

Effect of diethylstilbestrol on skin sterols of the male rat

L. HORLICK

Department of Medicine, University of Saskatchewan, University Hospital, Saskatoon, Saskatchewan, Canada

ABSTRACT Diethylstilbestrol (DES) was injected in doses ranging from 600 μg to 0.4 $\mu\text{g}/\text{kg}$ body weight into mature male rats over a 3 wk period. Profound effects on skin morphology and on sterol content of skin were noted. The sebaceous glands atrophied and the epidermis lost granularity. The concentrations of all skin sterols, with the exception of cholesterol, were reduced. At a dose level of DES of 4 $\mu\text{g}/\text{kg}$ there was still a perceptible reduction in the concentration of Δ^7 -cholestenol. Incubation of skin fragments with acetate- $2\text{-}^{14}\text{C}$ for 2 hr demonstrated a reduced uptake of ^{14}C into the non-saponifiable fraction of skin lipids at all dose levels studied. Preliminary thin-layer chromatography of the nonsaponifiable fraction revealed that the uptake of ^{14}C into cholesterol was only slightly decreased; uptake into cholesterol precursors was decreased somewhat more.

The epidermis and dermis were separated by incubation of skin with elastase and hyaluronidase. The epidermis contained at least three times as much sterol per mg dry weight as did the dermis. Unesterified cholesterol was the major sterol present in both layers; the other sterols were present mainly as esters. DES injection resulted in no change in the free sterol content but markedly reduced the ester content of the epidermis and dermis.

SUPPLEMENTARY KEY WORDS inhibition · sterol biosynthesis · epidermis · atrophy · sebaceous glands

THE SEBACEOUS GLANDS of the rat are known to be sensitive to both estrogens and androgens (1-3). Hooker and Pfeiffer (1) demonstrated that the administration of estradiol benzoate (83 $\mu\text{g}/\text{rat}$) twice weekly resulted in a retardation of body growth and a reduction in the size and number of sebaceous glands in both male and female rats. Ebling (2) demonstrated a reduction in the number of cells in the sebaceous glands of immature rats with subcutaneous injections of 1.0 μg of estradiol ben-

zoate for 30 days. A dose of 100 μg resulted in a marked decrease in the size of the sebaceous glands. The decreased fat content of the fur of castrated rats was further lowered by estrone or estriol in daily doses of 0.5 mg (4).

The composition of skin surface lipids of the rat was studied by Nikkari (5). He administered 10-12 μg of ethinyl estradiol by mouth to four rats daily for 11 wk. Two rats showed a definite drop in body weight and sebum secretion, and two were unaffected. Several changes in the composition of the surface lipid were noted, none of them statistically significant, among which was an increase in cholesterol from 22.7% of total sterols in the control group to 32.9% in the group receiving ethinyl estradiol. There was a corresponding decrease in the other sterols, especially those of the C_{28-29} group (to 19.4%) as compared with the control group (22.7 and 26.6%, respectively), but very little change in Δ^7 -cholestenol secreted. The changes in the composition of the sterols were greatest in the sebum of the two rats that also exhibited weight loss and decreased secretion of sebum.

Kandutsch (6) found that castration reduced the ratio of Δ^7 -cholestenol to cholesterol in the male mouse, and that this ratio was significantly increased by injection of 15 μg of estradiol benzoate three times a week for 2-3 wk. No histological studies were reported, and the dose may have been inadequate to suppress sebaceous secretion. Wilson (7) compared the effects of testosterone,

Abbreviations: GLC, gas-liquid chromatography; TLC, thin-layer chromatography; 7DH, 7-dehydrocholesterol; KR, Krebs-Ringer phosphate; DES, diethylstilbestrol.

Systematic names of sterols referred to in the text by their trivial names only are as follows. Cholesterol, 5-cholesten- 3β -ol; Δ^7 -cholestenol, Δ^7 -cholesten- 3β -ol; 7-dehydrocholesterol, $\Delta^5,7$ -cholestadien- 3β -ol; methostenols, 4α -methyl- Δ^7 -cholesten- 3β -ol and 4α -methyl- $\Delta^7,24$ -cholestadien- 3β -ol; lanosterols, 4,4,14 α -trimethyl- Δ^8 -cholesten- 3β -ol and 4,4,14 α -trimethyl- $\Delta^8,24$ -cholestadien- 3β -ol.

progesterone, and estradiol on sterol synthesis in preputial gland and skin of rats. In preputial gland both testosterone and progesterone enhanced ester sterol synthesis to about the same degree whereas estradiol had little effect. In skin, testosterone and progesterone markedly accelerated the synthesis of sterol esters, whereas estradiol treatment resulted in a marked inhibition. The influence on free sterol synthesis was inconstant.

We report here the effects of a wide range of dosage of diethylstilbestrol on the quantitative changes in skin sterols of the male rat and on the biosynthesis of the sterols.

METHODS

Male rats of the Sprague-Dawley strain weighing 200–250 g at the start of the experiment were used. They were housed in individual cages and given food (calf starter pellets) and water ad lib. Records of daily food intake, feces production, and body weight were kept. The experimental animals received diethylstilbestrol (DES) in peanut oil intramuscularly in doses ranging from 0.4 to 600 $\mu\text{g}/\text{kg}$. Control animals received peanut oil only (0.25 ml). At the end of 3 wk of treatment (five injections per wk) the rats were killed and the dorsal skin was shaved, removed, and scraped free of subcutaneous muscle and fat. Duplicate 1 g samples were taken for sterol analysis and a standardized biopsy specimen was taken for histological study. The skin samples were incubated with 10 μC of acetate-2- ^{14}C (specific activity 10 $\mu\text{C}/\mu\text{mole}$) in 6 ml of Krebs-Ringer phosphate (pH 7.4) for 2 hr. The samples were digested in alcoholic KOH and the sterols were extracted and quantified as previously described (8). In most of the analyses, the nonsaponifiable material extracted from skin was studied directly by GLC on 1.16% SE-52 (methyl phenyl silicone) on Anakrom 100–110 mesh. The major sterols were well separated, but 7-dehydrocholesterol (7DH) was not separated from Δ^7 -cholestenol and is included in this fraction.

For a few animals preliminary chromatography was carried out on 20 \times 40 cm plates coated with Silica Gel G into which Rhodamine 6G had been incorporated. The solvent was benzene-ethyl acetate 20:1 and the plates were run in the dark for 24 hr. Several zones could be distinguished under UV light (Fig. 1). In order of decreasing R_f , they were as follows. Zone 1, which contained only one substance, was identified as squalene. Zone 2 contained dihydrolanosterol and lanosterol. Zone 3 contained a number of fatty alcohols as well as methostenol and dehydromethostenol. Zone 4 contained cholesterol and desmosterol. Zone 5, which could not be clearly separated from zones 4 and 6, was dark in UV

light; it contained small amounts of cholesterol, desmosterol, and Δ^7 -cholestenol, and especially 7DH. Because of incomplete separation of 7DH from Δ^7 -cholestenol or GLC, we have grouped it with the Δ^7 -cholestenol. Zone 6 contained most of the Δ^7 -cholestenol. Zone 7 contained no sterol material. The zones were scraped off separately the sterols were eluted and quantified by GLC, and their radioactivity was counted in a liquid scintillation spectrometer. Quenching was corrected for with an internal standard.

To determine the relative contributions of epidermis and dermis (containing the sebaceous glands) to the changes in sterol content observed after DES treatment we separated these layers by the enzymatic method of Giovanella and Heidelberger (9) in three control and three treated rats (75 μg of DES per kg body weight for 3 wk). Skin strips were incubated in a medium containing the enzymes hyaluronidase and elastase for periods between 4 and 24 hr. At the end of that time, the epidermis could be lifted or scraped off the dermis as a single sheet. Unfortunately, the enzymatic digestion of the tissues interfered with biosynthetic capacity and the *in vitro* uptake of acetate- ^{14}C into the sterols of epidermis and dermis could not be determined.

Duplicate strips of epidermis and dermis were exhaustively extracted with acetone in a Soxhlet extractor and the dry weight of the epidermis and dermis obtained in this way was used as a basis for calculating the sterol content of the strips that were incubated with acetate- ^{14}C and subsequently digested with alcoholic KOH.

The acetone-soluble lipids were separated by TLC in hexane-ethyl ether-acetic acid 85:15:1.5. The free and esterified sterol fractions were scraped from the plate and eluted. The sterol ester fraction was saponified and the free sterols were extracted and rechromatographed away from the alcohols on Silica Gel G in benzene-ethyl acetate 20:1. Percentages of sterol components were determined by GLC on a 1% DC-560 (chlorophenyl methyl silicone) column, which gave results identical to those obtained on 1.16% SE-52. The data were corrected for losses during extraction and chromatography by the use of an internal standard of cholesterol- ^{14}C .

The skin biopsy samples were embedded in paraffin and stained with hematoxylin and eosin. Sections were cut from the front, middle, and end of each block.

RESULTS

Skin Sterols

Data from groups A, B, and C (600, 300, 75 μg of DES per kg body weight) were pooled (Table 1) after inspection had revealed the results to be very similar. Doses of DES ranging from 600 to 0.4 $\mu\text{g}/\text{kg}$ failed to produce any significant changes in the cholesterol content of skin

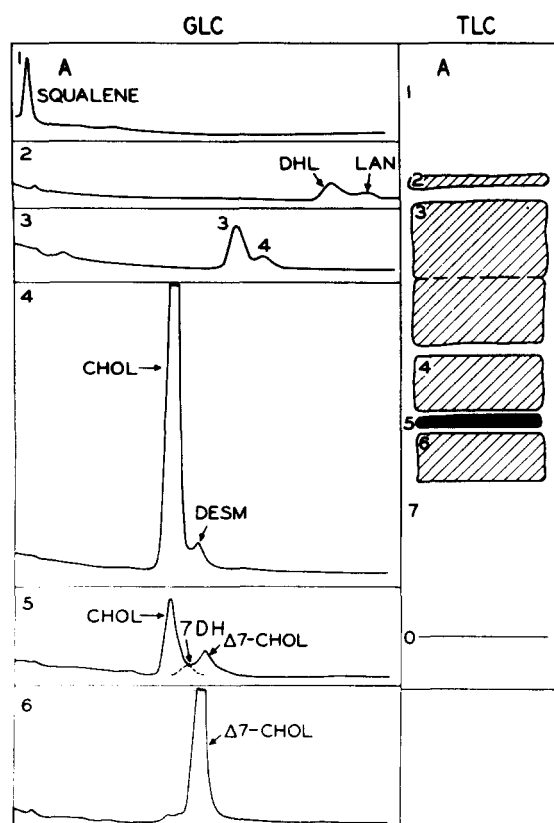


FIG. 1. TLC patterns and the corresponding GLC peaks of the nonsaponifiable lipids of skin in the normal rat (see text and reference 8). DHL, dihydrolanosterol; LAN, lanosterol; CHOL, cholesterol; DESM, desmosterol; Δ^7 -CHOL, Δ^7 -cholestenol. Fraction 3: peak 3, methestanol; peak 4, dehydromethostenol.

Δ^7 -cholestenol was by far the most sensitive indicator of DES effect; doses of DES from 600 down to 4 $\mu\text{g}/\text{kg}$ resulted in a highly significant ($P < 0.001$) reduction in the amount of Δ^7 -cholestenol per g of skin. Methostenols and lanosterols were less sensitive to the effect of DES, showing significant reduction in the dose range 600–18.75 $\mu\text{g}/\text{kg}$ only. Typical chromatograms are shown in Fig. 2.

Biosynthesis

The uptake of acetate- ^{14}C into the nonsaponifiable fraction of rat skin was significantly reduced at all dosage

levels studied (Table 2). The effect was least marked with the smallest dose (group F, 0.4 $\mu\text{g}/\text{kg}$). The inhibitory effect of DES on the uptake of radioactive acetate into skin sterols was also noted in vivo: in another group of animals, 200 μc of acetate- ^3H per 100 g body weight was injected intraperitoneally 24–48 hr before the animal was killed. The minced, 1 g samples of skin were incubated with 10 μc of acetate- ^{14}C . There was a significant reduction in the uptake of both ^{14}C and ^3H acetate into the nonsaponifiable fraction of the skin (Table 3).

The skin sterols were separated into seven fractions by TLC and the radioactivity of the individual fractions was assayed. There was a decrease in radioactivity of all sterol fractions both in vivo and in vitro. The decrease was least marked in F_4 (cholesterol) and most marked in F_2 (lanosterol) and F_{5-6} (cholesterol, 7DH, and Δ^7 -cholestenol) (Table 4).

Sterol Content of Separated Epidermis and Dermis (Table 5)

In the control (untreated) rats, the epidermis contained much more sterol per mg dry weight than did the dermis. Cholesterol was the major sterol present, and was mainly in the free form. Δ^7 -Cholestenol was almost as plentiful and there were lesser amounts of methostenols and lanosterols, all of these sterols being preponderantly in the ester form. The dermis contained lesser amounts of all of these sterols, and again cholesterol was the only one present mainly as free sterol.

After treatment with DES, sterol content of both epidermis and dermis was markedly reduced. There was no change in cholesterol in the epidermis, but all other fractions, especially the esterified portion, decreased. In the dermis all sterols, both free and ester fractions, decreased.

Body Weight and Organ Weight (Table 6)

600–18.75 $\mu\text{g}/\text{kg}$ doses of DES resulted in a very significant failure to gain weight; some of the treated rats weighed less at the end of the 3 wk than they did at the beginning. Doses of 4.0 and 0.4 $\mu\text{g}/\text{kg}$ did not prevent weight gain. Testes weight (expressed as gm/kg body-

TABLE 1 EFFECT OF DIETHYLSTILBESTROL ON SKIN STEROLS

Group	n	DES $\mu\text{g}/\text{kg}$ body wt.	Δ^5	Δ^7	M	L
			mg/g skin			
Control	8	0	1.27 \pm 0.19	1.20 \pm 0.12	0.70 \pm 0.19	0.40 \pm 0.20
ABC	9	600, 300, or 75	1.20 \pm 0.14	0.44 \pm 0.08*	0.19 \pm 0.06*	—†
D	6	18.75	1.10 \pm 0.14	0.54 \pm 0.11*	0.32 \pm 0.06*	0.15 \pm 0.12‡
E	8	4.0	1.03 \pm 0.10	0.84 \pm 0.12*	0.68 \pm 0.18	0.40 \pm 0.16
F	6	0.4	1.22 \pm 0.14	1.15 \pm 0.15	0.91 \pm 0.10	0.30 \pm 0.15

Values \pm SD. Δ^5 , cholesterol; Δ^7 , Δ^7 -cholestenol; M, methostenols; L, lanosterols.

* $P < 0.001$.

† None detected.

‡ $P = 0.02$.

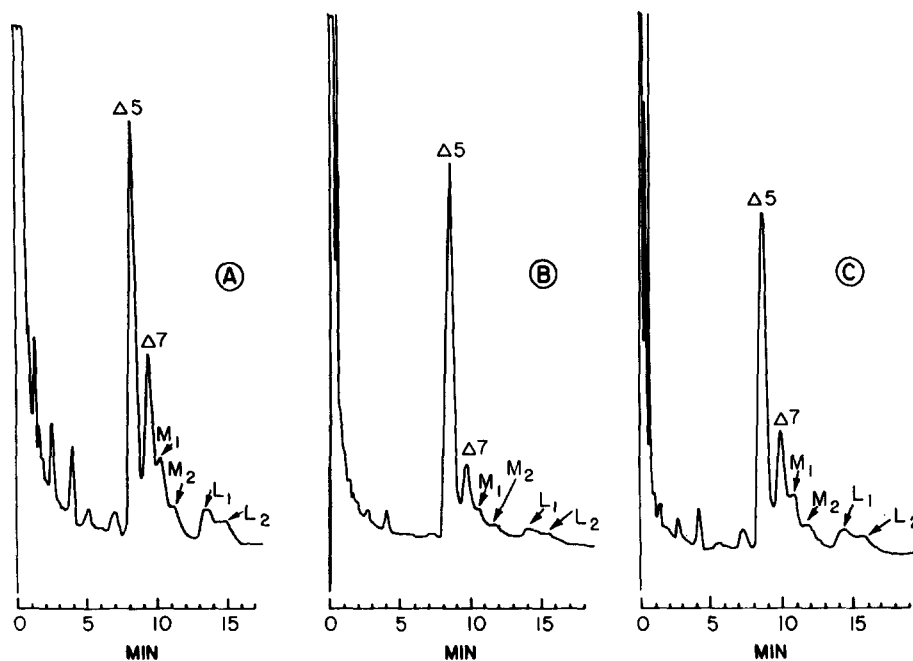


FIG. 2. GLC of skin sterols of control and DES-treated rats. Δ^5 , cholesterol; Δ^7 , Δ^7 -cholestenol; M_1 , M_2 , methostenols; L_1 , L_2 , lanosterols. F & M model 402 GC; columns 1.16% SE-52 (methyl phenyl silicone) on Anakrom (100–110 mesh), temp 230°C. A, control; B, DES 18.75 $\mu\text{g}/\text{kg}$ body weight; C, DES 4.0 $\mu\text{g}/\text{kg}$ body weight.

weight) was reduced at DES dosages ranging from 600 to 75 $\mu\text{g}/\text{kg}$.

Skin Morphology (Figs. 3–6)

Rats treated with the higher doses of DES (600–18.75 $\mu\text{g}/\text{kg}$) all showed gross changes relative to the controls. Their skin was often milky white and thinner than that of the controls. Specifically, the brownish deposit or discoloration commonly seen on the epidermal surface of the normal male rat was absent.

In the normal rat, the epidermis revealed a relatively thick, loose, and regular keratin layer. The granular layer was very prominent but the basal layers consisted only of one or two layers of cuboidal cells with occasional mitoses in the basal layer. The junction between epi-

dermis and dermis was a straight line with an almost inappreciable basement membrane. In the dermis, the appendages were seen in two layers. The hair follicles were most superficial and were associated with sebaceous glands. The bases of the hair follicles lay deeper in the dermis. The dermal connective tissue was well collagenized, dense, and eosinophilic.

In the highest dosage series (600–300–75–18.75 $\mu\text{g}/\text{kg}$) the keratin layer appeared irregular and somewhat more basophilic than in the controls, but parakeratosis was not seen. The granular layer was much reduced from its normal prominence. Some granules were still present but were not sufficient in number to obscure the details of the cells in which they lay. The viable layers of the epidermis were thinned and irregular but the basal layer was well preserved. In the dermis collagenization was good. Hair follicles were not reduced in number but they were smaller than normal. The sebaceous glands were reduced in number and their cells were small, with scanty cytoplasm.

There was considerable overlap between groups with the changes most marked in A, B, C, and least in D. Loss of epidermal granularity was the criterion easiest to grade on a dosage level. In groups E and F (4.0 and 0.4 $\mu\text{g}/\text{kg}$) the skin morphology did not differ from controls.

Treatment of the skin with elastase and hyaluronidase permitted the epidermis to be scraped or lifted off the dermis. Sections showed that epidermis only, and not its appendages, was removed. The sebaceous glands re-

TABLE 2 INCORPORATION OF ACETATE- ^{14}C INTO NON-SAPONIFIABLE FRACTION OF RAT SKIN

Group	Number	Dose of DES	Amount
			Incorporated
		$\mu\text{g}/\text{kg}$	$\text{cpm}/\text{g skin}$
Control	17	0	14,700 \pm 7,450
A	6	600	4,980 \pm 1,870*
D	12	18.75	3,810 \pm 1,730*
E	11	4.0	6,110 \pm 2,610*
F	5	0.4	8,010 \pm 854*

Duplicate 1 g samples of minced skin were incubated with 10 μC of acetate- ^{14}C in KR buffer, pH 7.4, for 2 hr.

* $P < 0.001$.

TABLE 3 INCORPORATION OF ACETATE-³H (IN VIVO)* AND ACETATE-¹⁴C (IN VITRO)† INTO NONSAPONIFIABLE FRACTION OF RAT SKIN

Group	n	Dose of DES	Nonsaponifiable Material	³ H Incorporated	¹⁴ C Incorporated
		μg/kg	mg/g	cpm/mg nonsaponifiable lipids	
Control	33	0	7.56 ± 3.05	760 ± 349	1,300 ± 549
Treated	33	250	5.45 ± 1.81	269 ± 149	397 ± 237
P			<0.01 > 0.001	<0.001	<0.001

Mean values ± sd.

* 200 μc of acetate-³H per 100 g body weight was injected intraperitoneally 24 hr before the animal was killed.

† 1 g of minced skin was incubated with 10 μc of acetate-¹⁴C in KR buffer, pH 7.4, for 2 hr.

TABLE 4 DISTRIBUTION OF RADIOACTIVITY AMONG THE SKIN STEROLS OF CONTROL AND STILBESTROL-TREATED RATS

Sterol Fraction	Incorporation into Skin Sterols							
	³ H*				¹⁴ C†			
	Control		Treated		Control		Treated	
	cpm	%	cpm	%	cpm	%	cpm	%
F ₁	100 ± 9.9	8.2	27.8 ± 7.2	5.3	17.5 ± 5.9	5.4	2.0 ± 2.2	1.9
F ₂	148 ± 72.7	12.2	35.7 ± 8.9	6.8	54.9 ± 22.5	16.8	4.1 ± 5.1	4.0
F ₃	142 ± 39.9	11.6	46.2 ± 6.8	8.8	41.5 ± 18.2	12.7	14.0 ± 3.2	13.5
F ₄	380 ± 58.5	31.1	284 ± 134	54.0	92.6 ± 22.9	28.4	56.0 ± 19.7	53.9
F ₅	216 ± 63.9	17.7	48.8 ± 7.8	9.3	74.2 ± 14.6	22.8	9.8 ± 6.4	9.4
F ₆	181 ± 45.6	14.9	42.2 ± 12.0	8.0	38.7 ± 10.4	11.9	11.0 ± 4.3	10.6
F ₇	52 ± 16.7	4.3	41.3 ± 5.7	7.8	6.7 ± 6.0	2.0	7.0 ± 4.0	6.7
Total cpm	1,219		526		326		104	

Mean values ± sd. n = six rats in control and stilbestrol-treated groups. F₁, squalene, hydrocarbons; F₂, lanosterol, dihydrolanosterol; F₃, methostenol, dehydromethostenol; F₄, cholesterol; F₅, cholesterol, 7-dehydrocholesterol, Δ⁷-cholestenol; F₆, Δ⁷-cholestenol; F₇, origin.

* ³H (in vivo) uptake: 200 μc of acetate-³H per 100 g body weight was injected intraperitoneally 24 hr before the animal was killed.

† ¹⁴C (in vitro) uptake: 1 g of minced skin was incubated with 10 μc of acetate-¹⁴C in KR buffer, pH 7.4 for 2 hr.

TABLE 5 FREE AND ESTER STEROL CONTENT OF EPIDERMIS AND DERMIS OF RAT SKIN

Group	n	Δ ⁵		Δ ⁷		M		L		Total	
		Free	Ester	Free	Ester	Free	Ester	Free	Ester	Free	Ester
<i>mg/g dry weight</i>											
<i>Epidermal sterols</i>											
Control	3	7.42	4.49	1.65	8.75	0.81	6.28	0.66	2.74	10.54	22.26
DES	3	8.22	4.24	1.25	3.10	1.03	1.18	0.89	1.15	11.39	9.67
<i>Dermal sterols</i>											
Control	3	2.03	1.26	0.13	3.19	0.21	2.16	0.19	0.98	2.56	7.59
DES	3	0.97	0.17	0.04	0.05	0.11	0.06	0.10	0.08	1.22	0.36

Δ⁵, cholesterol; Δ⁷, Δ⁷-cholestenol; M, methostenols; L, lanosterols.

TABLE 6 EFFECT OF DES ON BODY WEIGHT AND TESTIS WEIGHT

Group	n	DES	Body Weight		Testis Weight
			Initial	Final	
			μg/kg bw	g	g/kg bw
Control	17	0	256 ± 15	396 ± 36	8.8 ± 0.8
A, B	11	600 or 300	264 ± 10	246 ± 17*	3.4 ± 0.8*
C	6	75	258 ± 16	272 ± 21*	6.8 ± 1.6*
D	14	18.75	265 ± 23	272 ± 21*	8.0 ± 2.9
E, F	19	4.0 or 0.4	260 ± 12	367 ± 23	9.4 ± 1.0

Mean values ± sd; bw, body weight.

* P < 0.001 when compared to controls.

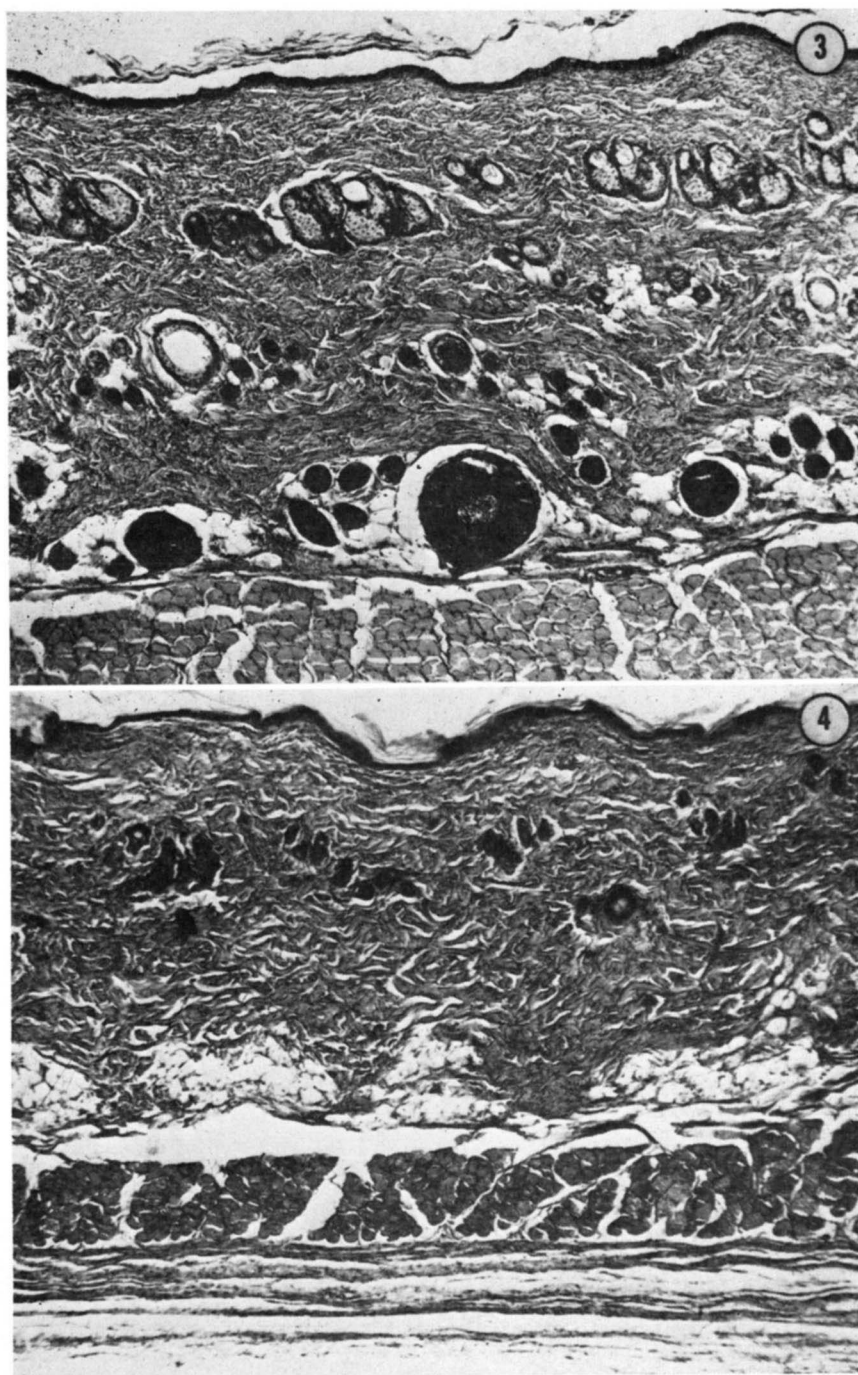


FIG. 3. Dorsal skin of male rat. Control (placebo) group. Hematoxylin-eosin, $\times 80$. Note well-defined sebaceous glands.

FIG. 4. Dorsal skin of male rat treated with stilbestrol ($18.75 \mu\text{g}/\text{kg}$ body weight) for 3 wk. Hematoxylin-eosin, $\times 80$. Sebaceous glands are almost completely atrophied.

mained behind. Histological detail was often poor because of digestion of tissue by the enzymes (Figs. 5 and 6).

DISCUSSION

DES over a wide dosage range ($600\text{--}0.4 \mu\text{g}/\text{kg}$ body weight) had profound effects on the structure and sterol

content of the dorsal skin of male rats.

Doses ranging from 600 to $18.75 \mu\text{g}/\text{kg}$ resulted in failure to gain weight and in testicular atrophy. They also produced marked morphological changes, atrophy of the sebaceous glands and loss of granularity in the epidermis being the most important. All skin sterols, with the exception of cholesterol, were reduced in concentra-

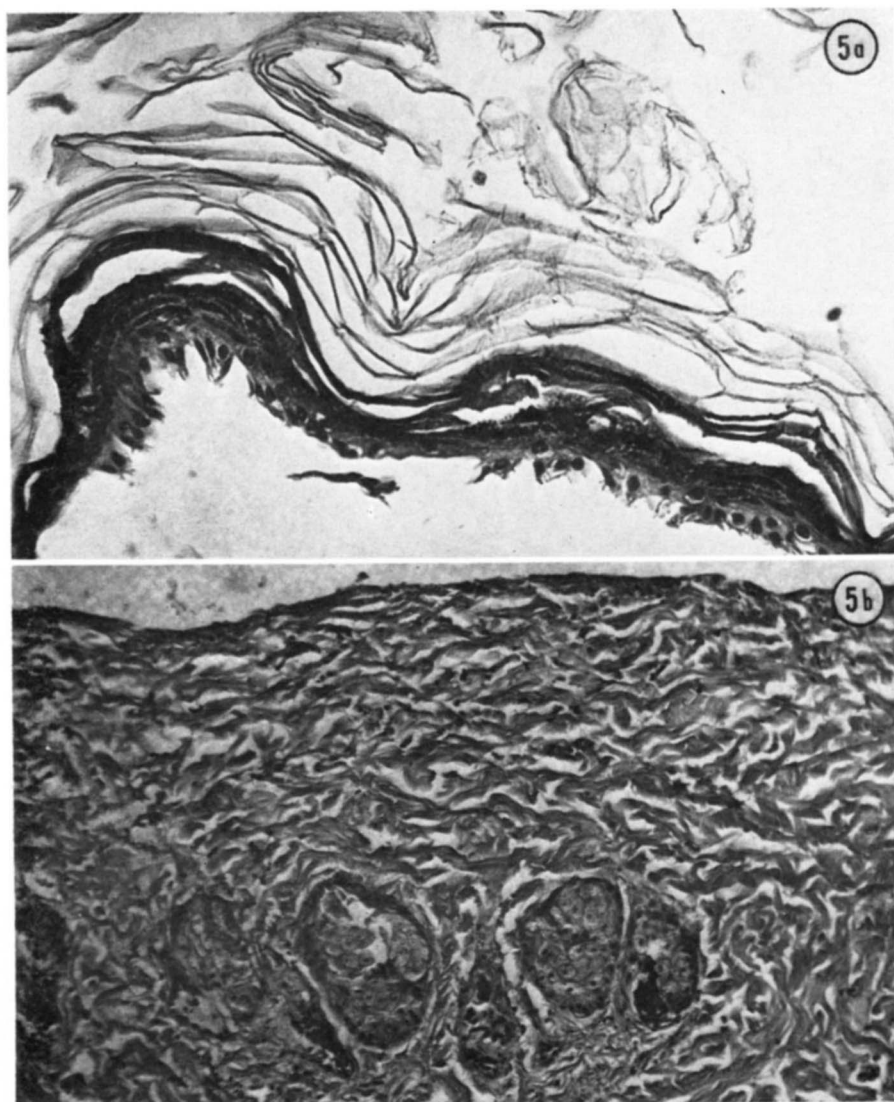


FIG. 5. Separated epidermis and dermis from control rat. a, epidermis (hematoxylin-cosin, $\times 325$); b, dermis (hematoxylin-cosin, $\times 135$). Note heavy keratin layer and pigment in epidermis and well-preserved sebaceous glands in dermis.

tion as was the *in vitro* uptake of acetate- ^{14}C into the nonsaponifiable fraction of skin lipids. At doses below $18.75 \mu\text{g}/\text{kg}$ a variable response was seen. Thus body weight and testicular weight were not affected, and gross and microscopic changes in skin structure were not seen. At a dose level of $4.0 \mu\text{g}/\text{kg}$ there was still a perceptible reduction in the concentration of Δ^7 -cholestenol. Reduced uptake of acetate- ^{14}C into skin nonsaponifiable lipid was still significant at the lowest dosage levels studied (4.0 and $0.4 \mu\text{g}$).

The most sensitive criteria of DES action on skin seem, then, to be the uptake of acetate- ^{14}C into the nonsaponifiable fraction and the content of Δ^7 -cholestenol in the skin.

Our biosynthetic studies showed a slight effect of DES on uptake of acetate- ^{14}C into cholesterol; this correlated

well with the failure to detect any major reduction in skin content of cholesterol. On the other hand significant reduction of uptake of acetate- ^{14}C into lanosterols, and into the combined 7DH and Δ^7 -cholestenol fraction was observed, together with a decline in the total amount of these sterols. Thus DES depressed the synthesis of sterols on the synthetic pathway up to and including Δ^7 -cholestenol, but did not affect the synthesis of cholesterol itself.

The experiment on separated epidermis and dermis indicated the higher sterol content of the epidermis. Cholesterol and Δ^7 -cholestenol were the major sterols present in both epidermis and dermis. Cholesterol was principally in the free form whereas Δ^7 -cholestenol, methostenols, and lanosterols were in the ester form. DES did not affect the free sterol content of epidermis but markedly

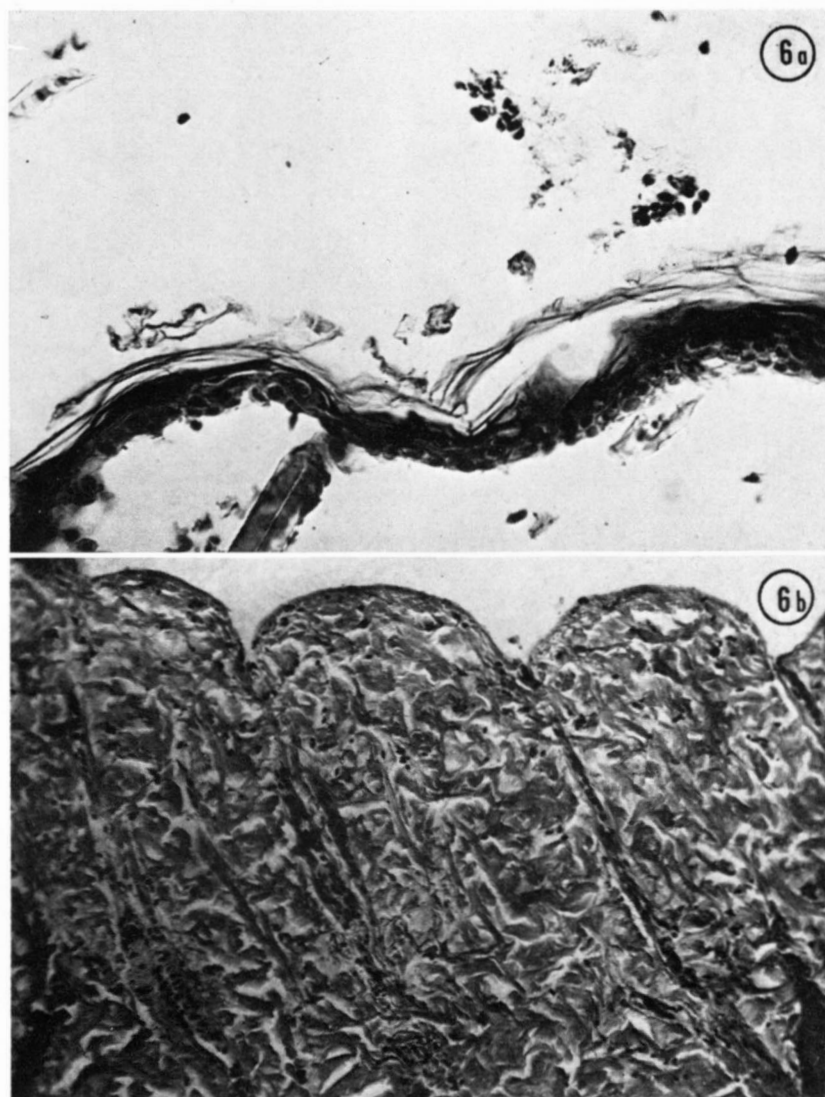


FIG. 6. Separated epidermis and dermis from DES-treated rat. a, epidermis (hematoxylin-eosin, $\times 325$); b, dermis (hematoxylin-eosin, $\times 135$). Note thin layer of keratin and relative absence of pigment in epidermis, as well as atrophic sebaceous glands in dermis.

decreased the ester sterols. These results are very similar to those previously reported by Wilson (7), who found that estradiol markedly inhibited sterol synthesis in rat skin, that this was most marked in the ester sterol fraction, and that Δ^7 -cholestenol was the sterol most affected.

Previous studies (7, 8) have indicated the presence of two pathways of cholesterol synthesis in rat skin: the Bloch pathway in which the nuclear transformations occur prior to reduction of the side chain of the sterol intermediates, and the Kandutsch-Russell pathway in which the side chain is reduced prior to nuclear transformations. The intermediates and end products in the Bloch pathway are in the free form, whereas those in the Kandutsch-Russell pathway are mainly in the ester form. Wilson (7) suggested that estradiol affected principally the Kandutsch-Russell pathway of biosynthesis,

that it acted somewhere between mevalonic acid and squalene, and that the latter might be a rate-limiting factor for sterol synthesis in skin.

Histologic examination of the skin of DES-treated rats revealed thinning of the epidermis, degranulation, and virtual atrophy of the epidermal appendages, especially the sebaceous glands. Thus one would expect a marked diminution of sebum secretion. Studies on isolated sebaceous glands in man indicate that the major constituents of sebum are squalene, triglycerides, and waxes (10). Only minute amounts of cholesterol have been recovered. No data are available on isolated sebaceous glands in the rat. Brooks, Lalich, and Baumann (11) observed with histological techniques the presence of Δ^7 -sterols in rat sebaceous glands. Gaylor and Sault (12) demonstrated that most of the 7DH and fast-acting

sterols of rat skin were localized in the sebaceous glands and layers of dead keratin, and that relatively little was present in the Malpighian layer of the epidermis and in the dermis (free of sebaceous glands). In their experiment the epidermis was removed with a keratotome. In our hands it proved impossible to remove the epidermis without some sebaceous contamination even when the keratotome was set for its finest setting, whereas the enzymatic method of separation resulted in a well-defined separation of epidermis from dermis, the sebaceous glands, and hair follicles remaining behind in the dermis.

Comparison of our data with those of Gaylor and Sault reveals a marked discrepancy with respect to the Δ^7 -sterol. We found the concentration of Δ^7 -sterol highest in the epidermis, whereas Gaylor and Sault found it in the dermis, specifically in the sebaceous glands.

DES produced histological changes in both epidermis (thinning, degranulation) and dermis (atrophy of sebaceous glands). The concentration of noncholesterol sterols, especially Δ^7 -cholestenol, was reduced in both skin layers. It is impossible from the evidence at hand to establish whether DES primarily affects sebaceous glands and has only a secondary effect on epidermis resulting from a deficiency of sebum. If the role of sebum is in fact to oil and protect the epidermis, and possibly to provide squalene, a precursor of sterols, then a deficiency of sebum could produce the changes we have noted. Therefore we must conclude that our results do not clearly identify the site of synthesis of the noncholesterol sterols of skin, which could originate in either epidermis or sebaceous glands. Whatever the ultimate source of these sterols, DES acts to reduce their synthesis by way of the Kandutsch-Russell pathway.

The precise manner in which estrogens induce a shrinkage of the sebaceous glands is not yet clear. Ebling's observations in the mouse suggest that estrogens do not compete directly with androgens at the sebaceous

gland. The increased mitotic activity due to androgens is not suppressed by simultaneous administration of estrogens (13, 14). Ebling believes that estrogens cause an increased breakdown of sebaceous cells, which reduces their time of passage through the gland and prevents their accumulation of lipids. The effect of estrogens, unlike that of androgens, does not depend on the presence of an intact pituitary. Since the estrogen effect may also be seen in adrenalectomized female rats (14) and in castrated males (15) it is unlikely to be due to interference with endogenous androgen production.

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