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ULTRASTRUCTURAL STUDY OF EMBRYONIC SEBACEOUS CELLS, ESPECIALLY OF THEIR SEBUM DROPLET FORMATION

H. Fujita, C. Asagami, S. Murota and S. Murozumi

From the Department of Dermatology, Yamaguchi University School of Medicine, Ube, Japan

Abstract. Sebaceous cells of human fetal skin were studied with special reference to their sebum droplet formation and compared with fat cells of the fetal skin, as we reported previously. In the present study, the embryonic sebaceous cells were divided into 1) immature, 2) progressively maturing, and 3) fully matured cells. The cytoplasm of progressively maturing cells exhibited numerous vesicles, vacuoles and/or tubular structures mostly containing probable glycogen granules, amorphous substances, myelin figures or cytoplasmic elements. The amorphous substances were presumed to represent a substance of lipid nature transformed from probable glycogen, and the latter two to represent stages of the lytic process leading to the transformation of cytoplasmic elements into lipid. The formation of most sebum droplets may be postulated to be initiated by the above transformation of probable glycogen into lipid inside the vesicles and to be promoted by an autophagic mechanism inside the vacuoles and/or tubular structures. Besides this main route, a secondary route seems possible via which some sebum droplets having no limiting membrane might be formed by a lytic process within either glycogen areas, or mixed clusters of free ribosomes and glycogen. This process is similar to the lipid droplet formation in fat cells. Numerous vesicles, vacuoles and/or tubular structures were located nearby or contiguous with sebum droplets and so suggesting the enlargement of the droplet by fusing with it.

There are a number of electron microscopic studies on sebaceous glands of the skin and the oral mucosa of postnatal organisms such as the human adult, rabbit and rodent (1–4, 7–9, 11, 12, 14, 15). However, except for the one by Breathnach (1), study of them in developmental skin has rarely been reported. In addition, the problem concerning the site and modus of their lipogenesis is not yet settled.

The present study is an attempt to investigate the ultrastructural features of the sebaceous cells in human fetal skin, with special reference to their sebum droplet formation compared with the lipid droplet formation of fat cells in fetal skin, which we reported previously (5, 6).

MATERIALS AND METHODS

The study was performed on skin samples obtained from limbs of freshly aborted human fetuses, 21 weeks (35 mm in foot length and 132 mm in crown–rump length) to 26 weeks (48 mm in foot length and 202 mm in crown–rump length) of menstrual age.

The samples were fixed in 4% glutaraldehyde for 2 hrs followed by post-fixation in 1% osmium tetroxide for 1 hr. Both fixatives were buffered at pH 7.4 with Millonig phosphate buffer (10). After dehydration in graded ethanols, the materials were embedded in Epon 812. Sections for electron microscopy were stained with 2% uranyl acetate and 0.4% lead citrate.

OBSERVATIONS

As in the adult glands, acinei undergoing sebaceous differentiation in fetal sebaceous glands consisted of cells in various phases of sebaceous transformation. They were bounded peripherally by a basal lamina (Fig. 1).

For ease of description, these acinar cells were divided into 1) immature, 2) progressively maturing, and 3) fully matured cells, according to the degree of sebum droplet formation in their cytoplasm.

Immature cells

The cells of this type seemed to be at a precursor stage to the inception of sebaceous transformation. They were usually adjacent to the basal lamina, contained no sebum droplet and were mostly oblong in shape, having their long axis parallel (occasionally oblique) to the basal lamina

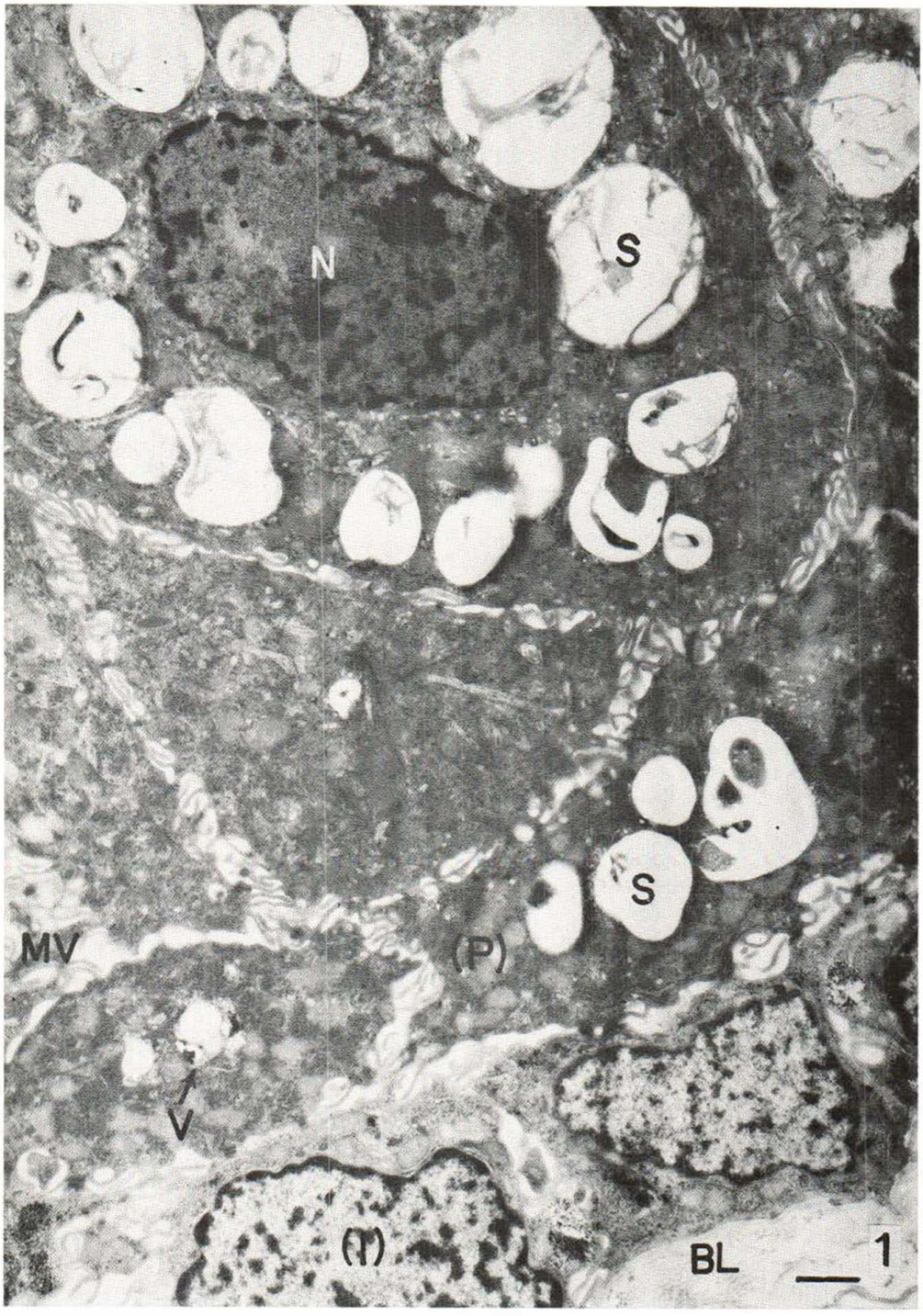

Fig. 1. Survey view of a sebaceous acinus consisting of immature cells (*I*) and progressively maturing sebaceous cells (*P*). *BL*, basal lamina between the acinus and surrounding mesenchyme; *MV*, prominent microvilli along apposing surfaces of sebaceous cells; *N*, nucleus of a progressively maturing sebaceous cell; Arrow *V* indicates a vacuolar structure presumably representing a form of glycogenosome in immature cells. *S*, sebum droplets. × 9 600.

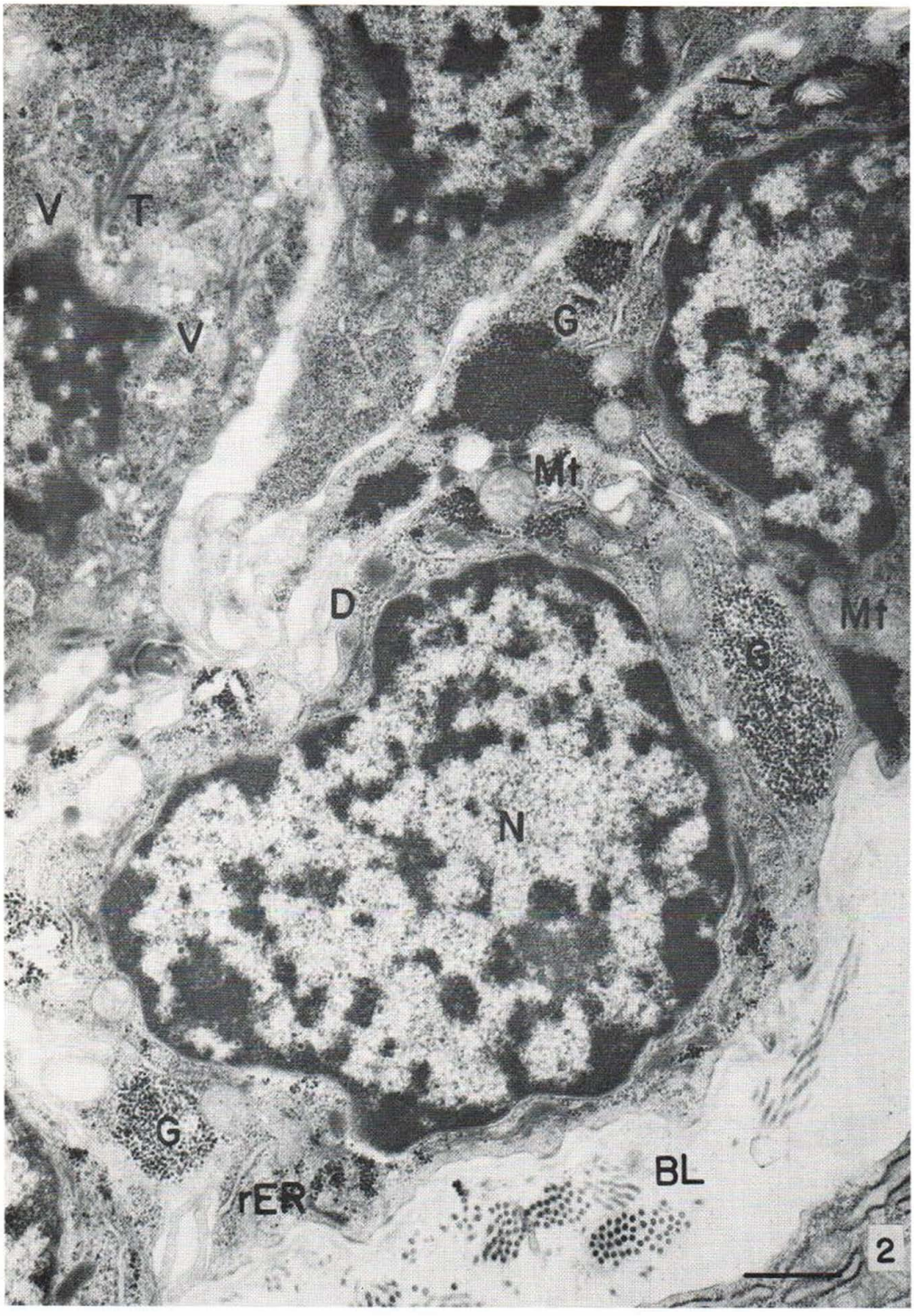

Fig. 2. Immature cells. *N*, nuclei; *Mt*, mitochondria; *rER*, tubular profiles of rough endoplasmic reticulum; *V*, vesicles appearing in the cytoplasm of an immature cell; *T*, many tonofibrils; *D*, dense body; *G*, glycogen areas; Arrow indicates a myelin figure presumably representing a form of glycogenosome; *BL*, basal lamina. × 14 200.

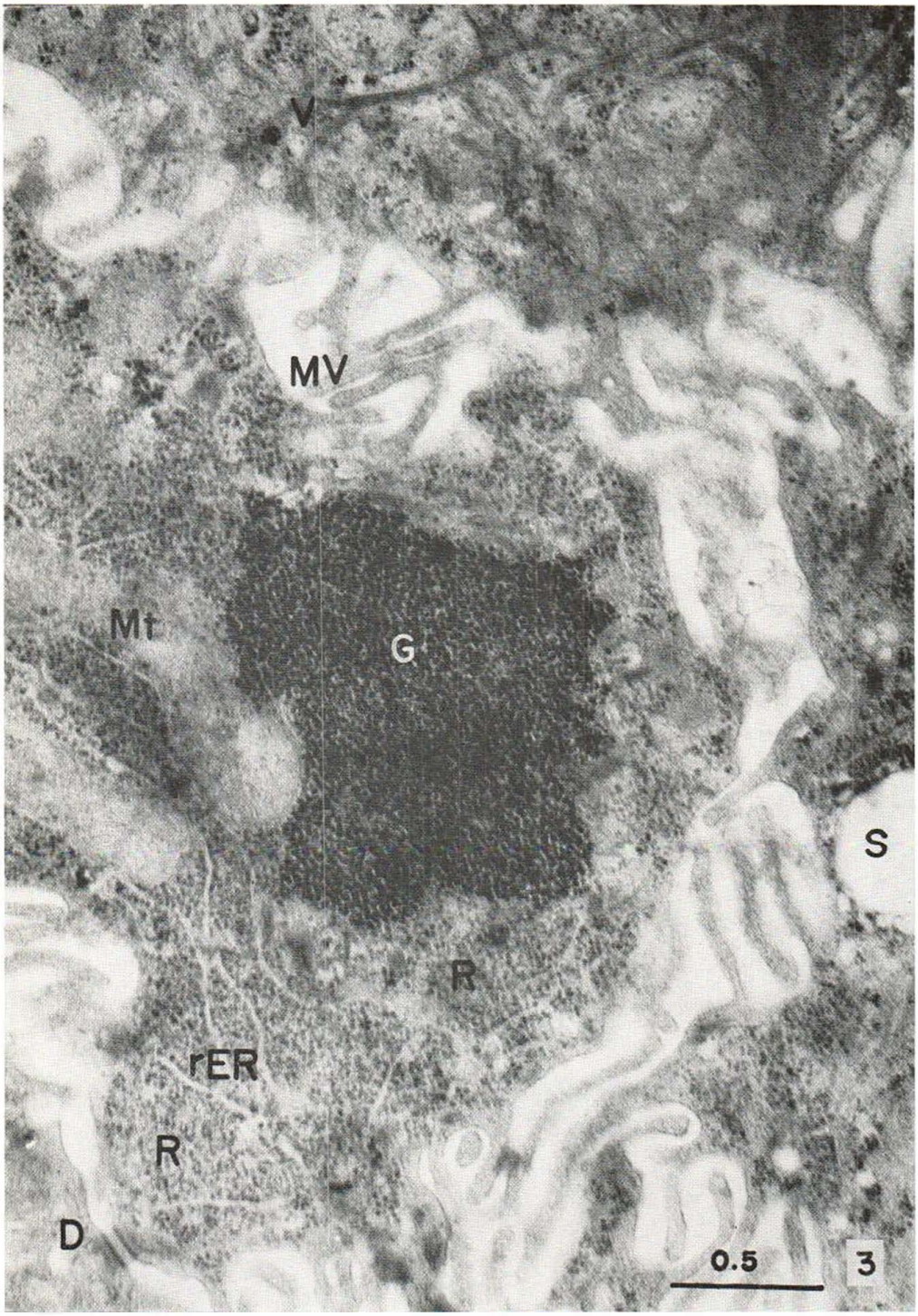

Fig. 3. Cytoplasm of immature cells. *R*, clusters of free ribosomes mixed with amounts of glycogen granules permeated by long tubular profiles of rough-surfaced endoplasmic reticulum (*rER*); *Mt*, mitochondria; *G*, glycogen area; *D*, desmosome; *MV*, prominent microvilli along the plasma membrane; *V*, a small number of smooth-surfaced vesicles in immature cells; *S*, sebum droplet studded with many vesicles. × 43 600.

(Fig. 2). The outer surfaces were mostly smooth but occasionally had a comparatively small number of microvilli or slight indentations while the surfaces apposing other sebaceous cells were covered by prominent microvilli. Apposed plasma membranes and the interlocking villous processes along their surfaces were connected by rather sparse desmosomes (Figs. 2, 3).

The nuclei often exhibited prominent nucleolei and patchy aggregates of chromatins often showing marginal condensation, and had a large nucleo–cytoplasmic ratio. Nuclear inclusion bodies were rarely found in them, thus differing from those of adult sebaceous cells reported by Ellis (4). The cytoplasm exhibited a moderately high electron density, numerous free ribosomes mostly aggregated into clusters, glycogen granules often forming variously sized glycogen areas, relatively small mitochondria and many tonofibrils (Figs. 2–4). It usually lacked a Golgi apparatus, though occasionally exhibiting a prominent one, and commonly contained many clusters of free ribosomes mixed with glycogen granules often encircled by long tubular profiles of rough endoplasmic reticulum (Figs. 3, 4). Among these clusters were seen certain amorphous areas that were possibly derived from clusters losing their granularity and becoming homogeneous in appearance, and suggestive of undergoing lytic process leading to their transformation into lipid (Fig. 4). The glycogen areas contained neither clustered vesicles having a foamy appearance, nor small areas enclosed by a lamella, but occasionally displayed a comparatively small number of dense bodies or of glycogenosomes such as vacuolar structures and myelin figures (Figs. 1, 2).

Progressively maturing cells

The cells of this type seemed to be actively synthesizing and accumulating sebum, and were located more centripetally than the immature cells. They were variable in size but usually larger than the latter (Figs. 1, 3). The nuclei were not always observable but were evidently smaller in nucleo–cytoplasmic ratio than those of the immature cells. This ratio seemed to be inversely proportional to the number and size of the sebum droplets. These droplets were mostly round to ovoid in shape and approximately 1 μ to 3.5 μ in diameter. The dense inclusions within mitochondria of the sebaceous cells reported by Rogers (14) were seldom found in the present study.

In contrast with the immature cells, tonofibrils were much less conspicuous and their cytoplasm developed more prominently vesicles approximately 60 nm and vacuoles approximately 650 nm in diameter (Figs. 5, 6). The vesicles were of unknown origin and differed from those observed in the fat cells (5, 6), in that they did not form a rosette-like structure and each of them contained one or more small electron-dense granules probably of a glycogen nature, or amorphous substances less electron-dense than glycogen. Each of the vacuoles contained mostly the above amorphous substances, myelin figures or cytoplasmic elements (Figs. 6, 7). Frequently, densely clustered vesicles coalesced, encircled cytoplasmic elements such as mitochondria and seemed to be in the process of developing into vacuoles by an autophagic mechanism (Figs. 5, 8).

Around most of the sebum droplets were numerous satellite-like vesicles or vacuoles containing the various aforementioned structures and some of them, being contiguous with the sebum droplet, appeared to enlarge it by their coalescence with it (Figs. 5, 7, 8). These vesicles and vacuoles seemed to gradually replace the glycogen areas by their increase in number.

The cytoplasm also contained long tubular structures that were fairly straight and seemed to come from the endoplasmic reticulum. They were usually smooth but occasionally rough surfaced (Fig. 9). They contained nearly the same substances as those in the vesicles and vacuoles, some of which might represent the cross sections of the tubular structures. Occasionally they enter directly into the sebum droplet.

Though it was difficult to decide clearly whether or not the sebum droplets were bordered by the unit-membrane, since they were mostly embedded in numerous densely aggregated glycogen granules or free ribosomes, occasional droplets appeared limited by single or multilayered membranes that seemingly came from profiles of the surrounding smooth endoplasmic reticulum, extremely flattened by the compressing effect of expanding sebum droplets (Fig. 10). On the other hand, there were certain forms of sebum droplets that did not appear limited by membrane and derived from the mixed cluster of free ribosomes and glycogen granules or from the glyco-

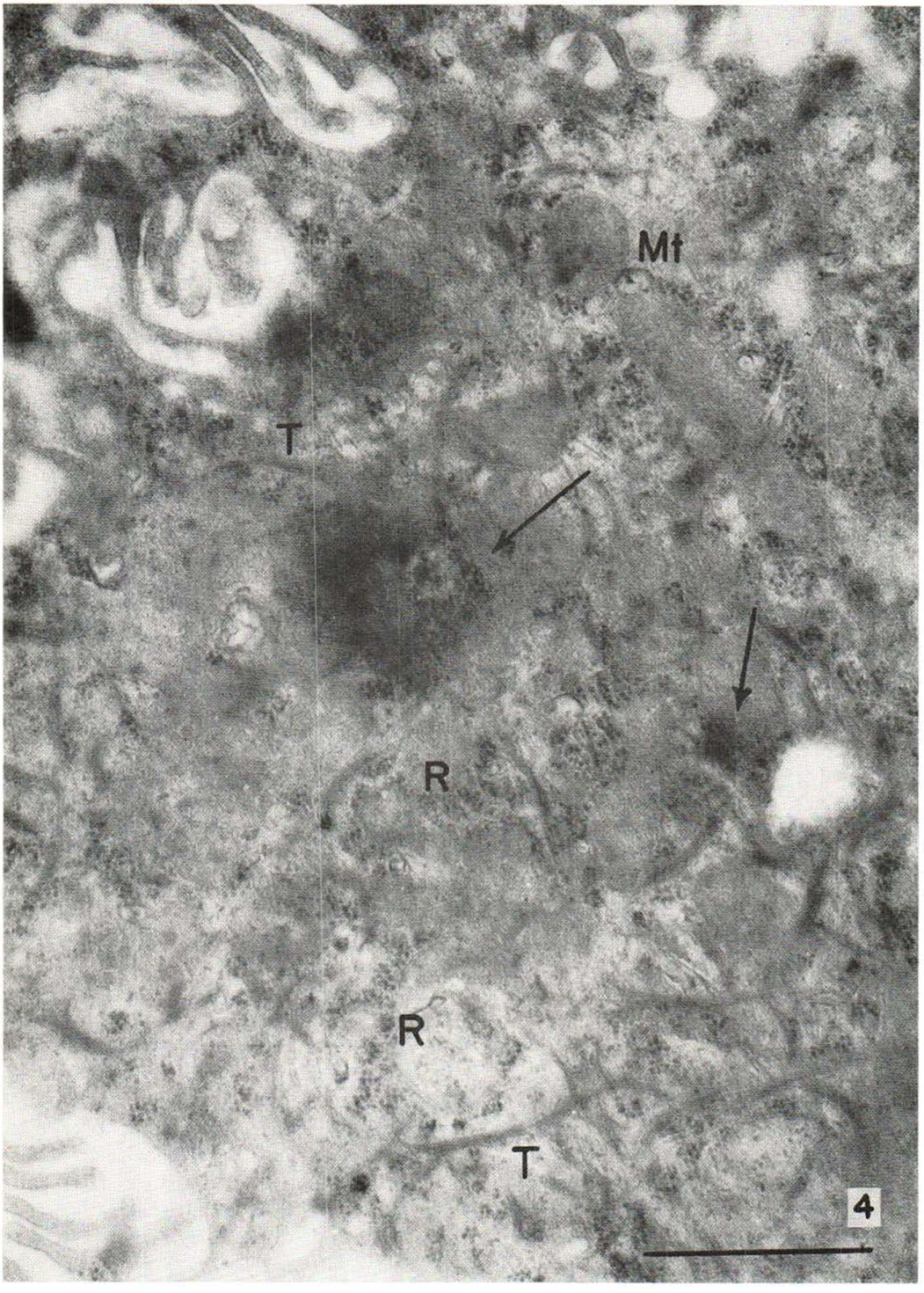

Fig. 4. Cytoplasm of an immature cell. *T*, many tonofibrils; *Mt*, mitochondria; *R*, many clusters of free ribosomes mixed with some glycogen; Arrows indicate amorphous areas under lytic process leading to the transformation of clusters into lipid, losing the granularity and becoming homogeneous in appearance. × 34 800.

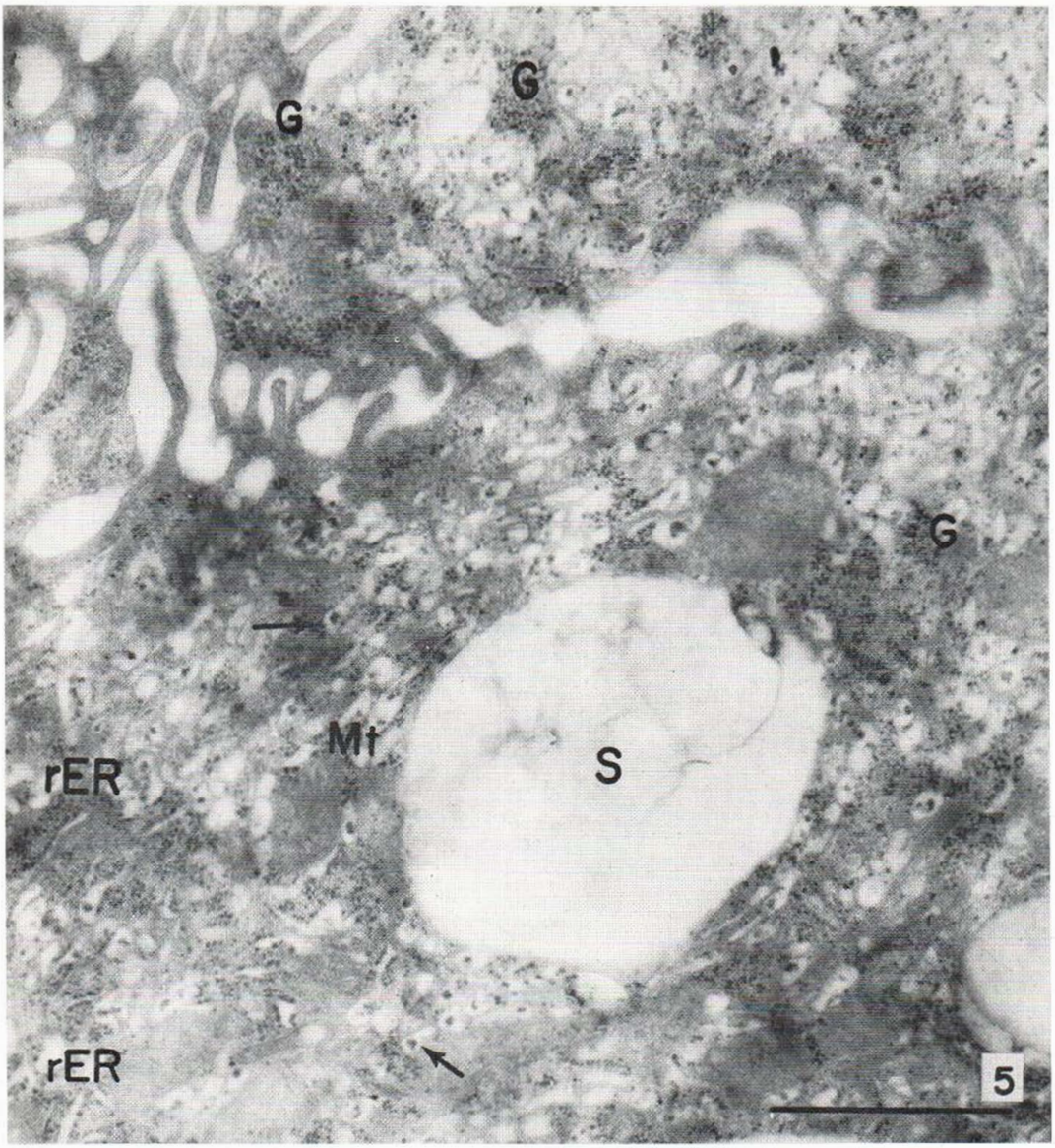

Fig. 5. Prominent vesicles surrounding a sebum droplet in a progressively maturing sebaceous cell. Arrows indicate vesicles containing electron-dense probable glycogen. *G*, cytoplasmic glycogen outside the vesicle; *Mt*, an electron-dense mitochondrion suggestive to be under lytic process, being encircled by many vesicles; *rER*, tubular profiles of rough endoplasmic reticulum. × 32 000.

genosome such as the vacuolar structure and the myelin figure observed in some immature cells (Fig. 10).

There were neither filamentous structures bordering the sebum droplet and orienting parallel to one another with regular spacing, nor a continuous lamella limiting the droplet, both of which were commonly observable in many lipid droplets of fat cells in the same fetal skin (5, 6).

Most of the droplets were completely hollow, probably due to extraction during tissue preparation, while, as reported previously, the lipid droplets of fat cells in the same fetal skin were not so conspicuous in such an extraction, which in-

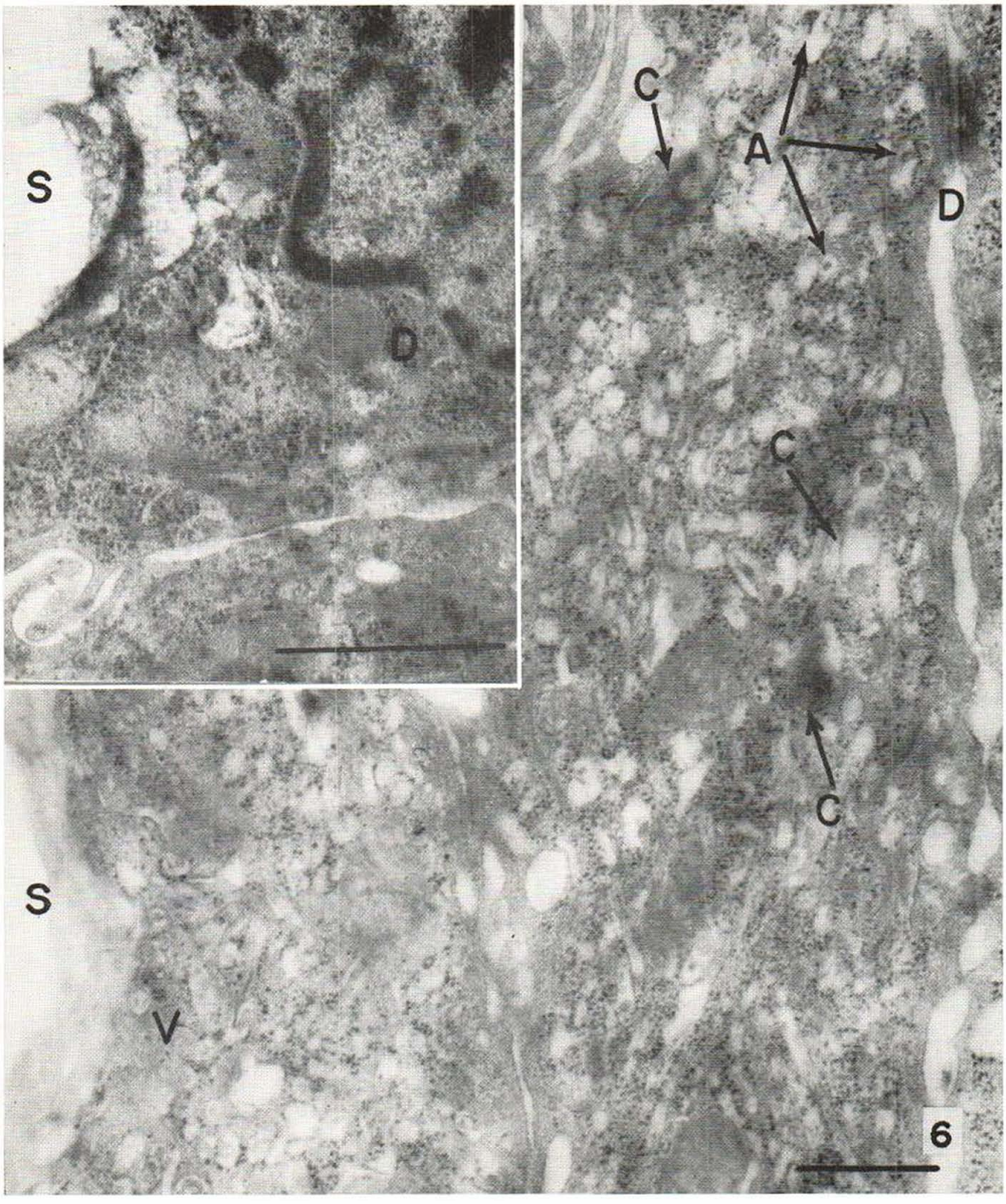

Fig. 6. Prominent vesicles in the cytoplasm of progressively maturing sebaceous cells. Arrows *A* indicate vacuoles containing amorphous substance of a lipid nature possibly transformed from probable glycogen. Arrows *C* indicate vacuoles containing cytoplasmic elements and appear to be in the process of forming a larger one by autophagy mechanism. *S*, a sebum droplet studded with many vesicles (*V*); *D*, desmosome. × 20 000. *Inset: D*, a single, membrane-limited, electron-dense body located near a sebum droplet (*S*). × 32 000.

dicates their different chemical nature. Some droplets contained amorphous substances mostly of low electron density, having a mottled appearance, or cytoplasmic elements reduced to a heavily condensed substance and so suggestive to be under transformation into a lipid (Figs. 7, 8).

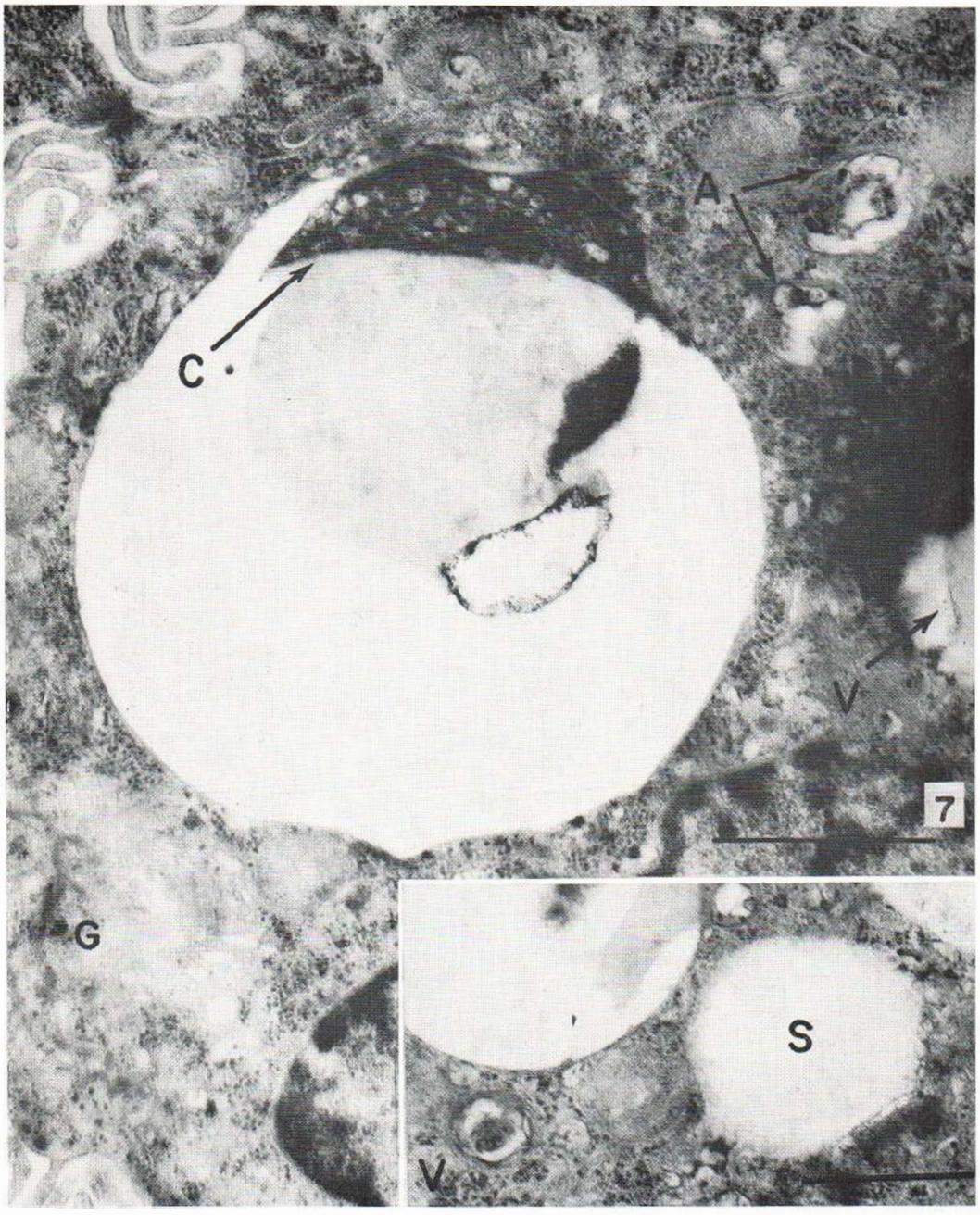

Fig. 7. Sebum droplet of a progressively maturing sebaceous cell. Arrow *C* indicates a cytoplasmic element heavily condensed but still containing many remnants of vesicle and falling into droplet. Arrows *A* indicate vacuoles containing amorphous substance mixed with probable glycogen. Arrow *V* indicates a vacuolar structure occurring within glycogen area and presumably developing into a sebum droplet. *G*, Golgi area. × 32 000. *Inset: S*, sebum droplet with border eroded by the fusion of many vesicles. *V*, a vacuole containing a myelin-figure. × 25 000.

Fully matured cells

The cells of this type were mostly located around the center of acinus and had the cytoplasm occupied by multiple large sebum droplets. They showed an evident increase in total volume compared with the more peripherally located younger

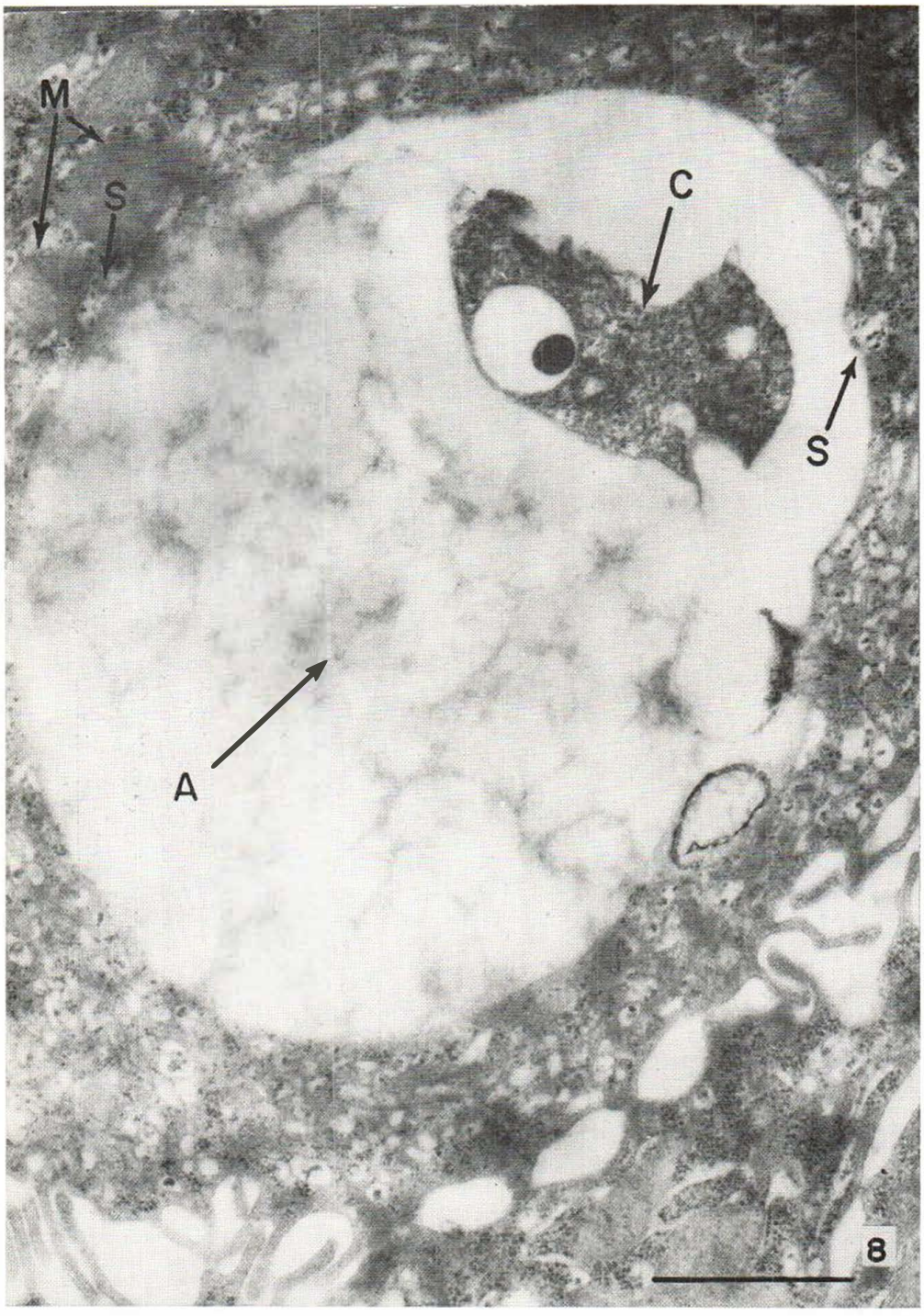

Fig. 8. Sebum droplet in a progressively maturing sebaceous cell. Arrow *C* indicates a cytoplasmic element engulfed by the droplet being separated from the cytoplasm possibly by autophagy. Arrows *S* indicate many satellite-like vesicles surrounding the sebum droplet and containing probable glycogen. Arrow *A* indicates many amorphous substances of a low electron density contained in the sebum droplet. Arrows *M* indicate mitochondria suggestive to be under lytic process, being encircled by many vesicles. × 29 000.

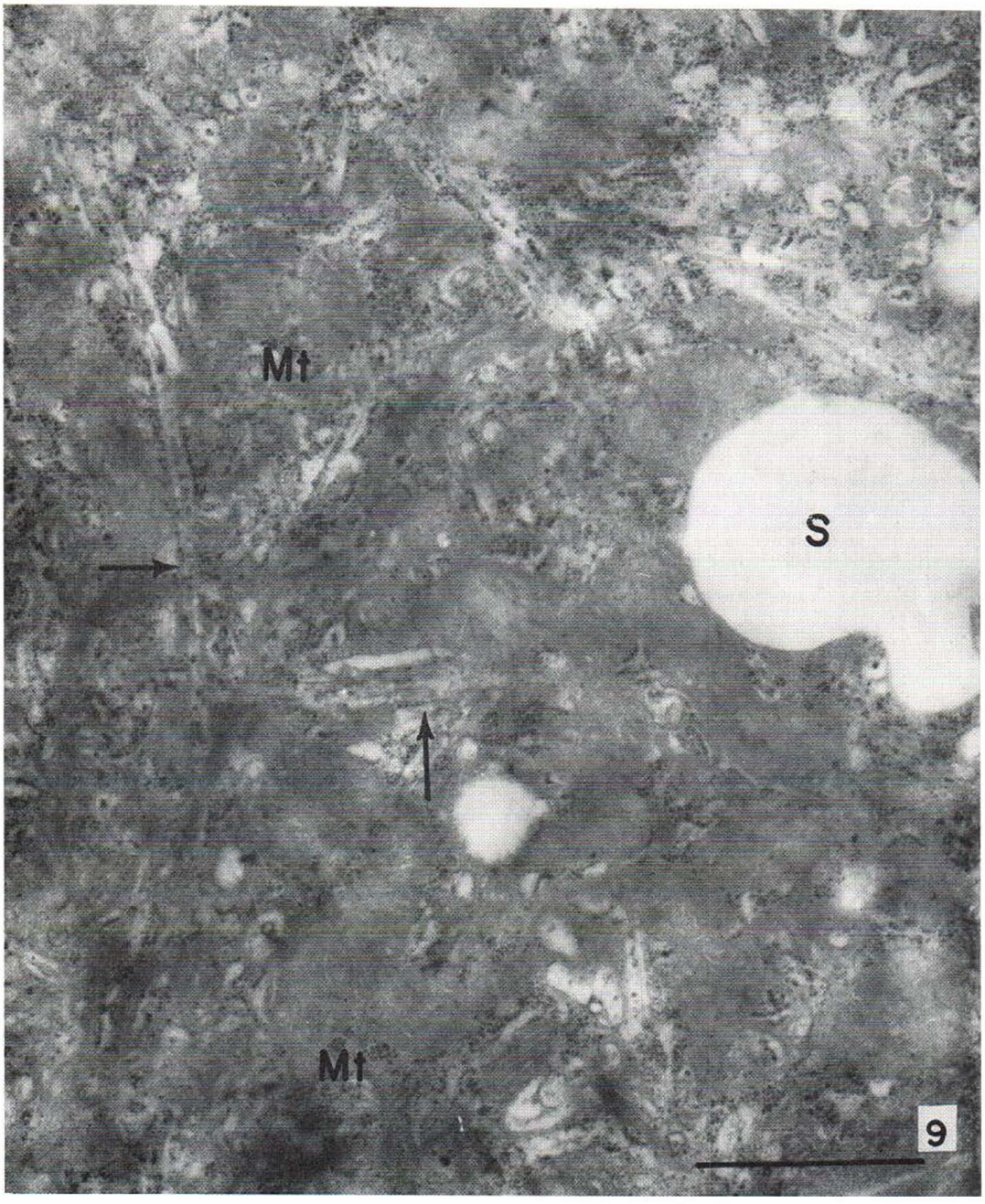

Fig. 9. Tubular structures in a progressively maturing sebaceous cell. *S*, a sebum droplet; Arrows indicate fairly straight long tubular structures presumably coming from the endoplasmic reticulum, being smooth or rough surfaced in places. *Mt*, numerous dense mitochondria. × 23 000.

sebaceous cells. Usually more than ten droplets were packed closely into each cell, having a nearly uniform size 0.5 to 3 μ in diameter, and occasionally showing evidence of fusion between juxtaposed droplets (Fig. 11). The nuclei were mostly small and mis-shapen by crowding sebum droplets having uniformly dark-staining chromatins. Varying numbers of vesicles, vacuoles and

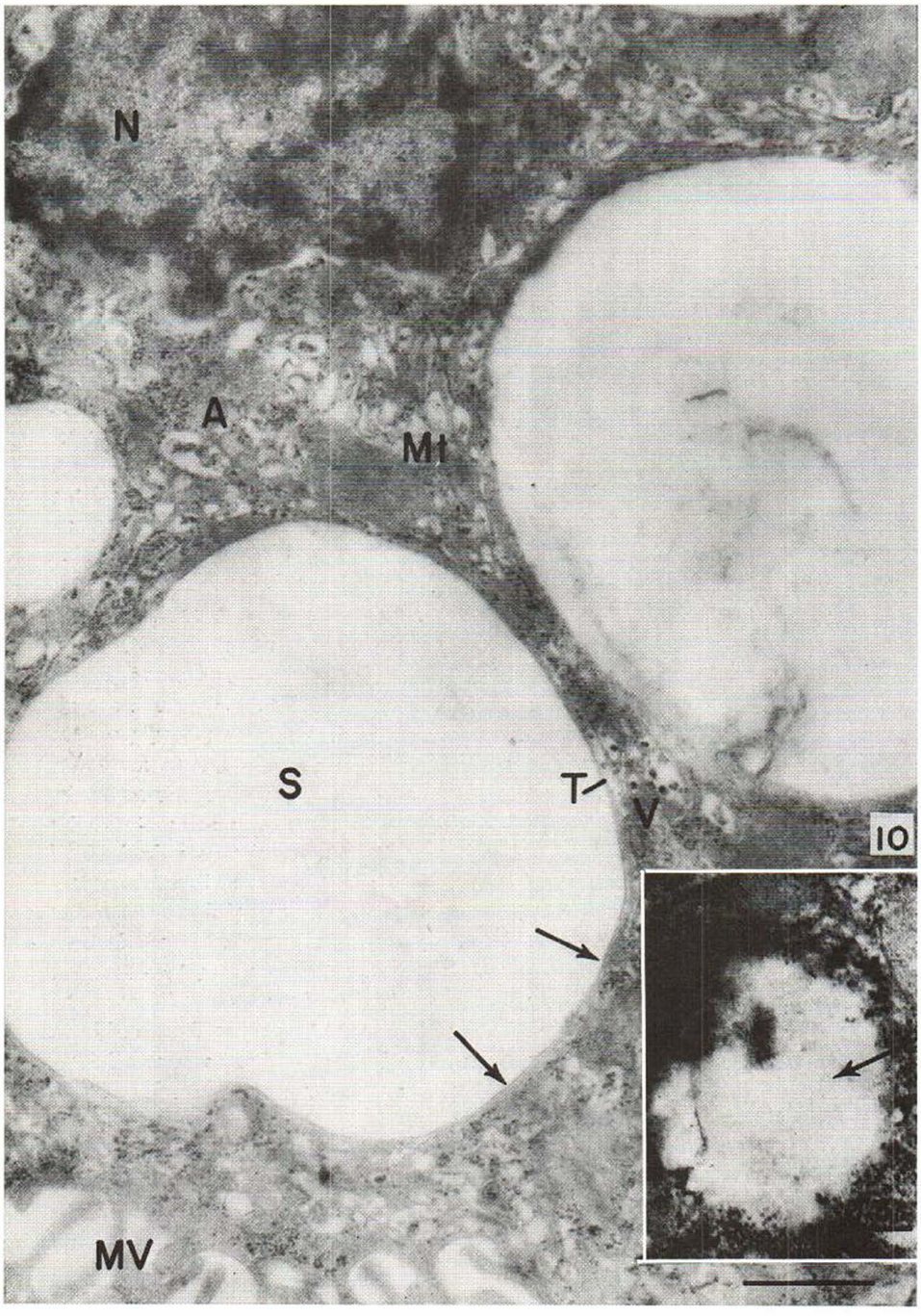

Fig. 10. Sebum droplets in a progressively maturing sebaceous cell. *S*, a droplet encircled by about two layers of membranes (*arrows*) seemingly contiguous, with extremely flattened profiles of smooth endoplasmic reticulum, being surrounded by numerous vesicles (*V*) or tubular structures (*T*) with probable glycogen; *A*, vacuole containing amorphous substances mixed with small amounts of probable glycogen. *Mt*, numerous electron-dense mitochondria; *N*, nucleus; *MV*, microvilli. × 18 000. *Inset:* Sebum droplet within a glycogen area. Arrow indicates a droplet displaying no limiting membrane and possibly representing an advanced form of the vacuolar structure withn glycogen area (marked by arrow in Fig. 1). × 23 400.

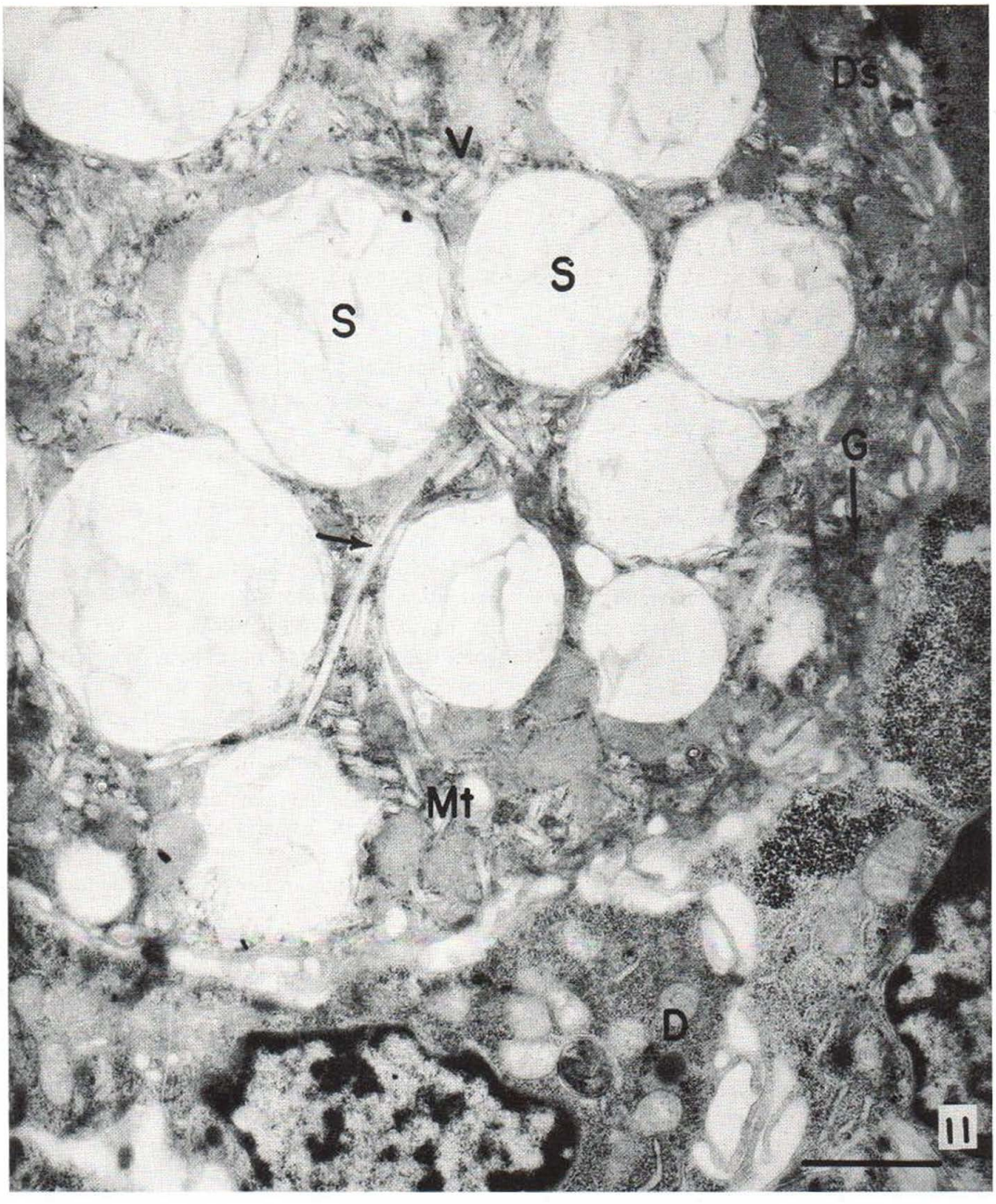

Fig. 11. Portion of an almost mature sebaceous cell. *S*, more than ten sebum droplets packed closely in the cytoplasm; Arrow indicates a tubular structure entering directly into a sebum droplet; *Mt*, fairly numerous dense mitochondria (*Mt*) and vesicles (*V*) and small amounts of probable glycogen (*arrow G*) in the cytoplasm among the sebum droplets; *Ds*, desmosome; *D*, dense body. x 20 000.

tubular or slit-like structures, a few glycogen granules and free ribosomes, and a comparatively small number of dense mitochondria were the dominant components of the cytoplasm among the sebum droplets. Tonofibrils were recognizable, though very few in number. A few desmosomes, but no tight junctions were seen between adjacent plasma membranes.

DISCUSSION

Fujita et al. (1971) in their study of the fat cells in human fetal skin (6) reported that 1) dense bodies, 2) clustered vesicles having a foamy appearance, 3) glycogen areas enclosed by a lamella, 4) vacuolar structures, and 5) myelin figures, were observed fairly numerously in glycogen areas of undifferentiated-type fat cells and that the latter three structures of the five were identical with the glycogenosomes postulated by Philips et al. (13) and by Sasaki (16) to be under lytic process leading to the transformation of glycogen into lipid. The present authors consider that the above five structures play an important role in a precursor stage prior to the inception of lipid droplet formation in the developing fat cells. The present investigation of glycogen areas of immature cells in sebaceous acinei of the same fetal skin did not reveal the structures listed above in 2) and 3), but rather, dense bodies and glycogenosomes such as vacuolar structures and myelin figures identical with those of the above authors. Therefore, analogous to the lipid droplet formation in the developing fat cell, these dense bodies (as well as glycogenosomes and amorphous areas derived from mixed clusters of free ribosomes and glycogen under lytic process) were presumed to be intimately concerned with a precursor stage at the beginning of sebum droplet formation in the immature cell, not as the main route of formation but possibly as a secondary route.

Progressively maturing cells had cytoplasm with more prominently developed vesicles and vacuoles, a small number of which had already appeared in occasional immature cells (Figs. 2, 3). The vesicles were of unknown origin and mostly contained probable glycogen or amorphous substances. The vacuoles contained amorphous substances, myelin figures, or cytoplasmic elements. The amorphous substance inside them was presumed to represent a substance of lipid nature transformed from the probable glycogen, although the relationships between the probable glycogen inside the vesicle and the ergastoplasmic glycogen, and between the former and its own vesicle, have not yet been resolved. The myelin figures and the cytoplasmic elements in the vacuoles were presumed to represent, respectively, some stages of the lytic process leading to the transformation of cytoplasmic elements into lipid. In addition to the vesicles and vacuoles, the cytoplasm often contained long tubular structures possibly coming from the smooth endoplasmic reticulum and occasionally encircling or entering the sebum droplet.

These vesicles, vacuoles and/or tubular structures were often found numerously near to or contiguous with the sebum droplet, and therefore suggestive of enlarging the sebum droplet by fusing with it and discharging their contents. Densely clustered vesicles often coalesced with one another and encircled cytoplasmic elements such as mitochondria were presumed to be in the process of fusing with their neighboring sebum droplet after developing into vacuoles by an autophagic mechanism. Rowden (15), by combined use of the electron microscope and cytochemistry, studied the sebaceous gland of mouse skin, and reported that lysosomes with aryl sulphate activity were demonstrated numerously in the sebaceous cells developing prominent vacuoles. This finding by Rowden appears to support our contention that autophagy may be involved in the formation of the above vacuoles deriving from the coalescence of clustered vesicles and encircling cytoplasmic elements. With the increase of these vesicles, vacuoles and tubular structures, the number of glycogen granules, free ribosomes and tonofibrils (each of which was most abundant in the immature cells) was progressively decreased. Based upon these results, sequences of the major steps in the process of formation and maturation of the sebum droplet are postulated, as depicted in Fig. 12.

The Formation of Sebum Droplets

Main route

The first detectable event that may contribute to the formation of most sebum droplets is a marked increase in vesicles of unknown origin containing probable glycogen granules in the cytoplasm of progressively maturing sebaceous cells. The second event is brought about by 1) the coalescence of these vesicles, resulting in the formation of vacuoles usually containing amorphous substances appearing of a lipid nature and probably transformed from the contained probable glycogen granule or myelin figures from cytoplasmic elements; and 2) the formation of autophagosomal structures such as vacuoles containing cytoplasmic elements and clustered vesicles coalescing with one another and encircling cytoplasmic elements.

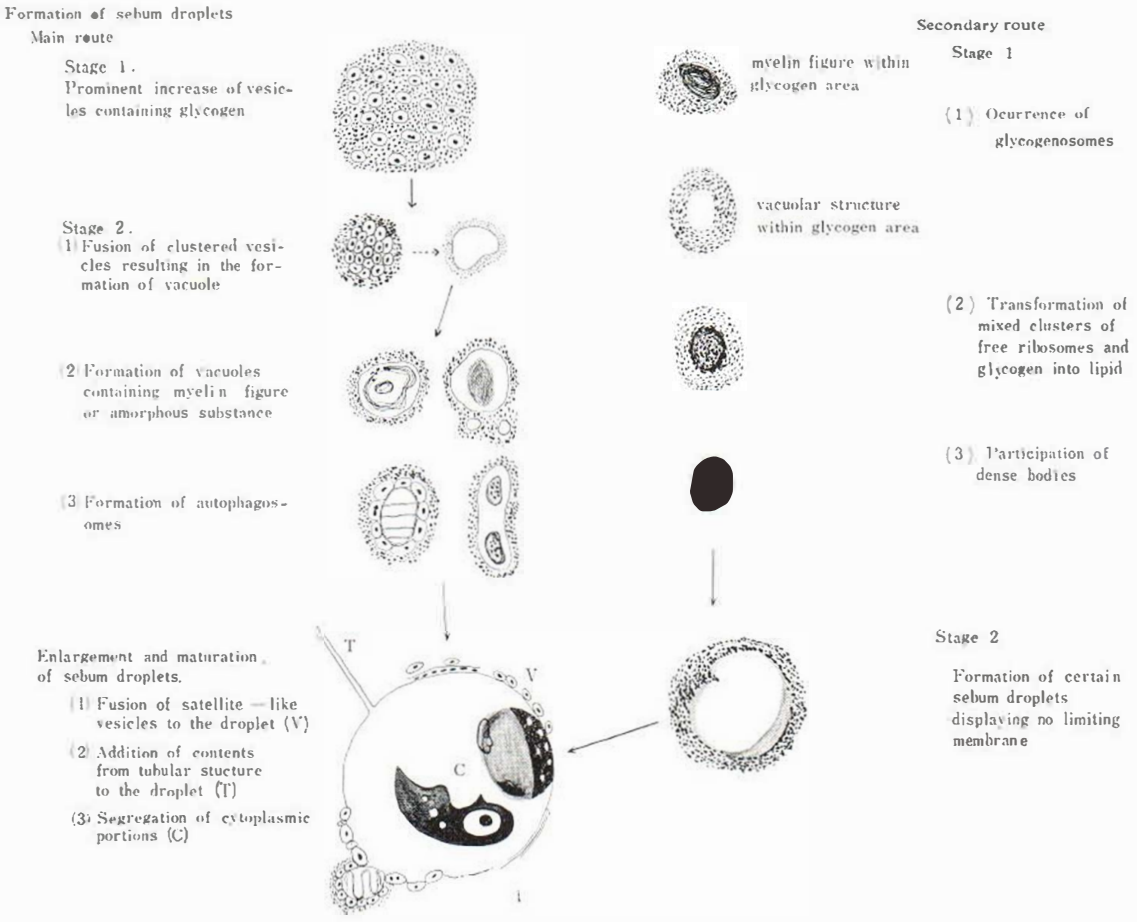

Fig. 12. A postulated sequence of events in formation and maturation of sebum droplets.

Vacuoles of autophagosomal structures formed thus may then develop into the sebum droplet. This transformation is probably caused by a lytic process in the vacuoles.

Secondary route

Besides the above (main) route of sebum droplet formation, there may be another via which certain sebum droplets having no limiting membrane are formed; it appears to be initiated by the lytic process resulting in the formation of glycogenosomes as well as amorphous areas in mixed clusters of free ribosomes and glycogen, and possibly by the participation of neighboring dense bodies in the cytoplasm of immature cells, analogous to the lipid droplet formation in the developing fat cell (6).

The Maturation and Enlargement of Sebum Droplets

The sebum droplets thus newly formed via the main route and also possibly those via the secondary route seem to be matured and enlarged by the following autophagic processes: 1) many satellite-like vesicles to vacuoles adjacent to the sebum droplet fuse with the droplet and discharge their contents into it; 2) the contents derived from the tubular structures are discharged into the sebum droplet by direct contiguousness with it; and 3) some cytoplasmic portions adjacent to the sebum droplet are segregated from the cytoplasm and transformed into a lipid substance after falling into the droplet. Breathnach (1), in describing sebaceous cells in the arm skin of human fetuses 21 weeks (169 mm C–R length) and 24

weeks (215 mm C–R), suggested that in ontogeny, sebum droplets appeared free in the cytoplasm, showing no intimate relationship to any cytoplasmic membrane system, since they could be quite numerous before many vesicles were evident. Most workers, however, were of the opinion that the vesicles represented the smooth endoplasmic reticulum and that the latter was intimately concerned with the synthesis of lipid. Breathnach based his suggestion on the finding that vesicles, either of Golgi or smooth endoplasmic reticular nature, were not apparent in the cytoplasm of fetal sebaceous cells containing a comparatively small number of sebum droplets. He also noted, in the cytoplasm of these sebaceous cells during an early stage of development, that there were certain circular structures consisting of ill-defined wispy whorled material and certain vesicular elements that appeared larger in size than the above vesicles (judging from the micrographs of Breathnach) and mostly containing amorphous substances. From this he proposed that these vesicular elements might represent early stages in sebum droplet formation and also that their appearance and size suggested that they might represent mitochondria in process of being transformed into lipid. In this connection, there had been a postulation by previous investigators such as Kurosumi (9) and Rogers (14) that the sebum droplets form directly in mitochondria. The present study did not reveal any evidence supporting this postulation, although mitochondria were frequently seen in close proximity to the sebum droplet throughout synthesis, even in the fully matured cell. We found, however, in the cytoplasm of progressively maturing sebaceous cells, vacuolar structures that contained amorphous substances, myelin figures or cytoplasmic elements, all of which presumably represented some stages leading to the transformation into lipid, and that some of these structures such as that marked *V* in the inset of Fig. 7 appeared to be somewhat similar to the circular structure of Breathnach, while others such as those marked by arrow *A* in Fig. 7 or by *A* in Fig. 10 look like his vesicular element. This seems to suggest that our postulation concerning the initial stage of sebum droplet formation would find in further study agreement with the idea proposed by Breathnach.

Palay (12) stated that the sebum droplets were bounded by a limiting membrane contiguous with the smooth endoplasmic reticulum. Breathnach, on the contrary, found no evidence that sebum droplets had a limiting membrane. The present investigation did not reveal any source to clearly decide this controversial question. However, from our postulation mentioned above, this question should be examined by dividing sebum droplets into two forms, namely the major form possibly formed via the main route, and the minor form possibly via the secondary. In this investigation we should also pay attention to the fact that some of the 'main route' droplets were apparently limited by single or multilayered membranes seemingly derived from extremely flattened profiles of the surrounding smooth endoplasmic reticulum (Fig. 10), while the 'secondary route' droplets (Fig. 10) representing an advanced form of the amorphous area derived from clusters of free ribosomes and glycogen (Fig. 4) as well as of the glycogenosome (Figs. 1, 2) displayed no limiting membrane.

REFERENCES

1. Breathnach, A. S.: An Atlas of the Ultrastructure of Human Skin, p. 337, Sebaceous Gland. J. & A. Churchill, London, 1971.
2. Cashion, P. D., Skobe, Z. & Nalbandian, J.: Ultrastructural observations on sebaceous glands of the human oral mucosa. J Invest Derm *53:* 208, 1969.
3. Charles, A.: Electron microscopic observations of the human sebaceous gland. J Invest Derm *35:* 31, 1960.
4. Ellis, R. A.: Ultrastructure of Normal and Abnormal Skin. *In* The Sebaceous Glands (ed. A. S. Zelickson), p. 144. Lea & Febiger, Philadelphia, 1967.
5. Fujita, H., Asagami, C., Oda, Y., Mori, T. & Suetomi, Y.: Electron microscopic studies of the differentiation of fat cells in human fetal skin. J Invest Derm *53:* 122, 1969.
6. Fujita, H., Asagami, C., Yamamoto, K. & Murota, S.: Ultrastructural study on lipid droplet formation in human fetal skin fat cells. To be published.
7. Henrikson, R. C.: Glycogen and sebaceous transformation. J Invest Derm *44:* 435, 1965.
8. Hibbs, R. G.: Electron microscopy of human axillary sebaceous glands. J Invest Derm *38:* 329, 1962.
9. Kurosumi, K., Kitamura, T. & Kano, K.: Electron microscopy of the human sebaceous gland. Arch Hist Jap *20:* 235, 1960.
10. Millonig, G.: Advantages of a phosphate buffer for OsO_4 solution in fixation. J Appl Physics *32:* 1637, 1961.
11. Montagna, W.: The Structure and Function of Skin, 2nd ed., p. 268. Academic Press, Inc., New York, 1962.

12. Palay, S. L.: Frontiers of Cytology, p. 305, cit. by R. A. Ellis, 1967. Yale University Press, New Haven, 1958.
13. Philips, M. J., Unakar, N. J., Doormewaard, G. & Steiner, J. W.: Glycogen depletion in the newborn rat liver. An electron microscopic and electron histochemical study. J Ultrastruct Res *18:* 142, 1967.
14. Rogers, G. E.: Electron microscope observations on the structure of sebaceous glands. Exp Cell Res *13:* 517, 1957.
15. Rowden, G.: Aryl sulfatase in the sebaceous glands of mouse skin. A combined electron microscope and cytochemical study. J Invest Derm *51:* 41, 1968.
16. Sasaki, M. & Takeuchi, T.: Electron-histochemical studies on the lysosome system in ascites hepatoma AH 13 cells. Acta Path Jap *19:* 443, 1969.

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H. Fujita, M.D.
Department of Dermatology
Yamaguchi University
School of Medicine
Ube, Yamaguchi-ken
Japan