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MORPHOMETRIC EVALUATION OF SEBACEOUS GLAND VOLUME IN INTACT, CASTRATED, AND TESTOSTERONE-TREATED RATS

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Starting at 19 weeks of age six male rats, castrated at 16 weeks, were injected intramuscularly three times per week with 0.25 mg testosterone propionate (dissolved in sesame oil) per 100 grams body weight. Four castrated control rats received sesame oil only. Samples of dorsal skin were taken under light ether anesthesia at the time of orchidectomy and at weekly intervals during treatment. Histologic sections were cut perpendicular to the skin surface, stained with hematoxylin and eosin, and analyzed morphometrically for their volume content of sebaceous glands. A square grid of 42 sampling points was superimposed on each microscopic field at a magnification of 300× and an average of about 1000 points were counted over the entire thickness of the skin in each section. The average thickness of the skin was also determined for each sample. Three weeks after castration the mm^3 of sebaceous glands per cm^2 of skin was reduced from 1.76 to 0.96, a difference that is statistically significant at $p < 0.005$. One week after starting testosterone injections, the volume of sebaceous glands was more than double that in the control animals: 3.0 vs 1.2 ($p < 0.02$). Even greater differences were found at 2 to 4 weeks of treatment.

Gonadal androgens are the primary hormones involved with the development of sebaceous glands in both rat [1] and man [2]. Administration of testosterone to the sexually mature rat further increases the size of sebaceous glands [3,4]. Quantitative determinations of the size of rat sebaceous glands after parenteral testosterone treatments have been accomplished by cell counts [1] and by planimetric measurements on projections of serial sections [3,5]. It has been shown that sebum production is correlated with the size of the holocrine sebaceous glands [6]. Thus, the measurement of ether-extractable hair fat in the rat [7] and skin surface lipids in man [8] may be considered indirect indices of sebaceous gland size, at least whenever the rate of cell breakdown is constant. Recently a newer approach to morphometry, based on the principles of stereology [9], was used to measure testosterone-induced changes in rat skin [4]. This morphometric method has the practical advantage that it can be applied to routine histologic sections both easily and with optimum efficiency. The present study is an application of this method to examine quantitatively the effects produced by orchidectomy and subsequent testosterone injections on the sebaceous glands of young sexually mature rats.

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MATERIALS AND METHODS

Ten young adult male Holtzmann rats weighing 410–430 gm were castrated at 16 weeks of age. Starting 3 weeks later, 6 of these animals were injected intramuscularly 3 times per week for 4 weeks with 0.25 mg of testosterone propionate dissolved in 0.05 ml of sesame oil per 100 gm body weight. Four control rats received sesame oil only. All rats were maintained on routine laboratory chow and 3 weeks after orchidectomy had gained an average of 10 gm each. During the 4 weeks of injections, testosterone-treated rats gained an average of 17 gm in weight while control animals gained an average of 1 gm.

Biopsies of dorsal skin, approximately 5 × 10 mm, were taken under light ether anesthesia at the time of orchidectomy and at weekly intervals during treatment. Histologic sections were carefully oriented perpendicular to the skin surface, stained with hematoxylin and eosin, and analyzed morphometrically for their volume content of sebaceous glands. The morphometric measurements were done by surveying contiguous microscopic fields covering the skin section at a magnification of 300×, as illustrated in Figure 1*A*. A typical sample contained cross sections of about 20 individual sebaceous glands. A square grid of 42 sampling points [10] was superimposed on each field by means of an ocular reticle (Wild Heerbrugg Instruments, Inc. #105844), as shown in Figure 1*B*. In each section an average of approximately 1000 points was counted over the entire thickness of the skin, including epidermis, dermis, and the thin layer of subcutaneous adipose tissue. The total number of such points, P_{skin}, and the number of those points overlying cross sections of sebaceous gland cells, P_{sg}, were recorded and then used to calculate the volume fraction, $V_{V_{sg}}$, of sebaceous glands in the skin:

$$V_{V_{sg}} = \frac{P_{sg}}{P_{skin}} \quad (1)$$

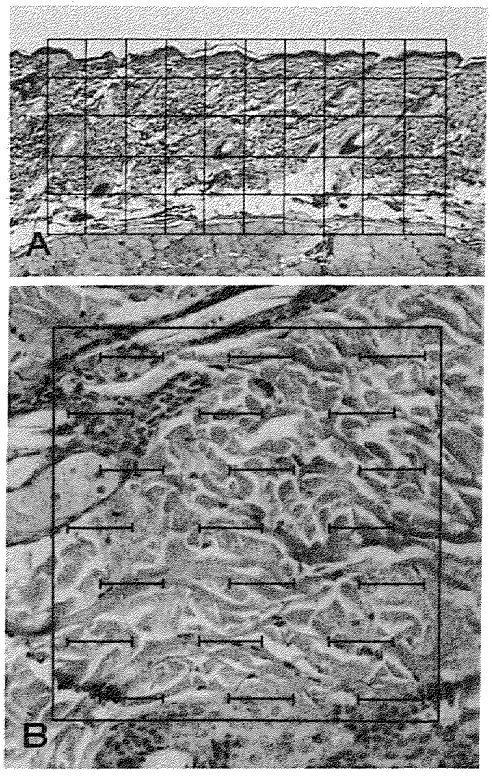

FIG. 1. *A*: Mid-dorsal skin cut perpendicular to the body surface from a rat treated 1 week with testosterone. Structures in the skin were measured morphometrically in contiguous microscopic fields, represented by the sequence of superimposed squares covering the entire dermal and epidermal layers. A typical square at the higher magnification used is shown in Fig 1*B* (× 16). *B*: Each area of skin cross section was assayed with this square array of 42 points, indicated by the marked ends of the line segments. The set of points was applied to contiguous fields, as illustrated in *A*, until approximately 1000 points overlying cutaneous structures had been counted. At this magnification one square field covers a tissue area of 90,000 μm^2. Thus, each point is equivalent to 2140 μm^2 and a total sample of 1000 points represents approximately 2.14 mm^2 of skin cross section (× 162).

The average thickness of the skin, t, in mm was also determined in each biopsy by means of an eyepiece micrometer. At least 10 measurements were made at intervals of 1 mm along the length of several sections and an average value was calculated for each tissue sample. Finally, the absolute volume of sebaceous glands, $V_{A_{sg}}$, in mm^3 per cm^2 of skin area was calculated from the equation:

$$V_{A_{sg}} = 100tV_{v_{sg}} \quad (2)$$

RESULTS

Table I shows the changes in the individual and average values measured in 10 adult male rats immediately before orchidectomy and 3 weeks later. The volume percent of sebaceous glands decreased to less than one-half the normal value. At the same time the average thickness of the skin increased approximately 16%. Both of these changes are statistically significant at the level of $p < 0.0005$. The figures in the last two columns of Table I are obtained by multiplying the corresponding numbers in the preceding columns of data, and show the calculated volume of sebaceous glands in mm^3 per cm^2 of mid-dorsal skin surface. These values are significantly different at $p < 0.005$.

The 10 orchidectomized rats were divided into groups of 6 and 4 to receive injections of testosterone propionate and carrier sesame oil, respectively. The volume percent of sebaceous glands measured at weekly intervals is shown in Table II. Testosterone injections produce a marked and continuous rise in the mean partial volume of sebaceous glands well above normal levels, while the values in control rats remain below normal. Much of the variation seen from week to week in individual rats, particularly among the controls, results from the small number, P_{sg}, of sebaceous gland points in each skin section. However, the difference between experimental and control animals is highly significant statistically at 1 week and becomes more pronounced in later biopsies. Table III shows that the changes observed in sebaceous gland volume during testosterone treatment were not paralleled by changes in skin thickness. Throughout the experimental period the average thickness of dorsal skin in all rats drifted toward lower values but no significant differences were found between testosterone-treated and control groups.

Calculated values of sebaceous gland volume per unit area of skin are summarized graphically in Figure 2. Vertical bars indicate plus and minus one standard error. The difference between testosterone-treated and control animals is significant at $p < 0.02$ after 1 and 2 weeks of injections and $p < 0.002$ after 3 and 4 weeks. During all 4 weeks of treatment the mean values are not significantly different from each other in either the steroid-treated or control groups. Thus, the full effect of the testosterone treatment is already observable after only 1 week of injections.

DISCUSSION

The principal value of the present studies is their demonstration of an efficient quantitative morphologic technique for evaluating the response of sebaceous glands to experimental treatment. Orchidectomy was shown to reduce sebaceous gland volume nearly 50% and the subsequent administration of testosterone induced a 3- to 5-fold increase above control levels. The latter effect was statistically significant after only 1 week and the injection of 0.75 mg of hormone. These results confirm the observation of Ebling [3] who administered 0.2 mg of testosterone per day to castrated male rats and found after 3 weeks that the volume of an average

TABLE I. *Effect of castration on the volume of sebaceous glands in the dorsal skin of adult male rats*

Rat	Volume percent		Skin thickness (mm)		$\frac{mm^2 \text{ Sebaceous glands}}{cm^2 \text{ skin}}$	
	Precastration	3 Weeks post castration	Precastration	3 Weeks post castration	Precastration	3 Weeks post castration
1	1.8	0.6	1.01	1.18	1.82	0.71
2	1.2	0.9	1.15	1.23	1.38	1.11
3	2.7	0.2	1.09	1.22	2.94	0.24
4	1.4	1.3	1.09	1.24	1.53	1.61
5	1.4	1.5	1.25	1.52	1.75	2.28
6	1.7	0.9	1.30	1.32	2.21	1.19
7	2.0	0.1	1.04	1.18	2.08	0.12
8	1.3	0.4	0.86	1.06	1.12	0.42
9	1.5	0.7	1.15	1.28	1.73	0.90
10	1.1	0.7	0.94	1.42	1.03	0.99
Mean	1.61	0.73	1.09	1.27	1.76	0.96
S.E.	0.15	0.14	0.04	0.04	0.18	0.21
	$p < 0.0005$		< 0.0005		< 0.005	

TABLE II. *Effect of testosterone injections on the volume percent of sebaceous glands in rat dorsal skin*

Rat	Treatment	Duration of treatment (age), in weeks				
		0 (19)	1 (20)	2 (21)	3 (22)	4 (23)
1	testosterone	0.6	2.4	2.6	2.7	3.3
2	"	0.9	2.7	4.2	3.0	2.8
3	"	0.2	2.4	3.6	3.5	3.3
4	"	1.3	2.6	3.7	3.4	4.7
5	"	1.5	3.2	4.2	3.7	4.5
6	"	0.9	1.8	1.1	3.6	3.5
Mean		0.90	2.52	3.23	3.32	3.68
S.E.		0.19	0.19	0.49	0.16	0.31
7	control	0.1	2.0	2.0	1.0	0.7
8	"	0.4	1.6	0.5	0.3	0.9
9	"	0.7	0.3	1.5	1.9	0.2
10	"	0.7	0.7	0.9	0.3	0.7
Mean		0.48	1.15	1.23	0.88	0.63
S.E.		0.14	0.40	0.33	0.38	0.15
p[a]		< 0.2	< 0.01	< 0.01	< 0.0005	< 0.0005

[a] Student's *t*-test of the difference between the means of testosterone-treated and control rats

sebaceous gland increased from 368×10^3 μm^3 to 686×10^3 μm^3. Similarly, Haskin et al [5] reported a 4-fold increase in the average volume of individual sebaceous glands in mature spayed female rats treated with intramuscular testosterone, 1.0 mg per day for 30 days. A recent autoradiographic study using ^{3}H-thymidine in the hamster indicates a significant decrease in the labeling of cells at the periphery of sebaceous glands of the flank organ following orchidectomy, and as much as a 4-fold increase in the cell proliferation rate 5 hr after a single massive injection of testosterone [11].

Two properties of skin were measured *directly* in these experiments: (1) the volume fraction (or volume percent) of sebaceous glands, which varied in response to testosterone injections, and (2) skin thickness, which also varied but apparently independently of the exogenous androgen. A quantitative evaluation of skin necessarily includes both groups of data. These two properties are combined in the equation for $V_{A_{sg}}$ to yield the absolute volume of sebaceous glands as a single numerical characteristic of rat dorsal skin. Furthermore, this characteristic is expressed with respect to the relatively stable parameter of skin surface area.

The technique of quantitative morphometry uti-

TABLE III. *Thickness measurements of rat dorsal skin during testosterone and control injections (mm)*

Rat	Treatment	Duration of treatment (age), in weeks				
		0 (19)	1 (20)	2 (21)	3 (22)	4 (23)
1	Testosterone	1.18	0.87	0.90	0.80	0.60
2	"	1.23	1.36	1.19	0.69	0.77
3	"	1.22	0.98	1.15	0.69	0.59
4	"	1.24	0.98	1.24	0.87	0.81
5	"	1.52	1.61	0.95	0.88	1.00
6	"	1.32	1.32	0.75	0.84	0.85
Mean		1.29	1.19	1.03	0.79	0.77
S.E.		0.05	0.12	0.08	0.05	0.06
7	control	1.18	0.94	0.93	0.66	0.74
8	"	1.06	0.96	1.04	0.94	0.81
9	"	1.28	1.36	1.07	0.87	1.24
10	"	1.42	1.47	0.81	0.75	0.90
Mean		1.24	1.18	0.96	0.81	0.92
S.E.		0.08	0.14	0.05	0.07	0.12

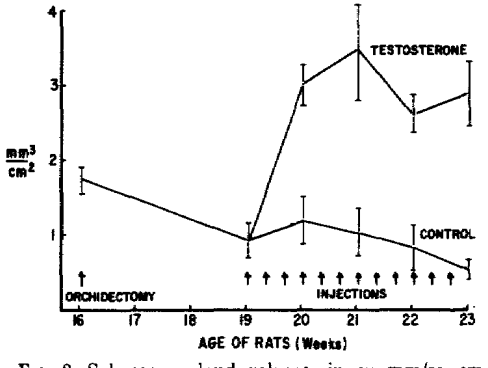

FIG. 2. Sebaceous gland volume, in cu mm/sq cm dorsal skin surface in the rat. Arrows indicate the initial event of orchidectomy and the triweekly injections of testosterone propionate in experimental animals or carrier sesame oil in the controls. Vertical bars represent plus and minus one standard error of the data.

lized here has been well justified in theory [12] and illustrated in previous applications to skin [4] and other tissues [13–15]. It is based on the principle that the volume fraction of a component in tissue is numerically equal to the fractional area of that component seen in random cross sections. Such areas are most easily measured by superimposing a regular point lattice over the tissue section, or its image in a microscope, and counting the fractional number of points that overlie transections of the component structure. As a method for measuring cross-sectional area, point counting is superior to planimetry in that it requires little apparatus and few special skills. It is an easily defined and executed operation, namely, the counting of those points associated with an identified structure. The results are numerical and subject also to statistical tests of significance.

In contrast to planimetry, the morphometric method enables one to reduce the precision of individual measurements in return for increased efficiency [16]. Because the point lattice constitutes a subsample of the tissue section, just as the section itself is a subsample of the tissue, the extent of sampling can be readily adjusted to obtain any degree of accuracy and to assure a truly representative assay. Accuracy is improved by increasing the number of points counted, preferably by increasing the number of fields to which the lattice is applied rather than by increasing the number of points in the lattice. It has been shown [17] that point counting efficiency is optimized by matching the standard deviation in each sample, as estimated by the square root of the total counts collected, to the standard deviation among equivalent samples. In the present study, for example, the standard deviation among the measurements of the volume percent of sebaceous glands in 10 rats at 16 weeks of age is 0.47 (S. E. $\sqrt{n}$ = 0.15 $\sqrt{10}$ = 0.47). This figure is 30% of the mean value of 1.61. Since approximately 1000 points were examined over each skin section, the number of sebaceous gland points collected per section was only about 16. Though this sample seems small, its standard deviation (given by $\sqrt{16}$) is 25% and is still less than the overall 30% variation. Thus, improved accuracy in the estimation of the mean will be obtained more efficiently by assaying tissues from additional animals than by expending effort to measure individual biopsies with greater precision. Increasing the number of animals assayed would have led to an improved estimate of the mean sebaceous gland volume as indicated by its standard error. However, with the large differences induced by castration and subsequent testosterone treatments the number of rats used and the number of points counted were quite sufficient to obtain results with a high degree of statistical significance.

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REFERENCES

1. Ebling FJ: Sebaceous glands. I. The effect of sex hormones on the sebaceous glands of the female albino rat. J Endocrinol 5:297–302, 1948
2. Strauss JS, Pochi PE: The human sebaceous gland: its regulation by steroidal hormones and its use as an end organ for assaying androgenicity in vivo. Recent Prog Horm Res 19:385–444, 1963
3. Ebling FJ: The action of testosterone on the sebaceous glands and epidermis in castrated and hypophysectomized male rats. J Endocrinol 15:297–306, 1957
4. Sauter LS, Weibel ER: Morphometric evaluation of skin structures by stereologic methods: application to testosterone-treated rats. Dermatologica 143:174–183, 1971
5. Haskin D, Lasher N, Rothman S: Some effects of ACTH, cortisone, progesterone and testosterone on

sebaceous glands in the white rat. J Invest Dermatol 20:207–211, 1953
6. Miescher G, Schonberg A: Untersuchungen über die Funktion der Talgdrüsen. Bull Schweiz Akad Med Wiss 1:101–114, 1944
7. Ebling FJ, Skinner J: The measurement of sebum production in rats treated with testosterone and oestradiol. Br J Dermatol 79:386–392, 1967
8. Strauss JS, Pochi PE: The quantitative gravimetric determination of sebum production. J Invest Dermatol 36:293–298, 1961
9. Weibel ER, Elias H (eds): Quantitative Methods in Morphology. New York, Springer-Verlag, 1967
10. Weibel ER, Kistler GS, Scherle WF: Practical stereological methods for morphometric cytology. J Cell Biol 30:23–38, 1966
11. Frost, P, Giegel JL, Weinstein GD, Gomez EC: Biodynamic studies of hamster flank organ growth: hormonal influences. J Invest Dermatol 61:159–167, 1973
12. Underwood EE: Quantitative Stereology. Reading, Mass, Addison-Wesley Publishing Company, 1970
13. Chalkley HW: Method for the quantitative morphologic analysis of tissues. J Natl Cancer Inst 4:47–53, 1943
14. Weibel ER: Morphometry of the Human Lung. New York, Springer-Verlag, 1963
15. Loud AV: A quantitative stereological description of the ultrastructure of normal rat liver parenchymal cells. J Cell Biol 37:27–46, 1968
16. Hilliard JE, Cahn JW: An evaluation of procedures in quantitative metallography for volume-fraction analysis. Trans AIME 221:344, 1961
17. Hennig A: A critical survey of volume and surface measurement in microscopy. Zeiss Werkzeitschrift 30:3, 1958