ACID NUMBER AND SPREADING INDEX OF THE HUMAN SKIN SURFACE LIPIDS

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Presented October 8, 1958, Seminar, New York City

In previous extensive investigations we studied the amount ("casual level") of the skin surface lipids under various conditions with special attention to their distribution in different areas of the human body surface (1, 2, 3, 4, 5). More recently we investigated the *free fatty acid* contents of the respective samples, as well as the *spreading index* (6, 7). The latter term was introduced for the cm.^{2} area of skin surface covered by 1 mcgm. of the lipid under investigation—when a given quantity of it is, under standardized conditions, placed on an aqueous surface (6). Assays of the lipid spread on water had been advocated by Jones and collaborators for determination of the lipid quantity (8, 9, 10), since they presumed that the free fatty acids control the spreading ability of the samples and that their proportion in the skin surface lipids is constant. Our investigations had revealed (1) that the spreading index differs from one individual to another for samples collected from a given site so that it is not generally feasible to utilize the index for determination of the lipid quantity: (2) that there are distinct regional differences for the lipids collected from different test areas; and (3) that for certain test areas the index tended to increase during the warmer season.

Our assays of the acid number which had been carried out on only a minor scale have been greatly expanded. It is the object of this presentation to report on the results obtained to date in addition to those previously published.

Experimental

Method. The methods of assaying lipid quantity and spreading index were the same as described in our previous reports (1, 2, 6, 7).

In order to obtain sufficient lipid quantities per sample for each of the

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three pertinent assays—weight, acid number (A.N.) and spreading index (S.I.)—every sample was processed as follows.

After the total volume (10 ml.) of fat free ethyl ether used to collect the material in our cylindrical receptacle had in a 10 ml. measuring flask slowly evaporated at 40°C., the residue was redissolved in 10 ml. of fat free petroleum ether. Under precautions necessary to avoid transfer of scales, etc. (6, 11), 5 ml. of this volume were used for gravimetric determination of the lipid quantity,*† 3ml. for assaying the acid number and the remainder for assaying the spreading index.

The A. N. was assayed in a manner similar to the procedure of Hodgson-Jones and Wheatley (12), who in turn rather closely followed the method described by Moyle, Baldwin and Scarisbruck (13). However, because of the much smaller lipid quantity obtainable from the different sites to be investigated separately, several modifications had to be introduced.

Thus, for the titration it was found necessary to reduce the concentration of KOH in methanol from 0.005 *N* to 0.002 *N*.‡ Smaller volumes, moreover, of solvent (chloroform) and of indicator had to be employed. Briefly, we proceed in the following manner:

The 3 ml. aliquot of the solution in petroleum ether is placed in a wide-necked 30 ml. Erlenmeyer flask and evaporated again at 40°C. After cooling, 2 ml. of chloroform and one drop of 0.04 per cent cresol red in methanol are added to the residue. The solution is carefully shaken. In order to remove the CO_2 present a stream of gaseous nitrogen is carefully bubbled through the chloroform solution for thirty seconds prior to titration. This is continued also through the period of titrating; it must be done slowly to avoid volatilization. The titration out of a microburette (one drop equals 0.01 ml. of the KOH solution in methanol) likewise is carried out slowly, since neutralization and color change, as a rule, do not occur immediately. The turn from greenish-yellow to a reddish-yellow is the end point. The addition of one drop in excess produces a distinctly purple color.

Under our laboratory conditions it is necessary to titrate about four "blanks" of chloroform, 2 ml. each, immediately prior to any assaying of samples. There are day to day variations of the blank titres from 0.02 to 0.03 or even 0.04 ml. of KOH, which must be deducted from the volumes required for neutralizing the solution under test.

From time to time it has been useful to perform control titrations of different known concentrations of stearic acid in chloroform.

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* TCY (Key Board) Balance, William Ainsworth & Sons, Denver, Colo., sensitivity 0.01 mg.

† Weighing of the residue was performed in 6 ml. aluminum foil cups, 3 cm. in. diameter, "Crinkle Cups," Muller Paper Goods Co., Inc., Long Island City, N. Y.

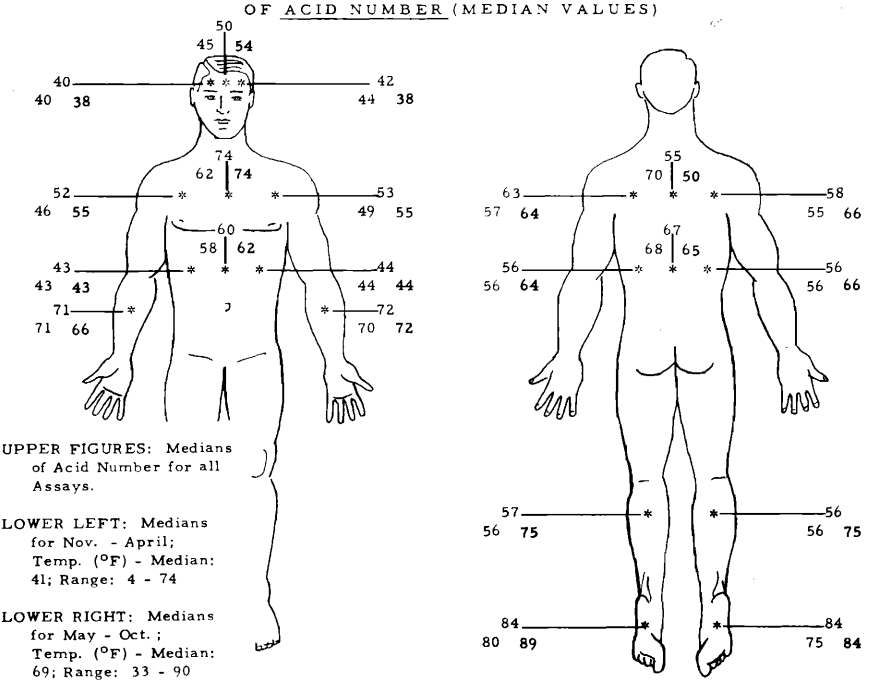
‡ Prepared by courtesy of Joseph R. La Vietes, La-Mar Laboratories, Inc., New York, N. Y.

TABLE 1—ACID NUMBER (A.N.)—MEDIANS OF SKIN SURFACE LIPIDS FOR (21) DIFFERENT TEST SITES AND TWO SEASONS (WARM AND COLD)

Site	Warm Period: May–October Temp., °F.—Median: 69; Range: 33–90				Cold Period: November–April Temp., °F.—Median: 41; Range: 4–74			
	No. of Subjects	No. of Assays	Median	Range	No. of Subjects	No. of Assays	Median	Range
I. Forehead								
a) Left	12	22	38	16– 71	15	47	44	7– 96
b) Middle	9	13	54	29– 76	15	21	45	21– 79
c) Right	12	37	38	21– 70	17	45	40	7– 77
II. Chest								
1. Upper (Level of 3d. Chondro-costal junction)								
a) Left (L. midclavicular line)	11	32	54	21– 85	15	46	49	18–112
b) Middle (Mid-sternum)	7	11	74	37– 83	12	23	62	27–116
c) Right (R. midclavicular line)	11	33	55	20– 86	15	46	46	16–112
2. Lower (Level of 7th. Chondro-costal junction)								
a) Left (L. midclavicular line)	14	41	44	19– 93	29	76	44	16–121
b) Middle (midsternal line, longitud.)	8	10	62	41– 89	14	15	58	21– 92
c) Right (R. midclavicular line)	14	41	43	19– 90	29	77	43	11–140
III. Forearms, volar surface, upper third								
a) Left	8	9	72	45–100	14	19	70	37–126
b) Right	8	23	66	26–100	14	19	71	34–126
IV. Back								
1. Upper (Level of T4)								
a) Left (L. midscapular line)	12	16	64	19– 87	13	17	57	32– 76
b) Middle (Vertebral line)	7	8	50	25–100	11	12	70	27– 98
c) Right (R. midscapular line, longitud.)	15	28	66	12–108	12	15	55	21– 75
2. Lower (Level of T9)								
a) Left (L. midscapular line, longitud.)	6	9	64	25–112	14	15	56	27–112
b) Middle (Vertebral line)	6	9	65	15–131	14	15	68	25–131
c) Right (R. midscapular line, longitud.)	6	10	66	25–112	14	15	54	25–112
V. Calves, upper third								
a) Left	3	7	75	47–137	16	24	56	26– 87
b) Right	3	8	75	47–137	16	24	56	30– 93
VI. Soles, arch								
a) Left	6	6	89	75–137	16	23	80	19–149
b) Right	6	6	84	74–137	16	24	75	19–140

GRAPH I

REGIONAL DIFFERENCES AND SEASONAL VARIATION OF ACID NUMBER (MEDIAN VALUES)



Subject and Test Sites. Forty-five healthy volunteers, twenty-two to thirty-eight years of age were subjected to the examinations. Thirty-seven of these were males, 35 white, 2 Negro and eight were females, 5 white, 2 Negro, 1 Chinese.

Most of the subjects were investigated on several occasions and whenever feasible, both during the warm and cold seasons of the year.

The same [21] skin areas were used for collecting the lipid samples which were standard test sites also in our previous studies (1, 2, 3, 6, 7). The sites are identified on tables as well as Graphs I and II.

Similarly, the precautions employed prior to testing, avoidance of washing of the sites for at least forty-eight hours, etc., were identical with those described previously (1, 2, 3, 6, 7). In all, 1214 A.N. assays have been performed to date.

Results

Upon the advice of our biostatistician* the results are presented as medians, rather than as mean values, since the median—representing the

* Dr. R. R. Freudenthal, Statistical Consultant, Jackson Heights, N. Y., evaluated our results mathematically and statistically.

TABLE 2—SPREADING INDEX (S.I.)—MEDIANS OF SKIN SURFACE LIPIDS, Cm.² AREA ON WATER/γ, FOR (21) DIFFERENT SITES AND TWO SEASONS (WARM AND COLD)

Site	Warm Period: May–October Temp., °F.—Median: 69; Range: 33–90 No. of Subjects	No. of Assays	Median	Range	Cold Period: November–April Temp., °F.—Median: 41; Range: 4–74 No. of Subjects	No. of Assays	Median	Range
I. Forehead								
a) Left	13	28	2.3	1.1– 6.2	17	37	1.9	0.8– 3.5
b) Middle	12	24	2.4	0.8– 5.6	15	28	2.0	0.7– 3.3
c) Right	13	27	2.4	1.0– 5.3	17	36	2.0	0.8– 4.0
II. Chest								
1. Upper (Level of 3d. Chondro-costal junction)								
a) Left (L. midclavicular line)	11	22	2.6	1.7– 4.8	15	30	2.4	0.8– 3.9
b) Middle (Midsternum)	10	20	2.6	1.5– 5.9	14	25	2.9	1.6– 3.9
c) Right (R. midclavicular line)	11	22	2.6	1.8– 5.0	15	30	2.7	0.8– 4.7
2. Lower (Level of 7th Condro-costal junction)								
a) Left (L. midclavicular line)	16	29	2.2	1.1– 5.9	29	69	2.1	0.6– 3.4
b) Middle (midsternal line, longitud.)	13	23	2.4	1.3– 5.3	16	27	2.2	0.6– 3.3
c) Right (R. midclavicular line)	14	25	2.5	1.1– 5.6	29	69	1.9	0.6– 3.5
III. Forearms, volar surface, upper third								
a) Left	14	22	2.8	1.4–10.8	18	37	2.1	1.1– 6.7
b) Right	14	23	2.6	1.5–10.2	18	37	2.2	1.1– 6.0
IV. Back								
1. Upper (Level of T4)								
a) Left (L. midscapular line, longitud.)	19	35	2.2	1.0– 4.2	16	29	1.8	0.5– 3.5
b) Middle (Vertebral line)	15	29	2.6	1.5– 4.1	14	23	2.5	0.8– 4.1
c) Right (R. midscapular line, longitud.)	21	45	2.6	1.0– 4.7	16	28	1.9	0.5– 4.5
2. Lower (Level of T9)								
a) Left (L. midscapular line, longitud.)	14	32	2.2	1.0– 5.6	17	26	1.9	0.5– 5.6
b) Middle (Vertebral line)	14	31	2.6	0.9– 6.2	17	23	2.1	0.5– 6.2
c) Right (R. midscapular line, longitud.)	14	33	2.2	1.2– 5.4	17	26	1.9	0.5– 5.3
V. Calves, upper third								
a) Left	7	11	3.3	1.8–12.5	17	22	2.7	1.3– 4.5
b) Right	7	12	3.3	1.8–12.5	17	22	2.6	1.2– 4.5
VI. Soles, arch								
a) Left	9	9	4.0	1.8– 4.5	17	20	2.9	1.6–11.0
b) Right	9	9	3.3	2.4– 6.7	17	20	3.2	1.6–12.5

midpoint in a numerical series—is not affected by exceptionally high or low values.

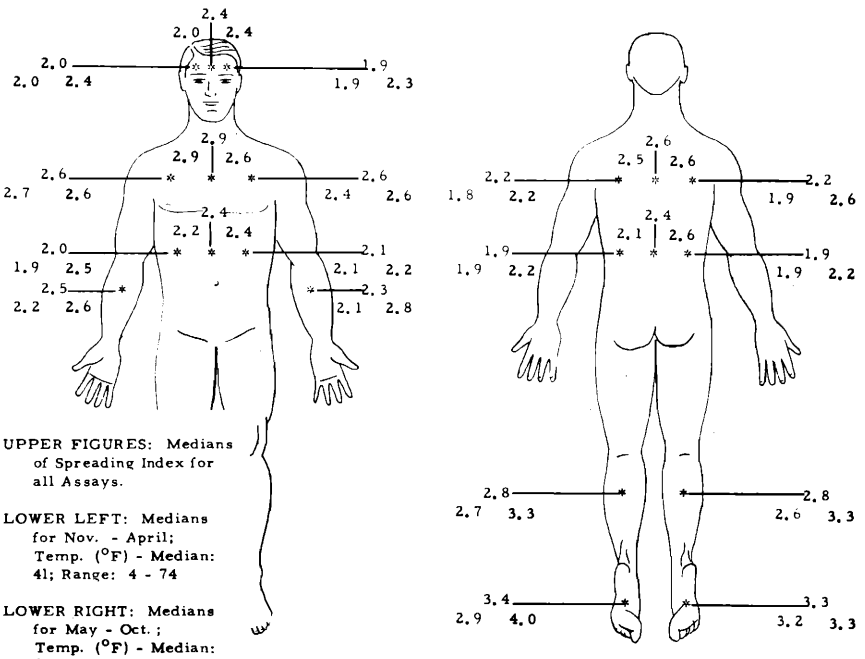
As is apparent from Graph I,† as well as from Table 1 the medians of the *acid number* for symmetrical sites are virtually identical. The wide ranges tabulated for each of the test areas are due to the fact that for any given site the values differed considerably among the subjects. The values are, moreover, by no means equal for our different test areas. The lipids from the three sites of the forehead, for example, have a lower acid number than those collected from mid upper chest, forearms or soles. For the soles the values are higher than for any of the other areas tested.

Furthermore, a tendency is apparent for the values of some of the areas to rise during the summer season. Only for the mid upper back an appreciable trend in the opposite direction seems to be apparent, but to date the number of subjects and assays is smaller for this site than for any other.

† The Graphs I and II and the results presented in Tables 1 and 2 were submitted for publication also to the Editors of "Der Hautarzt."

GRAPH II

REGIONAL DISTRIBUTION AND SEASONAL VARIATION OF SPREADING INDEX cm^2/mcgm (MEDIAN VALUES)



UPPER FIGURES: Medians of Spreading Index for all Assays.

LOWER LEFT: Medians for Nov. - April; Temp. (°F) - Median: 41; Range: 4 - 74

LOWER RIGHT: Medians for May - Oct.; Temp. (°F) - Median: 69; Range: 33 - 90

The median values for the *spreading index*, while being practically equal for symmetrical sites, again differ distinctly for different skin regions. We have high values for mid upper chest, the calf regions and the soles; and low values for temples and lateral aspects of the lower trunk. Again, a number of the medians are higher in the warmer season. This is even more pronounced than it was for the acid number. Thus, the difference between the means for the two seasons is statistically significant, or almost significant for forearms and calves (probability ranging from 0.05 to 0.1).

Comments

The fact that the acid number is not the same for every area of the body surface must be borne in mind for any comparative evaluation especially as regards results obtained by different investigators.

The fact that acid number and spreading index do not show a perfect correlation denotes a participation of components other than the free fatty acids in affecting the lipid spread. Free cholesterol, for example, is known to increase the index; unsaturated fatty acids promote the spreading more than saturated acids, whereas waxes producing multilayers rather than a monolayer interfere with the spread. Like ourselves, Wheatley and his group discarded the spreading method as unreliable for estimation of the lipid amount and pointed out that the hydrocarbons in sebum do not spread in the surface film (14).

We should like to know the cause of the differences, in particular in the acid number, obtained for the different skin areas. Like others, we believe that the presence of free fatty acids on the skin is largely due to esterase activity produced by the microbial flora, even though experiments presently in progress in our laboratory seem to suggest a liberation of acids also by other factors, such as enzymes preformed in the tissues. Microbial acitivity, nevertheless, can be regarded as crucial (15, 16). Microbial growth and activity usually being facilitated by moisture and impaired by dryness (17, 18, 19, 20, 21), liberation of fatty acids may be expected to be promoted in areas of increased sweating and/or under conditions of generally increased sweat delivery. Larger amounts of sweat, moreover, might render the triglycerides more susceptible to the attack by enzymes, since the lipids undergo spontaneous emulsification with the sweat, as was shown in our previous investigations (3, 4, 5). All this may well account for the higher acid numbers we obtained for such areas as the mid upper chest or the soles. Similar considerations would be justified regarding the increase of the values we observed for some of the test areas during the warm season. The seasonal differences should be the more distinct, the greater the contrast is for a given region between dryness in winter and moisture in summer. This would explain such differences as seen here for the forearms and soles (Graph II).

Summary

Acid number, as well as spreading index of the skin surface lipids differ for a given skin area from subject to subject. They both likewise distinctly differ for different skin areas and for some of the areas tend to rise during the warmer season. Some inferences of these results have been discussed.

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