STUDIES ON CARTILAGE

III. The Occurrence of Collagen within Vacuoles of the Golgi Apparatus

H. SHELDON, M.D., and F. B. KIMBALL, M.D.

From the Department of Pathology, Faculty of Medicine, McGill University, Montreal, Canada

ABSTRACT

Electron microscopic observations on chondrocytes from rabbit ear cartilage 72 hours after papain has been given intravenously show that many vacuoles in the Golgi apparatus contain a material with a speckled appearance. Some Golgi vacuoles also contain a material with a banded pattern which can be identified as a collagen. The significance of this observation is discussed.

Several recent publications have considered the possible cellular sources of the protein collagen (6, 8, 15, 17, 24, 25, 38, 47). There is evidence that collagen is synthesized in the cytoplasm of connective tissue cells in association with ribonucleoprotein-containing membranes, and it can be identified first from homogenized tissue in an unpolymerized form (22). We believe that these procollagen (31) and tropocollagen (43) macromolecules are liberated from the cell into the extracellular space, where they then aggregate to constitute recognizable collagen fibrils. Neither the precise mechanism whereby the cell achieves the synthesis of these molecules nor the manner in which the products of synthesis are discharged from the cell is entirely clear. This communication reports the occurrence of a material with the appearance of a collagen[1] within vacuoles of the Golgi complex of cartilage cells during the reconstitution of cartilage matrix. We do not know of any previous study on thin sections in which collagen has been identified within a specific compartment of the cytoplasm. The present observations seem to document a step in a likely pathway by which a protein macromolecule, monomeric collagen, may be transported from sites of cytoplasmic synthesis to the extracellular space.

[1] Collagen has been defined as any fibrous protein material which gives a characteristic wide angle x-ray diffraction pattern of 2.86 A along the fiber axis and 10 to 17 A perpendicular to it (26). The appearance in the electron microscope of proteins of the collagen class depends on the manner in which the macromolecules are aggregated (18). The feature which permits identification of collagen in the electron microscope is its repeating periodic structure (3).

MATERIALS AND METHODS

In an earlier communication we suggested that observations on rabbit ear cartilage cells during the period of recovery from the effect of papain might elucidate the relationship between components of the cytoplasm and components of the matrix (46). Recent refinements in techniques of fixation (28), embedding (29), and the staining of sections (53) have improved the reproducibility of sampling and the quality of sections so that we have been encouraged to extend our studies on this model, which was drawn to our attention by Thomas (52). The present observations were made on ear cartilage from six young rabbits each of which weighed between 600 and 1000 grams. Specimens were taken 72 hours after a single intravenous injection of 1 ml of 1 per cent papain in a 0.7 per cent sodium chloride solution. All observations were made on tissue fixed

in 2 per cent potassium permanganate buffered in veronal acetate to pH 7.4 (56), embedded in Epon 812, sectioned with either a Porter-Blum or an LKB microtome with a glass knife, stained with lead hydroxide, and examined in an RCA EMU-3E electron microscope.

OBSERVATIONS

Specimens of cartilage taken 72 hours after the injection of papain show chondrocytes which are larger than normal. Many of these cells have two nuclei, and most cells have plentiful cytoplasmic membranes, large mitochondria, abundant dense granules which have been identified as glycogen (12), and a well developed Golgi complex. The Golgi apparatus can be identified in normal cartilage cells from the ears of rabbits under normal conditions and soon after the injection of papain; however, it appears remarkably hypertrophied in many cartilage cells 72 hours after the injection of papain. The cartilage cells also contain the cytoplasmic feltwork we have described previously, although this appears less dense in sections fixed in permanganate than in specimens fixed in osmium tetroxide.

The Golgi apparatus was first identified in thin sections cut for electron microscopy by Dalton and Felix, who recognized three components in specimens from epithelial cells of the epididymis fixed in osmium tetroxide (11). They described (*a*) a horseshoe-shaped group of large vacuoles, (*b*) lamellar membranes arranged concentrically around the vacuoles, and (*c*) small granules approximately 400 A in diameter which are intimately associated with the lamellae. In general, these findings have been confirmed in a variety of vertebrate tissues (2, 4, 6, 10, 17. 19, 35, 36, 41, 45, 55, 56). The same elements have been seen also in the contractile vacuole of parazoa and protozoa (16), and Afzelius has affirmed the homology of invertebrate dictyosomes and the vertebrate Golgi complex (1). Buvat (5) and Whaley, Mollenhauer, and Kephart (54) have described a Golgi apparatus in plant cells.

A well developed Golgi complex with lamellar membranes, vacuoles, and smaller vesicles is shown within a chondrocyte 72 hours after papain in Fig. 1. Suggestions of a particulate material can be seen within the Golgi vacuoles even at low magnifications. Fig. 2 shows large arrays of lamellar membranes lying between two nuclei of a chondrocyte. From this section it is not clear whether this represents two separate areas of Golgi membranes and vacuoles or one very large complex.

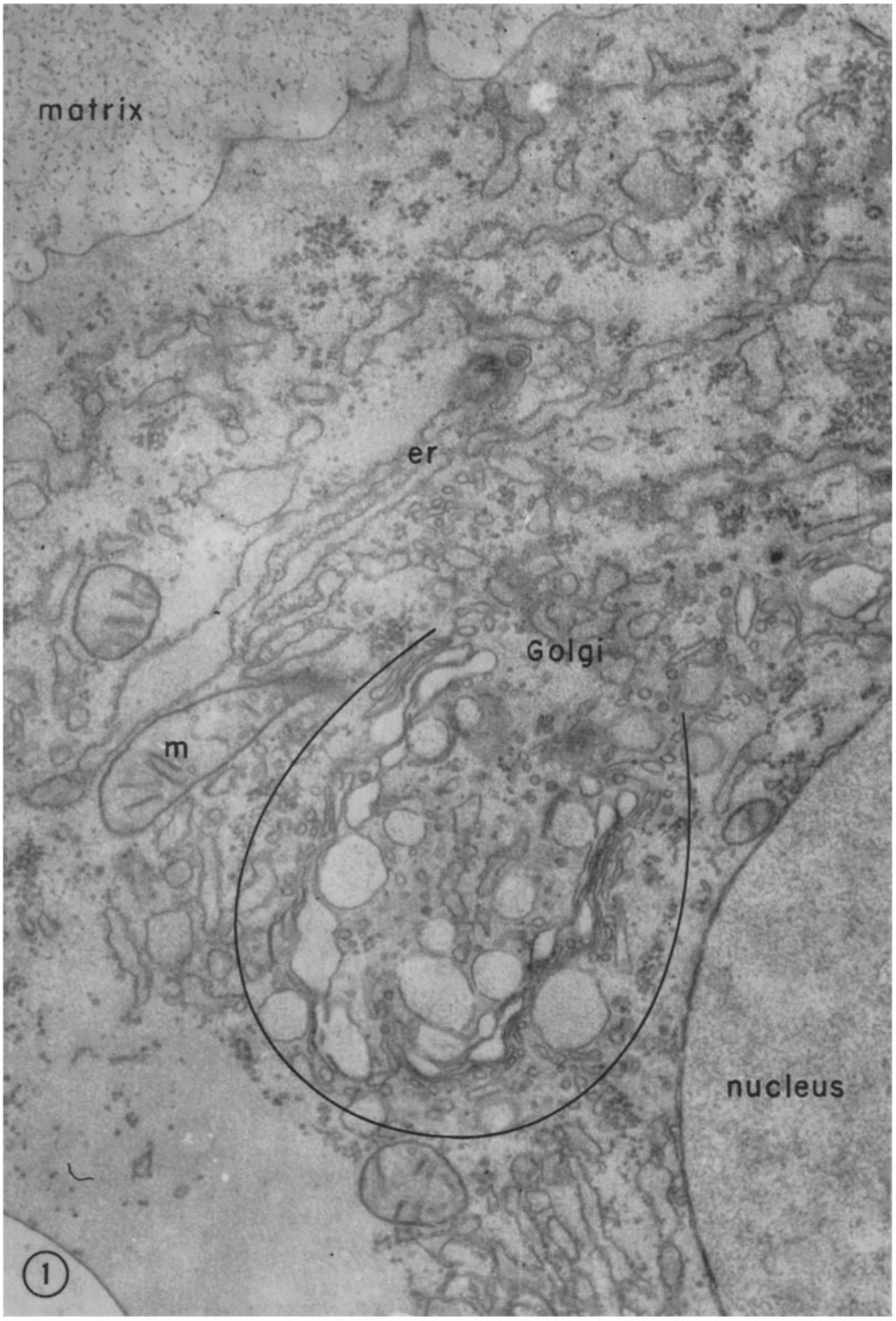
The close topographic association of the granular endoplasmic reticulum[2] and Golgi membranes is demonstrated in Fig. 3, which shows at a higher magnification what may be interpreted as continuity between the granular endoplasmic reticulum and membranes of the Golgi complex. Such anastomoses have been described by others, and commented on by Palade in a recent article (34). We wonder if the small

[2] Christiansen and Fawcett have suggested that the ribonucleoprotein-containing membranes of the cytoplasm, often referred to as rough surfaced components of the endoplasmic reticulum (32), be called more simply the *granular endoplasmic reticulum* (9). They suggest that membranes without granules be called the agranular reticulum. The membranes in the Golgi apparatus may be referred to simply as Golgi membranes. For a further discussion of nomenclature of terms used in electron microscopy the reader is referred to Haguenau review article (19)

All electron micrographs in this study are from thin sections of rabbit ear cartilage taken 72 hours after the administration of papain, fixed in 2 per cent potassium permanganate buffered in veronal acetate to pH 7.4, embedded in Epon 812, and stained with lead hydroxide for 30 minutes.

FIGURE 1

This electron micrograph shows part of a chondrocyte and its adjacent matrix. The cell surface membrane lies in the upper left corner, and a sector of an empty fat droplet lies in the lower left corner surrounded in part by the cytoplasmic feltwork which is characteristic of these chondrocytes. The horseshoe-shaped Golgi apparatus is defined by a thin black line. The lamellar membranes and vacuoles of different sizes are characteristic Golgi components. A mitochondrion can be seen to the left of the Golgi apparatus at *m*. The nucleus lies in the lower right corner. Endoplasmic reticulum, *er*. × 23,000.


matrix
er
Golgi
m
nucleus
1

Golgi vesicles may arise from the pinching off or "budding" of terminal portions of the granular endoplasmic reticulum, as has been suggested by Parks (37).

The large Golgi vacuoles often appear as dilatations of the paired Golgi membranes. They may arise either at the end of the lamellae, as in Fig. 3, or in the middle, as can be seen in Fig. 4. This phenomenon has been illustrated by Fawcett (14) and was anticipated by Hirsch (21) from light microscopic observations on the exocrine cells of the pancreas.

In the chondrocytes of rabbits which have been given papain, the material within the large Golgi vacuoles appears to differ from the material within the cisternae of the rough endoplasmic reticulum. In the Golgi vacuoles the moderately dense material shows a speckled appearance (Figs. 3 and 4), while in the cisternae of the endoplasmic reticulum the contents appear homogeneous and do not show the speckled appearance (Fig. 3).

In specimens from two animals, sections through large Golgi vacuoles show a material which has a different appearance. A pattern of banded structures can be seen in parts of several large Golgi vacuoles. The remaining area of these vacuoles contains the usual moderately dense material with its speckled appearance. In one vacuole, the major period of the banded structures is about 2000 A. In other vacuoles, the extent of the pattern is not great enough to permit accurate measurements to be made. Sufficient resolution is not achieved in the present study to permit complete characterization of the symmetry or dimensions of the components which contribute to the periodic structures shown. However, the banded structures which are demonstrated in Fig. 5 conform to descriptions of fibrous long-spacing (FLS) collagen (18).

Near the surface of many chondrocytes, there are smaller aggregates of moderately dense speckled material (Fig. 6). This material is often surrounded only in part by a membrane which appears the same as the membrane of the Golgi apparatus. In some sections the cell surface membrane has an appearance similar to that of the Golgi membrane. It may be relevant that Rose has observed with the phase contrast microscope in osteoblasts a movement of droplets from the Golgi zone to the cell surface (42). From the present study we have no additional direct evidence to support the hypothesis that the Golgi vacuolar membranes fuse with the cell surface membrane, thereby secreting their contents into the extracellular space, nor have we any evidence which would suggest or support any other hypothesis.

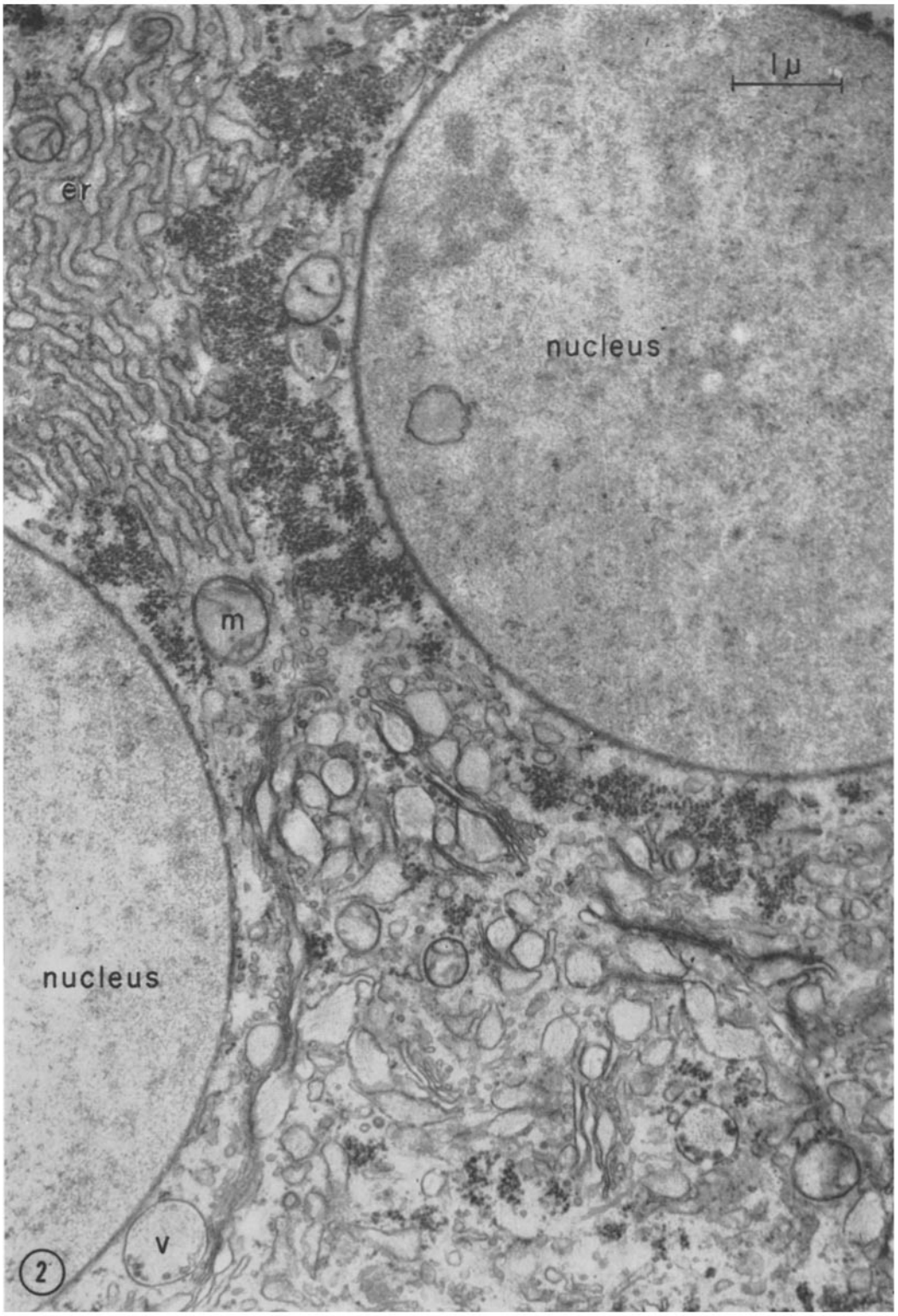
DISCUSSION

We have described previously the early effects of the proteolytic enzyme papain on the fine structure of rabbit ear cartilage (46). The early changes in the chondrocytes and matrix presumably reflect the disappearance of various constituents from this tissue (glycogen from the cells, and the amorphous component and elastic material from the matrix). Recent studies indicate that there is activation of the enzyme, papain, within the tissue before there is dissolution of the cartilage matrix (39). It is possible that the earliest vacuolization of the chondrocyte, which occurs before the cartilage collapses, is related to the activation of papain within the cartilage. Further observations soon after papain has been given may define this more clearly.

Thomas showed that when the ears have regained their erect position the cartilage matrix is restored to normal. The changes which take place after the phenomenon of collapse has occurred presumably reflect the processes which

FIGURE 2

This electron micrograph of a part of a chondrocyte shows two nuclei which are separated by membranes of the granular endoplasmic reticulum (*er*) in the upper left corner. A large array of lamellar Golgi membranes and Golgi vacuoles also lies between the nuclei in the middle and lower right part of the figure. The small dense particles between the granular endoplasmic reticulum and the nucleus on the right represent glycogen as it appears in sections prepared by this method. There is a vacuole which is larger than many Golgi vacuoles at the junction of the nucleus and cytoplasm at *v*. A periodic structure within the contents of this vacuole can be seen even at this low magnification. Mitochondrion, *m*. × 17,000.


1μ
er
nucleus
m
nucleus
v
2

restore the cartilage to normal. Glycogen could be demonstrated again in the chondrocytes 72 hours after papain was given (52). The appearance of glycogen in the present study corresponds to the appearance of glycogen as it has been identified recently in other tissues with similar methods of preparation for electron microscopy (12, 40).

The changes in cartilage cells during this process of reconstitution are analogous to those that occur during the development of cartilage. In a study with the electron microscope, Godman and Porter observed a striking transformation of the Golgi apparatus during differentiation of mesenchymal cells into chondroblasts and into mature hypertrophied chondrocytes in the epiphyseal apparatus (17). In their study the Golgi complex first appears as grouped elements of membranes and associated vesicles. Later, large numbers of vesicles appear to proliferate in the juxtanuclear region until the Golgi complex develops its maximum size and characteristic morphology in the late chondroblast or early chondrocyte stage. During cellular maturation, as the Golgi apparatus enlarges, the contents of Golgi vacuoles appear to change markedly in form and density. These vacuoles, limited by a single membrane, at first either appear empty or contain a material of low density. However, there are no previous descriptions of a material with a well defined pattern within the Golgi vacuoles of epiphyseal cartilage cells.

Godman and Porter also describe another similar but larger vacuole in chondrocytes which has not one but two encircling membranes. They refer to these larger vacuoles, which may be seen in the neighborhood of the Golgi complex, as "pools" or "lakes." With allowances made for differences in techniques of preparation, the material contained in the various Golgi vacuoles in the present study, particularly the large ones in Fig. 5, appears similar to the material shown in their study.[3]

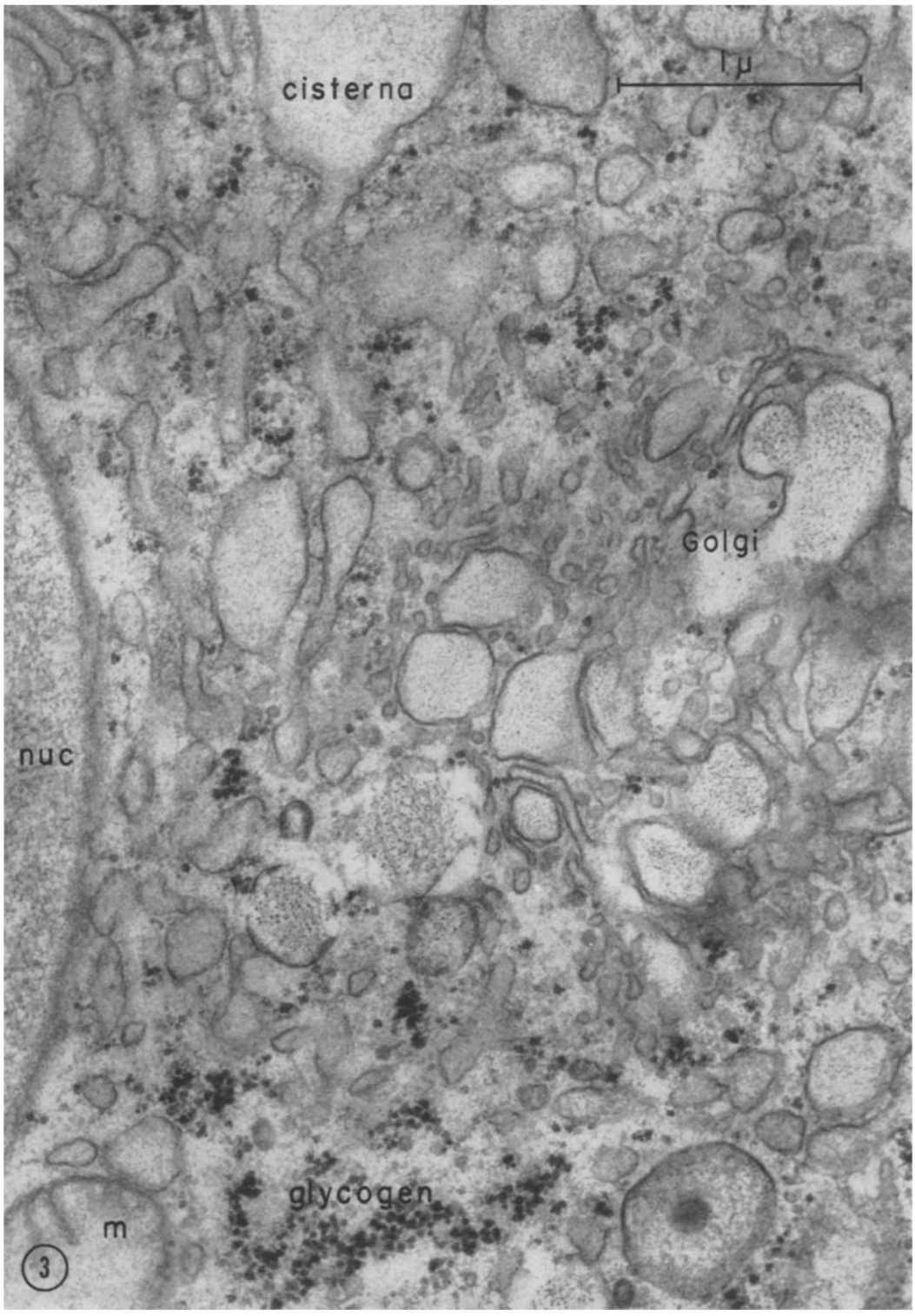
When we consider observations of the Golgi apparatus in other types of cells we find that there is considerable circumstantial evidence to support the hypothesis that the Golgi apparatus represents a portion of the cell which segregates the products of cell synthesis. The most extensive studies to support this contention are those of Palade and Siekevitz on guinea pig pancreas. Palade identified a small granule which first appears within the cavities or cisternae of the granular endoplasmic reticulum (33); subsequently, using correlated biochemical and electron microscopical methods, Siekevitz and he identified this material as chymotrypsinogen (48, 49). Sjöstrand and Hanzon had earlier described the close topographic relationships of the zymogen granules to the Golgi apparatus in their pioneering electron microscopic observations on pancreas (50). Most recently, using a method combining radioautography and electron microscopy, Caro has demonstrated that newly synthesized proteins of pancreas are concentrated in structures of the Golgi apparatus, especially in large vacuoles filled with dense material (7).

From studies on other cell types, the role of the Golgi complex has been likened to that of the "packaging and shipping department." Palay has commented on the possible origins of the neurosecretory granules in the goldfish hypothalamus. He states that "like other protein secretory products they originate in the basophilic Nissl substance or ergastoplasm and are then transported to the Golgi complex where they are segregated into droplets for export down the axons to the neurohypophysis" (36).

[3] We would refer the reader to their monograph for a further discussion of the possible mechanisms involved in secreting the contents of the cell into the extracellular space.

FIGURE 3

This electron micrograph shows part of a chondrocyte in which the relationships of the Golgi apparatus and endoplasmic reticulum can be seen at a higher magnification. In the upper part of the figure is a dilated cisterna of the endoplasmic reticulum with amorphous contents. The lowermost part of this cisterna is narrowed to a tubular structure. In the center of the figure are several small tubular structures which lie between the cisterna and the Golgi apparatus. In cross-section such tubules would appear as round vesicles or vacuoles. In the present preparation, the particles attached to the outer surface of the RNP-containing membranes are not visible. In the middle of the figures there are Golgi vacuoles which contain a material with a speckled appearance. Glycogen can be seen at the bottom of the figure. A mitochondrion (*m*) lies in the lower left corner. Nucleus, *nuc.* × 38,000.


cisterna
1μ
Golgi
nuc
glycogen
m
3

Farquhar and Wellings suggest that the granules of cells in the anterior hypophysis originate in the Golgi complex (13). Bargmann and Knoop show that the Golgi complex is involved in the elaboration of a material that is probably protein (casein?) in their study on milk secretion (2). In their pictures one sees in the Golgi vacuole a small dense granule surrounded by a larger, less dense space which is reminiscent of the appearance of the large vacuole which contributes to the formation of the acrosomal granule during spermatogenesis (4). Fawcett reviews the possible roles of the Golgi apparatus in a recent article (14), and suggests that the correct interpretation of the diverse observations on the contributions of the Golgi complex during spermatogenesis is that a composite material is secreted into it.

What, then, is the nature of the material which is demonstrated within the Golgi vacuoles in the present study, and what are the possible reasons for the patterns which have been shown? Is collagen synthesized and secreted as a pure protein in monomeric form? Or is a mixture of collagen and mucopolysaccharide the natural product of this connective tissue cell?

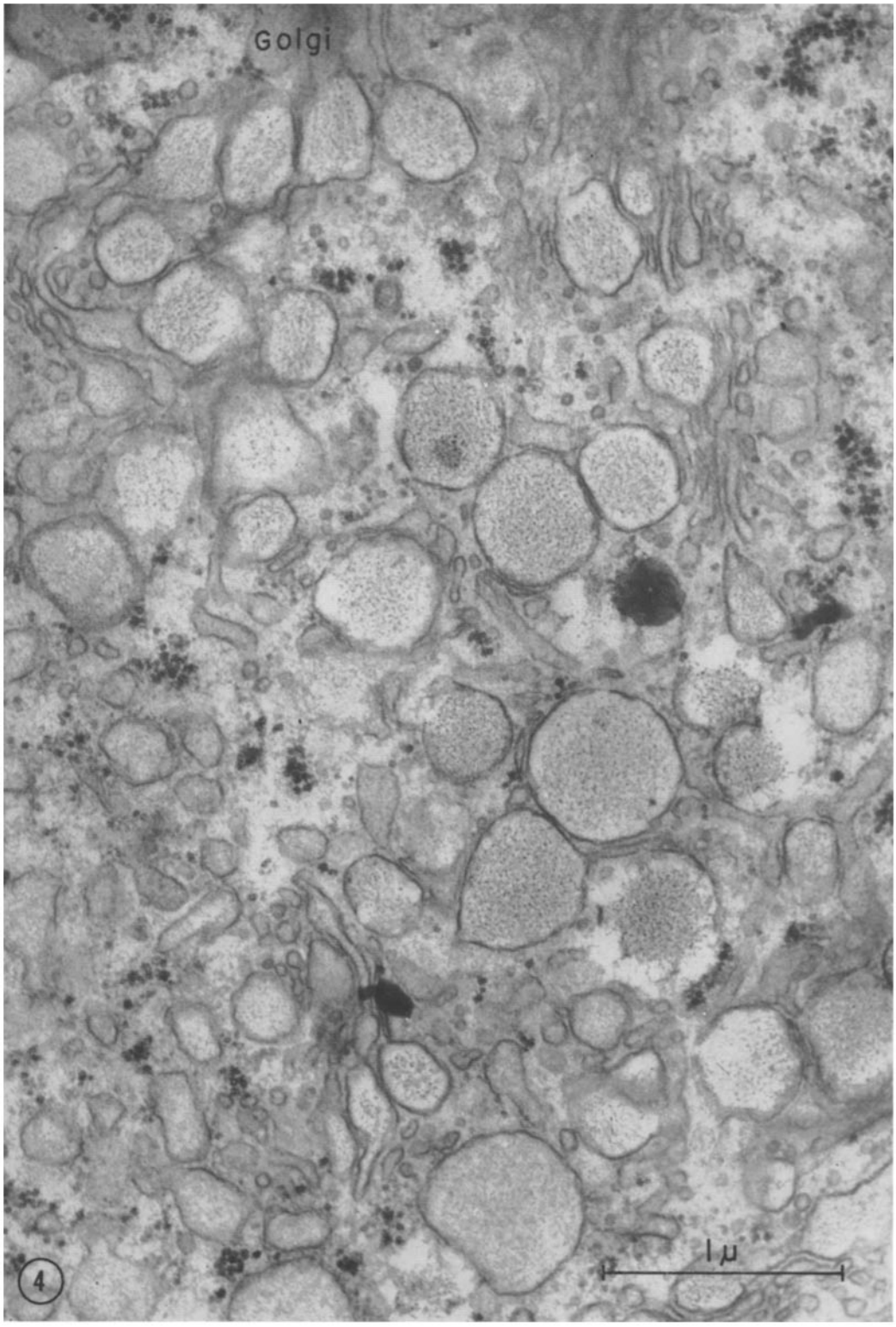
To explain why a collagen has been visualized in the present circumstances in what appears to be an unusual form, we can offer a variety of conjectures. The simplest explanation is that we have observed an accident which occurred during fixation, but this does not explain why the observation has not been made more often. From the studies of Schmitt, Gross, and Highberger it is well known that collagen may precipitate *in vitro* in several forms: native collagen, with a repeating asymmetric banding and major period of approximately 640 A; fibrous long-spacing (FLS) collagen, with an asymmetric period of approximately 2000 A; and segment long-spacing (SLS), with a symmetrical period of approximately 2000 A (18). In addition to these well known different forms, which have been demonstrated *in vitro*, Jakus has described another collagen arranged in another pattern as the principal constituent of Descemet's membrane, from observations on thin sections (23).

While it is obvious that changes in temperature, pH, and ionic strength of the solution may precipitate a protein, there are additional factors which affect the formation of a gel. For example, the setting of a gel is greatly dependent on the presence of other substances; the setting of gelatin is favored by the presence of sulfates. Has chondroitin sulfate contributed to the precipitability of the tropocollagen macromolecules in the present situation? From studies on colloidal solutions we know that there may be mutual coagulation of two colloids. They should be of opposite electrical sign but may have like charges. One might look for analogies between the system within the Golgi vacuole and various *in vitro* colloid systems.

If we suppose that the Golgi vacuole contains a solution of monomeric collagen macromolecules alone, rather than both chondroitin sulfate and collagen, we can consider those colloid phenomena which concern a single colloidal system. Liquid crystals tend to orient themselves on any adjacent solid surface. This tendency alone could serve to explain many observations on the preferential alignment of collagen fibrils in various connective tissues without invoking a specialized area of the cell surface to account for the orientation of the fibrils, as has been suggested by Porter and Pappas (38). Even without a solid surface, which could be represented by the membrane of the Golgi vacuole, long molecules in a solution tend to take up mutually oriented positions. The tropocollagen molecule has been shown to be approximately 2000 × 15 A by Hall and Doty (20). A tendency to orientation and alignment of long molecules is facilitated by various stresses, such as a mechanical force applied externally to any liquid in any capillary system. Finally, from analogy with the

FIGURE 4

This electron micrograph shows many Golgi vacuoles of different sizes and appearances. In the upper left corner four Golgi vacuoles appear as dilatations in the ends of lamellar Golgi membranes. Other vacuoles appear to be either completely or partially enclosed by a dense membrane which usually is irregularly arranged about the contents of the vacuole, as if it were composed of segments of straight lines joined at obtuse angles. In this figure, the contents of the Golgi vacuoles have a distinct speckling; no well defined pattern or array can be seen. The dense particles within the vacuoles which contribute to the speckled appearance are less than 75 A in diameter. × 38,000.


Golgi
1 μ
4

contractile vacuole of protozoa, it has been suggested that the Golgi complex is an area where water is conserved. If this is true of the vertebrate cell too, the contents of the Golgi vacuole may represent the progressively concentrated products of cell synthesis. The removal of water would favor the aggregation of the molecules which are in solution. The formation of crystals in association with the Golgi apparatus has already been reported in an invertebrate (51). (For further parallels between what may occur *in vivo* in the region of the Golgi zone and what is known from studies on artificial systems, the reader is referred to such texts as McBain (30).)

We might, in addition, consider the likely possibility that the substance within the Golgi vacuoles in the present study represents both a collagen (because of the periodic structure which has been demonstrated here) and a mucopolysaccharide (see Godman and Porter (17)). One might wonder what information is available about mixtures of these materials.

In a study on the effect of various biological substances on the polymerization of collagen *in vitro*, Keech has found that chondroitin sulfate greatly increases the rate of precipitation (25). When observed in the electron microscope, this precipitate consists of rigid, discrete fibrils of collagen which resemble those from fresh dermis. Other substances, such as heparin, may delay precipitation and prevent macroscopic gelation. On the basis of various staining reactions, Fitton-Jackson has concluded that fibroblasts may secrete a mucopolysaccharide (15). It has been known that a carbohydrate moiety is closely associated with collagen in connective tissue matrices, and it has been suggested many times that such substances as chondroitin sulfate or hyaluronic acid are involved in the biogenesis of collagen (44). It would seem worth while to look for further cytologic evidence that cells commonly secrete substances which are complexes, coacervates, or colloids rather than materials which conform to the rigid taxonomic scheme of either lipid, carbohydrate, or protein.

Several mechanisms for the transport of materials from within a cell to the extracellular space have been proposed, but few electron microscopical studies have enabled the assembling of data to show precisely how the membranes of the endoplasmic reticulum, granular or agranular, come into continuity with the cell surface membrane. One discrepancy in the general hypothesis is that with various methods of preparation the cell surface membrane has been shown to appear as a trilaminate structure, whereas high resolution studies have always shown that the membranes of the Golgi apparatus appear as a single dense structure. The identity of various cytoplasmic membranes and their relationships to one another require further examination before a conclusive scheme for the anatomy of intracellular transport can be decided on. At this time the general hypothesis for the handling of proteins for extra-

FIGURE 5

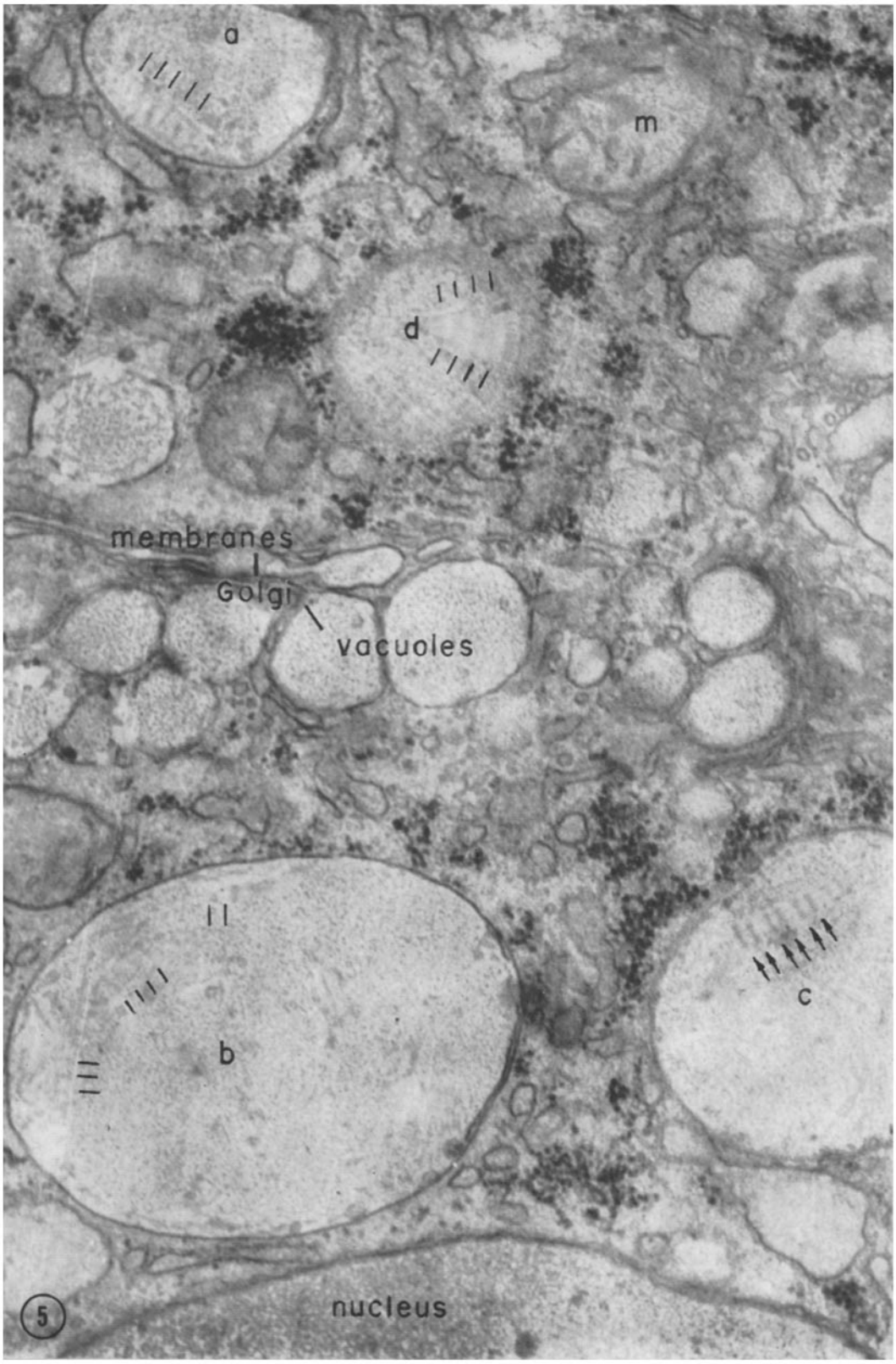
This electron micrograph shows several Golgi vacuoles which contain a material in which a pattern can be seen. In addition to this structured material (which has a periodic banding), speckled material similar to that seen in other Golgi vacuoles is present.

In the center of the figure are lamellar Golgi membranes; below them are typical Golgi vacuoles. Above this complex there is a poorly defined pattern in vacuole *a*. The black lines indicate the spacing of the major bands.

Just above the nucleus, which lies at the bottom of the figure, is a large vacuole (*b*) nearly 3 microns in diameter. This contains material in which a banded structure can be seen. A portion of this vacuole is bounded by two membranes reminiscent of the "lakes" or "pools" shown by Godman and Porter in their study of epiphyseal cartilage

A third vacuole (*c*) contains material with a clearly defined periodic structure which is best seen at the arrows. The distance between the major bands is about 2000 A.

At *d*, above the Golgi membranes, there is an area of cytoplasm with a banded structure but with no apparent enclosing membrane. Various other components of the cytoplasm can be seen. Mitochondrion, *m*. × 28,000.


a
m
d
membranes
Golgi
vacuoles
b
c
nucleus
5

cellular use includes synthesis in the granular endoplasmic reticulum, segregation and concentration in the Golgi apparatus, and secretion through the cell surface membrane via some mechanism which may be holocrine, apocrine, or merocrine in its micromorphology.

This investigation was supported by research grant A-4422 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service.

Dr. Sheldon is a Markle Scholar in Medical Science.

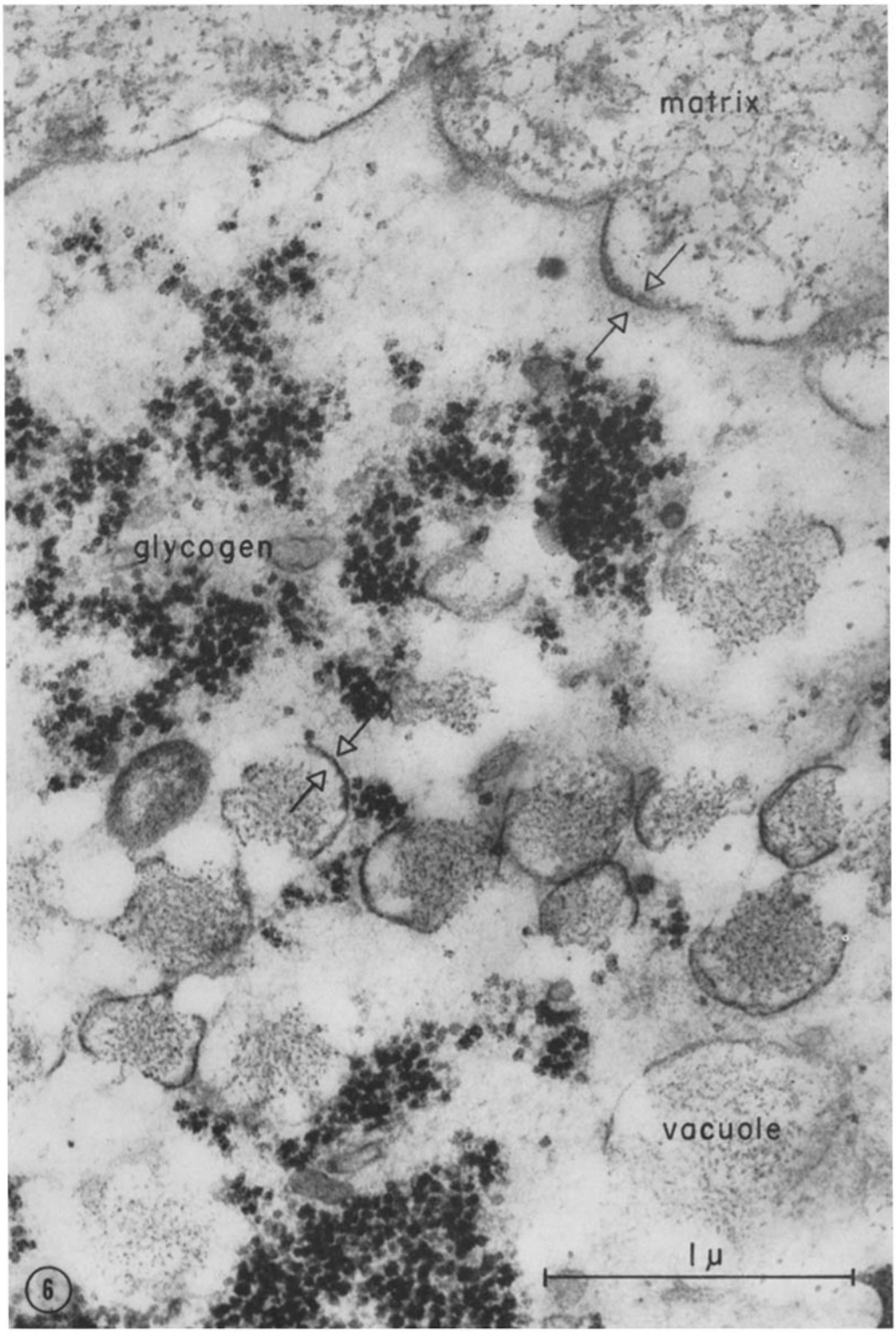
Received for publication, September 29, 1961.

REFERENCES

1. AFZELIUS, B. A., Electron microscopy of Golgi elements in sea urchin eggs, *Exp. Cell Research*, 1956, **11**, 67.
2. BARGMANN, W., and KNOOP, A., Über die Morphologie der Milchsekretion. Licht und Elektronen mikroskopische Studien an der Milchdrüse der Ratte, *Z. Zellforsch.*, 1959, **49**, 344.
3. BEAR, R. S., The structure of collagen fibrils, *Advances in Protein Chem.*, 1952, **7**, 69.
4. BURGOS, M. H., and FAWCETT, C. W., Studies on the fine structure of the mammalian testis. I. Differentiation of the spermatids in the cat, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 287.
5. BUVAT, R., Cytologie végétale—formations de Golgi dans les cellules radicules d'*Allium cepa* L., *Compt. rend. Acad. sc.*, 1957, **244**, 1401.
6. CAMERON, D. A., The fine structure of osteoblasts in the metaphysis of the tibia of the young rat, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 583.
7. CARO, L. G., Electron microscopic radio-autography of thin sections: The Golgi zone as a site of protein concentration in pancreatic cells, *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, 37.
8. CHAPMAN, J. A., Morphological and chemical studies of collagen formation. I. The fine structure of guinea pig granulomata, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 639.
9. CHRISTENSEN, A. K., and FAWCETT, D. W., The normal fine structure of opossum testicular interstitial cells, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 653.
10. CLERMONT, Y., The Golgi zone of the rat spermatid and its role in the formation of cytoplasmic vesicles, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 119.
11. DALTON, A. J., and FELIX, M. D., Cytologic and cytochemical characteristics of the Golgi substance of epithelial cells of the epididymis—in situ, in homogenates, and after isolation, *Am. J. Anat.*, 1954, **94**, 171.
12. DROCHMANS, P., Mise en évidence du glycogène, *Proc. 2nd European Regional Conf. Electron Micr.*, Delft, 1960, 645.
13. FARQUHAR, M., and WELLINGS, S. R., Electron microscopic evidence suggesting secretory granule formation within the Golgi apparatus, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 319.
14. FAWCETT, D. W., Changes in the fine structure of the cytoplasmic organelles during differentiation, *in* Developmental Cytology, New York, Ronald Press, 1960, 161.
15. FITTON-JACKSON, S., Structural problems associated with the formation of collagen fibrils *in vivo*, *in* Connective Tissue, a Symposium Organized by Council for International Organization of Medical Science, London, Blackwell, 1957, 77.
16. GATENBY, J. B., DALTON, A. J., and FELIX, M. D., The contractile vacuole of parazoa and protozoa and the Golgi apparatus, *Nature*, 1955, **176**, 301.

FIGURE 6

This electron micrograph shows a portion of chondrocyte cytoplasm, its cell surface membrane, and the neighboring extracellular matrix. Between the arrows in the upper right, the cell surface membrane shows a curious appearance in which small round structures are present. Between the arrows in the middle of the figure, a segment of membrane of a vacuole has a similar appearance. Most of the vacuoles which contain a speckled material are not completely encircled by a membrane. One could visualize a situation in which the membrane of the vacuole fuses with the cell surface and becomes a part of that membrane, much as two soap bubbles can fuse together. Alternately, reconstruction of serial sections might show a continuity between the Golgi membranes and invaginations of the cell surface membrane. × 47,000.


matrix
glycogen
vacuole
1 μ
6

17. GODMAN, G. C., and PORTER, K. R., Chondrogenesis, studied with the electron microscope, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 719.
18. GROSS, J., The behavior of collagen units as a model in morphogenesis, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 261.
19. HAGUENAU, F., The ergastoplasm, its history, ultrastructure and biochemistry, *Internat. Rev. Cytol.*, 1958, **7**, 425.
20. HALL, C. E., and DOTY, P., A comparison between the dimensions of some macromolecules determined by electron microscopy and by physical chemical methods, *J. Am. Chem. Soc.*, 1958, **80**, 1269.
21. HIRSCH, G. C., Form und Stoffwechsel der Golgikorpen, Berlin, Protoplasmologica Monographs, 1939.
22. JACKSON, D. S., and BENTLEY, J. P., On the significance of the extractable collagens, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 37.
23. JAKUS, M. A., Studies on the cornea. II. The fine structure of descemet's membrane, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 243.
24. KARRER, H. E., Electron microscope study of developing chick embryo aorta, *J. Ultrastruct. Research*, 1960, **4**, 420.
25. KEECH, M. K., The formation of fibrils from collagen solutions. IV. Effect of mucopolysaccharides and nucleic acids: An electron microscope study, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 193.
26. KENDREW, J. C., Structure proteins, *in* The Proteins, (H. Neurath and K. Bailey, editors), New York, Academic Press, 1954, 845.
27. KUFF, E. L., and DALTON, A. J., Biochemical studies of isolated Golgi membranes, *in* Subcellular Particles, (T. Hayashi, editor), New York, Ronald Press, 1959.
28. LUFT, J. H., Permanganate, a new fixative for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 799.
29. LUFT, J. H., Improvement in epoxy embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
30. MCBAIN, J. W., Colloid Science, Boston, D. C. Heath and Co., 1950.
31. OREKHOVITCH, V. N., and SHIPITIKER, V. O., Procollagens as biological precursors of collagen and the physio-chemical nature of these proteins, *in* Connective Tissue. A Symposium Organized by Council for International Organization of Medical Science, London, Blackwell, 1957, 281.
32. PALADE, G. E., The endoplasmic reticulum, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 85.
33. PALADE, G. E., Intracisternal granules in the exocrine cells of the pancreas, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 417.
34. PALADE, G. E., Functional changes in the structure of cell components, *in* Subcellular Particles, (T. Hayashi, editor), New York, Ronald Press, 1959, 64.
35. PALAY, S., and PALADE, G. E., The fine structure of neurons, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 69.
36. PALAY, S. L., Fine structure of neurosecretory cells in the preoptic nucleus of fishes, *Proc. 2nd European Regional Conf. Electron Micr.*, Delft, 1960, 831.
37. PARKS, H. F., personal communication.
38. PORTER, K. R., and PAPPAS, G. D., Collagen formation by fibroblasts of the chick embryo dermis, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 153.
39. POTTER, J. L., MCCLUSKEY, R. T., WEISSMANN, G., and THOMAS, L., The removal of cartilage matrix by papain. Factors affecting the distribution of crystalline papain *in vivo*, *J. Exp. Med.*, 1960, **112**, 1173.
40. REVEL, J. P., NAPOLITANO, L., and FAWCETT, D. W., Identification of glycogen in electron micrographs of thin tissue sections, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 575.
41. RHODIN, J. Correlation of Ultrastructural Organization and Function in Normal and Experimentally Changed Proximal Convoluted Tubule Cells of the Mouse Kidney, Stockholm, Aktiebolaget Godvil, 1954.
42. ROSE, G. G., The Golgi complex in living osteoblasts, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 463.
43. SCHMITT, F. O., Interaction properties of elongate protein macromolecules with particular reference to collagen (tropocollagen), *in* Biophysical Science—Study Program, (J. Oncley, editor), New York, Wiley, 1959., 349.
44. SCHMITT, F. O., GROSS, J., and HIGHBERGER, J. H., States of aggregation of collagen, *in* Fibrous proteins and their biological significance, *Symp. Soc. Exp. Biol.*, 1955, **9**, 148
45. SHELDON, H., An electron microscope study of the epithelium of the normal mature and immature mouse cornea, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 253.
46. SHELDON, H., and ROBINSON, R. A., Studies on cartilage. II. Electron microscope observations on rabbit ear cartilage following the administration of papain, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 151.
47. SHELDON, H., and ROBINSON, R. A., Studies on rickets. II. The fine structure of the cellular

components of bone in experimental rickets, *Z. Zellforsch.*, 1961, **53**, 685.

48. SIEKEVITZ, P., and PALADE, G. E., A cytochemical study on the pancreas of the guinea pig. IV. Chemical and metabolic investigation of the ribonucleoprotein particles, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 1.
49. SIEKEVITZ, P., and PALADE, G. E., A cytochemcal study on the pancreas of the guinea pig. V. *In vivo* incorporation of leucine-1-C^{14} into the chymotrypsinogen of various cell fractions, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 619.
50. SJÖSTRAND, F. S., and HANZON, V., Ultrastructure of Golgi apparatus of exocrine cells of mouse pancreas, *Exp. Cell. Research*, 1954, **7**, 415
51. STRUNK, S. W., The formation of intracellular crystals in midgut glands of *Limnoria lignorium, J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 385.
52. THOMAS, L., Reversible collapse of rabbit ears after intravenous papain, and prevention of recovery by cortisone, *J. Exp. Med.*, 1956, **104**, 245.
53. WATSON, M. L., Staining of tissue sections for electron microscopy with heavy metals. II. Application of solutions containing lead and barium, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 727.
54. WHALEY, W. G., MOLLENHAUER, H. H., and KEPHART, J. E., The endoplasmic reticulum and the Golgi structures in maize root cells, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 501.
55. ZELANDER, T., Ultrastructure of articular cartilage, *Z. Zellforsch.*, 1959, **49**, 720.
56. ZETTERQVIST, H., The Ultrastructural Organization of the Columnar Absorptive Cells of the Mouse Jejunum, *Aktiebolaget Godvil*, Stockholm, 1956.