Cytoplasmic Membranes and the Nuclear Membrane in the Flagellate *Trichonympha*

By A. V. GRIMSTONE, Ph.D.

(From the Zoological Laboratory, University of Cambridge, England, and the Biological Laboratories, Harvard University, Cambridge, Massachusetts)*

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ABSTRACT

The structure of the nuclear and cytoplasmic membranes of *Trichonympha*, a complex flagellate, has been studied in the electron microscope. The nuclear membrane consists of two 70 A membranes, penetrated by numerous pores. Small (100 A) granules occur on the outer surface, around the rims of the pores. Granule-bearing membranes, only 30 to 40 A thick, form long, ribbon-shaped sacs, with 100 A granules on their outer surface. They apparently form close to the nucleus, from which they probably derive their granules. Smooth membranes occur in the parabasal bodies, which consist of stacks of 70 A membranes, joined at their edges in pairs to form flattened sacs. These can inflate and form cytoplasmic vesicles. A protein fibre is applied laterally to the pile of sacs. New sacs, replacing those lost by inflation, appear to form by a process involving the granular membranes, and there may be a transformation of one into the other. Starving eliminates granular membranes and results in a failure in the formation of new parabasal sacs. Refeeding reverses these effects. A parabasal body is a steady-state system, in which the rates of loss and gain of sacs are normally approximately equal. Parabasal bodies resemble the Golgi apparatus.

Trichonympha is a large, wood-feeding flagellate which lives in the gut of termites. This is one of several papers on various aspects of its fine structure and cytoplasmic organisation; it is an attempt to describe certain features of the structure, behaviour, and functional interrelationships of the nuclear and cytoplasmic membranes. A preliminary account of some of the results has been given elsewhere (14).

Materials and Methods

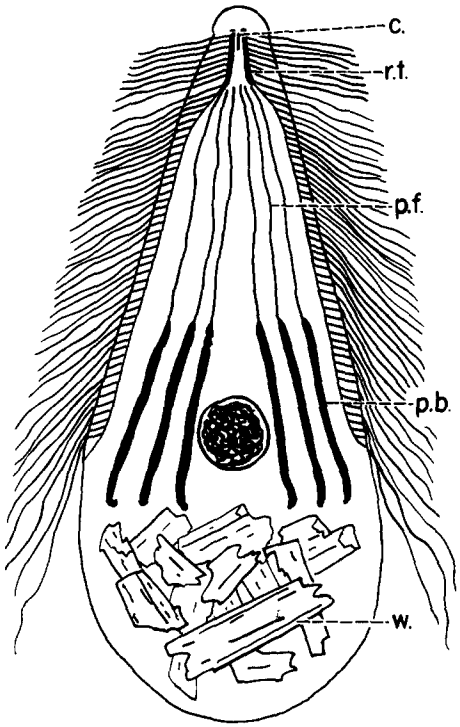
The present study is confined to the three species of *Trichonympha* (*T. campanula*, *T. collaris*, *T. sphaerica*) from the termite, *Zootermopsis angusticollis*.

Colonies of *Zootermopsis* were kept in Petri dishes at 18–20°C. and fed on moistened wood and filter paper. The flagellates are extremely abundant in the hind gut of the termite and can usually be readily obtained by gently squeezing its abdomen.

For electron microscopy material was usually fixed for 20 to 60 minutes at room temperature in the following solution: Michaelis' acetate-veronal stock solution, 2 ml.; 0.1 N HCl, 1.2 ml.; NaCl, 0.2 gm.; OsO_4, 1 gm.; distilled water, 97 ml. This has a pH of about 7.9 and is approximately isotonic with the termite gut fluid (about 0.1 M). Potassium permanganate (23) was also used as a fixative. After fixation specimens were washed briefly in 0.5 per cent NaCl or water, dehydrated in ethanol, and embedded in a mixture of butyl and methyl methacrylate (9:1) containing 0.2 per cent benzoyl peroxide. Polymerisation was carried out at 60°C. Sections, prepared by the usual techniques and supported on collodion films, were examined in a Siemens Elmiskop I electron microscope.

A study was made of the effects on the flagellates of starving the termite host. For these experiments groups of 3 to 6 termites (5th and 6th stage nymphs) were isolated in small Petri dishes without food. Water was supplied and faecal pellets were removed at frequent intervals. Samples of the flagellates were taken daily and studied by both light and electron microscopy. The reversibility of the effects of starving was examined by starving termites for 3 days and then returning them to their normal diet of moist wood and filter

* Present address.



TEXT-FIG. 1. Diagram of *T. campanula*. Only six of the sixty to seventy parabasal bodies are shown. (*c.*, centriolar apparatus; *p.b.*, parabasal body; *p.f.*, parabasal filament; *r.t.*, rostral tube; *w.*, ingested wood.)

paper. The flagellates were examined 1 and 2 days later, again by both light and electron microscopy.

RESULTS

The species of *Trichonympha* from *Zootermopsis* were described in detail by Kirby (18, 19). They have not been found to differ fundamentally from each other in fine structure and they are therefore usually considered together in the following account. Text-fig. 1 illustrates the essential features of the genus. *Trichonympha* is a large organism: *T. sphaerica*, the smallest of the three species studied, is about 150 μ long, and *T. collaris* may be twice that size. Pitelka and Schooley (27) have recently described certain aspects of the fine structure of the flagellar apparatus and fibre systems of these species.

Nuclear Membrane

In life the nucleus is spherical, smooth in outline, and about 20 μ in diameter. It is frequently somewhat irregular in shape after fixation (Figs. 3, 6).

Electron microscopy shows the nuclear membrane (or nuclear envelope, *cf.* 33) to be essentially typical in fine structure, though thicker than in many other cells. Sections normal to the surface (Fig. 1) reveal two component membranes, each about 70 A thick, separated by a space of rather variable width, averaging about 230 A. The total thickness of the whole structure is, therefore, between 350 and 400 A. Consistent differences between the inner and outer membranes have not been detected. At frequent intervals the two membranes approach and join each other, thereby delimiting pores (Fig. 1). These are about 420 A in diameter. A dark line, which may constitute a diaphragm, can sometimes be seen running across the middle of the pores (Fig. 1); if this is indeed a membrane then the cytoplasm and nuclear contents are not in direct connection.

Tangential sections of the membrane reveal numerous closely packed annuli (Fig. 2). There are about 80 of these per square micron and their maximum diameter is approximately 730 A. They are made up of dense rims, about 140 A wide, surrounding a central area (about 450 A in diameter), the density of which is slightly greater than that of the material between the annuli.

As a first approximation, the annuli can be interpreted as surface views of the pores. They agree in frequency and dimensions; and in places where the nuclear membrane is folded, so that the plane of the section changes rapidly from perpendicular to tangential, the "porous" appearance passes almost imperceptibly into the "annular" (Figs. 2, 5). Fig. 5 shows that the annuli are included within the thickness of the membrane, rather than superimposed upon its outer surface. The heavy rims of the annuli in surface view are also to some extent to be expected from the observed structure of the pores, for provided the thickness of the section is greater than that of the single membranes (which is certainly the case here), the latter must obviously present a greater thickness to the electron beam when they bend down to form the margin of a pore than when they run in the plane of the section.

However, this simple view of the structure of the annuli is incomplete, for examination of tangential sections sometimes shows that the rims are granular (Fig. 2 *a*). The granules apparently lie on the outer surface of the membrane and are about

100 A in diameter. There are ten to twelve around each annulus, often with one in the centre. Undoubtedly, they contribute to the density of the rims of the annuli. None of the micrographs provides any reason for supposing that the granules should be interpreted as sections of filaments or cylinders.

In addition to the granules lying around the margins of the pores there are sometimes others, also arranged in rings or whorls, which lie in the cytoplasm immediately outside the nuclear membrane, apparently unattached to it (Fig. 3). In a few micrographs whorls of granules can also be seen inside the nucleus, usually close to the inner surface of the inner membrane (Fig. 4). There are also regions of nuclear membrane where granules cannot be detected at all, even though the resolution on the micrographs would apparently be high enough to reveal them if they were present.

A final feature of the nuclear membrane is the frequent presence on the outer surface of a diffuse, somewhat irregular layer of amorphous material, 200 to 500 A thick (Figs. 1, 4).

Granular Cytoplasmic Membranes

I. Structure:

The cytoplasm contains many membranes bearing small granules. Examination of a large number of micrographs shows that these take the form of long, flattened, ribbon-shaped sacs, with the granules on their outer surfaces (Figs. 7, 8, 14). The sacs are 400 to 450 A thick (excluding the granules) and about 0.3 μ wide; their total length is difficult to estimate but profiles at least 3 μ long have been seen (Fig. 7), and the actual length is almost certainly much greater than this. Each sac appears to be a separate entity, for no convincing instance of anastomosis or branching has been observed in several hundred micrographs. The membrane which forms the basis of the sacs is apparently only 30 to 40 A thick and is seen clearly only in high-resolution micrographs in which it is cut transversely (Figs. 9 to 11). The lumen which it encloses is about 350 A wide and contains material of slightly higher density than the cytoplasmic ground substance. The granules attached to the membranes are about 100 A in diameter and are always arranged in rings or whorls (Fig. 8). These range in diameter from 450 to 750 A, they lie flat on the membrane, and there are ten to twelve granules in each, often with one in the centre. The arrangement in whorls seems to be more common than that in closed rings. The granules are dense and give these membranes a characteristic appearance, which enables them to be identified with certainty even in low-resolution micrographs in which the individual granules and their underlying membrane are not clearly resolved.

The granular membranes (this term is equivalent to Sjöstrand's "α-cytomembrane" (31)) occur throughout a broad zone of cytoplasm around the nucleus; they are less frequent towards the cell surface and are not found at all in the extreme anterior and posterior regions of the cell. They are often strikingly abundant immediately outside the nuclear membrane, where they are sometimes lined up side by side to form an almost continuous layer over the nuclear surface (Fig. 6). Both here and elsewhere they tend to be oriented with their long axes parallel to the length of the cell.

II. Origin:

It is not yet possible to give more than a tentative account of the manner in which new granular membranes are produced. The following observations, however, appear to be significant.

From the descriptions already given it is clear that the granules on the cytoplasmic membranes resemble those on the nuclear membrane. They agree closely in size and their arrangement in whorls or rings is strikingly similar. Given this similarity, the frequent abundance of granular membranes close to the nucleus suggests that this might be their site of formation. However, the membranes which underlie the granules in the two structures differ markedly in thickness (30 to 40 A, as against 70 A) and shape (the cytoplasmic membranes are not porous), which makes it difficult to suppose that one derives directly from the other. Furthermore, no blebs have ever been seen on the nuclear membrane. Hence, if there is any relationship between the two structures it could apparently involve only the granules.

It has been noted that detached whorls of granules may occur in the cytoplasm immediately adjacent to the nuclear membrane. In addition to these, also close to the nuclear membrane, there may be all kinds of intermediates between well defined granular membranes and what seem to be loose aggregates of whorls and rings of granules, not obviously supported by membranes (Figs. 2 to 5). The appearances are consistent with—though of course do not in any way prove—the hypothesis that the granular membranes are laid

down in close proximity to the nucleus, and that the latter is the source of the granules. The variable location of the granules on the nuclear membrane is in agreement with this suggestion: if they were formed inside the nucleus and passed out into the cytoplasm, they would not be expected to occupy a constant position. There is no evidence at present concerning the origin of the membranes which come to support the granules; conceivably they are synthesised on the spot.

The tentative nature of this hypothesis requires no emphasis. It may be noted, however, that no other structures have been found which could be interpreted as granular membranes in process of formation.

Parabasal Apparatus

I. Structure:

The parabasal apparatus consists of a number of long, cylindrical *parabasal bodies*. In *T. campanula* there are usually 60 to 70 of these, easily visible in the living cell, each a little under 1 micron in diameter and 50 to 70 μ long. They run longitudinally, extending for about equal distances in front of and behind the nucleus, and enclosing the latter in a kind of cage. Often there is an inner ring of parabasal bodies, more or less closely applied to the nucleus, and an outer, more numerous set, between nucleus and cell surface. The apparatus is essentially similar in the other two species, except that in *T. collaris* the parabasal bodies are grouped in bundles of about half a dozen. The parabasal bodies of all species (and, indeed, of almost all flagellates) are heavily stained by Bodian's silver-protein method. In a few preparations, as Kirby (19) noted, this technique also reveals the *parabasal filaments*, delicate fibres, one of which runs the length of each parabasal body. They continue anteriorly alone and can be traced in the light microscope to the base of the rostral tube (Text-fig. 1). They probably continue from there up to the centriolar apparatus (27).

In transverse section each parabasal body is seen in the electron microscope to consist basically of a pile of membranes (Fig. 12). These membranes, which are about 70 A thick and quite smooth, are joined at their edges in pairs, thereby forming flattened sacs. The interpretation of the appearance in transverse section as indicating sacs, and not tubes, is confirmed by the examination of longitudinal and oblique sections (Figs. 14, 15, 19). The length of the sacs is not known; in longitudinal sections they often run uninterruptedly for several micra and it is possible, though perhaps improbable, that they run the whole length of the parabasal body. The number of sacs per parabasal body (in *Trichonympha* from termites fed normally on wood) ranges from 11 to 21; the average is 15. The same average number is found after fixation with potassium permanganate.

The parabasal filament, known from light microscopy to run the length of each parabasal body, can be identified in electron micrographs by its size and position. It appears in longitudinal sections as a cross-striated fibre, 90 to 120 mμ in diameter (Fig. 13). The periodicity of the striations varies in different specimens between 360 and 540 A (the reason for this variation is not known (*cf.* 27)) and there are at least three minor bands within each major segment; obviously it is a protein fibre. It usually appears round in cross-section (Fig. 12), though there are occasional indications of angularity. The filament is applied laterally to the pile of sacs, thereby giving the parabasal body structural polarity. The sacs near the filament will be referred to as *proximal*, those furthest from it as *distal* (Fig. 12).

The most proximal sac is almost invariably greatly flattened and embraces the filament closely (Fig. 12), while the most distal one is usually, though not always, inflated. The intervening sacs are usually only very slightly swollen over most of their width but are frequently inflated at their edges, especially the more distal ones (Figs. 6, 15, 16).

Vesicles of different sizes commonly lie in the cytoplasm around the parabasal bodies (Figs. 14, 15). For the larger of these it is possible to find all stages between inflated distal parabasal sacs, still recognisably part of the parabasal body, and isolated vesicles lying some distance away from it (Fig. 14). Similarly, there are intermediates between the inflated margins of the less distal sacs and small cytoplasmic vesicles. The micrographs suggest rather strongly that either the vesicles are derived from the sacs, or *vice versa*. The conversion process, whichever way it goes, can apparently involve either whole sacs or merely their margins, and it is largely confined to the more distal sacs: almost without exception, the proximal sacs are totally uninflated.

II. Origin of New Parabasal Sacs:

It is proposed now to make the assumption (for which some justification will be offered later)

that the parabasal sacs give rise to the vesicles. The problem then arises of explaining how new parabasal sacs are formed.

It seems reasonable to postulate that, since the sacs are lost at the distal face of the parabasal body, their replacement must occur in the proximal region. In other words, it is suggested that new sacs are formed adjacent to the parabasal filament, displace the existing ones distally and ultimately, in the most distal position, inflate and transform into cytoplasmic vesicles. If this interpretation is correct, the following facts appear significant:

1. In all species of *Trichonympha* (and, indeed, in most of the termite flagellates) the parabasal apparatus is closely associated with the nucleus. The position of the parabasal bodies in the cell varies from species to species, but wherever they occur the nucleus is invariably in their midst. A form such as *T. magna*, in which the nucleus and parabasal apparatus have both migrated far posteriorly, demonstrates this spatial association of the two organelles rather strikingly (18).

2. The parabasal bodies are invariably oriented with their proximal (*i.e.* supposedly formative regions) directed towards the nucleus (Fig. 6).

3. The ribbon-shaped sacs of the granular membranes are similar in shape and orientation to the parabasal sacs. They are both elongated, flattened structures running longitudinally in the cell.

4. It is common to find granular membranes lying parallel and rather close to parabasal bodies (Fig. 7). In a few cases granular membranes have been found in very close proximity to the parabasal filament, and probably in direct continuity with supposedly newly forming, proximal parabasal sacs (Fig. 16).

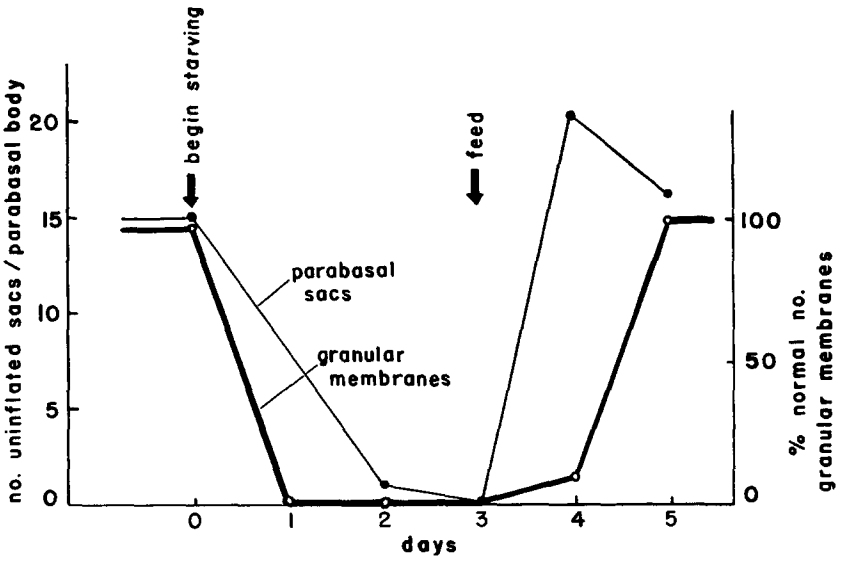
Taken in conjunction with the postulated formation of granular membranes at the nuclear surface, these facts lead to the further suggestion that granular membranes are closely implicated in the formation of new parabasal sacs. They could either be directly transformed into them, or merely concerned in some way in their formation. This point will be discussed later.

The Effects of Starving and Feeding

The effects on the flagellates of starving the termite host were first studied in the light microscope. Changes usually first become clearly visible after 2 days. At this time *Trichonympha* retains its normal activity but contains less ingested wood than usual and its glycogen reserves, normally abundant, are somewhat depleted. The parabasal bodies appear normal. More pronounced changes are visible after 3 days: most individuals still contain a little wood and a certain amount of glycogen, but on the whole they look empty by this time, and they are less active than usual. Many of the parabasal bodies are clearly thinner than normal. These changes continue progressively until the 5th or 6th day, by which time the flagellates are mostly dead or moribund.

The events at the electron microscope level during starving are of great interest. All granular membranes disappear within 1 day in the majority of individuals, and are completely absent after 2 days. They do not return as long as starving is continued. After 2 days the parabasal bodies, instead of consisting largely of uninflated sacs, are made up almost entirely of inflated sacs or vesicles (Fig. 17). The average number of uninflated sacs per parabasal body, normally 15, falls to one, with actual numbers ranging from four to none. (The averages given here and later are based on at least 20 parabasal bodies, taken from at least five different organisms.) After 3 days none of the parabasal bodies has any uninflated sacs left and many of them are reduced to nothing more than a filament and a few vesicles (Fig. 18). In some, only the filament remains. While these changes are going on the nuclear membrane still consists of its usual two membranes. The granules have not been seen, but it cannot be asserted that they are absent since they can normally only be demonstrated by taking high-resolution micrographs of sections cut in the correct plane, and this has not yet been done adequately with starved organisms.

The effects of starving are reversible at least up to 3 days. Termites were starved for this length of time (so that all granular membranes of the flagellates were lost and the parabasal bodies reduced to the condition shown in Fig. 18) and then fed. Twenty-four hours later many of the trichonymphas contained newly ingested wood fragments and a normal glycogen store. Others had apparently not fed by this time (there was some variation in the proportion of these in different experiments, probably caused by differences in the feeding behaviour of their hosts). Organisms that were known to have fed were examined in the electron microscope. The granular membranes had reappeared, but were very few in number; while the parabasal bodies consisted of unusually large numbers of almost entirely uninflated sacs. Instead of the usual average of 15, there was now an aver-



TEXT-FIG. 2. Graph showing the effects of starving and refeeding on the numbers of cytoplasmic membranes. The estimates of the numbers of granular membranes are approximate only and were obtained by counting the numbers of profiles occurring in randomly selected areas of cytoplasm in the electron micrographs.

age of *twenty* sacs per parabasal body, with actual numbers ranging from 18 to 25.

Forty-eight hours after refeeding the granular membranes were abundant and the parabasal bodies had almost regained their normal appearance (Fig. 19). The mean number of sacs, 16, was only slightly higher than usual.

Some of these results are presented graphically in Text-fig. 2. Their interpretation is considered in the Discussion.

DISCUSSION

The nuclear membrane of *Trichonympha* seems essentially similar in structure to that of many other cells. The present interpretation of the pores and annuli agrees on the whole with that of Watson (33); there is nothing to suggest that the pores are traversed by cylinders, such as have been described in amphibian and sea urchin oocytes (1, 34). Granules, often somewhat larger than those found here, have been seen on the nuclear membranes of a variety of other cells (7, 33). The number of pores, 80 per square micron, is within the range found in sea urchin oocytes (1).

The granular membranes also seem to be basically similar in structure to those of some other cells, though they differ markedly in shape and arrangement from the parallel arrays of granular membranes found in many metazoan cells (31). It is to be emphasised that in *Trichonympha* there is nothing to suggest the existence of an interconnected system of granular membranes (*cf.* 25). It has been shown that in some cells the presence of granule-bearing membranes is correlated with the presence of ribonucleic acid (RNA), and there are reasons for supposing that the latter is localised in the granules (26; but see also 21). In *Trichonympha* there are no obvious basophilic regions in the cytoplasm around the nucleus, and the ultraviolet microscope has so far failed to reveal any local concentrations of cytoplasmic nucleotides in frozen-dried material (unpublished observations). However, these negative findings may well result simply from the fact that in this organism the granular membranes, even when lined up as a layer over the nuclear membrane, are not dense enough to be demonstrated at the resolution obtainable with these methods; they are not taken to imply the absence of RNA from the granules.

The tentative suggestion that in this organism the granular membranes may originate at the surface of the nucleus, while not supported by particularly strong evidence, is not altogether implausible. Several workers have produced evidence that the nucleus is implicated in the formation of granular membranes, though it has usually been suggested that they originate as cast-off pieces of

nuclear membrane (1, 8, 28). This does not seem to be the case in *Trichonympha*, where only the granules are thought to originate from the nucleus. On the other hand, studies of the formation of new granular membranes in rat liver cells have not provided any evidence for their nuclear origin (3); the new membranes here were no more abundant around the nucleus than elsewhere. Quite possibly different cells produce this type of membrane in different ways, though without information on the rates of production and migration of the granules (or granular membranes), the absence of a gradient centred upon the nucleus cannot be held to rule out their origin from the latter.

The description given here of the fine structure of the parabasal bodies agrees closely with the accounts previously given by Grassé (11, 12) and by Grassé and Carasso (13) of these organelles in a variety of other termite flagellates. These workers showed that each parabasal body is made up of a pile of sacs applied to the parabasal filament, and suggested that the distal sacs may inflate and form cytoplasmic vesicles. They also drew attention to the fact that the organisation of each parabasal body is precisely similar to that of the dictyosomes of vertebrate and invertebrate spermatids, and of the Golgi apparatus in general. This important conclusion is entirely supported by the present study. It is becoming steadily clearer that for the electron microscopist the essential feature of the Golgi apparatus is a pile of flattened sacs, some of which are apparently capable of inflating and forming cytoplasmic vesicles (see 4, 6, 29, 32, and, for further references, 15). It has long been supposed that one of the functions of the Golgi apparatus is secretion, and there is now available a good deal of evidence to suggest that the inflation of a flattened sac to form cytoplasmic vesicles may be the fine-structural manifestation of the secretory process.

The parabasal apparatus of *Trichonympha* is brought further into line with the Golgi apparatus of other cells by cytochemical studies, which have shown it to contain acid phosphatase (24, 30) and also quantities of a polysaccharide (14; results to be published in detail elsewhere). There are several reports of the latter in the Golgi apparatus (2, 9) and also evidence that it is actually secreted by it in mammalian spermatids (4, 5). It is not unreasonable to suggest that the parabasal apparatus of *Trichonympha* may also secrete polysaccharide. (The possible functional significance of the polysaccharide, and the ultimate fate of the cytoplasmic vesicles in which it is apparently contained, will be considered elsewhere.)

Any hypothesis which suggests that Golgi sacs are converted into cytoplasmic vesicles presupposes a mechanism for the production of new sacs. Grassé (11) recognised this but could offer no suggestion as to the nature of the mechanism; apart from this the problem seems scarcely to have been appreciated. The present study does not allow a definite answer to be given to this question. Nevertheless, two lines of evidence appear to suggest that the granular membranes may be involved in the formation of new parabasal sacs. Firstly, there are the morphological observations: that the two kinds of membranes are similar in shape, that the parabasal bodies are oriented with their supposed formative regions facing the supposed source of granular membranes (the nucleus), and that the two kinds of membrane can probably be in direct continuity. Secondly, there are the starving and feeding experiments. The interpretation of these experiments, which throw light not only on the formation of the parabasal sacs but also on their subsequent fate, must now be considered.

It may be postulated that at the biochemical level one of the effects of starving is to stop synthetic processes. It is known that the wood on which *Zootermopsis* feeds may contain no more than 0.03 per cent nitrogen and that neither the termites nor their flagellates (nor their associated micro-organisms) are able to fix atmospheric nitrogen (16, 17). Obviously this nitrogen supply is very small, and it seems probable that it would be sufficient to meet the needs of the flagellates only if supplied continuously. *Trichonympha* appears to contain no appreciable nitrogen reserves: for example, it stains extremely feebly with Millon's reagent or bromphenol blue (unpublished results). On the other hand, feeding on wood, *Trichonympha* has available a great abundance of carbohydrate, a fact which is reflected in the large amount of glycogen which it normally contains. It therefore seems reasonable to suggest that starving will affect primarily the synthesis of nitrogen-containing substances, and in particular of proteins and nucleotides.

At the structural level a reduction in protein and nucleotide synthesis would be expected to result in failure to form new granular membranes, which, in turn, according to the present hypothesis, will stop the formation of new parabasal sacs. This is what happens in the starving experiments: new parabasal sacs fail to form following the disap-

pearance of their supposed precursors, the granular membranes. Sacs already formed when starving begins continue to inflate and be cast off, but they are not replaced, so that the parabasal bodies progressively diminish. The sequence in which structures disappear—granular membranes, uninflated parabasal sacs, inflated sacs—is precisely that in which they are supposed to be formed, which suggests that the sequence is probably being read in the right direction. This is the justification for the earlier assumption that the sacs give rise to the cytoplasmic vesicles, rather than *vice versa*. On refeeding, the production of granular membranes recommences and the parabasal bodies are rebuilt. The fact that fewer granular membranes are found 24 hours after refeeding, than after 48 hours, can be explained by postulating their rapid transformation into parabasal sacs as soon as they are formed during the 1st day; that is, surplus granular membranes do not appear until the parabasal bodies are fully "saturated."

Irrespective of the mechanism of sac formation (which is admittedly doubtful) these experiments provide strong evidence for the view that the loss of sacs in the distal region of a parabasal body is normally balanced by the acquisition of new ones proximally. Starving disturbs the equilibrium. One of the most important conclusions from this study, therefore, is that we have to think of a parabasal body not as a static structure but as a *dynamic, steady-state system*—it is the site of a flux of membranes.

The factors which determine the equilibrium size of a parabasal body are largely unknown. Clearly, the food supply is important. The "overshoot" in the number of sacs 24 hours after refeeding, although at present unexplained, will probably eventually be significant in revealing the controlling factors. *Trichonympha* cannot yet be cultured *in vitro*, which makes precise investigation of this point difficult.

The demonstration that a parabasal body is a steady-state system may well throw light upon its mode of formation at cell division. Given the formation of a new filament, the production of a new parabasal body need involve nothing more than the building up of the pile of sacs to the steady-state level. No special morphogenetic mechanisms need be postulated. Grassé (12) has published electron micrographs which are interpreted as showing division of the parabasal bodies of the flagellate, *Joenia*, but this organism has branched parabasal bodies (10) and a section through a region where branching occurs could easily be mistaken for an example of division. In the termite flagellates at least, there is no good evidence that parabasal bodies ever divide (20) and much to suggest that they always form *de novo*.

Little can be said at present about the role of the filament in the organisation of the parabasal body. Quite possibly it has something more than a mere supporting or skeletal function, and the knowledge that it is a centriolar derivative (27) recalls the frequent association of Golgi apparatus and centriole in other cells. It would be interesting to know whether the polarity which the filament so clearly imparts to the parabasal apparatus in the flagellates is present in less obvious form in other Golgi systems.

The extent to which the various ideas presented here can be applied to other types of cell remains to be determined. One may predict that the dynamic view of the Golgi apparatus will prove to be fairly generally applicable, though it will need to be modified in some instances. It is already clear that not all Golgi systems maintain a steady state—in the mammalian spermatid, for example, the processes of formation and inflation of Golgi sacs are dissociated in time. This, however, need not imply a fundamental dissimilarity in organisation. The hypothesis that granular membranes transform into Golgi membranes, advanced only tentatively here, perhaps finds some support in the fact that there have been several reports of continuity between granular and smooth membranes (25). It is true that the trend of current biochemical studies is to suggest a protein-synthesising function for the granule-bearing membranes, rather than a role as intermediary in the formation of Golgi sacs (22); however, so little is known about the granular membranes that at present there is no reason why we should suppose them to have only one function in all cells.

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References

1. Afzelius, B. A., *Exp. Cell Research*, 1955, **8**, 147.
2. Arzac, J. P., and Flores, L. G., *Stain Technol.*, 1952, **27**, 9.
3. Bernhard, W., and Rouiller, C., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 73.

4. Burgos, M. H., and Fawcett, D. W., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 287.
5. Clermont, Y., Glegg, R. E., and Leblond, C. P., *Exp. Cell Research*, 1956, **8,** 453.
6. Dalton, A. J., and Felix, M. D., *Symp. Soc. Exp. Biol.*, 1957, **10,** 148.
7. Gall, J. G., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 393.
8. Gay, H., *Cold Spring Harbor Symp. Quant. Biol.*, 1956, **21,** 257.
9. Gersh, I., *Arch. Path.*, 1949, **47,** 99.
10. Grassé, P. P., Traité de Zoologie, Tome I, fasc. 1, (P. P. Grassé, editor), Paris, Masson, 1952.
11. Grassé, P. P., *Compt. rend. Acad. sc.*, 1956, **242,** 858.
12. Grassé, P. P., *Compt. rend. Acad. sc.*, 1957, **245,** 1278.
13. Grassé, P. P., and Carasso, N., *Nature*, 1957, **179,** 31.
14. Grimstone, A. V., *Proc. XVth Internat. Cong. Zool.*, London, 1959, 480.
15. Grimstone, A. V., *Am. Naturalist*, 1959, in press.
16. Hungate, R. E., *Ann. Entomol. Soc. Am.*, 1941, **34,** 467.
17. Hungate, R. E., *Proc. Texas Acad. Sc.*, 1944, **27,** 91.
18. Kirby, H. W., *Univ. California Pub. (Zool.)*, 1932, **37,** 349.
19. Kirby, H. W., *Univ. California Pub. (Zool.)*, 1944, **49,** 185.
20. Kirby, H. W., *J. Morphol.*, 1944, **49,** 361.
21. Kuff, E. L., Hogeboom, M. D., and Dalton, A. J., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 212.
22. Lotfield, R. B., *Progr. Biophysics and Biophysic. Chem.*, 1958, **8,** 347.
23. Luft, J. H., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 799.
24. el Mofty, M. M., *Nature*, 1957, **180,** 1367.
25. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 85.
26. Palade, G. E., and Siekevitz, P., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 171.
27. Pitelka, D. R., and Schooley, C. N., *J. Morphol.*, 1958, **102,** 199.
28. Rebhun, L. I., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 93.
29. Rhodin, J., and Dalhamn, T., *Z. Zellforsch. u. mikr. Anat.*, 1956, **44,** 345.
30. Schneider, W. C., and Kuff, E. L., *Am. J. Anat.*, 1954, **94,** 209.
31. Sjöstrand, F. S., *Internat. Rev. Cytol.*, 1956, **5,** 456.
32. Sjöstrand, F. S., and Hanzon, V., *Exp. Cell Research*, 1954, **7,** 415.
33. Watson, M. L., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 257.
34. Wischnitzer, S., *J. Ultrastruct. Research*, 1958, **1,** 201.

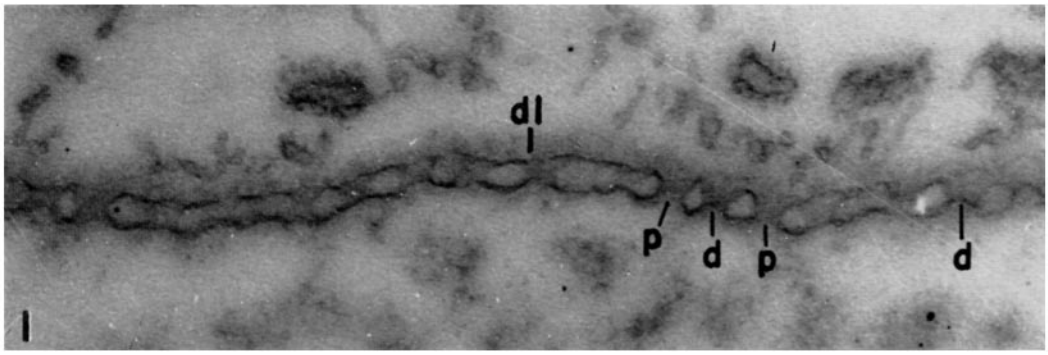
Explanation of Plates

Plate 170

Fig. 1. Perpendicular section of the nuclear membrane. The inner and outer membranes, interrupted by pores (*p*) are shown. Possible diaphragms (*d*) are indicated running across some of the pores. Note also the diffuse layer (*dl*) on the outer surface of the outer membrane. × 90,000.

Fig. 2. Tangential section of the nuclear membrane, showing the annuli (*a*) in surface view. Granules (*g*) occur around the rims of some of them. Note the granular membrane (*gm*) with whorls of granules (*gr*), and the irregular aggregates of granular material (*ig*) which also lie in the cytoplasm close to the nuclear membrane. × 50,000.

Fig. 2 *a*. Enlargement of annulus, showing granules (arrowed) around its rim. × 130,000.


dl
p
d
p
d
1

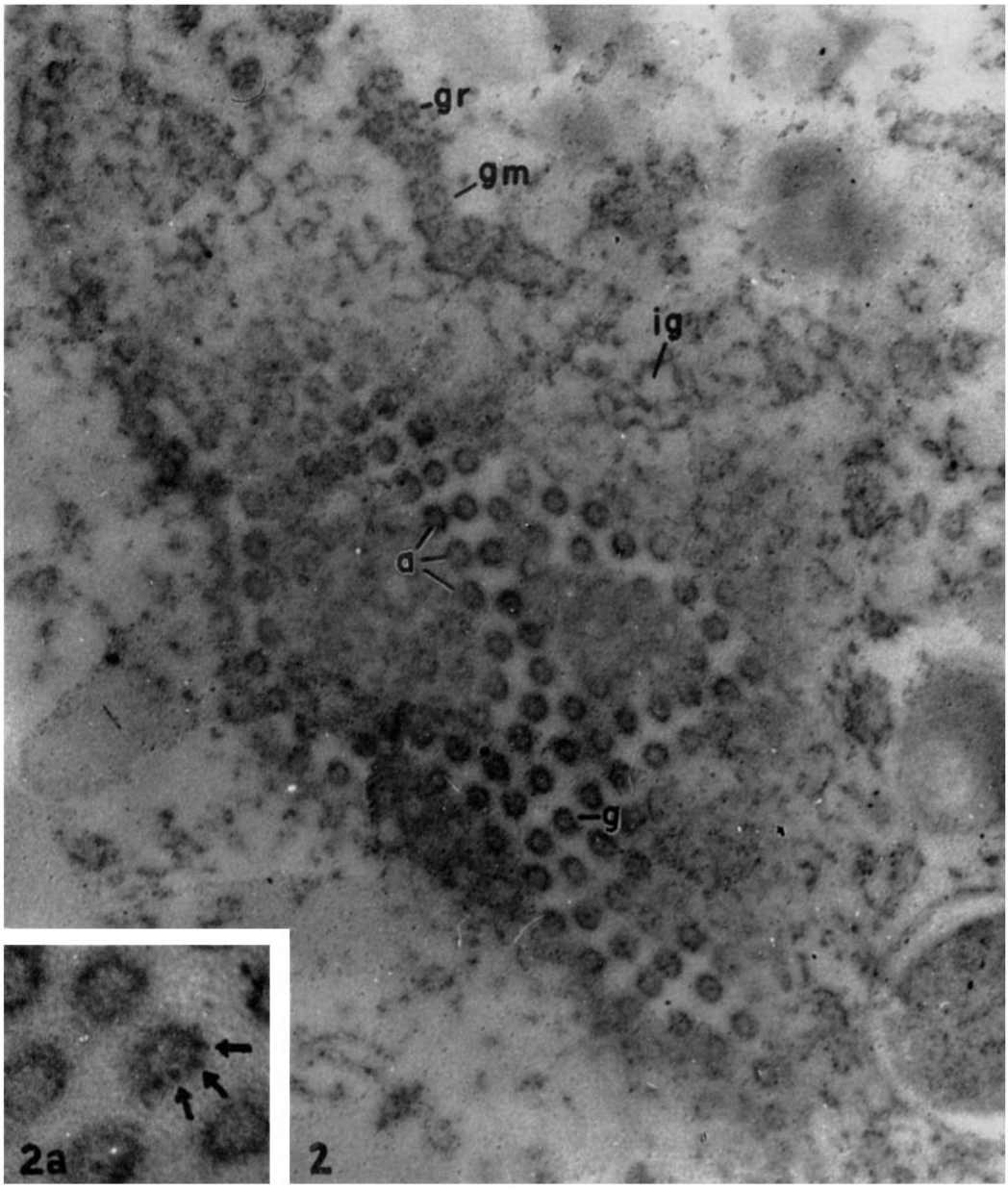
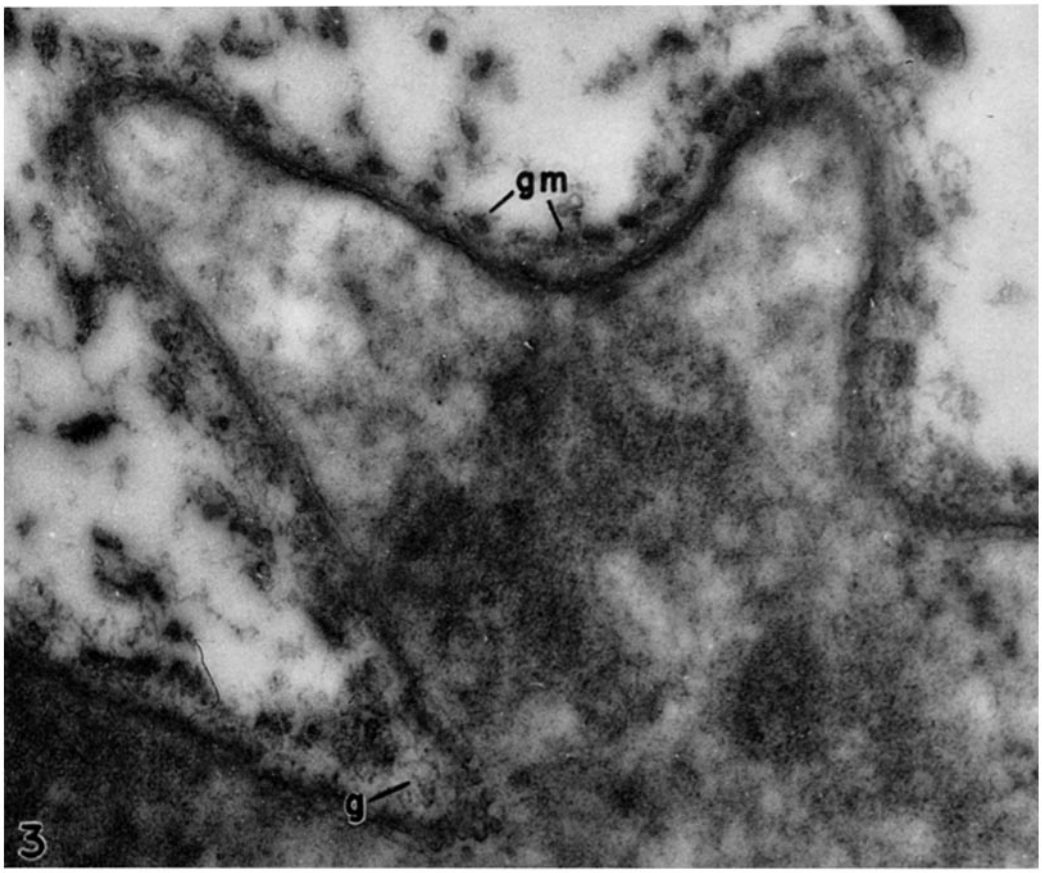

gr
gm
ig
a
g
2a
2

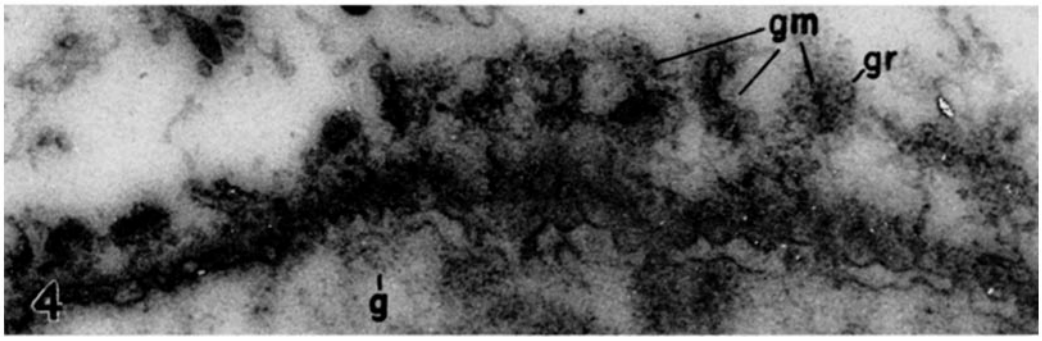
PLATE 171

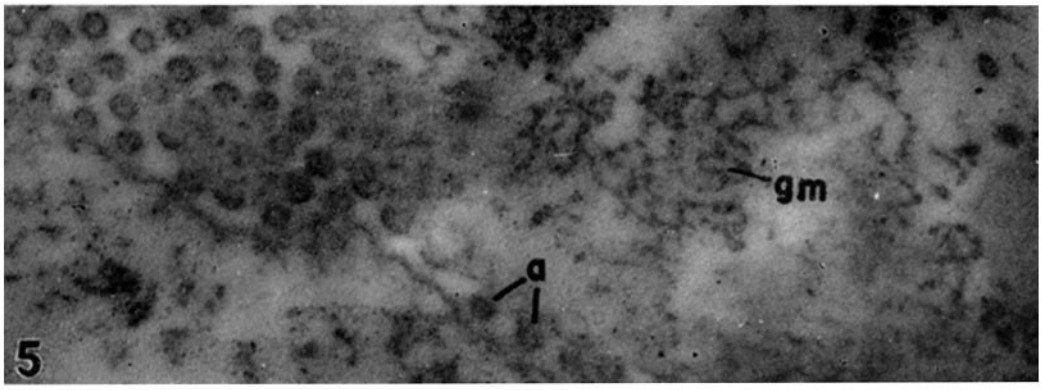
FIG. 3. Nuclear membrane and granular membranes. Shrinkage of the nucleus during fixation and embedding has resulted in an irregularly folded membrane. In the cytoplasm immediately adjacent to the nucleus are the structures (*gm*) interpreted as granular membranes in process of formation. Most of those shown here are small and rather irregular. Note also that some of the material between them and the nuclear membrane appears granular (*g*). × 30,000.

FIG. 4. Nuclear membrane and supposedly newly forming granular membranes (*gm*). Note the whorls of granules on the latter (*gr*), and also the amorphous and granular material between them and the nuclear membrane. A possible whorl of granules *inside* the nucleus is indicated at *g*. × 50,000.

FIG. 5. Part of a nuclear membrane runs from top, left (obliquely sectioned) to bottom centre (almost perpendicularly sectioned). Note annuli, which at *a* can be seen to lie within the thickness of the membrane. The loose aggregate of fibrous and granular material (*gm*), not obviously supported by a membrane, is interpreted as a granular membrane in process of formation. × 50,000.



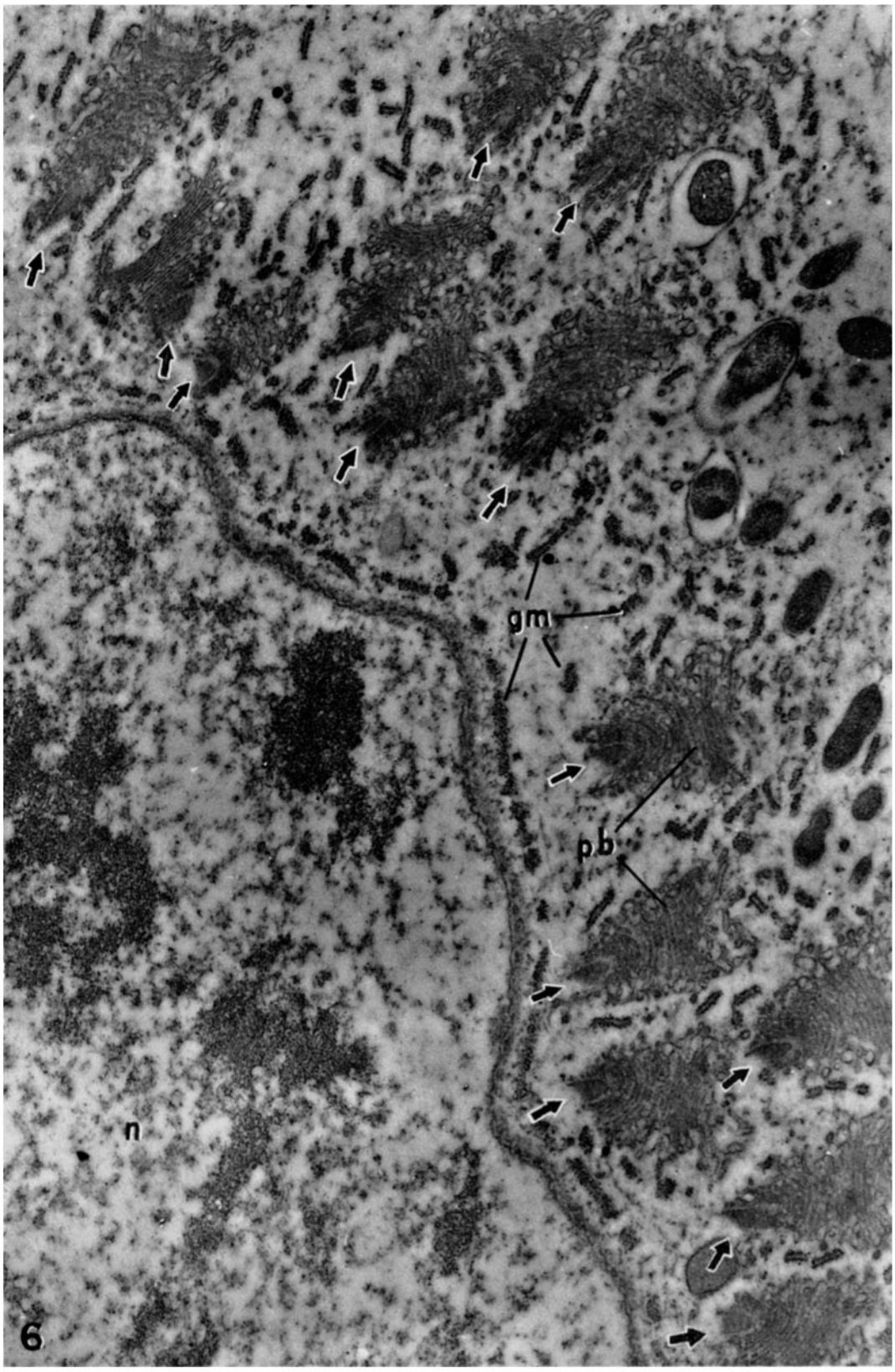




(Grimstone: Cytoplasmic and nuclear membranes)

PLATE 172

FIG. 6. Survey picture, showing nucleus (*n*), nuclear membranes, granular membranes (*gm*), and parabasal bodies (*pb*). Note that the granular membranes form an almost continuous layer over the nuclear membrane, as well as being scattered through the cytoplasm. The parabasal filaments of the parabasal bodies are indicated by arrows: note that they are all directed towards the nucleus. (The dense granules at the right are probably bacteria.) × 23,000.



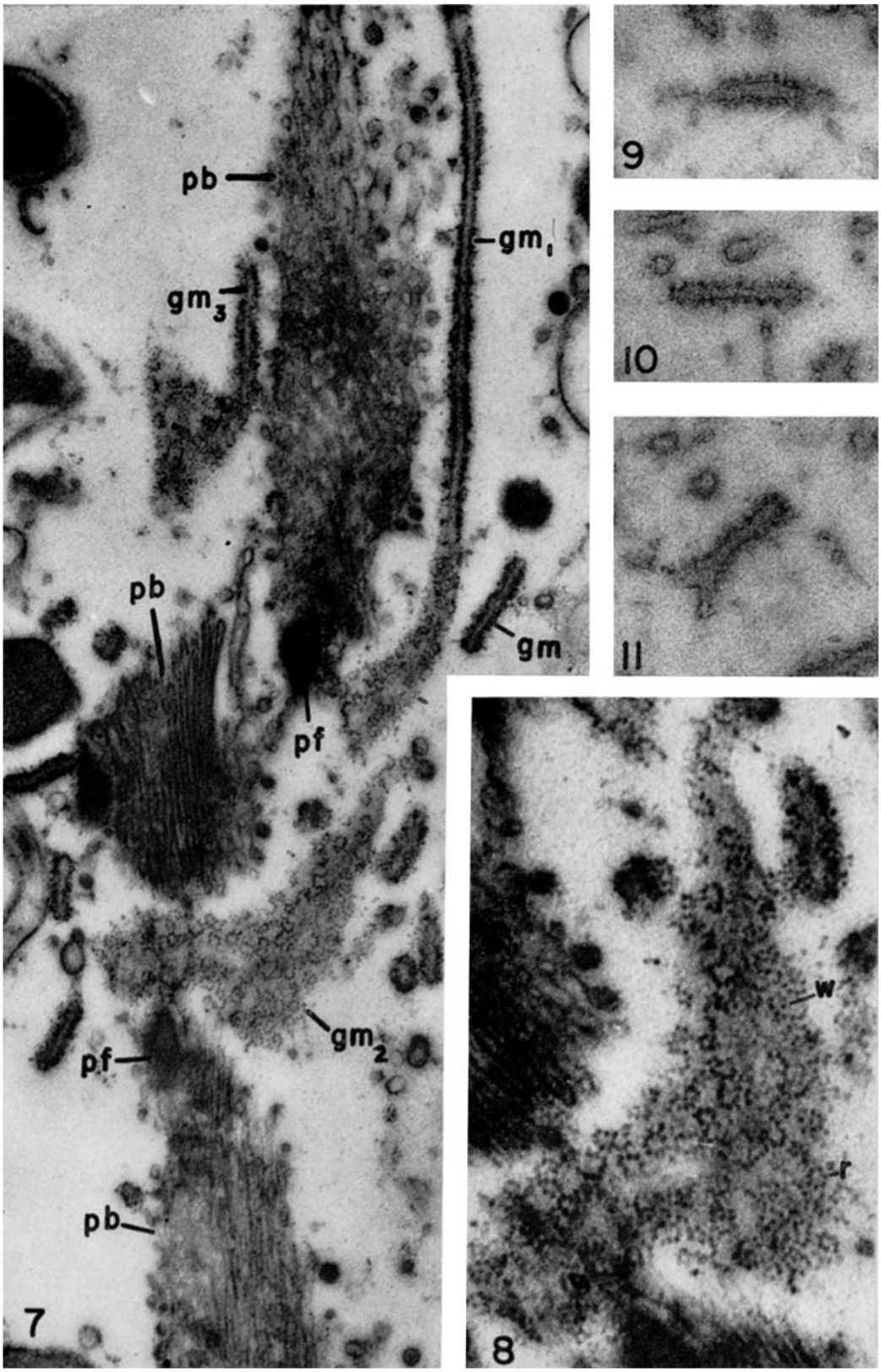
(Grimstone: Cytoplasmic and nuclear membranes)

PLATE 173

FIG. 7. Parts of three parabasal bodies (*pb*) are shown. The middle one, sectioned more transversely than the others, shows the sacs which form the parabasal bodies. The parabasal filaments (*pf*) are also indicated. Several granular membranes (*gm*) are also shown. One of them (gm_1) is sectioned longitudinally in the top half of the picture and then bends, so that it is seen in surface view. gm_2 may be a continuation of the same membrane. gm_3 also changes orientation. Note the whorls of granules in the surface views. × 45,000.

FIG. 8. Enlargement from Fig. 7 to show rings (*r*) and whorls (*w*) of granules on the granular membrane, gm_2 of Fig. 7. × 80,000.

FIGS. 9 to 11. Transverse sections of granular membranes, to show the extremely thin membranes and the granules on their outer surfaces. Note that the material within the membranes is more dense than the cytoplasmic ground substance. × 70,000.



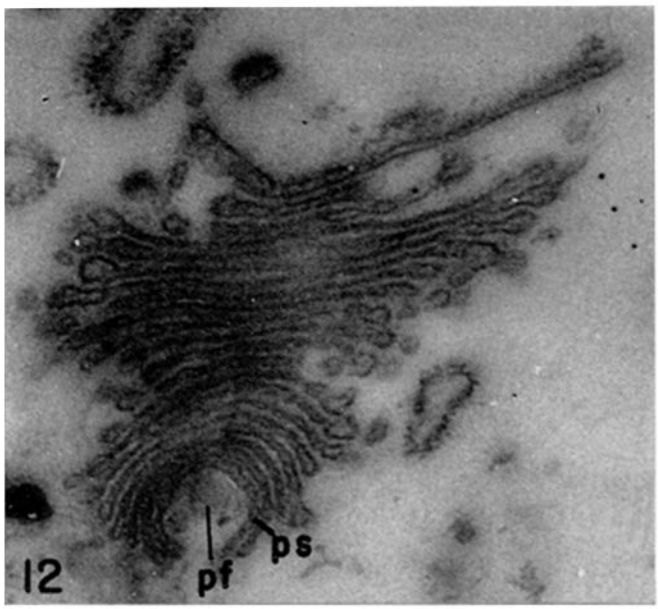
(Grimstone: Cytoplasmic and nuclear membranes)

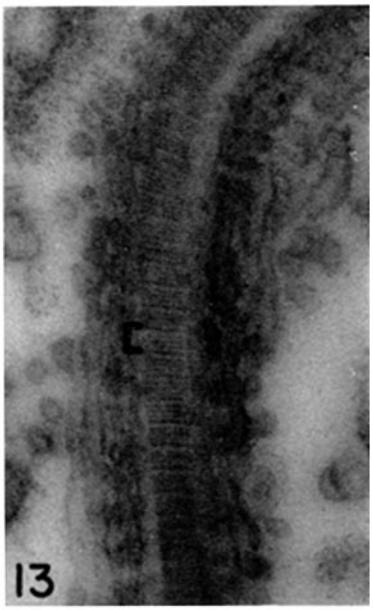
PLATE 174

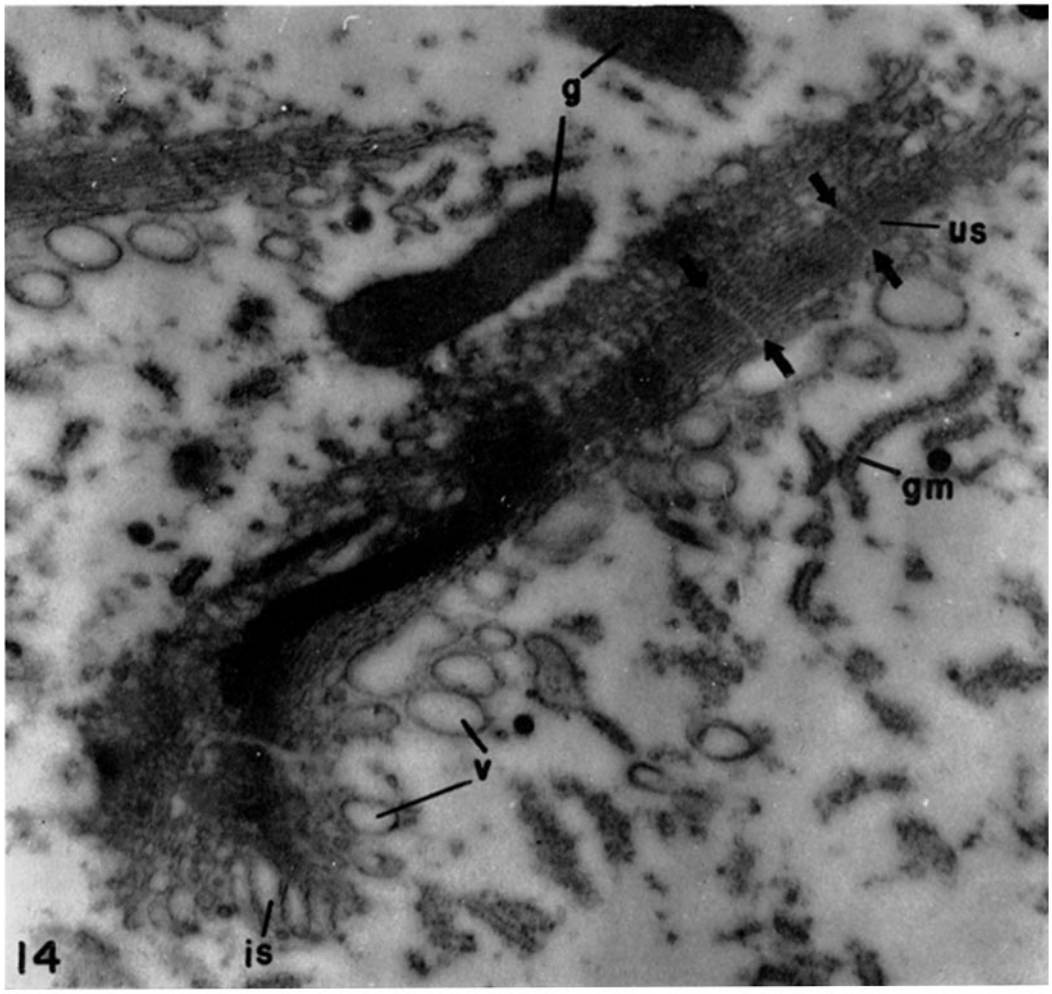
FIG. 12. Transverse section of a single parabasal body, showing the parabasal filament (*pf*) and the pile of sacs. The filament is closely embraced by the sacs (*ps*) nearest to it. These are the proximal sacs; the distal ones are at the top of the picture. This parabasal body is unusual in having an exceptionally large number of sacs, none of which is inflated. × 75,000.

FIG. 13. Longitudinal section of a parabasal filament, showing its cross-striations. There are three fine, dark bands within each main period. Membranes of the parabasal sacs lie on either side. × 80,000.

FIG. 14. Longitudinal section of a parabasal body, which shows both uninflated (*us*) and inflated sacs (*is*), together with cytoplasmic vesicles (*v*) apparently derived from the latter. The breaks in the membranes (arrowed) are thought to be artifacts. The dense bar on the parabasal body is caused either by dirt or a fold in the section. Part of another parabasal body, together with cytoplasmic vesicles, is seen at top, left. Note also the granular membranes (*gm*). The dense bodies (*g*) are probably not bacteria in this case, but a type of cytoplasmic granule characteristic of this organism. × 35,000.





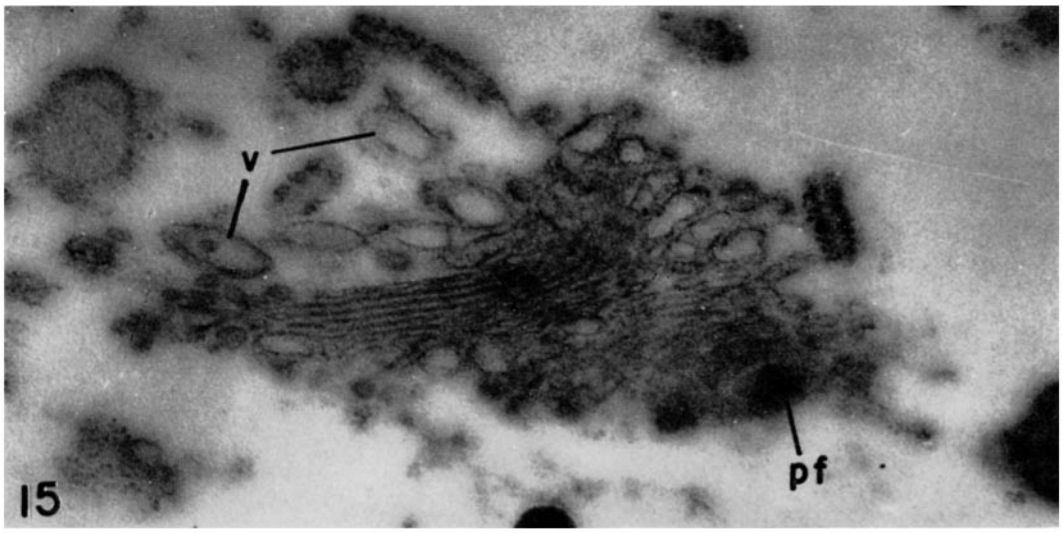


(Grimstone: Cytoplasmic and nuclear membranes)

PLATE 175

FIG. 15. Slightly oblique section of a parabasal body, showing the parabasal filament (*pf*), a pile of sacs, the more distal of which have swollen margins, and cytoplasmic vesicles (*v*). Note that there is very little inflation near to the filament. × 60,000.

FIG. 16. Oblique section of a parabasal body. The filament (*pf*) is embraced by uninflated, proximal sacs (*us*), and the most distal sac (*is*) is highly inflated. Two granular membranes occur, very close to the parabasal filament (gm_1, gm_2); gm_2 may be in continuity with one of the proximal parabasal sacs. Micrographs of this kind suggest that granular membranes are involved in sac formation. × 75,000.


v
pf
15

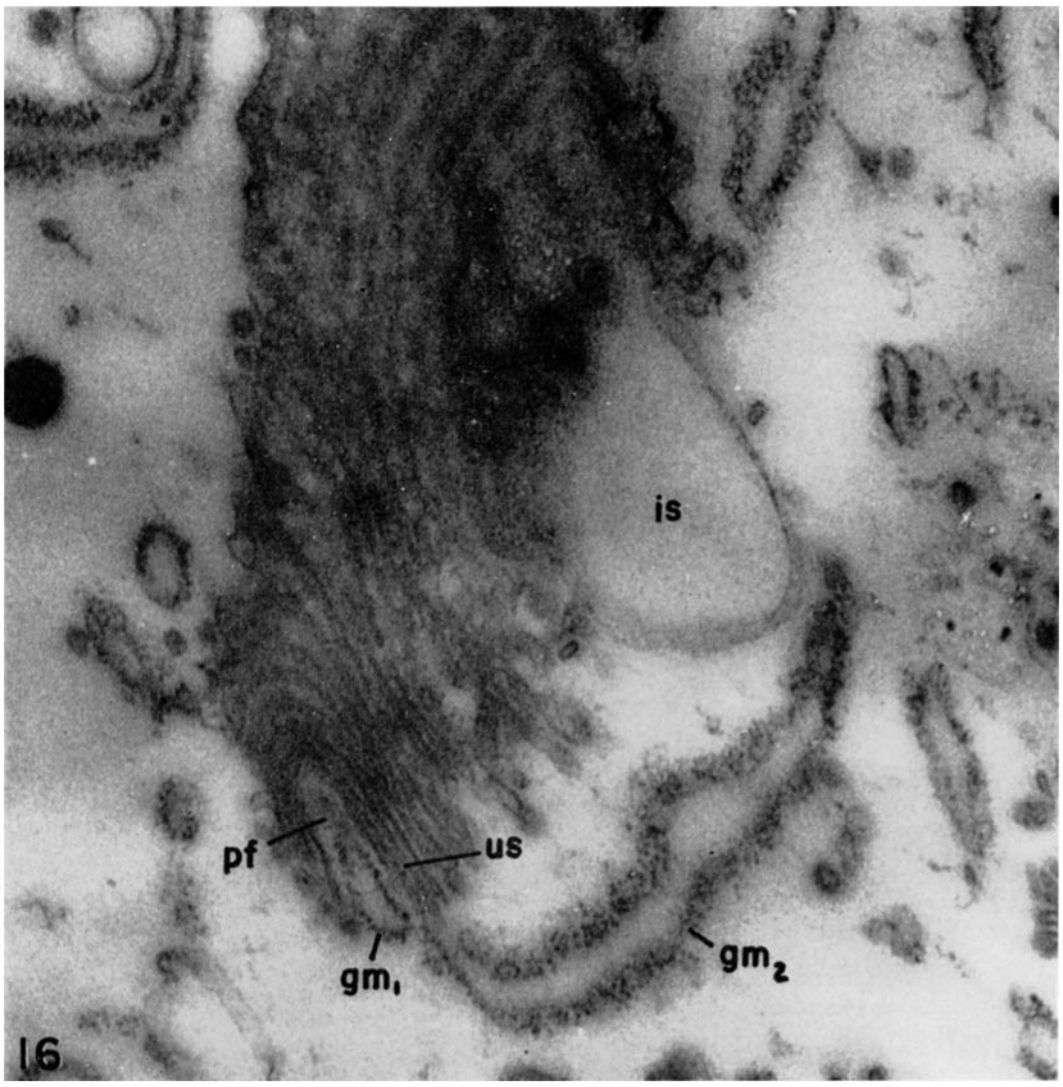
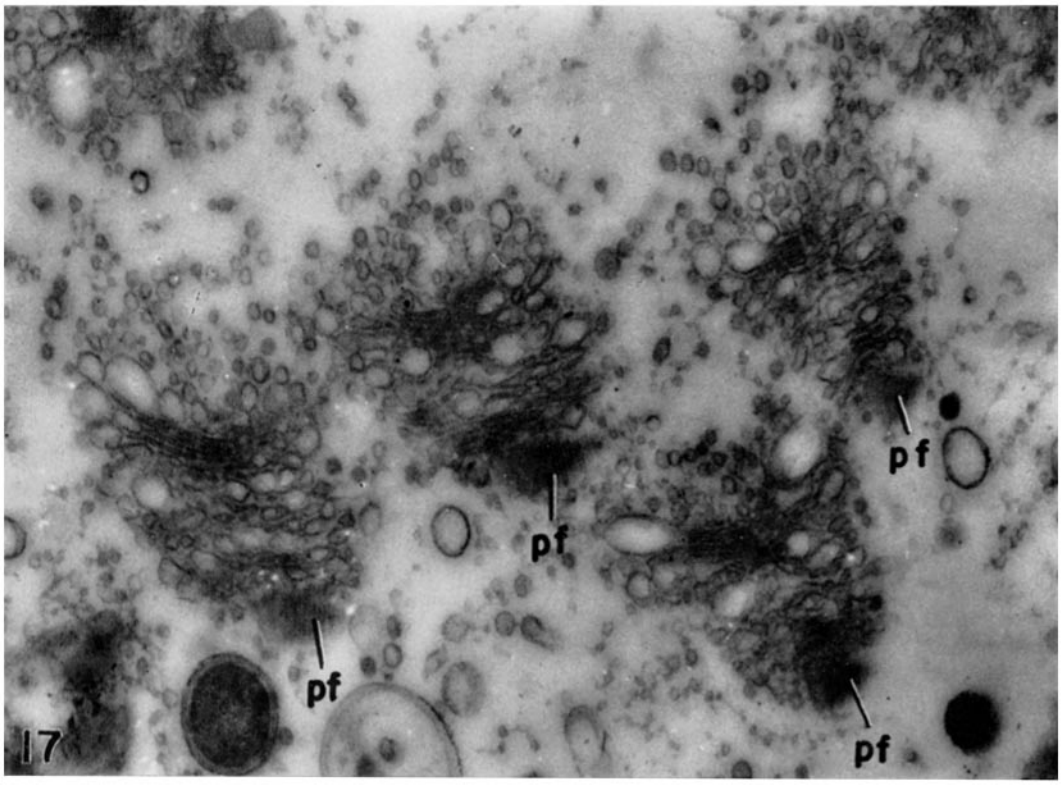

is
pf
us
gm1
gm2
16

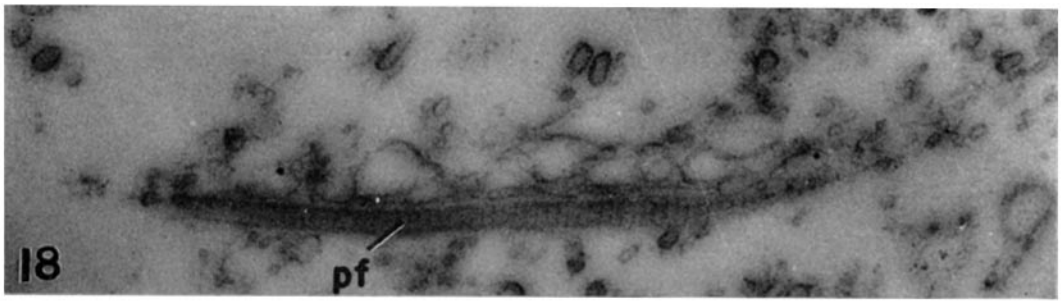
PLATE 176

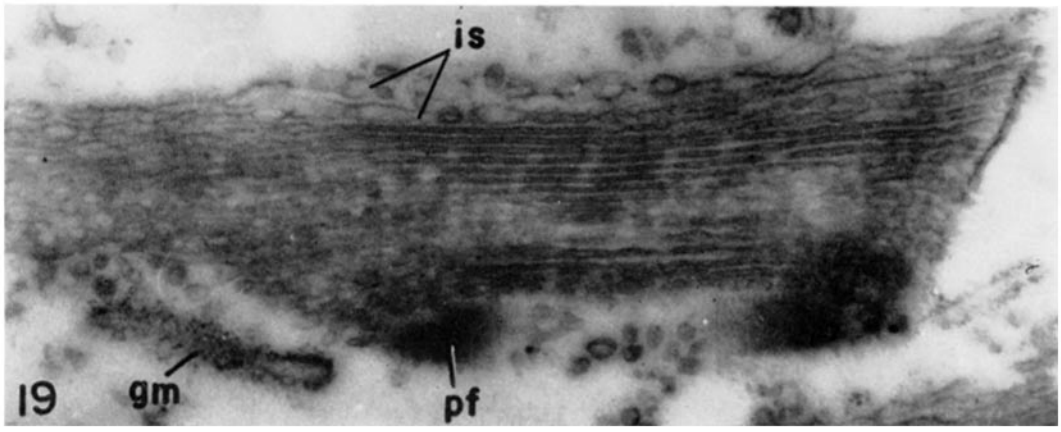
FIG. 17. Parabasal bodies after starving for 2 days. Almost no uninflated sacs are left. Most of the sacs are swollen and seem to be giving rise to either large or small cytoplasmic vesicles. The parabasal filaments (*pf*) are indicated. Note the entire absence of granular membranes. × 38,000.

FIG. 18. Parabasal body after 3 days' starving. Only the filament (*pf*) and a few inflated sacs remain. × 38,000.

FIG. 19. Parabasal body 2 days after refeeding, following 3 days' starvation. A pile of 17 sacs is present, of which the two most distal are just beginning to inflate (*is*). The remainder are wholly uninflated. Note the parabasal filament (*pf*) and the granular membrane (*gm*). × 60,000.







(Grimstone: Cytoplasmic and nuclear membranes)