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BIODYNAMIC STUDIES OF HAMSTER FLANK ORGAN GROWTH: HORMONAL INFLUENCES*

PHILLIP FROST, M.D., JOSEPH L. GIEGEL, PhD.†, GERALD D. WEINSTEIN, M.D.†, AND EDWARD C. GOMEZ, M.D., Ph.D.

ABSTRACT

The biodynamic response of flank organs of male and female hamsters to androgenic stimulation has been studied by autoradiographic and electron microscopic techniques, as well as by routine histology and gross observation. Intraperitoneal administration of 2.5 mg of testosterone on alternate days resulted in bilateral increase in palpable bulk, and pigmentation of flank organs of females, males, and castrated males. Topical application of testosterone, dihydrotestosterone (DHT), methyltestosterone, or androstenedione to one flank organ of females resulted in unilateral stimulation of sebaceous gland growth and pigmentation. Androsterone, epiandrosterone, and progesterone did not cause flank organ growth or increased pigmentation when applied topically.

Histologic changes in flank organs of castrated males following testosterone administration are described. Autoradiographic studies indicate a decrease in the labeling index of flank organ sebaceous gland cells within 20 hours after castration and a subsequent significant increase in the labeling index within 10 hours after a single intraperitoneal administration of 2.5 mg of testosterone. Pigmentation of flank organs is due to the presence of dendritic cells in the dermis which contain dense structures resembling stage IV melanosomes.

Previous studies in humans and various animals indicate that sebum production is related to sebaceous gland size [1] which, in turn, is related to androgenic stimulation [2–4]. Thus, sebum production rates increase in boys after puberty [4]; testosterone propionate administered to prepubertal boys increases sebaceous gland size [3]; methyltestosterone increases sebum production in prepubertal males and adult females [4]; adult men produce more sebum than adult women, corresponding to the higher circulating blood levels and production rates of androgens in males [4, 5]; and castration decreases sebum production in adult males [4]. In the rat [6–8], mouse [9], Mongolian gerbil [10], and golden hamster [11], androgens have also been shown either to stimulate sebum production or sebaceous gland growth. The golden hamster has, in addition to normal pilar units over most of its body, a pair of flank organs (costovertebral spots, scent glands) which contain dermal melanocytes, larger hair follicles and sebaceous glands, all of which are androgen-dependent [11].

Attempts to use the flank organs of male hamsters for the topical assay of antiandrogenicity [12] proved only partially successful since systemic effects upon the contralateral gland were observed. To determine the usefulness of the flank organ for the assay of androgens and antiandrogens, the biodynamics of the response of this tissue to androgenic stimulation have been studied in greater detail by autoradiographic and electron microscopic techniques, as well as routine histology and gross observation. The response of the flank organs to graded doses of testosterone, dihydrotestosterone (DHT), and other androgenic steroids has also been determined.

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* From the Skin and Cancer Unit, Mount Sinai Medical Center, Miami Beach Florida, and the † Department of Dermatology, University of Miami School of Medicine, Miami, Florida. (Reprint requests to: Dr. P. Frost, Skin and Cancer Unit, Mount Sinai Medical Center, 4300 Alton Road, Miami Beach, Florida 33140.)

MATERIALS AND METHODS

Adult hamsters (120–140 gm) were purchased from Manor Farms Inc. and kept on an ad lib diet of Purina Chow and water and exposed to 12 hr of light per day. Males and females were kept separately in large cages in groups of 8 until an experiment was begun, when they were placed in individual cages. Males were castrated by the scrotal route using intraperitoneally administered phenobarbital (8 mg/100 gm body weight) as an anesthetic agent. Testosterone in aqueous suspension (Oreton) purchased from Schering Corporation was diluted to a volume of 1 cc in normal saline and injected intraperitoneally. For topical application, Δ^4-androstenedione, androsterone, epiandrosterone, testosterone, 5α-dihydrotestosterone (DHT), methyltestosterone, and progesterone purchased from Mann Research Laboratories were dissolved in acetone and applied directly to the flank organ with a Hamilton microliter syringe after shaving the backs of the animals with an electric hair clipper. Initially, the status of the flank organ was determined by measuring the diameter and calculating the surface area showing pigmentation and thickness. Subsequently, it was found that accurate grading of the flank organ could be carried out by gross evaluation of the animal and assignment of an arbitrary grade of 0 to 4 (Table). In this gross evaluation of the flank organ area, grade 4 represents maximal stimulation (largest diameter

TABLE

Topical application of steroids

Preparation applied to right flank organ (μg)		Flank organ size (day 15)	
		Right	Left (control)
5α-Dihydrotestosterone	2	3.5	1.0
5α-Dihydrotestosterone	25	4.0	2.0
5α-Dihydrotestosterone	50	4.0	2.0
5α-Dihydrotestosterone	100	4.0	2.0
Testosterone	2	3.5	1.0
Testosterone	25	4.0	1.0
Testosterone	50	4.0	1.0
Testosterone	100	4.0	1.0
Methyltestosterone	100	3.2	1.0
Δ^4-Androstenedione	100	3.0	1.0
Androsterone	100	1.5	1.0
Epiandrosterone	100	1.2	1.0
Progesterone	200	1.0	1.0

Test compounds were applied in acetone with a Hamilton microliter syringe to the right flank organ of normal adult female hamsters; a similar volume of plain acetone was applied to the left gland. Applications were made 5 days a week (weekdays). Flank organ size was evaluated on a 1 to 4 scale on the basis of the widest diameter of the gland (1 = 0–2 mm; 2 = 2–4 mm; 3 = 4–6 mm; 4 = > 6 mm). Values are the means for two animals for all compounds except androstenedione, androsterone, and epiandrosterone for which values are the means of three animals.

greater than 6 mm) and grade 1, the appearance of the flank organ of an adult female (0 to 2 mm). All grading in an experiment was done by the same individual and the correlation of this grading system with sebaceous gland size was confirmed by examination of histologic sections of flank organs that had previously been graded by gross inspection.

For autoradiographic studies, tritiated thymidine (sp. act. = 1.9 Ci/mmole) purchased from Schwartz Bioresearch was injected intraperitoneally (0.75 μCi/gm body weight) 45 min prior to sacrificing animals by exposure to ether vapor. Tissues were then processed according to established procedures [13]. For determination of labeling indices in sebaceous glands, the perimeter of glands was measured with a map measure on projected images of histologic sections as described previously [13]. Since the germinative cells are at the periphery of glands, only labeled cells at the outer edge of the glands were counted, and results expressed per unit length of gland periphery. Only glands containing well-labeled cells were counted.

RESULTS

Gross appearance of flank organs

The gross appearance of the normal male hamster flank organ is shown in Figure 1. Female hamsters have flank organs which show minimal hyperpigmentation and thickening. The castration of male hamsters results in a decrease in pigmentation and palpable bulk of their flank organs.

Histologic appearance of flank organs

Normal adult males (*Fig. 2*). The stratum corneum is thin but similar in thickness to the adjacent general body skin. The epidermis consists of 3–4 cell layers. Epidermal mitoses are not more numerous than in the adjacent general body skin. In the dermis, there is a sharp demarcation between the flank organ region and adjacent skin. The flank organ pilosebaceous units are much larger, with sebaceous glands extending down to near the level of the hair bulbs, which are also larger than those in the adjacent skin, where sebaceous glands are miniscule. The hair bulbs are heavily pigmented and are about 2–3 times the diameter of those in adjacent skin; almost all are in the anagen phase of the hair cycle.

The basal cells of the sebaceous glands have round nuclei and scant cytoplasm. In cells of the parabasal layers, the cytoplasm contains many clear granules separated by septae of pink-staining material. There is a separation between healthy-appearing, more peripheral cells and cells toward the center of the gland which have pyknotic or absent nuclei, or are frankly necrotic and filled with clear granules. In the center of the gland,

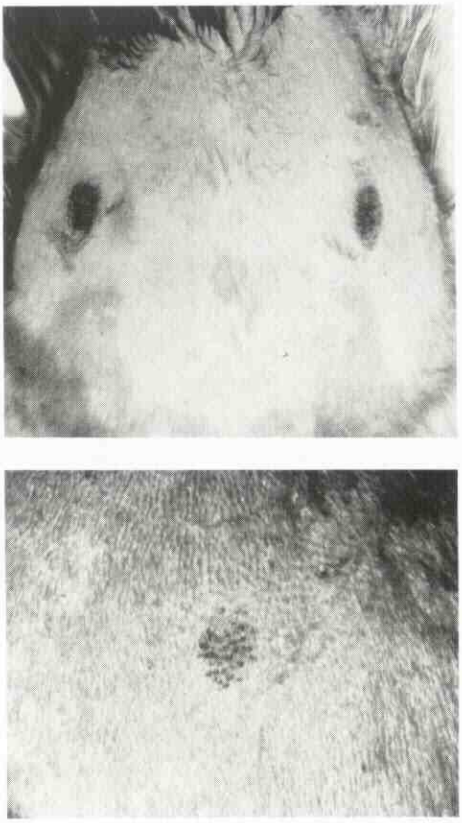

FIG. 1: Normal adult male hamster. Note dorsal location of flank organs. On close inspection it can be seen that pigment is concentrated in clusters of small distinct foci. Each focus surrounds the common orifice for several pilosebaceous units.

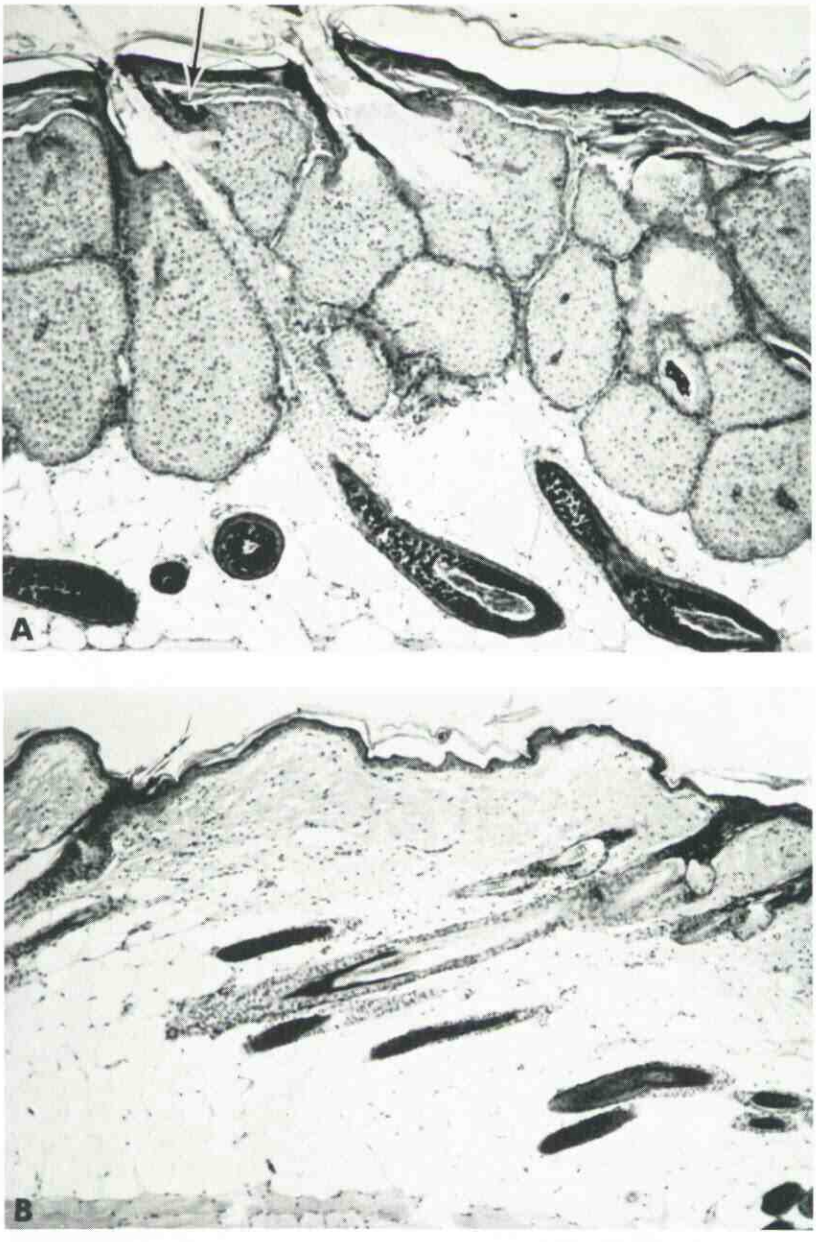

FIG. 2: Normal adult flank organ (A). Note large sebaceous glands and hair follicles compared to skin immediately adjacent to flank organs (B). In the flank organ, large sebaceous glands compress dermal melanocytes into small compact collections (arrows) and the hair bulb region of the follicles bends to lie parallel to the skin surface. H and E stain; × 100.

where cell outlines are no longer discernable, there is a necrotic mass of refractile material.

Large dendritic cells filled with brown granules are clustered in the dermis about the sebaceous glands near the sebaceous gland duct and the infundibulum of the hair follicle (Fig. 3). Electron micrographs of these cells reveal the ultrastructure of the granules to be similar to that of melanin granules (Fig. 4). Pigmented dendritic cells are not seen in the dermis of the skin adjacent to the flank organ.

Castrated adult males. Eight hr after castration, no morphologic changes are evident in histologic specimens of the flank organ. After 4 hr, nuclei of the sebaceous gland basal cells are flatter, and necrosis of sebaceous gland cells is present closer to the periphery of the glands (Fig. 5A). The infundibulum is slightly distended and filled with pink-staining debris.

By 72 hr after castration, the basal cell nuclei are quite flat and necrosis is evident from the center of glands to 4–5 cell layers from their periphery. The

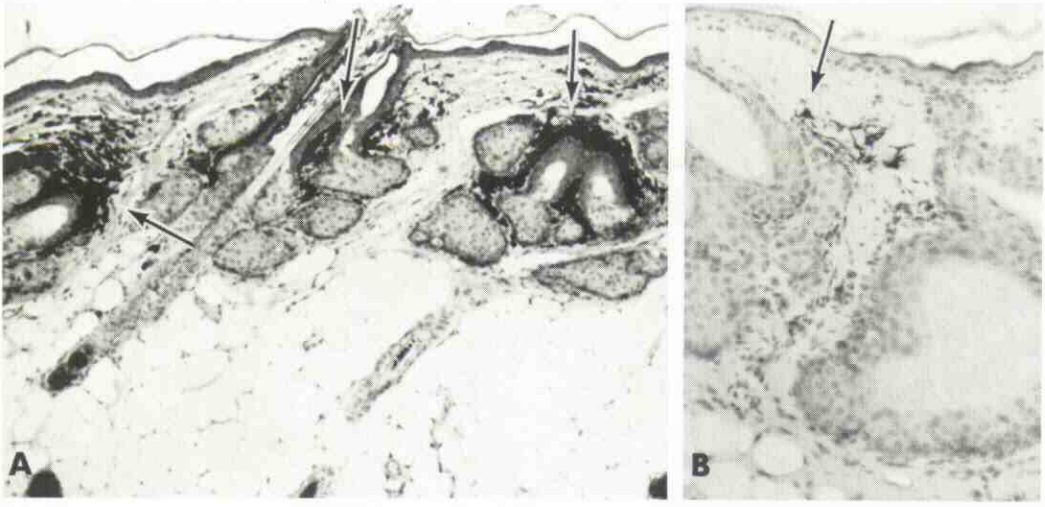

FIG. 3: Dendritic cells (arrows) containing brown granules are clustered about sebaceous gland. A: specimen from castrated male hamster taken after 5 days of testosterone administration (2.5 mg/day, intraperitoneally). B: Specimen taken from male hamster 5 days after castration.

pigmented dermal cells are less visible around the follicles at either edge of the flank organ.

In specimens taken 5 days after castration, there is clearly more atrophy of the sebaceous glands at the periphery of the flank organ. Only in the center of the flank organ are a few large pilosebaceous units left. In these, only a few layers of viable sebaceous gland cells remain at the periphery of the glands. A pink-staining hyaline band forms a sharp demarcation between these viable cells and the central mass of debris, which consists of clear refractile crystals and other necrotic material (Fig. 5B). Most hairs appear to be in telogen at this point.

By 8 days after castration, the appearance of the hair follicles of the flank organ is quite remarkable (Fig. 5C). The infundibulum is widely distended, filled with necrotic material, and resembles a comedo in most respects. The only glandular tissue remaining consists of small nubbins of sebaceous gland ducts. The enlarged, stuffed infundibulae extend downward to near the level of the hair bulbs. The remaining pigmented dermal cells are compressed under the epidermis by the upper part of the infundibulum which is now spherical.

By 6 weeks after castration, the follicular necks are no longer so distended and the pilosebaceous units are quite similar to those in the adjacent skin except for a few larger follicles in the center of the flank organ.

Autoradiographic studies of flank organ response to castration and testosterone administration

Labeling indices in the germinative population of flank organ sebaceous glands (Fig. 6) were determined at various times after castrating normal adult males and after the intraperitoneal injection of a single dose of 2.5 mg of testosterone in aqueous suspension to male hamsters castrated 6 weeks previously. Within 24 hr after castration the number of labeled sebaceous gland cells in autoradiographs of titrated thymidine-injected specimens decreased dramatically, indicating that cell replication activity was diminished (Fig. 7). By 8 weeks after castration, labeled indices increased slightly as seen in the zero time point in Figure 8, but remained less than half the normal levels (zero time point in Fig. 7). The systemic administration of testosterone to castrated male hamsters caused a rapid increase in labeling indices and, after 5 hr, labeling indices were greater than those in normal male hamsters and remained at these levels for at least 50 hr (Fig. 8).

Changes in gross appearance of flank organs

The effects of castration and testosterone administration on the hamster flank organ are shown in Figure 9. In evaluating flank organ size, the area of pigmented and palpably thickened skin was determined. After castration of normal males the area of flank organ pigmentation decreased and the sebaceous gland bulk began to decrease; by 20 days after castration, the pigmented area was maximally diminished. The intraperitoneal administration of 2.5 mg of an aqueous suspension of testosterone on alternate days to castrated male, normal female, or normal male hamsters resulted in maximal increases in flank organ area to roughly twice the normal gland area. There were minor variations in size of the flank organs in untreated females; it is not known whether these were related to the estrous cycle. Although the volume of the underlying sebaceous glands and the area of pigmentation in the flank organ region were related in these studies, palpable thickening, due to increase in bulk of the sebaceous glands, was considered a better indicator of sebaceous gland growth than area of pigmentation. The relationship of the gross

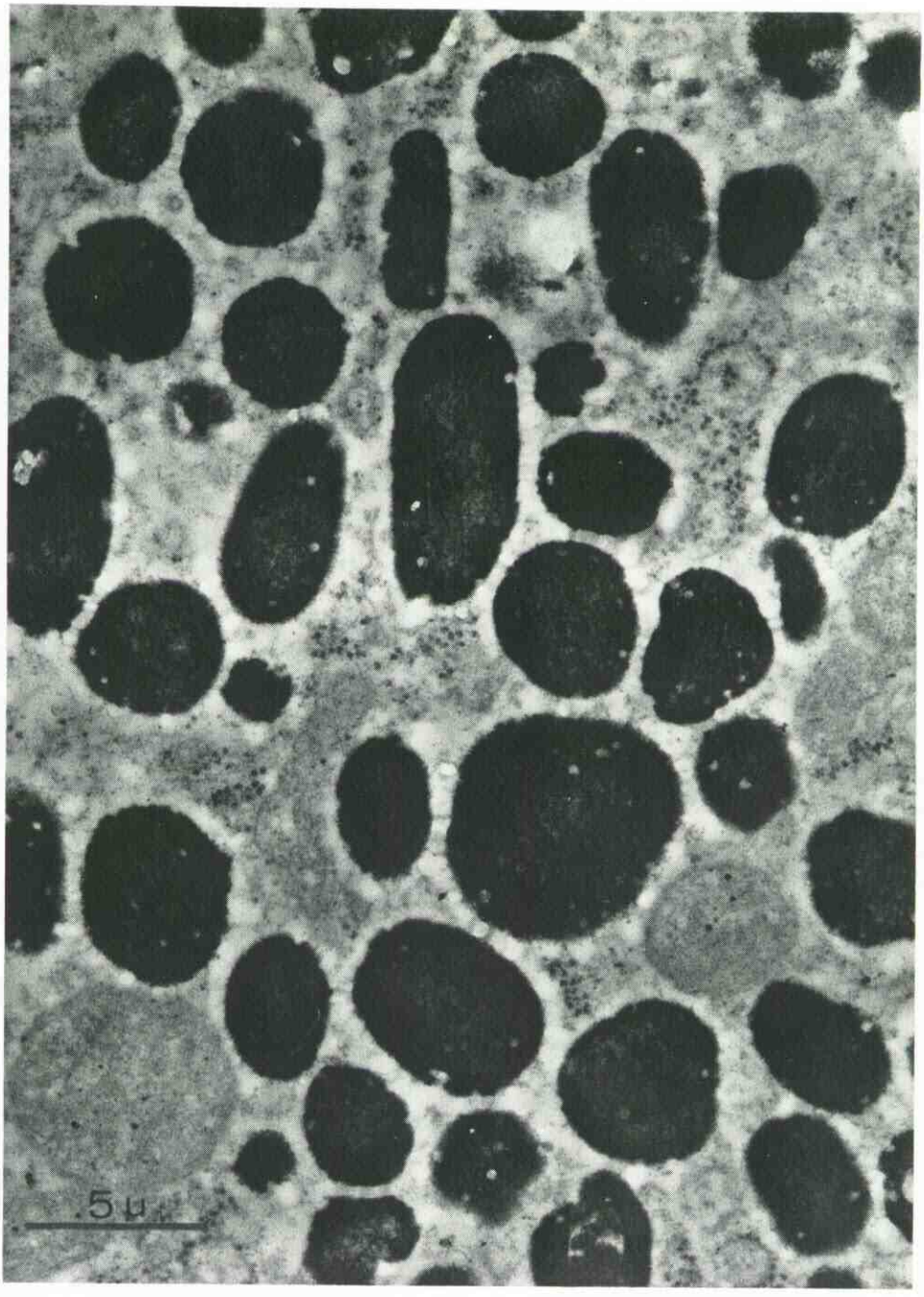

FIG. 4: Electron micrograph of dendritic dermal cell in hamster flank organ. Granules are similar in appearance to those of melanocytes. × 49,000. Specimen was fixed in osmium and embedded in araldite.

evaluation of flank organ area (see *Materials and Methods* and Table) to sebaceous gland size was demonstrated by measurements of histologic sections of flank organs showing various degrees of stimulation by gross examination (Fig. 10).

The topical administration of small doses of testosterone and DHT to only one flank organ of an adult female hamster resulted in rapid growth of only the gland to which the steroids were applied (Fig. 11). With larger doses, slight growth of the contralateral gland occurred with DHT; with testosterone only the gland receiving the hormone grew (Table).

The increase in area of the flank organ was gradual during the first 15 days, after which further growth did not occur. The applications

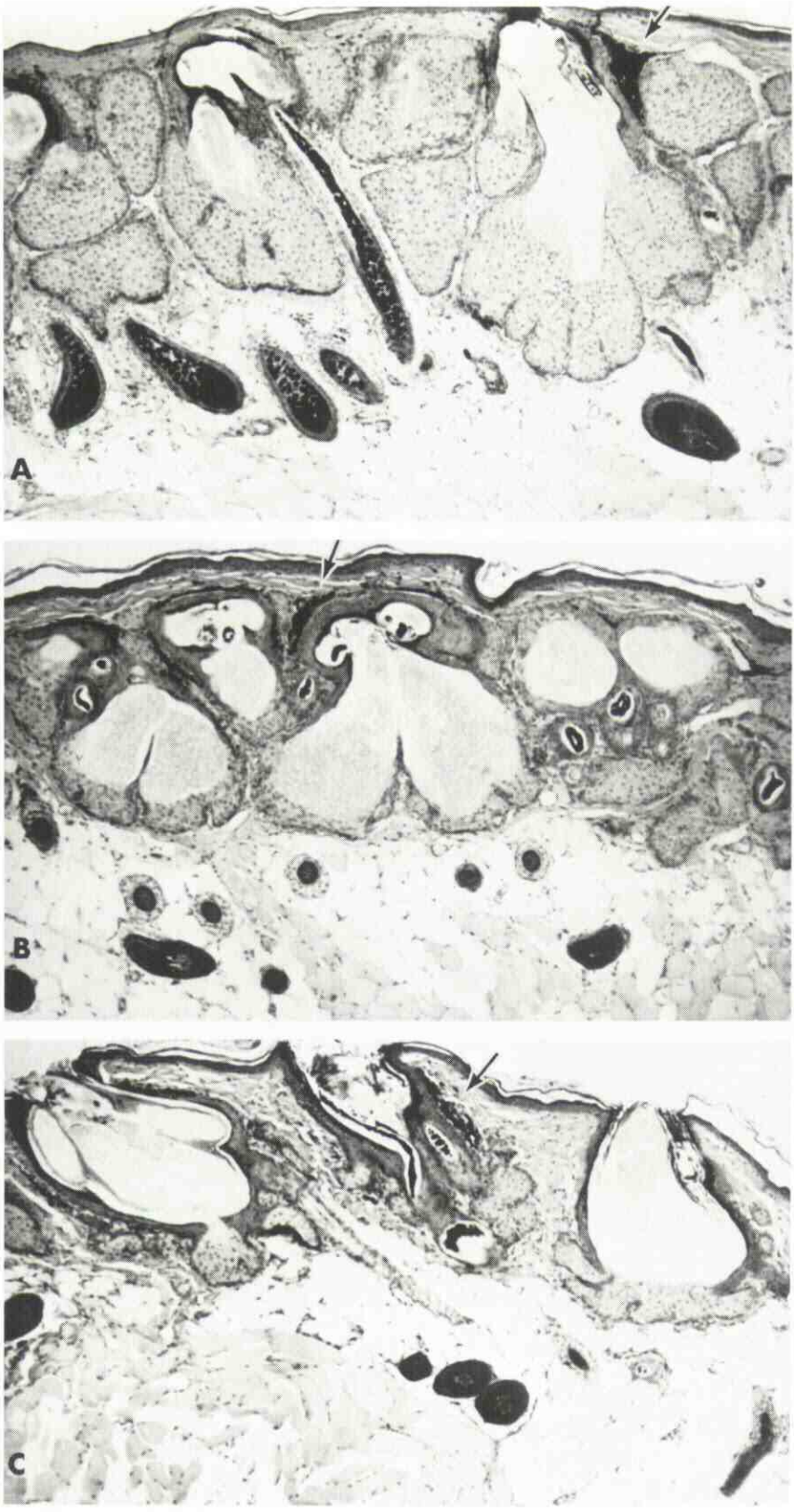

FIG. 5: Adult male hamster flank organs. A: 48 hr after castration; B: 5 days after castration; C: 8 days after castration. Note progressive atrophy of sebaceous glands and distension of follicular necks by necrotic debris. Pigmented dendritic cells remain compressed under the epidermis (arrows). H and E stain; × 100.

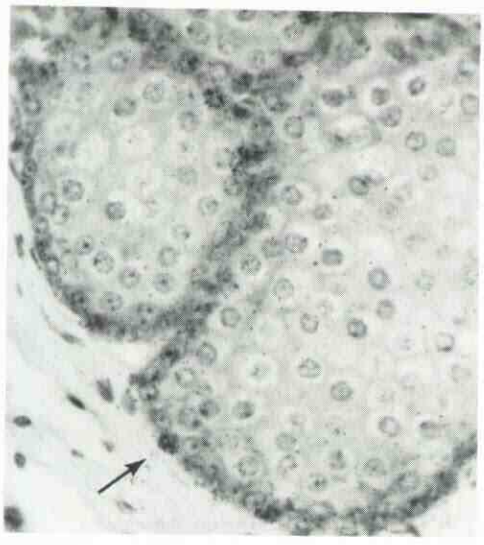

FIG. 6: Autoradiograph of sebaceous glands of hamster flank organs. Labeled cells appear only at periphery of acini (arrow). These represent cells which were in DNA synthesis (S) phase of the cell cycle at the time of injection of the tritiated thymidine. H and E stain; × 400.

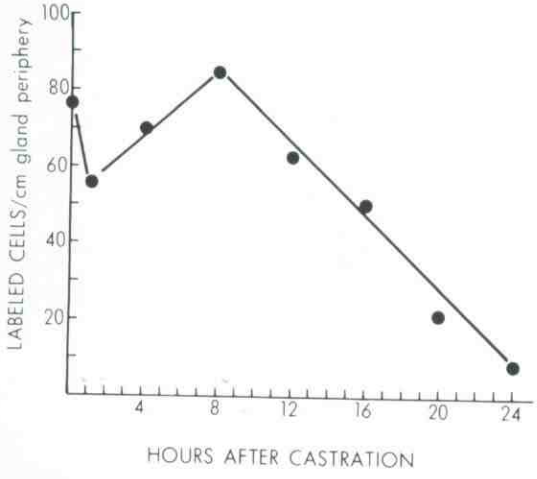

FIG. 7: Labeling index of proliferative cells of sebaceous glands in hamster flank organs after castration. Each point represents an average of counts from six sections of each of four flank organs from two hamsters.

were discontinued after day 15 but the animals were examined for two more weeks during which time the flank organ size decreased slowly. At the end of this time flank organ size had not decreased to normal in either group and the flank organs of the DHT-treated animals diminished in size more slowly than those treated with testosterone.

Methyltestosterone and Δ^4-androstenedione applied topically were almost as effective as testosterone in stimulating flank organ growth. Androsterone and epiandrosterone caused minimal flank organ growth. Topically applied progesterone caused no growth of flank organ.

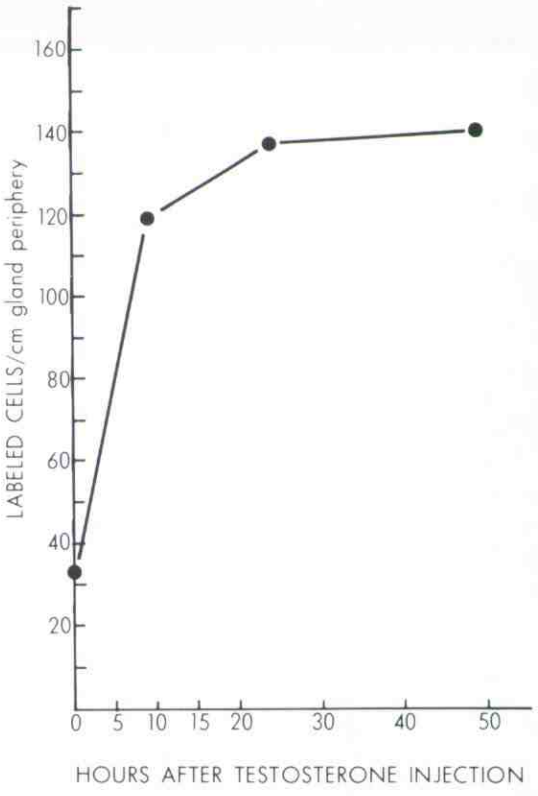

FIG. 8: Labeling index of proliferative cells of sebaceous glands of flank organs. Animals castrated 8 weeks previously were given a single intraperitoneal injection of 2.5 mg of an aqueous suspension of testosterone and sacrificed at the indicated intervals 45 min after the intraperitoneal injection of tritiated thymidine. Each point represents an average of counts from six sections of each of four flank organs from two hamsters.

DISCUSSION

The flank organs of the golden hamster are unique, specialized, cutaneous organs of uncertain biologic function. The coordinated androgen-dependent changes in sebaceous glands, hair follicles, and pigment cells suggest a sex-related function but these organs have also been thought to play a role in territorial marking. Despite our lack of knowledge concerning their exact purpose, their hormone dependence provides a useful means of studying the physiologic effects of androgens on skin. These organs differ from other specialized hormone-dependent cutaneous structures such as the preputial gland of rats, the cock's comb, or the preening gland of the duck, in that the glandular structures of the flank organs are morphologically very similar to sebaceous glands in human skin and retain their association with functioning hair follicles. Unlike the cock's comb, in which hormonally induced changes are largely in the dermal connective tissue, the sebaceous gland growth induced in flank organs appears to be merely an accentuation of the normal response of sebaceous glands on other parts of the body to androgens.

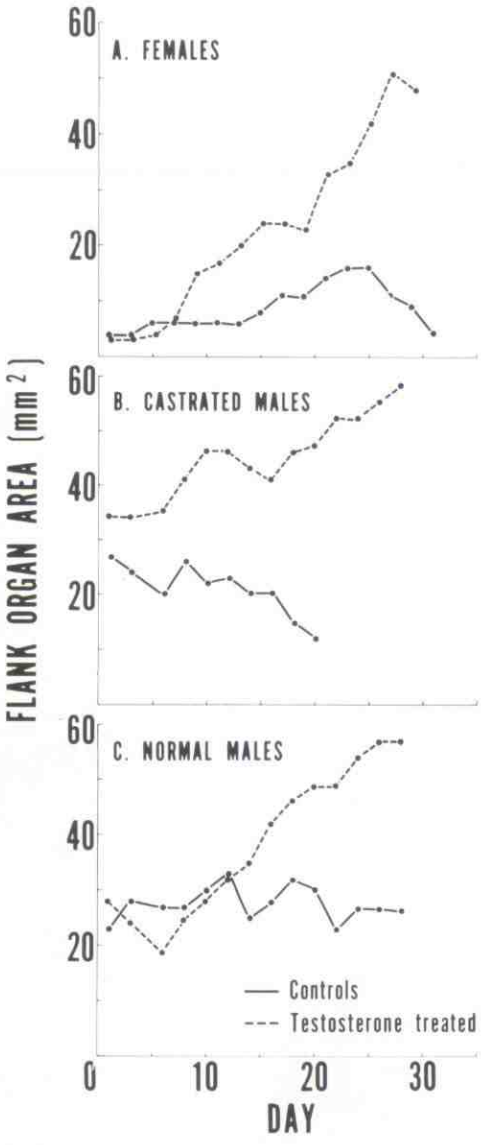

FIG. 9: The effect of testosterone on hamster flank organ growth. 2.5 mg of an aqueous suspension of testosterone were injected intraperitoneally on alternate days into 4 normal adult female, 4 normal adult male, and 4 castrated male hamsters. A: Adult female hamsters; B: adult male hamsters castrated on day 1 of study; C: adult males. The control animals in each group received injections of the vehicle for testosterone on alternate days. The backs of the animals were shaved weekly, the diameters of the flank organs measured with calipers, and their areas determined on each day.

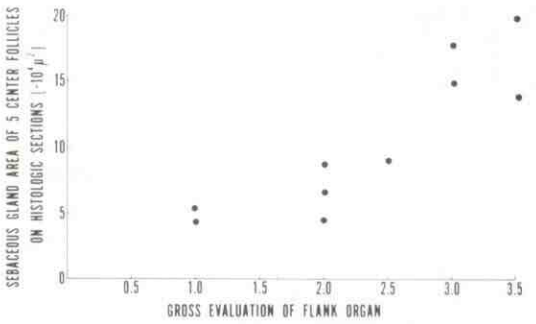

FIG. 10: The flank organs of 10 female hamsters who had been treated for varying times with topical applications of testosterone were assigned a 1 to 4 rating according to their size (pigmented diameter and fullness). "1" corresponded to the size of the flank organs of untreated female hamsters and "4" to their maximal size (see legend to Table). The animals were then sacrificed and H and E stained histologic sections of the flank organs were prepared. Projected images of the sebaceous glands of the five center follicles in the flank organ were traced and their areas measured with a planimeter as described previously for epidermal areas [13].

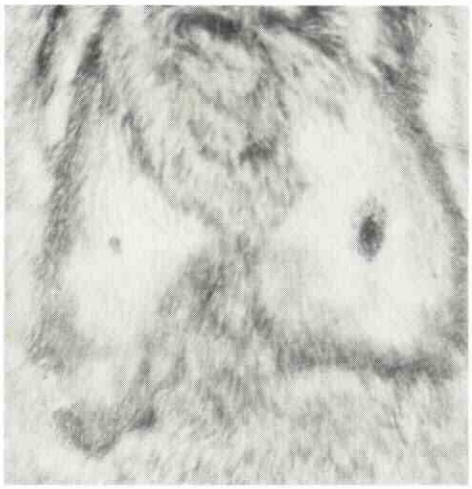

FIG. 11: Normal adult female hamster. Fifteen days after the application 5 days/week of 25 μg of testosterone in acetone to the right flank organ and acetone to the left flank organ.

The response of intact adult male hamsters to exogenous androgens clearly shows that their flank organs are not maximally stimulated by androgen levels obtained by endogenous production. Nevertheless, the rapid decrease in the labeling index of sebaceous glands following castration confirms the necessity for continual androgen stimulation for the maintenance of their characteristic size and rate of cell replacement. This is in accord with the gross morphologic changes observed and with the previously described gross observations of Hamilton and Montagna [11]. The present studies confirm that the female flank organ is as responsive as that of the male to hormonal stimulation [11], and that females can easily be used in place of castrated males to study the effects of topically applied androgens. This may prove useful in the study of hormonal inhibitors since it will be possible to vary the hormone/inhibitor dose ratio, resulting in a more controlled situation than with the use of intact males [12].

The rapid increase in the labeling index of the flank organ of male castrates on administration of androgens is consistent with previously reported biochemical findings [14] and confirms the ex-

pected rapid increase in cell turnover despite the previously reported paucity of mitotic figures after 5 days of androgenic stimulation [11]. Increases in labeling indices of sebaceous gland germinative cells following systemic androgen administration have also been demonstrated in humans [15]. As in the present work on hamsters, proliferative activity in the human sebaceous gland has also been shown to be limited to cells in the periphery of the acini [16].

In addition to the sebaceous gland hyperplasia, stimulation of flank organs by androgens results in increased pigmentation and hair growth. The dendritic pigment cells, present in the dermis around hair follicles, contain dense structures resembling stage IV melanosomes. These contain translucent areas similar to those described by Jimbow and Kukita [17]. Further studies are necessary to determine whether these pigment-containing cells are indeed dermal melanocytes.

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