

We have observed earlier that total body lipid is diminished in scorbutic guinea pigs and that insulin treatment has a beneficial effect(14). The decreased plasma triglycerides in scorbutic guinea pigs might be due to combined effects of decreased total body lipid and increased mobilization of NEFA from fat depots. Insulin and ascorbic acid corrected the diminished plasma triglycerides probably by increasing lipid synthesis(14) and by re-esterifying the fatty acids.

The role of plasma phospholipids in scurvy is rather obscure. The increased value obtained in scurvy could not be corrected either by insulin treatment or by ascorbic acid supplementation. Since lipid synthesis is decreased in scurvy, the available fatty acids are transported more as phospholipids. After insulin treatment or ascorbic acid supplementation, though there was increased fat synthesis it is possible that there was diversion of fatty acids towards phospholipid formation in a preferential way. Insulin and ascorbic acid seem to have no specific role in the level of plasma phospholipids.

Summary. The different fractions of plasma lipids were estimated in normal, scorbutic, insulin treated scorbutic and ascorbic acid supplemented scorbutic guinea pigs. Beta lipoprotein : alpha lipoprotein ratio, beta lipoprotein cholesterol, phospholipids and NEFA of plasma were increased and plasma triglycerides diminished in scorbutic animals. While insulin treatment of the scorbutic animals corrected only the plasma

NEFA and triglyceride values, ascorbic acid supplementation brought back all these values to normal levels. Total plasma cholesterol did not change in scurvy. Ascorbic acid supplementation lowered the plasma cholesterol value below the value seen in normal guinea pigs, indicating hypocholesteremic effect of ascorbic acid. Neither insulin nor ascorbic acid seem to have specific roles in the metabolism of phospholipids.

1. Banerjee, S., Singh, H. D., *J. Biol. Chem.*, 1958, v233, 334.
2. Banerjee, S., Ghosh, P. K., *Am. J. Physiol.*, 1960, v195, 1064.
3. Waddell, Wm. R., Geyer, R. P., *Proc. Soc. Exp. Biol. and Med.*, 1957, v96, 251.
4. Banerjee, S., *Nature*, London, 1944, v153, 344.
5. Banerjee, S., Ghosh, N. C., *J. Biol. Chem.*, 1947, v168, 207.
6. Dole, V. P., Bierman, E. L., Roberts, T. N., *J. Clin. Invest.*, 1957, v36, 884.
7. Banerjee, S., *J. Biol. Chem.*, 1945, v159, 327.
8. Abell, L. L., Levy, B. B., Brodie, B. B., Kendall, F. E., *ibid.*, 1952, v195, 357.
9. Dole, V. P., Meinertz, H., *ibid.*, 1960, v235, 2595.
10. Youngberg, G. E., Youngberg, M. V., *J. Lab. Clin. Med.*, 1930, v16, 158.
11. Banerjee, S., Ghosh, P. K., Bandyopadhyay, A., *Proc. Soc. Exp. Biol. and Med.*, 1962, v109, 313.
12. Van Handel, E., Zilversmit, D. B., *J. Lab. Clin. Med.*, 1957, v50, 152.
13. Raben, M. S., Hollenberg, C. H., *J. Clin. Invest.*, 1960, v39, 435.
14. Banerjee, S., Kawishwar, W. K., *J. Sci. Indust. Res.*, 1961, v20C, 141.

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Low Values for Sebum in Eunuchs and Oöphorectomized Women.* (28050)

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Androgenic stimulation has been shown to induce acne and is believed to be the incitant of this condition in the human male(1,2).

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Testicular secretions seem to be the usual source of acne-inciting androgens in males, since unpublished observations showed that acne did not develop after castration in any of 91 males. Testicular secretions and androgenic treatment enhance sebaceous secre-

tions, and the amounts of ether-soluble materials on the surface of skin and hair have tended to be subnormal in the few eunuchs studied thus far(1-4). In view of the relationships of sebum to acne(5) and of testicular secretions to sebaceous function and acne, sebum was measured in a large series of castrated and intact males and in smaller groups of oöphorectomized and intact females.

Materials and methods. Sebum was measured by a technic essentially similar to that described by Jones(6). While subjects remained supine and immobile for 30 minutes, a brass cup (0.5×1.0 cm) was pressed against the center of the forehead and the skin around the cup was wiped with a cotton swab moistened with ether. The cup was removed and a spatula drawn across the skin which had been shielded. The spatula was dipped into a film formed by a drop of oil (previously subjected to high temperature) spread on the surface of water. The sebum spread as a monomolecular layer to form a circle, the area of which was calculated in cm^2 . Sebum that spread to cover an area of 0.2 cm^2 or more was measurable; from Jones' calculations(6) it appears that amounts of sebum as small as 0.00005 mg could be measured. Values for the initial scraping of the test area of skin were more variable than those obtained when the process was repeated after 15 minutes; therefore only replacement values, obtained 15 minutes after the first scraping, were reported.

Lately Strauss and Pochi(7) have recommended that adhesive tape be used to circumscribe the area under study and prevent the inflow of sebum from surrounding skin; since the inflow can be considerable in the presence of sweat, we compared values for sebum in skin isolated by tape with those for non-isolated skin under the conditions employed in the present study. Two symmetrical areas of the forehead, one circumscribed by tape, were studied simultaneously in 82 intact men in tests made during June when the temperature in this laboratory varied between 24 and 32°C . Data for isolated and non-isolated areas were 22 ± 3.3 and $20 \pm 3.5 \text{ cm}^2$, respectively; the difference was not significant ($P = .71$), indicating that data obtained by

TABLE I. Low Values for Sebum in Absence of Testes or Ovaries. Subjects are mentally-retarded.

Status	Age, yr	No.	Sebum, $\text{cm}^2/15 \text{ min}$
MALES			
Castrate	20-29	14	$*1 \pm 1.0$
Intact		12	8 ± 1.3
Castrate	30-39	26	$*1 \pm .5$
Intact		21	7 ± 1.4
Castrate	40-49	20	$*1 \pm .1$
Intact		12	7 ± 1.8
Castrate	50-80	9	$*0 \pm .0$
Intact		4	$4 \pm .6$
FEMALES			
Oöphorec- tomized	19-30	11	$\dagger 0 \pm .0$
Intact		21	$8 \pm .7$

P of difference from intact group: $* < .001$, $\dagger .01$.

our routine procedures were not invalidated by sweating.

The chief group of subjects were inmates of a home for the mentally-retarded. Operated subjects consisted of 70 eunuchs and 11 oöphorectomized women. Sebum was measured in males who had been orchiectomized for 3-59 years and in females oöphorectomized for 4-19 years. The control series of intact subjects consisted of 65 male and 21 female inmates, comparable to the operated subjects in age, sex, ethnic group (Caucasian) and mental status.

A supplementary study was made of mentally-normal subjects, 6 oöphorectomized and 17 intact women who were 38-44 years old.

Results. Castrate vs intact males. Values for sebum were significantly lower ($P < .001$) in the 70 eunuchs than in the 65 intact males at all ages shown in Table I. Sebum in excess of 1 or more cm^2 characterized only 20% of the eunuchs but 86% of the intact males; values in excess of 3 cm^2 were observed in 6% of the eunuchs as compared to 62% of the intact. Amounts of sebum greater than in any eunuch were observed in 12% of the intact males.

1) *Castration prior to sexual maturation.* Four men had been orchiectomized at 11-13 years of age and were studied when 32-71 years old. They received no androgenic treatment and had genitalia which were infantile in appearance. In all 4 eunuchs the amount of sebum on the test area of the forehead was too small to be quantitated with

TABLE II. Values for Sebum in Relation to Age at Castration and Duration of Castrate Status.

Age at castration	No. of subjects			Mean age at study			Sebum, cm ² /15 min			Years since castration			% of subjects with sebum, <1.0 cm ²			% of subjects with sebum, <1.0 cm ²		
	Range	Mean	No. of subjects	Mean age at study	Range	Mean	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median
11-15	13.9	13.9	12	33.8	0-13	1.2	0-13	1.2	0	3-11	7.5	7.5	83	83	0	0-13	.9	0
16-18	16.9	16.9	20	36.0	0-12	1.0	0-12	1.0	0	12-21	17.3	17.3	75	75	0	0-12	1.1	.1
19-29	21.5	21.5	31	37.2	0-4	.5	0-4	.5	0	22-59	27.1	27.1	84	84	0	0-3	.2	0
30+	38.6	38.6	7	53.7	0-2	.4	0-2	.4	0				71	71	0			

the sensitive method employed. Another eunuch had been castrated when 14 years old and subsequently received daily intramuscular injections of 25 mg of testosterone propionate for 30 consecutive days and similar dosage for unknown periods of time on 2 other occasions; although no androgens had been administered for 1 year prior to measurement of sebum at 42 years of age, a replacement value of 1 cm² was observed.

This suggests that sebum is not present on the test area of the forehead in amounts large enough to measure with the methods employed, if the sebaceous glands were never stimulated by androgens.

2) *Castration during or after sexual maturation.* The pronounced increase in sebaceous secretions which accompanies sexual maturation(2), was not maintained in subjects whose testes had been removed during or after puberty (Table II). Of 65 subjects who had not been castrated until 15 or more years of age, the great majority (63%) had amounts of sebum that were non-detectable or too small to be measured; only 17% had values as high as 1 cm². In the control series of intact men, only 11% had replacement values too small to be measured; 83% had values of 1 or more cm².

Prolonging the interval between puberty and orchiectomy did not permanently enhance the amount of sebum. Values of 1 or more cm² were observed in 16% of the 31 men not orchiectomized until 20-59 years old, and in 24% of the 34 men who had been castrated at 15-19 years of age. In data restricted to 24 eunuchs tested when 30-39 years old, values of 1 or more cm² were found in 12% of those castrated when 20 or more years old and in 21% of those orchiectomized at 15-19 years of age.

3) *Duration of castrate state.* Duration of the castrate state over a range of 3-59 years had no discernible effect upon values for sebum (Table II) other than that some subjects in both castrate and intact control groups were old at the time of study and had relatively low values.

4) *Studies of 9 males before castration and 4 weeks afterwards.* The amounts of sebum

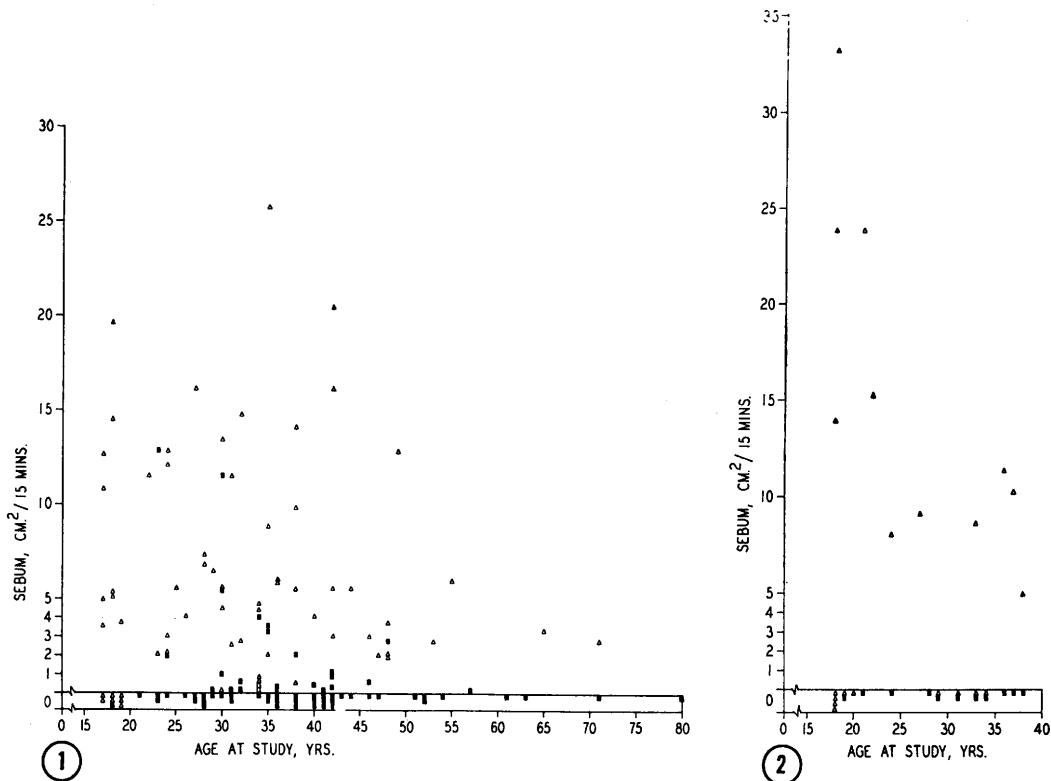


FIG. 1. Values for sebum in intact and castrate males. Orchiectomies were done at least 3 years before the study of sebum. Squares represent eunuchs, triangles the intact males. Subjects were mentally-retarded.

FIG. 2. Values for sebum in intact and oöphorectomized females. Oöphorectomies were done at least 4 years before the study of sebum. Squares represent oöphorectomized females and triangles the intact. Subjects were mentally-retarded.

found 4 weeks after orchiectomy were not significantly different from that prior to castration in any of these subjects ($P = .12$ or more). The mean value for all subjects was 9 cm^2 in 71 tests made 4 weeks after castration, whereas it had been 4 cm^2 in 37 tests of the same subjects one week before orchiectomy; the difference was not statistically significant. A delayed decline after cessation of androgenic treatment has been reported in a prepubertal boy(7).

5) *Low values for sebum in old eunuchs.* Values of 1 or more cm^2 were found in 23% of the 47 eunuchs tested when 18 to 48 years of age but in none of 9 other eunuchs tested when 50 years of age or older (Fig. 1); none of these males had been castrated until 14 or more years of age.

Oöphorectomized vs intact females. In the mentally-retarded subjects, values for sebum

were significantly lower ($P = .01$) in oöphorectomized than in the intact females (Table I).

In the smaller series of mentally-normal subjects, the mean amount of sebum was also less in oöphorectomized than in intact women; the difference tended to be significant ($P = .04$). Only 6 of the 17 intact women had values low enough to be in the range observed in the oöphorectomized women.

Discussion. Surgical deprivation of testicular or ovarian secretions resulted in subnormal amounts of sebum. Immature children also have low values for sebum(3); unpublished studies showed that only 4 of 44 boys and girls, 4 to 10 years of age, had sebum in amounts sufficient for evaluation with the method employed in the present investigation.

There is evidence that the low values for sebum in eunuchs are due primarily to a lack of testicular secretions and not to concomitant reduction in steroids of extragonadal origin(8,9). Urinary metabolites of adrenal cortical secretions, which serve to assess these secretions, have been measured in most subjects utilized in the present study(8,9); daily titers of 17-hydroxycorticoids, and of a chromatographic fraction containing dehydroepiandrosterone, may be noted in particular to have been as high in castrate as in intact men. Since the amounts of these substances were not reduced as a result of castration, the subnormal sebaceous values in eunuchs can not be attributed to a decrease in adrenocortical secretions.

Summary and conclusions. Comparisons of intact and castrate males indicate that testicular secretions or their metabolites are responsible for the increased amount of sebum observed in males after sexual maturation. Values for sebum were less in oöphorectomized than in intact women, which suggests that ovarian secretions or their metabo-

lites may also enhance sebaceous function. Sebum values remained high in tests made 4 weeks after orchiectomy. A long latent period before sebum values decline markedly upon reduction in androgenic stimulation (even with the sudden and drastic decrease afforded by castration) should be considered in evaluating therapy designed to reduce sebaceous secretions and acneform responses.

1. Hamilton, J. B., *J. Clin. Endocrinol.*, 1941 v1, 570.
2. Rothman, S., *Physiology and Biochemistry of the Skin*, Univ. Chicago Press, 1954.
3. Emanuel, S., *Acta Dermat.-Vener.*, 1936, v17, 444.
4. Hamilton, J. B., *Am. J. Anat.*, 1942, v71, 451.
5. Prose, P. H., Baer, R. L., Hermann, F., *J. Invest. Dermat.*, 1955, v25, 423.
6. Jones, K. K., *Science*, 1950, v111, 9.
7. Strauss, J. S., Pochi, P. E., *J. Invest. Dermat.*, 1961, v36, 293.
8. Hamilton, J. B., Bunch, L., Mestler, G. E., *J. Clin. Endocrinol. and Metab.*, 1959, v19, 535.
9. ———, *ibid.*, 1962, v22, 1103.

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Effect of Egg Yolk Size on Yolk Cholesterol Concentration.* (28051)

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Recent workers(1,4,5) have shown no change in egg yolk cholesterol concentration due to dietary differences in fat, choline or addition of a hypercholesteremic agent to the ration. It has been reported(6) that the membranes, theca interna and granulosa, surrounding the developing ovum in the chicken synthesize fatty acids and cholesterol, depositing them in the yolk.

Since cholesterol concentration per gram of dry ova of developing ovarian follicles has been shown to increase as dry weight of the follicle increases(3) it was thought that a study of mature yolks of different sizes would

be a next logical point to investigate.

Procedure. In each of the 4 experiments presented here, the hens were kept in community cages and fed a basal corn-soy laying ration.

In Exp. 1, eggs were collected over a 14-day period from a group of 15 crossbred Rhode Island Red hens.

In Exp. 2, eggs were collected from 2 groups of 20 linebred, Single Comb White Leghorn hens. Hens designated as line "B" had been selected through several generations for an individual trait of large body size, and line "C" had been selected for small body size.

Experiment 3 was conducted to determine the dry matter of the boiled egg yolks of both line "B" and line "C" eggs so that the choles-

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