THE QUANTITATIVE GRAVIMETRIC DETERMINATION OF SEBUM PRODUCTION*

JOHN S. STRAUSS, M.D. AND PETER E. POCHI, M.D.

The accuracy of measurement of skin lipids has progressed considerably from the days when our forefathers extracted the lipid collected in long-johns worn for varying periods of time (1–3). Unfortunately, in the past, many methods have not distinguished between the amount of lipid normally found on the skin surface and lipid production, the amount of sebum elaborated per unit area per unit time. The present report is is concerned with a simplified quantitative technic for measuring sebum production adapted from the technics of Miescher and Schönberg (4), and Kligman and Shelley (5). The principle is simply to absorb the sebum on paper as soon as it reaches the surface, thus trapping the sebum before it can flow away.

TECHNIC

1) The surface lipid of the forehead is wiped off thoroughly with dry gauze sponges.

2) A square area measuring 2.54 × 2.54 cm. is defined by strips of cloth adhesive tape. (Figure 1). In some patients with a low frontal hairline, the length of the vertical borders must be shortened.

3) All collections are made onto cigarette papers. These very thin papers, measuring approximately 7 × 3.5 cm. in size, are used by those who roll their own cigarettes. Various brands have different absorbing qualities; we prefer those marketed as "Top" gummed cigarette papers manufactured by the R. J. Reynolds Tobacco Company. The gummed edges are cut off before use.

4) Four ether washed papers† are placed over the demarcated area, the edges overlapping onto the tape (Figure 2). They are held in place by folded gauze squares. The papers and gauze squares are maintained in position by a three inch wide rubber bandage encircling the head (Figure 3).

5) The papers are allowed to remain in place for 15 minutes and are discarded. This is repeated once. These two fifteen minute collections, which are not used, assure uniform prehandling of the skin.

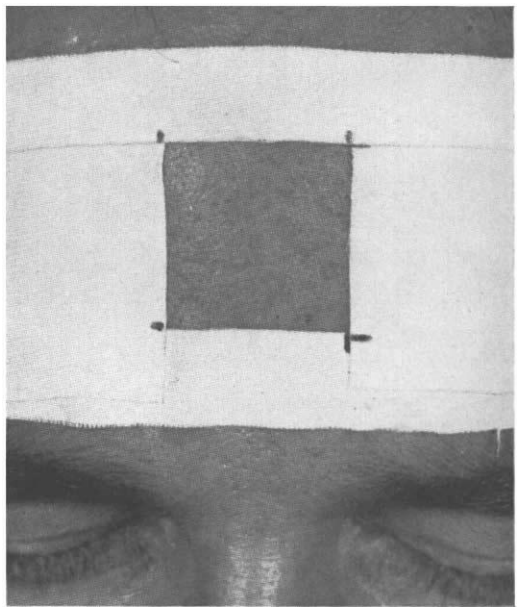

FIG. 1. Square collection area of the forehead outlined by cloth adhesive strips.

6) For the actual test, a stack of four papers is put down and held in place for three hours.

7) At the termination of the test period the central area of the papers which has been in contact with the skin and which contains the lipid is cut out with scissors (Figure 4). (Visualization of this area is facilitated by holding the papers up to a bright light).

8) The papers are placed in 20 cc. of anhydrous ethyl ether in a tared aluminum weighing cup for 5 minutes. The papers are subsequently washed with three aliquots of 10, 10, and 20 cc. of ether. The washings are combined and the cup taken to dryness.

* From the Department of Dermatology, (Herbert Mescon, M.D., Professor), Boston University, School of Medicine, and Evans Memorial of Massachusetts Memorial Hospitals, Boston 18, Massachusetts.

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† By separate analysis of each paper, we found slight amounts of oil in the second paper in individuals with extremely high lipid output. No oil was present in the third and fourth papers. Thus four layers of cigarette papers are used to make certain that no oil is lost.

9) The cups are weighed after temperature equilibrium has been reached. The necessary correction for the weight of control cups which contain the same amount of ether and control clean papers is applied.

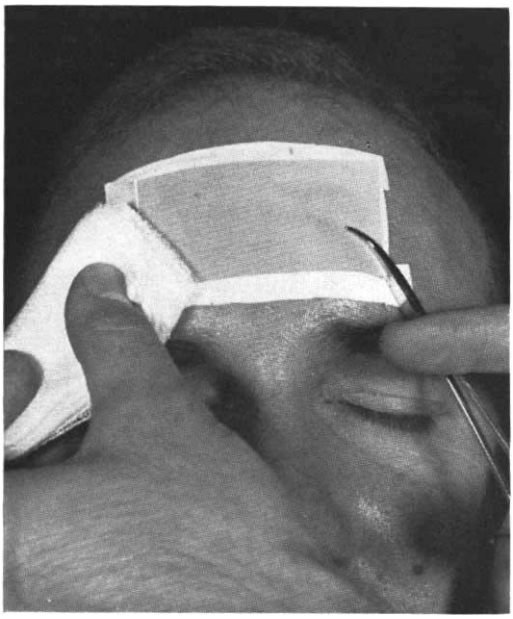

FIG. 2. Ether cleaned cigarette papers are placed over the collection site, overlapping the adhesive strips.

RESULTS

The results are given as milligrams of lipid per ten square centimeters per three hours. Although the results are reproducible, as shown in Table I, occasional values will be out of line. However, the standard deviation, averaging about fifteen per cent, is certainly within acceptable limits for biological test procedures. That this method of collection is a valid one is indicated in Figure 5 which shows that sebum production is linear with the length of the collection period.

While the variation in individual tests may make the interpretation of a single test difficult, repeated testing of any one subject allows accurate evaluation. To demonstrate this, the sebum production in two subjects respectively receiving methyl testosterone and ethynyl estradiol experimentally is illustrated in Figures 6 and 7.‡ The measurable change in sebum production is obvious in both cases.

DISCUSSION

The tendency of sebum to be lost by flowing away from the collection area has been the major obstacle to accurate measurement of sebum production. Herrmann and Prose (6) found that

‡ Hormones supplied by G. Kenneth Hawkins, M.D., Schering Corporation, Bloomfield, New Jersey as Oreton M® (methyl testosterone) and Estinyl® (ethynyl estradiol).

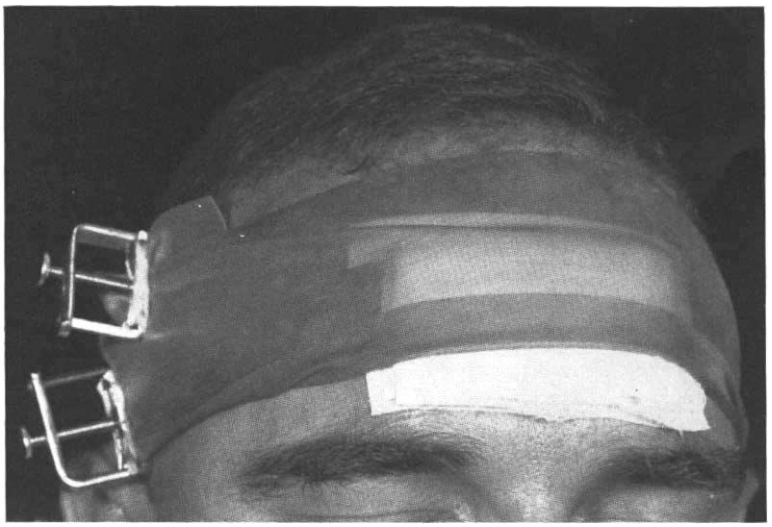

FIG. 3. The cigarette papers, held in place by gauze squares, are secured with a three inch wide rubber bandage. In this picture the lower border of the bandage has been reflected to show the underlying gauze.

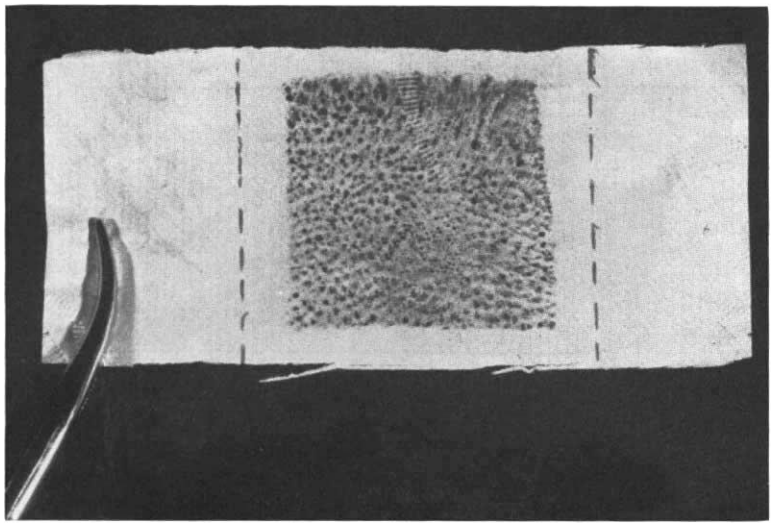

FIG. 4. After the three hour collection period, the central lipid containing area is cut for extraction and gravimetric determination. For photographic purposes *only*, the lipid has been stained with osmic acid vapors and the dotted lines have been drawn to demonstrate where the paper is cut with scissors.

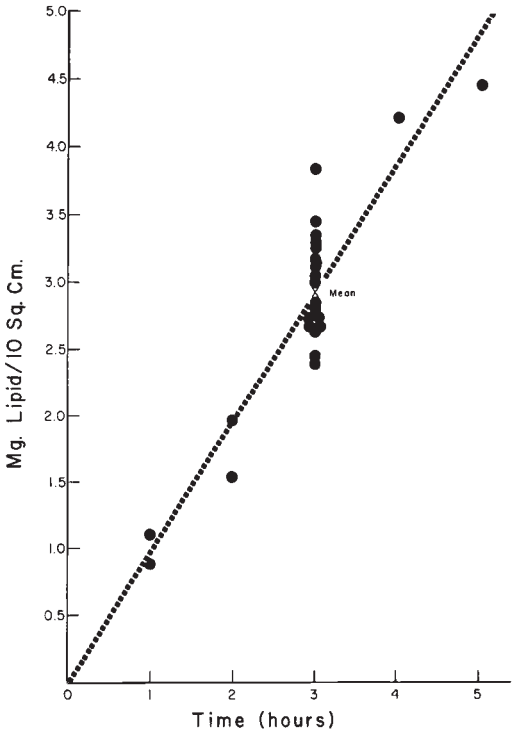

FIG. 5. Sebum, or lipid production, as a function of the collection time. Three hours has been the usual collection time. Lipid values for other collection times lie close to a straight line drawn through the mean for the three hour collection.

they could collect twice as much sebum if the test area was protected by a cup. This whole problem has been adequately analyzed by Kligman and Shelley (5). If a fixed cup is used to completely isolate the test area, the technic becomes almost impractical because the patients must be absolutely immobilized. The chief advantage of the absorption technic is that the sebum is trapped as soon as it comes to the surface and none can escape during collection. Furthermore, the delineating adhesive tape prevents the inflow of lipid into the area.

Another obstacle to the accurate measurement of sebum production has been the definition of what the baseline should be before the collection is started. It is not our purpose to discuss the problem of the "stored sebum" in the capillary reservoir of the stratum corneum and the follicular reservoir; these have been adequately detailed by Kligman and Shelley. We have found that if the skin is defatted with lipid solvents before testing, the results show a wide variation. However, when the skin is wiped free of sebum and the two fifteen minute control collections are run, the results tend to be more uniform.

As regards methods of measuring what has been collected, the simplest and least susceptible to error is a gravimetric determination (5, 7). The method of Jones, *et al.* (8), based upon the

TABLE I

Representative values for sebum production of the forehead in four adult mates tested repeatedly at approximately one week intervals

Subject.....	A	B	C	D
	Milligrams of lipid/ten square centimeters/three hours			
	4.04	2.74	2.96	2.93
	5.25	2.80	2.23	2.60
	4.10	3.02	2.53	3.29
	5.50	3.84	2.02	3.10
	4.80	3.38	2.42	3.42
	5.02	3.28	1.22	3.08
	6.16	3.18	1.93	3.70
	4.74	3.09	2.00	4.08
	3.66	2.82	2.38	4.02
	4.88	3.24	2.60	2.67
	4.64	3.16	2.12	3.91
	4.72	2.43	2.22	3.91
	4.56	2.68	2.42	4.97
	4.77	2.70	2.03	4.77
	4.99	3.47	1.88	4.78
	4.51	2.48	2.22	4.23
	4.18	2.65	1.77	
		3.06	2.20	
		2.48	2.02	
		2.46	2.22	
			2.04	
			2.09	
Mean & S.D.	4.47 ±.58	2.95 ±.38	2.15 ±.36	3.74 ±.74

spread of the sebum dissolved in a solvent as a monolayer film over water, is dependent upon the surface area occupied by the various components of sebum. The method is unsatisfactory because of the variable composition of sebum. In Kvorning's technic (9), the quantity of sebum is determined by measuring the carbon dioxide liberated after combustion of a sebum sample. A correction factor determined for mixed plasma lipids is used to convert this to weight of sebum. Once again, the varied composition of sebum, along with its great dissimilarity to plasma lipids lessens the accuracy of his determinations. The nephelometric method, used by Emanuel (10) also bears no fixed relationship to the actual quantity of sebum.

Recently, Brun *et al.* (11) and Smith (12) have attempted to put the classical method of visualizing sebum by osmic acid discoloration on a quantitative basis. Transmission of light through a blackened sebum print was determined photoelectrically. The relationship of the values obtained to the amount of sebum present on the paper was not determined so that the accuracy of the method cannot be evaluated. Moreover, the use of successive five minute collection periods involves the problem of tapping the follicular reservoir of sebum which tends to increase the flow of sebum to the surface. The rationale for using such short collection periods is not given.

Finally, the correlation between sebum production and sebaceous gland size has already been pointed out (4, 5). Our results are in complete agreement with those of these other investigators. As an example, after the administration of hormones, the decrease or increase in sebum production as shown in Figures 6 and 7, is a reflection of the changes in size of the sebaceous glands seen by biopsy (13). As a matter of fact, our newer data indicates that sebum production is probably a more sensitive method of determining changes in the sebaceous glands than histologic technic.

SUMMARY

A simple quantitative method of measuring sebum is described. The lipid is absorbed into a fixed trap of cigarette papers and is gravimetrically determined after extraction with ether.

ACKNOWLEDGMENTS

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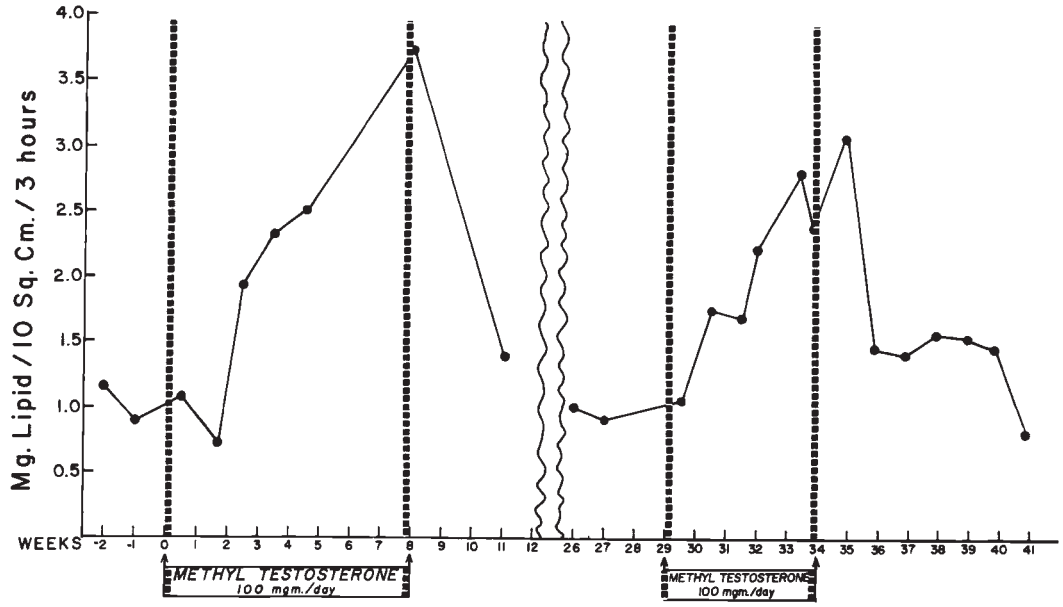

FIG. 6. Increased sebum production following the administration of methyl testosterone to a twelve year old prepuberal boy. The tremendous rise in sebum production reproduced on two occasions is far greater than the inherent variation in the test procedure.

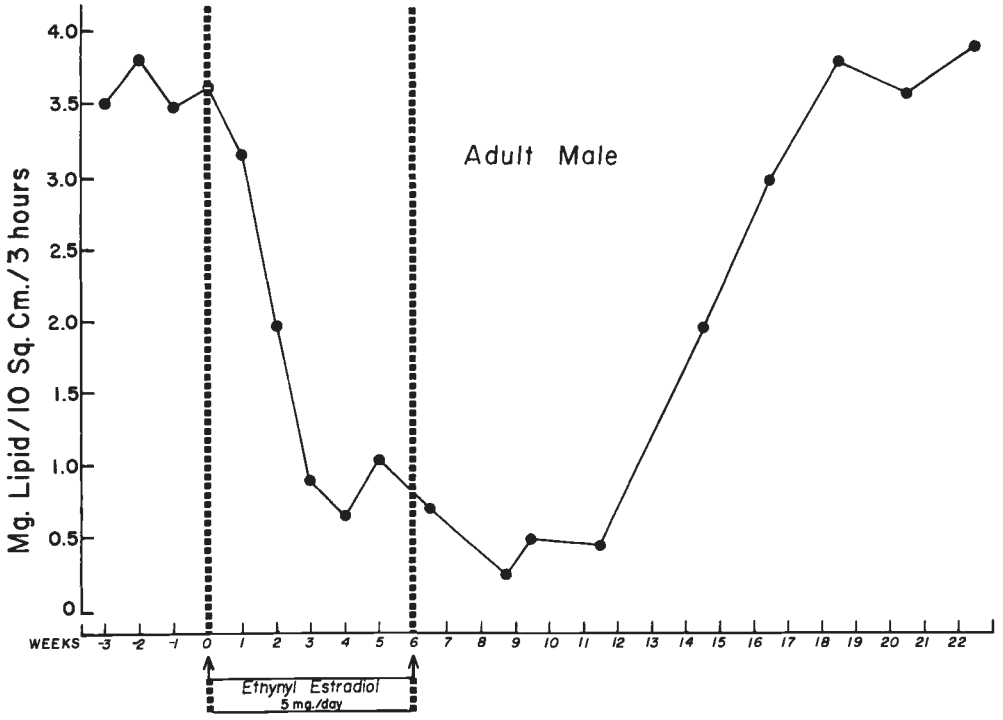

FIG. 7. Depression of sebum production in an adult male given ethynyl estradiol. Once again the changes are much greater than the variations on repeated testing of the same individual.

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FIRST ANNUAL HERMAN BEERMAN LECTURE

MOLECULES, SOCIAL SYSTEMS AND DERMATOLOGY

RENÉ J. DUBOS, M. D., Sc. D.

Member and Professor, The Rockefeller Institute
New York, N. Y.

At the 22nd Annual Meeting of The Society for Investigative Dermatology, in New York, June 27–29, 1961, Dr. René J. Dubos, Member and Professor, The Rockefeller Institute, New York, will deliver the first annual Herman Beerman Lecture. Dr. Dubos's topic will be "Molecules, Social Systems and Dermatology," and the lecture will be given on Wednesday, June 28th, at 2:00 P. M., at the Barbizon-Plaza Hotel, New York.