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EFFECT OF ANDROGEN ON THE ULTRASTRUCTURE OF THE SEBACEOUS GLAND IN TWO SPECIES*

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The discovery of specific effects produced on a given tissue by a biochemical molecule provides a potentially important base for further studies. Thus the finding that some hormones produce various effects on sebaceous glands adds another dimension to the growing store of information about these glands. Among the hormones from the anterior pituitary, thyroid, adrenal cortex, and gonads reported to affect the structure and function of sebaceous glands, androgens clearly are the most potent (Ebling, 1957; Strauss and Pochi, 1963). These effects vary according to the types and sites of the glands. In most species, many glands, especially those of the general body skin, are androgen sensitive and increase in size, and the individual cells become larger. The glands are slightly larger in males than in females. When androgen sources are removed, the glands become somewhat, but not substantially, smaller.

Some specialized glands are also androgen dependent: they are larger in males than in females but decrease substantially in size when their sources of androgen are depleted. When the costovertebral (CV) organs of hamsters, large aggregates of sebaceous glands associated with coarse hair follicles and numerous pigment cells (Hamilton and Montagna, 1950), are deprived of testicular androgens, their levels of several enzymes, particularly glycolytic enzymes, are significantly altered (Adachi and Takayasu, 1972). The ultrastructure of these sebaceous cells is profoundly altered by androgen stimulation and depletion, and some of the ultrastructural changes that occur during stimulation are reported here.

The sebaceous glands of the general body surface of *Galago crassicaudatus* (the greater bushbaby) are an example of an androgen-stimulated rather than androgen-dependent system. The size of the glands does not decrease significantly after castration. However, androgen stimulation induces unique patterns of cytoplasmic filaments in their cells (Bell, 1971b); these formations can be detected with certainty only with the electron microscope. The structures in these cells will be described in detail as an example of specific cellular change in an androgen-stimulated system.

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MATERIALS AND METHODS

Costovertebral Glands of Hamsters

Costovertebral (CV) glands were obtained from (1) normal male and female hamsters, (2) both sexes at 5, 12, 13, 19, and 31 days after gonadectomy, and (3) animals, gonadectomized, then injected 54 days later with 8 mg testosterone propionate in 0.4 ml sesame oil and sacrificed after 1 week. The tissues were cut into tissue blocks 1 × 2 × 2 mm, fixed overnight in 2% paraformaldehyde–2.5% glutaraldehyde buffered with 0.03 M phosphate buffer,† washed overnight in 0.16 M phosphate buffer, postfixed in phosphate-buffered 1% OsO_4, dehydrated through an ascending series of ethanols to propylene oxide, and embedded in Araldite (Luft, 1961) or according to Spurr (1969). Sections were cut on a Porter–Blum MT-2 ultramicrotome and viewed in a Philips 200 electron microscope operating at 60 KV. For light microscopy, sections (1 μ) were dried on slides and stained with 1% toluidine blue–1% borax.

Sebaceous Glands of Galago crassicaudatus

Biopsy specimens of the scalp and back skin of six brown males and one black (melanotic) male were removed at the time of castration. Three months later, four of the brown males and the melanotic male were injected daily for 5 weeks with 2 mg testosterone propionate in 0.1 ml sesame oil; the other two brown males were used as controls and injected with 0.1 ml sesame oil only. More biopsy specimens were removed from the back skin of each animal 1, 2, and 5 weeks after the start of androgen treatment. Three months later, additional biopsy specimens were taken from the back; then four brown males, two of which had been controls in the earlier experiment, and the melanotic animal were similarly injected with testosterone propionate daily for 2 weeks. The remaining two brown animals, which had been androgen injected in the first test, served as controls in this second experiment. Biopsy specimens were obtained from the backs of all animals at the end of the 2-week androgen period. The animals were then kept 6 months without androgen and later rebiopsied.

Pieces of the back skin were removed from three spayed females, the animals were injected daily with testosterone propionate for one week, and more back skin was removed from each animal.

All tissue specimens were fixed for 2 hr either in buffered 1% OsO_4 (Palade, 1952) or in 4.6% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2)–8% sucrose. Aldehyde-fixed specimens were washed overnight in 0.1 M cacodylate buffer (pH 7.2)–8% sucrose, postfixed in 1% OsO_4 in cacodylate buffer, and processed according to routine procedures for electron microscopy.

† Russell, N. J. (1972). Epidermal ultrastructure during wound healing in chick embryos. A Thesis submitted to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

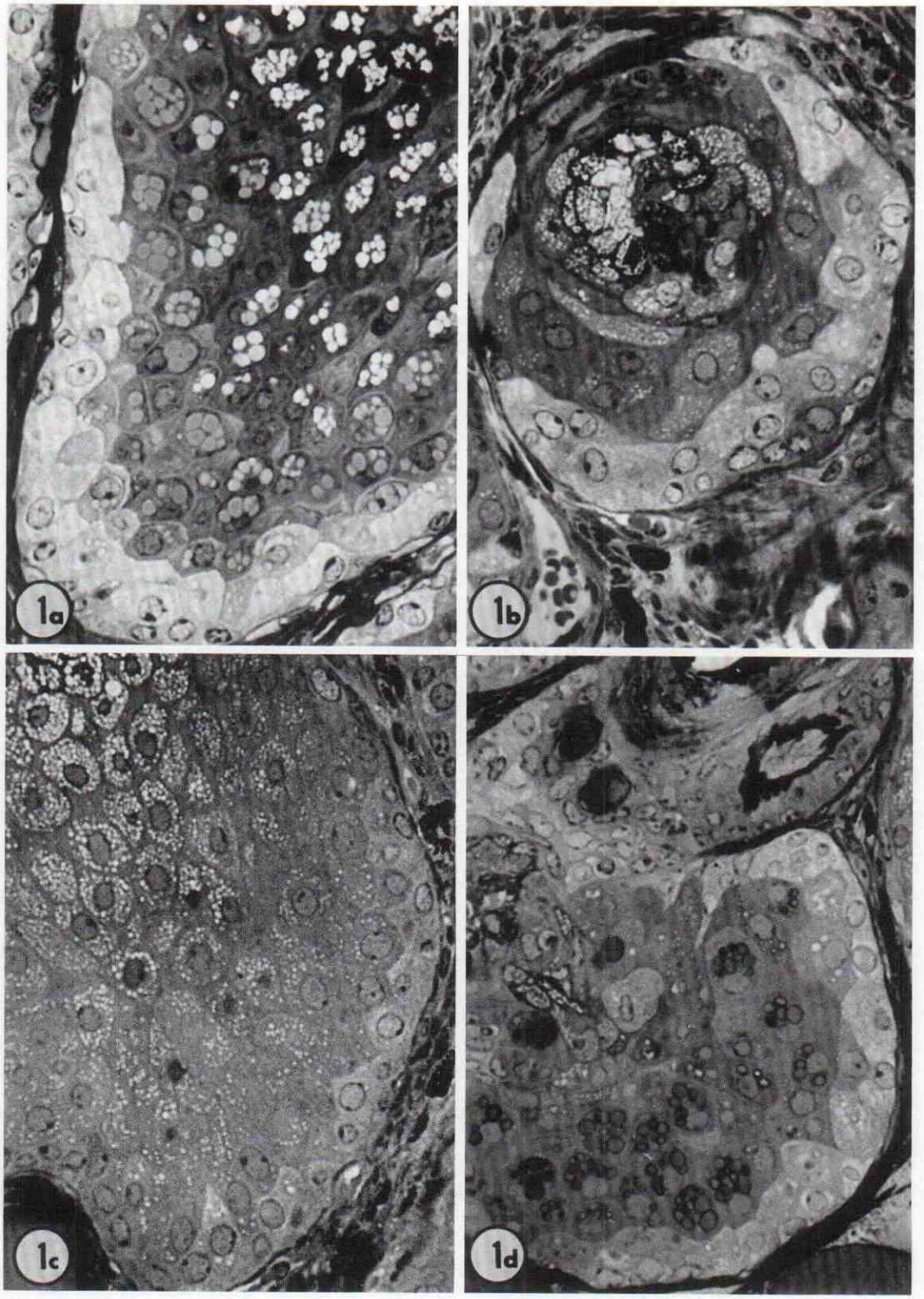

FIG. 1: (a) Light micrograph of sebaceous cells from the costovertebral gland of a normal male hamster. Note that the sebum vesicles are large and closely associated with the nuclei. This association is particularly evident in the cells that are just beginning to differentiate. × 540.

(b) A costovertebral gland from a normal female hamster. Note that the sebum vesicles are small and randomly distributed in the cytoplasm of the cells. × 540.

(c) A costovertebral gland from a male hamster castrated 12 days before sacrifice. The size and distribution of the sebum vesicles mimic the pattern seen in the normal female. × 540.

(d) A costovertebral gland from a spayed female hamster treated with testosterone propionate. The size of the sebum vesicles and their close relationships with the nuclei are similar to the pattern seen in the normal male. × 540.

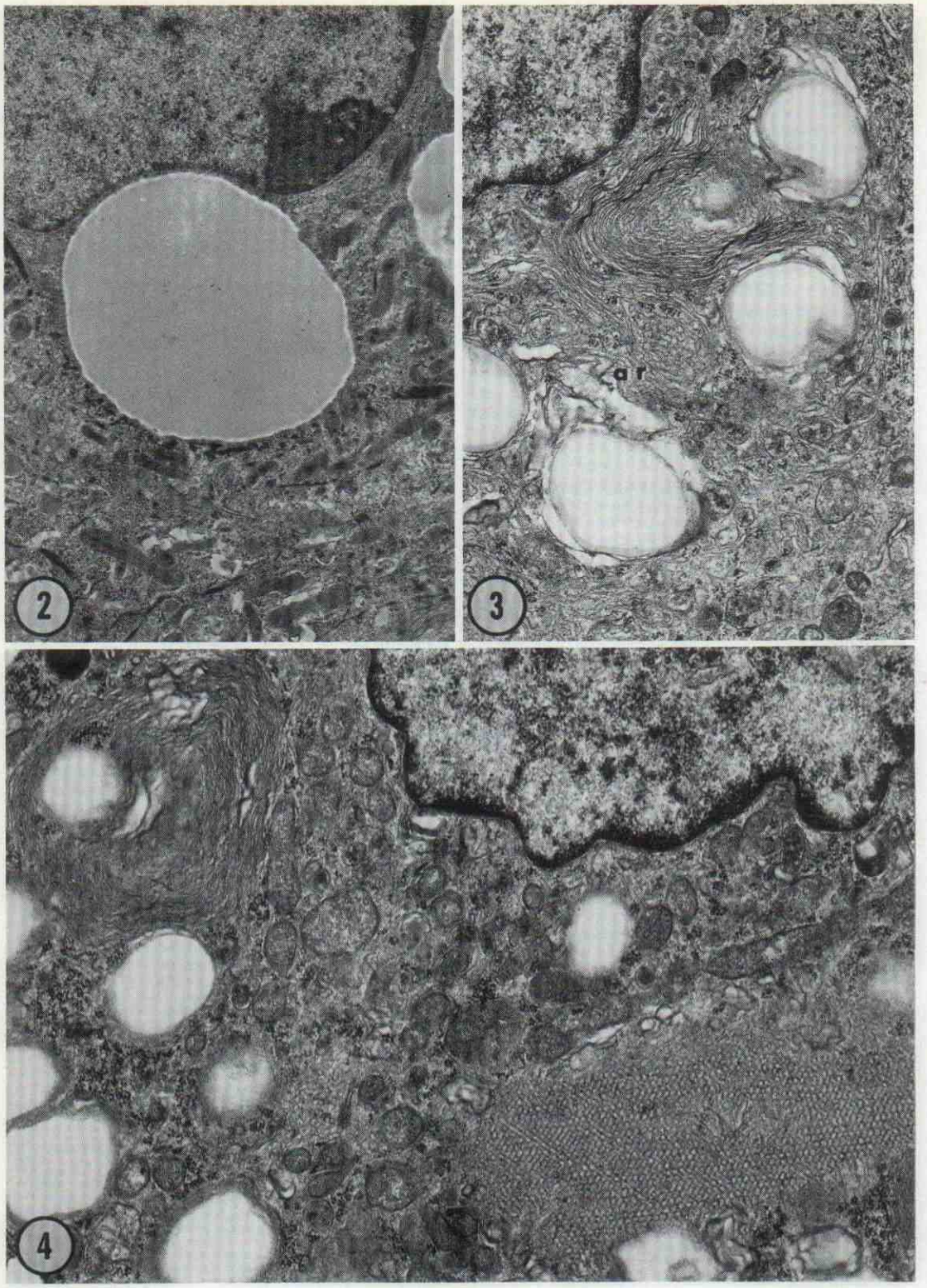

FIG. 2: Electron micrograph of costovertebral sebaceous cells from a normal male hamster. In cells at early stages of differentiation, the first sebum vesicles are large and maintain an extremely close association with the nucleus. Cytoplasmic membranes (agranular and granular endoplasmic reticulum and Golgi) are dispersed throughout the cytoplasm and membranes do not substantially accumulate at the circumferences of the sebum vesicles. × 9,450..

FIG. 3: Electron micrograph of a differentiating sebaceous cell from a normal female hamster. Golgi membranes are closely associated with the sebum vesicles, which remain small in comparison with those seen in males. Other cytoplasmic membranes, particularly agranular endoplasmic reticulum (ar), are more conspicuous than in the cells of normal males. × 19,500.

RESULTS

Light Microscopy of Sebaceous Cells in CV Glands

The differentiating sebaceous cells of normal male hamsters and of gonadectomized males and females treated with testosterone propionate are ~ 23 μ in diameter and contain large sebum vesicles (Figs. 1a,d). In normal females, however (Fig. 1b), and in gonadectomized males and females (Fig. 1c), the sebum vesicles are small (~ 1.4–1.6 μ), and the differentiating cells have a smaller diameter (~ 20 μ) than do those of normal males.

Ultrastructure of Sebaceous Cells in CV Glands

Normal male hamsters. The differentiation of sebaceous cells follows the general pattern described in the glands of all species. The peripheral cells have a high nucleo:cytoplasmic ratio and contain several small Golgi zones, numerous mitochondria, large concentrations of free ribosomes, and some profiles of granular endoplasmic reticulum. The differentiating cells, with a lower nucleo:cytoplasmic ratio, also have many mitochondria and free ribosomes (Fig. 2). Unlike the sebaceous cells in the glands of the general body skin, where sebum vesicles form randomly in the cytoplasm and remain small (1.4–1.6 μ), the sebum vesicles in the CV glands of intact male animals begin to form right next to the nuclei (Fig. 2) and become comparatively large (~ 4 μ). The numerous Golgi zones are continuous with other cytoplasmic membranes, including the numerous profiles of agranular and granular endoplasmic reticulum widely dispersed in the voluminous cytoplasm. In mature cells, which have a very low nucleo:cytoplasmic ratio, the sebum vesicles are so enormous that most of the other cytoplasmic contents are compacted and difficult to identify. Irregularly shaped and nonhomogeneous, electron-opaque lysosomes are found in cells in all stages of differentiation.

Normal female hamsters. The pattern of differentiating sebaceous cells in the CV glands is different from that in the glands of normal males. Regardless of sex, the peripheral cells are comparable and mature cells are almost so, but in the latter sebum vesicles are smaller (~ 1.4–2 μ) in females. In differentiating cells, the vesicles are distinctly surrounded by whorled membranes; the sebum vesicles are never as large (1.4–1.6 μ) as in males (4 μ) (Fig. 3). The patterns of these membranes and the distances (~ 275 Å) between adjacent lamellae suggest that they are derived from Golgi zones. Agranular reticulum tubules are often insinuated at intervals along the whorls and are continuous with them, but they are always discrete and easily distinguishable from the Golgi elements. Most of the cores of the whorls where sebum accumulation occurs contain small dense bodies. These cells have many more profiles of agranular reticulum throughout the cytoplasm than the cells of intact males (Fig. 3).

Gonadectomized females and males. In male hamsters 5 days after castration, the structure of the differentiating sebaceous cells of the CV glands is different from that of intact males and resembles that of normal females. The sebum vesicles form randomly in the cytoplasm and remain small (~ 1.4 μ); numerous whorled membranes surround the sebum droplets. The cytoplasm abounds throughout with granular reticulum profiles and also has some agranular profiles. In females spayed 5 days previously, the cells do not differ appreciably from those in intact females, but there are more whorled membranes around the sebum vesicles and more agranular reticulum.

The most significant change after gonadectomy is the larger number of highly organized agranular reticulum profiles in the differentiating cells. In females spayed 12–13 days previously, the agranular reticulum occurs as grids (Fig. 4) similar to those described by Palay (1958), but in males, grids are not seen until about 19 days after castration (Fig. 5). In males castrated 5 days before sacrifice, whorled membranes begin to occur around the sebum vesicles, but the agranular reticulum is dispersed. Twelve days after castration, however, the agranular reticulum is as abundant as in females spayed 5 days before sacrifice. The whorled membranes often enclose more than one vesicle. These changes are best seen in minimally differentiated cells. After all the cytoplasmic changes have occurred (after 12 days in spayed females, 19 days in castrated males), the cells maintain these newly acquired characteristics and do not revert to their normal modes of differentiation.

Gonadectomized animals treated with testosterone. One week after treatment with testosterone propionate, many of the differentiating cells in gonadectomized females and males are like those of normal males (Fig. 6). The sebum vesicles, which are as large as those of males, have no whorled membranes around them. The voluminous cytoplasm has abundant agranular reticulum but these profiles are widely dispersed. Ribosomes frequently adhere to the membranes around the sebum vesicles. The formation of the vesicles is similar to that of normal males. The large lipid-containing vesicles first form so near the nucleus that they often indent it. In the testosterone-treated, gonadectomized animals, the mature cells still have small vesicles, but the exaggerated cytoplasmic membranes described above are no longer abundant.

Ultrastructure of Hormonally Stimulated Cytoplasmic Formations in Galago crassicaudatus

The sebaceous cells of the scalp and back skin of all except one of the brown male animals contain

FIG. 4: Electron micrograph of a sebaceous cell from a female hamster spayed 12 days before sacrifice. A whorled Golgi zone surrounds a developing sebum vesicle at the left of the field, and a large grid of agranular reticulum is also present. All cytoplasmic membranes are more conspicuous than in normal females. × 19,550.

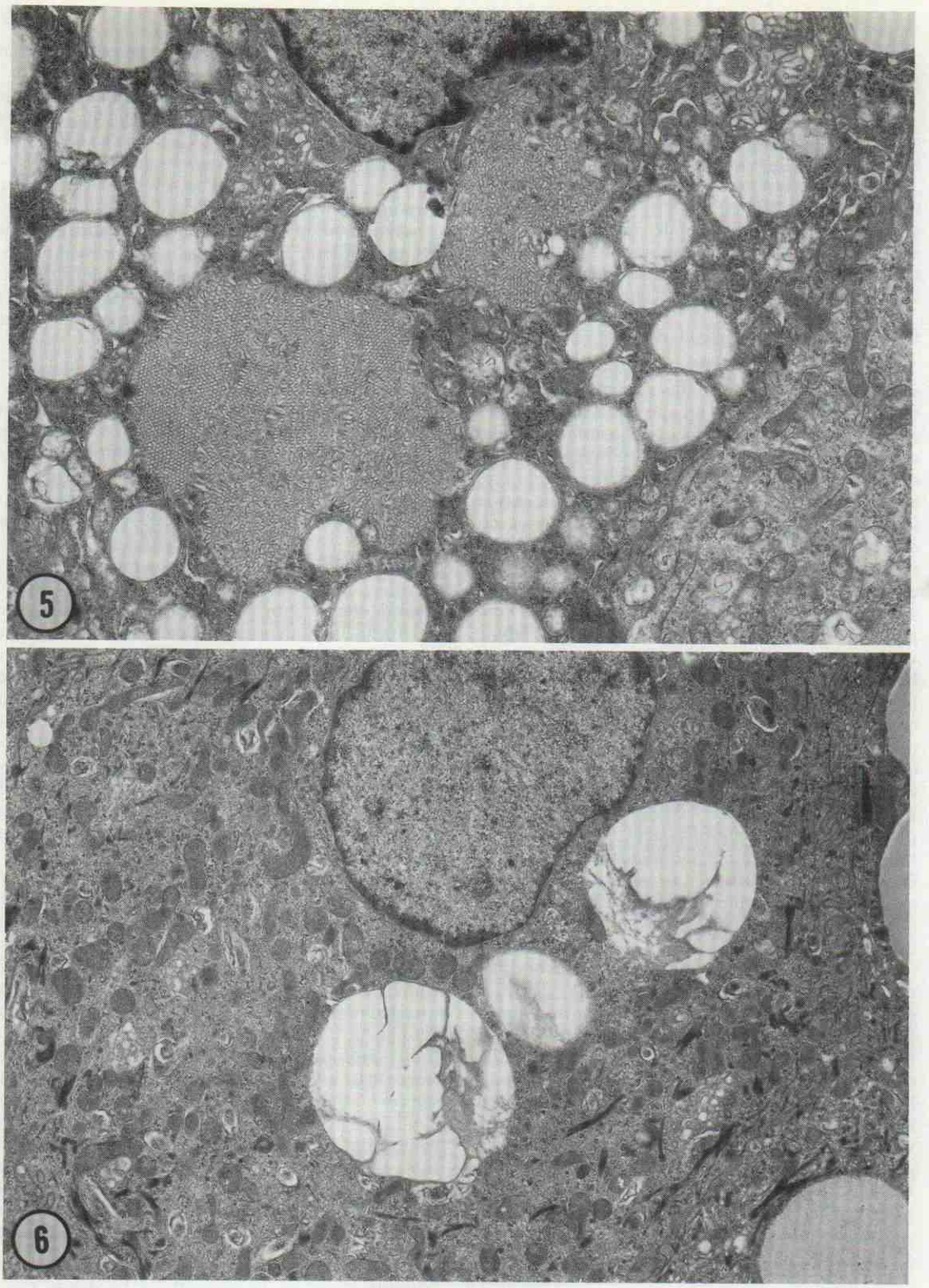

FIG. 5: A costovertebral sebaceous cell from a male hamster castrated 19 days before sacrifice. Several grids of agranular endoplasmic reticulum are prominent in the cytoplasm. Note the random distribution of small sebum vesicles in this cytoplasm compared with that of normal males. × 12,000.

FIG. 6: Costovertebral sebaceous cells from a spayed female treated with testosterone propionate. As in the normal male, the sebum vesicles are large and located near the nucleus; the cytoplasmic membranes are dispersed and do not accumulate at the circumferences of the sebum vesicles as they do in the gonadectomized animals without testosterone. × 9,450.

unique formations of cytoplasmic filaments (Bell, 1970). These 4000 Å long formations occur as parallel lamellae. When several of them are associated, they are separated by vesicles 0.2 μ in diameter. In cross-section, these formations intersect in three planes to form hexagrams that sometimes extend up to 8 μ. Since tubules and vesicles are often associated with the filaments, they are probably an integral part of the structures.

Figures 7 to 10 compare the sebaceous cytoplasm in a single animal at the stages described above. Although all six brown animals exhibited the cytoplasmic filaments, two consistently had more than the others. Large formations occurred as soon as 1 week after the onset of androgen treatment; somewhat larger formations had developed after 5 weeks. When, 6 months after the second androgen regimen, the animals were rebiopsied, minute formations were found only in the two males that consistently had had the most formations. No formations were ever found in the one melanotic animal.

The filament formations are always associated with the Golgi zones close to the nuclei of cells that have just begun to form sebum vesicles (Fig. 7). They first appear as parallel arrays associated with small vesicles; subsequently, more parallel arrays form and become more electron opaque. Ultimately, the unique patterns become randomly distributed throughout the cytoplasm. The integrity of these cytoplasmic filament formations is lost in the mature cells.

Of the three spayed females only one had a few small formations in its sebaceous cells; when these same animals were injected with 2 mg testosterone propionate daily for 1 week, all three had developed cytoplasmic filament patterns in their sebaceous cells. In one animal, the structures became as large (~7.5 μ) as those of males.

Organized grids of agranular endoplasmic reticulum, unlike the cytoplasmic filament formations, occur in normal males and females and in gonadectomized animals with or without androgen treatment (Figs. 7–10). Grids of agranular reticulum appeared more often in castrated animals without androgen treatment than in normal or testosterone-treated animals.

DISCUSSION

The modifications in both the androgen-dependent and androgen-stimulated systems described here provide the basis for further studies of the target sites of hormonal action in sebaceous cells. In both systems, the effects of stimulation or depletion occur rapidly in cells that either have not yet differentiated or have just begun to differentiate. Cells already differentiated are not appreciably altered by hormonal stimulation.

It is not known whether in both systems the effects described can be obtained by androgen analogues other than testosterone. Nor is it known whether antiandrogens, which affect metabolism in costovertebral glands (Burdick and Hill, 1970; Hsia and Voigt, this issue), produce changes identical with those that occur after castration.

The high concentration of 5α-reductase in the costovertebral glands of intact male hamsters converts testosterone to dihydrotestosterone (Takayasu and Adachi, 1970, 1972). Castration significantly reduces the activity of this enzyme and testosterone increases it. Voigt et al. (1970) have demonstrated the transformation of testosterone to dihydrotestosterone by microsomal fractions of human skin. It is still not known whether the decrease in the conversion of testosterone to dihydrotestosterone is related to the apparent increase in the cytoplasmic membranes after gonadectomy. Since the glands of normal or treated animals have more granular endoplasmic reticulum than do those of castrated animals, the ribosome–membrane relationship may be essential not only for 5α-reductase synthesis but also for maintaining its activity.

The persistence of agranular reticulum and its accumulation in highly organized arrays in both these systems, but particularly in the costovertebral glands of castrated hamsters, suggests the relationship of this organelle to lipid synthesis. Although more membrane systems are seen after castration, we do not know whether active synthesis or mere accumulation is occurring. Since the membrane systems become larger after gonadectomy, however, they may be synthesizing precursors that cannot be incorporated into sebum.

What the Golgi zones contribute to the holocrine secretory process is still not clear. In gonadectomized hamsters, whose Golgi zones are prominent, the expansion of sebum vesicles seems to be inhibited. Whether Golgi functions relate to this process remains to be determined. Membranous whorls similar to those observed in the sebaceous gland Golgi zone but derived from agranular endoplasmic reticulum have been reported in adrenal glands (Nickerson, 1972; Black, 1972) and in testicular interstitial cells (Christensen, 1965) but, at least in the adrenals, their occurrence is not regulated by testosterone (Nickerson, 1972). In costovertebral glands, however, these whorls are probably Golgi associated because they are immediately adjacent to the circumferences of the sebum vesicles that are elaborated by the Golgi zones (Bell, 1971a; Breathnach, 1971).

The role of the cytoplasmic filaments peculiar to the sebaceous cells of *Galago crassicaudatus* is not known, but these structures are easily identifiable with the electron microscope and further studies may elucidate their function.

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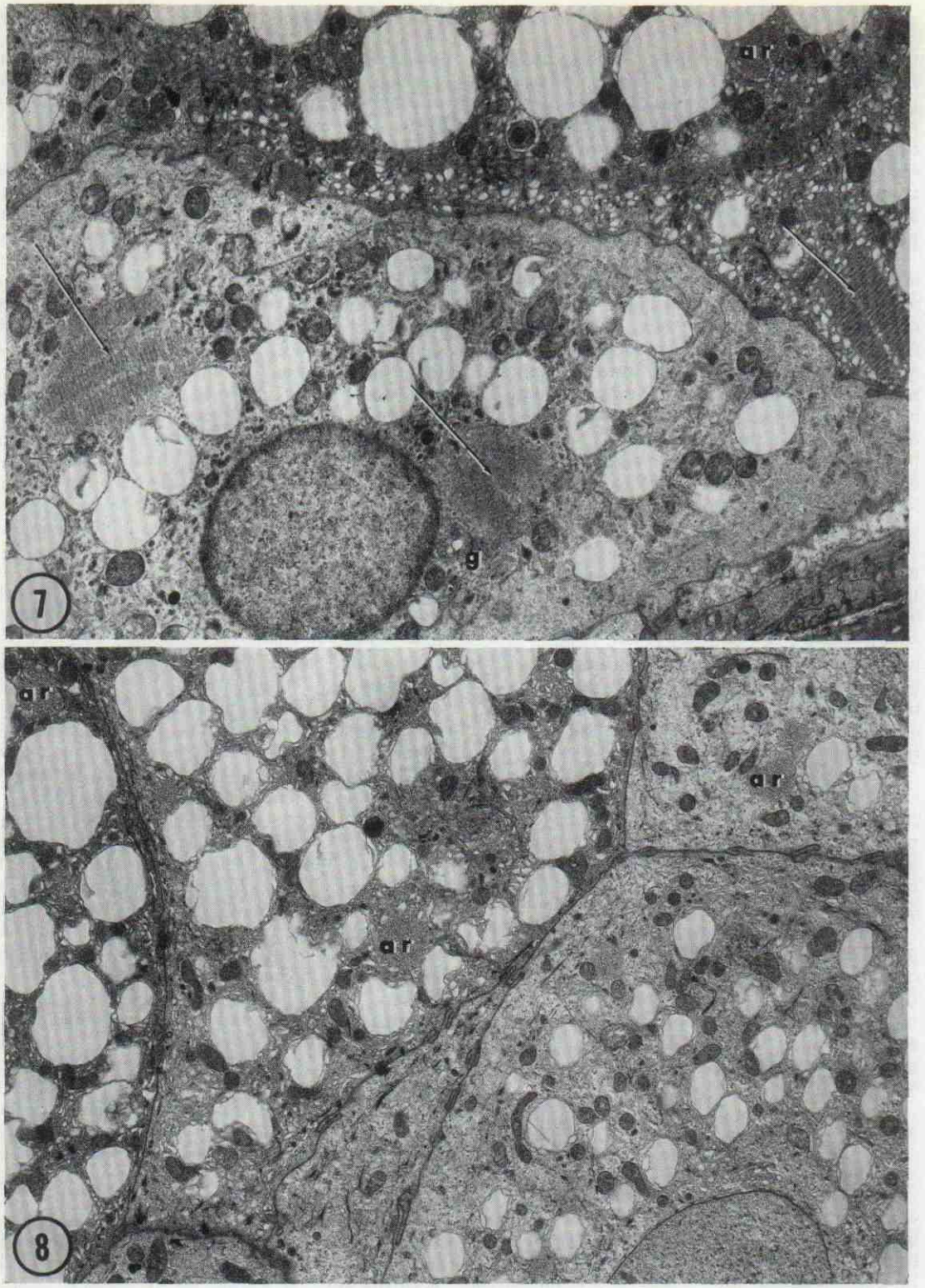

FIG. 7. Electron micrograph of sebaceous cells from a normal male *Galago crassicaudatus* (#2748). Several cytoplasmic filament formations (arrows) are present in the differentiating cells. When these structures first appear, they are associated with the Golgi zones (g), but later they occur randomly in the cytoplasm, usually associated with sebum vesicles. Grids of agranular reticulum (ar) are also commonly seen. × 7,500.

FIG. 8: Sebaceous cells from *G. crassicaudatus* (#2748) after castration. Although many grids of agranular reticulum (ar) are present, no cytoplasmic filament formations occur. × 6,000.

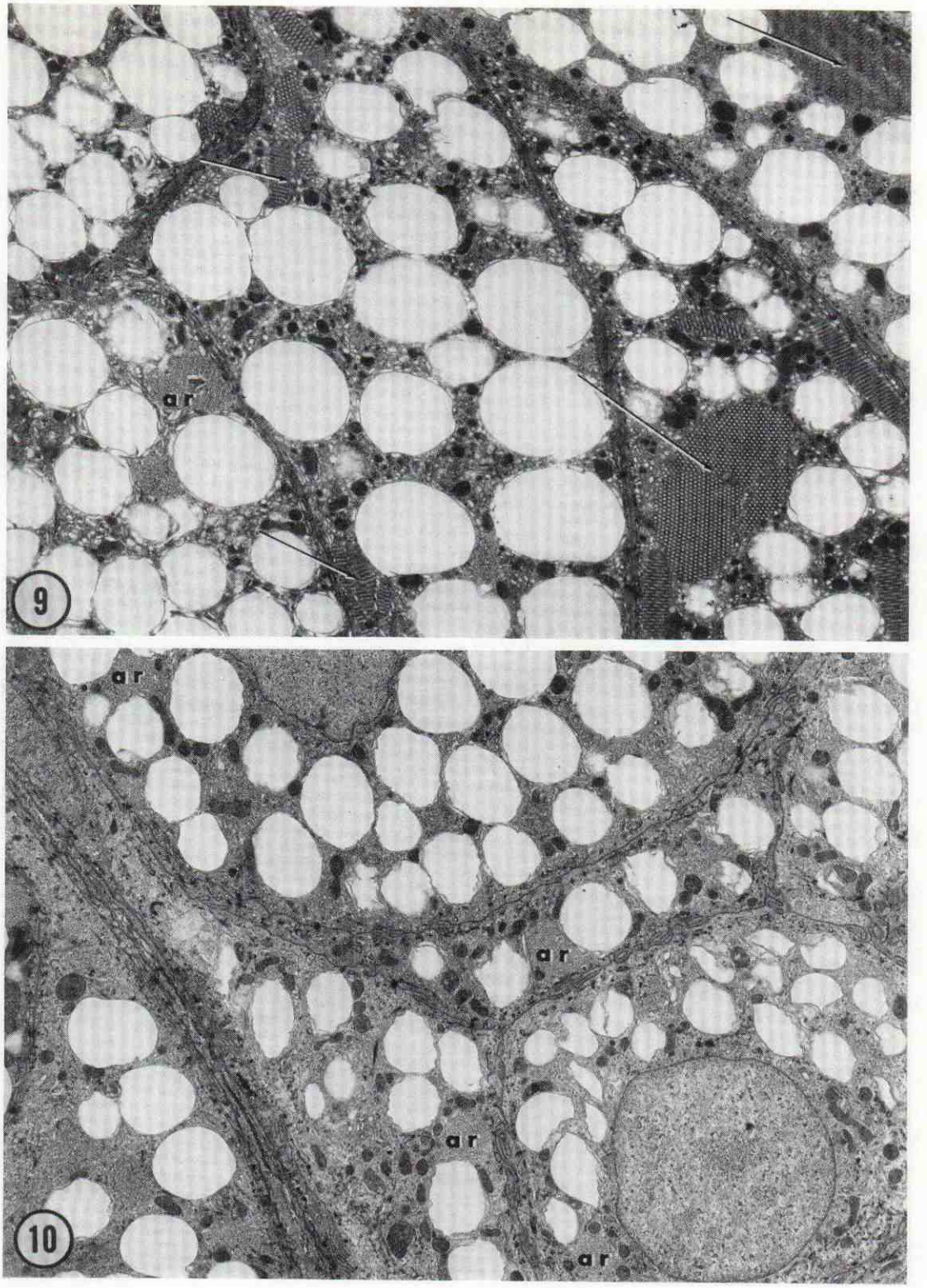

FIG. 9: Sebaceous cells from *G. crassicaudatus* (#2748) biopsied 3 months after castration and subsequent androgen treatment. Many well-developed cytoplasmic filament formations (arrows) are present, together with grids of agranular reticulum (ar). × 6,000.

FIG. 10: Sebaceous cells from *G. crassicaudatus* (#2748) after castration and subsequent treatment with only sesame oil. No cytoplasmic filament formations are present, but grids of agranular reticulum (ar) persist. × 6,000.

REFERENCES

Adachi, K., and Takayasu, S. (1972). The mechanisms of testosterone action on the sebaceous glands of the Syrian hamster. In: *Advances in Biology of Skin. Vol. XII. Pharmacology and the Skin* (ed. by Montagna, W., Stoughton, R. B., and Van Scott, E. J.). Appleton-Century-Crofts, New York, pp. 381–401.

Bell, M. (1970). A comparative study of sebaceous gland ultrastructure in subhuman primates. I. *Galago crassicaudatus, G. senegalensis,* and *G. demidovii.* Anat. Rec., *166:* 213–224.

Bell, M. (1971a). A comparative study of sebaceous gland ultrastructure in subhuman primates. III. Macaques: Ultrastructure of sebaceous glands during fetal development of rhesus monkeys (*Macaca mulatta*). Anat. Rec., *170:* 331–341.

Bell, M. (1971b). Comparative ultrastructure of sebaceous glands in primates. Demonstration presented at the Eleventh Annual Meeting, The American Society for Cell Biology, New Orleans, p. 343. (Abstract.)

Black, V. H. (1972). The development of smooth-surfaced endoplasmic reticulum in adrenal cortical cells of fetal guinea pigs. Am. J. Anat., *135:* 381–417.

Burdick, K. H., and Hill, R. (1970). The topical effect of the antiandrogen chlormadinone acetate and some of its chemical modifications on the hamster costovertebral organ. Br. J. Dermatol., *82*(Suppl. 6)*:* 19–25.

Breathnach, A. S. (1971). In: *An Atlas of the Ultrastructure of Human Skin. Development, Differentiation, and Post-Natal Features.* J. & A. Churchill, London, p. 340.

Christensen, A. K. (1965). The fine structure of testicular interstitial cells in guinea pigs. J. Cell Biol., *26:* 911–935.

Ebling, F. J. (1957). The action of testosterone and oestradiol on the sebaceous glands and epidermis of the rat. J. Embryol. Exp. Morphol., *5:* 74–82.

Hamilton, J. B., and Montagna, W. (1950). The sebaceous glands of the hamster. I. Morphological effects of androgens on integumentary structures. Am. J. Anat., *86:* 191–233.

Luft, J. H. (1961). Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol., *9:* 409–414.

Nickerson, P. A. (1972). Effect of testosterone, dexamethasone and hypophysectomy on membranous whorls in the adrenal gland of the Mongolian gerbil. Anat. Rec., *174:* 191–204.

Palade, G. E. (1952). A study of fixation for electron microscopy. J. Exp. Med., *95:* 285–298.

Palay, S. L. (1958). Morphology of secretion. In: *Frontiers in Cytology* (ed. by Palay, S. L.). Yale University Press, New Haven, pp. 305–342.

Spurr, A. R. (1969). A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res., *26:* 31–43.

Strauss, J. S., and Pochi, P. E. (1963). The human sebaceous gland: Its regulation by steroidal hormones and its use as an end organ for assaying androgenicity in vivo. Recent Progr. Horm. Res., *XIX:* 385–444.

Takayasu, S., and Adachi, K. (1970). Hormonal control of metabolism in hamster costovertebral glands. J. Invest. Dermatol., *55:* 13–19.

Takayasu, S., and Adachi, K. (1972). The in vivo and in vitro conversion of testosterone to 17β-hydroxy-5α-androstan-3-one (dihydrotestosterone) by the sebaceous gland of hamsters. Endocrinology, *90:* 73–80.

Voigt, W., Fernandez, E. P., and Hsia, S. L. (1970). Transformation of testosterone into 17β-hydroxy-5-androstan-3-one by microsomal preparations of human skin. J. Biol. Chem., *245:* 4495–4499.