POLYRIBOSOMES AND CISTERNAL

ACCUMULATIONS IN ROOT CELLS OF RADISH

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ABSTRACT

The zone of root hair formation of seedling radish roots, *Raphanus sativus* L., was studied by phase-contrast and electron microscopy. Localized dilations of the endoplasmic reticulum, which contained a moderately dense proteinaceous material, were found to be a common component of the cytoplasm in cells of the epidermis and cortex. The surfaces of these dilations were covered with polyribosomes in discrete coils commonly composed of 15 to 17 ribosomes. The function of these structures and the fate of the material accumulated in them are unknown. Their similarity to structures described in some types of animal cells is discussed.

INTRODUCTION

Root hairs, because of their ease of accessibility and unique growth pattern, have long provided attractive experimental material for light microscopy, but their fine structure has as yet been little explored. In the course of a study designed to correlate the fine structure of the cytoplasm and that of the wall during growth of the root hair, striking configurations of ribosomes and swollen cisternae of the endoplasmic reticulum were observed. This paper reports the results of a further investigation of the characteristics and distribution of these structures.

MATERIALS AND METHODS

Seeds of radish, *Raphanus sativus* L., variety "White Icicle," were surface sterilized in a 10 per cent solution of Clorox and germinated at 22 to 23°C in Petri plates containing moist filter paper. 44 hours after placing the seeds in the Petri plates, the seedling radicles were fixed, at which time a large number of root hairs had developed, representing a range of lengths from small protuberances to hairs 1 or 2 mm in length. Fixation, rinsing, and postfixation in osmium tetroxide were carried out at room temper-

ature in 0.025 M phosphate buffer at pH 6.8. Intact seedling radicles were immersed in 3 per cent glutaraldehyde (13), segmented into 2-mm portions, and fixed for 1.5 hours. Following washing, segments were treated with 2 per cent osmium tetroxide for 2 hours, dehydrated in an acetone series, and embedded in Araldite-Epon (9). Silver sections were cut on a Servall MT-1 ultramicrotome, stained with uranyl acetate and lead citrate, and viewed in a Hitachi HU-11A microscope at 50 kv. For light microscopy, 250-mμ sections were stained with mercuric–bromophenol blue (8).

RESULTS

In the initial observations of radish root tissue, structures were apparent which did not conform to the organelles commonly described in plant tissues. These consisted of a granular or somewhat fibrous material enveloped by a unit membrane with attached ribosomes, and thus appeared to represent localized dilations of the endoplasmic reticulum. This conclusion was directly confirmed by occasional observations of profiles such as that illustrated in Fig. 1, which shows an enveloping

membrane continuous with undilated membranes of the endoplasmic reticulum. The infrequent appearance of profiles of endoplasmic reticulum leading directly to dilated cisternae, even though the dilations are common in this material (Fig. 7), suggests that the endoplasmic reticulum at the edge of these structures is constricted to a tubular neck, or that these structures are budding off the endoplasmic reticulum while retaining their affinity for ribosomes. A section (Fig. 2) closely successive but not consecutive to that shown in Fig. 1 reveals that there is now a discontinuity in the endoplasmic reticulum at the neck of the dilated cisterna, whereas the extent of the structure itself is essentially unchanged. A continuity of endoplasmic reticulum at both ends of a dilated cisterna has never been observed, although the probability of obtaining such a fortunate section is slight. Seen in longitudinal view the dilations are oblong in shape; in transection they appear circular (Fig. 7).

In median longitudinal sections of the dilated cisternae, the membranes of the endoplasmic reticulum are sectioned normally and appear studded with ribosomes. In tangential sections of the cisternae, a characteristic arrangement of the ribosomes on the surface is revealed. Figs. 3 to 6 show a series of closely successive sections through a portion of an epidermal cell with a large cisterna. A median, longitudinal section is shown in Fig. 3, and successive sections, showing planes of view closer to the periphery of the cisterna, are illustrated in Figs. 4, 5, and 6. The position of a pair of mitochondria in Fig. 3 may be followed for reference to location of the cisterna. Fig. 4, because of a slight indentation near the middle of the structure, shows a region where a surface view of the limiting membrane can be seen. The ribosomes are clearly seen to be arranged in spirals (Pr). Figs. 5 and 6 show that the entire structure is covered with discrete ribosomal coils (arrows). Counts of the number of ribosomes in the polyribosomes on a number of dilated cisternae, yielded a peak at 15 to 17 ribosomes, and a sharp drop in numbers beyond 17. Many and perhaps all of the polyribosomes with fewer than 15 to 17 ribosomes may represent sectioned portions of coils.

There is little that can be said at the present time about the function of these structures. They are found not only in cells bearing root hairs, but also in hairless epidermal cells and in cortical cells. Stelar tissue has not been examined to establish whether these cisternae are prominent in all differentiated living cells of the primary root structure. They are not present in cells of the apical meristem which have little endoplasmic reticulum, but are present in cells near the apical meristem which have developed a fairly extensive endoplasmic reticulum. They have been observed in epidermal cells located at least as far as 2 cm from the root apex.

The dilated cisternae are present not only in tissue fixed in glutaraldehyde followed by osmium tetroxide, but also are present and have a similar morphology in tissue fixed in glutaraldehyde alone, in osmium tetroxide alone, and in acrolein followed by osmium tetroxide.

Although dilated cisternae up to 10 μ long have been observed, they are generally only a few microns in length and about 1 μ in diameter. Since they are large enough to be resolved by the light microscope, cytochemical tests were carried out on thick sections so that stained structures could be compared by location with structures evident in electron micrographs of adjacent thin sections. The cisternae were negative to the periodic acid–Schiff's reagent for carbohydrates. Fig. 8 shows a photomicrograph of a portion of cytoplasm stained with mercuric–bromophenol blue. Fig. 9 shows an electron micrograph of a serial thin section in which identification of the cellular components can be made. Among the structures which have stained (Fig. 8) are three dilated cisternae, shown in transection (Fig. 9), indicating that the material within the dilated endoplasmic reticulum is proteinaceous.

The preceding figures should not be taken to indicate that all polyribosomes observed in these

FIGURE 1 Section near the base of a root hair showing at the arrow the relationship of a dilated cisterna (DC) to the endoplasmic reticulum. X 33,000.

FIGURE 2 A section closely successive to that of Fig. 1 showing a discontinuity in the profile of the endoplasmic reticulum at the neck of a dilated cisterna (arrow). X 33,000.
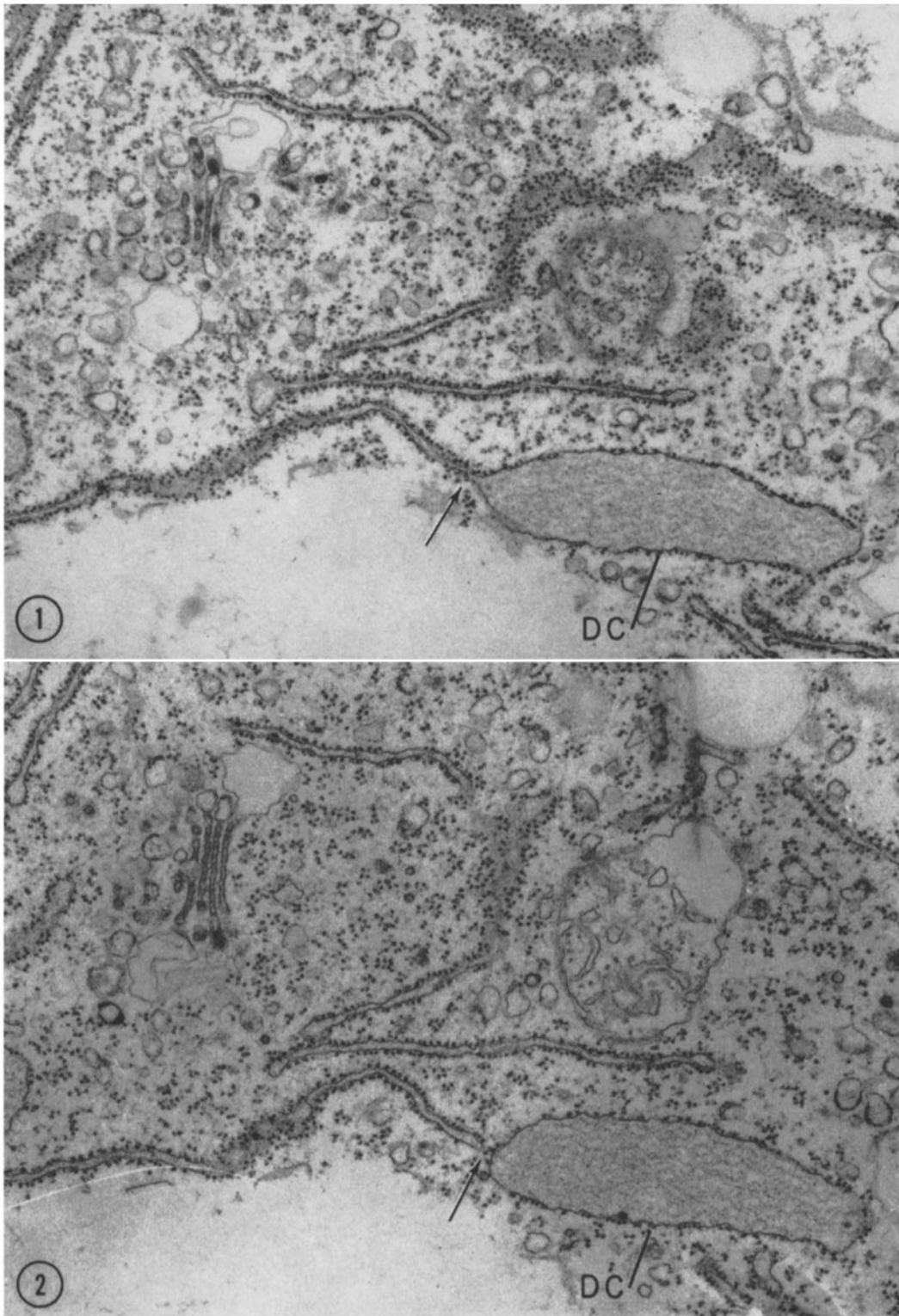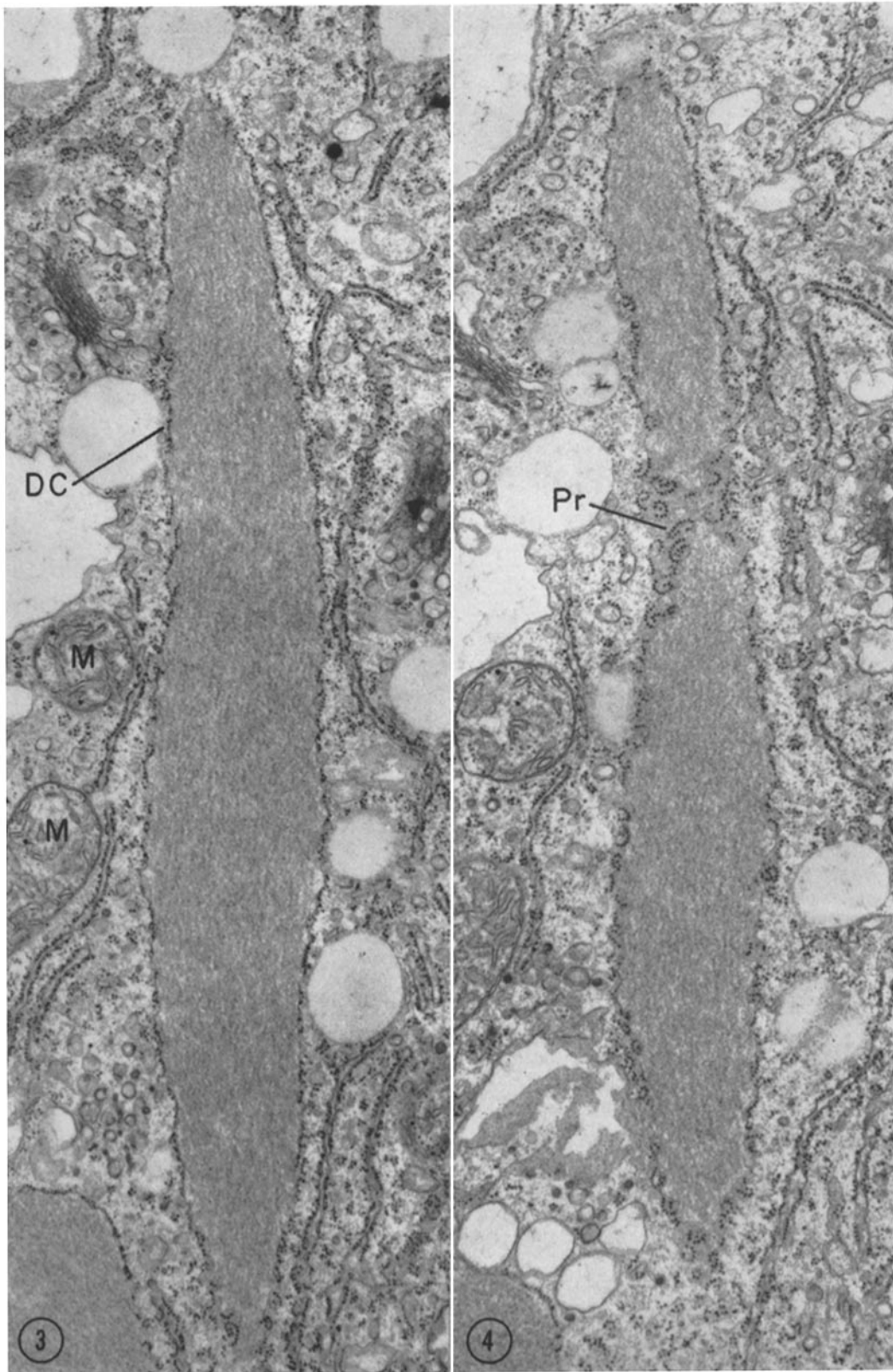
FIGURE 3 A median longitudinal section of a dilated cisterna of the endoplasmic reticulum in a hairless epidermal cell. Labeled mitochondria (*M*) may be followed through the closely successive sections shown in Figs. 4, 5, and 6. Because of the shape of the cisterna or the orientation of the cut, each section of the dilated cisterna is slightly displaced from the previous one with respect to the surrounding organelles.
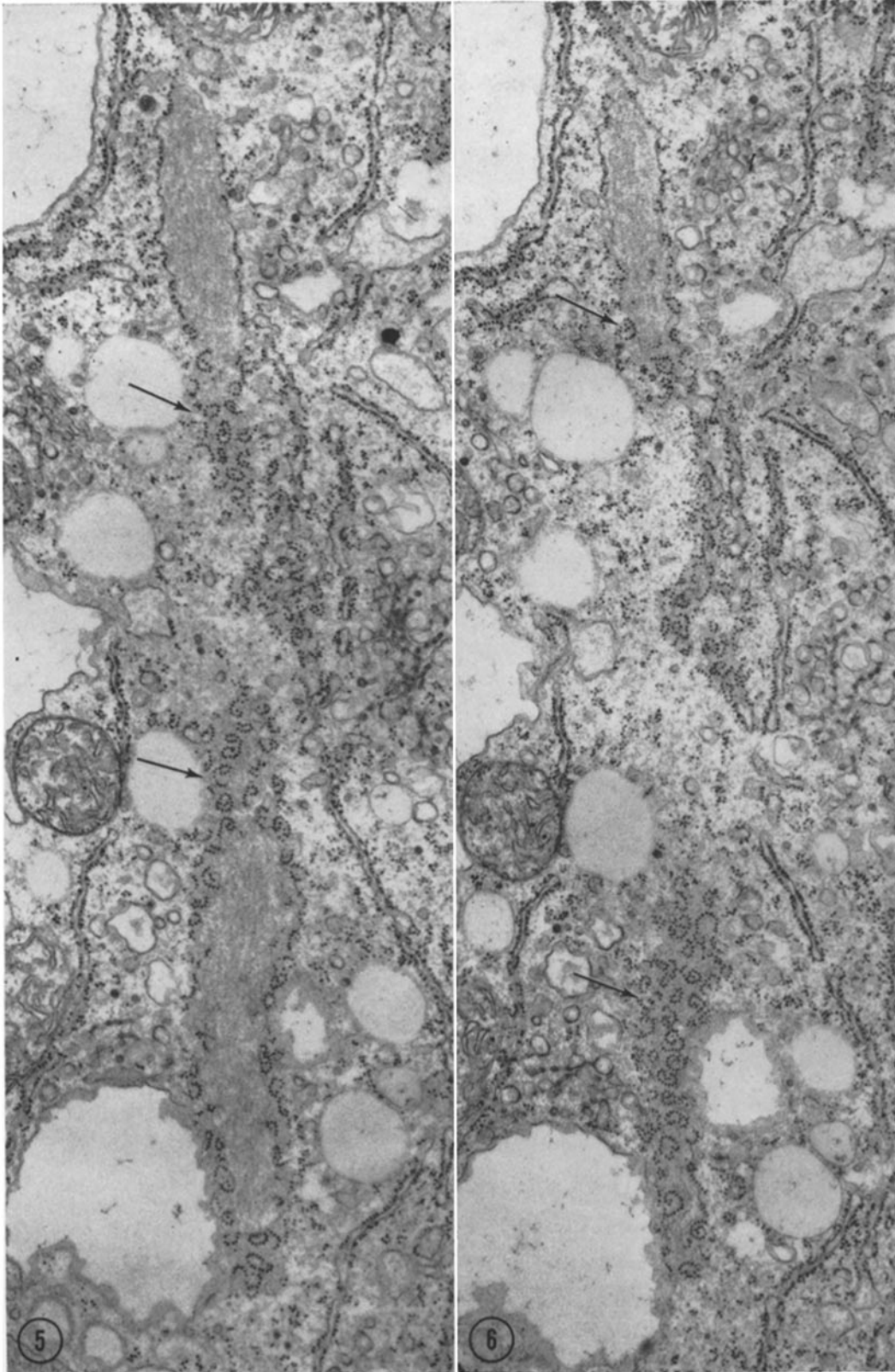
This is only readily apparent when comparing Fig. 3 to the others in the series. To include the entire cisterna in Fig. 3, it has been lowered slightly with respect to Fig. 4. *DC*, dilated cisterna. × 23,500.

FIGURES 4, 5, and 6 Successive sections progressively nearer the periphery of the dilated cisterna. Arrows show polyribosomes (*Pr*) seen when the endoplasmic reticulum of the dilated cisterna is sectioned tangentially. × 23,500.

cells are on the surface of dilations of the endoplasmic reticulum. The dilated cisternae occur with a frequency considerably less than that of mitochondria and slightly less than that of plastids, and their enveloping membranes comprise only a minor proportion of the rough endoplasmic reticulum in the cells. Large fields of polyribosomes can be observed which are not on dilated portions of the endoplasmic reticulum. Fig. 10 shows a tangential view of the endoplasmic reticulum in the basal portion of an epidermal cell bearing a root hair. In such sections ribosomal aggregates can regularly be distinguished comprising as many as 20 to 25 ribosomes (arrows).

Measurements of ribosome dimensions have been made by projecting an image of the polyribosomes, shown in Fig. 10, on a wall at a magnification of 380,000. The lengths of several polyribosomes were measured in centimeters by tracing with the wheel of a map-measurer. Ribosomal dimensions were measured in millimeters with a millimeter ruler. Individual ribosomes are about 185 A in width and 250 A in length, and are separated in polyribosomes by an average distance of about 90 A. The latter value was obtained by measuring the total length of the polyribosome, subtracting from this the proportion occupied by ribosomes, and dividing by the total number of spaces. Closer examination of the polyribosomes shows that the distance between adjacent ribosomes is not constant, being of about ribosomal width in some regions and less in others. The inset in Fig. 10 shows a magnified view of portions of several polyribosomes. A cleft or small gap is often visible within individual ribosomes, which may represent the juncture of the two main ribosomal subunits.

DISCUSSION

Dilations of the endoplasmic reticulum of the type described here have not, to our knowledge, been reported in plant tissue. Buvat (1) has described dilations of the endoplasmic reticulum, which he believes give rise to vacuoles during cell maturation. However, these dilated regions occur in the smooth endoplasmic reticulum and are completely electron-transparent. Several workers have described structures in animal tissues similar to those reported here. Weibel and Palade (16) reported their occurrence in the vascular endothelia of rat tissues, but not in other tissues examined (e.g., endothelium of alveolar capillaries). They established that the dilations contained protein but drew no conclusions regarding their function. Goldberg and Green (3) reported distentions of the endoplasmic reticulum by large amounts of material of moderate density in cells which were undergoing in vitro collagen synthesis. Tangential sections of these structures revealed that they were covered with coils consisting of 10 to 12 ribosomes.

The clearest evidence permitting functional significance to be ascribed to particular aggregations of ribosomes has been obtained from reticulocytes synthesizing hemoglobin. In thin sections of these reticulocytes, most ribosomes are in aggregates of tetramers and pentamers but are not bound to membranes (6, 7). When labeled amino acids are incorporated into intact reticulocytes and the polyribosomes are then isolated by differential centrifugation, the activity is found to be concentrated in the pentamer fraction (2, 15). The length of a pentamer is about 1500 A, or approximately that of a messenger RNA molecule large enough to code for the peptide chains mak-

FIGURE 7   Portion of a transection near the base of a root hair, showing a cluster of dilated cisternae (DC) seen either in cross-, oblique, or longitudinal section. X 42,000.

FIGURE 8   Photomicrograph of a portion of a cytoplasmic process projecting into the large central vacuole of an epidermal cell. Section is stained with mercuric-bromophenol blue and photographed in phase-contrast. X 8000.

FIGURE 9   Electron micrograph of a section serial to that shown in Fig. 8. DC, dilated cisterna; N, peripheral lobe of the nucleus; P, plastid; M, mitochondrion; D, dictyosome; V, vacuole; T, tonoplast of the large central vacuole. X 8000.
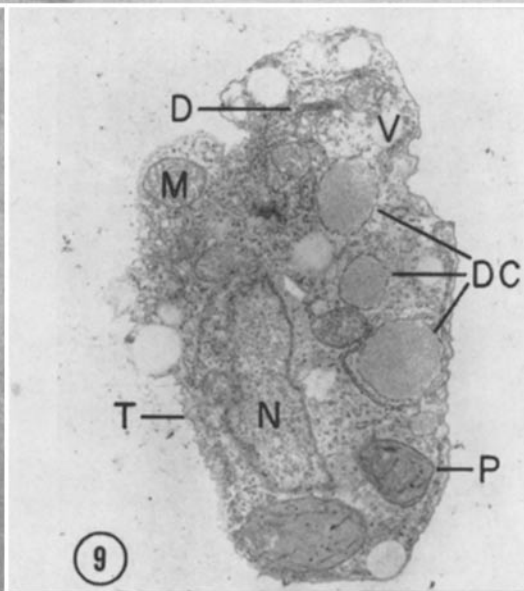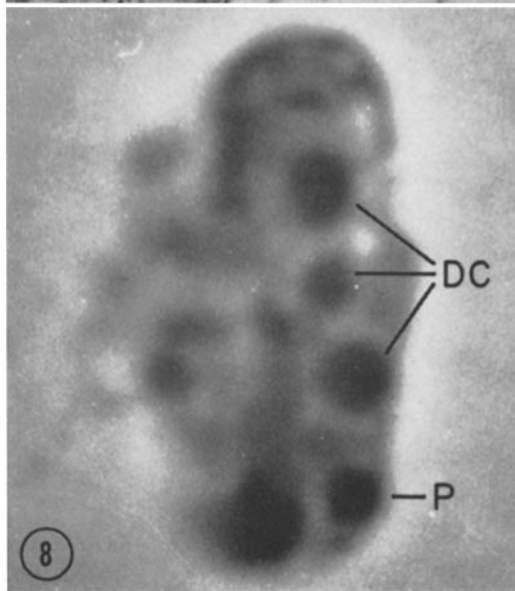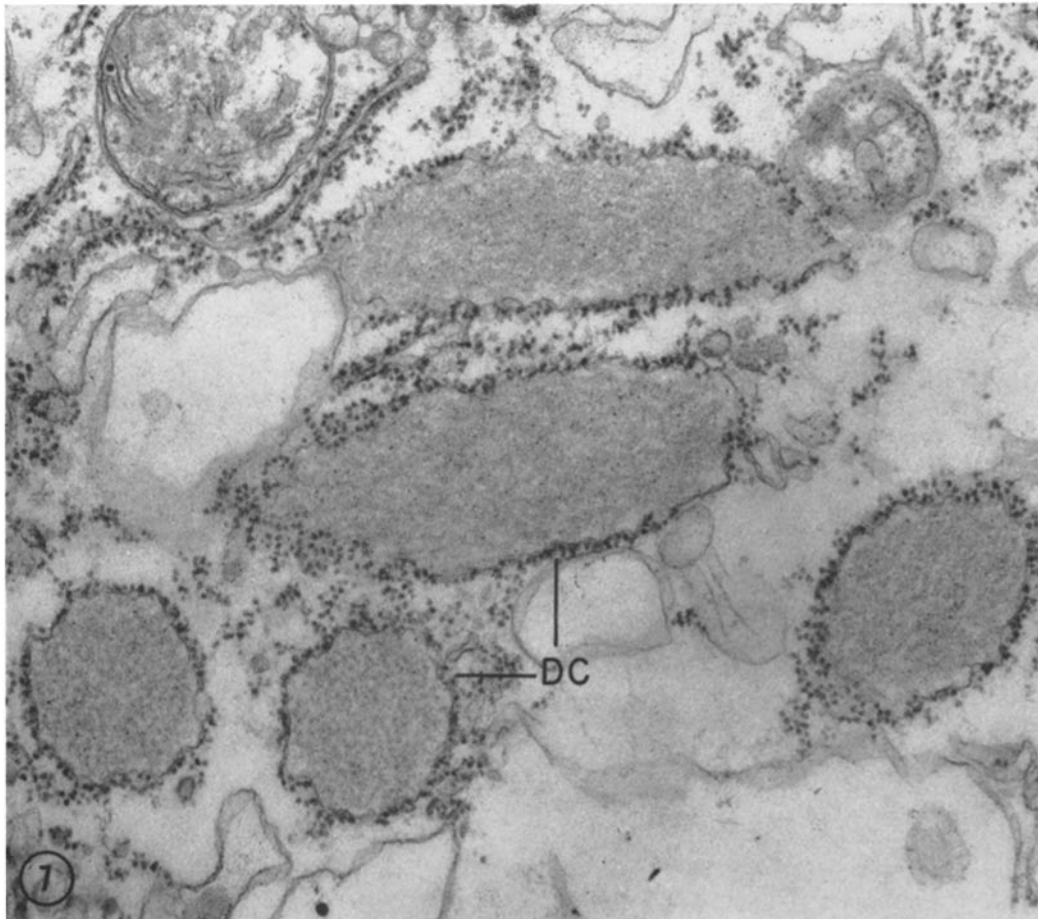
ing up the hemoglobin molecule. A fine strand, 10 to 15 A in diameter, running between ribosomes in reticulocyte polyribosomes (7, 14) is believed to be messenger RNA, since RNase at low concentrations destroys the integrity of the polyribosomes, reducing them to monomers (2, 15).

As yet, there are no such correlations relating membrane-bound coils of ribosomes to protein synthesis. Cells undergoing collagen synthesis exhibit a marked development of endoplasmic reticulum which is covered with spirals of ribosomes (3, 12), but at present it is not possible to bring into accord the results concerning the number of ribosomes in these spirals as seen with the electron microscope, the size of polyribosomes isolated by differential centrifugation (4), and estimates of the molecular weight of the polypeptide subunits of the collagen molecule (10). If the assumptions made in correlating polyribosome length with peptide synthesis in reticulocytes are applied to the polyribosomes on dilated cisternae of radish roots, in which 15 to 17 ribosomes are separated by a mean center-to-center distance of about 275 A, then a protein of about 50,000 mol. wt. would be synthesized.

In undilated regions of the endoplasmic reticulum, there are numerous polyribosomes which are made up of variable numbers of ribosomes significantly in excess of 15 to 17. The functional significance of the specific arrangement of ribosomes in different polyribosomes, leading to a variety of configurations, is at present little understood. In sectioned material in which the membrane of the endoplasmic reticulum underlies the polyribosomes (Fig. 10), it has not been possible to see a strand connecting the ribosomes.

The appearance and content of the cisternae of the endoplasmic reticulum, and the configuration of the ribosomes on the cisternal surfaces, are remarkably similar in radish root cells and collagen-synthesizing cells (3, 12). Furthermore, like cells synthesizing collagen, growing plant tissues synthesize an extracellular structural protein high in hydroxyproline. This protein is tightly bound to the cell wall (5). Although dilated cisternae in fibroblasts and in radish root cells may be homologous structures in cells producing a hydroxyproline-rich structural protein for export, it seems more likely that the similarities are coincidental. The dilated cisternae in radish root cells are not primarily located in regions of most active wall growth, nor have they been observed in growing root cells of *Melilotus alba* or *Phaseolus vulgaris* (unpublished results). Hydroxyproline-containing protein, on the other hand, has been observed in the cell wall fraction of practically all plant tissues investigated.

In fibroblast cells, Goldberg and Green found that rough-surfaced elements of the endoplasmic reticulum were continuous with smooth-surfaced membranes of this system. Vesicles produced irom the smooth-surfaced regions could be seen arrayed near the cell surface. The authors concluded that this was the pathway of movement of collagen precursors from dilated cisternae to the outside of the cell. Revel and Hay (11), using H^3-proline, traced the movement of radioactivity from the rough endoplasmic reticulum to vesicles in the Golgi zone, and finally to collagen fibrils outside the cell. In radish root cells, examination of a large number of micrographs showing dilated cisternae has given no hint of the fate of the proteinaceous content. No consistent associations between the cisternae and other cellular components, such as dictyosomes or vesicles, have been observed.

FIGURE 10 Section near a radial wall of an epidermal cell bearing a root hair, showing profiles of the endoplasmic reticulum sectioned tangentially. Arrows indicate some of the polyribosomes. Inset shows a magnified view of ribosomes with indications of a subunit structure (arrows). Fig. 10, $\times$ 57,000; inset, $\times$ 130,000.
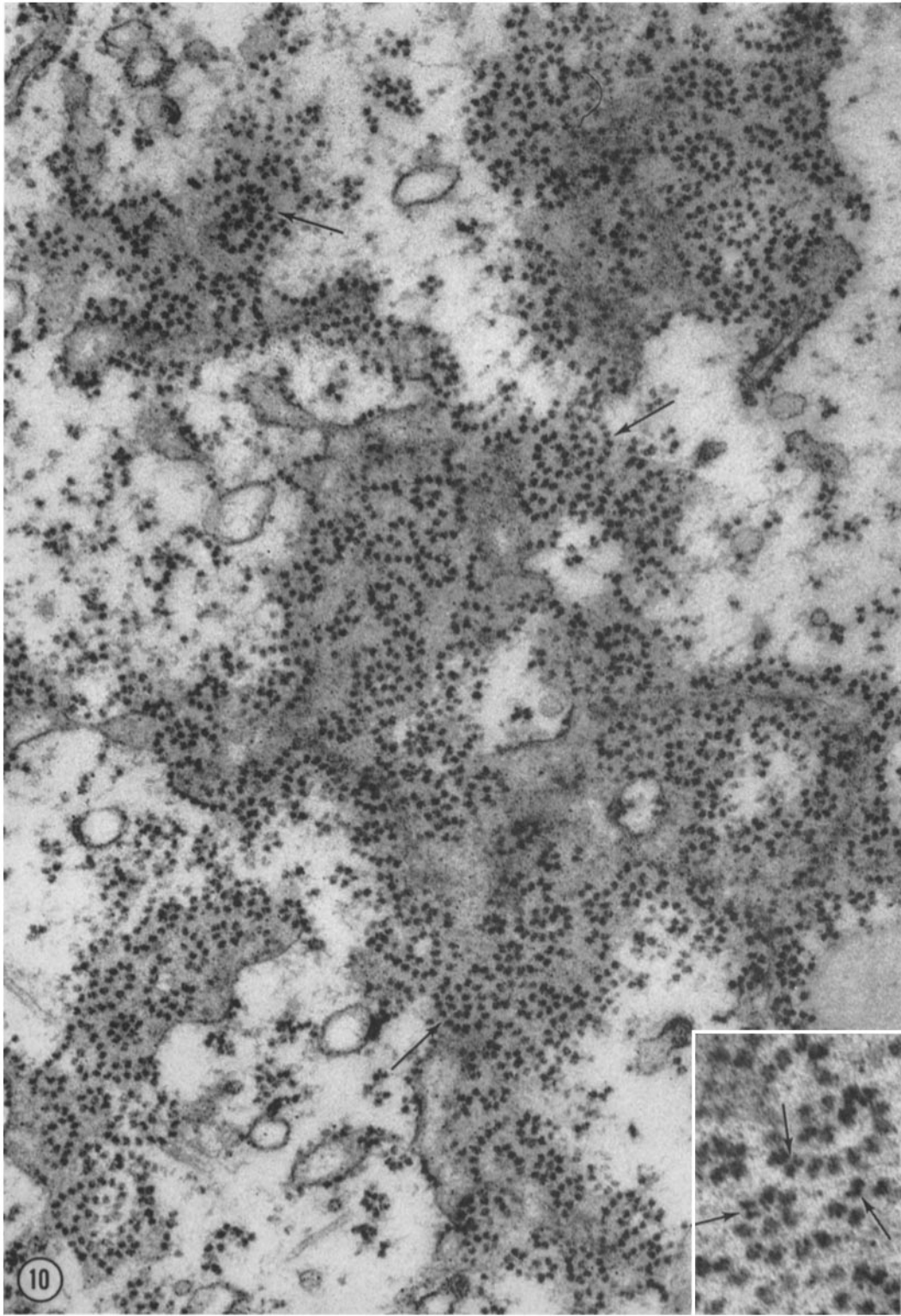
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