Brit. J. Ophthal. (1963) **47**, 222.

TARSAL (MEIBOMIAN) GLANDS OF THE RAT*

BY

T. S. LEESON

Department of Anatomy, University of Toronto, Canada

THE tarsal glands first adequately described by Meibomius (1666), are regarded as being enormously developed and complex sebaceous glands. Duke-Elder and Wybar (1961) describe them as consisting of long tubes embedded in the tarsal plate and opening onto the surface at the lid margin. From the main duct, numerous lateral branches lead off into acini which may be single or compound. The acini are filled with glandular epithelium, the peripheral cells being cubical and fat-free and the central cells polygonal in shape and loaded with fat. Each acinus is bounded by a basement membrane and embedded in connective tissue. The secretion is formed by the debris of the central fatty cells, *viz.* the gland is holocrine in type. This description is more extensive but in no way disagrees with that given in the standard textbooks of histology (*e.g.* Maximow and Bloom, 1957; Finerty and Cowdry, 1960; Ham and Leeson, 1961).

To date, no work on the fine structure of these glands has been published and, indeed, there is little information on the fine structure of the ordinary sebaceous glands of the skin. Two papers on this subject have been published by Rogers (1957a, b). The first described the formation of sebaceous glands from the outer root sheaths of developing hair follicles in neonate mice (Rogers, 1957a). In the acini, Rogers distinguished two cell types: the sebaceous cell showing numerous, nearly circular profiles, and numerous mitochondria containing granules; and the non-sebaceous cells, situated at the periphery of the acinus, showing no circular profiles and no mitochondrial granules. In neither cell type was endoplasmic reticulum obvious and Rogers emphasized that, in all differentiated layers of the hair follicle, endoplasmic reticulum is sparse. In his second paper, Rogers (1957b) described the arrangement of collagen fibrils around sebaceous glands and hair follicles in the mature animal. Both the sebaceous and duct cells of the human sebaceus gland have been described by Charles (1960), who made observations on the origin of lipid in these cells. Recently, a more extensive study on human axillary sebaceous glands has been published by Hibbs (1962), in which cells at the base of an acinus and superficial or centrally located cells are described in some detail. Hibbs attempted to correlate the appearance of these cells

* Received for publication September 10, 1962.

as seen with the electron microscope with previously reported light microscope studies. He concluded that the basophilia of sebaceous cells was due to the presence of large numbers of ribonucleoprotein (RNP or Palade) granules which were presumably involved in the synthesis of sebaceous material. However, there are numerous theories concerning the formation of lipid in these glands and the present investigation was undertaken in an attempt to clarify this problem and to establish the fine structure of tarsal glands. A brief note has already been published on the relationship between ribonucleoprotein, cell division, and lipid formation (Leeson, 1962).

Methods

Upper eyelids from seven adult albino rats were excised rapidly after killing the animals with an overdose of ether. One eyelid was fixed in 10 per cent. buffered formalin and prepared for light microscopy. The other was cut into small pieces (1 mm. a side) after removing the skin from the outer surface of the eyelid. Most of this tissue was fixed for 1 hour at 0°C. in 1 per cent. buffered osmium tetroxide, pH 7·4 (Palade, 1952). Pre- and post-fixation with 10 per cent. buffered formalin at pH 7·0 was used also but did not improve the results. After rapid dehydration through graded ethanols, most of the tissue was infiltrated and embedded in a methacrylate mixture (12·5 per cent. methyl: 97·5 per cent n-butyl), containing 1 per cent. benzoyl peroxide as catalyst, and polymerized overnight at 60°C. The rest of the tissue was infiltrated and embedded in a Selectron mixture (Low and Clevenger, 1962). Ultra-thin sections were cut on a Cambridge ultramicrotome (Huxley pattern) and examined in a Philips 100B/15 electron microscope. Sections were stained as required with 1 per cent. uranyl acetate, 1 per cent. phosphotungstic acid, 1 per cent. potassium permanganate (Lawn, 1960), and lead hydroxide (Watson, 1958). For light microscopy, sections were cut at 6μ and stained by various standard methods.

Results

Light Microscopy.—The tarsal glands of the rat lie embedded in the conjunctival side of the tarsal plate and are arranged in a single row with their ducts opening at the lid margin (Fig. 1, overleaf). The main duct of a gland is widely patent and is lined by a cubical or squamous epithelium of two or more layers with epithelial debris at the surface. The cytoplasm of these cells is basophilic. Numerous side branches of the main duct open into sebaceous acini (Figs 1 and 2). The acini are limited by a periodic acid Schiff-positive basement membrane, supported by relatively dense fibroconnective tissue in which numerous small blood capillaries and small nerves are present. The basal (peripheral) cells of an acinus are cubical or much flattened, with intensely basophilic cystoplasm and elongated nuclei. In this layer of cells, mitotic figures are often apparent. In cells adjacent to the basal layer ("second-layer" cells), the nuclei are spherical and stain darkly. The cytoplasm, whilst still basophilic, has a speckled or foamy appearance due to the presence of lipid. The more central cells in the acinus are polygonal in shape, often

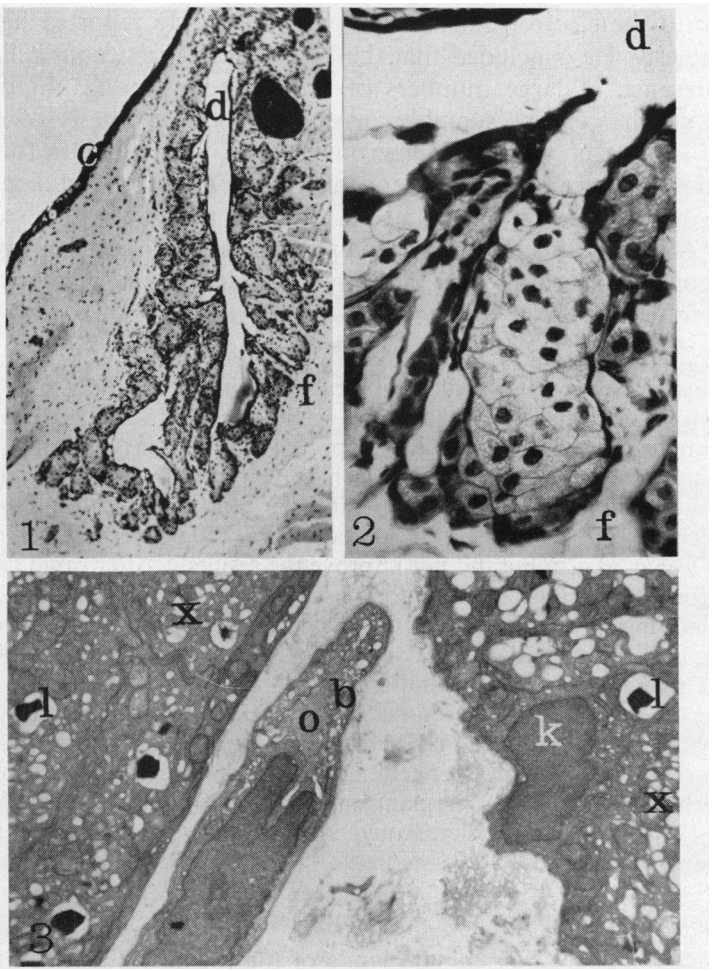

FIG. 1.—Photomicrograph of 6μ section stained with haematoxylin and eosin. The conjunctiva (c) and dense fibrous tissue (f) of the tarsal plate are shown with the main duct (d) lying vertical in the section. Note the side branches of this duct and numerous acini. ×116.

FIG. 2.—Higher power of one acinus from the above. ×560.

FIG. 3.—Small capillary with lumen (o), lined by attenuated endothelial cytoplasm (b), lying between the bases of two acini. Cells in the acini contain lipid (l) and vacuoles (x). The nucleus (k) of a basal cell is seen. ×5,333.

with irregular pyknotic nuclei, and have an eosinophilic foamy cytoplasm because of the presence of many lipid droplets.

Electron Microscopy.—Low-power survey micrographs (Figs 3, 4, 5) confirm the light microscope findings. The supporting tissue of an acinus is composed of irregularly arranged, densely-packed unit fibrils of collagen containing slips of fibroblast cytoplasm, small blood capillaries (Fig. 3), and

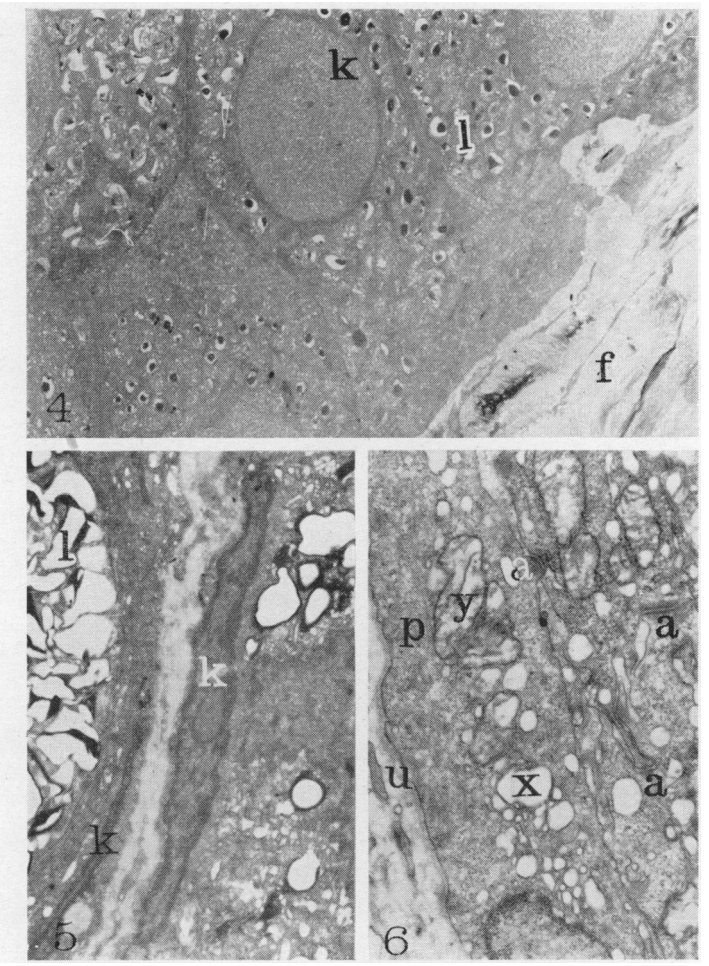

FIG. 4.—Base of one acinus embedded in dense fibrous tissue (f). ×3,000.

FIG. 5.—Bases of two adjacent acini with elongated nuclei (k) of basal cells. ×7,000.

FIG. 6.—Base of one acinus. The basal cell contains mitochondria (y), vacuoles (x), and many free RNP granules (p). Between cells of the basal and "second" layers and adjacent cells of the second layer are desmosomes (a). An extracellular basement membrane (u) limits the acinus. ×18,000.

small nerves (Fig. 9). Often, extravasated eosinophilic leukocytes are found in this fibro-connective tissue and, occasionally, mast cells. The basal cells of the acinus are limited by a homogeneous, electron-dense, extracellular basement membrane (Figs 6, 8, 9). They are usually flattened with elongated nuclei and in the cytoplasm are numerous mitochondria and many Palade (RNP) granules scattered singly and in small rosettes (Figs 6, 8, 9). Endoplasmic reticulum is poorly developed or not identifiable. Elements of the

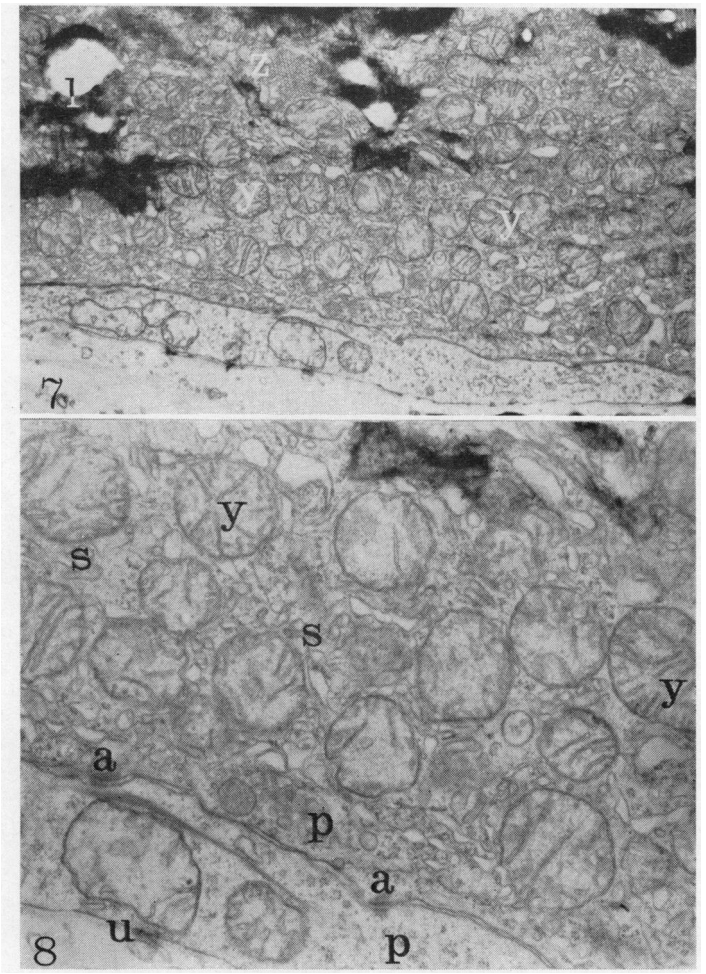

FIG. 7.—Part of one basal and one second-layer cell. In the latter, mitochondria (y) are numerous and lipid (l) is present. Note the geometric, membranous figure (z). (Selectron embedded, lead stained.) ×8,333.

FIG. 8.—An enlargement of part of the above. Note desmosomes (a) and many smooth-surfaced vesicles and tubules (s). ×20,000.

Golgi apparatus are small and few in number or totally absent, as reported by previous investigators (Rogers, 1957a, b; Hibbs, 1962).

Small lipid droplets are occasionally seen in these cells, and, in tissue embedded in methacrylate, small vesicles are obvious (Figs 3, 4, 6). The latter are not found in Selectron-embedded tissue (Figs 7, 8, 9).

In "second-layer" cells, nuclei are spherical or ovoid with obvious nucleoli (Fig. 4). The cytoplasm of these cells closely resembles that of cells of the basal layer, but the lipid droplets are larger and more numerous (Figs 4, 5, 7, 10) and vesicles too are more obvious. In Selectron-embedded tissue, the cells

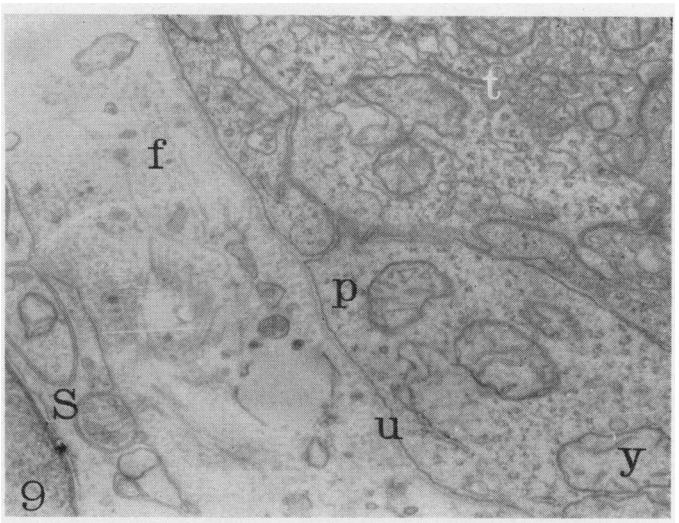

FIG. 9.—Base of an acinus. One second-layer cell contains a Golgi complex (t). Part of a Schwann cell with nerve axons is seen lower left (S). (Selectron embedded, lead stained). × 20,000.

of the second layer differ from those of the basal layer quite dramatically. They contain more mitochondria and numerous small vesicles and tubules which are smooth-surfaced. In some areas, these smooth-surfaced membranous elements form small collections identifiable as parts of the Golgi apparatus (Figs 8 and 9). Like the basal cells, the cells of the second layer contain numerous RNP granules unassociated with membranes. Between adjacent cells of this layer and between cells of the two outer layers, desmosomes are apparent (Figs 6, 8, 9). In the central cells of an acinus, the nuclei are irregular in outline, often show clumping of chromatin, and many are frankly degenerate. The cytoplasm too looks degenerate with numerous irregular vacuoles, some completely empty, others containing irregular masses of intensely osmiophilic lipid (Figs 10, 11, 12). (Much of the lipid is extracted by the preparative techniques.) Between these irregular vacuoles and lipid droplets, smaller vesicles and mitochondria are present, the latter often distended and occasionally with an incomplete outline. In grossly degenerate cells (Figs 12 and 13) and, sometimes, in others (Fig. 7), interesting membranous whorls and geometric patterns of membranes are seen. Plasma membranes of degenerate central acinar cells often are difficult to identify.

Cells lining the duct of a tarsal gland are arranged in two or more layers (Fig. 14), and they merge at the mouth of an acinus with the basal cells of that acinus. Nuclei are irregularly ovoid, usually without visible nucleoli. In the cytoplasm, a few mitochondria with small, sparse cristae, some RNP granules, both free and associated with membranes of the endoplasmic reticulum, and a few small elements of the Golgi apparatus are seen. Characteristically, desmosomes are numerous on the interfaces between duct cells. The luminal

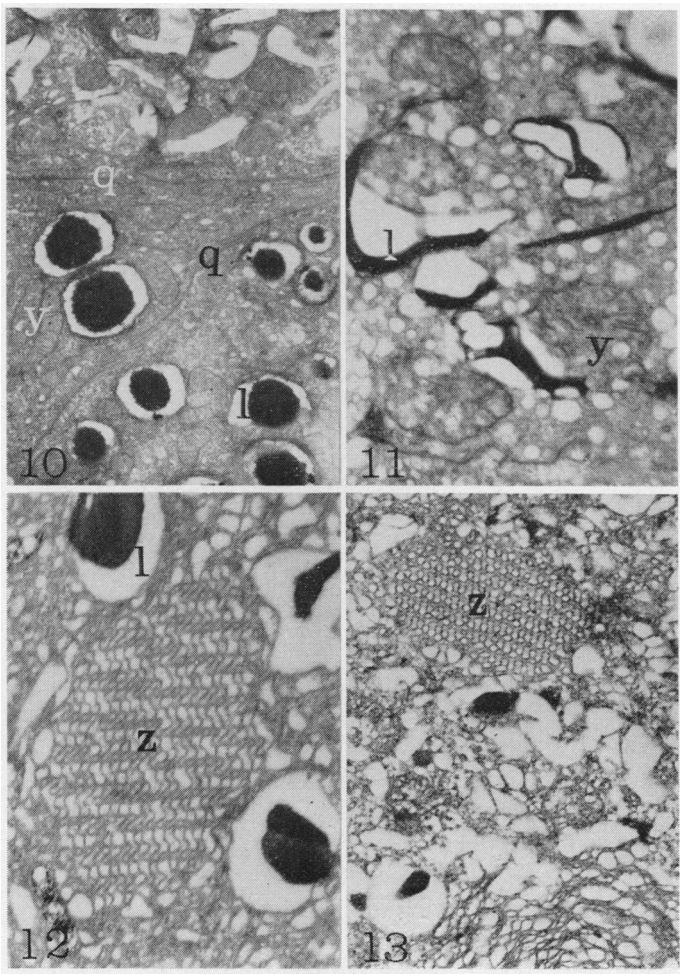

FIG. 10.—Junction of two second-layer cells (below) and a more central degenerate cell (above). Plasma membranes (q) and mitochondria (y) are intact. ×8,333.

FIG. 11.—Part of a central degenerate cell. ×16,666.

FIG. 12.—Geometric membranous figure (z) in a central cell. ×25,000.

FIG. 13.—Another geometric figure (z) in a more completely degenerated cell. ×12,333. (Stained with potassium permanganate.)

cells of the duct are much flattened and are covered by membranous squames of cornified material (Fig. 14, opposite).

Discussion

This investigation confirms the description of the tarsal glands given by Duke-Elder and Wybar (1961), based on light microscopy. As indicated previously regarding the ordinary sebaceous glands of skin (Hibbs, 1962), the basophilia of the basal cells in an acinus is due to the enormous number of

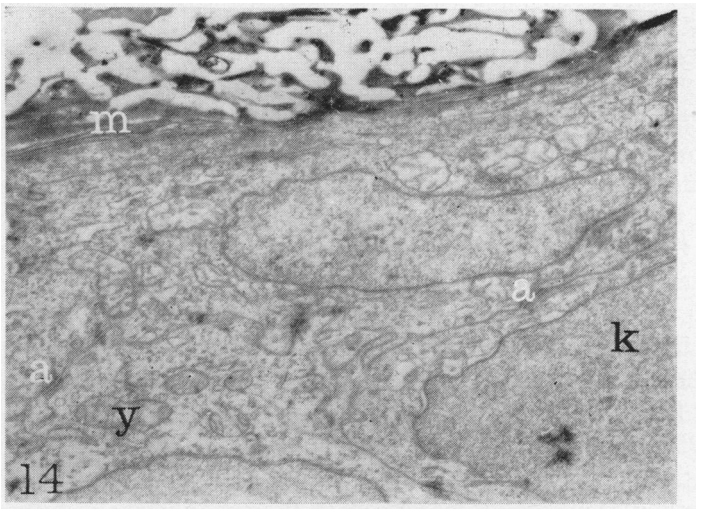

FIG. 14.—Part of the wall of a main duct. Note keratinized squames (m) on luminal surface. (Selectron embedded.) ×10,000.

RNP granules. This at once raises the problem of the formation of lipid in these glands. Sebum secreted by the glands is rich in fat, fatty acids, and cholesterol, and differs in composition from other body lipid (Schmidt-Nielsen, Suskind, and Taylor, 1951). For some time it has been accepted that there is a progressive centripetal loss of basophilic substance (RNP) in the cells of an acinus, the amount of RNP being inversely proportional to the amount of lipid, which increases as cells pass to the centre of an acinus (Montagna, 1956). Thus, it was suggested that ribonucleoprotein is involved in lipid formation, a suggestion which recently received support from Hibbs (1962) working with the electron microscope. However, Hibbs also suggests that, whilst RNP granules are involved in lipid formation, it is probable that lipid droplets originate in Golgi vesicles, although he found it surprisingly difficult to locate Golgi material in his electron-microscopic preparations. That mitochondria may be involved in the production of lipid has been suspected for some time (Nicolas, Regaud, and Favre, 1914) and this theory received some support recently from Rogers (1957a). Montagna, Chase, and Lobitz (1952) found glycogen in the sebaceous glands of the skin and suggested that sebaceous lipids are accumulated principally by a conversion of glycogen, although previous workers (Lombardo, 1907; Sasakawa, 1921) had found no glycogen in this site. During this investigation, both methacrylate and Selectron sections have been stained with lead hydroxide for periods of 2-5 minutes after the method of Revel, Napolitano, and Fawcett (1960) but no glycogen was demonstrable in the sebaceous cells of the tarsal gland. Staining with lead hydroxide for brief and long periods (20 to 60 min.), however, emphasizes the large number of RNP granules in the basal layers of these acini (Figs 8 and 9). One investigator, again with respect to the skin

sebaceous gland, has suggested that lipid droplets do not arise within cytoplasmic membranes, nor in mitochondria, but appear directly in the cytoplasm (Charles, 1960). This investigation does support his statement that mitochondria probably are not involved in lipid formation. Finally, Melczer and Deme (1942) have suggested that sebaceous material is formed by an accumulation of "haemoconia" from the blood. Obviously, material involved in lipid formation must be transported by the blood to the secretory cells and the richness of the capillary bed around acini of the tarsal glands is impressive. It is worth noting here that the attenuated endothelium of these capillaries contains many small micropinocytotic vesicles (Fig. 3), indicative of active fluid transport between the blood and the surrounding cells.

Before further discussion of the role of RNP granules in lipid formation, the other functions of these tarsal gland cells should be considered. Bertalanffy (1957), using the colchicine technique, has shown that all cells of the sebaceous gland are renewed within 8 days and that the vast majority of the cells undergoing mitosis are basal cells. Presumably, the mitotic rate of the tarsal gland is similar. Howatson and Ham (1955), working with malignant cells, and Leeson (1960), working with embryonic cells, have correlated the presence of free cytoplasmic RNP granules with a capacity, in these immature, relatively undifferentiated cells, for growth and cell division. (It is generally accepted, of course, that RNP granules attached to membranes of the endoplasmic reticulum are associated with protein synthesis, *e.g.* of zymogen in pancreatic acinar cells (Caro, 1961).) It seems reasonable, therefore, to suggest that the presence of large numbers of free RNP granules within the cytoplasm of basal cells in tarsal gland acini is associated with a capacity for cell growth, differentiation, and division. However, lipid formation may involve both Golgi membranes and RNP granules, as suggested by Hibbs (1962), and certainly Golgi membranes are demonstrable in Selectron-embedded tissue, particularly in the "second-layer"cells (Figs 8 and 9). It is in these cells that lipid first appears in any quantity.

Studies of holocrine glands such as the tarsal gland provide some information regarding the normal process of cell death and degeneration. Even in the central degenerate cells, nuclei are identifiable, mitochondria are present and often appear normal (Figs 10 and 11), and plasma membranes are intact. However study of the various organelles is made difficult by the presence within the cytoplasm of enormous quantities of lipid, which factor also contributes to the relatively poor quality of the fixation in these cells. In the tarsal gland, it is obvious that degenerative changes are present in all but the most basal cells, *i.e.* in those which are still capable of cell division. It is concluded, therefore, that once a cell has differentiated to the point where capability for cell division is lost, then degeneration soon occurs.

It has been reported that the tarsal glands of rabbits and guinea-pigs may be emptied forcibly by a subcutaneous injection of physostigmine (Buschke and Fränkel, 1905), and that their secretion is increased after section of the

cervical sympathetic. Numerous small nerves between the acini have been seen in thin sections (Fig. 9), and it is interesting to postulate their relation to secretion by the glands. The degree to which these modified sebaceous glands are developed is most impressive, and one can only conclude that their function in preventing adhesion of the lids and loss of tears from the conjunctival sac is an important one. Finally, the compactness of the fibroconnective tissue in which the tarsal glands are embedded is an obvious factor in the common pathology of the glands, *i.e.* blockage and infection. No regular arrangement in the layers of collagen fibrils was found, such as was described around hair follicles by Rogers (1957b).

Summary

Tarsal glands, when examined with the electron microscope, have a similar appearance to sebaceous glands. The possible mechanism of lipid formation in these glands is discussed and it is suggested that both RNP granules and Golgi membranes are involved. However, the majority of the RNP granules are probably associated with the capacity of the relatively immature basal cells for cell differentiation and division.

This investigation was aided by a grant from the Medical Research Council of Canada.

N.B. All illustrations are electron micrographs of material embedded in methacrylate unless otherwise stated.

REFERENCES

BERTALANFFY, F. D. (1957). *Anat. Rec.*, **129**, 231.
BUSCHKE, A., and FRÄNKEL, A. (1905). *Berl. klin. Wschr.*, **42**, 318.
CARO, L. G. (1961). *J. biophys. biochem. Cytol.*, **10**, 37.
CHARLES, A. (1960). *J. invest. Derm.*, **35**, 31.
DUKE-ELDER, S., and WYBAR, K. C. (1961). "System of Ophthalmology". vol. 2, "The Anatomy of the Visual System", p. 519. Kimpton, London.
FINERTY, J. C., and COWDRY, E. V. (1960). "A Textbook of Histology", 5th ed., p. 510. Lea and Febiger, Philadelphia.
HAM, A. W., and LEESON, T. S. (1961). "Histology", 4th ed., p. 905. Lippincott, Philadelphia.
HIBBS, R. G. (1962). *J. invest. Derm.*, **38**, 329.
HOWATSON, A. F., and HAM, A. W. (1955). *Cancer Res.*, **15**, 62.
LAWN, A. M. (1960). *J. biophys. biochem. Cytol.*, **7**, 197.
LEESON, T. S. (1960). *Norelco Reporter (N.Y.)*, **7**, 45.
——— (1962). *Exp. Cell Res.*, (in press).
LOMBARDO, C. (1907). *G. ital. Mal. vener.*, **48**, 292.
LOW, F. N., and CLEVENGER, M. R. (1962). *J. Cell. Biol.*, **12**, 615.
MAXIMOW, A. A., and BLOOM, W. (1957). "A Textbook of Histology", 7th ed., p. 579. Saunders, Philadelphia.
MEIBOMIUS, H. (1666). "De vasis palpebrarum novis epistola". Muller, Helmstadt.
MELCZER, N., and DEME, S. (1942). *Dermatologica (Basel)*, **86**, 24.
MONTAGNA, W. (1956). "The Structure and Function of Skin", p. 241. Academic Press, New York.
———, CHASE, H. B., and LOBITZ, W. C. (1952). *Anat. Rec.*, **114**, 231.
NICOLAS, J., REGAUD, C., and FAVRE, M. (1914). "17th int. Congr. Med. London, 1913. Sect. 13: Dermatology and Syphilography", Part 2, p. 101.
PALADE, G. E. (1952). *J. exp. Med.*, **95**, 285.
REVEL, J. P., NAPOLITANO, L., and FAWCETT, D. W. (1960). *J. biophys. biochem. Cytol.*, **8**, 575.
ROGERS, G. E. (1957a). *Exp. Cell Res.*, **13**, 517.
——— (1957b). *Ibid.*, **13**, 521.
SASAKAWA, M. (1921). *Arch. Derm. Syph. (Berl.)*, **134**, 418.
SCHMIDT-NIELSEN, K., SUSKIND, R. R., and TAYLOR, E. (1951). *J. invest. Derm.*, **17**, 281.
WATSON, M. L. (1958). *J. biophys. biochem. Cytol.*, **4**, 727.