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OBSERVATIONS ON THE FINE STRUCTURE OF THE PYRAMIMONAS STAGE OF *HALOSPHAERA* AND PRELIMINARY OBSERVATIONS ON THREE SPECIES OF *PYRAMIMONAS*

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(With 47 Figures on Plates I–XIV)

It has recently been established (Parke & Adams, 1961) that some *Pyramimonas*-like monads, isolated from the marine plankton near Plymouth and maintained for many cell-generations in culture, were nevertheless part of the life history of the non-motile green alga *Halosphaera* Schmitz (1879). In reporting this discovery these authors described salient structural features of the monads from one particular culture (no. 205 in the Plymouth collection), as far as this was possible by means of the light microscope. In extending the inquiry by means of the electron microscope the same culture, among others, has been utilized, but in addition three species already recorded in the literature as true members of the genus *Pyramimonas* Schmarda (1850) have been investigated in a preliminary way in order to establish at once the reality or otherwise of the apparent resemblance.

As is usual in such studies, the inquiry in each case began with observations on external morphology from shadow-cast whole mounts. For the *Pyramimonas* species these were sufficient to demonstrate a very close similarity to the *Halosphaera* zooids in several elaborate and unusual details and only these will be quoted. The initial elucidation of the nature of the details nevertheless required sections. In attempting to secure sections from all the cultures under examination very great differences in fixability were at once encountered. Fortunately culture 205 proved to be relatively favourable in this respect and the main emphasis for the present purpose will be on this.

A complete description of all aspects of fine structure will not, however, be attempted here, even for culture 205. There are several anatomical characters as well as general problems regarding the changes involved in the transition to the *Halosphaera* condition which must receive intensive separate study later. It will be sufficient at present to concentrate attention on those internal features most necessary for understanding the external morphology. This, in

effect, will virtually limit the inquiry to fundamental observations on scales, their arrangement and their mode of origin. Other topics will only be introduced incidentally if they happen to be included in the micrographs selected for reproduction.

MATERIAL AND METHODS

As previously reported (Parke & Adams, 1961) culture 205 was obtained from a sea-water sample taken at the surface at position lat. N. 50° 06′, long. W. 04° 21′ on 9 July 1958. Similar monads have subsequently been established in culture many times from *Halosphaera* individuals which liberated their zooids after capture. One particular culture obtained in this way from the contents of one *Halosphaera* cell brought in from the sea near Plymouth on 10 May 1960, and known as no. 247 (1960) in the Plymouth collection, has been included in the present inquiry because of the exceptional ease with which it will reconstitute the *Halosphaera* condition in culture at appropriate times of year (mainly spring and early summer). The reasons for the differences in the behaviour of these two cultures are unknown, but because they exist it will be necessary to specify individually which isolate is being used in the account which follows.

The three *Pyramimonas* species were all originally obtained from the English Channel. *P. grossii* was first brought into culture by the late Dr F. Gross and described by Parke (1949) though it was subsequently lost. It was re-isolated (no. 78 in the Plymouth collection) in May 1950 from a sea-water sample taken at position lat. N. 49° 21′, long. W. 04° 54′ at the surface, but was again lost in 1962. Fortunately, however, a subculture had been sent to the Zoology Department, University College, Swansea (Dr J. Moyse), for use as a food organism, and we were able to re-establish it for the present purpose from there. *P. amylifera* Conrad (1939), no. 246 in the Plymouth collection, was obtained from an inshore water sample taken off Plymouth on 20 Jan. 1961. The third species, no. 280, has recently been encountered in a mixed sample brought in on 13 June 1962 and identified as resembling, though perhaps not identical with, *P. obovata* Carter (1937). It will be referred to as *P. aff. obovata* Carter for the present purpose.

The technical methods employed offer nothing worthy of special comment except for the use of a light stain with uranyl acetate for sections. Fixation for these was with 2% osmium tetroxide buffered to pH 7 with acetate veronal and used for 1 h or less, followed by methacrylate embedding. Sections were cut with a glass knife on a Porter–Blum microtome and mounted on carbon films. When dry they were floated section-side downwards on a freshly prepared saturated solution of uranyl acetate in 50% alcohol in the dark for 30 min, followed by thorough rinsing in distilled water. The preparative treatment for whole mounts (fixing with osmic vapour on a prepared carrier, drying, rinsing and shadow-casting with gold-palladium) is standard.

Most of the preparative treatment involved in whole mounts and embedding was carried out at Plymouth during a 4-day visit by the Leeds collaborators in July 1961. Supplementary preparations, notably those used for Plates XI and XIV, representing

the two smaller species of *Pyramimonas*, were completed in the summer of 1962 on material posted to Leeds. All the electron microscopical observations were carried out in Leeds using a Siemens Elmiskop I.

OBSERVATIONS ON THE *PYRAMIMONAS*-LIKE ZOOIDS OF *HALOSPHAERA*

(Cultures 205 and 247, Pls. I–X)

Pl. I sufficiently illustrates the range of cell types commonly encountered among *Halosphaera* zooids when grown in culture. Fig. 1. represents the normal quadriflagellate condition in culture 205. Figs. 2–4 are taken from culture 247 at a time of year (July) at which reconstituted *Halosphaera* cells were abundantly present in it (Fig. 4). Such cultures also contain a considerable array of different sizes and shapes among the monads. Fig. 3 illustrates a pyramidal quadriflagellate cell, but when division has been rapid the body size may become greatly reduced, the pyramidal shape may be lost and the number of flagella per cell restricted to two or even one (Fig. 2). An individual in this last condition could readily be mistaken for a *Micromonas* (Manton & Parke, 1960) if its origin were not known.

When shadow-cast whole mounts of the kind used for Pl. I are examined at higher magnifications a profusion of scales is always encountered, though by no means all the scale types produced are at once detectable in this way. That the flagella are scale-covered to their tips is a most striking characteristic and examples included in Pls. II and III will show, for both cultures, some of the varied appearances manifested according to the degree to which the superficial scales are undisturbed or partially shed. There is some indication that the flagella may also carry hairs of a peculiar kind though these are exceedingly fragile and are more conspicuous as separate objects (Fig. 11, Pl. IV); their exact relation to the flagella and flagellar scales has not yet been clarified.

The flagellar scales

The scales characteristic of flagella in both cultures and in cells of all sizes (quadri-, bi- or uniflagellate) are rounded or slightly hexagonal plates each with a longitudinally arranged central ridge resembling a bar on the outer side. When in position on the flagellum the ridges of successive scales are strictly aligned, giving an appearance as of regular longitudinal striations along the length of an intact flagellum (Figs. 5 and 8, Pls. II and III). When disturbed, these scales can be found either scattered singly or in compact sheets of various sizes with their imbricated arrangement still preserved. Fig. 9, Pl. III, illustrates a specimen in which the under-face of such an imbricated sheet is exposed. When undisturbed each scale overlaps the hind edge of the scale in front, direction being assessed in relation to the distal tip of the flagellum which is regarded as forward.

Beneath the superficial scales lies a rugose or papillate surface (Figs. 6 and 9, Pls. II and III) which at first was interpreted as that of the flagellar membrane. This is not, however, the correct interpretation. In decomposing specimens (Fig. 7), or still more clearly in sections (Fig. 10, Pl. III), the papillae can be separately seen as a second layer of tiny scales of wholly different design underlying the superficial scales, but attached so firmly to the flagellar surface that they appear to be embedded in some kind of cementing material. This material has either dissolved or become electron-transparent in sections embedded in methacrylate (Fig. 10), but in whole mounts its presence is necessarily postulated to explain the deceptively homogeneous appearance of the surface of this

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Explanation of Plates I–IV

Pyramimonas stages of *Halosphaera*

I

Fig. 1. A characteristic cell from culture 205 (with a rod-shaped bacterium at the base of the 4 flagella); micrograph B3597, ×3000.

Fig. 2. The smallest type of cell produced by culture 247; more highly magnified details in Pl. V, fig. 13. Micrograph S734.27, ×3000.

Fig. 3. The most usual type of cell produced by culture 247; more highly magnified details in Pl. III, fig. 9. Micrograph B3584, ×3000.

Fig. 4. Cell of the *Halosphaera* type produced by culture 247. Micrograph B3576, ×3000.

II. Culture 205

Fig. 5. Parts of two flagella with their surface coverings intact, from a quadriflagellate cell off field left. Overlapping imbricated scales arranged in longitudinal rows. Micrograph B3602 reversed print, ×30,000.

Fig. 6. Part of a flagellum with the outer scales disarranged and exposing the rugose surface of the underlayer; micrograph B4931 reversed print, ×30,000.

Fig. 7. Fragment of surface from a damaged flagellum showing the small scales of the underlayer beginning to separate, scales of the outer layer also present and one square body-scale at the bottom of the field; micrograph B4924, ×30,000.

III. Culture 247

Fig. 8. Part of a flagellum with undamaged surface for comparison with fig. 5, direction of the subtending body off field right; a few flagellar hairs still present; detached flagellar scales (bottom right), detached scales from the flagellar underlayer (inset left) and a single body-type scale (bottom centre). Micrograph B3560 reversed print, ×33,000.

Fig. 9. Tip of a flagellum from the cell of Pl. I, fig. 3, showing part of the outer layer of scales folded back and exposing the rugose underlayer. Micrograph B3592, reversed print, ×30,000.

Fig. 10. Oblique longitudinal section of a flagellum showing the flagellar axis (left) covered by the flagellar membrane with the two superposed layers of scales outside it, the close-packed spiral arrangement of the small scales of the underlayer clearly visible (right). Micrograph B7427, ×50,000.

IV

Fig. 11. Detached flagellar hairs from the cell of Pl. I, fig. 2; micrograph B3591 reversed print, ×30,000.

Fig. 12. Field of scattered scales showing the range of patterns including two scales from the flagella, the remainder body-type scales, culture 205; micrograph B4920, reversed print, ×30,000.

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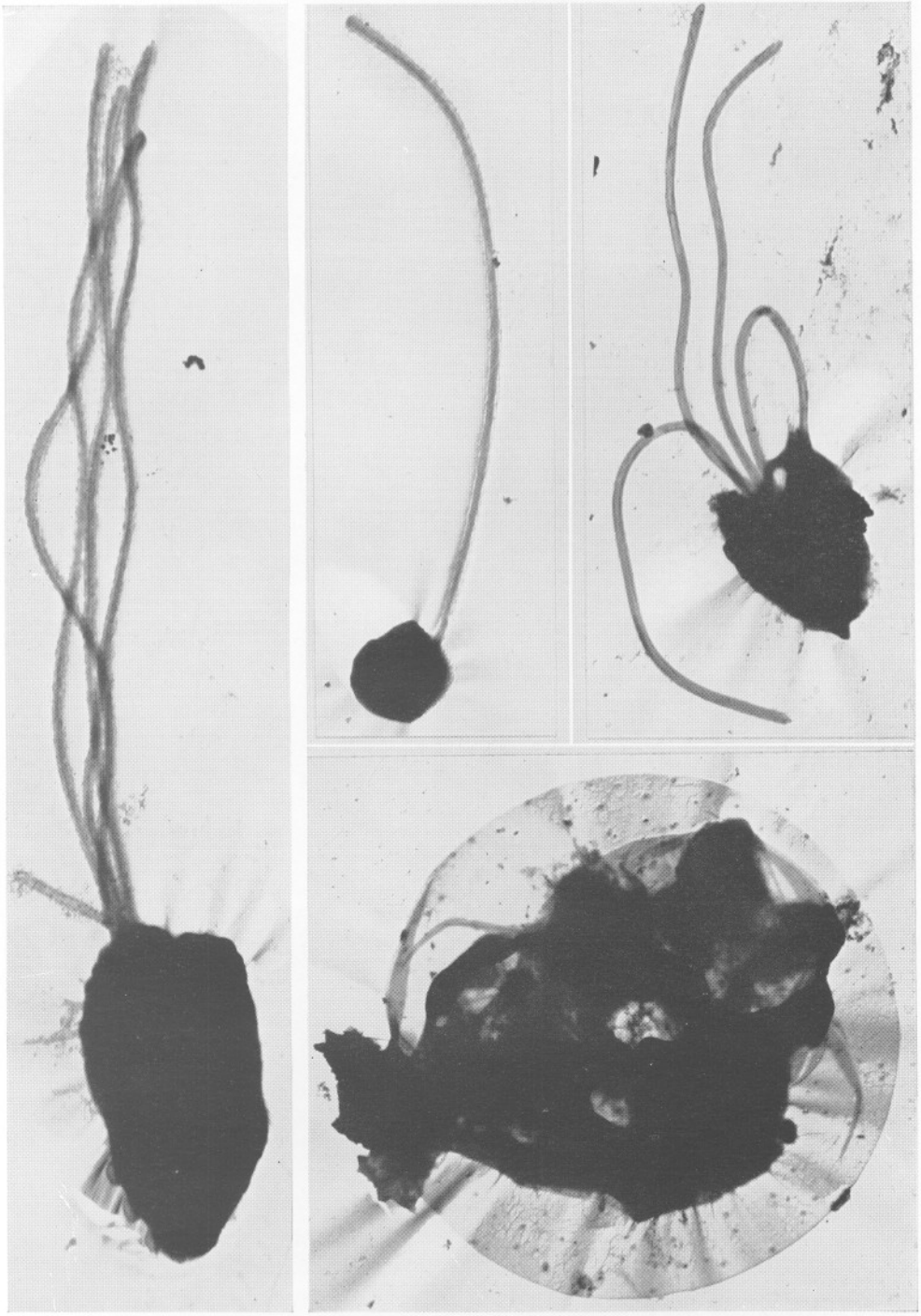

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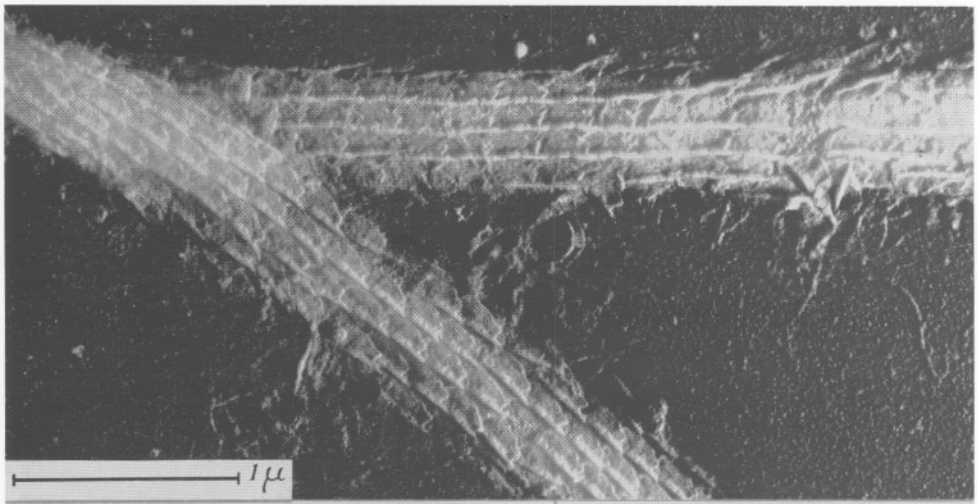

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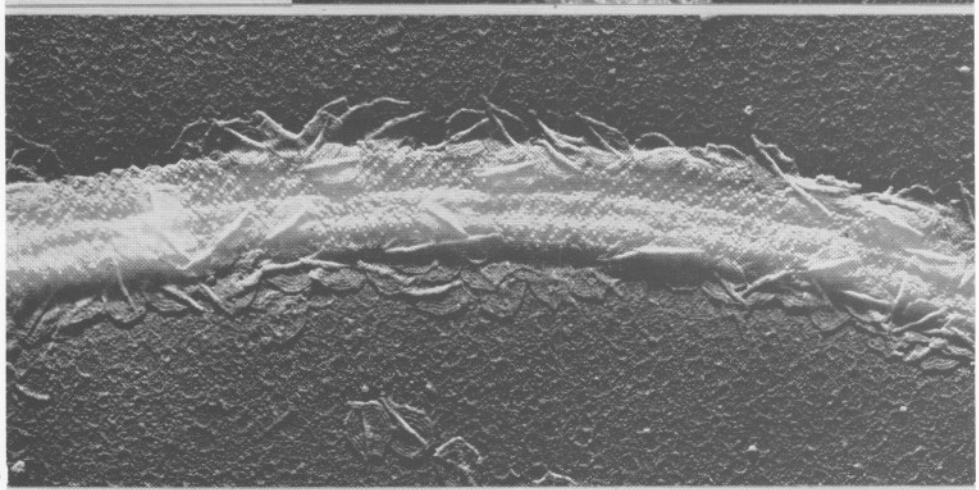

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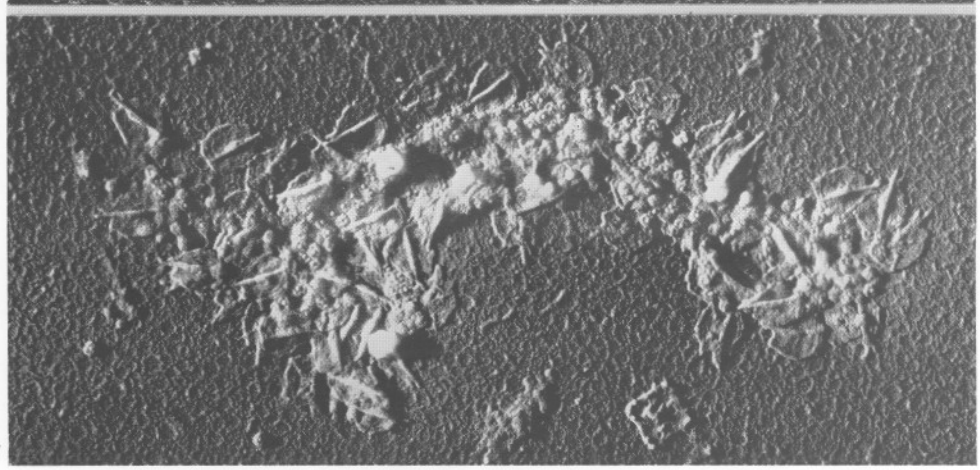

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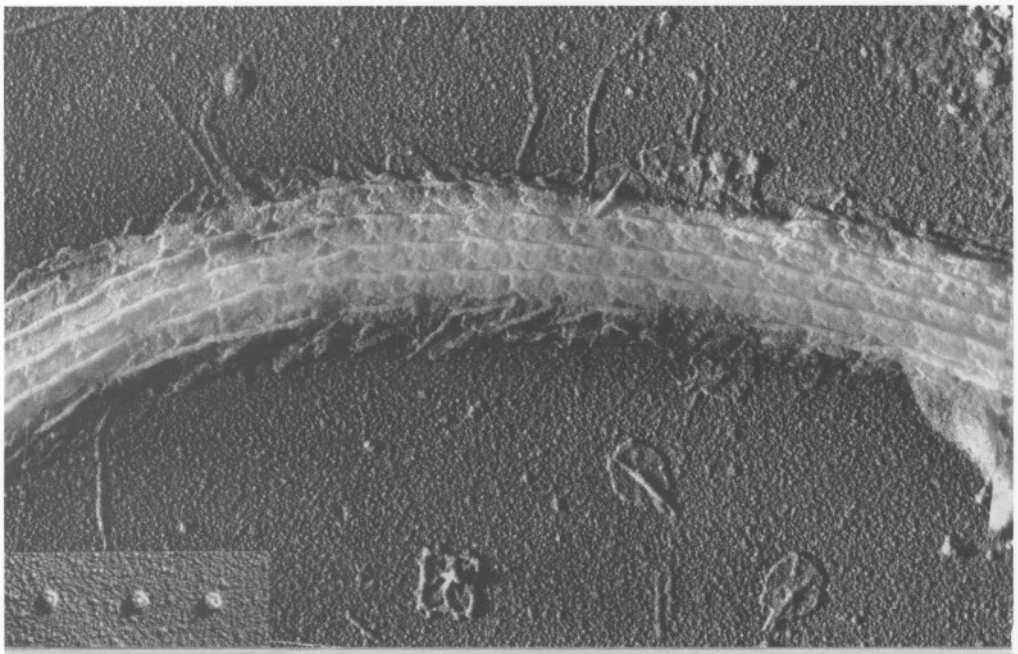

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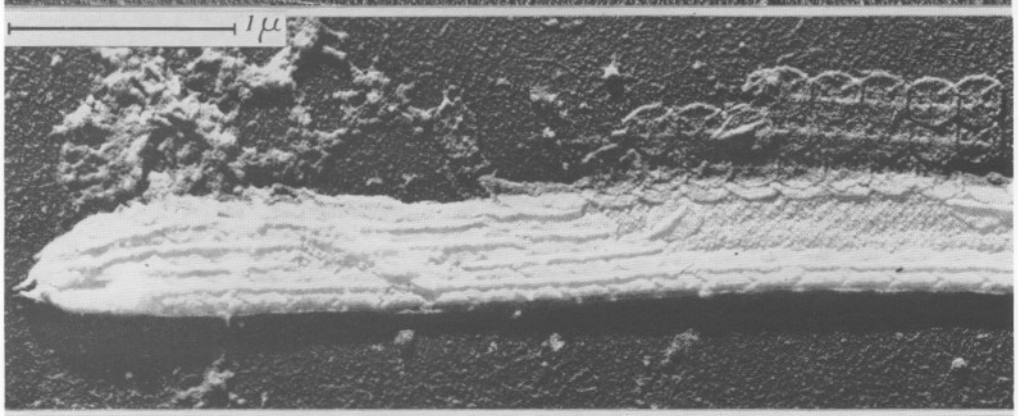

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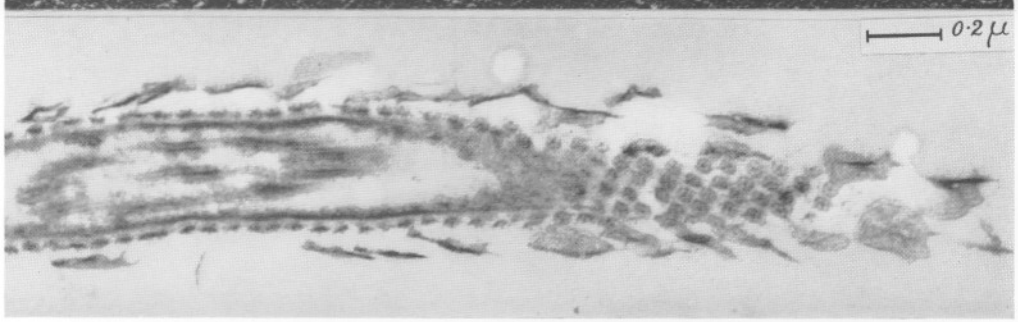

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The most difficult type of scale to encounter in position on the cell are the flat lace-like plates. These are always present in abundance in whole mounts and their scarcity from sections suggests that here we have the converse of the situation regarding the underlayer, namely an absence of cementing material and a resulting loss of these scales from surfaces when handled. In favourable cases (e.g. Fig. 18) the flat scales can be identified as a third layer on the outer side, but whether this is an invariable position is still uncertain.

Origin of the scales

All the remaining sections except one (Fig. 28) will be from culture 205, since this alone gave tolerable fixation of the protoplasmic structures with the methods employed. The cell size is unfortunately too large to permit reproduction of complete sections except at very low magnifications. Two are,

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Explanation of Plates V–VII

Pyramimonas stages of *Halosphaera*

V

Fig. 13. Part of the surface of the body and base of a flagellum, from the cell of Pl. I, fig. 2 (culture 247) to show the different character of the scales on the two regions; micrograph S734.32, ×20,000.

Fig. 14. Slightly oblique transverse section of a flagellum (culture 247) to show the layering of the scales, and the stellate outline denoting 9 longitudinal rows of outer scales; micrograph B7955, ×40,000.

Fig. 15. Oblique transverse section of a flagellum and adjacent part of the subtending cell surface, from culture 205, other details as in fig. 14; micrograph B5027, ×40,000.

Fig. 16. Tangential section of the surface of a cell near the flagellar pole showing parts of a striated flagellar root and square scales of two sizes (culture 205); micrograph B5107, ×40,000.

Fig. 17. Tangential section through the two layers of body scales (culture 247), the small scales of the underlayer arranged in irregular lines, the larger outer scales showing a somewhat rounded outline with distinct rims (compare with fig. 13); micrograph B7885, ×40,000.

Fig. 18. Tangential section showing parts of three layers of body scales, an underlayer of small squares, an outer layer of square plates with rims and traces of an apparently outermost layer of lace-like plates without rims. Culture 247, micrograph B8063, ×50,000.

VI. Culture 205

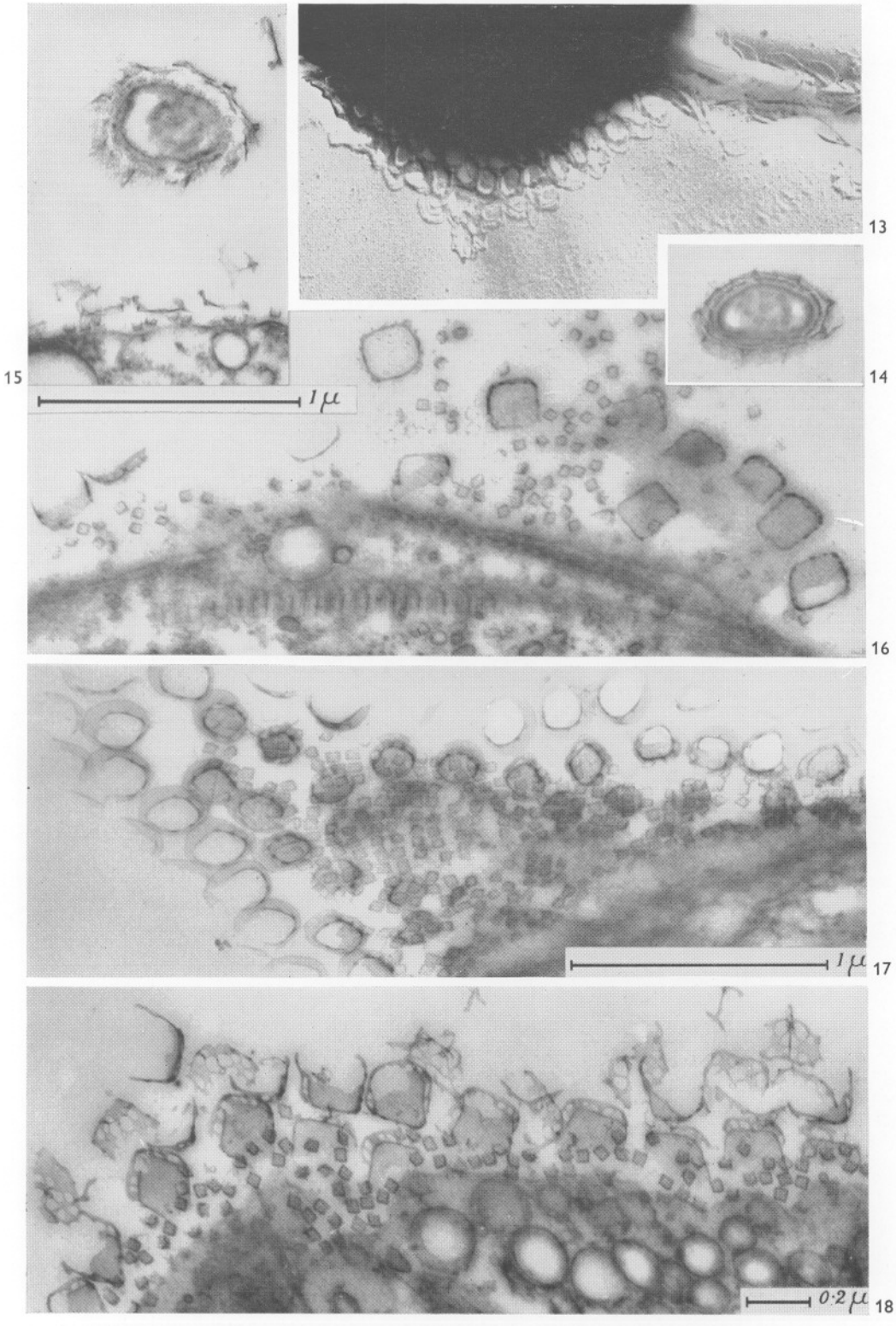
Fig. 19. The central area of the section of fig. 20 between the plastid (*P*), the nucleus (*N*) and the reservoir (*R*), showing mitochondria (*m*) and four Golgi bodies (*g*), two of them with traces of scale production, for further details see Pl. VII; micrograph B8172, ×20,000.

Fig. 20. The section from which fig. 19 is taken; letters as in that figure. Micrograph B8171, ×5000.

VII. Culture 205

Fig. 21. More highly magnified details from another cell showing scale production within a Golgi body (arrows); also a mitochondrion (*m*), a fat body (*f*), parts of two plastid lobes (*P*), and the reservoir (*R*); micrograph B8025, ×40,000.

Fig. 22. The cell of fig. 21 but another section; the field of fig. 21 includes the top margin of the central cavity (reservoir) and the Golgi body immediately above it. Micrograph B7986, ×5000.



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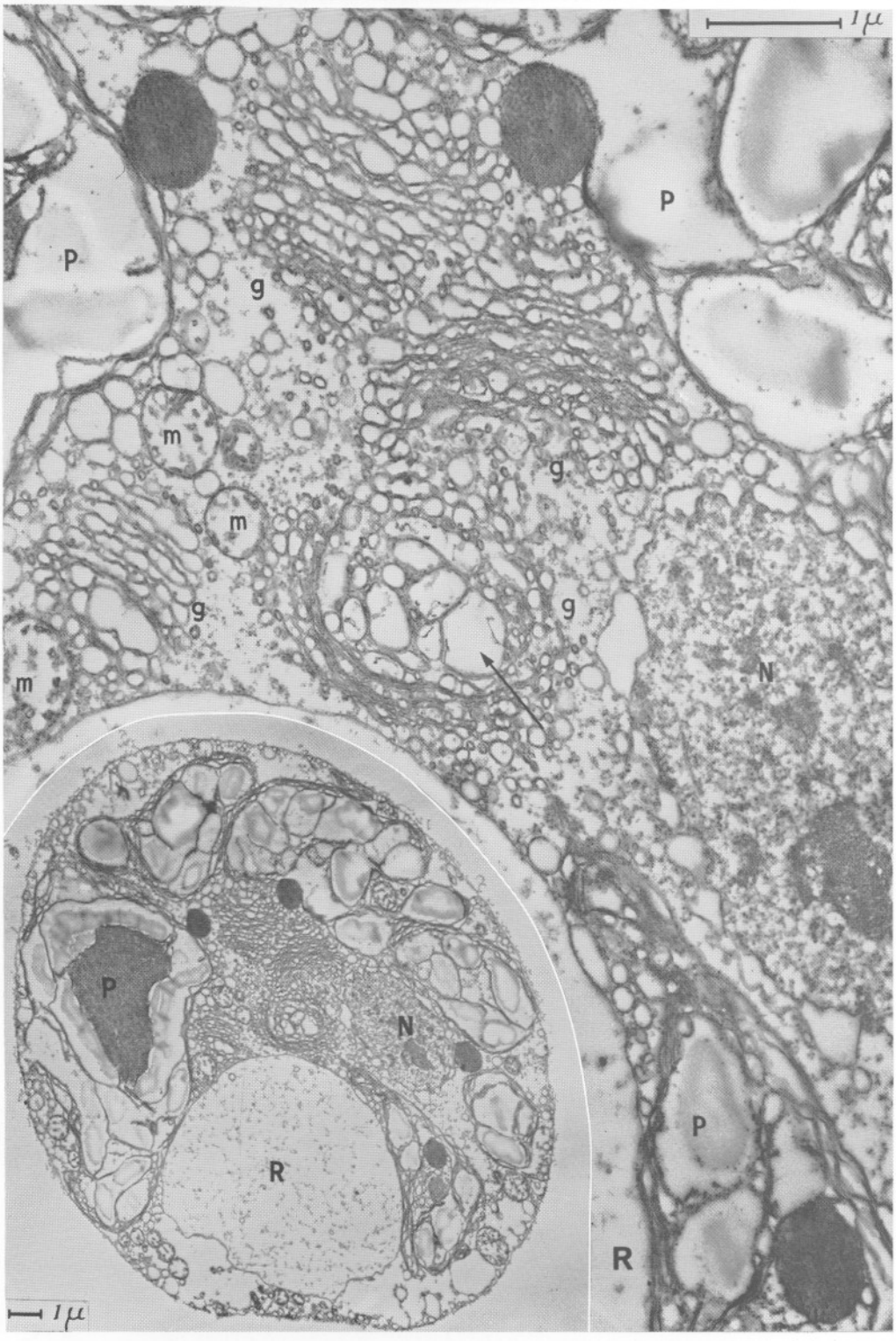

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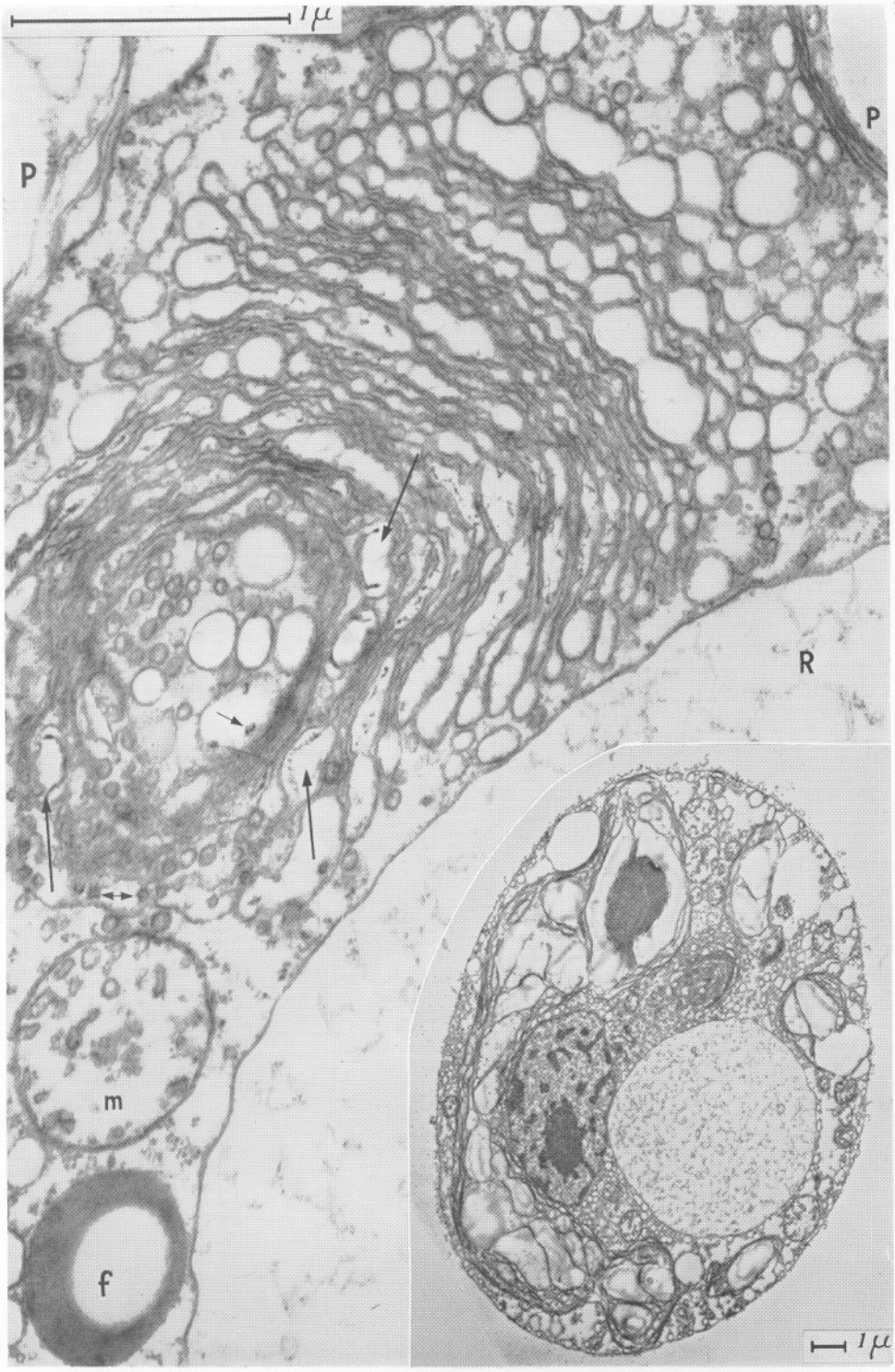

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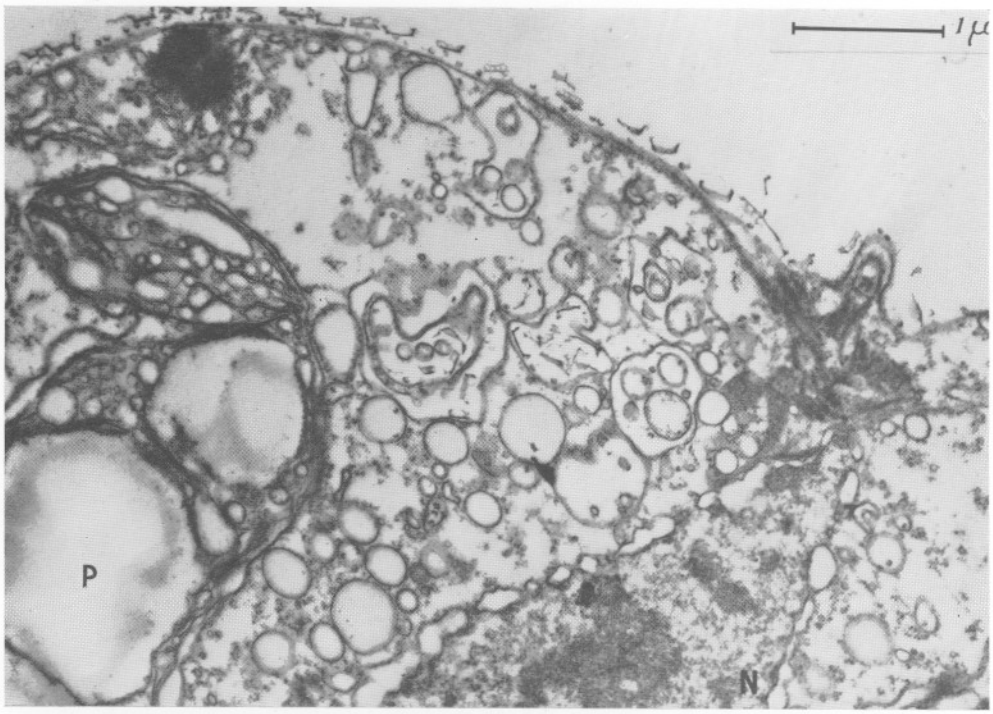

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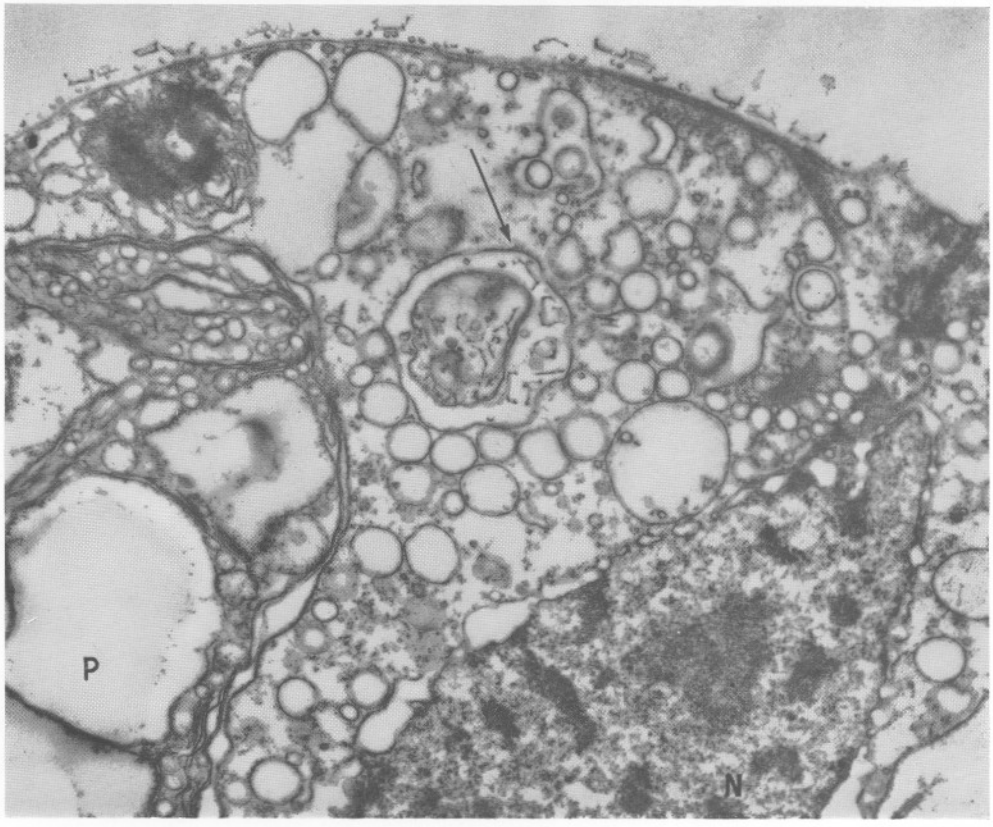

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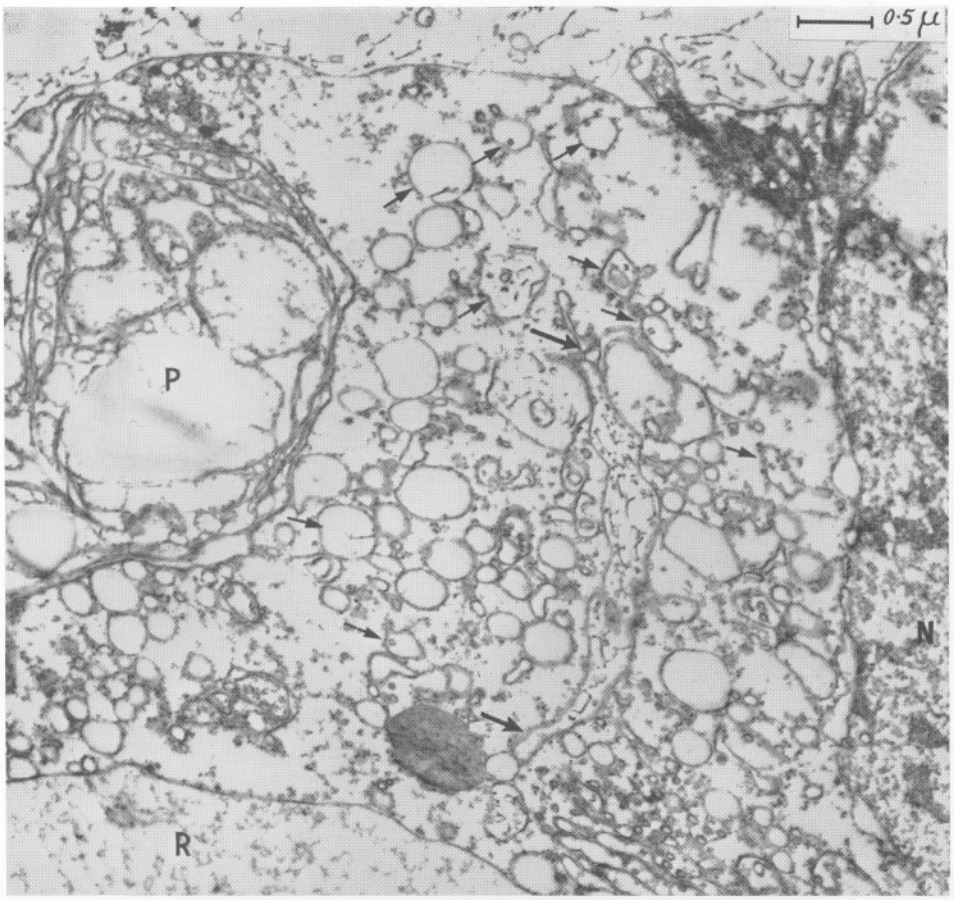

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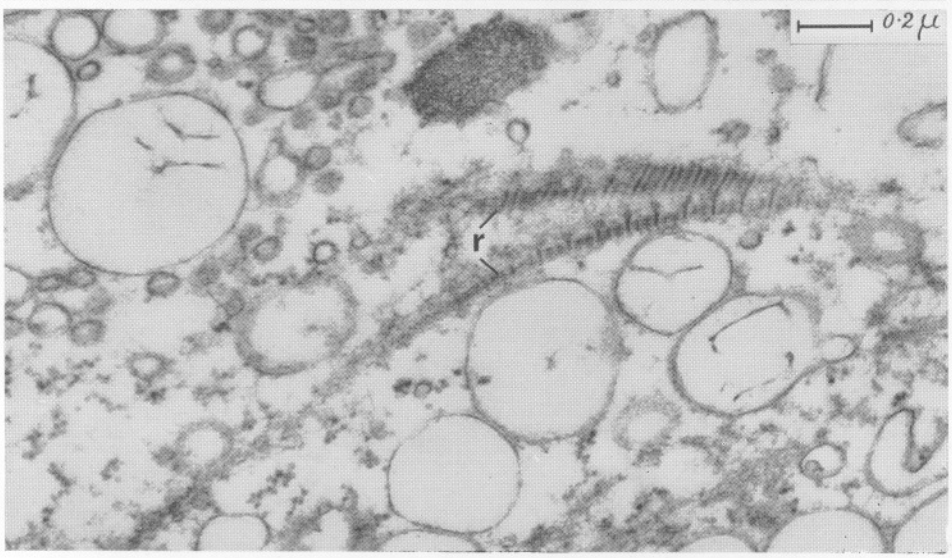

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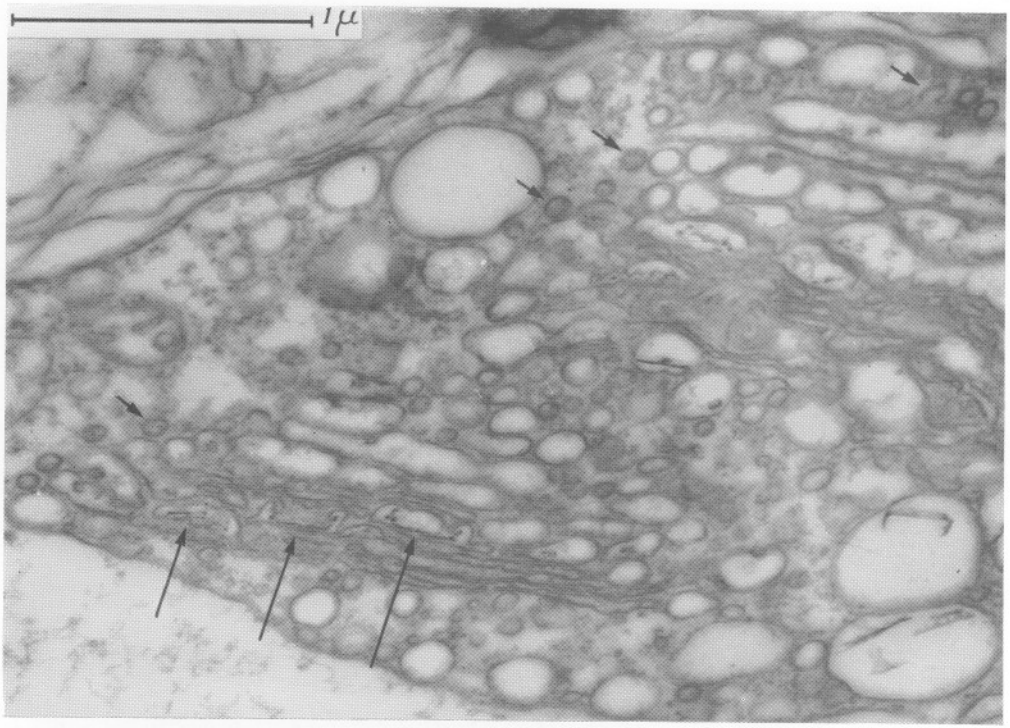

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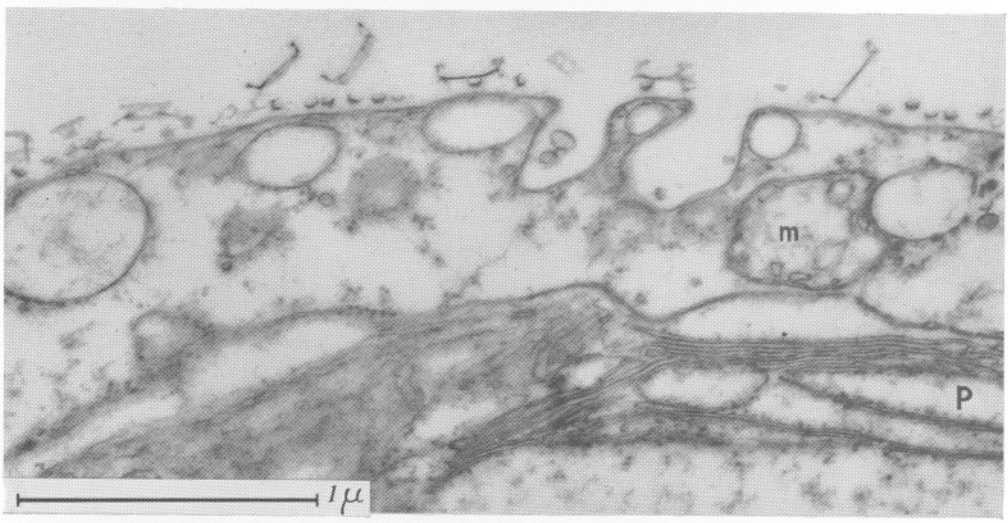

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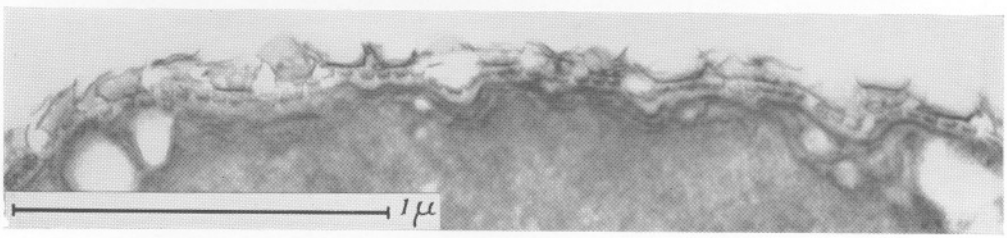

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however, provided (Figs. 20 and 22, Pls. VI and VII) as background to more highly magnified details, and these sufficiently illustrate the nature and arrangement of the various internal parts as they appear in sections not transecting the flagellar bases and therefore somewhat uncertain in exact plane. Both the specimens illustrated are probably in oblique transverse section. Near the periphery is a large much dissected chloroplast containing several pyrenoids of characteristic structure, one of which is contained in each section. The nucleus lies on the inner side of the chloroplast and the large spherical cavity resembling a vacuole is an organ of uncertain nature known as the reservoir (Parke & Adams, 1961). Mitochondria and small vesicles are scattered throughout the cytoplasm, while several Golgi bodies and fat bodies are commonly present in the central region near the nucleus and reservoir.

A detail which can only be seen in another plane of section is that the nucleus is pear-shaped and attached to the flagellar bases at its pointed end. In addition, cross-banded 'roots' pass out from between the flagellar bases to lie immediately under the body surface at the anterior end of the cell for considerable distances. Some of these details are illustrated incidentally in Pls. VIII and IX in sections primarily selected for another purpose, but it will perhaps help understanding if these details are enumerated here.

Explanation of Plates VIII–X

Pyramimonas stages of *Halosphaera*

VIII. Culture 205

Figs. 23 *a*, *b*. Two successive sections through the surface of a cell near the point of attachment of the flagellar bases to the nucleus (*N*), flagellar roots visible under the plasmalemma in both sections, numerous vesicles with scales visible in the cytoplasm between the nucleus and the plastid (*P*), a specially large one marked by the long arrow; micrographs B 8034 and B 8022, × 20,000.

IX. Culture 205

Fig. 24. Section from the same region as that of figs. 23 *a*, *b* another specimen showing part of the reservoir (*R*) and numerous scale-filled cavities (arrows) in the cytoplasm including one of considerable length (thick arrows); micrograph B 8053, × 20,000.

Fig. 25. Tangential section near the surface at the flagellar pole of a cell showing parts of two striated flagellar roots (*r*) and several scale-containing vesicles; micrograph B 7971, × 50,000.

X

Fig. 26. Golgi cisternae between a plastid (top left) and the edge of the reservoir (bottom left), with scales in process of formation (long arrows); concurrent production of small dense-walled vesicles by abstriction from the cisternal edges (short arrows), and completed scales in large rounded vesicles nearby (especially bottom right). Culture 205, micrograph B 8017, × 40,000.

Fig. 27. Surface of a cell with indentations strongly suggesting an early stage in the opening of scale-bearing vesicles to the outside. Culture 205, micrograph B 7983, × 40,000.

Fig. 28. Surface of a cell from culture 247 with corrugations strongly suggesting a late stage in the deposition of scales from vesicles; for further explanation see text. Micrograph B 7956, × 50,000.

The presence of multiple Golgi bodies is an important difference from the condition in several other monads recently studied (*Micromonas*, *Paraphysomonas*, *Chrysochromulina*) which characteristically possess only one. Exactly how many are contained in one *Halosphaera* zooid cannot be certainly known though it is not difficult to find sections showing as many as six. Since it is clearly a matter of chance what proportion of the total contents will be traversed in any one plane of section the most that can be claimed is that the four Golgi bodies visible in the specimen of Pl. VI are more fully representative of the cell as a whole than the single Golgi body which happens to be included in the sections reproduced for other reasons in Pl. VII.

The special importance of the Golgi bodies in the present context will at once become apparent by close scrutiny of Figs. 19, 21 and 26 on Pls. VI, VII and X. In each micrograph, though most conspicuously in the last two, the Golgi cisternae themselves can be seen to contain scales in process of formation. Completed scales are also visible in smaller or larger vesicles, sometimes present in a compact group round which the Golgi cisternae are curved (Fig. 19, arrow), at other times apparently free in the neighbourhood (Fig. 26, right). Fig. 26 (short arrows) also illustrates the concurrent abstriction of small dense-walled vesicles from the edges of the Golgi cisternae in a manner which we have found to be characteristic of Golgi bodies in several other flagellates (e.g. *Paraphysomonas vestita*, Manton & Leedale, 1961; *Chrysochromulina parva*, Parke, Lund & Manton, 1962); the identity of the organelle seems therefore not to be open to question.

Stages in the passage of completed scales towards the cell surface are illustrated in Pls. VIII and IX, which incidentally also include demonstration of the attachment of the flagellar bases to the nucleus and cell surface as mentioned on p. 231. As the surface is approached (Figs. 23*a*, *b*) scale-containing cavities of very irregular shape and often of large size become conspicuous, their shapes frequently suggesting an origin by recent coalescence of smaller vesicles. Sometimes these cavities may take the form of irregular channels almost spanning the entire width of the cytoplasm between the reservoir and the cell exterior (Fig. 24, thick arrows). When viewed from above (Fig. 25) the contours of scale-containing cavities are predominantly circular, although an occasional U shape (Fig. 25, bottom right) again suggests recent coalescence. The proximity of this field to the surface is attested by the cross-banded flagellar roots included in the section (Fig. 25) and which we know to lie immediately beneath the plasmalemma (cf. Figs. 23*a* and 23*b*).

We have no reason at present to suppose that the so-called reservoir plays any part in these happenings though negative evidence is not in itself conclusive. We have so far failed to find any permanent opening of the reservoir to the outside in sections examined with the electron microscope, and though the inner ends of scale-containing channels can obviously be in close proximity to the reservoir boundary (as in Fig. 24) the difference in contents, scales

being virtually absent from the reservoir itself, seems to imply a continuing structural separation.

That the scale-containing cavities eventually open to the exterior by fusion of their bounding membranes with the plasmalemma can scarcely be doubted. It is indeed difficult to interpret a specimen such as that of Fig. 27, Pl. X, in any other way. Indentations of the cell surface, of the type illustrated, if present at all, are very numerous; in the next section to that of Fig. 27 two additional openings were revealed in a closely adjacent field. Such openings can at once be distinguished from accidental surface damage by the smooth continuity of the bounding membranes at the point of junction. It is also found that in suitable places at higher magnifications a tripartite structure can be resolved equally in the plasmalemma and in the bounding membranes of at least the larger scale-bearing cavities. There seems little doubt therefore that these membranes do indeed become confluent.

Whether at a later stage such openings close up again having liberated their scales to the outside, or whether the lining membrane of a cavity flattens out and becomes incorporated into the plasmalemma, cannot easily be ascertained. The latter is, however, inherently probable and the shallow convolutions on the surface of the cell illustrated in Fig. 28, Pl. X, might then be interpreted as a final stage in this process. Be that as it may the conclusion seems unavoidable that here we have direct evidence that the Golgi bodies themselves are concerned with the scale-production cycle and from them additions to the outermost covering of the cell are repeatedly being contributed.

OBSERVATIONS ON SPECIES OF *PYRAMIMONAS*

The three species of *Pyramimonas* to be discussed in a preliminary way in this section will be presented in an arbitrary order determined primarily by cell size. *P. grossii* Parke and *P. aff. obovata* Carter both possess small cells which lend themselves to effective illustration in one plate (Pls. XI and XIV respectively). The cells of *P. amylifera* Conrad, on the other hand, are so large that we have felt impelled to spread the micrographs on to two facing pages (Pls. XII and XIII), which have necessarily to be placed centrally within the group, and we have also been prevented from using the electron microscope to obtain a complete low power view, since for a cell of this size the only means of doing so would have been in the form of a surface replica which we have not attempted to make. For *P. amylifera* therefore we have used the light microscope (Fig. 35) to provide the only general view of the cell to be reproduced here. We have, however, added photographs of each of the other two species (Figs. 29 and 42) taken with the light microscope at the same magnification to maintain comparability.

Explanation of Plates XI–XIV

XI

XI. *Pyramimonas grossii* Parke

Fig. 29. Two living cells photographed with the light microscope, × 1000.
Fig. 30. Dried cell, shadow-cast, micrograph B 8220, × 3000.
Fig. 31. Bases of four flagella on a cell, with scales and broken tubular pieces of a discharged trichocyst; the right-hand side of the cell protected from shadowing by the bulge of the body; micrograph B 8239, × 20,000.
Fig. 32. Tip of a flagellum with hairs and attached scales; other scales, including some of the body type on the field; micrograph B 8236, reversed print, × 30,000.
Fig. 33. Body-type scales of both layers with a flagellar type scale (bottom left), and a single small scale from the flagellar underlayer (left-hand corner inset) all from parts of one field; micrograph B 8218, reversed print, × 3000.
Fig. 34. Large scales of the three main shapes more highly magnified, from parts of one field; a square body-type scale (right), a lace-like plate scale from the body and two flagellar scales (left). Micrograph B 8236, reversed print, × 40,000.

XII. *Pyramimonas amylifera* Conrad

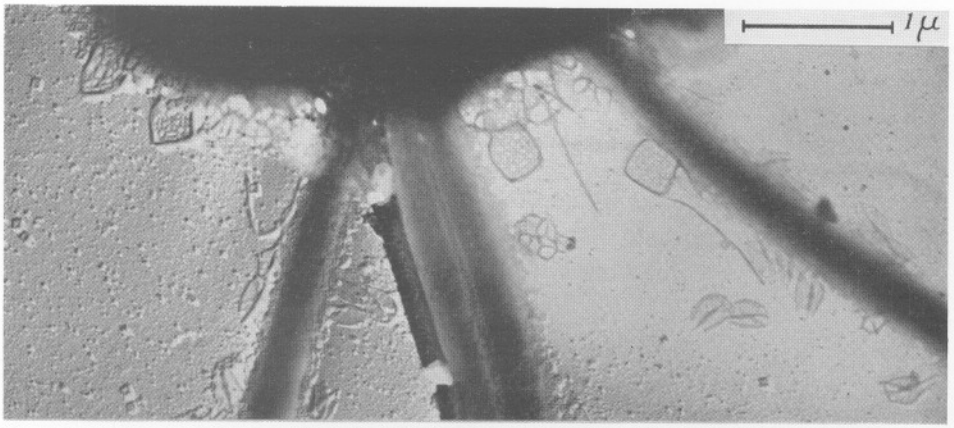
Fig. 35. Two cells killed with iodine and photographed with the light microscope, × 1000.
Fig. 36. Field of scales (unshadowed) near a flagellum (bottom) showing body scales and flagellar scales in a general way. Micrograph B 3762, × 30,000.
Fig. 37. Field of scales similar to those of fig. 36 more highly magnified; one flagellar scale in the centre; rimmed and rimless square scales elsewhere; three small scales from the underlayer at extreme right. Micrograph B 3756, × 40,000.

XIII. *Pyramimonas amylifera* Conrad

Fig. 38. Tip of a flagellum with scales, including some from the body, and with the rugose pattern caused by the small scales of the underlayer visible though not distinct; micrograph B 8154, reversed print, × 30,000.
Fig. 39. Parts of two flagella showing longitudinal ridges caused by aligned outer scales as in fig. 9, Pl. III, the number of ridges corresponding to a total of nine longitudinal rows of scales as in the *Halosphaera* zooids; micrograph B 8152, reversed print, × 30,000.
Fig. 40. Field of square, rimmed, scales from the body, mixed with flat spined scales from the flagella; micrograph B 3766, reversed print, × 30,000.
Fig. 41. Field of rimless body scales with loops, a few other scale-types present including many small scales from the underlayer; micrograph B 3755, reversed print, × 30,000.

XIV. *Pyramimonas aff. obovata* Carter

Fig. 42. Two living cells photographed with the light microscope, × 1000.
Fig. 43. The four flagella with hairs and scales and a subtending body with scales of a different kind; micrograph B 8118, × 10,000.
Fig. 44. Field of large flagellar scales, almost all exposing their dorsal surface; a few small scales of the underlayer also present, the body underlayer (top left inset), the flagellar underlayer (central). Micrograph B 8131, reversed print, × 30,000.
Fig. 45. Somewhat damaged flagellar scale (left) with a round rimmed body scale of an infrequent type (right); compare, however, with Pl. IV. Micrograph B 8143, reversed print, × 30,000.
Fig. 46. Flagellar scale with the ventral surface exposed (contrast with fig. 44); micrograph B 8131, reversed print, × 30,000.
Fig. 47. Three large square body scales, the middle one probably exposing the ventral surface and the other two the dorsal surface, and with a square rimless meshwork scale from the body at extreme right; micrograph B 8141, reversed print, × 30,000.



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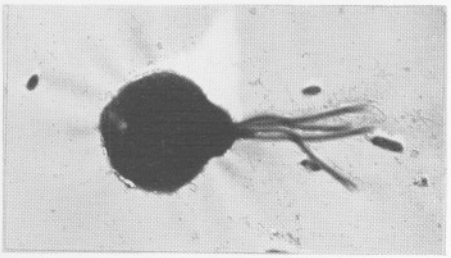

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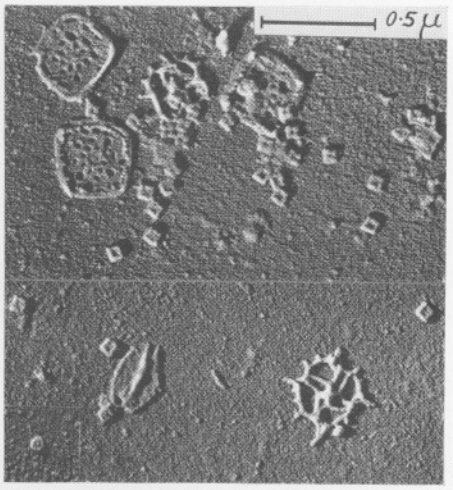

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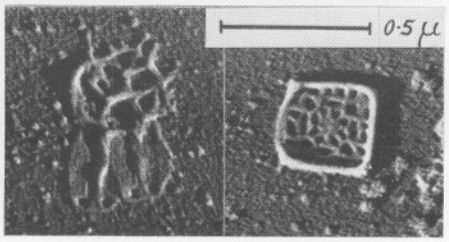

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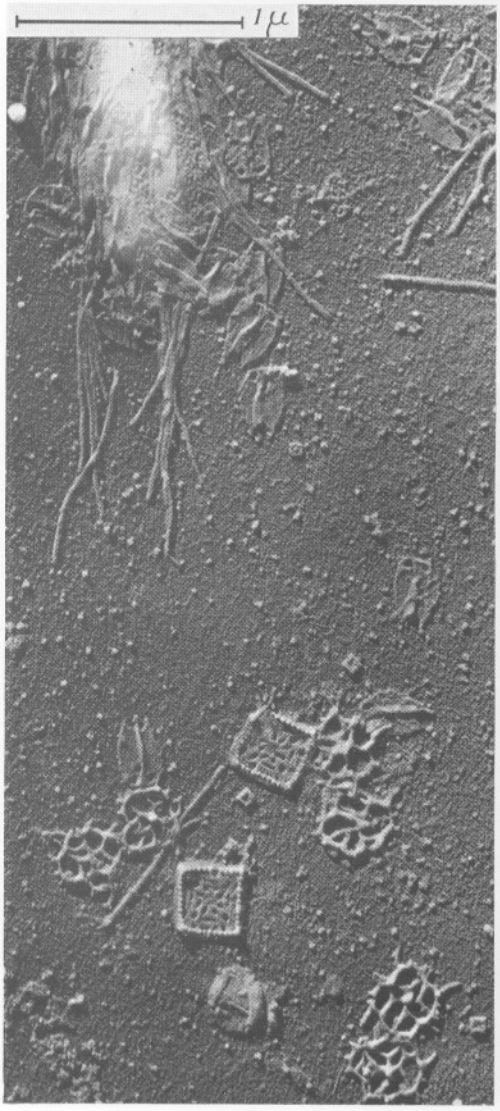

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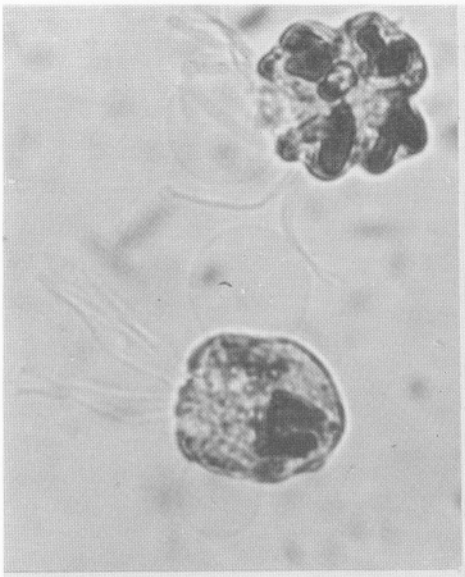

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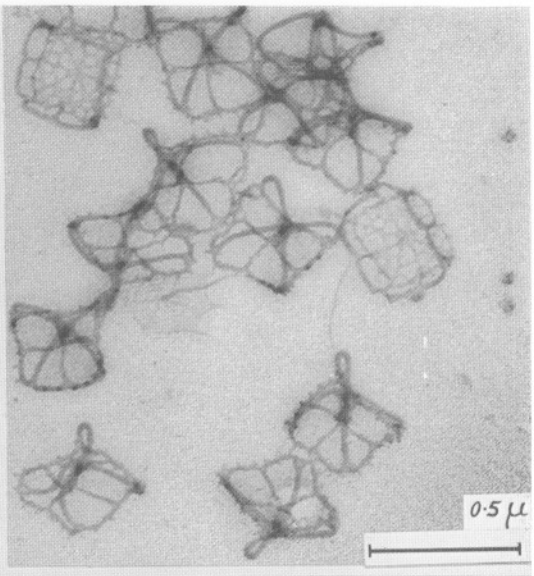

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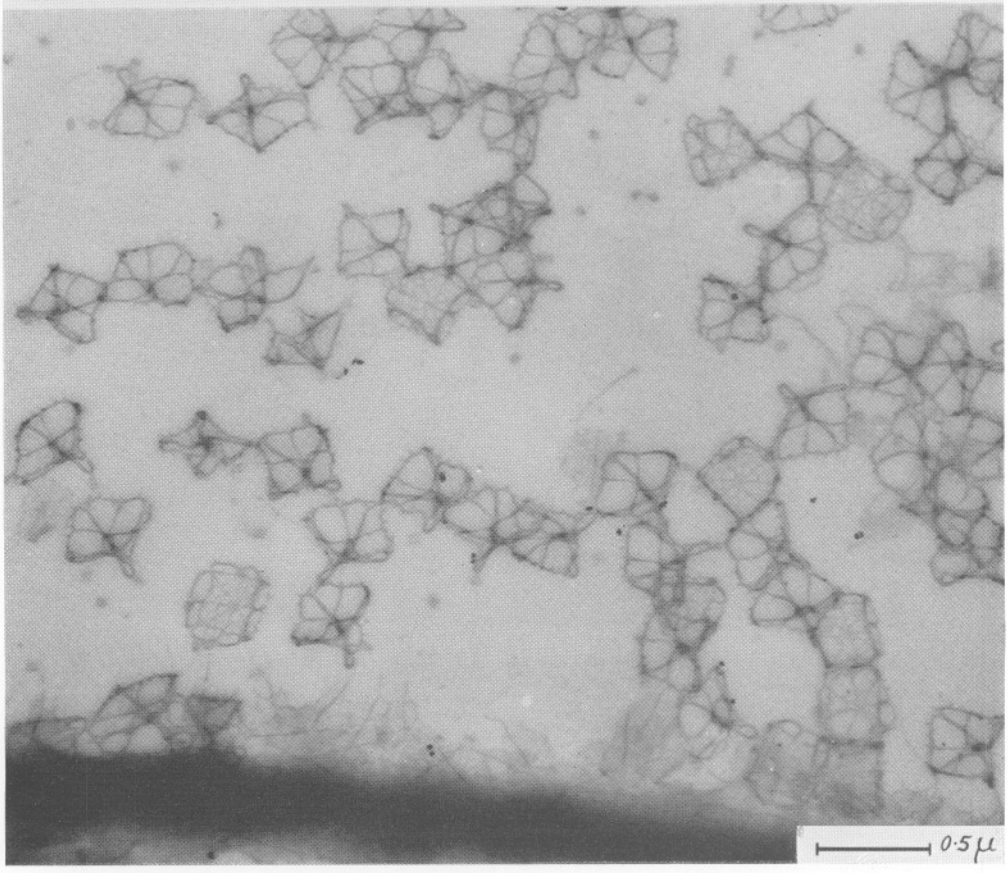

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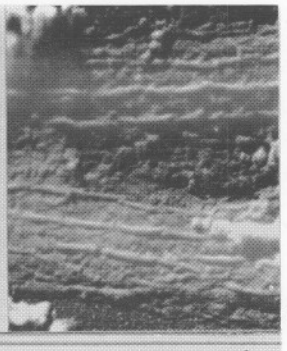

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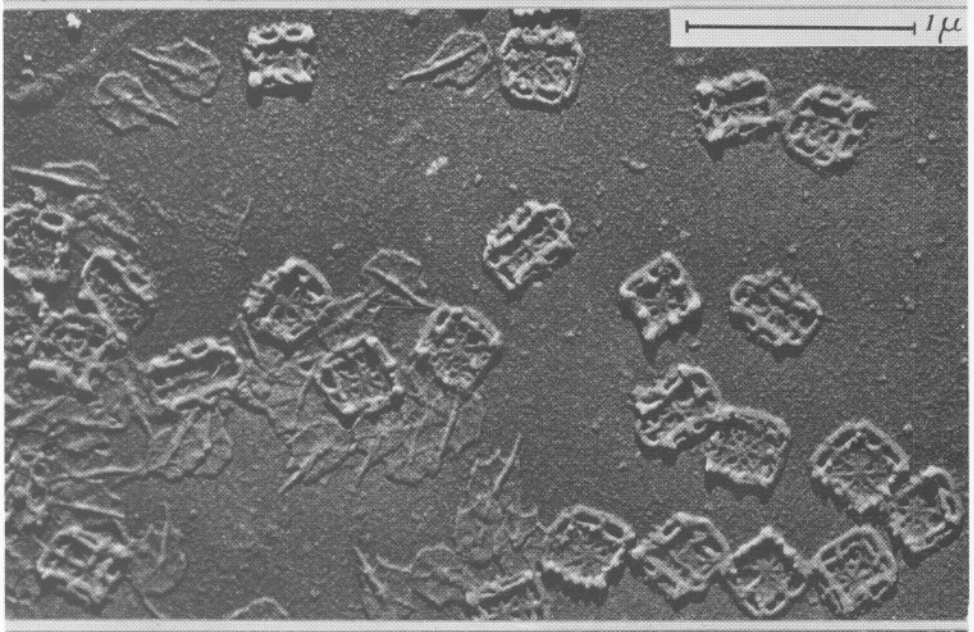

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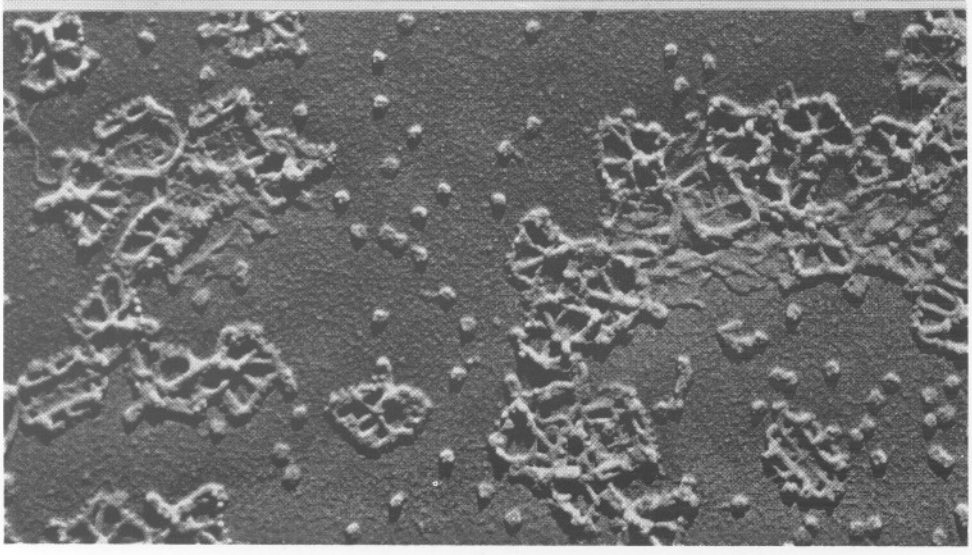

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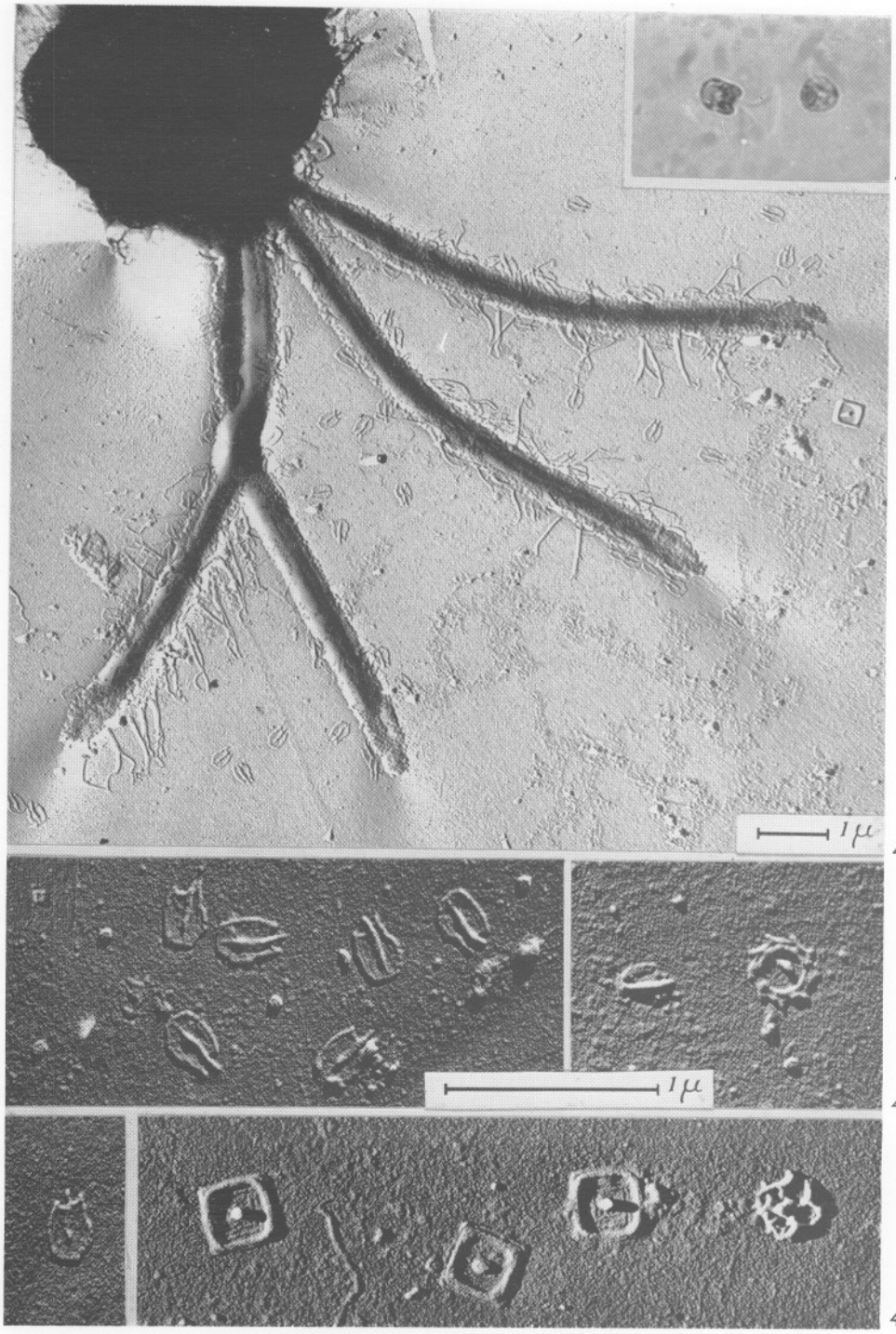

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Pyramimonas grossii Parke (no. 78 in the Plymouth collection, Pl. XI)

The very small size of the cells of this species can at once be appreciated by comparing Fig. 30 with Pl. I. For comparison with the other species of *Pyramimonas* it will be necessary to use the light microscopy of Fig. 29. A silhouette view of the surface of the body and bases of the four flagella (Fig. 31) may be directly compared with that of Fig. 13, Pl. V, although it should be noted that Fig. 31 includes parts of both shadowed and unshadowed sides of the specimen, a detail which will explain the difference in treatment of the micrographs reproduced for the next species, *P. amylifera*, in Pls. XII and XIII.

Details of the other types of surface garniture are reproduced in Figs. 32–34. The flagella are conspicuously hairy, though whether these hairs should or should not be interpreted as Flimmer of the ordinary kind remains problematical; we suspect that they should not. The individual scales immediately recall those of the *Halosphaera* zooids though they are somewhat larger. The outer flagellar scales fall off very readily, exposing a papillate surface beneath (Fig. 32). Detached flagellar scales can at once be recognized as such since they differ from those previously encountered only in having the forward edge indented and the hinder edge marked by two perforations (Figs. 33 and 34, bottom left); the character of the scale surface and the possession of a dorsal bar marking the long axis is as before.

The larger body-scales are of two main types, namely rectangular plates with rims and flat lace-like plates without rims. Details of shape and marking are characteristic of the species and could almost certainly be used to distinguish it. They do not need further verbal description at present.

Finally the tiny scales of the underlayer are present in profusion (see especially Fig. 33). One may recognize the small rectangles with depressed centres as belonging to the body underlayer. More nearly circular small scales with a raised central hub (Fig. 33, bottom left inset) may with equal certainty be referred to the flagellar underlayer.

Pyramimonas amylifera Conrad (no. 246 in the Plymouth collection, Pls. XII and XIII)

In the very large cells of this species the characteristically U-shaped starch 'shields', on which the name is based can be readily demonstrated with the light microscope after iodine fixation (Fig. 35). Apart from this the large size is in itself disadvantageous since it seriously impedes electron-microscopical observation even of the flagella, owing to the increase of opacity with thickness; only short lengths will therefore be illustrated. Fig. 38 is perhaps sufficient to illustrate the rugose surface of a flagellar tip from which the outer scales have fallen off, while the specimen of Fig. 39 still retains the longitudinal rows of outer scales exactly as in Figs. 5 and 8, and similarly denoting 9 rows in all though with the details less elegantly displayed.

Detached scales are commonly present in vast numbers and since each scale is slightly larger than those previously examined they make convenient and rewarding objects. Some sample fields, with and without shadow-casting, are reproduced in Figs. 36–41. All the various categories previously recorded are again encountered, in this case with even closer resemblance to the situation in *Halosphaera*. Apart from scale-size, a characteristic morphological detail is the presence of a peculiar spine-like loop (or loop-like spine) present on the flat rimless scales (see especially Fig. 37), though exactly how this is arranged in life can scarcely be worked out from flattened specimens. The outer flagellar scales are characterized by the presence of two indentations (leading to a three-pronged appearance) at the morphologically anterior edge and by the dorsal spine apparently starting in the centre of the scale before projecting forward. The tiny scales of the underlayer from both body and flagella are likewise of the familiar types (see especially Fig. 41). Hairs apparently detached from the flagellar surfaces as in the *Halosphaera* zooids are also easily encountered but are not illustrated.

Pyramimonas aff. obovata Carter (no. 280 in the Plymouth collection, Pl. XIV)

This very elegant small species is probably sufficiently demonstrated by a glance at Pl. XIV. The flagellar hairs are as conspicuous (Fig. 43) and as firmly attached as in *P. grossii*. The flagellar scales (Figs. 44 and 46) also resemble those of *P. grossii* though the perforations found near the hind edge of each scale in that species are here lacking. The body scales are more distinctive and in this case are so firmly attached to the cell that very small groups or even single scales have to be used to illustrate their structure (Figs. 45 and 47). We have the same general categories of round and square, rimless or rimmed, though the individual shapes are again characteristic of the species. In particular, the box-like rectangular body-scales (Fig. 47) could at once be used to separate this species from any others that we have examined. The small scales of the underlayer are easily distinguished in position on the flagella (Fig. 43) but those of the body would not have been detected had their presence not been expected; one small square scale probably representing this layer is however included in a corner of the field of Fig. 45 (top left).

DISCUSSION

It will already be obvious that without the knowledge of the special relation of flagellates 205 and 247 to the genus *Halosphaera* Schmitz the most natural conclusion to draw from these observations would have been that all the species studied, though individually distinct, were closely related members of one homogeneous group. It may be suspected that this conclusion is indeed the right one, but without much more information about life histories among species of *Pyramimonas* we cannot make it. The most that can be claimed is

that the generic concepts of both *Halosphaera* and *Pyramimonas* have been rendered uncertain by these findings and both should for the time being be regarded as form-genera only but not as satisfactorily defined taxa.

A wider problem is the relationship, if any, of *Pyramimonas*-like organisms to other examples of flagellates with scales on their flagella. It is premature to discuss this in detail since only one example is at present known to us from the literature, namely our own *Micromonas squamata* Manton & Parke, but we are already aware of the existence of several others and it is clearly desirable to place some of these on record before more general comparisons are attempted. It is, however, fair comment on the change which the observations recorded here have brought about to recall that when *Micromonas squamata* was first described (Manton & Parke, 1960) the presence of scales on its flagellum was a new and apparently unique phenomenon and we had therefore no means of ascertaining whether it should properly be treated as a peculiar specific character or as an indicator of higher phyletic significance. In the light of our present information there seems little doubt that the latter will prove to be the case since we can now see that *Micromonas squamata* resembles the *Pyramimonas* group of organisms in several important respects though its total organization is at a much simpler level.

From the point of view of fine structure, however, by far the most important observations are those connecting scale production for the first time securely with the activity of the Golgi bodies. Such a relation has been suspected before, most clearly in a recently described species of *Chrysochromulina* (*C. polylepis* Manton & Parke, 1962) though in that case on evidence which was circumstantial but not complete. It need not be assumed that the details will be the same everywhere, though it can scarcely be coincidence that in two very different groups (*Chrysochromulina*, a member of the Chrysophyceae; *Halosphaera* and *Pyramimonas* sharing the possession of chlorophyll *b* with the green algae) the same cytoplasmic organelle has been implicated. That there are still important gaps in the morphological information is unimportant since further work can scarcely fail to rectify this. The mode of production of flagellar-type scales versus body-type scales in the Pyramimonas stage of *Halosphaera* is a gap of this kind which an attentive reader will already have noticed since only the latter have been clearly demonstrated in the various cisternae, vesicles and cavities illustrated here. Such gaps notwithstanding, the information now before us has brought an investigation which began as a strictly algological study into a much wider field of relevance. Insight into the metabolic or other functional significance of individual cell components is being actively sought at the present time by plant and animal cytologists at many different levels, since it is already clear that very similar categories of organelles can be encountered in exceedingly different types of cells in both kingdoms. Our findings under this head have therefore much more than strictly algological significance and we hope to give considerable further attention to them in the very near future.

SUMMARY

The entire cell surface, including the flagellar surfaces, of three species of *Pyramimonas* and of two different strains of *Pyramimonas*-like zooids of *Halosphaera* are shown to be covered with scales in at least two layers, namely, an underlayer of very small scales arranged directly on the flagella membrane or plasmalemma and one or more outer layers of much larger scales of different character. On one and the same cell the shapes of the scales of all layers are different on the flagella from those on the body. Among all the taxa studied the flagella scales are very similar but those of the bodies are specifically differentiated; details are illustrated in the plates. In two taxa (*Halosphaera* and *Pyramimonas amylifera*) the outer scales on the flagella have been shown to be consistently arranged in 9 longitudinal rows; numerical information is not available for the other taxa studied but they are not expected to be different. The presence of an unusual type of flagellar hair has also been noted though those have not yet been studied in detail.

In the *Halosphaera* zooids for which sections are reproduced a major finding is the presence of clearly recognisable scales in process of formation withint he cisternae of the Golgi bodies and the passage of completed scales thence to the cell surface in vesicles has been traced in detail.

The implications of these various findings are discussed in a preliminary way.

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