A COMPARATIVE STUDY OF SEBACEOUS GLAND ULTRASTRUCTURE IN SUBHUMAN PRIMATES

II. Macaques: Crystalline Inclusions in the Sebaceous Cells of *Macaca mulatta, M. nemestrina, M. speciosa, M. fascicularis*

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Sebaceous cells usually contain lysosomes. Since these are most numerous and best developed in mature cells and disappear from those cells that are disintegrating, Brandes et al. (7) have suggested that they are involved in the autolytic phase of holocrine secretion.

Lysosomes in the sebaceous cells of rodents have been described as spherical bodies about 0.2 μ, homogeneously electron-opaque, bounded by single membranes, and containing acid phosphatase and aryl sulfatase (7, 12). In the sebaceous cells of macaques, however, lysosomes have such elaborate and distinct structural features that one can easily trace their formation and ultimate fate. The most distinctive of these features is a high content of crystalline material, which is described in this report.

MATERIALS AND METHODS

Electron Microscopy

Specimens of scalp skin were obtained from adult animals of four species of macaques: *Macaca mulatta* (rhesus), *M. nemestrina* (pigtail) *M. speciosa* (stumptail), and *M. fascicularis* (crab-eating); from rhesus monkeys 75–149 days gestation (total gestation period, 164 days); and from infant rhesus monkeys (3 days and 6 months). Small blocks (1–2 mm^3) were fixed either in ice or at room temperature for 1–2 hr in buffered aldehyde or OsO_4 solutions as previously described (5). Aldehyde-fixed specimens were washed overnight in buffer solutions and postfixed for 2 hr in buffered 1% OsO_4. Standard embedding and staining techniques were applied. Sections were viewed in a Philips 200 electron microscope operating at 60 kv.

Histochemistry

The specimens used for histochemistry were obtained from the scalp of fetal, infant, and adult rhesus monkeys and from adult stumptail and pigtail macaques. They were cut into small blocks (1–2 mm^3) or strips (2 × 2 × 15 mm), fixed in 3.1% or 4.6% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, + 8% sucrose, and washed overnight in 0.1 M cacodylate buffer, pH 7.2, + 8% sucrose. Before histochemical incubation, skin strips were sectioned

at 40 or 80 μ on a freezing microtome. Frozen sections and small blocks were incubated for 30 min in histochemical media for the demonstration of acid phosphatase (10, 2) and thiolacid esterases (6). Controls included tissue incubated in media lacking only substrate and in media to which 0.01 M NaF had been added; for the latter reactions, tissues were preincubated for 15 min in 0.01 M NaF in 0.1 M cacodylate buffer, pH 7.2. After histochemical incubation, tissues were washed for 30 min–1 hr in 0.1 M cacodylate buffer, pH 7.2, + 8% sucrose. They were then dehydrated and embedded as described above. Stained sections were compared with unstained counterparts to ascertain the validity of the deposition and localization of the reaction product.

RESULTS

Crystalline inclusions with lysosomal properties occur in the sebaceous cells of *Macaca mulatta*, *M. speciosa*, *M. nemestrina*, and *M. fascicularis*. These inclusions are found in all sebaceous cells regardless of the stage of differentiation, but they are most numerous in mature and disintegrating cells. They are also present in cells of the glands of fetal rhesus monkeys (*M. mulatta*), even in the earliest stages of sebaceous gland differentiation.

The architecture of the inclusions is similar in the four species (Fig. 1). The largest bodies, usually dispersed throughout the cytoplasm, are ~1.5 μ long. They are irregular masses containing both crystalline and moderately electron-opaque, amorphous material (Figs. 1, 2); the range of periodicities (~55 A to ~130 A) does not vary with their size. The larger inclusions often contain complexes of cuboidal, crystalline masses (Fig. 1), sometimes with different periodicities even when the masses are adjacent to and confluent with each other.

The smaller inclusions are spherical, measure ~0.13 μ, and consist exclusively of crystalline material, the smallest concentrations of which occur in Golgi-associated vesicles (Fig. 2). None of the different fixatives and fixation temperatures affected the morphology of the crystalline inclusions.

In preparations for the demonstration of acid phosphatase activity, the reaction product (lead phosphate) was precipitated on the crystalline inclusions (Figs. 3, 4), in Golgi zones, and in residual sebum adhering to the peripheries of sebum vesicles. Within both small and large inclusions, the precipitate often appeared on the linear arrays of the crystals. The largest inclusions had some crystalline zones that contained no precipitate (Fig. 4), whereas amorphous zones had a heavy deposition of reaction product. Tissues incubated without substrate, or with 0.01 M NaF added to the incubating media, showed no reaction product in the cytoplasm or the crystalline inclusions; some residual product occasionally appeared in the sebum.

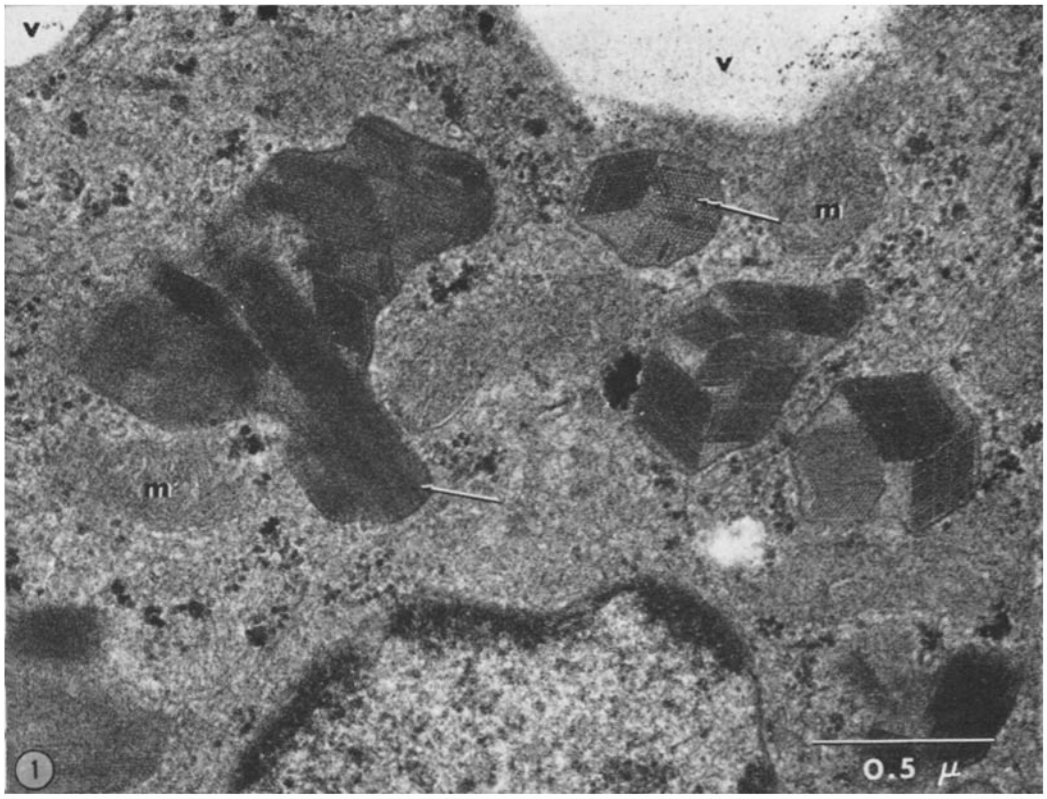
When tissues were incubated for the demonstration of thiolacid esterase activities, heavy deposits of reaction product (lead sulfide) appeared in the crystalline inclusions, the agranular endoplasmic reticulum, the periphery of the sebum vesicles, and the Golgi zones. The crystalline inclusions were intensely reactive when both thiolacetic and thiobutyric acids were used as substrates; the agranular endoplasmic reticulum was more reactive with thiobutyric than with thiolacetic acid. Tissues incubated without substrate showed no precipitate. Deposition of reaction product was not completely suppressed in tissues incubated in media that contained 0.01 M NaF. In these controls, the crystalline inclusions demonstrated considerably less reaction, whereas the reaction in the agranular endoplasmic reticulum was comparable to that in tissues incubated in standard thiolacid-containing media.

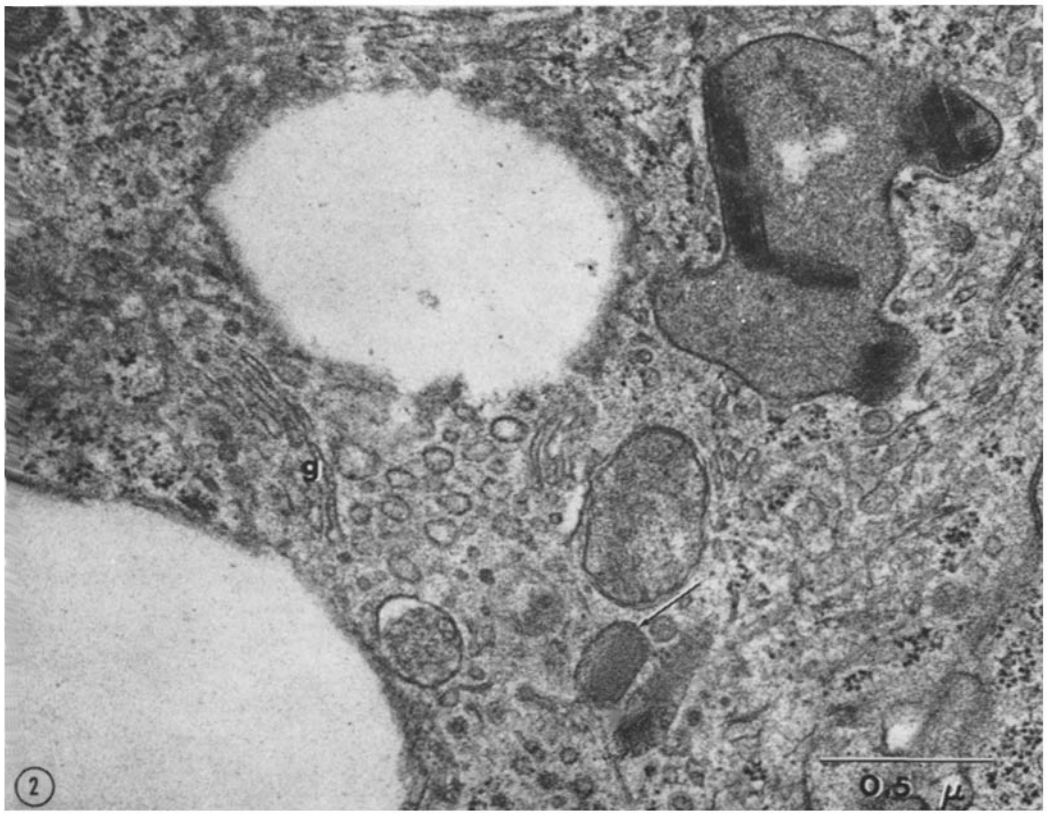
DISCUSSION

The inclusions described here contain acid phosphatase and cathepsin-like enzymes and therefore have lysosomal properties. Since they are most numerous and largest in mature sebaceous cells, they probably function in the autolytic phase of holocrine secretion, as postulated by Brandes et al. (7). Unlike the lysosomes of rodents, which are small and homogeneously electron-opaque (7, 12), those of macaques have a striking crystalline structure.

Inclusions that contain crystalline material are usually categorized as microbodies, but microbodies do not contain acid phosphatase (11). The inclusions described here, therefore, are best considered as lysosomes. They resemble two other distinctive types of lysosomes, the perinuclear granules of the preputial gland of the rat and the specific granules of eosinophils.

Crystalline inclusions have not previously been reported in sebaceous glands, but Beaver (3, 4) and Dangelo and Munger (8) have described complex perinuclear granules in the preputial gland of the rat. These granules vary in size and shape, are bounded by single membranes, and contain faintly granular material; some of them





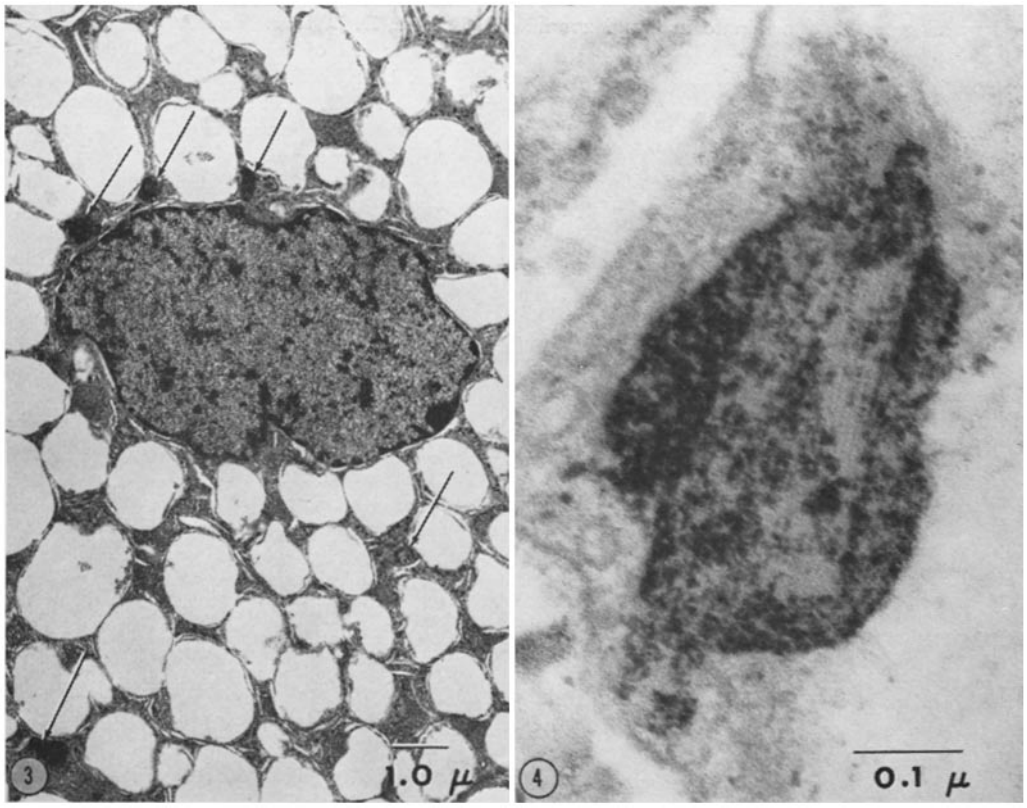


FIGURE 3 An electron micrograph of a sebaceous cell incubated for the demonstration of acid phosphatase. Several inclusions (arrows) have deposits of reaction product (lead phosphate). × 7200.

FIGURE 4 A higher magnification of a crystalline complex demonstrating acid phosphatase activity in a sebaceous cell from a stumptail macaque. Part of the crystalline zone is nonreactive. In more reactive sites, reaction product is precipitated on the linear arrays of the crystalline material. × 150,000.

contain one or more crystalloid masses (8). Preliminary histochemical tests of these granules have demonstrated that some of them contain acid phosphatase activity (Bell, unpublished observation); it is unlikely that they are composed of "keratin" as Dangelo and Munger (8) have suggested.

The crystalline material in the sebaceous inclusions appears morphologically like that in eosinophil granules, which are also bounded by

FIGURE 1 An electron micrograph of part of a sebaceous cell from a pigtail macaque. Portions of two sebum vesicles (*v*) are visible at the top of the field. Several inclusions in this field are complexes composed of both crystalline and amorphous material and bounded by single membranes. The periodicity of the crystalline material varies from ~55 A (single arrow) to ~130 A (double arrow). Mitochondria (*m*) are small in comparison with the crystalline bodies. Fixed in cacodylate-buffered glutaraldehyde, followed by cacodylate-buffered OsO_4. × 48,000.

FIGURE 2 An electron micrograph of part of a sebaceous cell from a pigtail macaque. Small concentrations (arrow) of crystalline material are present in vesicles closely associated with a Golgi zone (*g*). A large complex of crystalline and amorphous material is at the upper right. Fixed in veronal acetate-buffered OsO_4. × 45,000.

single membranes (13), originate in Golgi zones (1, 9), and contain acid phosphatase until they become mature (13). In contrast to eosinophil granules, which have uniform morphology, the bodies in the sebaceous glands have varying dimensions and concentrations of crystalline and amorphous material; in addition, some acid phosphatase remains associated with the crystals even when they have reached full development.

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REFERENCES

1. BAINTON, D. F., and M. G. FARQUHAR. 1967. Segregation and packaging of granule enzymes in eosinophils. *J. Cell Biol.* **35** (2, Pt. 2): 6A (Abstr.)
2. BARKA, T., and P. J. ANDERSON. 1963. *In* Histochemistry, Theory, Practice and Bibliography. Harper & Row, Publishers, New York. 240.
3. BEAVER, D. L. 1960. A re-evaluation of the rat preputial gland as a "dicrine" organ from the standpoint of its morphology, histochemistry and physiology. *J. Exp. Zool.* **143**:153.
4. BEAVER, D. L. 1963. Electron microscopy of the acinar cell of the rat preputial gland. *Anat. Rec.* **146**:47.
5. BELL, M. 1970. A comparative study of sebaceous gland ultrastructure in subhuman primates. *I. Galago crassicaudatus, G. senegalensis* and *G. demidovii. Anat. Rec.* **166**:213.
6. BELL, M., and R. J. BARRNETT. 1965. The use of thiol-substituted carboxylic acids as histochemical substrates. *J. Histochem. Cytochem.* **13**:611.
7. BRANDES, D., F. BERTINI, and E. W. SMITH. 1965. Role of lysosomes in cellular lytic processes. II. Cell death during holocrine secretion in sebaceous cells. *Exp. Mol. Pathol.* **4**:245.
8. DANGELO, J. G., and B. L. MUNGER. 1964. The ultrastructure of the rat preputial gland. *J. Ultrastruct. Res.* **11**:230.
9. FEDORKO, M. E. 1968. Formation of cytoplasmic granules in human eosinophilic myelocytes: an electron microscope autoradiographic study. *Blood.* **31**:188.
10. GOMORI, G. 1950. An improved histochemical technic (sic) for acid phosphatase. *Stain Technol.* **25**:81.
11. HRUBAN, Z., and M. RECHCIGL, JR. 1969. Microbodies and related particles. *Int. Rev. Cytol. (Suppl. 1)*: Ch.1.
12. ROWDEN, G. 1968. Aryl sulfatase in the sebaceous glands of mouse skin. A combined electron microscope and cytochemical study. *J. Invest. Dermatol.* **51**:41.
13. SEEMAN, P. M., and G. E. PALADE. 1967. Acid phosphatase localization in rabbit eosinophils. *J. Cell Biol.* **34**:745.