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Some Recent Advances in Studies of Silicon in Higher Plants [and Discussion]

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Some recent advances in studies of silicon in higher plants

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[Plates 1–4]

In comparison with elements commonly associated with the nutrition of higher plants, silicon has received relatively little attention. Recently developed techniques have, however, demonstrated its occurrence in a wide range of tissues and species. Grasses are heavy accumulators, but considerable variation occurs between and within species. The factors involved in uptake, translocation and deposition in different species are not fully understood. Deposition has been investigated in the roots of a number of species. Active or passive uptake or almost complete exclusion has been observed. While deposits most frequently occur in cell wall layers or in cell lumina of the root endodermis, the major influx remains in a soluble form and is translocated to the shoot. Deposition is heavy in grass and cereal inflorescence bracts. Silica has also been detected in the epicarp hairs of cereal grains, and evidence is presented regarding the time course of its accumulation in these hairs. It is suggested that such deposition cannot be entirely attributed to a passive transpiration mediated mechanism. The significance of these deposits is discussed in relation to plant growth and development, and to wider aspects associated with human health.

1. Introduction

In higher plants, silicon deposits are derived from silicates present in soils. Uptake and translocation occurs as monosilicic acid (Barber & Shone 1966), and deposition is in the form of amorphous silica gel $SiO_2 \cdot nH_2O$. Once deposited the silicon is immobile and the process appears to be irreversible.

Among the early land plants, species of the Equisetaceae are heavy silicon accumulators (Chen & Lewin 1969; Hoffman & Hillson 1979), while Dengler & Lin (1980) have shown that silica is deposited in the leaves of *Selaginella* species.

The dicotyledons have traditionally been thought of as plants that absorb relatively small amounts. However, several recent investigations have indicated that this view is an oversimplification. Scurfield *et al.* (1974) found silica deposits in 32 woody dicotyledons, and Postek (1981) has shown deposition to occur in the leaves of *Magnolia grandiflora*. Examples of herbaceous dicotyledon families in which silica deposits have been located include members of the Cannabaceae (Dayanandan & Kaufman 1976) and Urticaceae (Sowers & Thurston 1979). Despite these recent observations it is probably still true to say that the monocotyledons generally take up and deposit more silicon than the dicotyledons. According to Dahlgren & Clifford (1982) silica deposition is most common in certain monocotyledon families, including the Restionaceae, Arecaceae, Zingiberaceae, Bromeliaceae, Orchidaceae and Cyperaceae. It is, however, in the grass family (Poaceae), the most highly evolved family and a very successful

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family ecologically, that the heaviest and most characteristic deposits occur. In the leaves and culms of these species, deposited silica has been located in special idioblast cells, epidermal cells (Metcalfe 1960; Parry & Smithson 1964), and in mesophyll cell walls (Dinsdale *et al.* 1979). Grass inflorescence bracts (Parry & Smithson 1966; Parry & Hodson 1982), caryopses (Hodson & Parry 1982) and roots (Sangster 1978*a*) have also been investigated and shown to possess silica deposits.

This paper summarizes some recent studies on silica deposition in higher plant tissues. The results are discussed in relation to silicon deposition mechanisms, and to the possible involvement of plant silica in human health problems.

2. Root uptake and deposition

Clearly the root determines the amount of silica ultimately accumulating in the plant shoot. Angiosperm roots, depending on the species, may exhibit active uptake, passive uptake or active exclusion of silicon (Birchall 1978). Roots of the same species may show a gradual transition from active absorption to active exclusion as the external silicon concentration rises (Van der Vorm 1980). Active uptake is dominant in rice, sugar cane and wheat (*op. cit.*), while in oats uptake is thought to be a passive process (Jones & Handreck 1967). Root exudates that prevent silica polymerization may aid in active uptake, thereby allowing more transmembrane movement (Birchall 1978). The compound poly-2-vinyl pyridine-1-oxide may act in this manner, which explains its effect of increasing the amount of leaf silicification in rice (Parry 1975). In contrast, Van der Vorm (1980) found that active exclusion predominated in two dicotyledon species, except at very low concentrations.

Silicic acid available for uptake is affected by physicochemical factors of the soil (Jones & Handreck 1967; Birchall 1978). Plants of semi-arid regions, where little leaching occurs, have been shown to take up much silicon (Geis 1978), but less silicification would be expected in plants from areas with highly weathered soils. Kowalski & Davies (1982) have recently demonstrated that increasing soil organic matter leads to enhanced shoot silica concentrations in wheat.

After uptake, dissolved monosilicic acid moves across the cortex of the root until it reaches the endodermis. The endodermis has long been considered to control water and solute movement into the vascular tissues. The Casparian bands block apoplastic flow in young root tissues, and only flow through the symplast is possible. The secondary stage of endodermal development involves the formation of suberin lamellae around the inside of the cell. It has recently been suggested that this layer is permeable to water (Sanderson 1983). If this is so then apoplastic flow occurs within this layer, *inside* the Casparian bands in older roots. Nevertheless, the endodermis undoubtedly constitutes the major resistance to monosilicic acid flow through the grass root, and it is here that many of the root silica deposits are found.

The literature on the silicification of grass roots has been reviewed previously (Sangster 1978*a*; Sangster & Parry 1981). While silica contents of less than 1% (by mass) of dry matter have been recorded in the roots of dicotyledons, this figure can be as high as 15% in some monocotyledons. Within the monocotyledons, it is only in the Poaceae that amorphous silica deposition is known to occur in the roots. Deposits most frequently occur in cell wall layers, but are also found in cell lumina and in intercellular spaces.

Table 1 summarizes the results of investigations into silica deposition in roots. Despite the

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few species investigated, several tentative generalizations may be in order. (i) A phylogenetically determined silica distribution pattern may exist in rice, sorghum and bamboo species as well as the temperate cereal group, in both of which deposition is restricted to the endodermal walls. (ii) Cultivar differences are apparent. (iii) Deposition may be age related; compare old and young roots in *Oryza sativa* and *Molinia caerulea*. (iv) Root deposits were lacking in *Phragmites*, *Zea* and one wheat cultivar, and yet these are fairly heavy silica accumulators in their shoots (Lau *et al.* 1978; Lanning *et al.* 1980).

Table 1. Silica distribution patterns in the roots of grasses and cereals

	location	type of deposit	tribe	genera
1 *a*	endodermis	silica aggregates and inner tangential wall (i.t.w.)	Andropogoneae	eight genera, including *Miscanthus* (e)
b	endodermis	cell walls only (i) i.t.w.	Paniceae	*Digitaria*; *Panicum virgatum* forming 'plate phytoliths' (c)
		(ii) i.t.w. and radial	Oryzeae	*Oryza*, rice (young)
			Bambuseae	*Sasa*, bamboo
		(iii) all four walls	Triticeae	*Triticum aestivum*, wheat, two cultivars; *Hordeum sativum*, barley (a)
			Aveneae	*Avena sativa*, oats (a)†
c	probably endodermis	chemical analysis only	Chlorideae Eragrosteae	
2	intercellular spaces (cortex)	extracellular	Danthonieae	*Molinia* (young)
3	all tissues – epidermal sclerenchyma, xylem vessels	cell walls	Danthonieae Oryzeae	*Molinia* (young) *Oryza* (old)
4	no distributional pattern detected‡	—	Triticeae Maydeae Arundineae	wheat (one cultivar) (a) *Zea mays* (five cultivars) (b) *Phragmites australis* (one biotype only) (d)

Techniques employed were light and scanning electron microscopy and X-ray analysis. With the exception of the following, the original references are in Sangster (1978*a*) or Sangster & Parry (1981): (a), Bennett (1982); (b), Bennett & Sangster (1982); (c), Geis (1978); (d), Sangster (1978*b*); (e), Sangster (1983).

† Seminal roots only; in adventitious roots Si was confined to radial and i.t.w.

‡ Tentative; few specimens and cultivars were examined.

Silica aggregates are conical deposits projecting from the inner tangential wall of the endodermis, and to our knowledge are confined to the tribe Andropogoneae (for example *Miscanthus sacchariflorus* – Sangster 1983). Ultrastructural studies have revealed that silicon is associated with specific wall layers in the endodermis of sorghum (Sangster & Parry 1976*b*) and sugar cane (Parry & Kelso 1977). Amorphous root deposits in sorghum are composed of 'primary spherical units' about 100 nm in diameter. Similar ultrastructure has been demonstrated for deposits in the root intercellular spaces of *Molinia caerulea* (Montgomery & Parry 1979). Despite the presence of silica deposits in the roots of some grass species, much of the monosilicic acid absorbed is translocated to the shoot.

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3. The development of silicification in wheat epicarp hairs

Silica deposition in the grass caryopsis (grain) has been little studied, but recent investigations have indicated its presence in certain tissues of some species. In the caryopsis of *Setaria italica*, silica deposition takes the form of a granular electron-opaque layer external to the outer aleurone cell wall (Hodson & Parry 1982).

The epicarp hairs present on the mature caryopses of the four cereals, barley, oats, rye and wheat, have been investigated by Bennett & Parry (1981). In all four cases silicon was found to be present along the whole length of the hairs, but was most concentrated in the extreme tips. This study did not indicate the precise cellular location of the silicon within the hair. Moreover, only hairs from mature grains were examined, and the timing of silicification and its relation with hair development were not determined. Accordingly, deposition in the epicarp hairs of wheat (*Triticum aestivum* L. cv. Highbury) was investigated further, and several stages in hair development were examined.

(*a*) *Materials and methods*

Wheat plants were grown in a water culture containing $50/10^6$ SiO_2, in a growth cabinet, as described by Sangster *et al.* (1983*b*). Harvests of the ovary and caryopsis were taken at the following stages.

e.0, Emergence. At this stage the tips of the upper awns of the panicle were just emerging from the enclosing flag leaf sheath. The whole of the inflorescence was enveloped by the leaf sheaths.

e.+1, One week after the emergence of the awns. The inflorescence is fully exposed, but the florets have yet to open (pre-anthesis).

e.+3, Three weeks after the emergence of the awns, with the florets at anthesis.

e.+6, Milk ripe stage. Caryopsis has filled out, but is still green and fleshy.

e.+10, Mature grain. Caryopsis is yellow, dry and hard.

The epicarp hairs and their associated tissues were investigated with standard light microscope, scanning electron microscope and transmission electron microscope techniques (Parry & Hodson 1982; Hodson & Parry 1982). Unstained transections of wheat epicarp hairs were analysed for silicon in a Philips 301 transmission electron microscope fitted with an EDAX energy dispersive X-ray analyser. For elemental analyses the microscope was operated in the scanning transmission mode (s.t.e.m.). The operating voltage was 80 kV with a take off angle of 45°. A window at 0.15 keV was centred at the silicon Kα peak at 1.74 keV, and silicon counts were made for 100 s in selected tissue areas. Alternatively, results were obtained as X-ray spectra and the vertical scales of the spectra were varied according to the sample. Blank resin was analysed to check for contamination, and to give an estimate of background.

(*b*) *Epicarp hair development*

(i) *At awn emergence*

The wheat ovaries showed a range of developmental stages at the e.0 harvest, depending on their position on the inflorescence axis. The most immature stage of development is illustrated in figure 1 (*a*), plate 1. Here the young spikelet is completely devoid of all hairs. Epicarp hairs first develop as papillae on the ovary wall, but soon the papillae begin elongation. The most mature florets at this stage contain ovaries with many epicarp hairs developing on their surfaces

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(figure 1 *b*). These hairs range considerably in length from small papillae a few micrometres long to hairs that reach 800 μm. The developing feathery stigmatic hairs are also fairly prominent at the apex of the ovary.

Figure 2, plate 2, shows some light and transmission electron micrographs of the wheat hairs and their associated tissues at the e.0 harvest (ovary similar to that depicted in figure 1 (*b*); i.e. one of the more mature ovaries at this harvest). At this stage the unicellular epicarp hairs were about 9.5 μm in diameter, and their cell walls were approximately 0.5–0.6 μm thick. Most of the hairs had large central vacuoles with a thin layer of cytoplasm adpressed to the wall (figures 2 (*a*), (*b*), (*e*), (*f*)), but in a few, the cytoplasm filled the lumen (figures 2 (*a*), (*c*)). The cytoplasm was rich in organelles; nuclei (figures 2 (*a*), (*b*)), mitochondria, endoplasmic reticulum and golgi bodies were all observed. The extreme tip of the epicarp hair was characterized by the presence of electron opaque material in the cell wall (figure 2 (*g*)). This material was densely packed at the outer edge of the wall, and was also present as discrete particles in the inner zone. It was absent from the area of wall adjacent to the cell lumen. The stigmatic hairs (figure 2 (*a*), (*d*)) had an entirely different structure from that of the epicarp hairs. They were multicellular and irregular in shape, had thin cell walls, very prominent nuclei, active cytoplasm and only small vacuoles.

(ii) *One week after emergence*

At this stage the epicarp hairs were well developed in most florets (figures 1 (*c*), (*e*)). Bennett & Parry (1981) noted that the mature epicarp hairs of wheat had smooth tips, but sub-apical areas had surface striations. This pattern was already evident one week after emergence (figure 1 (*e*)). The feathery stigmatic hairs were fully expanded by this stage (figure 1 (*d*)) and their cells now had large vacuoles (figure 3 (*a*), plate 3).

In the week after inflorescence emergence great internal changes took place in the epicarp hairs (figure 3 (*a*)). The walls became considerably thickened (up to about 5.0 μm thick), the cytoplasm in the most advanced hairs was already breaking down, and a thin electron opaque layer formed at the extreme outer edge of the hair (figure 3 (*d*), (*e*)). This layer was present along the entire length of the hair, except in the extreme tip. Figure 3 (*b*) shows that by this stage the extreme tip of the hair became completely infilled with electron-opaque material. In an area approximately 0.5 μm below this (figure 3 (*c*)) the electron-opaque material was present at the edge of the wall, but not in the centre.

(iii) *Three weeks after emergence to maturity*

The external morphology and length (up to 1 mm) of the epicarp hairs changed little after the e. + 1 harvest, but the ovary, and later, the caryopsis tissues fill out considerably. At maturity the epicarp hairs are only present on the tip of the grain (figure 1 (*f*)), the stigmatic hairs having degenerated.

At anthesis (*ca.* 3 weeks after emergence) the epicarp hairs had completed their internal development, and their cytoplasmic contents had broken down. The cell wall had thickened, resulting in a small lumen. Hairs at the e. + 6 and e. + 10 harvests had a very similar internal structure to those at the e. + 3 harvest. Figures 3 (*f*) and 3 (*g*) illustrate some features of the mature hairs. The electron-opaque layer, formed by the e. + 1 harvest at the outer edge of the hair, was easily visible even in unstained sections, and was about 100–250 nm thick. The cell wall appeared to be laid down in a series of concentric rings.

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(*c*) *Silicon deposition*

The results of energy dispersive X-ray analysis of the wheat hairs are shown in table 2 (the length of the hair excluding the tip) and figure 4 (the tip). These results can only be considered to be reliable for solid deposited silicon, as most, if not all, of the soluble monosilicic acid present in the hairs will probably have been lost from the material during the preparative procedures. Table 2 shows that the vacuole and the middle of the wall had silicon count rates either at or just above background levels. In the e.0 harvest the outer edge of the wall (similar to that

TABLE 2. LEVELS OF SILICON DEPOSITED IN WHEAT EPICARP HAIRS

harvest tissue	vacuole	middle of wall	outer edge wall
e.0	541 ± 33	n.d.	1228 ± 164
e. + 1	1517 ± 59	1369 ± 108	8896 ± 2740
e. + 3	n.d.	566 ± 51	6422 ± 1362
e. + 6	n.d.	999 ± 103	5618 ± 1970
e. + 10	n.d.	1322 ± 574	5311 ± 2007

Silicon counts per 100 s obtained from transections of wheat epicarp hairs, sampled at random along the length of the hairs, but not including the tips (± standard deviation, $n = 6$). The overall background Si level was 821 ± 301 ($n = 28$); n.d., not determined. Details of harvest codes and of the method employed are given in §3 (*a*).

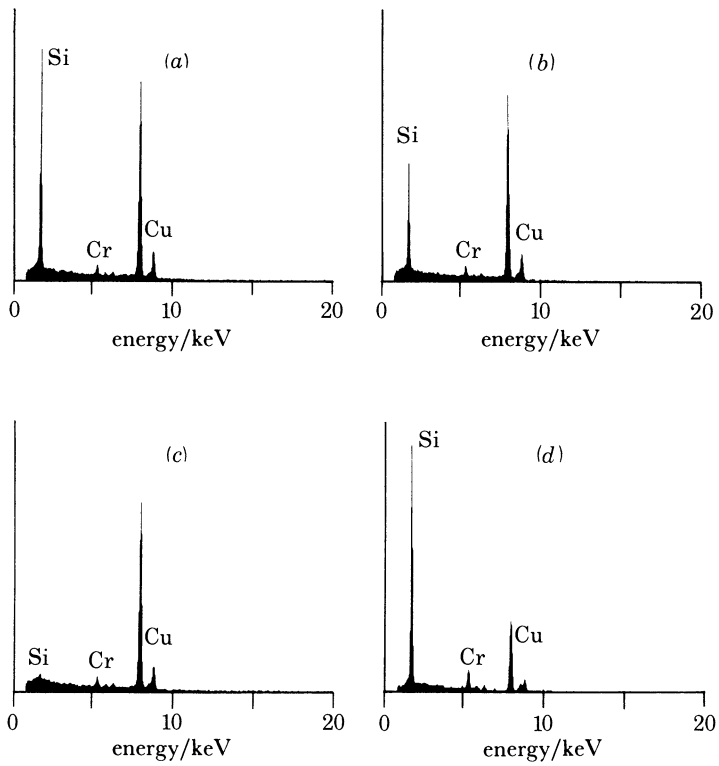

FIGURE 4. All diagrams are X-ray analysis spectra of transections through the tips of wheat epicarp hairs. The vertical scale is in counts. Horizontal scales are in keV. The silicon peak is at 1.74 keV. The other peaks present are due to copper (grid) and chromium (specimen holder).

(*a*), (*b*), (*c*) Harvest e.0. Tip similar to that shown in figure 2 (*g*); (*a*) outer edge of wall; (*b*) area of discrete particles; (*c*) area free from electron opaque material next to lumen. (*d*) Harvest e. + 1. Tip similar to that shown in figure 3 (*b*). The vertical scale for each of (*a*)–(*c*) is 5000 counts; for (*d*) it is 25000 counts.

shown in figure 2 (*f*)) had a similar silicon count to background. By e.+1, however, the silicon count rates were much above background in this zone. This increase in count rate coincided with the appearance of an electron-opaque layer at the outer edge of the wall (figures 3 (*d*), (*e*)). It can therefore be assumed that the electron-opaque material is deposited silica. The layer at the outer edge of the wall also yielded high silicon counts in the e.+3, e.+6 and e.+10 harvests.

Analyses of the extreme tips of the epicarp hairs are depicted as X-ray spectra (figure 4). For the e.0 harvest these were made at three points in a transection similar to that in figure 2 (*g*). A high silicon peak was detected at the outer edge of the wall (figure 4 (*a*)), a lower peak from the area of discrete particles (figure 4 (*b*)), and silicon was not detected above background level in the area free from electron-opaque material adjacent to lumen (figure 4 (*c*)). The silicon peak detected from a transection of an epicarp hair tip at the e.+1 harvest (figure 4 (*d*)) was the highest recorded for this investigation (see figure 3 (*b*) for a similar tip).

(*d*) *Discussion*

The results obtained from this investigation confirm those of Bennett & Parry (1981) for mature epicarp hairs; silicon is present along the whole length of the hair and is concentrated in the tip. However, it is now clear that deposition takes place in the tips of the hairs before inflorescence emergence, and that for the rest of the length of the hair silicon is deposited in a thin layer on the outer edge of the wall, within a week of inflorescence emergence. Epicarp hair development is remarkably rapid, and is nearing completion by one week after emergence when the other ovary tissues are still immature.

The early deposition of silicon in the tips of the hairs requires some consideration. Silicon transport has often been associated with the movement of water in the transpiration stream (Sangster & Parry 1976*a*; Kaufman *et al.* 1981; Raven 1983). In this case, however, transpirational water loss would probably be considerably impeded by the inflorescence bracts and the enclosing leaf sheaths. The glume macrohairs, prickle hairs and papillae of *Phalaris canariensis* also became silicified before inflorescence emergence (Sangster *et al.* 1983*b*), and it seems that a mechanism other than passive transpirational flow is required to account for silicon deposition in both cases. The growing hairs take in water to maintain turgor and to allow cell extension. It must be presumed that this water contains monosilicic acid. This is deposited as amorphous silica wherever it reaches a sufficient concentration. Whether silicon deposition in the tips of the epicarp hairs occurs via an active or a passive process cannot be determined from the present data.

In the week after inflorescence emergence, silica deposition continued in the epicarp hair tips, and it was also deposited in a thin layer over the entire length of the hair wall. Again a parallel exists with the work of Sangster *et al.* (1983*b*) on *Phalaris canariensis*. The lemma macrohairs of this species also accumulated much of their silicon in the week after emergence. Deposition in such cases may be due to increased transpiration on exposure to the atmosphere.

Long distance transport of monosilicic acid into the developing grains is almost certainly via three provascular bundles, two of which terminate very near the brush end. Figure 2 (*h*) shows a transection through one of these bundles in which three xylem elements are clearly visible. These bundles presumably supply both the epicarp hairs and the stigmatic hairs with ions, and yet it is only in the epicarp hairs that deposition of amorphous silica takes place. This would suggest that either the stigmatic hairs actively exclude silicon from their cells or that the epicarp

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hairs have an affinity for silicon. The mechanism of silicon deposition in the epicarp hairs cannot be determined until much more is known of water movement within the developing caryopsis.

The epicarp hairs are the only cells of the wheat caryopsis in which silica deposition has been located. The functional significance of this is unclear. It has been suggested that the hairs could be involved in defence against insect pests (Bennett & Parry 1981). Another possibility, which is not exclusive of the first, is that they are repositories for excess silicon, which might otherwise interfere with cellular metabolism in the developing grain.

4. Aspects of plant silicon and human health

It is considered beyond the scope of this paper to discuss disorders of the human respiratory tracts caused by siliceous material. However, in the context of our investigations on the silica deposits in the epicarp hairs of wheat, it is relevant that Simmonds *et al.* (1970) found that these hairs were a major component of dust arising from the handling of wheat grains. The authors suggested that such hairs could have highly irritant properties if inhaled.

Description of plate 1

Figure 1. Scanning electron micrographs illustrating some features of the development of wheat epicarp hairs.

(*a*) Harvest e.0. An abaxial view of an early primordial stage of a wheat spikelet, showing no sign of epicarp hair development. The glume (gl.), lemma (le.) and palea (pa.) are visible in the lower floret together with two developing anthers (a.). The gynaecium (gy.) is barely discernible within. (Magn. × 204.)

(*b*) Harvest e.0. One of the most mature ovaries (o.) at this harvest is surrounded by anthers (a.) and epicarp hairs (e.h.) are developing. Also developing at this stage are the feathery stigmas (st.). (Magn. × 37.)

(*c*) Harvest e. + 1. A group of epicarp hairs. (Magn. × 161.)

(*d*) Harvest e. + 1. A group of stigmatic hairs. (Magn. × 365.)

(*e*) Harvest e. + 1. The epicarp hair tip has a smooth surface, but for most of their length the hairs show striations. (Magn. × 1610.)

(*f*) Harvest e. + 10. Mature brush end of the caryopsis showing profusion of epicarp hairs (e.h.), the remains of the feathery stigmas (st.) and the crease (cr.) (Magn. × 27.)

Description of plate 2

Figure 2. All Harvest e.0. Transmission electron micrographs of wheat epicarp hairs and their associated tissues, except (*a*) and (*h*), which are light micrographs.

(*a*) The epicarp hairs (1, 2, 3, 4) have thin walls and most have large vacuoles. (1) Transection. (2, 3, 4) Oblique sections; (3) shows the nucleus and (4) is darkly stained due to cytoplasmic contents. Stigmatic hairs (st.h.) have dense cytoplasm and many nuclei are visible. Part of the stigma (st.) is also visible. (Magn. × 412.)

(*b*) Oblique section of epicarp hair with large nucleus (n.), large vacuole (v.), wall (w.) and a thin layer of cytoplasm (cy.). A transection of an epicarp hair tip (t.) (see 2 (*g*) for a higher magnification) is also shown (Magn. × 2000).

(*c*) Transection of epicarp hair. The wall is labelled w. The cytoplasm (cy.) is dense with only small vacuoles (v.) (Magn. × 8000).

(*d*) Transection of stigmatic hair. The cytoplasm is dense with small vacuoles (v.). In one cell a nucleus (n.) is visible (Magn. × 5000).

(*e*) Transection of epicarp hair. The labels are the wall (w.), thin layer of cytoplasm (cy.) and large vacuole (v.). (Magn. × 8000).

(*f*) Higher magnification of epicarp hair wall (w.) and cytoplasm (cy.). The vacuole is labelled v. (Magn. × 50000).

(*g*) Transection of epicarp hair tip showing a very electron-opaque layer around the edge of the wall (1); an area of dispersed electron dense granules (2); and a wall free from electron-dense material (3). The lumen is labelled l. (Magn. × 20000).

(*h*) Transection of ovary showing crease (cr.) and vascular bundle (v.b.) with three xylem vessels present. (Magn. × 412.)

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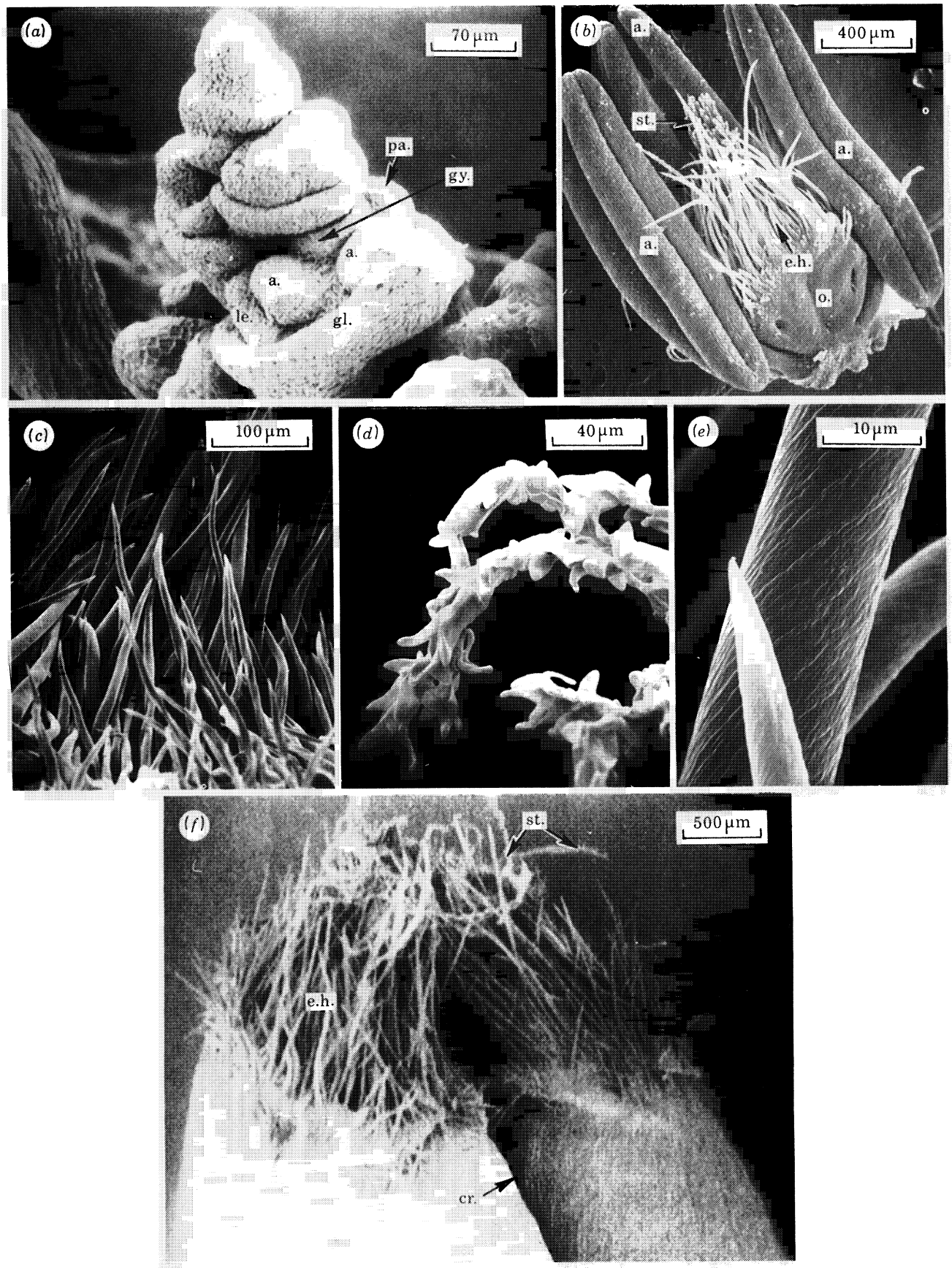

FIGURE 1. For description see opposite.

(*Facing p.* 544)

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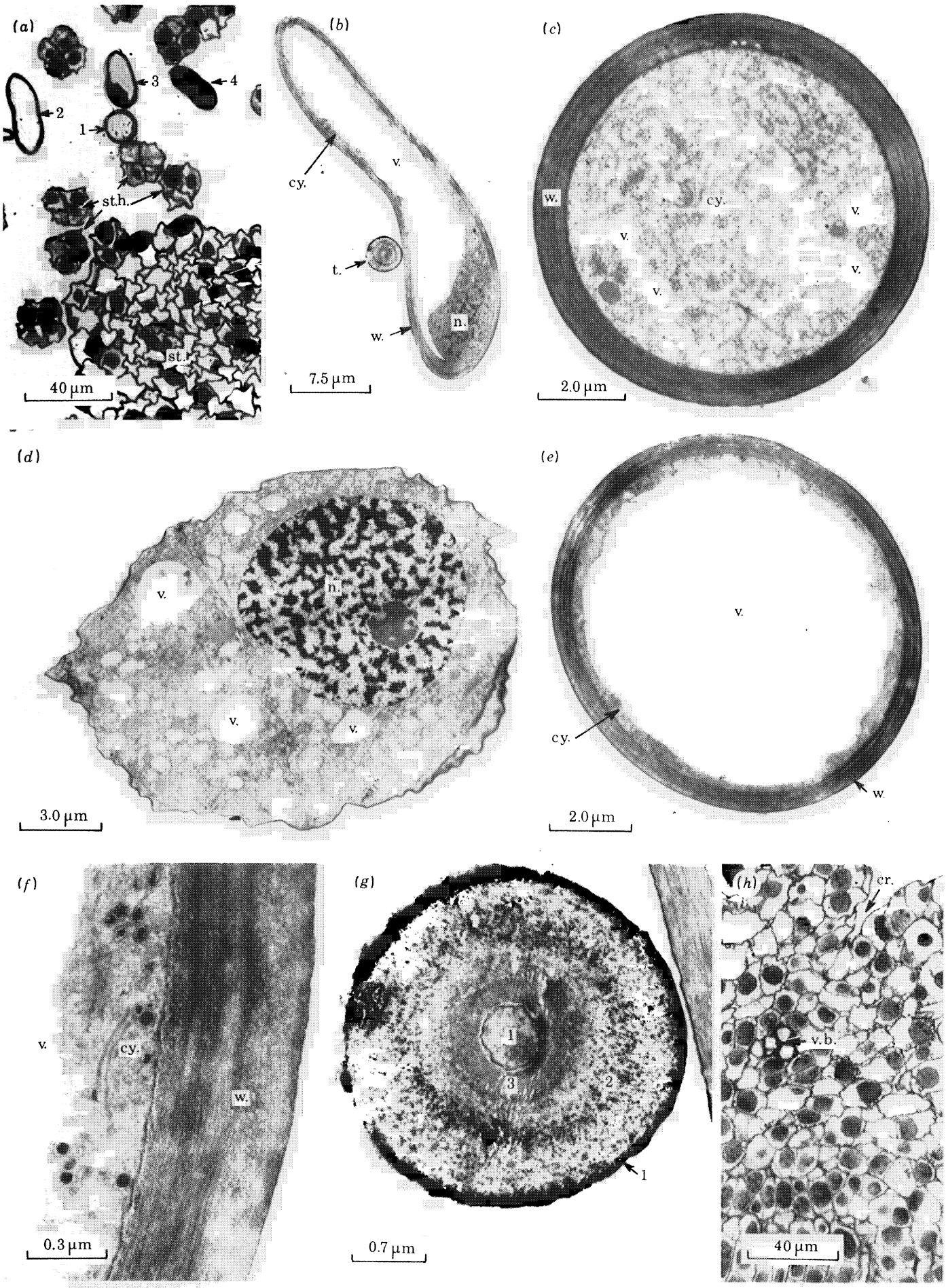

FIGURE 2. For description see p. 544.

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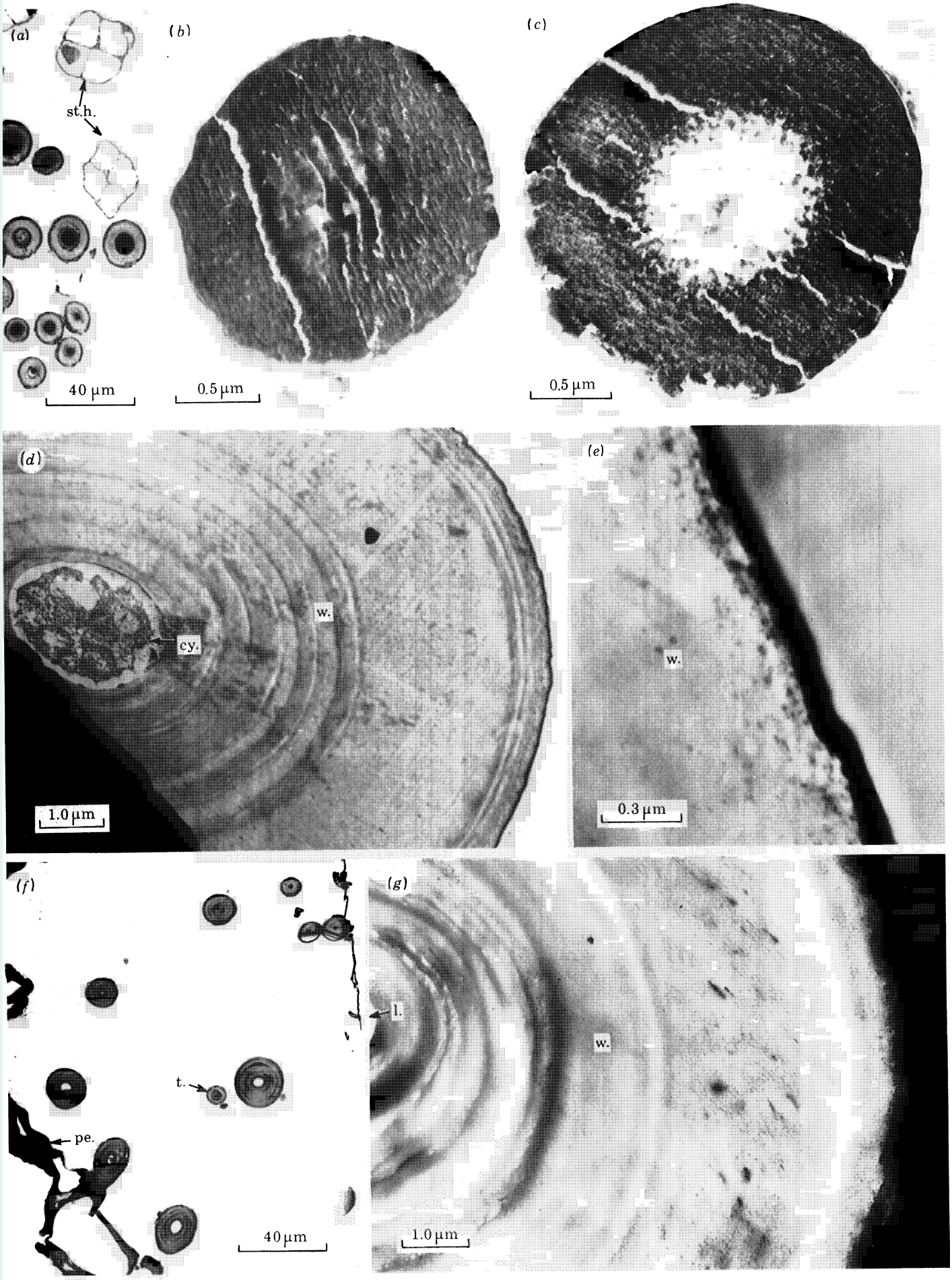

FIGURE 3. For description see p. 545.

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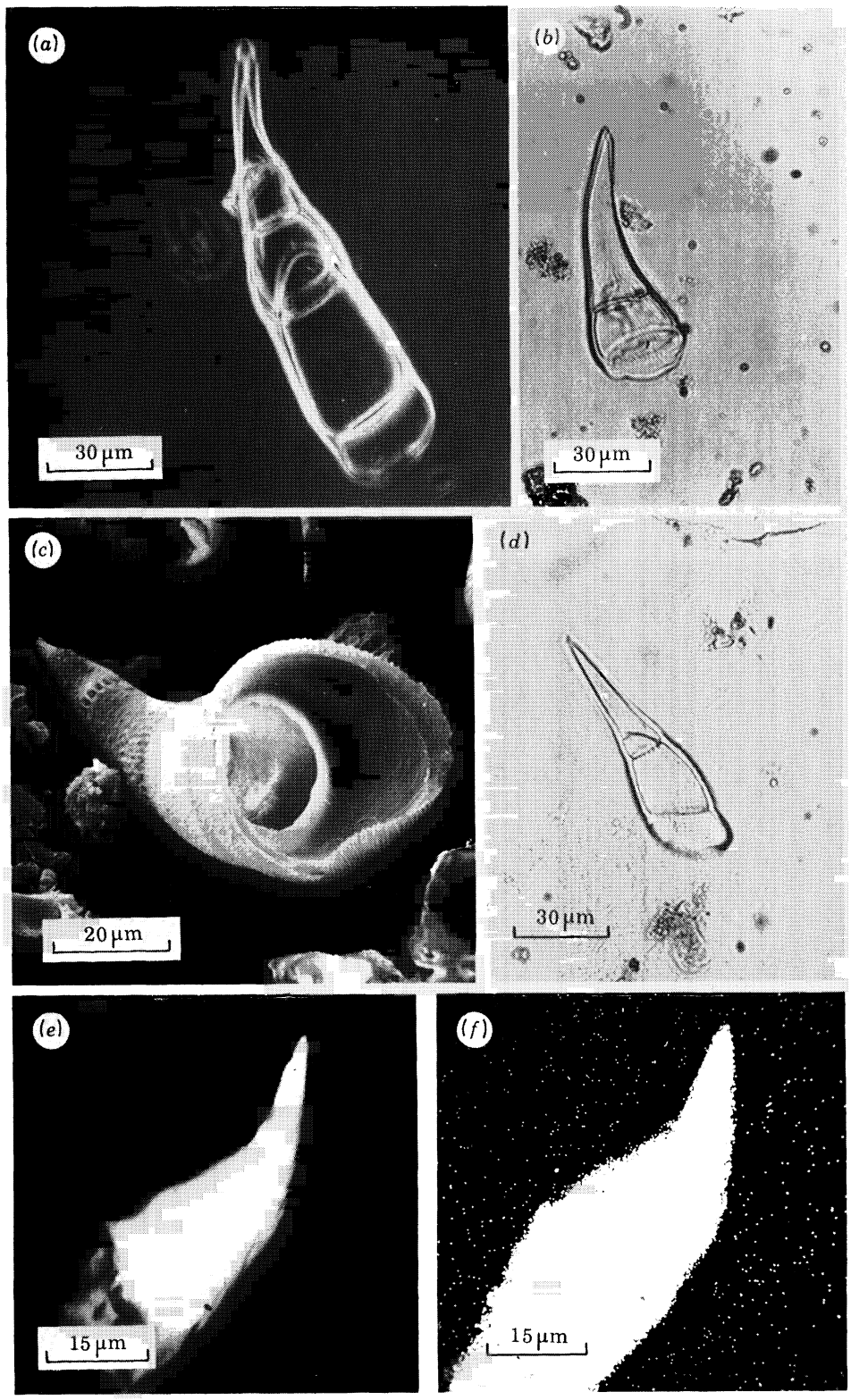

FIGURE 5. For description see opposite.

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(*a*) *Human kidney deposits*

Although siliceous uroliths are rare in humans, Dobbie & Smith (1982*b*) have shown that silicic acid is freely diffusible throughout human tissue fluids. Bendz & Lindqvist (1978) have also indicated that the human gut wall is permeable to silica particles. These are known to pass through the lymphatic and circulatory systems to reach other tissues, notably the kidneys. Relatively high levels of silicic acid have been encountered in the urine. Accordingly, Dobbie & Smith (1982*a*) have expressed concern that the increase in the use of silicates in manufactured foods and medicines may result in nephrotoxicity. High fibre diets could be a major source of silicon uptake.

(*b*) *Silicon and oesophageal cancer*

One of the most interesting recent developments relating to plant silica has been its linking with human oesophageal cancer. According to Li Mingxin *et al.* (1980), the three major areas of high incidence are the Henan, Hebei and Shanxi provinces of northern China, the north-eastern areas of Iran, and the southern Transkei region of South Africa. Although the disease probably arises as the result of an interaction between many variables, it has often been suggested that diet is an important factor (Rose 1972, 1973; Van Rensburg 1981).

(i) *Northeast Iran*

In their extensive investigations into the causes of oesophageal cancer in north east Iran, O'Neill *et al.* (1980) found that the diet of the local community was based largely on locally

Description of plate 3

Figure 3. Transmission electron micrographs of wheat epicarp hairs and their associated tissues, except (*a*) and (*f*), which are light micrographs.

(*a*) Harvest e.+1. Transections of epicarp hairs and stigmatic hairs (st.h.). The epicarp hairs have thick walls; in some the lumen is filled with darkly staining cytoplasmic material, while in others the cytoplasm is beginning to break down. (Magn. × 412.)

(*b*) Harvest e.+1. Transections of epicarp hair tip filled with electron-opaque material. (Magn. × 30000.)

(*c*) Harvest e.+1. Transections of epicarp hair tip approximately 0.5 μm below that depicted in (*b*). Electron-opaque material is confined to the outer edge of the wall. (Magn. × 30000.)

(*d*) Harvest e.+1. Transection of epicarp hair. Thick wall (w.) showing concentric rings of thickening and electron-opaque layer at the outer edge. The cytoplasm (cy.) is breaking down within the lumen (Magn. × 12000.)

(*e*) Harvest e.+1. Electron-opaque layer at edge of wall (w.). Some granular material is present just inside this layer. Section not post-stained (Magn. × 50000.)

(*f*) Harvest e.+10. Transections of epicarp hairs. For most of their length hairs have a thick wall and a thin empty lumen. Near the hair tip (t.) this lumen is less prominent. One hair base is attached to the caryopsis pericarp (pe.). (Magn. × 412.)

(*g*) Harvest e.+10. Transection of mature epicarp hair. Wall (w.) shows concentric rings of thickening, an electron-opaque layer is present at the outer edge and some granular material is present in the wall. The lumen (l.), just visible, is now empty. (Magn. × 12000.)

Description of plate 4

Figure 5. Ashed shoot material from some Compositae growing in the Transkei, South Africa. (*a*) Light micrograph, phase contrast. Multicellular *Sonchus oleraceus* hair (Magn. × 500.) (*b*) Light micrograph. Multicellular *S. oleraceus* hair. Note surface striations. (Magn. × 500.) (*c*) Scanning electron micrograph. *S. oleraceus* hair, viewed from its base. (Magn. × 85.) (*d*) Light micrograph. Multicellular *Bidens pilosa* hair (Magn. × 500.) (*e*) Absorbed electron image from an electron-probe microanalyser. *S. oleraceus* hair. (Magn. × 1000.) (*f*) Silicon X-ray distribution image from an electron-probe microanalyser. Same *S. oleraceus* hair as in (*e*). Silicon is present in all parts of the hair. (Magn. × 1000.)

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grown wheat and its products. The grains were contaminated by weed seeds of many different species, and the bread eaten contained large numbers of fine siliceous hairs. These fine hairs originated largely from the inflorescence bracts of grass species in the genus *Phalaris*. The anatomy and silica depositional patterns of the four *Phalaris* species involved have been studied by Sangster *et al.* (1983*a*), while the chemistry of the hairs has been investigated by Mann *et al.* (1983). The siliceous hairs proved to be powerful stimulants of the growth of cultured fibroblast cells (O'Neill *et al.* 1980), and also promote skin tumours in mice (O'Neill, personal communication). The mechanism by which these hairs stimulate cell growth and division is not fully understood, but they have some resemblances to the carcinogenic mineral fibres (Stoker *et al.* 1968). According to Selikoff & Lee (1978) and Wagner *et al.* (1980) mechanical strength, insolubility, and a narrow diameter are important factors required for carcinogenesis by fibres. The silicified macrohairs described by O'Neill *et al.* (1980) and Sangster *et al.* (1983*a*) fulfil these requirements.

(ii) *Northern China*

Statistics for the period 1973–77 indicate that the Henan province in northern China had the highest mortality rate for oesophageal cancer in the whole country. It was the most lethal cancer in the province and accounted for 41.7% of all malignant tumours (Li Mingxin *et al.* 1980). Comparative studies revealed that the incidence rate of upper digestive tract cancer among chickens parallels the rates of human oesophageal cancer in the high and low cancer areas (Cancer Institute, Chinese Academy of Medical Sciences 1976).

As plant silica had already been implicated in the aetiology of oesophageal cancer in north east Iran, O'Neill *et al.* (1982) initiated investigations on plant foods consumed in northern China. Foxtail millet (*Setaria italica* (L.) Beauv.) is considered to be the most important millet in northern China (Rachie 1975), and O'Neill *et al.* (1982) have estimated that the adhering bracts of this millet contain up to 20% silica on a dry mass basis. The inhabitants of the area include these fractions in a persimmon cake. Although this habit is now declining the cake once formed a major part of the diet, and all adult members of the population were likely to have eaten it. The distribution of silica deposits within the *S. italica* inflorescence has been investigated by Parry & Hodson (1982), Hodson & Parry (1982) and Hodson *et al.* (1982). These studies have revealed heavy silica deposition in the inflorescence bracts, particularly associated with papillae on their outer surfaces. Silica deposits were also found in a layer external to the outer aleurone cell wall in the caryopsis, in bristles subtending the inflorescence bracts, and in unicellular macrohairs covering the inflorescence branches.

O'Neill *et al.* (1982) recorded an unusual contamination of silica fragments in the oesophageal mucosa surrounding tumours of cancer sufferers in the Henan province. Domestic chickens from the area were also found to be contaminated in this manner. The evidence suggests a possible connection between the silica deposits of *Setaria italica* and the incidence of oesophageal cancer. This millet has a long history of cultivation in China.

(iii) *The Transkei*

Over 4000 cases of oesophageal cancer were reported in the Transkei population of 1.4 million over a thirteen year period from 1955 (Rose 1968). Oesophageal cancer is the most common single cancer in males, and is also prevalent in females. The staple diet of the population consists of maize mixed with a great variety of locally grown plant material. The plants are known to

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include dicotyledonous species in the genera *Amaranthus*, *Bidens*, *Chenopodium*, *Solanum* and *Sonchus*. Studies of the occurrence of silica in these foodstuffs obtained from the Transkei have been initiated. Material ashed at 500 °C for 12 h was examined with light and scanning electron microscopes, and by electron-probe microanalysis. *Sonchus oleraceus* L. and *Bidens pilosa* L. had large numbers of hairs. Some of the features of the *S. oleraceus* hairs are shown in figures 5 (*a*), (*b*), (*c*), plate 4. These hairs were all multicellular, and they frequently had striations on their walls. The lengths of the hairs varied from 30–170 μm with a breadth of 25–50 μm at their bases. Electron-probe microanalysis (figures 1 (*e*) and (*f*)) revealed that all of the hairs were highly silicified. *B. pilosa* had similar hairs to those of *S. oleraceus* (figure 5 (*d*)), but also has some silicified unicellular hairs up to 650 μm long by 25–50 μm in breadth. *Amaranthus hybridus* L. also contained siliceous deposits, but to date these have not been associated with specific trichomes.

S. oleraceus and *B. pilosa* are species of the Compositae, a group not normally associated with heavy silica deposition. The arid growth conditions prevalent in the Transkei may be an important factor in this respect. This needs further investigation. These studies must be regarded as preliminary because laboratory carcinogenicity tests have yet to be conducted, and there is no data to suggest that plant silica is associated with the tumours of Transkei patients. It has, however, now been established that the inhabitants of the Transkei consume food that contains large numbers of siliceous hairs.

The authors are grateful to Dr C. H. O'Neill (Imperial Cancer Research Fund Laboratories, London) for his continued interest in this work and to Dr E. F. Rose, who kindly supplied us with material from the Transkei. We would also like to thank the following colleagues in U.C.N.W. for their assistance in various aspects of these investigations: Mr W. Neville (School of Plant Biology), Mrs A. V. Buckland, Mr D. A. Davies (School of Animal Biology), Mr J. A. Rees (School of Electronic Engineering Science) and Dr D. J. M. Lane (Department of Marine Biology). Grateful acknowledgement is made for grants from the Agricultural Research Council, U.K., and from York University, Toronto, Canada, which made this work possible.

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Discussion

W. C. Jones (*School of Animal Biology, University College of North Wales, U.K.*). Can Dr Parry or anyone in the audience explain how silica induces carcinoma in humans? In 1968 Allison (1968) suggested that silica is toxic because silicic acid binds to membrane protein, causing damage to cell membranes. It is ingested by vertebrate macrophages and enters lysosomes. It would be interesting to hear the current views on this or other possible explanations, particularly in view of the absence of silica as a skeletal material (excluding the teeth of molluscan radulae) in all animal phyla higher than the sponges.

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C. H. O'Neill (*Imperial Cancer Research Fund Laboratories, London, U.K.*). Several types of biogenic silica fibre have been associated epidemiologically with human cancer. In particular, the diets of two regions of high incidence of oesophageal cancer are contaminated with fibres from species of either *Phalaris* (North Eastern Iran) or *Setaria* (North China). We are confident that work on these fibres will give important insights into the causes of cancer, since it is now clear they can cause tumours experimentally; we have found that *Phalaris* fibres can promote the appearance of tumours in the skin of mice at least as effectively as pharbol esters.

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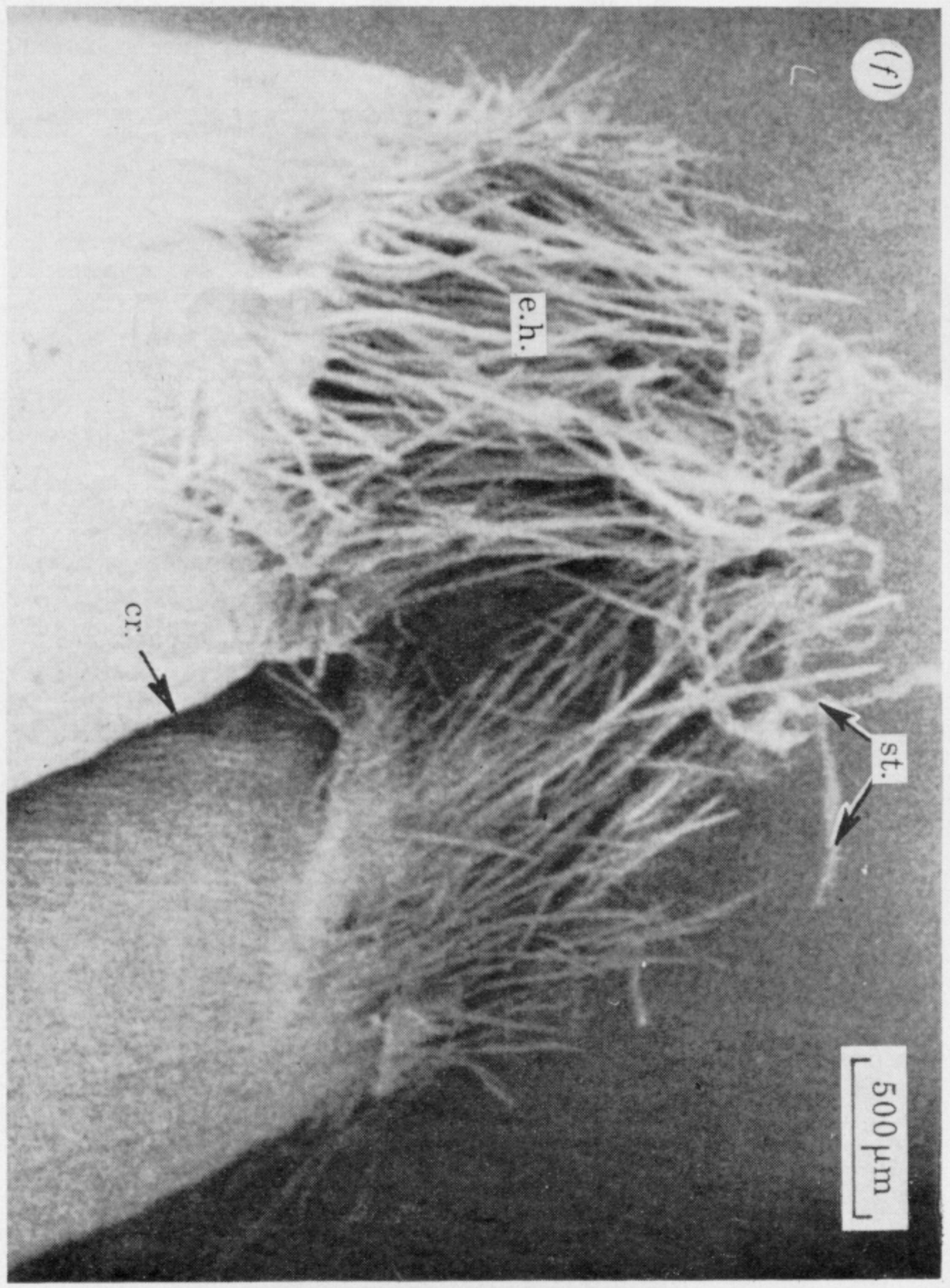
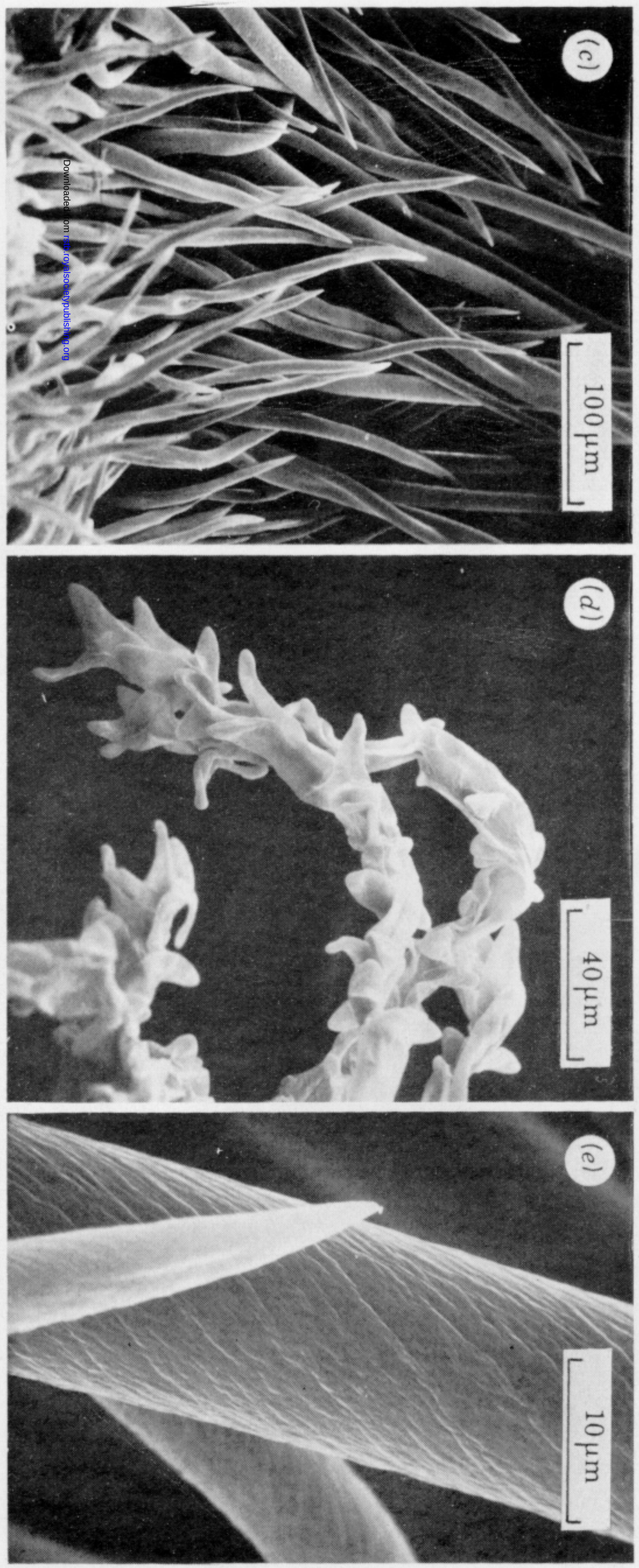




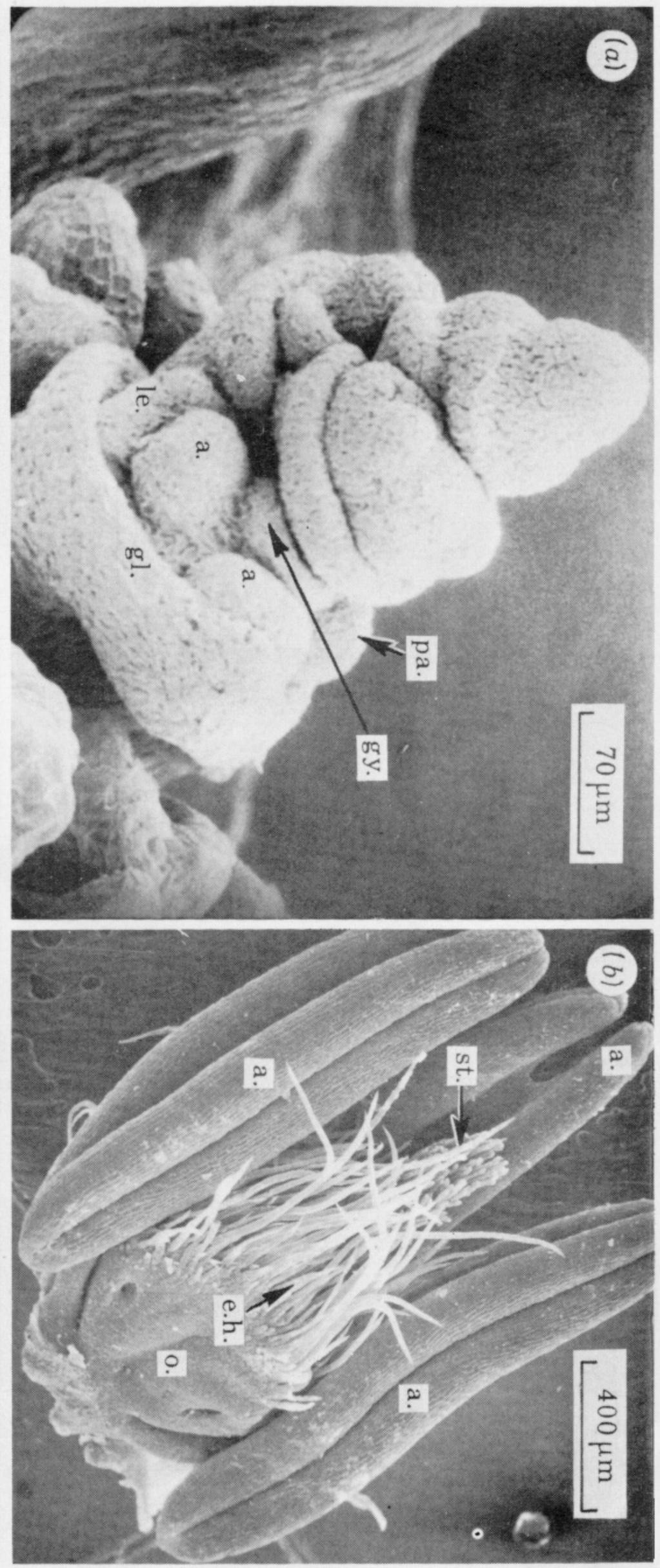
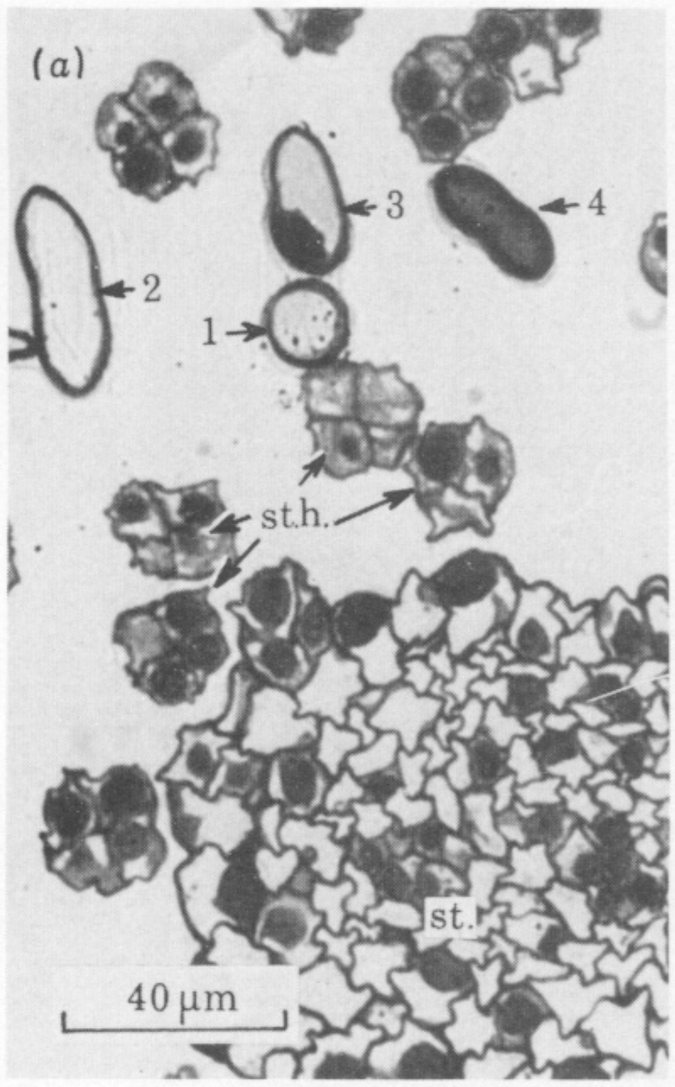
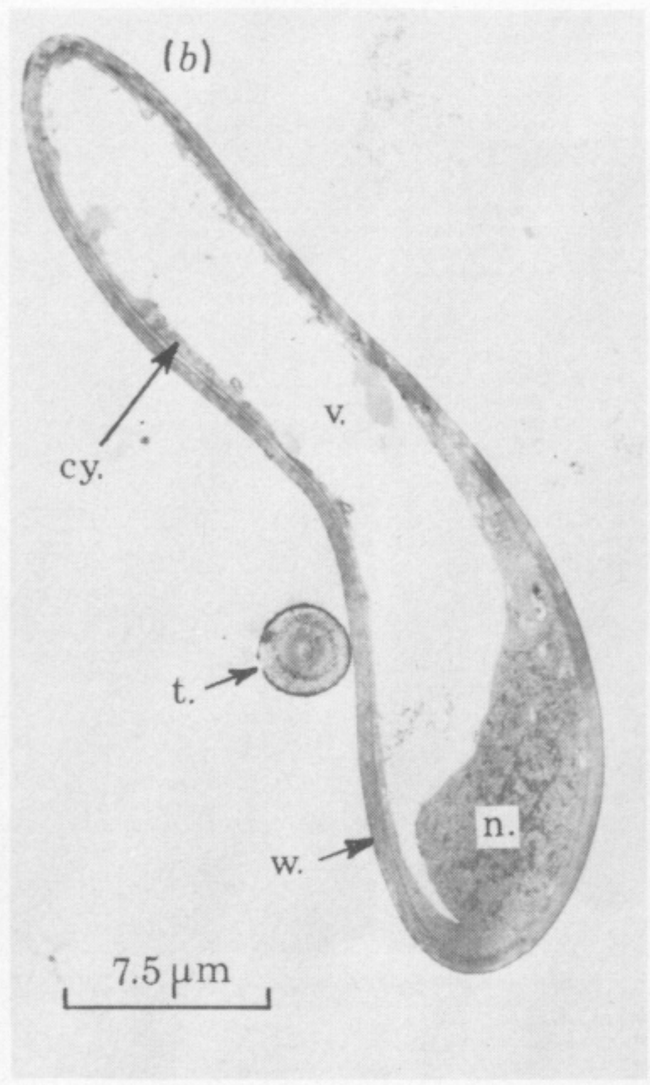
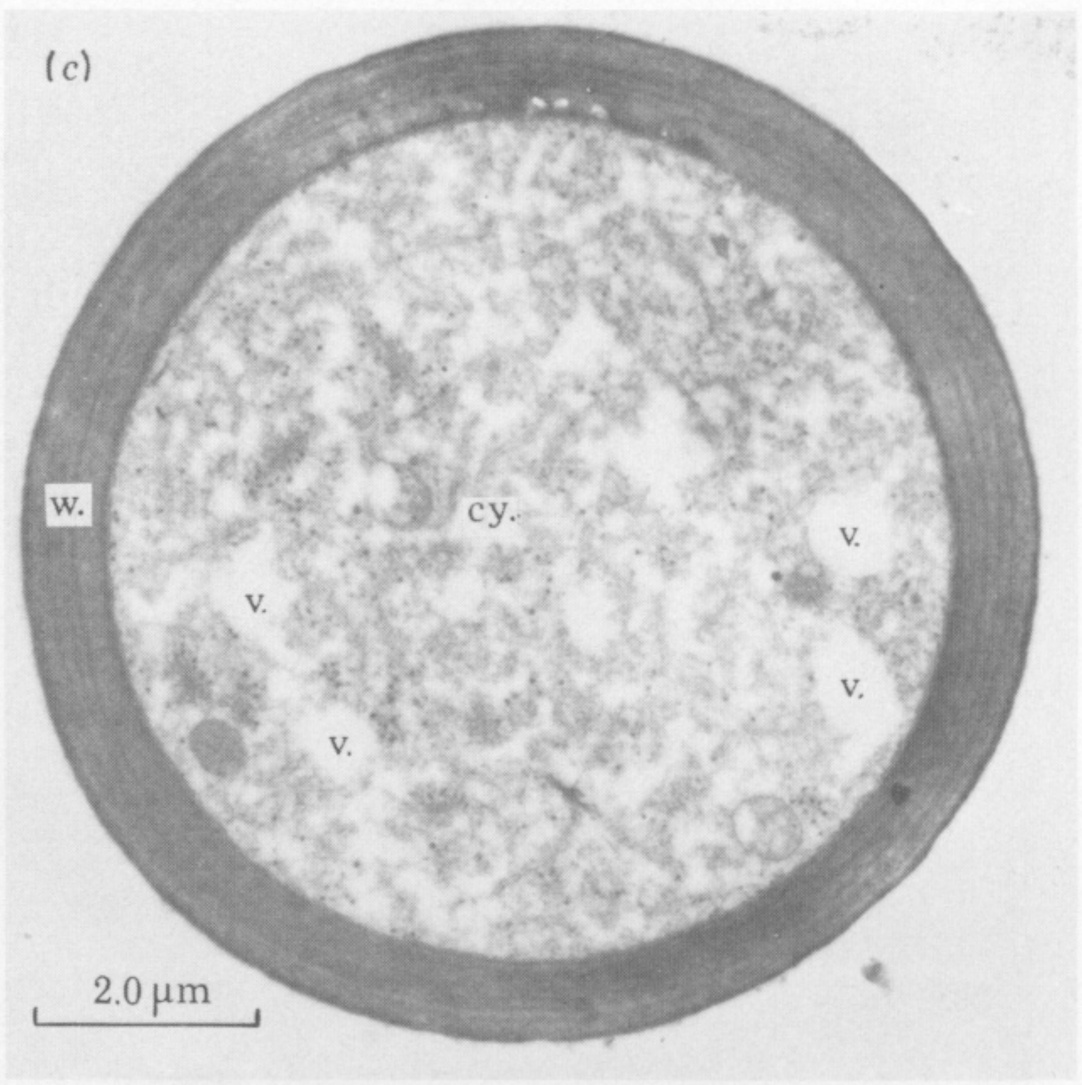
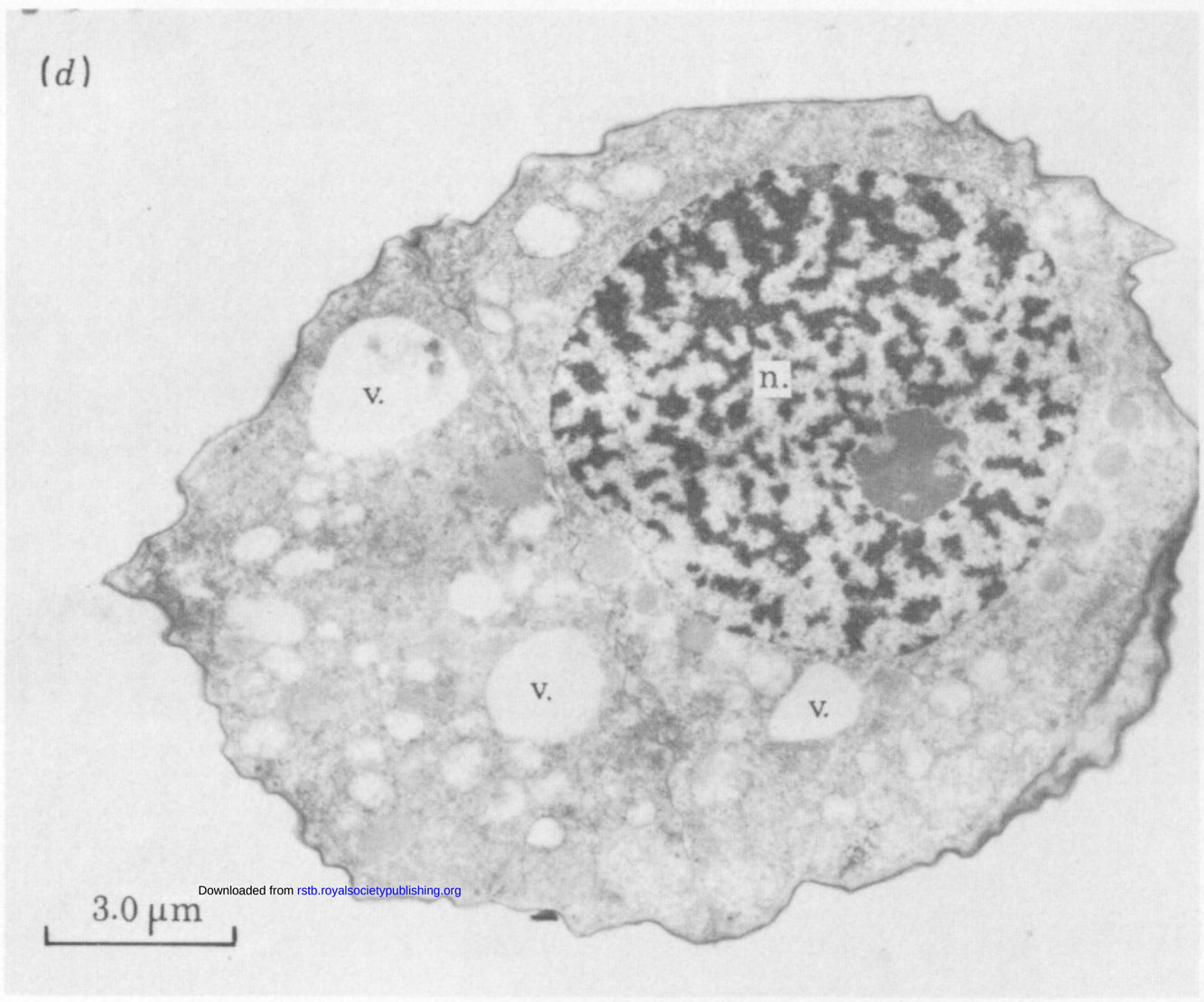


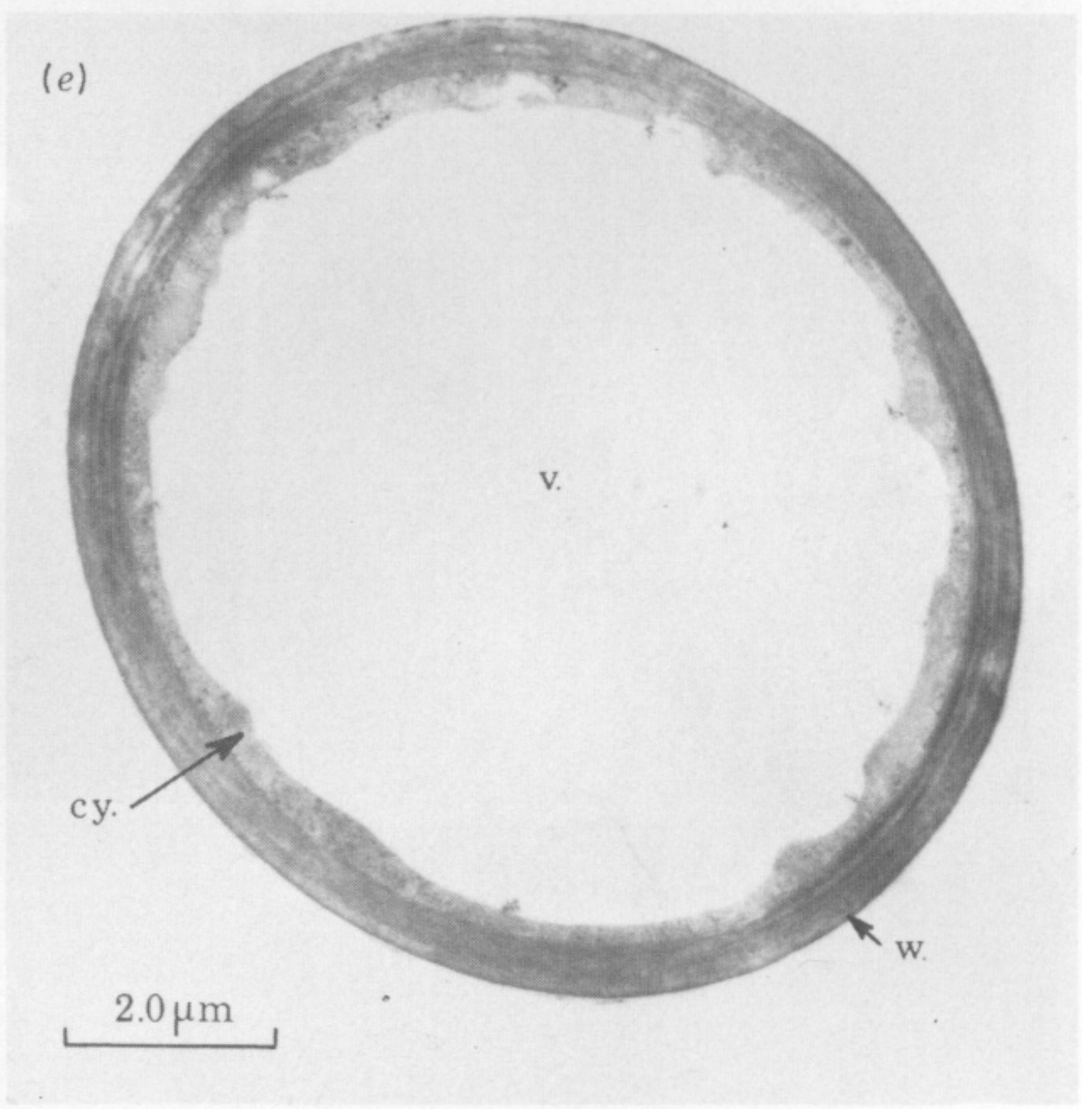
FIGURE 1. For description see opposite.

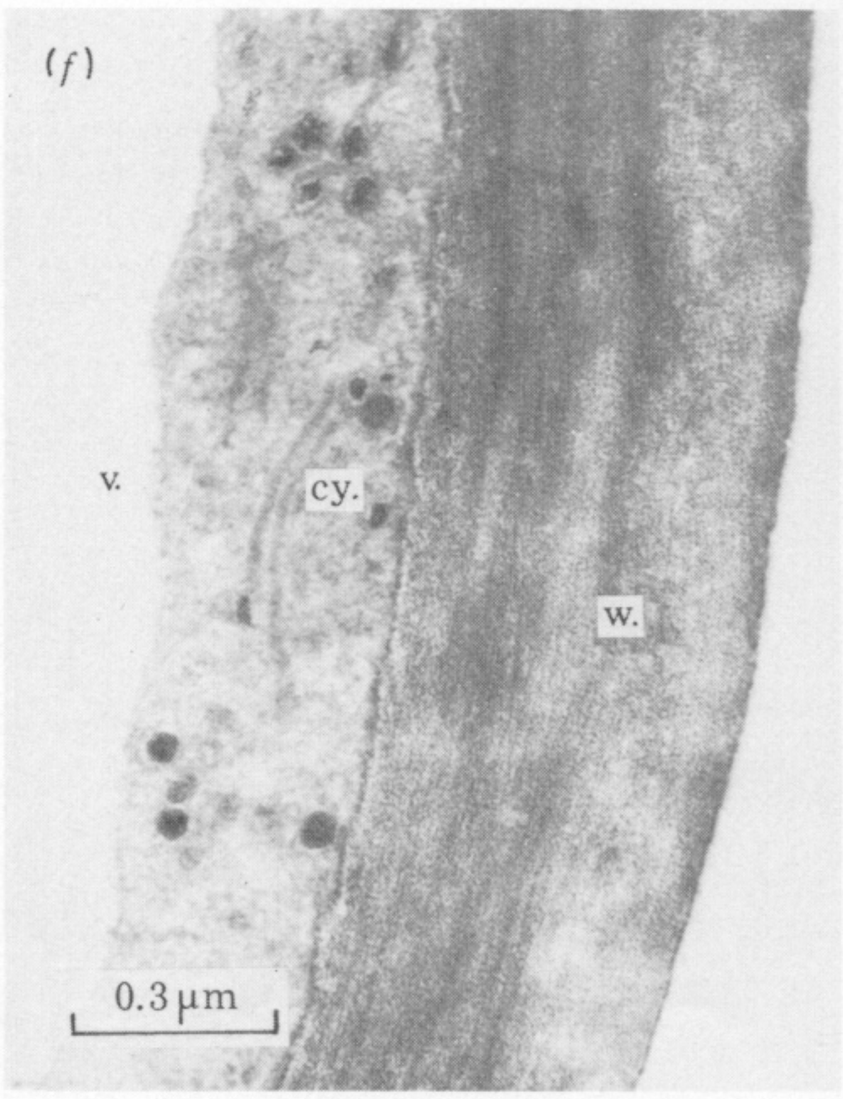


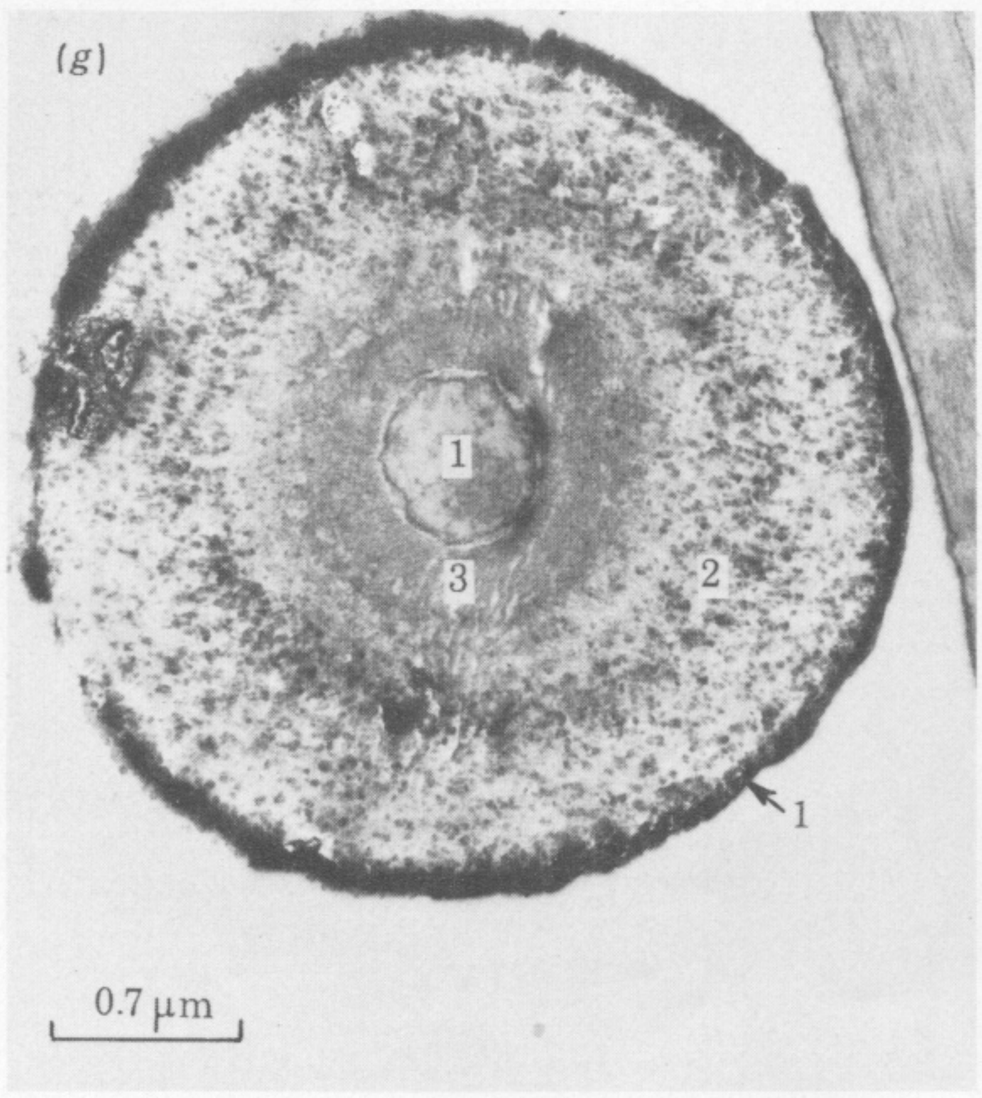












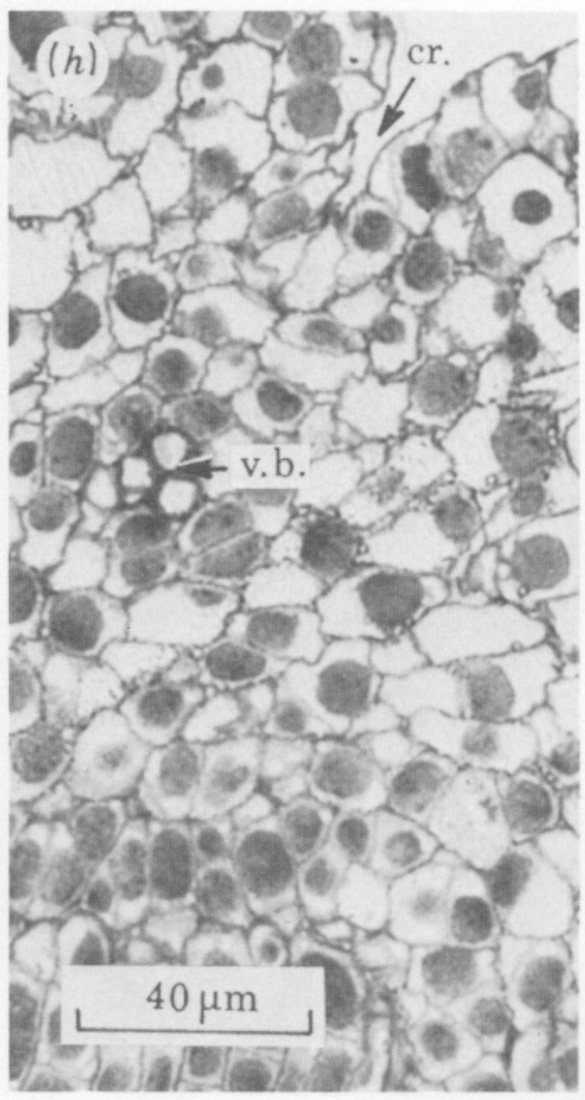


FIGURE 2. For description see p. 544.

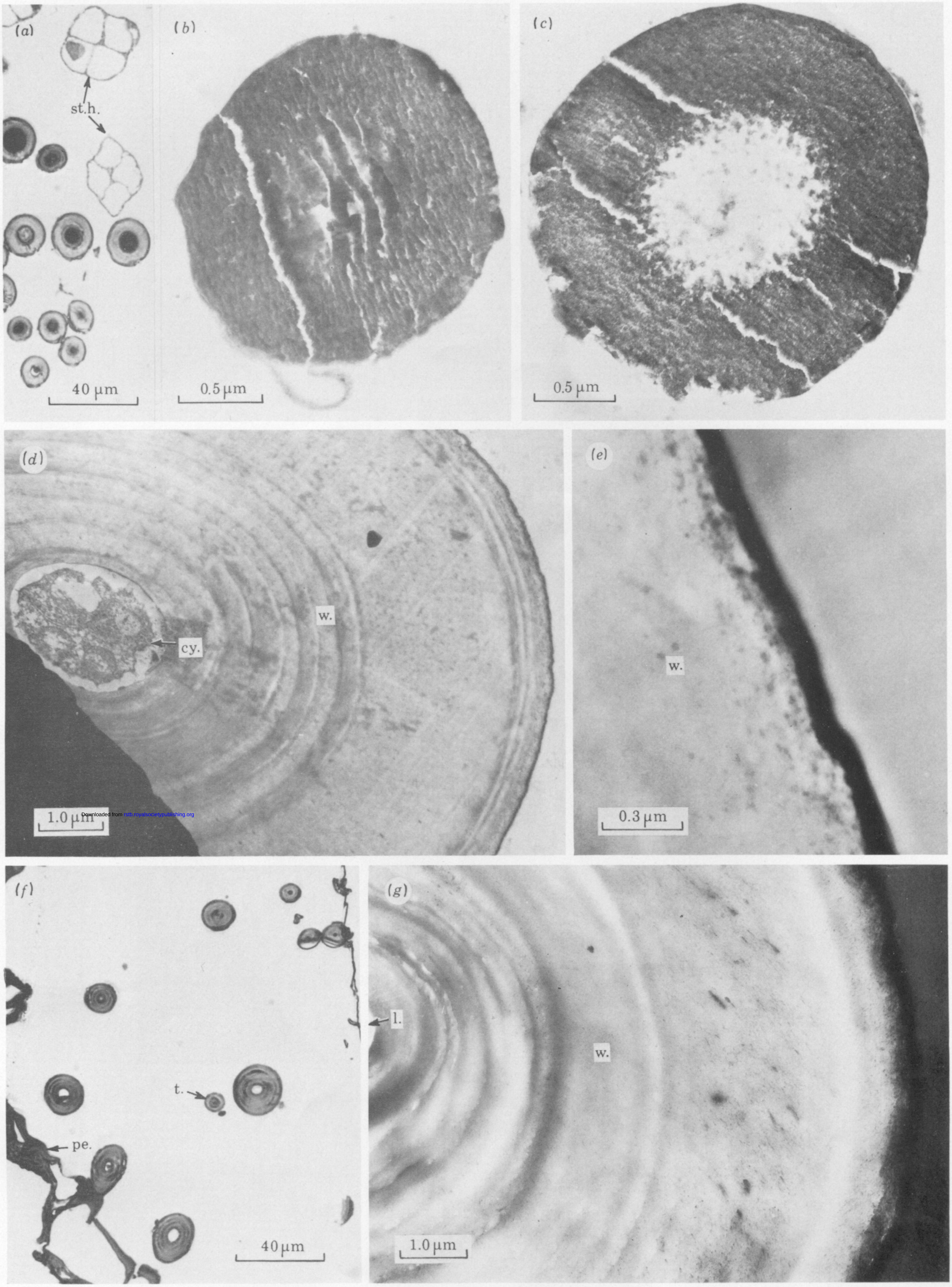
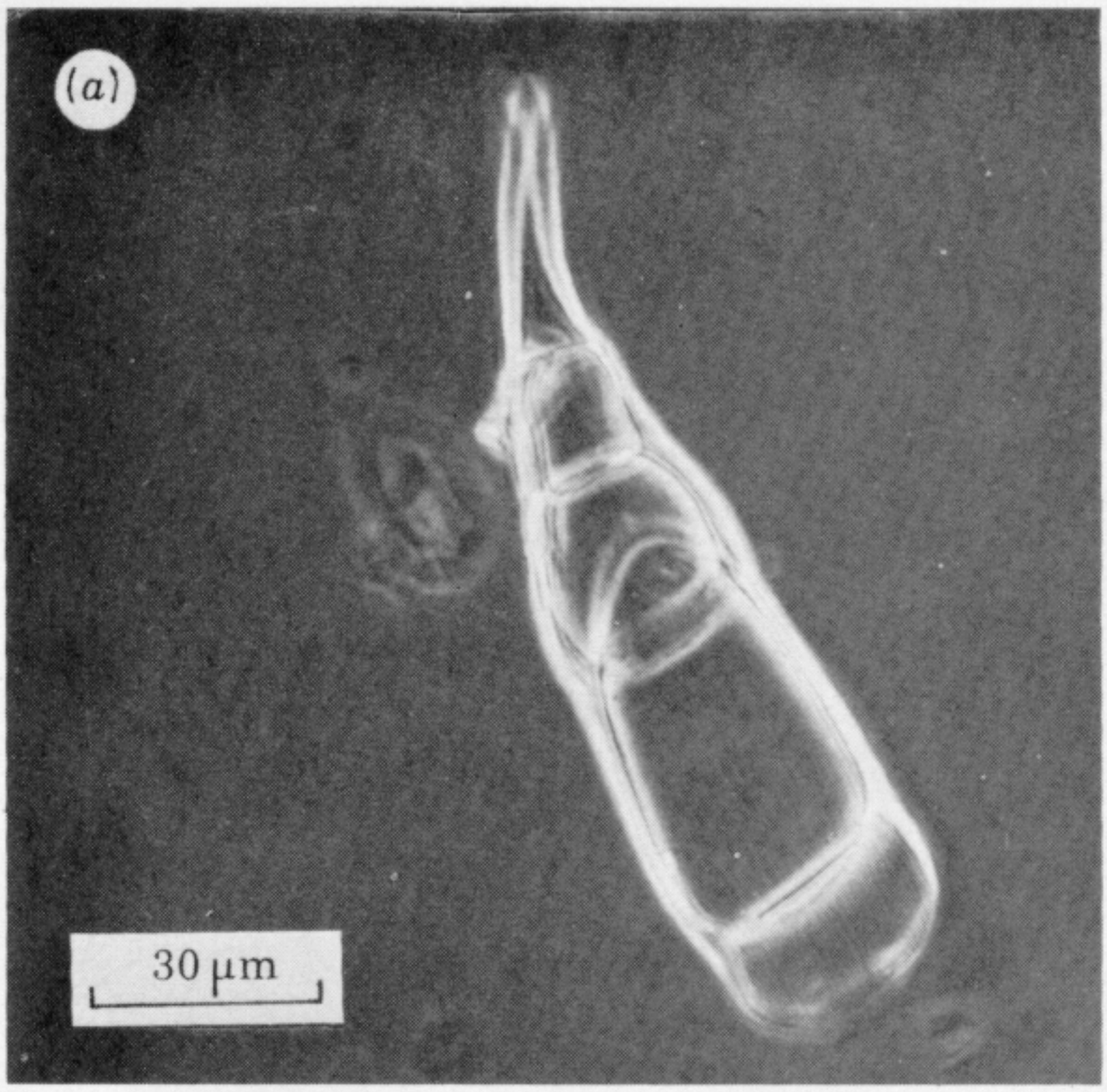
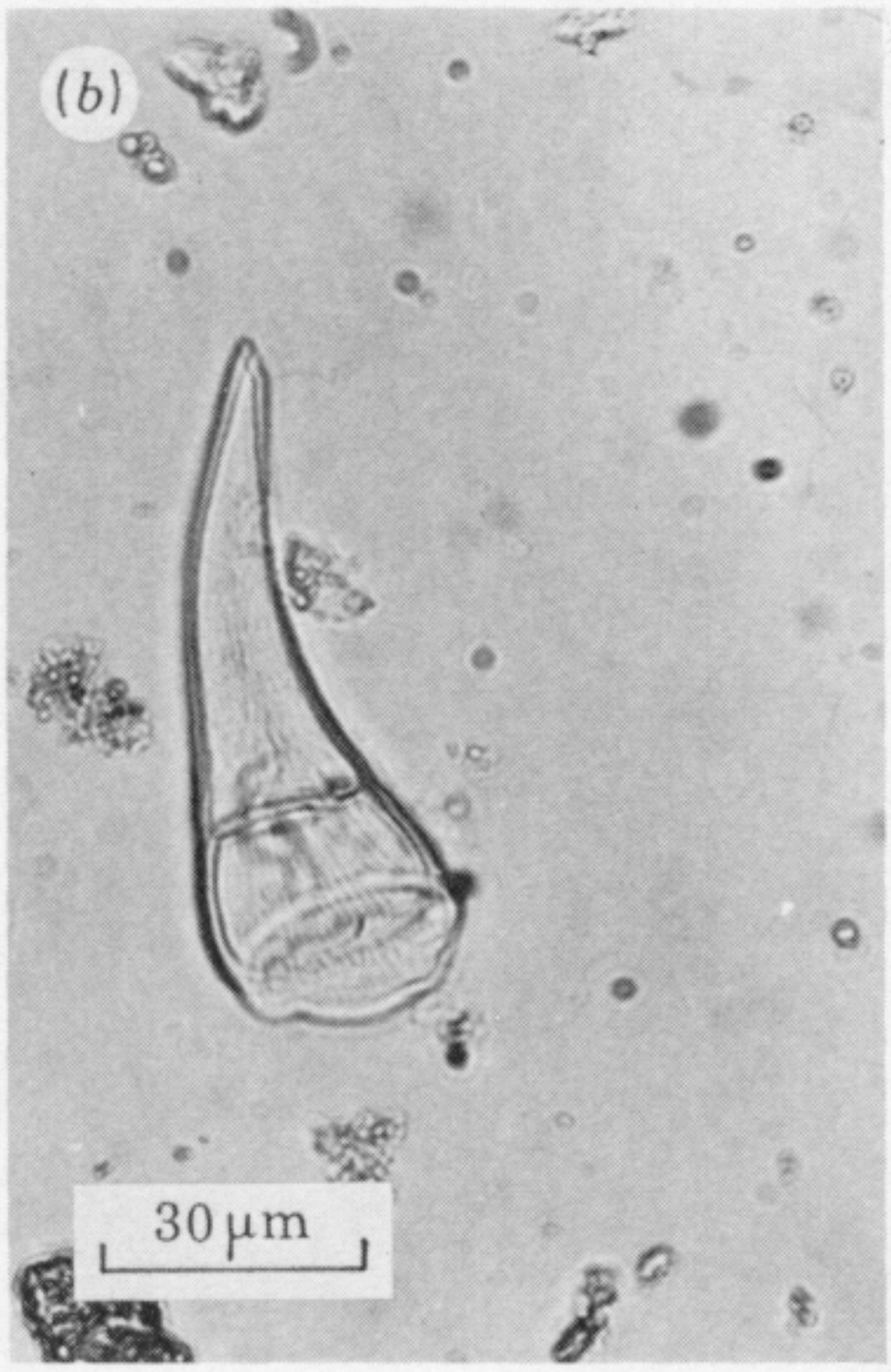
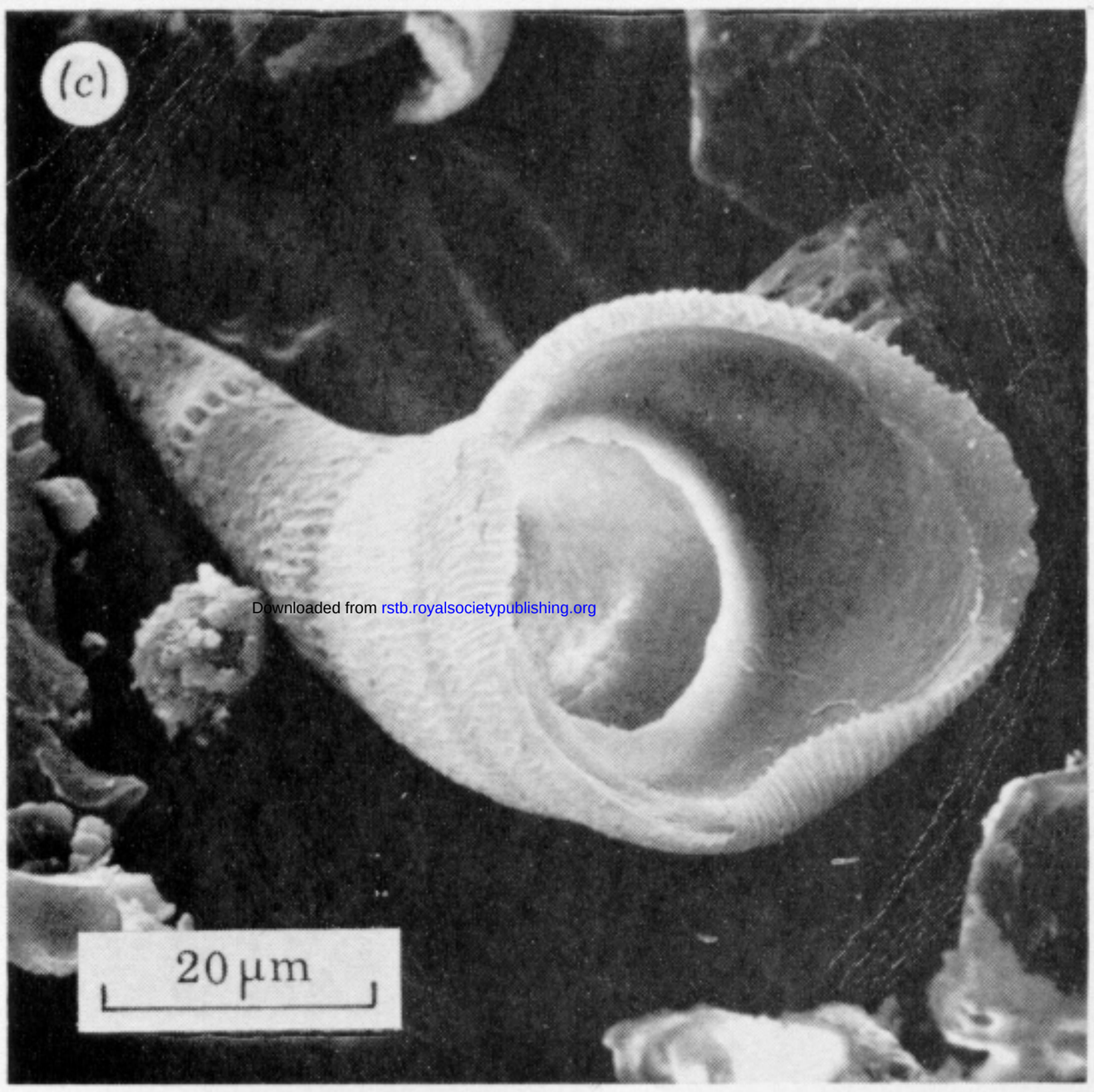
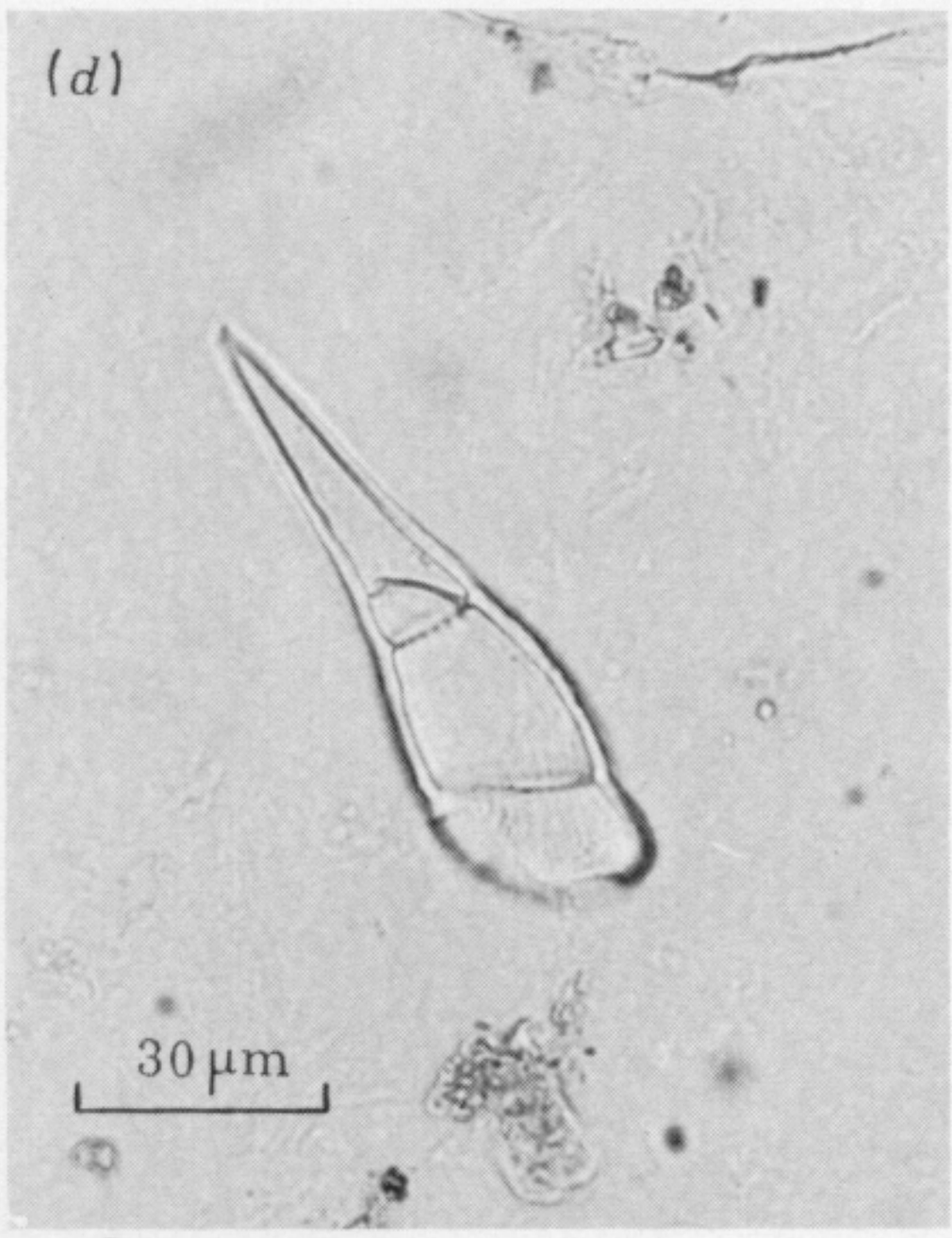


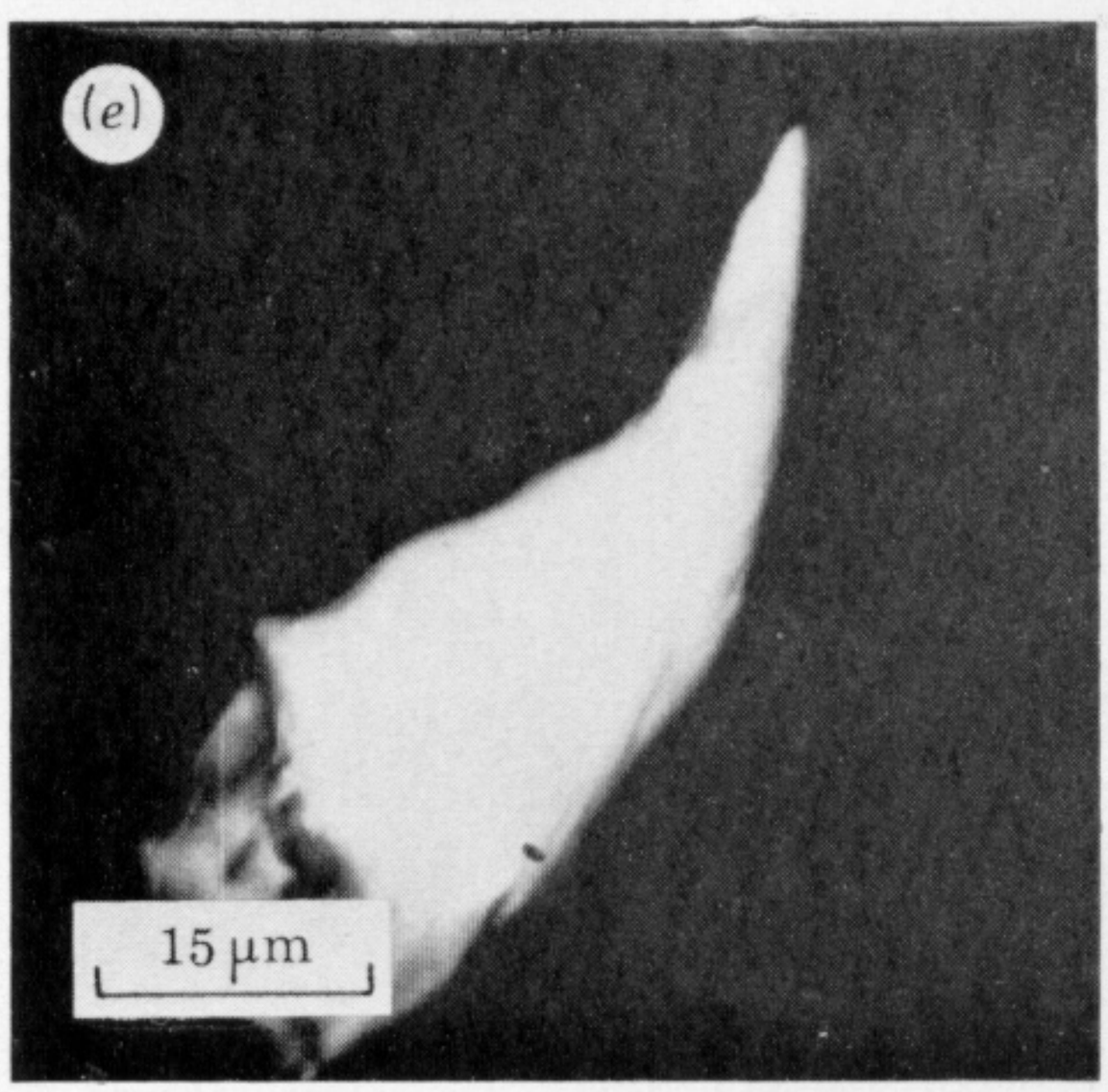
FIGURE 3. For description see p. 545.











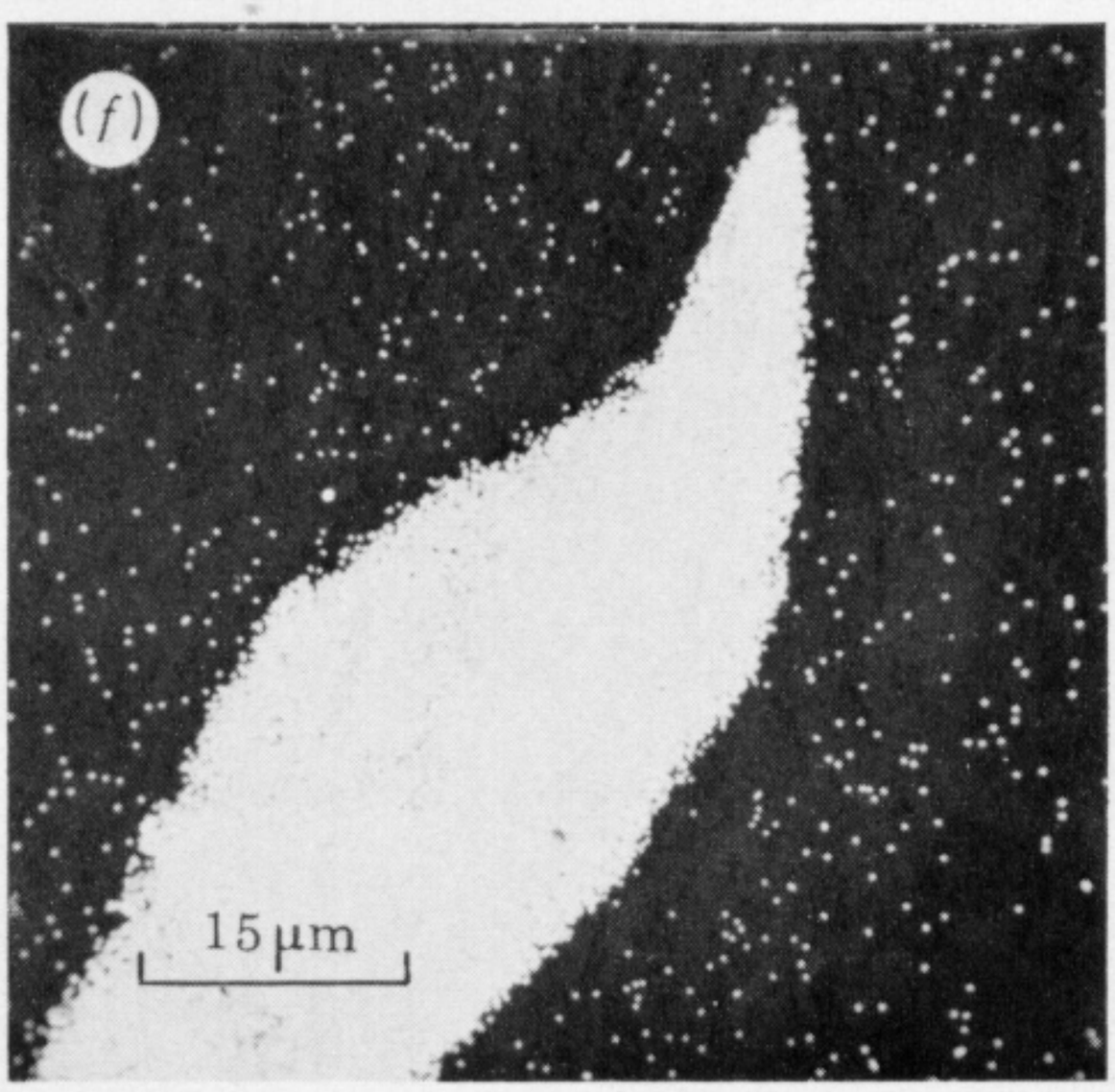


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FIGURE 5. For description see opposite.