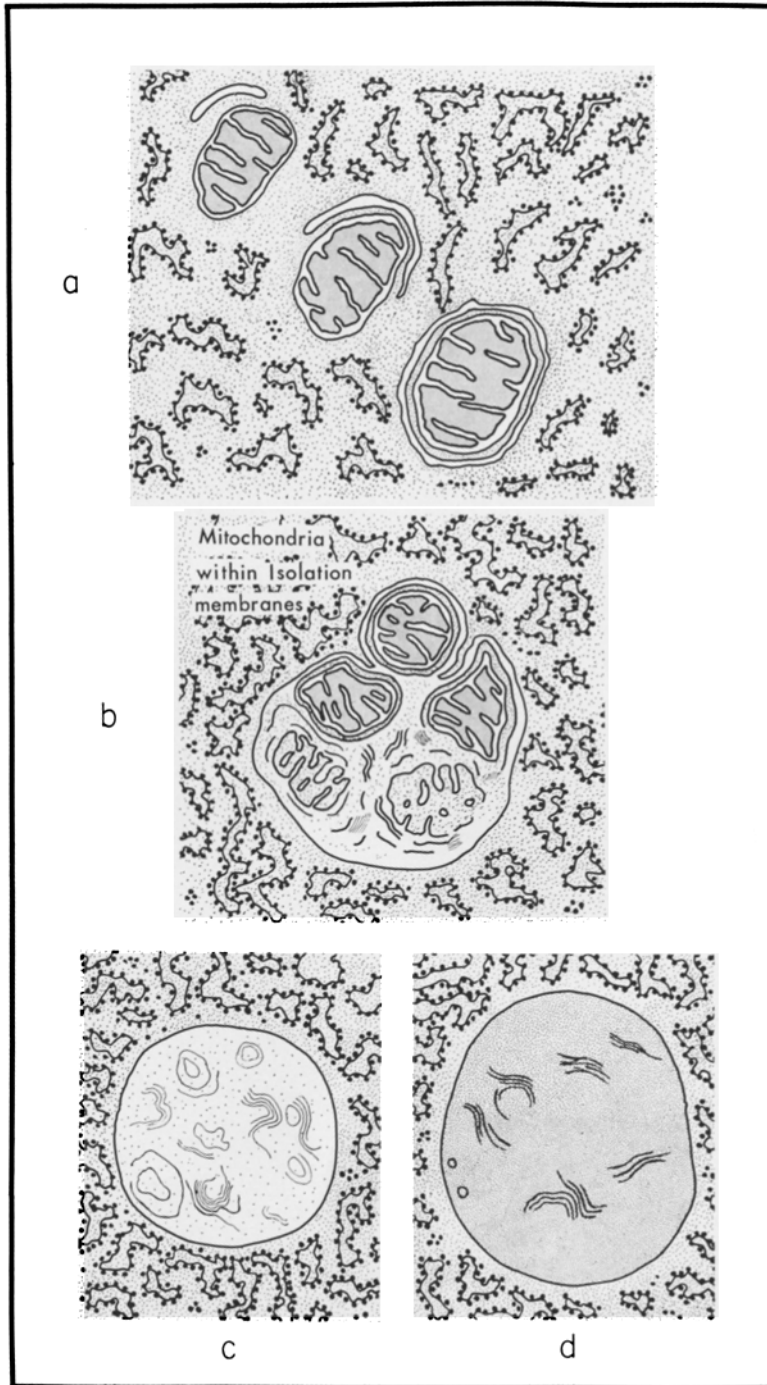


FIGURE 29 Diagram summarizing the events leading to the destruction of mitochondria. Fig. 29 *a*, the formation of isolation membranes enclosing individual mitochondria. Fig. 29 *b*, the aggregation of the mitochondrial isolation bodies within the outer isolation membrane. Fig. 29 *c*, lysis of the contents. Fig. 29 *d*, some of these structures may add material by microvesiculation to become protein granules, but this is uncertain and probably rare. See Figs. 25 to 28, 24 and 7.