EFFECT OF HAND WASHING ON SKIN LIPID REMOVAL

The studies of hand washing effects on surface lipid removal were performed under standardized and carefully controlled conditions. In the majority of the experiments a 1-minute or a 15-minute washing period was used, these tests being classified as ordinary hand washing and simulated dish washing, respectively. A limited number of investigations was also conducted in which special hand washing conditions were employed to supply additional information about the factors involved in skin lipid removal. The lipid content present on the skin surface before hand washing is described as the casual level and the concentration observed after washing is termed the residual level. These values are expressed in milligrams of ether-petroleum ether soluble carbon (EPESC) per cm^2 of skin surface. The general hand washing experiments and the special washing studies will be described in separate chapters.

GENERAL HAND WASHING EXPERIMENTS

The hand washing procedure commonly used for skin cleansing consists of washing under tap water for a brief period with a milled bar. Definite emphasis has therefore been placed on this type of ordinary hand washing, and experiments have been made with various types of commercially available washing agents, using a 1-minute washing period. Since household washing and laundering may expose the skin to solutions of surface-active agents for a considerably longer time, studies on skin lipid removal under such conditions also deserve consideration, and the greater part of the investigations reported in the present book are simulated dish washing tests performed over a 15-minute period. The particular value of this type of hand washing study is that the washing agent concentration can be carefully controlled under such experimental circumstances, thus permitting several decisive conclusions from the results.

TECHNICAL PROCEDURES

I. PERSONS USED FOR HAND WASHING STUDIES

Only reliable adult subjects without skin diseases were included in the investigations; over 95 % of the persons ranged in age between 20 and 60 years. It was requested that at least 3 hours elapse since the last conventional hand washing;

an interval of one week was usually arranged between experimental hand washing tests. Persons using nail polish were not considered suitable for hand washing studies because of the solubility of nail polish in ethyl ether and petroleum ether. Jewelry was systematically removed from the hands before the washing experiments. The persons are listed under individual numbers in all the tables; for comparative purposes the race and sex of the subjects are also recorded.

II. HAND WASHING TECHNIQUES

Every washing test was conducted under constant supervision.

A. *Ordinary Hand Washing Procedure (1-Minute Washing Period)*

The hands are wetted with lukewarm water from a faucet, and a milled bar is used for 15 seconds, the time being checked with a stop watch. The bar is then deposited, after which the washing is continued for another 45 seconds, one hand washing the other with ordinary movements. During this period, small amounts of lukewarm water are added to the hands from the faucet so that a good lather is worked up. Following this, the hands are rinsed for 20 seconds in running tepid water from faucet and are subsequently dried for 15 to 20 seconds in a clean paper towel. A new bar is used for each experiment.

In some of the studies, powdered material was used instead of a milled bar. In these experiments 10 ml. of tepid water was placed in the cupped hands and the washing material added to the water; if more suitable, 10 ml of water in which the preparation already had been dissolved was placed directly in the hands. The washing was otherwise conducted in a similar way as with the milled bar.

B. *Simulated Dish Washing Procedure (15-Minute Washing Period)*

1. *Preparation of washing solution*

Two liters of distilled water are poured into a metal basin and the washing agent is dissolved in the water by whipping the contents of the basin with a metal stirrer. The wash basin is heated on an electric plate until the solution reaches a temperature of 40° C. For some compounds it is necessary to heat the wash water to a temperature of 65° C. to bring the washing material into solution; in those instances the water is subsequently cooled to 40°C. before the washing experiment is started. A porcelain dish (diameter, 14 cm; weight, 230 grams) is inserted into the basin. pH measurements of the solution are made with a Beckman pH meter at 40° C. before and after the washing period. The average pH value for each set of washing experiments was calculated on the basis of hydrogen ion concentrations.

2. *Washing period*

Dish washing is carried out for exactly 15 minutes. The porcelain dish is handled by the person with manipulations imitating the motions of ordinary dish washing. It is ascertained that the hands are covered with water nearly all the time. The

temperature of the washing solution is maintained at a 38°C. to 40°C. level during the washing period.

3. *Rinsing and drying of hands*

Following the 15-minute washing period, the hands are withdrawn from the basin and are rinsed by slowly pouring 400 ml of distilled water over them (into another basin); they are subsequently dried for 15 to 20 seconds in a clean paper towel.

III. METHODS FOR LIPID COLLECTION AND DETERMINATION

The methods used for lipid collection, isolation, and quantitative determination are essentially those described by Kirk, Page, and Van Slyke (82), Van Slyke, Page, and Kirk (157) and Kirk (78). Before use, glassware is regularly cleaned with combustion fluid.

A. *Casual Skin Lipids*

1. *Collection of lipids*

Casual skin lipids are collected from a 4-cm^2 area in the central region of the palm and the back of each hand by applying a glass cylinder with an opening of 4 cm^2; the ground edge of the cylinder is pressed firmly against the skin to prevent leakage of the solvent under the edge into adjoining skin areas. The lipids are extracted from the delimited 4-cm^2 skin surface by two successive additions of 5 ml redistilled ethyl ether to the cylinder, each portion being left in contact with the skin for 1 minute. At the end of the contact period, the ether is transferred by suction to a collection tube; the transfer is made by means of an all-glass apparatus (Fig. 4). After transfer of the second 5-ml portion, the absorption tubing of the apparatus is rinsed with a few ml of redistilled ether which are sucked into the collection tube.

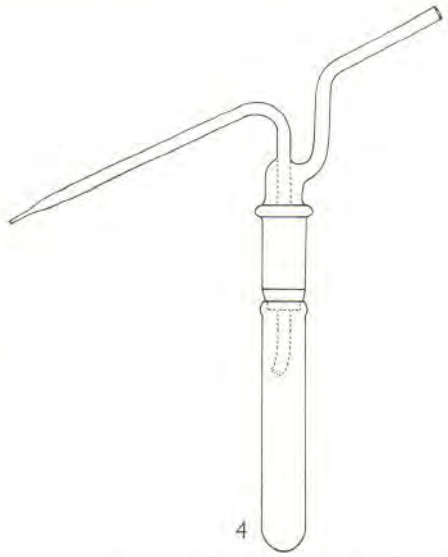
The efficiency of this procedure for quantitative removal of lipids from a skin area has been verified by Kvorning (88) who in a third 5-ml portion of ether applied to the skin surface found less than 0.5 % of the total amount of lipids removed by the first two treatments with ether. Similar results have been reported by Kirk (78) and by Johnsen and Kirk (73).

2. *Evaporation of ethyl ether from collected sample*

The ether is evaporated from the tube at room temperature or at a temperature which does not exceed 60° C. (82). During evaporation the sample is protected against dust. The residue after evaporation consists of lipids, epithelial scales and a certain amount of water-soluble organic and inorganic substances.

3. *Dissolution of lipids in petroleum ether*

The lipid material present in the sample residue in the collection tube is dissolved by application of successive portions of redistilled, water-free petroleum



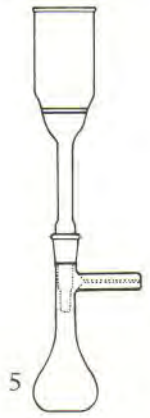


Fig. 4. Cylinder for Collection of Casual Skin Lipid Ethyl Ether Samples.
Fig. 5. Filter and Volumetric Flask for the Petroleum Ether Extract.

ether (Fisher Sc. Co., certified, A.C.S.) purified as described by Kirk, Page, and Van Slyke (82). Three ml. of petroleum ether is pipetted into the tube which is then immersed for a few seconds into hot water contained in a beaker. When boiling starts, the petroleum ether in the tube is shaken so as to come into contact with the whole inside of the tube. The petroleum ether is then poured into the cup of a sintered glass filter attached with a ground joint to a 10-ml volumetric flask provided with a side outlet (Fig. 5); it is passed through the filter by gentle suction. Two additional petroleum ether portions are successively added to the tube and similarly passed through the glass filter. The filter is subsequently rinsed with a few ml of petroleum ether. The sample in the volumetric flask has now reached a volume of 8 to 9 ml; the filter is then removed and the sample is made up to the 10-ml mark by addition of petroleum ether. The glass stopper is inserted and the content of the volumetric flask is mixed by repeated inversion; during the mixing of the sample, the side outlet of the flask is directed upward and is covered. A 5.0-ml aliquot of the lipid-containing petroleum ether extract is pipetted into a combustion tube for manometric carbon determination. The dimensions of the combustion tube are those described by Van Slyke, Page, and Kirk (157).

The filtration of the petroleum ether extract through a sintered glass filter as used in the present technique for removal of cellular debris suspended in the solvent is essential for accurate lipid measurement (64, 65, 66, 78, 88) but is omitted by many investigators according to the literature.

4. *Evaporation of petroleum ether in combustion tube*

The 5.0-ml petroleum ether sample in the combustion tube is maintained for several hours at room temperature for partial evaporation; during this period

the combustion tube is placed in a beaker protected from dust by a Petri dish. For final evaporation, the combustion tube is immersed in hot water placed in another beaker, the height of the water not exceeding 3 cm. At this stage it is not necessary to insure that the evaporation takes place below 60° C. When the residue in the tube appears to be dry, one small drop of redistilled water is added and the tube is heated carefully in a beaker placed on a slightly heated electric plate. When distilled water vapor no longer is observed on the inside of the neck of the combustion tube, the tube is cooled to room temperature and the sample is then ready for manometric carbon determination. The treatment of the residue with one drop of redistilled water serves to remove possible traces of petroleum ether present in the tube.

B. *Residual Skin Lipids on Surface of Whole Hand*

1. *Collection of lipids*

Before collection of residual skin lipids present on the surface of the whole hand, a copper wire is placed around the wrist. Each hand is then submerged to the evel of the wire into 450 ml of redistilled ethyl ether contained in a 1000-ml beaker, 18.5 cm tall and 9 cm wide; a separate beaker is used for each hand. After 30 seconds' immersion, the hands are withdrawn from the ether but are held over the beakers until all excess ether adhering to the skin has dripped into the beakers. Because repeated immersions produce skin irritation, the hands in each experiment are immersed only once in ethyl ether. A blank is performed on the whole ether sample (450 ml) after each preparation of redistilled ethyl ether.

2. *Evaporation of ethyl ether from collected samples*

The ether is transferred quantitatively in successive portions from the beaker into a 500-ml Florence flask with flat bottom and a 24/40 joint. The samples from the right and left hand are handled separately. The Florence flasks are placed in a water bath maintained at a temperature of 55° C and the ether is removed by distillation. The beakers are rinsed with ethyl ether which is added to the flasks.

3. *Dissolution of lipids in petroleum ether*

The lipid content of the sample residue in the Florence flask is dissolved in successive portions of 10 ml redistilled, water-free petroleum ether (Fisher Sc. Co., certified, A.C.S.), purified as described by Kirk, Page and Van Slyke (82). After addition of petroleum ether, the flask is heated to a temperature not to exceed 60° C. and is swirled to bring the solvent into contact with all the deposited residue. The petroleum ether portions are subsequently passed through a sintered glass filter attached with a ground joint to a 50-ml volumetric flask with a side outlet; they are passed through the filter by gentle suction. When a volume of about 40 ml has been reached, the sample is transferred to a measuring cylinder. The volumetric flask is rinsed with petroleum ether which is added to the cylinder. The volume is finally brought up to 50 ml, after which a glass stopper is

inserted and the sample is thoroughly mixed by repeated inversion of the measuring cylinder. One-ml aliquots are pipetted into combustion tubes for manometric carbon determination.

4. *Evaporation of petroleum ether in combustion tube*

The evaporation of the petroleum ether sample in the combustion tube is performed as described for the casual lipid sample, except that preliminary partial evaporation at room temperature is not necessary because of the smaller volume used.

C. *Manometric Carbon Determination*

The manometric carbon determinations were made by the procedure of Van Slyke, Page, and Kirk (157). This method is 99.5 % accurate (88, 157). A detailed description of the manometric method is available from the author on request; this description includes directions for preparation of reagents.

IV. STATISTICAL ANALYSIS OF RESIDUAL LIPID LEVELS

The casual and residual EPESC values observed in the various hand washing tests have been reported in separate Appendix Tables. In addition, mean residual levels have been calculated for each experiment on the basis of a casual skin lipid concentration of 0.0050 mg EPESC/cm^2 (representing an average value for the palm and back of each hand). These statistical analyses were performed by the R & D Statistical Section of The Procter & Gamble Company and were based on the slopes of the least squares straight line relating the residual and casual data for all subjects in a given series of experiments. Common slopes were computed separately for ordinary hand washing and simulated dish washing tests because of the difference in experimental procedure. These values are presented in Appendix Table 103, in which the 95 % confidence limits associated with the calculations are also listed.

V. MEASUREMENT OF HAND AREA

The outline of the whole hand, including the lateral aspects of the hands and fingers, is traced on graph paper and the hand area calculated by adding up the areas of tracing; the measured area includes the total surface of the hand to the level of the wrist.

To establish the efficiency of hand area determination, repeated measurements were made on 15 subjects. The mean recorded difference between these duplicate tracings and planimetric assays was 1.24 %.

SURVEY OF CASUAL SKIN LIPID LEVELS

In connection with the 1520 tests performed for the purpose of measuring the degree of removal of cutaneous surface fat by various washing agents, determi-

Table 3. *Summary of Casual Skin Lipid Levels*

Values expressed in mg EPESC per cm^2 of skin surface

	N	Right Back	Right Palm	Left Back	Left Palm	Mean
White Men	346	0.0531	0.0492	0.0485	0.0483	0.0498
White Women	570	0.0573	0.0513	0.0543	0.0488	0.0529
Colored Men	134	0.0604	0.0585	0.0539	0.0524	0.0563
Colored Women	470	0.0636	0.0541	0.0566	0.0509	0.0563
Average Value for 1520 Individual Studies (6080 Analyses) = 0.05333						

nations of casual lipid concentrations present on the skin before hand washing were made systematically on both the back and palm of the right and left hand. This represents a total of 6080 casual skin lipid analyses.

The mean values recorded for white and colored men and women are presented in Table 3, the average EPESC level being 0.05333 mg per cm^2. On the basis of this observation, the approximate thickness of the surface lipid film can be estimated. If such a calculation is made without consideration of the influence of skin folds on the actual size of measured areas, the recorded EPESC value corresponds to a surface lipid layer of 0.76 micron thickness. The percentage proportions of casual EPESC values, ranging from 0.010 to 0.125 mg/cm^2, are presented in Figs. 6 to 21.

After completing these extensive studies, it was considered desirable to compare the casual surface lipid levels recorded for various seasons. The average monthly EPESC values are listed in Tables 4, 5, 6, and 7. Although the seasonal variation in absolute values is not very pronounced, a distinct tendency was noted for the lipid level on the palm to increase during the summer months and for the level on the back of the hand to decrease during this period of the year. This conspicuous seasonal variation in the proportional distribution of the skin lipids on the two surfaces of the hands is listed in Table 8 and presented graphically in Fig. 22. The seasonal change in sweating and the associated spreading ability of fats on moist and dry skin may at least to some extent account for the proportional difference in the distribution of lipids on the palm and back of the hands as demonstrated in the present investigation.

The significance of these calculations was confirmed by The R & D Statistical Section of The Procter & Gamble Company who in addition made comparisons of values recorded for the same persons throughout the year. The average lipid values on the palms expressed in % of lipid levels on the backs of the hands for this group of subjects were:

December, January, February	78.27 %
March, April, May	86.38 %
June, July, August	111.71 %
September, October, November	88.33 %

These computed data are in good agreement with those presented for the total number of subjects in Table 8 and Fig. 22.

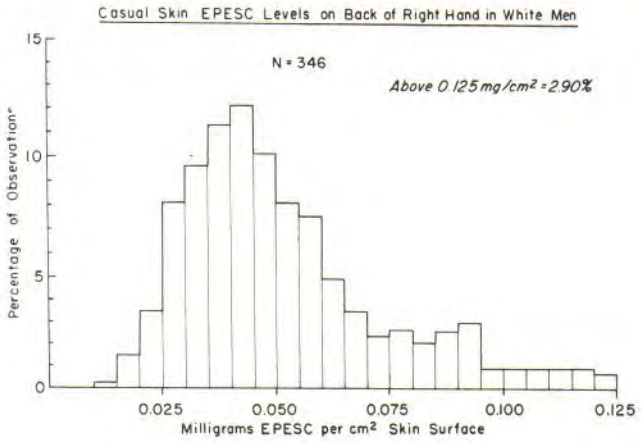


Fig. 6.

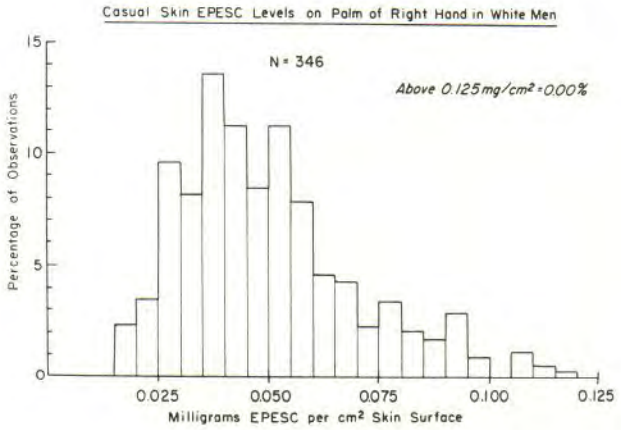
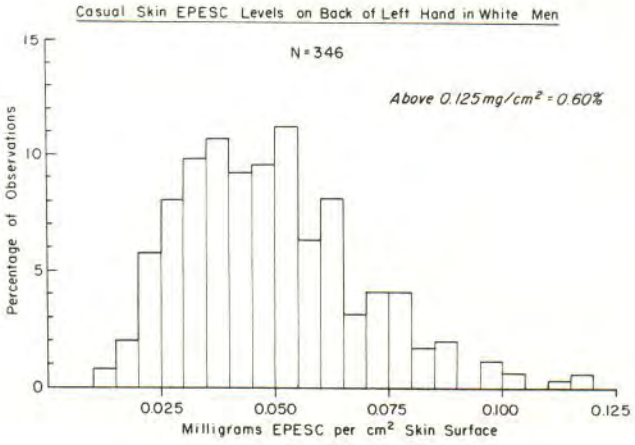


Fig. 7.



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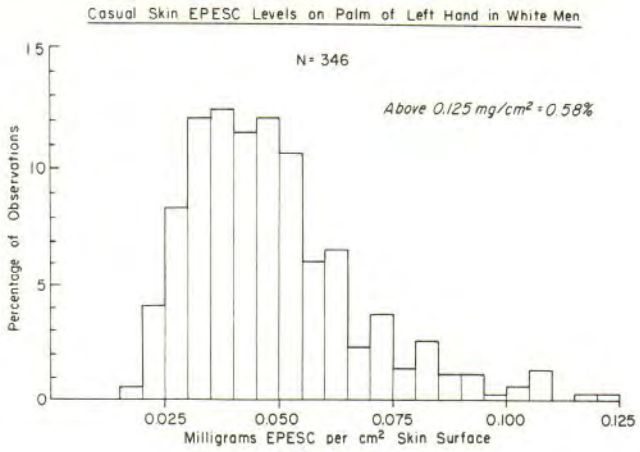


Fig. 9.

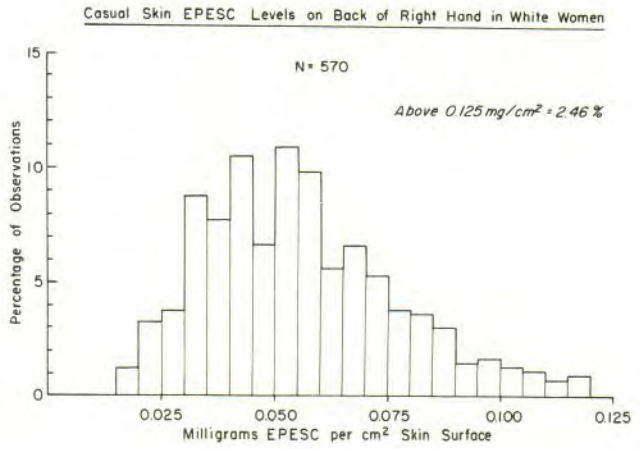


Fig. 10.

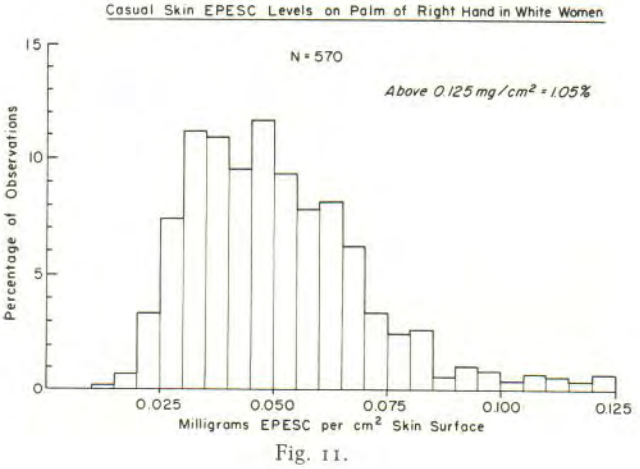


Fig. 11.

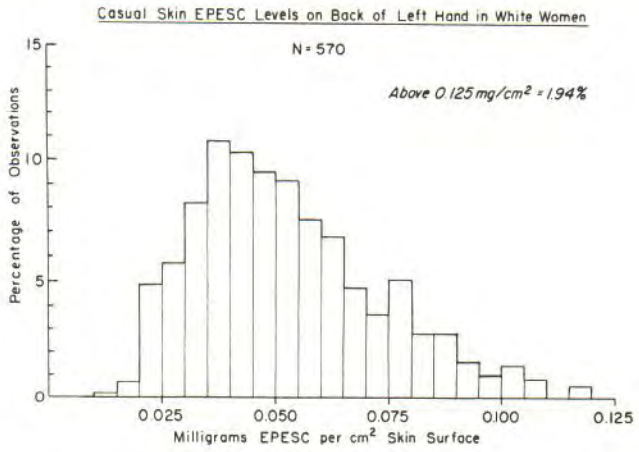


Fig. 12.

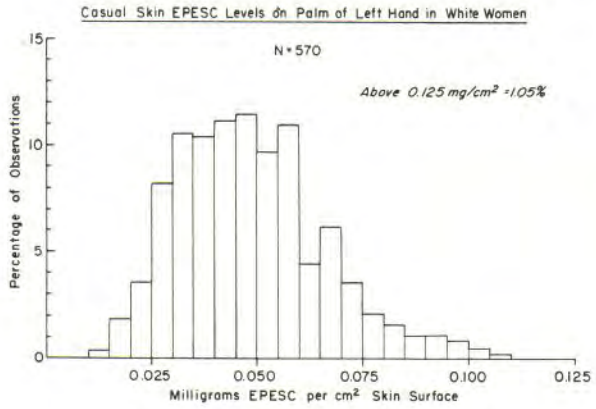


Fig. 13.

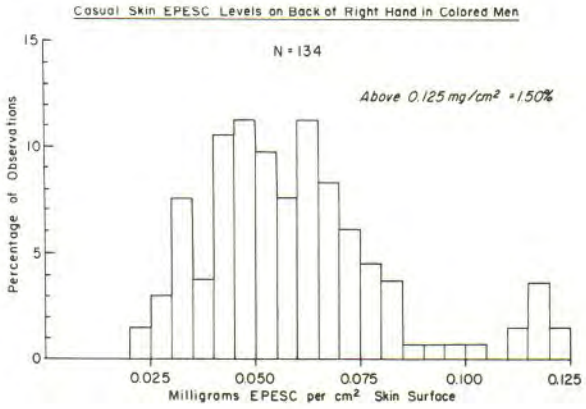


Fig. 14.

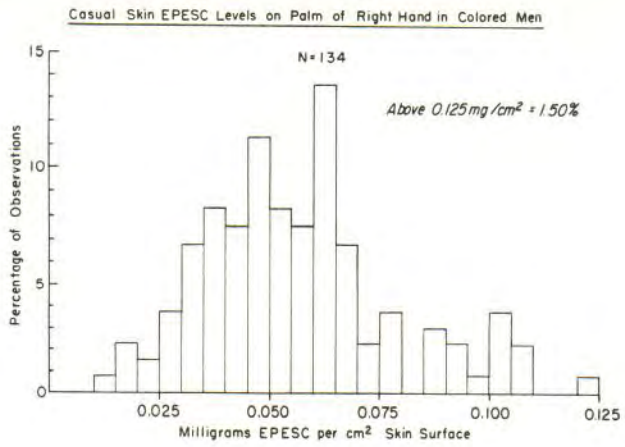


Fig. 15.

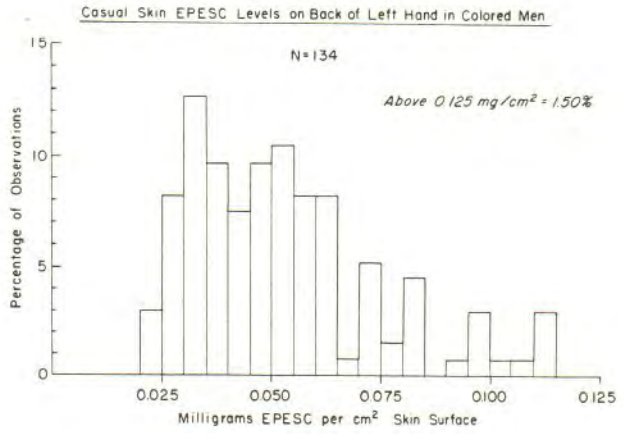


Fig. 16.

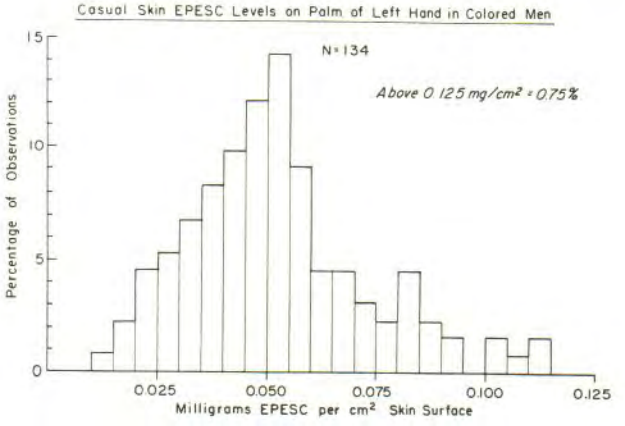
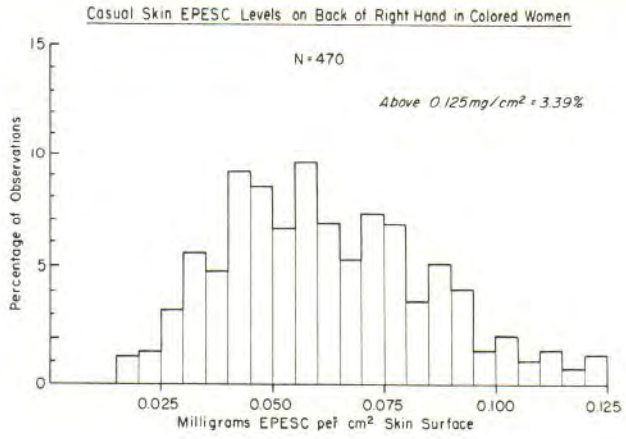


Fig. 17.



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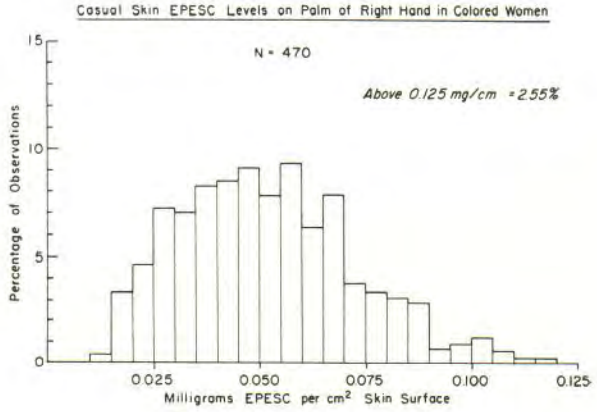


Fig. 19.

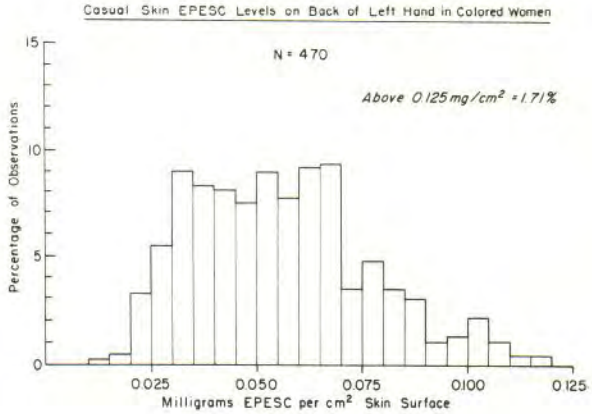


Fig. 20.

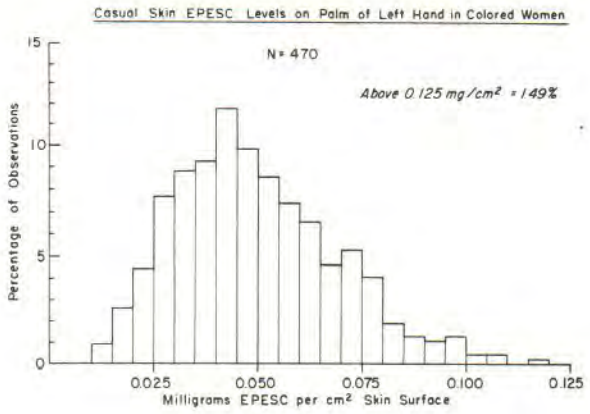


Fig. 21.

Table 4. *Casual Skin Lipid Values Observed in White Men* ($N = 346$)
Values expressed in mg EPESC per cm² of skin surface

Month	N	Right Back	Right Palm	Palm Value in % of Back Value	Left Back	Left Palm	Palm Value in % of Back Value	Average % Value
January	20	0.0571	0.0456	79.9	0.0480	0.0442	92.1	86.00
February	28	0.0575	0.0477	83.0	0.0511	0.0432	84.5	83.75
March	21	0.0622	0.0505	81.2	0.0575	0.0489	85.0	83.10
April	34	0.0515	0.0420	81.5	0.0453	0.0451	99.8	90.65
May	40	0.0472	0.0502	106.5	0.0400	0.0495	124.0	115.25
June	25	0.0467	0.0582	124.5	0.0430	0.0598	138.8	131.65
July	40	0.0400	0.0538	134.5	0.0400	0.0510	127.5	131.00
August	38	0.0513	0.0497	97.0	0.0482	0.0487	101.3	99.15
September	12	0.0568	0.0465	81.9	0.0565	0.0459	81.2	81.55
October	38	0.0566	0.0479	84.3	0.0543	0.0452	83.2	83.75
November	31	0.0595	0.0482	81.1	0.0555	0.0525	92.9	87.00
December	19	0.0684	0.0455	66.6	0.0586	0.0436	74.3	70.45

Table 5. *Casual Skin Lipid Values Observed in White Women* ($N = 570$)
Values expressed in mg EPESC per cm² of skin surface

Month	N	Right Back	Right Palm	Palm Value in % of Back Value	Left Back	Left Palm	Palm Value in % of Back Value	Average % Value
January	44	0.0688	0.0535	77.7	0.0665	0.0529	79.5	78.60
February	45	0.0659	0.0561	85.3	0.0656	0.0506	77.1	81.20
March	51	0.0678	0.0564	83.2	0.0597	0.0554	92.6	87.90
April	48	0.0631	0.0528	83.7	0.0587	0.0488	82.9	83.30
May	27	0.0500	0.0511	102.3	0.0457	0.0505	110.4	105.35
June	23	0.0426	0.0450	105.3	0.0437	0.0455	104.1	104.70
July	56	0.0466	0.0474	102.1	0.0452	0.0474	105.0	103.55
August	55	0.0431	0.0490	113.6	0.0432	0.0456	105.8	108.70
September	40	0.0493	0.0467	95.0	0.0440	0.0453	102.9	98.95
October	74	0.0631	0.0542	85.9	0.0579	0.0492	85.1	85.50
November	50	0.0600	0.0502	83.6	0.0579	0.0476	82.5	83.05
December	57	0.0600	0.0492	81.9	0.0552	0.0465	84.2	83.05

Table 6. *Casual Skin Lipid Values Observed in Colored Men (N= 134)*
Values expressed in mg EPESC per cm² of skin surface

Month	N	Right Back	Right Palm	Palm Value in % of Back Value	Left Back	Left Palm	Palm Value in % of Back Value	Average % Value
January	16	0.0749	0.0568	75.8	0.0591	0.0446	75.6	75.70
February	10	0.0607	0.0545	89.9	0.0553	0.0482	87.1	88.50
March	15	0.0623	0.0567	91.0	0.0620	0.0535	86.4	88.70
April	18	0.0626	0.0615	98.0	0.0634	0.0534	84.2	91.10
May	13	0.0593	0.0524	88.4	0.0524	0.0517	99.1	93.75
June	0	—	—	—	—	—	—	—
July	10	0.0615	0.0759	123.5	0.0442	0.0629	142.0	132.75
August	20	0.0448	0.0591	132.2	0.0382	0.0489	128.0	130.10
September	6	0.0524	0.0593	113.5	0.0369	0.0485	131.5	122.50
October	9	0.0660	0.0588	89.4	0.0590	0.0588	99.8	94.60
November	13	0.0637	0.0620	97.2	0.0660	0.0648	98.1	97.65
December	4	0.0583	0.0452	77.7	0.0466	0.0413	88.6	83.15

Table 7. *Casual Skin Lipid Values Observed in Colored Women (N= 470)*
Values expressed in mg EPESC per cm² of skin surface

Month	N	Right Back	Right Palm	Palm Value in % of Back Value	Left Back	Left Palm	Palm Value in % of Back Value	Average % Value
January	37	0.0702	0.0555	79.1	0.0635	0.0527	83.0	81.05
February	44	0.0702	0.0558	79.4	0.0590	0.0475	80.5	79.95
March	43	0.0699	0.0490	70.4	0.0616	0.0525	85.2	76.45
April	38	0.0674	0.0567	84.2	0.0605	0.0552	91.2	87.70
May	30	0.0757	0.0607	80.1	0.0680	0.0576	84.7	82.40
June	16	0.0525	0.0636	121.2	0.0500	0.0653	130.7	125.95
July	35	0.0515	0.0515	100.0	0.0427	0.0474	110.0	105.50
August	47	0.0495	0.0501	101.5	0.0436	0.0468	107.0	104.25
September	38	0.0470	0.0441	93.8	0.0425	0.0411	96.8	95.30
October	54	0.0612	0.0522	85.5	0.0525	0.0479	91.2	88.35
November	47	0.0650	0.0582	89.8	0.0594	0.0510	86.2	88.00
December	41	0.0799	0.0609	76.3	0.0733	0.0585	79.9	78.10

Table 8. *Summary of Seasonal Variations in Lipid Distribution on Back and Palm of Hands*
Calculations based on 1520 individual observations (6080 analyses)

Month	N	Palm Value in % of Back Value
January	117	80.34
February	127	83.35
March	130	84.04
April	138	88.19
May	110	99.19
June	64	120.76
July	141	119.26
August	160	110.55
September	96	99.58
October	175	88.05
November	141	88.92
December	121	78.69

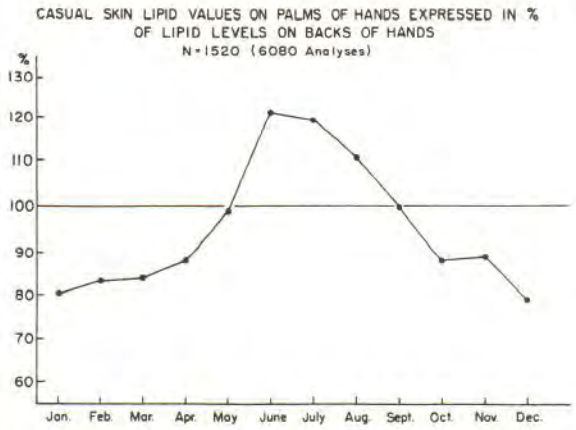


Fig. 22. Seasonal Variations in Casual Skin Lipid Distribution on Palms and Backs of Hands

SURVEY OF MEASURED HAND AREAS

The average hand surface areas observed for white and colored men and women are listed below. The hand areas include the total surface of fingers and of palm and back of the hand to the level of the wrist. The reported values are not corrected for the presence of skin folds and sulci.

Mean Hand Areas Observed in Various Groups of Adult Subjects

	N	Right Hand cm^2	Left Hand cm^2
White Men	45	504.5	503.3
White Women	51	416.0	413.8
Colored Men	5	546.8	529.5
Colored Women	24	436.0	432.8

Because of the rather large number of white men and women included in the study, the percentage distribution of hand areas recorded for these groups is presented graphically in Figs. 23 and 24.

RESULTS OF ORDINARY HAND WASHING STUDIES

I. WATER

Water is an essential factor in hand washing. Investigations by Szakall (144) on 8 individuals have shown that ordinary hand washing with water removes an average of 24.2 % of the skin surface lipids. In experiments conducted by the present author (Appendix Table 1) the mean residual level was 74.7 % of the original casual level, indicating a 25.3 % surface fat removal. These findings are in close agreement with those reported by Szakall.

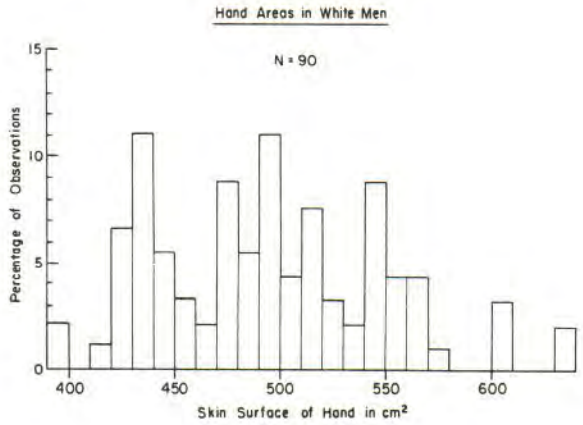


Fig. 23.

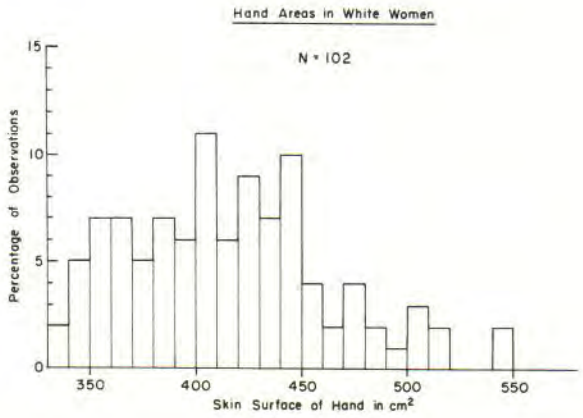


Fig. 24.

Ordinary Hand Washing with Water

No. of Persons	Mg EPESC/cm²		% of Lipids Removed
	Mean Casual Level on Both Hands	Mean Residual Level on Both Hands	
20	0.0445	0.0333	25.3

II. SOAP

By definition, conventional soap is the salt of an alkali and a fatty acid. In the soaps most commonly used, the alkali is either sodium or potassium hydroxide, both of which are strongly alkaline. The fatty acids are weak acids; they vary with the types of oil included in the soap.

Soap manufacturers use a large number of fats and oils of both animal and vegetable origin in the commercial production of soaps. The animal fats contain chiefly stearic, palmitic, oleic, and linoleic acids. In addition to these, certain of

the vegetable oils may contain lauric, myristic, capric, and small quantities of other fatty acids.

A fatty acid molecule is composed of a long hydrophobic chain and a hydrophilic group, COOH. This structure gives it a polar-nonpolar character and places it in the class of anionic surface-active compounds. Conventional soap is therefore an anionic detergent.

When dissolved in water, soaps are ionized and partially hydrolyzed.

$$\text{Ionization} : RCOONa \rightleftarrows RCOO^- + Na^+$$
$$\text{Hydrolysis} : 2\,RCOONa + H_2O \rightleftarrows (RCOO)_2NaH + Na^+ + OH^-$$

The principal product of the hydrolysis is a type of compound known as acidic soap : $(RCOO)_2NaH$. In this equilibrium reaction, small amounts of sodium hydroxide are formed with the result that all aqueous soaps are alkaline even though the soap contains chemically equivalent amounts of alkali and acid. Soap solutions usually show a pH of about 10. The amount of available alkali is increased in several commercial products through addition of builders.

When soap is put into hard water containing calcium and magnesium, soap reacts with these salts to form "lime" soap, which are insoluble compounds. Since the sweat secretions contain dissolved calcium and magnesium salts, some "lime" soap is always formed on the skin surface during washing. However, if soap is present in the washing solution in sufficient concentration to form suds, the "lime" soap precipitate may be brought into suspension. A special investigation on the adsorption of soap by the skin surface will be reported in a subsequent chapter.

A. *Conventional Soap*

Studies performed by Szakall (144, 145) on the effect of washing with soap on skin surface fats revealed a 37 to 40 % lipid removal. The effect of washing with soap bars of various composition on the cutaneous lipid level has previously been studied in the author's department (93); an average removal of 65 % of casual hand lipids was observed with the use of ordinary milled bars.

The results of 80 washing experiments with soap powder and with unperfumed soap bars are presented in detail in attached tables (Appendix Tables 2, 3). A summary of these findings shows mean removals of 50.1 and 55.2 % of casual hand surface lipids.

No. of Persons	Mg EPESC/cm²		% of Lipids Removed
	Mean Casual Level on Both Hands	Mean Residual Level on Both Hands	
1 gram soap powder (80 % tallow, 20 % coconut)			
20	0.0527	0.0264	50.1
Milled, unperfumed soap bar (80 % tallow, 20 % coconut)			
60	0.0477	0.0215	55.2

Significantly higher residual EPESC levels were found in hand washing investigations conducted with a conventional soap bar (80 % tallow, 20 % coconut) to which 0.9 % perfume had been added. The mean residual EPESC value observed in these experiments (Appendix Table 4) was 0.0354 mg per cm^2 of skin surface. Since this EPESC concentration is even higher than that recorded for hand washing with water only, the findings suggest that a definite deposit of perfume takes place in connection with the use of the perfumed bar. This is in accordance with observations made by The Procter & Gamble Company (Personal communication).

B. *Superfatted Soap*

Superfatted soaps are those which contain an excess of unsaponified fat. Addition of non-saponified fats is made with the purpose of replacing part of the skin surface lipids which are removed by the detergency of the soap. Hand washing studies with various types of superfatted milled soap bars (Appendix Tables 5, 6, 7) do not indicate significantly higher residual EPESC levels than those recorded for washing with unperfumed soap.

No. of Persons	Mg EPESC/cm^2		% of Lipids Removed
	Mean Casual Level on Both Hands	Mean Residual Level on Both Hands	
Conventional milled soap bar (80 % tallow, 20 % coconut) with 5 % added cottonseed oil			
20	0.0600	0.0230	61.7
Conventional milled soap bar (80 % tallow, 20 % coconut) with 5 % added lanolin			
20	0.0495	0.0204	59.2
Conventional milled soap bar (80 % tallow, 20 % coconut) with 5 % added mineral oil			
20	0.0542	0.0192	64.4

C. *Soap with Added Builder*

The addition of builders enables the soap to cleanse more efficiently. One of the builders most commonly present in laundry soaps is sodium tripolyphosphate (STP). The marked effect of this builder to increase the action of conventional soap in removing skin lipids has been clearly demonstrated in experiments on 40 persons (Appendix Tables 8, 9). The differences between results observed with 1 gram soap powder and with a mixture of soap and STP are presented in Fig. 25.

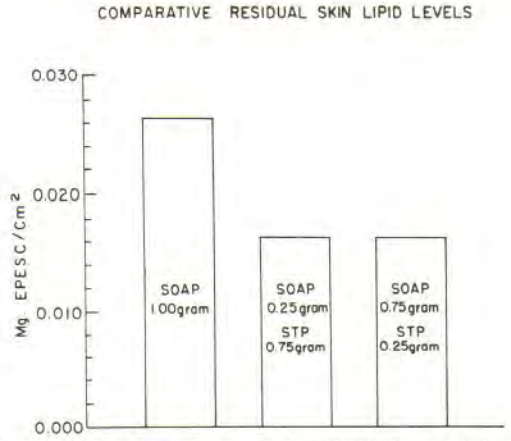


Fig. 25. Residual EPESC Levels After Ordinary Hand Washing With Soap and Soap + STP

No. of Persons	Mg EPESC/cm²		% of Lipids Removed
	Mean Casual Level on Both Hands	Mean Residual Level on Both Hands	
0.25 gram soap powder (80 % tallow, 20 % coconut), 0.75 gram STP			
20	0.0478	0.0164	65.8
0.75 gram soap powder (80 % tallow, 20 % coconut), 0.25 gram STP			
20	0.0588	0.0164	72.1

III. SYNTHETIC DETERGENTS

The use of synthetic detergents, particularly anionic detergents, has increased markedly during the last 15 years (Fig. 26). These compounds are used extensively both as toilet washing agents and as household cleansing and laundering products.

A. *Anionic Detergents*

Several of the anionic detergents are somewhat similar to conventional soaps in that they are salts of sodium or potassium. The remainder of the molecule is usually a sulfuric acid derivative of an organic compound containing a long carbon chain or a slightly modified hydrocarbon group. In aqueous solutions these substances ionize in much the same manner as do soaps. In contrast to soaps, the synthetic detergents do not hydrolyze when dissolved in water, for which reason the solutions are neutral in reaction. Their calcium and magnesium salts are soluble in water and deposit of these salts therefore does not occur.

The hydrophilic group of the more commonly used anionic synthetic detergents are the sulfates and the sulfonates. The lipophilic group is the hydrocarbon portion of the molecule.

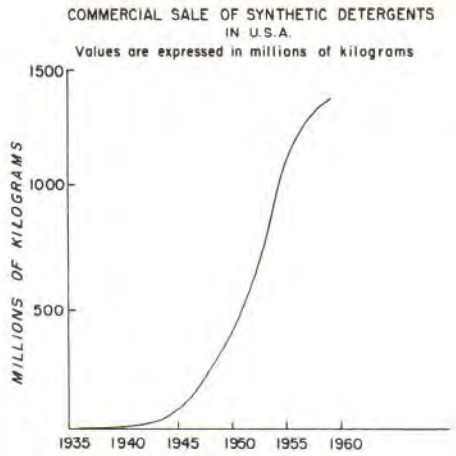


Fig. 26. The curve was constructed on the basis of data reported by Logue (96).

1. *Alkyl sulfates*

a. Fatty Alcohol Sulfates

The detergents classified as alkyl sulfates are fatty alcohol sulfates which are characterized by the presence of the following type of structure in the molecule:

$$R - O - SO_3\,Na$$

These synthetic detergents are manufactured by catalytic hydrogenation of fatty acids to high-molecular-weight alcohols; treatment of the alcohols with sulfuric acid yields alkyl sulfates which are neutralized with a base to form salts.

Hand washing experiments have been made with Coconut Alkyl Sulfate (CS = $C_{12}H_{25}OSO_3Na$), Tallow Alkyl Sulfate (TS = $C_{16-18}\,H_{33-37}\,OSO_3Na$) plus added builder, and with milled bars of Fatty Alcohol Sulfates (Appendix Tables 10, 11, 12).

No. of Persons	Mg EPESC/cm²		% of Lipids Removed
	Mean Casual Level on Both Hands	Mean Residual Level on Both Hands	
	Coconut alkyl sulfate (CS), 0.25 gram powder		
20	0.0553	0.0225	59.2
	Tallow alkyl sulfate (TS), 0.25 gram powder; STP 0.75 gram powder		
20	0.0538	0.0221	58.9
	Unperfumed milled bar of fatty alcohol sulfates		
20	0.0473	0.0203	57.2

Additional investigations were made in which a perfumed milled bar of fatty alcohol sulfates was used for hand washing; a quantity of 0.9 % perfume was present in this type of bar. The average residual skin surface lipid concentration observed in 20 experiments (Appendix Table 13) was 0.0313 mg EPESC per cm²

of skin, which is significantly higher than that found after hand washing with corresponding unperfumed bars. These findings are in agreement with the difference in EPESC levels obtained after hand washing with the perfumed and unperfumed soap bars.

b. Coconut Alkyl Oxyethylene Sulfate

This anionic synthetic detergent (COS) distinguishes itself by having three ethylene oxide groups interposed between the fatty acid chain and the sulfate portion of the molecule:

$$C_{12}H_{25}(OCH_2 - CH_2)_3\ OSO_3\ Na$$

Hand washing studies performed on 20 subjects (Appendix Table 14) revealed an appreciable degree of casual skin lipid removal by this agent.

No. of Persons	Mg EPESC/cm²		% of Lipids Removed
	Mean Casual Level on Both Hands	Mean Residual Level on Both Hands	
	Coconut alkyl oxyethylene sulfate (COS), 0.25 gram powder; Na_2SO_4, 0.75 gram powder		
20	0.0485	0.0187	61.4

2. *Alkyl sulfonates*

The synthetic detergents classified as alkyl sulfonates differ from the alkyl sulfates in that sulfonates contain a direct carbon-to-sulfur linkage, whereas in the sulfates, carbon is connected to sulfur through an oxygen atom. The following sulfur-containing group is therefore present in the sulfonates:

$$R - SO_3\ Na$$

The anionic sulfonated synthetic detergent most frequently used is alkyl benzene sulfonate which is also termed alkyl aryl sulfonate. A commerical production in 1958 of 1,920,000,000 pounds (= 870,000,000 kg) alkyl benzene sulfonate in U.S.A. has been recorded (155).

a. Alkyl Benzene Sulfonate

The structure of alkyl benzene sulfonate (ABS) is illustrated by the following formula:

Because of the wide use of this agent, hand washing studies were performed both with powdered ABS, and with ABS to which inorganic sulfate or sodium tripolyphosphate builder (STP) had been added (Appendix Tables 15 to 22). Investigations with the addition of Na_2SO_4 to the ABS compound were considered advisable because most commercial preparations contain an appreciable quantity of sodium sulfate, resulting from neutralization of sulfuric acid employed in the sulfonation process of the manufacturing procedure. With the purpose of increasing the cleansing action of alkyl benzene sulfonate, sodium tripolyphosphate (STP) is added to many commercial ABS products.

No. of Persons	Mg EPESC/cm²		% of Lipids Removed
	Mean Casual Level on Both Hands	Mean Residual Level on Both Hands	
Alkyl benzene sulfonate (ABS), 0.25 gram powder			
20	0.0550	0.0305	44.6
Alkyl benzene sulfonate (ABS), 0.125 gram powder; Na_2SO_4, 0.375 gram powder			
20	0.0628	0.0338	46.1
Alkyl benzene sulfonate (ABS), 0.25 gram powder; Na_2SO_4, 0.75 gram powder			
20	0.0438	0.0258	41.3
Alkyl benzene sulfonate (ABS), 0.50 gram powder; Na_2SO_4, 1.50 gram powder			
20	0.0543	0.0312	42.5
Alkyl benzene sulfonate (ABS), 0.0625 gram powder; STP, 0.1875 gram powder			
20	0.0615	0.0268	56.4
Alkyl benzene sulfonate (ABS), 0.125 gram powder; STP, 0.375 gram powder			
20	0.0543	0.0220	59.5
Alkyl benzene sulfonate (ABS), 0.25 gram powder; STP, 0.75 gram powder			
20	0.0418	0.0150	64.0

Additional experiments were made in which the quantities of alkyl benzene sulfonate and sodium tripolyphosphate powders used were 0.50 and 1.50 grams, respectively. The mean residual EPESC level observed in this experimental series (Appendix Table 22) was 0.0112 mg per cm^2. The notable correlation between amounts of washing agents used and resulting skin surface lipid concentrations is presented in Fig. 27.

b. Sulfonated Ethers

The important representative of this type of anionic detergent is the sulfonated glyceryl ether shown in the following formula:

$$\begin{array}{l} CH_2O - C_{12}H_{25} \\ | \\ CHOH \\ | \\ CH_2SO_3\,Na \end{array}$$

This product, termed Coconut Alkyl Glyceryl Ether Sulfonate (CGES) also gives neutral reaction. Hand washing studies were made with the surfactant and inorganic sulfate powder (Appendix Table 23).

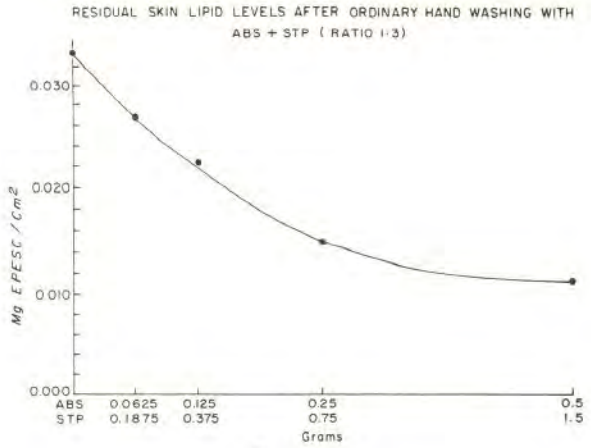


Fig. 27.

No. of Persons	Mg EPESC/cm²		% of Lipids Removed
	Mean Casual Level on Both Hands	Mean Residual Level on Both Hands	
Coconut alkyl glyceryl ether sulfonate (CGES), 0.25 gram powder; Na_2SO_4, 0.75 gram powder			
20	0.0518	0.0193	62.7

The cleansing action of the CGES compound seems rather effective.

3. *Combinations of synthetic anionic detergents*

Hand washing experiments were also performed with a mixture of a sulfated (TS) and a sulfonated (ABS) anionic detergent, both without and with an added builder (Appendix Tables 24, 25).

No. of Persons	Mg EPESC/cm²		% of Lipids Removed
	Mean Casual Level on Both Hands	Mean Residual Level on Both Hands	
TS, 0.25 gram powder; ABS, 0.25 gram powder			
20	0.0567	0.0274	50.8
TS, 0.25 gram powder; ABS, 0.25 gram powder; STP, 0.75 gram powder			
20	0.0532	0.0221	58.5

A comparison of the lipid removal in ordinary hand washing by ABS; ABS + STP ; TS + ABS ; TS + STP ; and TS + ABS + STP is shown in Fig. 28. These values indicate that higher defatting of the skin surface is achieved in the presence of the sodium tripolyphosphate builder.

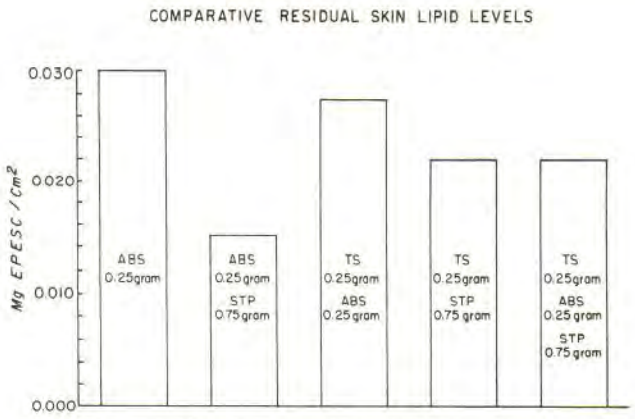


Fig. 28. Residual EPESC Levels After Ordinary Hand Washing With Anionic Detergents in the Absence and Presence of the STP Builder.

B. *Non-ionic Synthetic Detergent*

The synthetic detergents which do not ionize in the presence of water are consequently called non-ionic detergents. Although such compounds are not used to the same extent as anionic detergents, a hand washing investigation was made with a non-ionic detergent, classified as Polyoxyethylene Ester (Appendix Table 26).

$$R - \overset{\overset{O}{\parallel}}{C} - (OCH_2 - CH_2)_n\, OH$$

No. of Persons	Mg EPESC/cm²		% of Lipids Removed
	Mean Casual Level on Both Hands	Mean Residual Level on Both Hands	
	Polyoxyethylene ester, 0.25 gram powder; Na_2SO_4, 0.75 gram powder		
20	0.0500	0.0201	60.1

The degree of lipid removal recorded for this non-ionic detergent is rather great, an observation which is of definite interest.

IV. BUILDER

Of the various builders added to soaps and synthetic detergents, sodium tripolyphosphate (STP) is considered the most efficient. The production of this compound has increased markedly since 1947 (96), the annual sale in U.S.A. now exceeding 500 million kilograms (Fig. 29). Detailed descriptions about the chemistry and reaction of this agent have been made (59, 96, 142). Sodium tripolyphosphate is a good buffer (59); through its alkaline reaction the builder forms

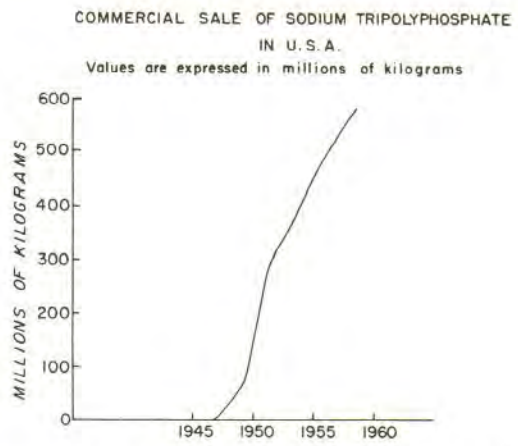
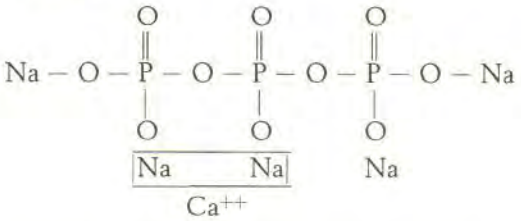


Fig. 29. The curve was constructed on the basis of data reported by Logue (96).

soap on the skin surface which to some extent may enhance its cleansing effect. Another important feature of this compound is that it reacts with calcium and magnesium in hard water to form unionized soluble salts; this reaction is indicated in the following formula:



Investigations of skin lipid removal in ordinary hand washing experiments with 1.00 gram of STP (Appendix Table 27) revealed a residual EPESC level of only 0.0199 mg/cm^2.

No. of Persons	Mg EPESC/cm^2		% of Lipids Removed
	Mean Casual Level on Both Hands	Mean Residual Level on Both Hands	
Sodium tripolyphosphate (STP), 1.00 gram powder			
20	0.0448	0.0199	55.5

RESULTS OF SIMULATED DISH WASHING STUDIES

The concentration of washing agent used in dish washing and household cleaning (a few tenths of 1 %) is much lower than that employed in ordinary hand washing (159), but the time of contact is considerably longer. Because of this prolonged exposure to aqueous solutions of surfactive agents, in the author's opinion, it is of great significance to determine the effect of concentration on the extent of

skin lipid removal. This is the reason for the very extensive dish washing studies reported in the present book. It is fortunate that the experimental procedures used in such investigations permit careful control of the washing agent concentration and therefore supply valuable and correct information.

I. WATER

In order to establish reliable measurement of the residual EPESC level remaining on the skin surface after 15 minutes' washing with redistilled water, experiments were conducted on a total of 40 individuals (Appendix Table 28). These investigations showed an average residual EPESC value of 0.0306 mg/cm², corresponding to a 42 % removal of the casual lipid concentration.

Washing Agent	No. of Persons	Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
		Mean Casual Level on Both Hands	Mean Residual Level on Both Hands		Before Washing	After Washing
Water	40	0.0526	0.0306	41.9	6.67	5.01

II. SOAP

A. *Conventional Soap*

The simulated dish washing investigations with aqueous solutions of conventional soap (80 % tallow, 20 % coconut) were carried out over a wide soap concentration range, from 0.05 % to 1.00 %. A total of 100 experiments were included in the study (Appendix Tables 29 to 33).

The CMC values reported by Shinoda (135) for potassium palmitate, stearate, and oleate are 0.0017, 0.00045, and 0.0015 moles per liter, respectively. This corresponds to percentage concentrations of approximately 0.050, 0.015, and 0.048 for these compounds which are main constituents of conventional soap. Somewhat higher values have been suggested by Stüpel (142), sodium palmitate, 0.080 %; sodium stearate, 0.050 %. On the basis of these CMC data, the lowest soap concentration used in the present investigation (0.050 %) seems to be close to the CMC levels.

Washing Agent / Soap	No. of Persons	Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
		Mean Casual Level on Both Hands	Mean Residual Level on Both Hands		Before Washing	After Washing
0.05 %	10	0.0540	0.0301	44.3	9.45	8.86
0.125 %	20	0.0537	0.0197	63.3	9.73	9.54
0.25 %	20	0.0494	0.0191	61.4	9.90	9.65
0.50 %	30	0.0513	0.0161	68.7	9.90	9.78
1.00 %	20	0.0591	0.0190	67.6	10.11	9.95

According to the results listed in the summarized table, there appears to be no increase in the skin surface fat removal above a soap concentration of 0.50 % (Fig. 30). The reasons for the higher residual skin lipid levels observed in washing

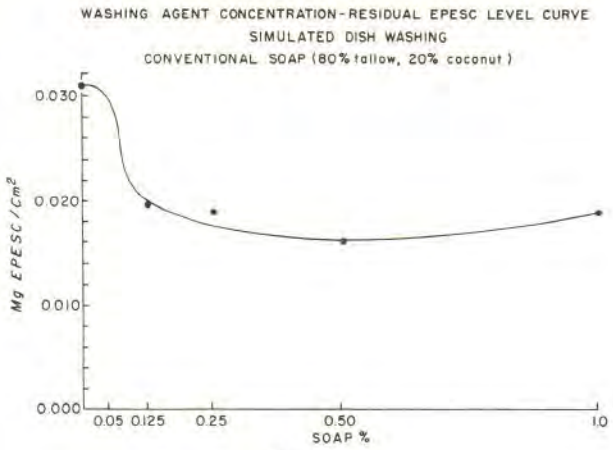


Fig. 30.

experiments with 1.00 than with 0.50 % soap solution have not been definitely established. Some factors which might be considered are 1) an increased deposit of soap on the skin with the use of a 1.00 % solution, and 2) the excessive lathering which occurs in dish washing experiments with the higher soap concentration. A study on the deposit of ether-petroleum ether soluble material on the skin from soap preparations will be outlined in a subsequent chapter.

The considerable lathering exhibited by the 1.00 % soap concentration offers a different approach to the explanation of the observed higher residual levels in these washing tests. Because a great amount of air is present in the sudsing part of the solution, this may bring about less contact between the washing agent and the skin surface and thus account for reduced removal of the cutaneous lipid film.

B. *Soap with Added Builder (STP)*

Addition of sodium tripolyphosphate (STP) to a conventional soap preparation increases the defatting effect on the skin surface lipids (Appendix Tables 34 to 39). These findings are in accordance with the observations reported in the ordinary hand washing experiments. The great cleansing activity exhibited by low concentrations of Soap + STP washing solutions is particularly noteworthy.

Washing Agent		No. of Persons	Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
Soap	STP		Mean Casual Level on Both Hands	Mean Residual Level on Both Hands		Before Washing	After Washing
0.01 %	0.03 %	10	0.0678	0.0185	72.7	8.88	8.30
0.03125 %	0.09375 %	10	0.0450	0.0113	74.8	9.50	9.14
0.0625 %	0.1875 %	10	0.0558	0.0125	77.6	9.22	8.90
0.125 %	0.375 %	20	0.0438	0.0096	78.2	9.39	9.26
0.25 %	0.75 %	10	0.0495	0.0139	71.9	9.30	9.24
0.375 %	0.125 %	20	0.0475	0.0105	77.9	9.95	9.68

A comparison of the defatting effect of 0.125 % Soap, 0.125 % Soap + 0.375 % STP, and 0.375 % STP solutions is presented in Fig. 31. The residual EPESC levels observed in the hand washing tests in which STP was present were only half as high as those recorded for the soap solution without added builder. It will further be seen from this illustration that a solution consisting only of the builder (0.375 % STP) had essentially the same skin defatting effect as that of the washing solution containing 0.125 % Soap + 0.375 % STP.

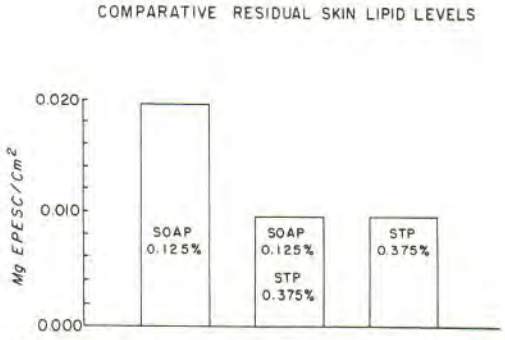


Fig. 31. Residual EPESC Levels After Simulated Dish Washing With Soap, Soap + STP, and STP Alone.

III. SYNTHETIC DETERGENTS

It is a substantiated fact that surface-active washing agents have a denaturing effect on proteins (2, 3, 17, 18, 39, 40, 60, 75, 121, 122, 156, 166). Excessive removal of cutaneous surface lipids may to an increased extent bring these agents into contact with epidermal cells. The detergent concentrations responsible for too great a removal of skin surface lipids should therefore be established. For this reason, in the present research investigation, great emphasis has been placed on construction of washing agent concentration—residual skin lipid (EPESC) curves.

A. *Anionic Detergents*

1. *Alkyl sulfates*

a. Fatty Alcohol Sulfates

Fifty simulated dish washing experiments conducted with Coconut Alkyl Sulfate (CS) solutions ranging in concentrations from 0.025 to 0.25 % (Appendix Tables 40, 41, 42, 43) show a gradual consistent reduction in residual EPESC values with increasing CS content of wash water. This observation is clearly presented in the CS concentration—residual EPESC curve (Fig. 32).

The very low EPESC level recorded for hand washing with an 0.25 % CS solution may be ascribed to the fact that the critical micelle concentration (CMC) of this washing agent ($C_{12}H_{25}OSO_3Na$) is 0.00865 moles per liter (135), corresponding to a 0.23 % CS solution.

Washing Agent CS	No. of Persons	Mg EPESC/cm² Mean Casual Level on Both Hands	Mg EPESC/cm² Mean Residual Level on Both Hands	% of Lipids Removed	pH of Wash Water Before Washing	pH of Wash Water After Washing
0.025 %	10	0.0635	0.0279	56.0	7.01	6.64
0.0625 %	10	0.0598	0.0167	72.1	6.15	6.09
0.125 %	20	0.0568	0.0146	74.3	7.03	7.06
0.25 %	10	0.0698	0.0080	88.5	7.03	6.59

Results recorded for another fatty alcohol sulfate compound, Tallow Alkyl Sulfate (Appendix Tables 44, 45, 46) show a TS concentration—residual EPESC curve quite similar to that of CS (Fig. 33). When estimated on the basis of molar concentrations, the hand washing efficiency of these two fatty alcohol sulfates is therefore apparently of the same order of magnitude. This is somewhat unexpected because the critical micelle concentration of TS with a 16-carbon chain

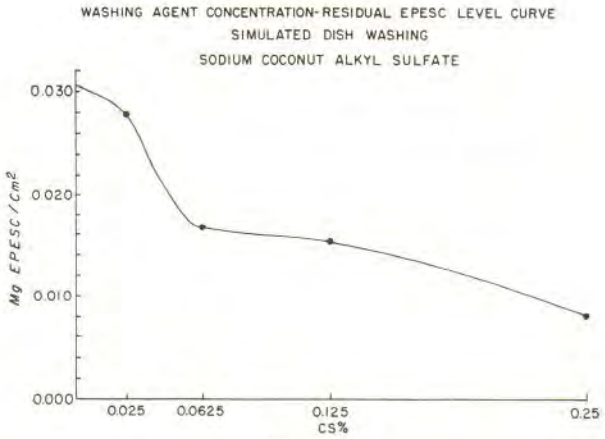


Fig. 32.

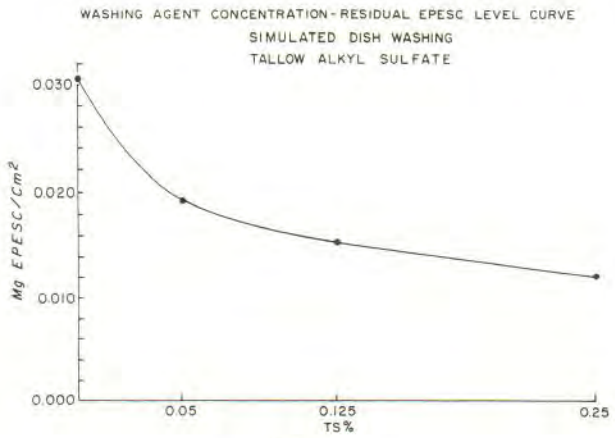


Fig. 33.

length is reported (135) to be 0.00058 moles per liter. Since this molarity is equivalent to a TS concentration of 0.019 %, all TS solutions used in the simulated dish washing studies have concentrations above the CMC.

Washing Agent	No. of Persons	Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
TS		Mean Casual Level on Both Hands	Mean Residual Level on Both Hands		Before Washing	After Washing
0.05 %	10	0.0698	0.0193	72.3	7.38	7.02
0.125 %	10	0.0723	0.0156	78.4	8.42	7.00
0.25 %	10	0.0718	0.0122	83.1	9.17	7.61

Addition of sodium tripolyphosphate (STP) builder to the TS solution (Appendix Table 47) resulted in distinctly lower EPESC values (Fig. 34). It will further be seen from this illustration that a solution consisting only of the builder (0.375 % STP) had essentially the same skin defatting effect as that of the washing solution containing 0.125 % TS + 0.375 % STP.

Washing Agent		No. of Persons	Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
TS	STP		Mean Casual Level on Both Hands	Mean Residual Level on Both Hands		Before Washing	After Washing
0.125 %	0.375 %	20	0.0538	0.0101	81.3	9.26	9.04

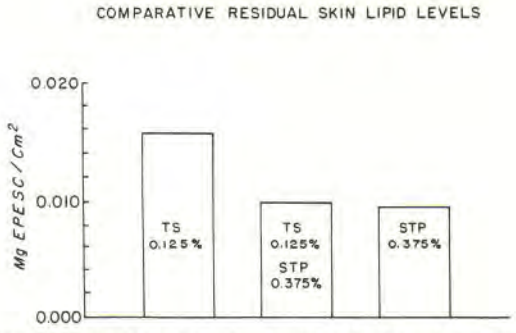


Fig. 34. Residual EPESC Levels After Simulated Dish Washing With TS, TS + STP, and STP Alone.

b. Coconut Alkyl Oxyethylene Sulfate (COS)

The efficiency of this anionic detergent sulfate to remove skin surface lipids at wash water concentrations of 0.125 % COS, 0.375 % Na_2SO_4 (Appendix Table 48) is even greater than that observed for the 0.125 % solutions of the studied fatty alcohol sulfates (CS, TS), the residual EPESC levels recorded for these three compounds being: COS, 0.0097; CS, 0.0146; and TS, 0.0156. The CMC for Coconut Alkyl Oxyethylene Sulfate has not been reported in the literature.

Washing Agent		No. of Persons	Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
COS	Na_2So_4		Mean Casual Level on Both Hands	Mean Residual Level on Both Hands		Before Washing	After Washing
0.125 %	0.375 %	20	0.0493	0.0097	80.4	7.28	6.90

2. *Alkyl sulfonates*

a. Alkyl Benzene Sulfonate (ABS)

As most other synthetic detergents, alkyl benzene sulfonate is a neutral compound which has no buffering capacity. In the manufacture of this washing agent, neutralization of the sulfuric acid used for sulfonation is sometimes overdone, leading to an alkaline preparation. Since ABS is the most widely used anionic synthetic detergent (155), it was considered desirable to study its defatting efficiency in solutions of uncorrected pH, at pH initially adjusted to 7.0, in orthophosphate buffer of pH 9.6, and in sodium carbonate—bicarbonate buffer of pH 10.0. Investigations on the effect of electrolyte (Na_2SO_4) and STP addition to the ABS solutions were also conducted. Research on the washing effect of this synthetic detergent under simulated dish washing conditions included a total of 260 tests (Appendix Tables 49 to 65).

The CMC for purified sodium dodecyl benzene sulfonate (MW 349) is reported to be 0.0012 (135) or 0.00245 (59) moles per liter, which corresponds to ABS solutions of 0.034 % and 0.070 %, respectively. Although all the ABS concentrations used in experiments without addition of builder were above the CMC level, the skin surface defatting increased with the washing agent concentrations used. This effect was also recorded in dish washing experiments where inorganic sodium sulfate had been added to the ABS solution.

The gradual decrease in residual EPESC skin values with increasing ABS concentration is presented in Fig. 35.

The extensive simulated dish washing studies conducted with ABS revealed that various compounds added to the ABS solution have a pronounced influence on its defatting capacity. The recorded effect of the STP builder on skin lipid removal (Fig. 36) is in accordance with observations made in other washing studies, but the fact that a low residual EPESC level of only 0.009 mg/cm² is found in the experiments with a 0.125 % ABS solution in orthophosphate buffer with a pH of 9.6 deserves special consideration; this value is about one-third of that resulting from simulated dish washing with a pure 0.125 % ABS solution. Since the 0.125 % ABS concentration is above the CMC level of 0.00245 moles per liter (0.070 %), the addition of electrolytes is apparently not of significance with regard to lowering of CMC under these circumstances, but the possibility exists that such addition generates formation of larger and higher charged ABS micelle aggregates.

b. Sulfonated Ethers

In accordance with the ordinary hand washing experiments with Coconut alkyl glyceryl ether sulfonate (CGES), the lipid cleansing capacity of this anionic

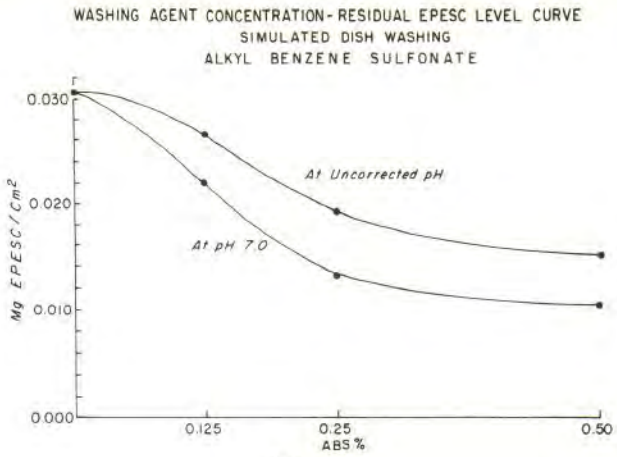


Fig. 35.

Washing Agent		No. of Persons	Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
			Mean Casual Level on Both Hands	Mean Residual Level on Both Hands		Before Washing	After Washing
ABS at uncorrected pH							
ABS							
0.125 %		20	0.0568	0.0266	53.0	8.54	7.19
0.25 %		10	0.0410	0.0192	53.3	9.22	7.80
0.50 %		10	0.0438	0.0149	66.0	9.90	8.09
ABS at neutral reaction (initial pH, 7.0)							
ABS							
0.125 %		10	0.0610	0.0220	64.0	7.00	6.84
0.25 %		10	0.0668	0.0130	80.5	7.00	6.95
0.50 %		10	0.0580	0.0103	82.5	7.00	7.10
ABS with added inorganic sulfate (Na_2SO_4)							
ABS	Na_2SO_4						
0.062 %	0.187 %	20	0.0600	0.0237	60.4	7.01	6.89
0.125 %	0.375 %	20	0.0408	0.0128	68.5	7.22	7.20
0.25 %	0.75 %	20	0.0500	0.0113	77.4	8.50	7.45
ABS in 26.4 mM orthophosphate buffer solution, pH 9.6							
ABS							
0.125 %		10	0.0613	0.0090	85.4	9.60	8.87
ABS in 0.10 % sodium carbonate-0.05 % sodium bicarbonate solution, pH 10.0							
ABS							
0.125 %		20	0.0489	0.0149	69.2	9.90	9.89
ABS with added builder (STP) at uncorrected pH							
ABS	STP						
0.005 %	0.015 %	10	0.0633	0.0252	60.1	9.14	7.85
0.0165 %	0.050 %	10	0.0683	0.0151	77.9	9.67	9.17
0.0315 %	0.0935 %	20	0.0575	0.0147	73.4	9.34	8.80
0.0625 %	0.187 %	20	0.0560	0.0133	76.3	9.30	8.99
0.125 %	0.375 %	30	0.0527	0.0064	87.9	9.28	9.01
ABS with added builder (STP) at neutral reaction (initial pH, 7.0)							
ABS	STP						
0.125 %	0.375 %	10	0.0505	0.0101	80.0	7.00	7.02

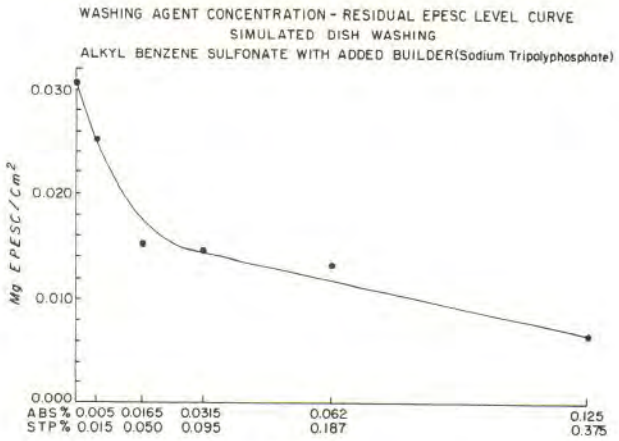


Fig. 36.

synthetic detergent also proved very efficient in simulated dish washing studies performed with a 0.125 % CGES solution to which 0.375 % inorganic sulfate had been added (Appendix Table 66)

Washing Agent		No. of Persons	Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
CGES	Na_2SO_4		Mean Casual Level on Both Hands	Mean Residual Level on Both Hands		Before Washing	After Washing
0.125 %	0.375 %	20	0.0503	0.0090	82.1	6.82	6.45

3. *Combinations of synthetic anionic detergents*

Because many commercial household washing products are combinations of various synthetic detergent compounds, simulated dish washing experiments were made with a mixture of Tallow alkyl sulfate (TS) and Alkyl benzene sulfonate (ABS), both without and with added Sodium tripolyphosphate (STP) builder (Appendix Tables 67, 68).

Washing Agent			No. of Persons	Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
TS	ABS	STP		Mean Casual Level on Both Hands	Mean Residual Level on Both Hands		Before Washing	After Washing
0.125 %	0.125 %	—	20	0.0555	0.0188	66.2	8.84	7.58
0.125 %	0.125 %	0.375 %	20	0.0538	0.0099	81.7	9.22	9.0

These studies show that addition of the STP builder reduces the residual EPESC level to such an extent that the resulting surface lipid concentration is half as great as that observed with the washing solution containing only TS and ABS.

B. *Non-ionic Synthetic Detergent*

Experiments conducted with a 0.125 % solution of Polyoxyethylene Ester to which 0.375 % inorganic sulfate had been added revealed a residual EPESC level of 0.0155 mg/cm² (Appendix Table 69). The extent of skin defatting with this non-ionic detergent compound is quite similar to that observed with 0.125 % solutions of CS and TS, the residual levels recorded for those two compounds being 0.0146 and 0.0156, respectively.

Washing Agent		No. of Persons	Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
Polyoxyethylene ester	Na_2So_4		Mean Casual Level on Both Hands	Mean Residual Level on Both Hands		Before Washing	After Washing
0.125 %	0.375 %	20	0.0483	0.0155	67.9	6.74	5.90

IV. BUILDER

The great enhancement of the cleansing effect of washing agents in the presence of sodium tripolyphosphate (STP) has been described in previous sections of this book. Because of these conspicuous results, the defatting capacity of pure STP solutions deserves definite consideration. For that reason, a total of 89 simulated dish washing experiments were performed with STP solutions of different concentrations (Appendix Tables 70 to 76); these investigations therefore represent a supplement to the 220 tests in which sodium tripolyphosphate was added as a builder to various washing products.

Washing Agent	No. of Persons	Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
STP		Mean Casual Level on Both Hands	Mean Residual Level on Both Hands		Before Washing	After Washing
		STP at uncorrected pH				
0.010 %	10	0.0540	0.0284	47.5	9.26	7.42
0.025 %	10	0.0435	0.0187	57.0	9.24	7.64
0.050 %	10	0.0418	0.0145	65.4	9.77	8.87
0.125 %	10	0.0525	0.0126	76.0	9.56	8.86
0.375 %	10	0.0505	0.0096	80.9	9.11	9.05
0.50 %	20	0.0495	0.0075	85.0	9.25	9.04
		STP at neutral reaction (initial pH, 7.0)				
0.125 %	19	0.0661	0.0261	60.5	7.00	6.77

The consistent gradual decline in residual EPESC skin levels with increasing STP concentrations is clearly presented in Fig. 37. A comparison of values observed with 0.125 % solutions at uncorrected pH and at a neutral reaction indicates that the alkaline reaction of the aqueous STP solution is a factor of great importance.

V. ELECTROLYTES

The effect of electrolytes on CMC of washing agents and on the size and charge of micelle aggregates has been established through many physicochemical studies

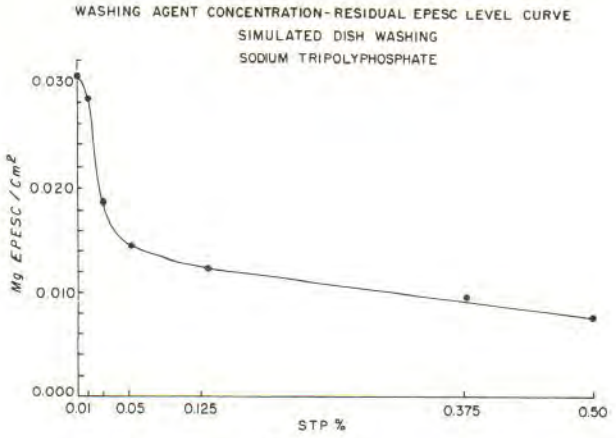


Fig. 37.

which have been extensively reviewed (45, 59, 91, 109, 135, 140, 143). In the present hand washing research project, several comparative investigations were performed in which inorganic sodium sulfate was added to the surface-active washing products. Additional simulated dish washing experiments were made with solutions of pure Na_2SO_4, the sodium sulfate concentrations ranging from 0.10 % to 0.50 % (Appendix Tables 77, 78, 79). The selection of sodium sulfate for such electrolyte studies was based on the fact that this inorganic compound is present in the majority of commercial washing agents, resulting from neutralization of employed sulfuric acid with NaOH. On the basis of the values reported in the summarized table, it appears that this electrolyte in the absence of a detergent exhibits a certain cleansing action, the residual EPESC values recorded for the 0.50 % solution being lower than those observed in the investigations with the 0.10 % solution.

Washing Agent Na_2So_4	No. of Persons	Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
		Mean Casual Level on Both Hands	Mean Residual Level on Both Hands		Before Washing	After Washing
0.10 %	10	0.0730	0.0368	49.5	7.38	6.32
0.25 %	10	0.0423	0.0270	36.1	7.17	5.68
0.50 %	10	0.0438	0.0238	45.5	7.16	5.62

VI. BUFFER SOLUTIONS

In several of the simulated dish washing studies reported in the present research program, the pH of various washing solutions seems to exhibit a decisive effect on the cleansing capacity. Many investigations have been reported in the literature on the effect of alkaline wash water on the epidermis. It appears to have been definitely established that exposure of the skin to an alkaline solution enhances swelling of the cornified epidermal cells (29, 132). This subject has also received a great deal of consideration during the last 30 years in connection with the pro-

blem of occupational dermatosis. As a supplement to the simulated dish washing experiments with surface-active agents, it was considered desirable to make some investigations on the cleansing capacity of neutral and alkaline buffer solutions (Appendix Tables 80 to 83). The results listed in the summarized table indicate that a greater defatting of the skin is accomplished by washing with alkaline buffer solutions than with neutral buffer solutions.

Washing Agent		No. of Persons	Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
			Mean Casual Level on Both Hands	Mean Residual Level on Both Hands		Before Washing	After Washing
Sodium carbonate-sodium bicarbonate buffer solution, pH 10.0							
Na_2CO_3 0.10 %	$NaHCO_3$ 0.05 %	10	0.0468	0.0162	65.3	9.98	9.88
Orthophosphate buffer solution, pH 9.6							
Orthophosphate 8.8 millimolar		10	0.0470	0.0184	61.0	9.60	8.71
26.4 millimolar		10	0.0570	0.0159	72.1	9.60	9.04
Orthophosphate buffer solution, pH 7.0							
26.4 millimolar		10	0.0728	0.0279	61.6	7.00	7.00

COMPARISON OF SKIN LIPID REMOVAL BY WASHING AGENTS IN ORDINARY HAND WASHING AND SIMULATED DISH WASHING TESTS

The 1-minute and 15-minute washing tests used in the present studies represent the types of hand washing generally employed by most persons. A comparison of the degree of skin lipid removal observed in these tests was therefore considered of definite importance. It is a well known fact that the washing agent concentration in the ordinary hand washing procedure is rather high and that it cannot be absolutely controlled since it depends on the amount of water added from the faucet and the size of the hand surface over which the agent is distributed. In the simulated dish washing tests the concentration of the washing product is lower but is very carefully controlled.

For purposes of relating the two procedures, comparisons were made between ordinary hand washings using Y grams of agent and simulated dish washings in which $\frac{Y}{2}$ % aqueous solutions of the compound were employed. A rather large series of tests was conducted for such comparative evaluation. The results of these investigations (Table 9) show consistently higher percentages of lipid removal in the simulated dish washing experiments. On the average, the degree of skin defatting in the 15-minute washing experiments was 1.36 times higher than in the corresponding ordinary hand washing tests. Since the agent concentration is much higher in the ordinary hand washing procedure, the longer exposure of the skin to the washing agent and the prolonged mechanical action in the simulated dish washing tests undoubtedly account for the greater lipid removal.

Table 9. *Comparison of Lipid Removal Percentages in Corresponding Ordinary Hand Washing (1-Min. Period) and Simulated Dish Washing (15-Min. Period) Hand Washing Experiments*

Washing Agent	Ordinary Hand Washing	Simulated Dish Washing	Lipid Removal Percentages	
	Washing Agent Amount gram	Washing Agent Concentration %	Ordinary Hand Washing %	Simulated Dish Washing %
Water			25.3	41.9
Soap	1.00	0.50	50.1	68.7
Soap, STP	0.25; 0.75	0.125; 0.375	65.8	78.2
Soap, STP	0.75; 0.25	0.375; 0.125	72.1	77.9
CS	0.25	0.125	59.2	74.3
TS, STP	0.25; 0.75	0.125; 0.375	58.9	81.3
COS, Na_2SO_4	0.25; 0.75	0.125; 0.375	61.4	80.4
ABS	0.25	0.125	44.6	53.0
ABS, Na_2SO_4	0.125; 0.375	0.062; 0.187	46.1	60.4
ABS, Na_2SO_4	0.25; 0.75	0.125; 0.375	41.3	68.5
ABS, Na_2SO_4	0.50; 1.50	0.25; 0.75	42.5	77.4
ABS, STP	0.0625; 0.1875	0.0315; 0.0935	56.4	73.4
ABS, STP	0.125; 0.375	0.0625; 0.187	59.5	76.3
ABS, STP	0.25; 0.75	0.125; 0.375	64.0	87.9
CGES, Na_2SO_4	0.25; 0.75	0.125; 0.375	62.7	82.1
TS, ABS	0.25; 0.25	0.125; 0.125	50.8	66.2
TS, ABS, STP	0.25; 0.25; 0.75	0.125; 0.125; 0.375	58.5	81.7
Polyoxyeth.est., Na_2SO_4	0.25; 0.75	0.125; 0.375	60.1	67.9
STP	1.00	0.50	55.5	85.0

For statistical purposes, in each hand washing series the correlation coefficient (r) between the casual and residual skin lipid levels was calculated. As expected, the coefficient of correlation between casual and residual values showed a tendency to decrease with increasing rate of defatting. A summary of these statistical evaluations recorded for simulated dish washing tests is presented in Table 10.

Table 10. *Average Coefficients of Correlation Between Casual and Residual Levels Observed in Simulated Dish Washing Studies with Various Degrees of Defatting*

Percentage of Defatting	Number of Experimental Series	Average Coefficient of Correlation
35—49	8	0.53
50—59	7	0.49
60—69	19	0.46
70—79	18	0.38
80—95	14	0.23

SPECIAL COMPARATIVE HAND WASHING STUDIES

With the purpose of investigating the relationship between time of hand washing and degree of removal of surface skin lipids, a limited number of simulated dish washing tests were made in which, instead of a 15-minute washing period a shorter (2.5 minutes) or longer (30 minutes) period of hand washing was used. In addition, studies were performed in which after 15-minute simulated dish washing the hands were dried with hot air from a hair dryer instead of the use of a

towel. Finally, a few tests were conducted (non-agitation experiments) in which the hands were merely submerged in washing solution for 15 minutes without movements and subsequently were dried with hot air.

In all these experiments, both casual and residual skin lipid levels were consistently determined.

TECHNICAL PROCEDURES

I. 2.5-MINUTE SIMULATED DISH WASHING TEST

The washing procedure is similar to that described in the conventional 15-minute simulated dish washing test except that only a 2.5-minute washing period is used. Following this period, the hands are withdrawn from the basin and are rinsed by slowly pouring 400 ml of distilled water over them; they are subsequently dried for 15 to 20 seconds in a clean paper towel.

II. 30-MINUTE SIMULATED DISH WASHING TEST

The washing procedure is similar to that employed in the ordinary 15-minute simulated dish washing experiments except that 3.5 liters of wash water and a 30-minute washing period are used. To maintain the temperature of the solution at a 38° C. to 40° C. level during the long washing period, the metal basin is placed on an electric plate and intermittent heating of the basin is made. Following the 30-minute washing period, the hands are withdrawn from the basin and are rinsed by slowly pouring 400 ml of distilled water over them; they are subsequently dried for 15 to 20 seconds in a clean paper towel.

III. 15-MINUTE SIMULATED DISH WASHING TEST WITHOUT TOWEL DRYING OF HANDS

The washing procedure is similar to that employed in the conventional 15-minute simulated dish washing tests. At the end of the 15-minute washing period, the hands are withdrawn from the basin and are rinsed by slowly pouring 400 ml of distilled water over them. The drying of the hands is accomplished by exposure of the skin to warm air. The hand drying is made in the following way:

1. Two portable electric hair dryers are turned on to hot position.
2. Each hand is placed opposite a dryer at a 30-to 35-cm distance. This exposes the hands to a temperature of about 42° C. (Fig. 38).
3. Effective drying is accomplished by a simple rotatory movement of the hands.

IV. NON-AGITATION, NON-TOWEL DRYING EXPERIMENT (15-MINUTE SUBMERSION PERIOD)

A. Preparation of washing solution.

Three liters of washing solution (prepared with distilled water) are used in these experiments to assure complete covering of the hands during the test. A separate wash basin is employed for each hand. After the washing solution has

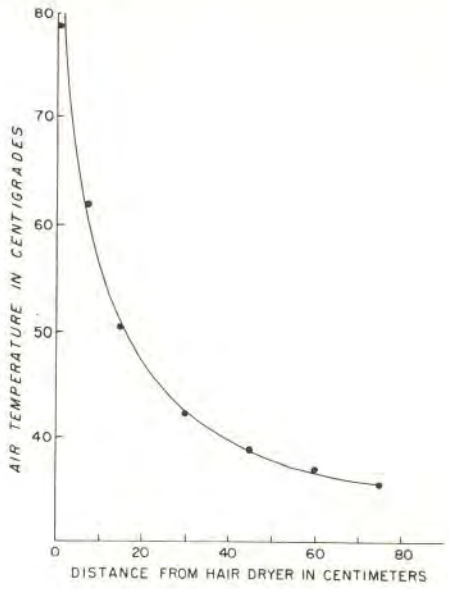


Fig. 38.

been prepared and the wash basins have been heated to a temperature of 40° C., the basins are carefully placed in a large water bath (temperature of water bath 45° C.) to maintain a constant 40° C. temperature of the washing solution during the 15-minute non-agitation period. To permit more complete contact of the solution with the hand surface, a plastic ring (diameter, 30 cm; height, 3 cm) is inserted into each basin.

B. Submersion period.

1. The person's hands are placed on the plastic rings in the two basins. No movements are made during the submersion period.
2. At the end of the 15-minute period, the hands are withdrawn from the basins.

C. Rinsing.

1. Distilled water is placed in two additional separate wash basins.
2. The person's hands are placed on plastic rings in these rinsing basins. No movements of the hands are made.
3. After 1 minute, the hands are withdrawn from the rinsing basins.

D. Drying of hands.

Drying of the hands is made by exposure of the skin to hot air from two portable electric hair dryers as described above.

RESULTS

I. BRIEF SIMULATED DISH WASHING TEST (2.5-MINUTE WASHING PERIOD)

Brief simulated dish washing tests were performed with ABS plus added inorganic sulfate, and with ABS and added builder (STP) (Appendix Tables 84, 85).

Washing Agent		No. of Persons	Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
			Mean Casual Level on Both Hands	Mean Residual Level on Both Hands		Before Washing	After Washing
ABS 0.125 %	Na_2SO_4 0.375 %	10	0.0765	0.0290	62.4	7.04	6.74
ABS 0.125 %	STP 0.375 %	10	0.0665	0.0167	74.8	9.19	8.97

A comparison of residual skin lipid levels recorded for the 2.5- and 15-minute simulated dish washing experiments shows the following values:

Washing Agent	Mean Residual Levels (Mg EPESC/cm²)	
	2.5-Minute Washing Period	15-Minute Washing Period
0.125 % ABS, 0.375 % Na_2SO_4	0.0290	0.0128
0.125 % ABS, 0.375 % STP	0.0167	0.0064

It will be seen from these data that the residual levels after 15 minutes of hand washing are markedly lower than those present on the skin surface after a 2.5-minute washing period.

II. LONG SIMULATED DISH WASHING TEST (30-MINUTE WASHING PERIOD)

The long simulated dish washing tests include separate hand washing experiments with 0.50 % conventional soap solution (80 % tallow, 20 % coconut) and 0.50 % ABS solution (Appendix Tables 86, 87).

Washing Agent	No. of Persons	Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
		Mean Casual Level on Both Hands	Mean Residual Level on Both Hands		Before Washing	After Washing
0.50 % Soap	10	0.0540	0.0187	65.9	9.35	9.22
0.50 % ABS	10	0.0500	0.0104	79.2	9.30	8.05

The corresponding residual skin lipid values recorded for 15-minute hand washing tests are presented in the following table:

Washing Agent	Mean Residual Levels (Mg EPESC/cm²)	
	30-Minute Washing Period	15-Minute Washing Period
0.50 % Soap	0.0187	0.0161
0.50 % ABS	0.0104	0.0149

These results show essentially the same residual values after 15 and 30 minutes' washing with the 0.50 % soap solution, whereas greater skin defatting was recorded after prolonged washing with the synthetic ABS detergent.

III. 15-MINUTE SIMULATED DISH WASHING WITHOUT TOWEL DRYING OF HANDS

An experiment was conducted in which the hands after 15-minute simulated dish washing in an 0.125 % ABS + 0.375 % Na_2SO_4 solution were dried with hot air dryers (Appendix Table 88).

Washing Agent		No. of Persons	Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
ABS	Na_2So_4		Mean Casual Level on Both Hands	Mean Residual Level on Both Hands		Before Washing	After Washing
0.125 %	0.375 %	10	0.0678	0.0206	69.5	7.75	6.84

Since the mean residual level recorded for the similar 15-minute test with towel drying was much lower (0.0128 mg EPESC/cm²), these findings indicate that the process of towel hand drying contributes greatly to the removal of the skin surface lipids.

IV. NON-AGITATION, NON-TOWEL DRYING EXPERIMENT (15-MINUTE SUBMERSION PERIOD)

Several studies were carried out to investigate the degree of skin defatting resulting merely from submersion of the hands in water or washing agent solution followed by drying of the skin with hot air dryers (Appendix Tables 89, 90, 91). The findings in these experiments are very conspicuous, because they clearly demonstrate that under such conditions the removal of skin surface lipids is very small. This strongly emphasizes the importance of the mechanical factors involved in hand washing.

Washing Agent	No. of Persons		Mg EPESC/cm²		% of Lipids Removed	pH of Wash Water	
			Mean Casual Level	Mean Residual Level		Before Washing	After Washing
Water	10	Right	0.0490	0.0460	6.0	7.01	6.61
Water	10	Left	0.0500	0.0452	9.5	7.11	6.85
ABS Na_2So_4							
0.125 % 0.375 %	10	Right	0.0555	0.0444	20.0	8.40	7.40
Water	10	Left	0.0495	0.0423	14.5	6.83	5.80
STP							
0.125 %	10	Right	0.0640	0.0389	39.2	9.21	9.22
0.125 %	10	Left	0.0550	0.0363	34.0	9.30	9.22

Comparisons of residual levels recorded in non-agitation, non-towel drying experiments with those observed for the same washing agents in conventional

15-minute simulated dish washing tests with towel drying are presented graphically in Fig. 39.

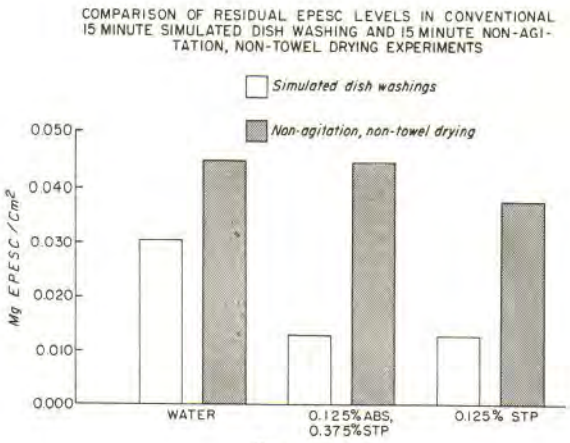


Fig. 39.

CONTRIBUTION OF SOAP AND DETERGENT TO RESIDUAL SKIN LIPID LEVEL

Since the main objective of the present research work is to study the effect of hand washing on removal of the cutaneous lipid film, specific investigations were made with the purpose of determining to what extent the ether-petroleum ether soluble material (EPESC) remaining on the skin surface after washing is derived from soap and detergents. Experiments were conducted separately with radiotagged and non-radioactive washing preparations.

I. RADIOTRACER STUDIES OF SOAP AND DETERGENT RETENTION ON HANDS

These investigations include ordinary hand washing experiments with 1) radiotagged soap (consisting of 5 % sodium palmitate-1-C^{14}, 76 % tallow soap, and 19 % coconut soap) and with 2) S^{35}-labelled sodium alkyl sulfate.

In addition to measurements of radioactivity exhibited by ether-petroleum ether soluble material extracted from the skin surface in connection with determination of residual skin lipid values, assays of the amount of washing agent deposited on the skin were made by a direct counting technique. In that procedure a delimited skin area on the back and palm of each hand is applied to the window of the Geiger tube; these counting values are compared with those of standards prepared by smearing a known amount of the washing agent over a skin area of similar dimensions. In using the direct count method, the hand radioactivity level was determined on the hands immediately after washing and drying and again after immersion of the hands in ethyl ether.

TECHNICAL PROCEDURE

A. *Casual Lipid Levels*

Casual skin lipid levels are determined as previously described on the back and palm of each hand before washing.

B. *Hand Washing*

In the radioactive experiments, hand washing is carried out over a special metal wash basin. A bottle (with a base outlet) containing lukewarm distilled water is placed over the basin.

1. Ten ml of tepid water is placed in cupped hands.
2. One gram of soap powder or of detergent pellets is added.
3. Hands are lathered for 30 seconds.
4. Five to 10 ml of distilled water is added, and hand washing continued for another 45 seconds.
5. Hands are rinsed in tepid, running distilled water for 30 seconds.
6. Hands are dried for 15 to 20 seconds in a clean paper towel.

C. *Quantitative Measurement of Radioactive Material Present on Surface of Hands Immediately After Washing and Drying*

1. An area 2.5 cm in diameter has been selected on the back and palm of each hand for radioactivity determination.
2. The radioactivity exhibited by these four areas is measured by direct application of the skin to an aluminum cover cap placed over the window of a Geiger counter tube (window thickness < 2 mg/cm^2).

D. *Collection of Residual Skin Surface Lipids*

The collection of lipids from the skin surface is made in the conventional way:

1. Each hand is immersed to the wrist for 30 seconds into a separate beaker containing 450 ml of redistilled ethyl ether.
2. The ethyl ether is evaporated, the residue redissolved in petroleum ether, and the sample filtered through a sintered glass filter; final volume of petroleum ether sample: 25 ml.

E. *Determination of Radioactive Material Remaining on Skin Surface After Submersion of Hands in Ethyl Ether*

These monitoring measurements are made in a way similar to that performed before submersion of the hands in ether by direct application of the delimited skin areas to the aluminum cover cap over the Geiger counter window.

F. *Measurement of Radioactivity of Lipid Material Present in Petroleum Ether Extract*

1. One ml of petroleum ether is pipetted into a cupped counting planchet and is subsequently carefully evaporated.
2. Radioactivity exhibited by the sample is measured by Geiger counter.

G. *Decontamination of Hands*

1. The hands are rinsed with two successive portions of 0.2 N HCl – 95 % ethyl alcohol solution. Each hand is immersed into a separate beaker containing 1 liter of this reagent. Both separate portions are kept for measurement of radioactivity.
2. The efficiency of the decontamination is checked by redetermination of the skin surface radioactivity. If the removal of radioactive washing material has not been sufficient, additional hand rinsings with 0.2 N HCl—95 % ethyl alcohol are performed.

RESULTS

The results of the soap and detergent radiotracer experiments (Appendix Tables 92, 93) after hand washing show an average adherence of 0.0253 mg soap and 0.0723 mg sodium alkyl sulfate per cm^2 skin surface. For a hand with a skin area of 450 cm^2, these values indicate a total retention by each hand of 11.4 mg soap and 32.5 mg synthetic detergent. Somewhat higher values (approximately 0.10 mg washing agent per cm^2) have been reported by Hopf and Burmeister (67).

No. of Persons	Region	Washing Agent Adhering to Skin After Hand Washing	Total Radioactive Ether-Petroleum Ether Soluble Material Extracted From Whole Hand		Radioactive EPESC Material Expressed in % of Total EPESC Determined Gasometrically
			Calculated*	Found	
		Mg per cm^2	Mg	Mg	%
		SOAP			
10	Right Hand	0.0266	2.14	2.25	36.6
	Left Hand	0.0240	1.96	2.01	34.7
	Average	0.0253	2.05	2.13	35.7
		*SODIUM ALKYL SULFATE***			
10	Right Hand	0.0789	0.00	0.00	0.0
	Left Hand	0.0656	0.00	0.00	0.0
	Average	0.0723	0.00	0.00	0.0

* Calculated on the basis of hand area, difference between radioactivity exhibited by 1.0 cm^2 of skin surface before and after ether extraction of hands, and carbon content of employed radiotagged soap preparation.
** One radioactive EPESC determination omitted from summarized table.

In these hand washing studies performed with radiotagged washing agents, the most important finding is that in the experiments with soap, 35.7 % of the ether-petroleum ether soluble material extracted from the hand surfaces after washing was radioactive; in contrast to this, no radioactivity in petroleum ether was found in similar washing tests with the alkyl sulfate detergent. These observations indicate a considerable contribution of the conventional soap agent to the residual skin lipid level. On the basis of the measured soap material retained on the skin surface it can be calculated that 27.2 % of the soap deposited on the hand after washing was soluble in ether-petroleum ether; the corresponding value calculated on individual percentages is 28.6.

The solubility in petroleum ether of the soap product adhering to the skin after washing deserved consideration. Direct measurement of the dissolution of the radiolabelled soap preparation in petroleum ether showed a solubility of only 0.27 %. This finding was subsequently confirmed by The Procter & Gamble Company. Since the original soap compound is only slightly soluble in petroleum ether, other factors must be responsible for the rather large quantity of radioactive material found in the petroleum ether extract of the skin. Although the main reasons for this solubility have not as yet been established, the following factors should be considered:

1) In accordance with investigations by Ramsey and Jones (123), a portion of the soap may be converted instantaneously to free fatty acids through action of the acid skin mantle, the Na-palmitate-C^{14} soap being hydrolyzed to free fatty acids.

2) An interchange may take place between the radiotagged fatty acids in the soap and the free fatty acids on the surface of the skin.

3) As previously outlined, when soap is dissolved in water, it undergoes hydrolysis. A principal product of the hydrolysis is the type of compound known as acidic soap: $(RCOO)_2NaH$. It has been demonstrated (26) that acidic soap is soluble in organic solvents. Acidic soap resulting from soap hydrolysis in aqueous solution may probably be deposited on the skin surface and subsequently be dissolved in ether-petroleum ether.

The rather high solubility in petroleum ether of soap material present on the skin after hand washing may to some extent account for the increase in residual skin lipid levels observed in the simulated dish washing experiments with increasing soap concentration in the wash water.

It is further of particular interest that in 9 of the 10 hand washing experiments with the synthetic detergent (sodium alkyl sulfate), no radioactivity was observed in the ether-petroleum ether extracts obtained from the skin surfaces after hand washing. This negative finding with detergent pellets suggests that the presence of radioactive material in the petroleum ether extracts in experiments with C^{14}-tagged soap cannot be due to contamination of the petroleum ether extract with moisture.

The notable degree of solubility in ether-petroleum ether of soap material present on the skin after hand washing was confirmed in subsequent experiments conducted by the author with The Procter & Gamble Company in which a chemical tracer, dichlorinated sodium stearate, instead of radiotagged soap was used as washing agent.

II. NON-RADIOACTIVE STUDIES ON THE CONTRIBUTION OF WASHING AGENTS TO THE EPESC LEVEL PRESENT ON THE SKIN SURFACE AFTER HAND WASHING

To acquire further information about the contribution of washing agents to the residual skin lipid level, a number of additional hand washing studies were made with non-radioactive washing preparations. These special investigations include the following experiments:

A. *Defatting of Skin before Hand Washing*

1. Measurement of EPESC levels on the dorsal side of the hand immediately following hand washing and drying. These determinations were devised with the purpose of eliminating the influence of the casual skin lipid layer on the EPESC material present on the skin after washing.
2. Assay of EPESC concentrations on protected skin areas 4 hours after washing. The results of these measurements provide information about the release of ether-petroleum ether soluble material from the washing agent deposited on the skin surface over a 4-hour period.

B. *Comparative Studies*

For comparative purposes, hand washing experiments were performed with the same washing agents but *without* preliminary defatting of the skin. In accordance with the defatting washing tests, EPESC values were measured on the dorsal region of the hand immediately after washing and on a protected skin area 4 hours later. To determine the reproducibility of the results, an extensive study was made in which this type of washing test was conducted 4 times on each of 10 individuals with a 1-week interval between experiments.

The dorsal side of the hand was chosen for these investigations because protection of a skin area with an attached celluloid hemisphere can be made more efficiently on the dorsum than on the palm. In addition, since studies have previously been made on the rate of sebaceous secretion in the dorsal hand region (68, 71, 73), measurements of the replenishment of skin lipids following hand washing on protected skin areas of this part of the hand can be compared with the secretion values.

TECHNICAL PROCEDURES

I. HAND WASHING EXPERIMENTS FOLLOWING PRELIMINARY DEFATTING OF HANDS, INCLUDING DETERMINATION OF SKIN LIPID VALUES IMMEDIATELY AFTER WASHING AND ON A PROTECTED SKIN AREA 4 HOURS LATER

For preliminary defatting of hands, each hand is placed for 30 seconds in a tall beaker containing 600 ml of redistilled ethyl ether. The hands are immersed to the middle of the lower arm; this is done to prevent casual lipids present on skin areas near the wrist from interfering with the washing test. Following ordinary hand washing (washing period 15 + 45 seconds; rinsing: 30 seconds; drying of hands in paper towel: 15 to 20 seconds), the residual lipids present on the dorsal aspects of the hands are immediately collected from a delimited 4-cm^2 area on the proximal part of one hand and on the distal part of the other hand. For protection against contamination, the opposite skin areas in the dorsal regions of the hands are then covered with perforated, celluloid hemispheres which are attached to the skin by means of adhesive tape. At the end of the 4-hour period,

the hemispheres are removed and lipid collection is made from a 4-cm² area in the center of the field which has been protected by the hemisphere. Standard procedures are employed for quantitative lipid collection, isolation from ethyl ether samples, and for subsequent manometric carbon determination.

II. COMPARATIVE TESTS WITHOUT PRELIMINARY SKIN DEFATTING

The procedures employed in these experiments are similar to those outlined above except that preliminary defatting of the hands is not made.

RESULTS

Hand washing studies of this type were made with conventional soap and with various synthetic detergent compounds (Appendix Tables 94 to 101). To facilitate comparison between residual EPESC values observed in hand washing tests with or without preliminary defatting of the skin, the mean values for these experiments have been listed in a summarized table. This table also includes EPESC concentrations recorded for samples collected from protected skin areas 4 hours after hand washing.

No. of Persons	Hand Washing Tests Following Preliminary Defatting of Skin Mg EPESC/cm²		Hand Washing Tests Without Preliminary Defatting of Skin Mg EPESC/cm²	
	Immediately After Washing	4 Hours Later on Protected Skin Area	Immediately After Washing	4 Hours Later on Protected Skin Area
Milled, perfumed soap bar (80 % tallow, 20 % coconut)				
10	0.0050	0.0058	0.0354	0.0352
Milled, perfumed bar of fatty alcohol sulfates				
20	0.0029	0.0054	0.0313	0.0291
Powder: CS, 0.065 gram; ABS, 0.195 gram; Na_2SO_2, 0.740 gram				
10	0.0021	0.0040	0.0190	0.0195
Powder: ABS, 0.50 gram; STP, 1.50 gram				
10	0.0027	0.0044	0.0112	0.0150

As seen from the summarized data, the average EPESC level present on the skin following defatting of the skin surface and subsequent hand washing averaged only 0.0032 mg/cm², an EPESC value which is markedly lower (mean concentration, 14.6 %) than observed in the corresponding ordinary hand washing tests in which preliminary defatting of the hands was not made. These findings indicate the appreciable contribution of casual skin lipids to the residual lipid concentration.

The low level of 0.005 mg EPESC/cm² recorded in the washing experiments with conventional soap after defatting of the hands may be due to preliminary removal of acids (including lactic acid) from the skin surface through exposure of the hands to ethyl ether; the acid skin mantle presumably contributes to re-

lease of free fatty acids from soap deposited on the skin. However, it should be noted that no significant increase in ether-petroleum ether soluble material was found after the soap had been retained on the skin for 4 hours.

The replenishment of skin lipids on protected hand areas (average, 0.009 mg EPESC /cm²/ 4 hours) is much lower than that previously reported by Laursen (92) for unprotected areas. This finding confirms the contention that the skin lipids present on the hand surface are primarily derived from extraneous sources.

The moderate increase in lipid concentration on protected skin areas is further demonstrated in the extensive repeated studies conducted on 10 subjects (Appendix Table 102); the mean values observed in this 4-week investigation are listed below. The average lipid replenishment recorded in 80 measurements is 0.004 mg EPESC /cm²/ 4 hours. In addition, these data show the reproducibility of hand washing tests.

Mean Residual Skin Lipid Levels
Observed in Repeated Tests Immediately After Hand Washing and on Protected Skin Area 4 Hours Later

Milled, perfumed bar of fatty alcohol sulfates

No. of Persons	Weeks	Right Hand		Left Hand	
		Mg EPESC/cm²		Mg EPESC/cm²	
		Immediately After Washing	4 Hours After Washing	Immediately After Washing	4 Hours After Washing
10	1	0.0259	0.0293	0.0244	0.0274
10	2	0.0247	0.0262	0.0236	0.0246
10	3	0.0219	0.0282	0.0255	0.0308
10	4	0.0222	0.0282	0.0225	0.0263
Total Average		0.0237	0.0280	0.0240	0.0273