THE ESTIMATION OF THE RATE OF SECRETION OF SEBUM IN MAN*

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Many attempts have been made during the past fifty years to estimate the rate and character of the secretion of sebum, since this is an important factor in the preservation and health of the skin. The results of these investigations have varied widely and have been admittedly inaccurate. The reason for this has been mainly the lack of a method sufficiently sensitive to determine the amount of fat on small, discrete areas for short, definite durations of time. Since a suitable method is now available, this question may be reinvestigated.

This paper is concerned mainly with methods for removing and estimating the fat on the skin and its secretion by the sebaceous glands. In order to keep all experiments comparable, these have been restricted to tests on one area of skin of a single individual, the volar aspect of the forearm, roughly five centimeters below the elbow.

Linser (1) and Schur and Goldfarb (2) claimed that the original amount of fat on the skin would return in fifteen minutes following its removal by means of petroleum ether. On this basis they concluded that if the fat were removed continuously, from one to two grams could be obtained daily from the entire surface of the body. This rapid secretion of fat has not been verified by later investigators.

Zehender and Dünner (3) found rates of 0.2–0.8 microgram per square centimeter per minute on the skin of the forehead, with a return to the original amount in three to four hours. Emanuel (4) found the rate of secretion to depend on the number and size of the sebaceous glands in a given area. The restoration of fat, after removal, was found to take longer than three hours.

Pritchard, Edwards and Christian (5), using a colorimetric method estimating cholesterol esters, found the rate of secretion to be one microgram per square centimeter per minute, with the fat returning to normal within three hours of removal. Mackenna, Wheatley and Wormall (6) found the skin of the forearm to secrete fat at the rate of 14–70 micrograms per minute for the entire forearm, the area of which was not given. Using an approximate computed figure of 980 sq. cm. for the forearm, the rate became .014 to .07 microgram per square centimeter per minute.

Kvorning (7) gives figures for the four hour secretion of 10 square centimeters of the forehead skin. From these a rate of 0.74 microgram per square centimeter per minute can be computed.

Methods that have been used to remove fat from the skin fall into three categories:

1. *Absorption methods*, where blotting or filter paper is placed on the skin for determined lengths of time and then is extracted with solvents to obtain the fat.

2. *Scrubbing methods*, where wads of fat-free cotton or gauze are soaked in solvent, rubbed on a definite area of skin, and extracted to obtain the fat.

3. *Cup methods*, where small containers are fastened to the skin, in which solvent can be held directly in contact with the area to be measured, for a determined duration of time.

There are objections to all of these methods. The paper absorption method is limited by the capacity of the paper to take up the fat. The scrubbing method requires the initial

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purification of the cotton, which is difficult. The last traces of fat are almost impossible to remove without wrecking the texture. No other material has been effective. It is difficult to keep the scrubbing constant from one operation to another. Another objection is the relatively large amount of skin scales and debris removed by the scrubbing. This material amounts to as much as 90 micrograms per sq. cm. of skin area. This material must be filtered off and thoroughly washed to avoid loss of fat.

The cup method must keep the solvent on a definite area, without loss in application or recovery. The solvent must be thoroughly agitated and brought into intimate contact with the skin or the fat will not be adequately removed.

The solvent for skin fat must remove both fat and water and do so without irritation. A mixture of alcohol and ether satisfy these requirements. This mixture is an excellent solvent when hot, but at body temperature anhydrous ethyl ether alone is superior to mixtures with alcohol. Petroleum ether will not remove fat effectively from moist skin. Acetone is relatively non-irritating and an excellent solvent but is difficult to purify and to keep from polymerizing after purification.

The amount of fat removed by any of these methods is extremely small. Micro methods must be used for its determination. Many different kinds have been tried. The ones most used may be grouped under four heads:

1. Gravimetric, usually by means of a micro balance (8, 9, 10).
2. Oxidation with chromic acid mixtures, followed by an estimation of the carbon dioxide released or back titration of chromic acid reduced (2, 11, 7).
3. Nephelometric determinations of fatty acid precipitates (12, 13).
4. Microtitration of free fatty acids after saponification (14).

None of these methods has been sensitive to less than .01 milligram and most of them are not sensitive to less than a milligram.

The method used by us is different from those listed above and is based on the spreading properties of fat on a water surface. It is sensitive to less than a microgram and accurate to one microgram. Since the method is new, it will be described in some detail. More details will be found in the original papers (15, 16).

METHOD OF FAT ANALYSIS

A trough, 6 x 28 x $\frac{1}{2}$″ in dimension, is filled with redistilled water. On the surface of this water is placed a small drop (.02 − .03 ml.) of "piston oil", which spreads over the surface. By means of a paraffin covered slide, the film is compressed until it becomes blue in color—changing to green. At this point the surface pressure is 20 ± 2 dynes per centimeter. This oil is called "piston oil" because the film it forms opposes other films with a constant pressure. It is made by heating a heavy mineral oil (Motor oil No. 20 or 30) (Cenco Hyvac No. 93050 C) in a shallow dish just below the smoking point until it becomes dark brown in color and spreads without lensing.

As soon as the piston oil film is stabilized, a measured quantity of a petroleum ether solution of the skin fat is placed on the blue-green piston oil film. The ether evaporates and the fat spreads to a colorless monolayer film, the outline of which is distinct against the piston oil film. A glass plate is placed directly over the trough and on this the figure can be traced. The outline on the plate is transferred to paper on which it can be traced with a planimeter, giving the area of the figure.

Each molecule of a saturated fatty acid occupies an area of 21 square Angstrom units at a pressure of 20 dynes per centimeter. Cholesterol occupies 40 square Angstrom units and other lipids from 20 to 120 square Angstroms. Skin fat is a

mixture of lipids, seemingly quite constant in composition. Samples of this fat obtained from the same individual used in these experiments, large enough to be weighed accurately when spread, gave a constant factor 0.19 micrograms of fat spreading over the area of one square centimeter. Hence multiplying the area obtained by the planimeter readings by this factor gives the amount of fat represented.

Using this technic for quantitating fat, different methods of removing fat from the skin have been tested and compared.

Methods for Removing Fat from the Skin

1. The Paper Method

Experiment 1. Following the technic of Miescher and Schönberg (17), a fat-free filter paper 7 cm. in diameter was placed on the forearm and covered with aluminum foil. Over this was placed a pressure cuff distended to 30 mm. of

TABLE I

Fat removed by paper method

Experiment 1

TIME	FAT
min.	*mcgm./sq.cm.*
0	4.68
6	3.13
12	4.68
18	1.82
24	1.99
30	2.11

mercury. Every six minutes the paper was replaced with a fresh piece. The paper removed was extracted with petroleum ether and the fat estimated. The results are shown in Table I.

It is evident that the results are variable and compared with the figures in Tables II, III, IV and V, low in amount. They seem to represent the ability of the paper to absorb fat rather than the amount of fat on the skin.

2. The Scrubbing Method

A mask was made by cutting a hole 32 mm. in diameter in the top of an ointment tin. This was fastened firmly on the skin. One hundred mg. of fat-free cotton held in an applicator was dipped in ethyl ether and rubbed on the area of skin exposed by the mask. This rubbing was continued for 2 minutes with frequent rinsings of the cotton in the ethyl ether. The cotton was then placed in a 5 ml. syringe and the ether pressed into an acetylization flask (Corning Glass Co. No. 4040). The ether used for the rinsing (10 ml.) was poured through the cotton in the syringe, pressed out, and the cotton rinsed with 5 ml. of fresh ethyl ether in the same way.

The ethyl ether was evaporated at low temperature, the last few drops removed under nitrogen gas, and the flask thoroughly dried. The skin fat in the flask was dissolved in 2 ml. petroleum ether and transferred to a 5 ml. volumetric flask. Subsequent rinsings of the acetylization flask with petroleum ether brought the contents of the volumetric flask to 5 ml. The amount of fat in the petroleum ether was then estimated by the monolayer technic described above. Subsequent scrubbings of the skin were made with the same cotton so that the effect of traces of fat originally in the cotton were minimized. Skin scales accumulated in the

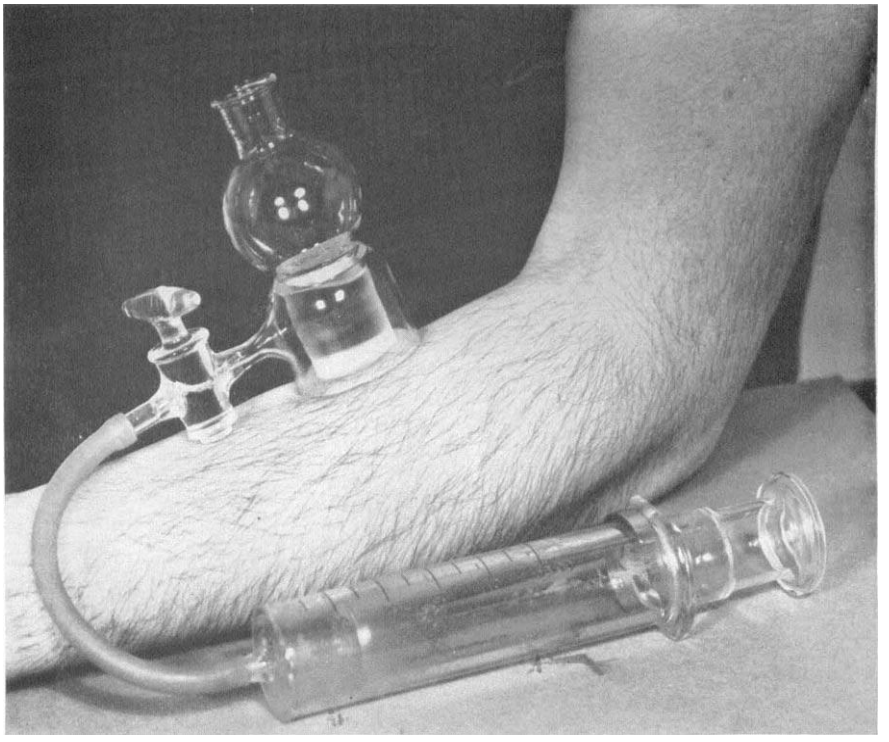

FIG. I

cotton and eventually interferred with the fat extraction. These scales form an opalescent cloud in the ether, difficult to remove and would be measured as fat in oxidation or nephelometric methods of analysis.

With care, uniform and reproducible results may be obtained on short series of tests, but a long series on the same area produces a definite irritation of the skin.

Experiment 2.

In Table II are the results of five scrubbings, each of two minutes duration.

Curve A, Chart I shows this original data plotted as a broken curve, showing the amount of fat removed each time it was scrubbed. Curve B shows the loga-

rithmic curve that would have been obtained from leather or dead skin. From Curve B it can be surmized that three scrubbings on such skin would remove practically all the fat, leaving only an infinitessimal quantity. The difference between Curve A and Curve B is believed to be due to the fat reappearing on

TABLE II

Fat removed by scrubbing method

Experiment 2

NUMBER OF SCRUBBINGS		TIME	FAT REMOVED			
Number	Log.		mcgm./sq.cm.	Log.	Log. Rectified	Computed
		min.				
1	0	2	58.00	1.76	1.76	58.00
2	.301	2	12.10	1.08	1.08	12.10
3	.477	2	8.35	.92	.65	4.46
4	.602	2	6.75	.83	.35	2.24
5	.699	2	7.13	.85	.20	1.59

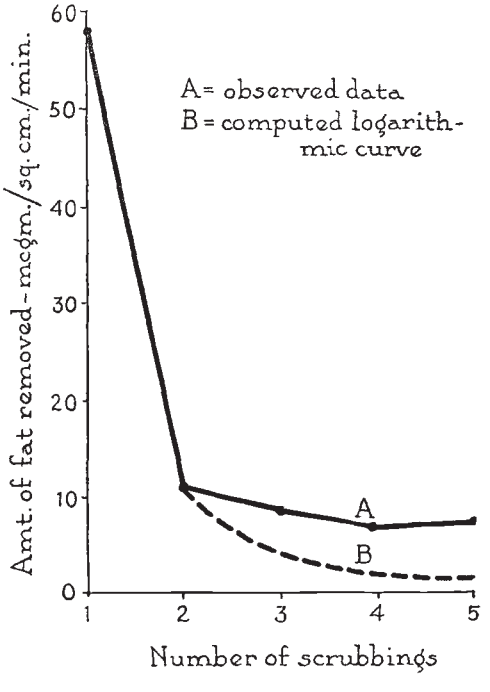

CHART I

the scrubbed surface from sebaceous glands or possibly spreading from adjacent areas.

Using this same technic, another experiment (Experiment 3) was made to estimate this return of fat. In the first place three separate scrubbings of the

test area were made and the fat removed was pooled. The analysis of this gave the fat initially on the surface. Then ten minutes were allowed to pass with the skin area protected, then a two minute scrubbing was made. The result is shown in column 3 of Table III. Ten more minutes passed and a determination of the fat was made. The result is in column 4 of this table. Following this, two twenty-minute periods tests were made in the same way, followed in order by 30, 40

TABLE III

Fat removed by scrubbing method

Experiment 3

COLUMN						
1	2	3	4	5	6	7
Intervals		Fat Removed			Am't. y	Rate y/x
Min. x	Min. x	mcgm./sq.cm.		Ave. y		
0	0			37.58	20.4	
10	10	9.05	4.98	7.02	10.0	.702
20	30	7.71	5.51	6.61	8.3	.331
30	60	6.95	7.71	7.33	7.3	.257
40	100	5.71	7.94	6.83	6.8	.170
50	150	5.65	6.53	6.09	6.3	.122

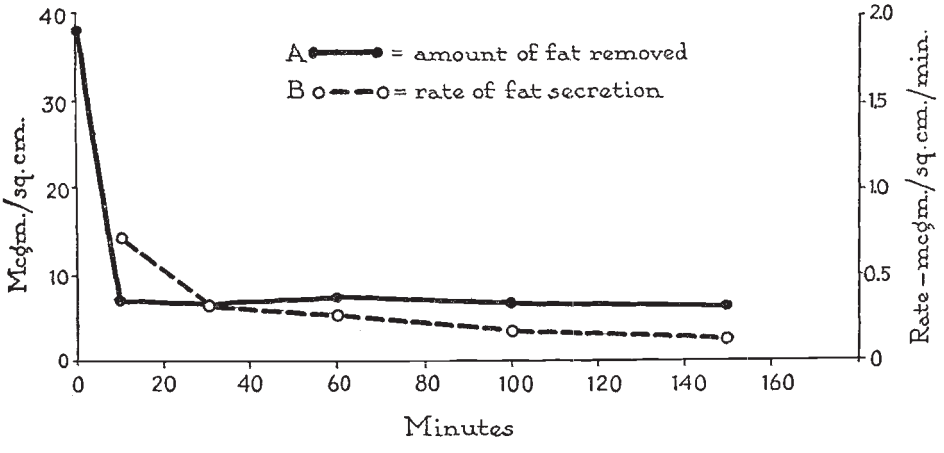

CHART II

and 50 minute periods. Considering the small amounts and the unknown factors involved, the agreement is considered good. The average values in column 5 were plotted against the total time involved (column 2) to give Curve A in Chart II. This indicates a constant amount on the skin despite the increasing time intervals between scrubbings. When these average amounts are divided by the time of each interval, an apparent rate for this time interval is obtained. Succeeding intervals show a decreasing rate, as listed in column 7.

In Curve B the rates for each time interval (column 7) are plotted against the elapsed time of this interval (column 2). This curve indicates that the rate is both diminishing in value and tending to reach a constant level.

3. The Cup Method

The cup method used by us was essentially the method used by Emanuel (12). This cup is shown in the accompanying figure (Fig. I). It has an opening 5 sq. cm. in contact with the skin. Around this is an annular space 1 cm. in width with an outlet by which this space can be evacuated. Suction is applied by means of a 50 ml. syringe. A vacuum of 30 cm. of mercury can be obtained which pulls the skin into the evacuated space and holds the cup firmly on the skin. When solvents are placed in the cup there is no loss. There is, however, tension on the skin and some reddening. No injury is produced.

A simpler cup and one often useful dispenses with the vacuum. It is essentially a cylindrical funnel with a stopcock. In this is placed the solvent, with the stopcock closed. The open end of the cylinder is placed against the skin and the body moved so that the cup is tilted. As the solvent clears the stopcock, the latter is opened to release the vapors of the solvent. With the stopcock open, the solvent is agitated in contact with the skin a definite length of time. Then the stopcock is closed and the cup immediately tilted back to the original position. The solvent flows back to the bottom of the cup and can now be removed for analysis. A little practice makes this maneuver possible without loss of solvent. Kvorning (7) uses an open cup without vacuum and removes the solvent by means of suction.

One advantage the open style cup has over a closed cup is the use of a flattened glass rod which can be rubbed gently over the skin while it is covered with the solvent. With this, the cup method is as efficient in removing fat as the scrubbing technic.

In Experiment 4 the same area of skin was washed by means of the cup method. The skin was washed for two minutes for the first test, then after two minutes, washed for the second test. Eleven such tests were made in succession. The results are shown in Table IV, column 2.

In Chart III, Curve A, these data are plotted against the number of washings. As this suggested an hyperbolic function, the data were fitted to the function $y = a/xb + c$ (21), giving the equation $y = 12.02/x^{1.04} + 2.42$. Except for the first figure, the data are well represented by Curve B. From this it may be seen that after a large number of washings, the amount of fat that can be removed becomes constant at 2.42 micrograms per square centimeter. As four minutes elapsed from one washing to another, this figure represents a rate of 0.6 microgram per square centimeter per minute.

The following eight experiments were made to substantiate this. As in a previous experiment, the area covered by the cup was washed three times, 2 minutes each time, and the results combined to give the initial figure. Intervals of time were allowed to elapse before the fat on the skin was again estimated. These intervals were 5, 10, 20, 40, 60, 80 and 100 minutes, each increasing in the order

listed. The results are given in Table V. The average figures in column 12 show very nearly the same amount of fat for each interval. When plotted as Curve A, Chart IV, there is seen a very slight tendency to rise with time. If, however, the

TABLE IV

Fat removed by cup method

Experiment 4

NUMBER WASHINGS	AM'T. FAT REMOVED MCGM.	AM'T. FAT COMPUTED $y = 12.02/x^{1.2} + 2.42$ mcgm.
	Column	
1	2	3
1	26.60	15.02
2	8.20	8.28
3	6.70	6.23
4	4.04	5.28
5	4.25	4.67
6	3.24	4.28
7	5.06	3.99
8	3.49	3.82
9	4.10	3.65
10	3.30	3.52
11	3.74	3.42

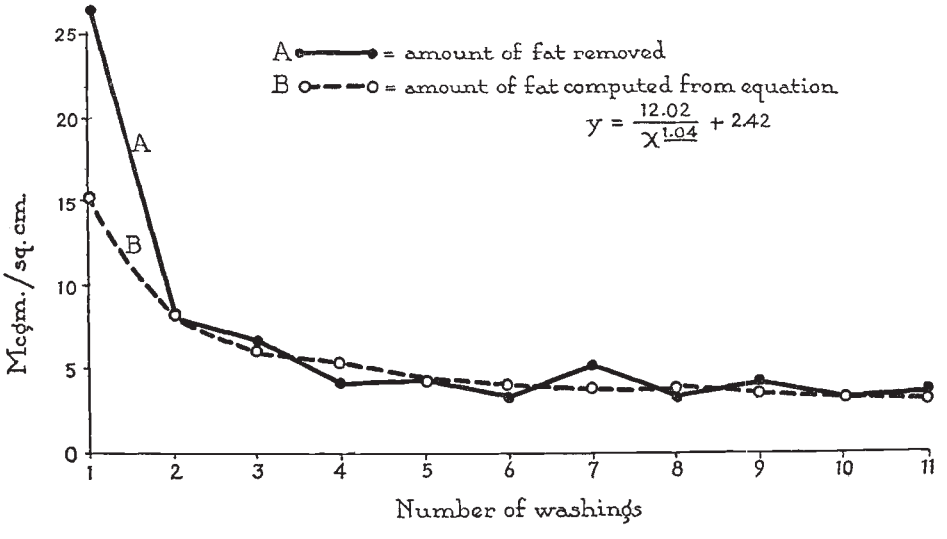

CHART III

amount of fat at each interval is divided by the time involved, a figure representing the rate of secretion is obtained (column 13). If these data are plotted against the time intervals, an hyperbolic type curve is obtained, tending to

approach a limit as the intervals are extended (Curve B, Chart IV). Fitting these data to the same function as used on preceding data, an equation of $y = 13.76/x^{1.2} + .081$ is obtained. This is plotted as Curve C, Chart IV, with excellent agreement. This curve indicates a steady rate of secretion of .081 microgram per

TABLE V

Fat removed by cup method

COLUMN													
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Number of Washings	Elapsed time	Total time	Individual Experiments			Micrograms per square centimeter				8	Ave.	Rate Column 12/Column 2	Rate Computed from equation
			1	2	3	4	5	6	7				
	min.	*min.*											
1	0	0	37.58	36.54	40.94	53.64	39.96	54.14	54.54	44.10	45.18		2.07
2	5	5		4.95	8.06	11.47	11.34	11.25	10.67	10.35	9.73	1.95	1.95
3	10	15	9.05	5.81	4.83	11.35	8.28	16.92	12.38	9.86	9.81	.98	.46
4	20	35	14.03	7.95	6.08	8.48	8.91	7.15	11.97	9.96	9.31	.47	.24
5	40	75	14.66	4.80			7.92	7.16	12.29	10.29	9.52	.24	.18
6	80	155	15.48				10.08	11.66	12.87	11.55	12.33	.15	.15
7	100	255					11.88	10.98	13.05	13.92	12.46	.08	.14

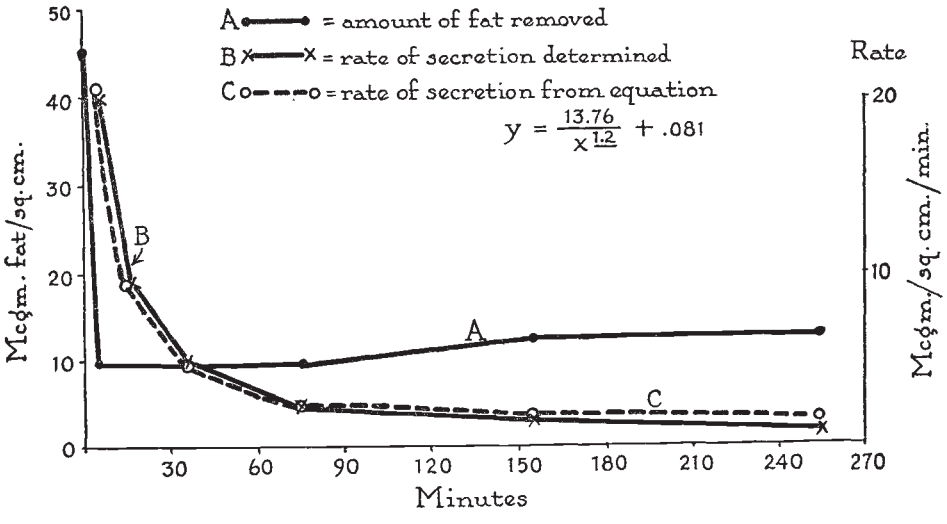

CHART IV

square centimeter per minute for this area. In Experiment 4 over a time of 4 minutes, a rate of 0.6 microgram per square centimeter per minute was found, while in the above experiment over a time of 260 minutes, a rate of 0.08 microgram per square centimeter per minute was found.

If the data given in Table V is arranged as a summation of the fat removed, the data as given in Table VI assumes a parabolic form when plotted (Curve A, Chart V).

TABLE VI

Summation of fat removed

TIME	AMOUNT OF FAT	
	Mcgm./sq.cm. as obtained	Computed from equation
min.		
0	45.18	45.2
5	54.91	57.3
15	54.72	63.6
35	74.03	71.7
75	83.55	82.6
155	95.88	96.5
255	108.34	109.2

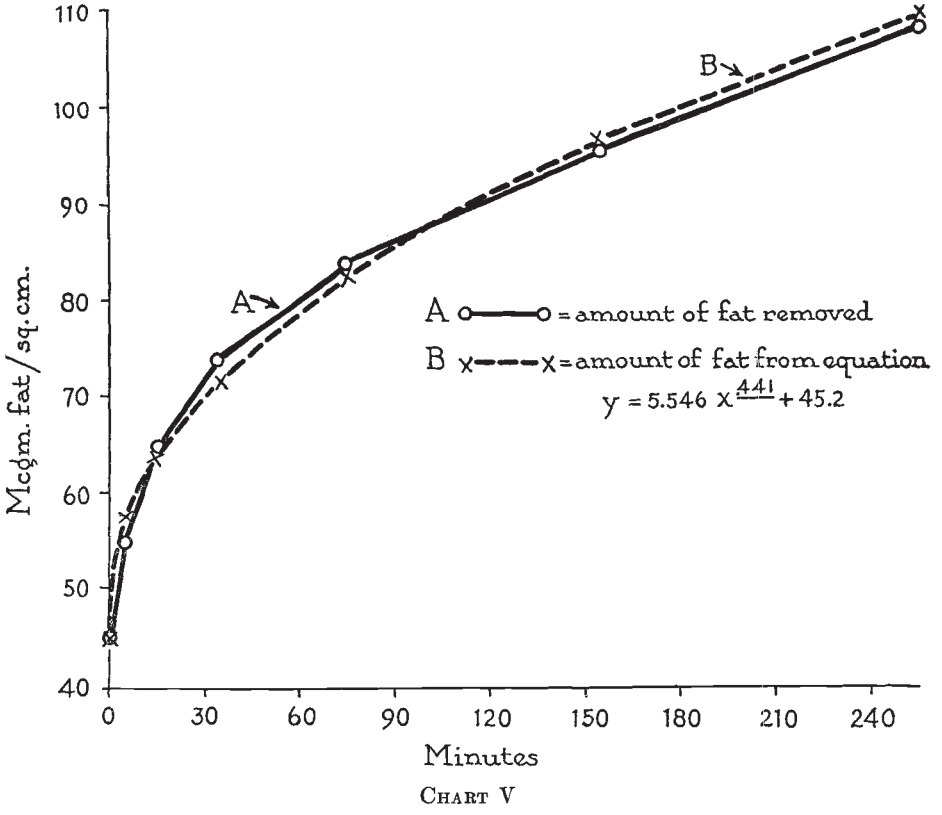

CHART V

The equation, $y = 5.546 \times ^{0.441} + 45.2$ fits this data closely as shown by Curve B, Chart V. The slope of this curve at any point is the rate of secretion at the

corresponding time. At 255 minutes the rate is .118 microgram per square centimeter per minute which agrees well with .112 microgram per square centimeter per minute found as an average of four independent determinations over periods of 3–4 hours.

Since the curve computed in this way is a parabola, the limiting slope at infinity would be zero. It is not known whether the curve would continue to be a parabola if the experiments were extended. Obviously, if the minimal rate is constant, the rate must become constant and be represented by a straight line. At approximately 8 hours the rate would be .08 microgram per square centimeter per minute or the same as the limiting rate as computed from the hyperbolic function.

It can be seen from the variance in these figures and variance in data reported in the literature, that factors other than experimental errors of analysis are present. In all experiments on the rate of secretion, when this is found for short intervals, the rate is high and when determined over an extended interval, the rate is low. Obviously, if the sebaceous glands continue to secrete at a constant rate, fat should accumulate on a given area if not removed. Observation has shown that such accumulation is slow and irregular.

It has been suggested that the tension of fat on the skin limits the secretion. The evidence of sebaceous cysts, comedones and the formation of vernix caseosa indicates a flow of sebum against pressures many times greater than those found on these cleansed areas.

One factor that has been ignored in experiments such as these is the spreading ability of fat on moist skin. It was evident in our experiments that such fat would flow rapidly from skin onto a water surface. Touching the skin to a piston oil film gives a spread equivalent to the amount that can be removed from the same area with solvents, 30 micrograms per square centimeter. This spread takes place within a few seconds. The following experiment shows the speed with which skin fat can flow on a water surface. A hydrophil balance (Cenco No. 70551) was set at zero reading on a clean water surface. At zero time the hand was gently touched to the water surface 50 centimeters from the float. At this moment the stop-watch was started and stopped the second the float moved. That this movement was due to the tension of the film of fat was shown by the return to zero of the pointer when the film was removed. The average speed was 3.3 centimeters per second, which was slow compared to the 20 cm. per second found by Cary and Rideal (20) for the spread of myristic acid.

Fat spreads rapidly over moist skin but seems to spread slowly or not at all over dry skin. This is shown by carefully cleansing the skin of the hand and fingers with anhydrous alcohol and ether. No fat is then found on touching the piston oil film. Ten minutes later .025 microgram per square centimeter was all that could be found. This low figure was not surprising since the finger tip has few sebaceous glands. The hand then was immersed in pure double distilled water and immediately (inside of 15 seconds) the same finger tip again was touched to the film. A spread of 3.5 micrograms per square centimeter was obtained, 140 times as much in 15 seconds as was found in 10 minutes on dry skin. This fact, that skin fats spread over moist skin, makes the determination of the

rate of secretion of a given area not only difficult but of mere academic importance. Fat undoubtedly flows from areas of high secretion, such as the scalp, to areas of low secretion and even to such areas as the palms of the hands where there are no sebaceous glands. This may help to explain the difference in the apparent rates of secretion over varying time intervals. The observed fact, that fat tends to come back rapidly at first and then seems to return slower and slower over extended intervals of time, was interpreted as due to fat stored in the glands. It could also be that fat flowed from contiguous areas even though these areas were supposedly isolated by the masks and cups. This point requires further study.

CONCLUSION

In conclusion, it is evident that a method sensitive to one microgram is necessary to measure the fat on limited areas of skin and to follow the rate of secretion of sebum on these areas. Such a method is described here, in the monolayer film method which not only gives accurate results but gives them in a short space of time and may be used to measure the fat on the skin by direct contact.

The total removal of fat is difficult to accomplish since fat is constantly returning to the cleansed surface. Removal of fat in repeated cleansings follows a logarithmic curve, but approaches a limit which is considered the rate of secretion.

The amount of fat on a given area, such as the forearm, in these experiments was fairly constant at 45.18 $\pm$ 2.73 micrograms per square centimeter. This is undoubtedly a resultant of the rate of secretion, rate of flow over the skin and removal by contact with clothing and other objects. Anything which increases secretion and decreases removal should increase the fat on the skin and vice versa.

Immediately after the removal of surface fat, stored fat in the glands flows to the surface giving an apparent rapid rate of secretion. Depletion of fat in the glands is accompanied by a decreasing rate until this falls to the level of the continuous rate of fat formation. The time necessary to establish this is dependent on the viscosity of the sebum. This minimal rate of secretion can be shown as limiting rate when the intervals between removals are extended indefinitely. For the forearm of a single individual this has been determined to be .081 microgram per square centimeter per minute. It is to be emphasized that this figure while it fits the facts as obtained is only a means of expressing this rate as a single value and indicates the difficulty in establishing such a value. Skin fat from the hand has been shown to flow over water from wet skin at an average rate of 3.3 centimeters per second. The flow over completely dry skin appears negligible.

With all these observations in mind, the estimation of the true rate of secretion requires:

1. Isolation of the test area from other areas of skin so completely that fat cannot flow either in or out.

2. Three washings immediately to reduce the surface fat to the lowest possible amount.

3. Determination of the return of fat over sufficient intervals of time that the glands are depleted of stored fat and the amount removed represents the true secretion of the glands.

SUMMARY

1. A method for the determination of fat in sebaceous secretion is described which is sensitive to less than microgram levels.

2. A comparison between absorption methods, scrubbing methods and direct solvent action shows that the absorption method is inadequate, the scrubbing method effective but irritating and the solvent method non-irritating and adequate, if combined with gentle rubbing.

3. The amount of fat on the skin of the forearm of a single individual was found to be 45.18 ± 2.73 micrograms per square centimeter initially.

4. The removal of fat from the skin requires repeated washings. The removal follows a logarithmic curve. After three washings, the amount being secreted affects the readings.

5. The interval of the time between determinations affects the amount of fat removed and the apparent rate of secretion. Over extended intervals, the rate of secretion tends to fall. A hyperbolic curve can be fitted to these rates obtained over intervals of increasing duration, giving a computed limit of .081. This indicates that a steady rate of secretion is in the order of 0.1 microgram per square centimeter per minute.

6. Skin fat flows with a speed of 3.3 centimeters per second over a water film and presumably over wet skin. It may be considered, therefore, that fat flows from areas of high sebaceous secretion to areas of low secretion and is constantly being removed by objects in contact with the skin.

7. Sebum flows very slowly over dry skin.

8. A procedure is suggested for the estimation of the true glandular secretion of sebum.

REFERENCES

1. Linser, P. Uber den Hauttalg beim Gesunden und bei einigen Hauterkrankungen Deutsches Archiv. fur klin Med. Leipzig **80:** 201, 1904.
2. Schur, H. and Goldfarb, L. Zur Physiologie und Pathologie der Talgsecretion; untersuchungs methodik und allgemeiner Sekretions mechanismus Wiener klinische Wochenschrift **40:** 1255, 1927.
3. Von Zehender, F. and Dunner, M. Untersuchungen uber die Methoden zur Messung der menschlichen Hauttalg Sekretion, Dermatologica **93:** 355, 1946.
4. Emanuel, S. Mechanism of sebum secretion. Acta Dermato-Venereorologica **19: 1,** 1938.
5. Pritchard, J. E., Edwards, L. D. and Christian, J. E. A Study of the surface lipids of skin. J. Am. Pharm. A. **38:** 546, 1949.
6. Mackenna, R. M. B., Wheatley, V. R. and Wormall, A. The composition of the surface skin fat (sebum) from the human forearm. J. Invest. Dermat. **15:** 33, 1950.
7. Kvorning, S. A. Investigations into the pharmacology of skin fats and ointments; I. The collection and quantitative determination of lipids on the skin, Acta Pharmacologica et Toxicologica **5:** 248, 1949.
8. Perutz, A. and Lustig, B. Zur Physiologie der Fettauscheidung an der Haut Oberflache, Biochemische Zeitschrift **249:** 370, 1932, **261:** 128, 1933.

9. KUZNITSKY, ERICH, Experimental und klinische Beitrage zur Frage der Hauttalg sekretion, Archiv. fur Dermatologie und Syphilis **114:** 691, 1913.
10. RABBENO, A. Contributo allo Studio della Secrezione Sebacea Giornale Italiano delle Malattie Veneree 65 (II): 1509, 1924.
11. PACHUR, REINHOLD, Die Talgabsonderung auf der menschlochen Haut Oberflache Archiv. fur Dermatologie und Syphilis **162:** 253, 1930.
12. EMANUEL, S. Quantitative Determinations of Sebaceous Gland's Secretion with Particular Mention of Method Employed, Acta Dermato-Venereologica **17:** 589, 1936.
13. BUTCHER, E. O. AND PARNELL, J. P. The distribution and factors influencing the amount of sebum on the skin of the forehead, J. Invest. Dermat. **10:** 31, 1948.
14. CARRIE, C. UND OTTOFRICKENSTEIN, H. Uber die Fette, die Saurezahlen und das Alkalineutralizations vermogen der Haut, Archiv fur Gewebe Pathologie Gewerbe Hygiene **11:** 345, 1941.
15. JONES, K. K. A micromethod for Fat Analysis based on formation of monolayer films, Science **111:** 9, 1950.
16. JONES, K. K. A micromethod for fat analysis based on formation of monolayer films. Quarterly Bulletin, Northwestern University Medical School **24:** 253, 1950.
17. MIESCHER, G. AND SCHONBERG, A. Untersuchungen uber die Funktion der Talg Drusen. Bulletin der Schweizerischen Akademie der Medizinschen Wissenshaften **17:** 444, 1936.
18. LANGMUIR, I. The constitution and fundamental properties of solids and liquids II. Liquids. J. Am. Chem. Soc. **39:** 1848, 1917.
19. ADAM, N. K. The Physics and Chemistry of Surfaces, London, Oxford University Press, 1941.
20. CARY, A. AND RIDEAL, E. K. The behavior of crystals and lenses of fats on the surface of water. Proceedings Royal Society, London A **109:** 301, 1925.
21. DAVIS, DALE S. Emperical Equations and Nomography, pg. 11, McGraw-Hill, New York, 1943.