The Germicidal Activity of Rosin Soap and Fatty Acid-Rosin Soap as Indicated by Hand-Washing Experiments

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PREVIOUS studies (1, 2, 7, 8) have indicated that rosin soap and mixed soaps of fatty acids and rosin have greater germicidal activity "in vitro" than equivalent concentrations of soaps made from fats, oils, or individual fatty acids alone. It is generally recognized that the results of "in vitro" studies cannot be interpreted directly in terms of actual usage. Therefore, supplementary data have been sought through the study of a more practical application such as in hand-washing.

In recent hand-washing studies, Price (5) grouped the microbial flora of the hands as transient and resident. The former are readily removed by soap and water, whereas the latter are quite resistant to removal. He reported that none of the commercial toilet soaps used by him had any germicidal activity on the resident population of the skin. The only action attributed to the soap was to assist, possibly, in the mechanical removal of some of the organisms.

The method employed by Price has been thoroughly examined in this laboratory (4). Although certain variables were encountered, it is believed that by this method definite conclusions can be drawn regarding the germicidal activity of soaps in the lather state on the organisms removed and those remaining on the hands.

The following modification of Price's procedure was used: A series of ten large enameled basins, each having a capacity of about 7 liters, was used for hand-washing. Before making a series of tests the basins were washed with soap and water and thoroughly rinsed with hot water. Distilled water was used in all the tests so that the hardness of the water would not have to be considered. Each basin contained 2 liters of distilled water ranging in temperature from 23° to 28° C.

Granular soap containing no rosin soap, and equivalent to 2 grams of anhydrous soap, was used at each basin for washing the hands in what will be called the normal procedure. The concentration of soap in the basins after washing was 0.1 percent.

To determine the control count per basin, a series of ten basins, each containing 2 liters of distilled water and 2 gms. of soap, was allowed to stand in the laboratory for at least an hour before taking samples for plating. The count varied from 0 to 6 with an average of less than 2 bacteria per milliliter. This gave an average control count of 4,000 per basin and was disregarded, since it is of an insignificant order as the data will show.

The hands were moistened, the granular soap was poured into the palms, and a good lather was worked up, adding water as needed. This was accomplished in 20 to 30 seconds. The soap was worked thoroughly on the hands for about 75 seconds, water being added to maintain a good lather. The last 15 or 20 seconds of the two-minute period allotted for washing in each basin were spent in rinsing the lather from the hands. This procedure was repeated in the next basin without wiping the hands and so on, until ten washings had been completed. This gave a total washing period of 20 minutes. The individuals were requested to wash in the same manner in each basin and to exercise care not to increase the area being washed.

When testing a germicidal solution, it was put into one or more of basins 4, 5 and 6. The hands were washed, without the granular soap, in the solution for two minutes, keeping them immersed as much as possible while washing. The solid or liquid soaps were tested by replacing the granular soap at basins 4, 5 and 6 with a measured quantity of the material, the washing procedure being otherwise the same.

After the subject had washed in all ten basins, two 1-ml. aliquots were transferred from each basin with a sterile pipette into sterile petri dishes. About 15 ml. of nutrient agar (4) at 45°C. was poured into each dish, thoroughly mixed with the sample, and allowed to solidify. As soon thereafter as possible the plates were placed in an incubator and held at 30°C. for 48 hours. Counts were then made using a Quebec colony counting chamber. The plate count was multiplied by 2,000 to obtain the bacterial count per basin.

Price (5) has shown that there is little or no killing by this procedure and that the count per basin gives the number of organisms removed with each washing. The number of organisms removed after the second or third washing is a function of the number of organisms remaining on the hands (5, 6). The organisms remaining on the hands after two or three washings are classed by Price as resident flora.

The bacterial count per basin plotted against the number of the basins, in the order used, gave consistent and characteristic curves for individuals. Under apparently normal routine the removal curves of individuals showed some variations. These have been discussed in a previous report (4). More consistent results were obtained when curves were plotted using

[1] L. S. Stuart was employed in the Industrial-Farm Products Research Division when this work was done.

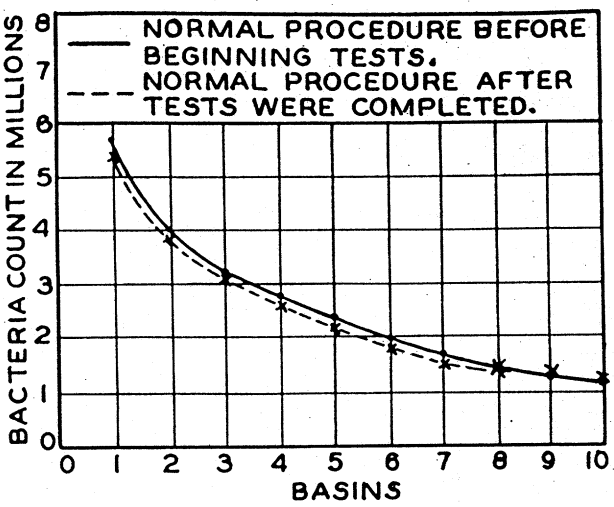

FIGURE 1

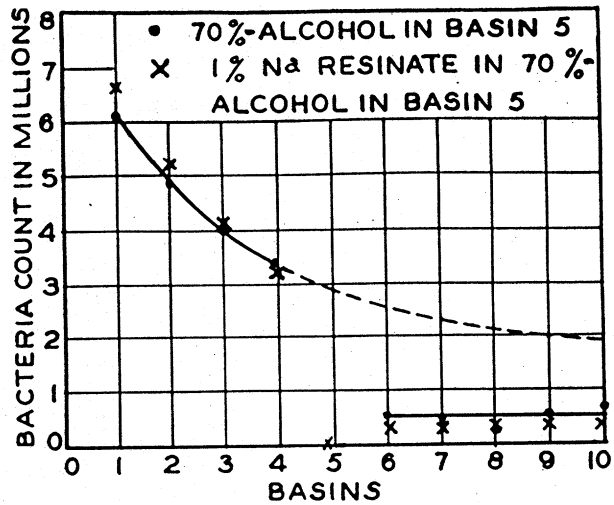

FIGURE 2

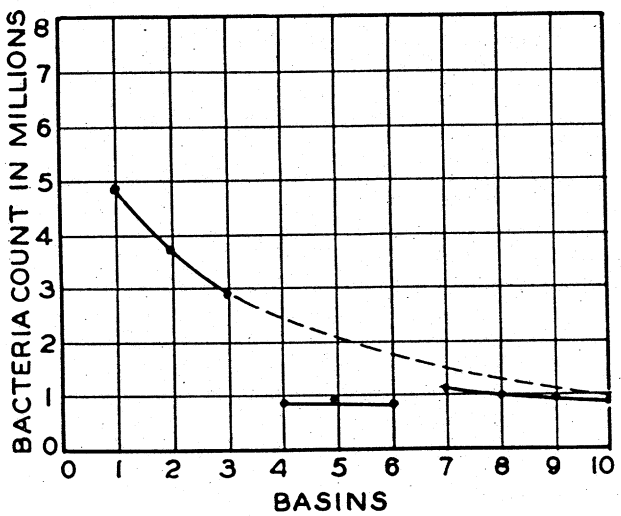

FIGURE 3

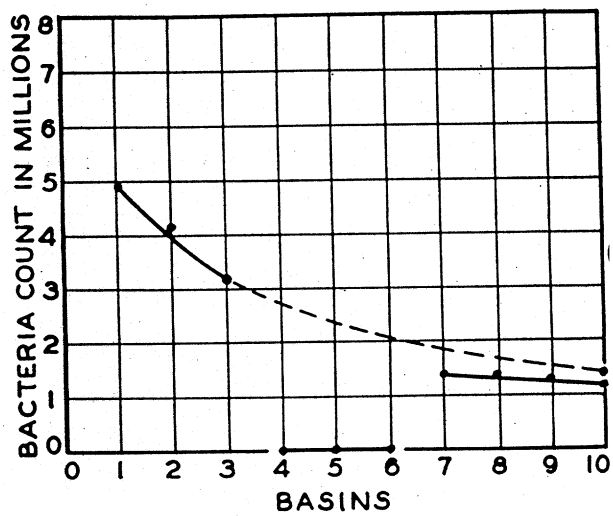

FIGURE 4

Plate 1

Fig. 1—Curves for normal procedure before and after completion of the tests reported

Fig. 2—Effect of using 70%-alcohol in the fifth basin

Fig. 3—Effect of substituting 20 ml. of a 10%-coconut oil soap solution for the granular soap at basins 4, 5 and 6

Fig. 4—Effect of using 1% rosin soap solution in basins 4, 5 and 6

TABLE I.—BACTERIAL COUNT OBTAINED WITH NORMAL PROCEDURE

Individuals	Bacterial Count per Basin, in Thousands									
	1	2	3	4	5	6	7	8	9	10
				Normal Procedure at Beginning of Series						
A	1,796	1,520	1,452	1,112	1,172	984	876	872	843	864
B	10,696	8,876	7,680	6,976	5,248	4,928	4,012	2,560	2,432	2,432
G	3,200	2,688	2,242	1,856	1,728	1,600	1,216	1,152	1,218	1,090
Q	5,632	2,084	1,856	1,600	1,344	1,282	768	612	612	584
R	4,932	3,944	2,176	2,176	1,600	1,856	1,536	1,346	1,328	1,160
W	7,912	5,056	4,032	3,136	3,072	1,792	1,600	1,536	1,216	1,152
Averages	5,695	4,028	3,240	2,809	2,361	2,074	1,668	1,346	1,275	1,214
				Normal Procedure at End of Series						
A	4,352	3,198	2,432	1,668	1,124	952	768	665	606	507
B	10,560	8,210	6,592	5,568	4,736	3,776	3,072	3,136	2,880	2,560
G	2,624	696	628	608	548	440	428	416	384	384
Q	5,184	3,648	2,244	1,360	1,152	1,000	812	858	722	744
R	3,905	3,520	3,456	3,264	2,560	2,240	2,048	1,806	1,664	1,472
W	5,184	3,968	3,200	2,880	2,816	2,368	1,920	1,792	1,472	1,408
Averages	5,302	3,873	3,092	2,558	2,156	1,796	1,508	1,446	1,288	1,179

the averages of the counts obtained with three or more individuals.

In selecting subjects for the hand-washing tests, care was taken to use only those who washed in a uniform manner and whose hands did not come in contact with organic solvents, such as alcohol, turpentine, and acetone, in the course of their daily work.

A procedure essentially the same as that suggested by Price (5) was followed in evaluating the germicidal action of materials and solutions. The count for the first three or four basins was plotted and the curve extrapolated to the tenth basin to conform to the slope of a previously plotted curve for the same individuals using the granular soap at each basin. The point or points for the bacterial counts of the basins at which the product was tested were then plotted, followed by the bacterial count per basin for the last four or five basins at which the use of granular soap was resumed.

A low count for the basins at which the soap being tested was used, may be due to a killing action of the soap in the lather state or the removal of fewer organisms. The position of the curve for the bacterial count of the last four or five basins, with respect to the extrapolated curve, provides an index of the effect of the test material on the organisms remaining on the skin of the hands.

The bacterial counts obtained at the beginning and at the completion of these studies with six individuals using the normal procedure are given in Table 1. The removal curves obtained by plotting the averages of these counts for the two intervals are given in Plate 1, figure 1. From these curves and the counts in Table 1, the uniformity of the average curve for a number of individuals, and the variation of an individual, may be determined.

The average bacterial counts for the normal pro-

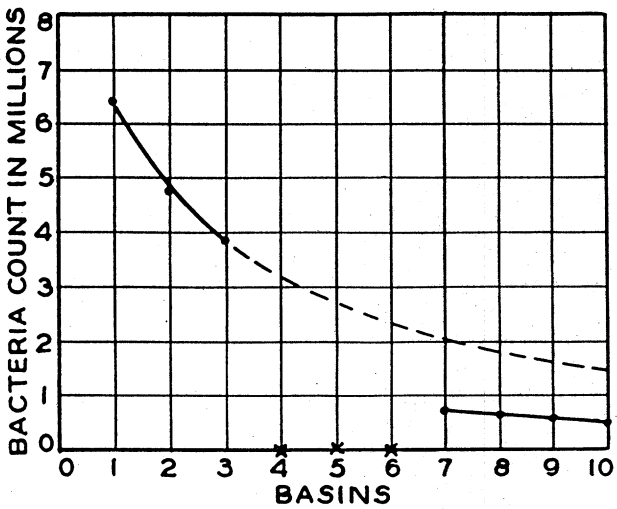

FIGURE 1

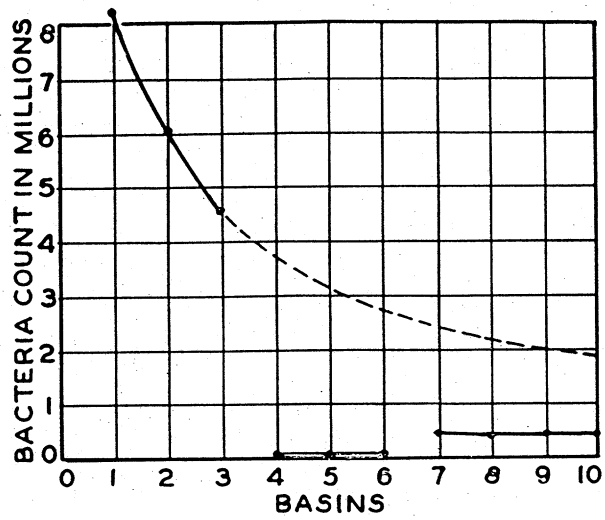

FIGURE 2

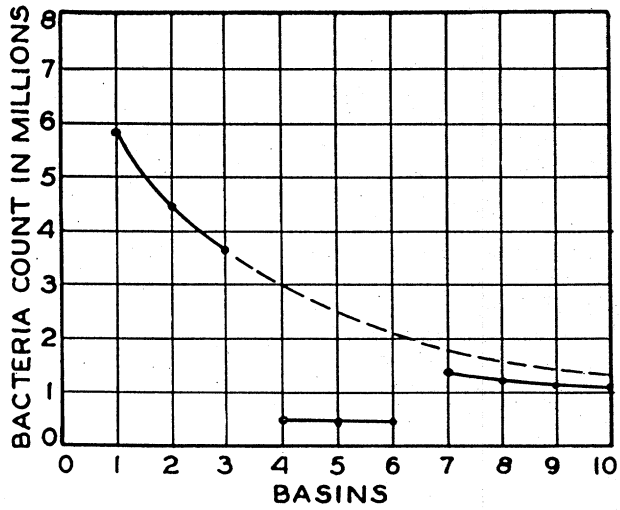

FIGURE 3

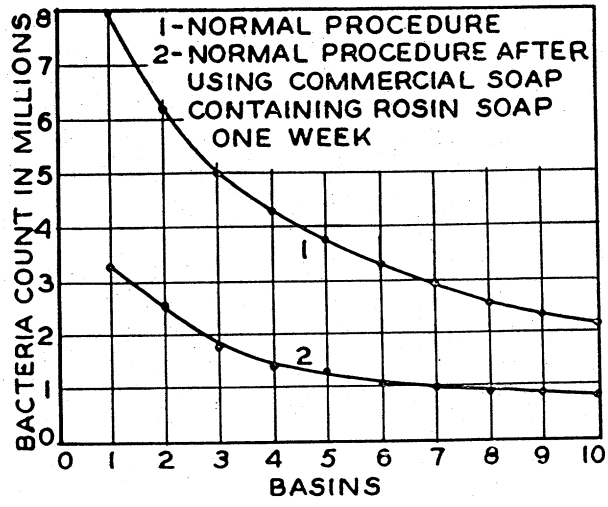

FIGURE 4

Plate 2

Fig. 1—Effect of using a solution containing 1% rosin soap and 0.1% palm oil soap in basins 4, 5, and 6

Fig. 2—Effect of substituting 20 ml. of a 10% rosin soap solution for the granular soap at basins 4, 5 and 6

Fig. 3—Effect of substituting a commercial soap containing rosin for the granular soap at basins 4, 5 and 6

Fig. 4—Effect on the flora of the hands of using a commercial soap containing rosin for washing the hands for one week

cedure and for germicidal tests with 70-percent alcohol, 70-percent alcohol plus rosin soap, coconut oil soap, coconut oil-rosin soap, rosin soap, rosin-palm oil soap, and a commercial soap containing rosin are given in Table 2.

The curves for the average counts obtained in the experiment in which 70-percent alcohol with and without the addition of 1 percent of rosin soap were tested, are shown in Plate 1, figure 2. This figure shows that 70-percent alcohol has a definite germicidal effect on the resident flora of the hands. One-percent rosin soap[1] added to 70-percent alcohol appears to somewhat incerase its bacterial action on the resident flora.

Since the "in vitro" studies (7) indicated that a coconut oil soap had greater germicidal activity than normal commercial soaps, a test was made using 20 ml. of a 10-percent coconut oil soap solution in place of the granular soap on the hands at basins 4, 5 and 6. Normal washing procedure was resumed for the last four basins. The low count for basins 4, 5 and 6 (Table 2, test 2) appears to be due to the killing action of the coconut oil soap in the lather state on the organisms removed. The similarity both in position and shape of the extrapolated curve and the one for the last four basins (Plate 1, figure 3) indicates that coconut oil soap had little effect on the resident flora. The fact that the latter curve is lower might indicate a slight reduction in the resident flora by the action of coconut oil soap, but the difference in the two curves is only slightly greater than some deviations found between normal curves. Similar results were obtained when 20 ml. of a 10-percent coconut oil-rosin soap solution was substituted for the granular soap at basins 4, 5 and 6 (see Table 2, test 3).

As "in vitro" tests (7) also indicated that a 1-percent sodium resinate solution was germicidal, 2 liters of a 1-percent rosin roap solution were used in basins 4, 5 and 6 (Table 2, test 4). The 1-percent rosin soap solution was germicidally active toward the organisms removed, but the effect of the solution on the resident flora was about equivalent to that of the coconut oil soap in the previous experiment. The averages of counts obtained in this test are plotted in Plate 1, figure 4.

Since surface tension may play a part in the germicidal activity of soap solutions, a solution containing 0.1 percent of palm oil soap and 1 percent of sodium resinate was tested as outlined above (Table 2, test 5). A 0.1-percent palm oil soap solution has a lowe rsurface tension than a 1-percent rosin roap solution (3) and would tend to lower the surface tension of the rosin soap solution. Palm oil soap in this concentration has little or no germicidal activity (7). This combination of sodium resinate and palm oil soap appears to be more germicidal in the basins than equivalent concentrations of rosin soap. At the same time it appears to have reduced the number of bacteria on the hands beyond that of a cleaning action alone. The effect on the organisms removed and the reduction of the resident flora of the hands both indicate that the sodium resinate-palm oil

[1] Rosin soap or sodium resinate is the product obtained by neutralizing the rosin acids of rosin with sodium hydroxide. Rosin contains about 5 percent of neutral material or resenes which is not affected by the neutralization and is part of the sodium resinate or rosin soap.

TABLE 2.—RESULTS OF GERMICIDAL TESTS WITH ALCOHOL, ROSIN SOAP, COCONUT OIL SOAP, AND FATTY ACID-ROSIN SOAPS

Test No.	Individual used in test	Average Count per Basin, in Thousands									
		1	2	3	4	5	6	7	8	9	10
		Normal Procedure									
	T, O, F and E	7,104	5,108	4,016	3,280	2,818	2,774	2,352	2,224	1,986	1,972
		70-percent Alcohol Used in Basin 5									
1	T, O, F and E	6,089	4,800	3,978	3,409	1	554	472	314	502	616
		1 percent Rosin Soap in 70-percent Alcohol Used in Basin 5									
	T, O, F and E	6,896	5,325	4,083	3,263	0	381	396	368	329	331
		Normal Procedure									
2	A, B and Q	6,370	4,589	3,709	3,047	2,463	2,154	1,718	1,451	1,349	1,282
		20 ml. of 10 percent Coconut Oil Soap Substituted for Granular Soap at Basins 4, 5 and 6									
	A, B and Q	4,907	3,725	2,922	927	947	903	1,236	974	969	927
		Normal Procedure									
3	B, P and V	6,999	5,642	4,405	3,753	3,086	2,651	2,275	1,939	1,687	1,587
		20 ml. of 10 percent Coconut Oil-Rosin (3-1) Soap Substituted for the Granular Soap at Basins 4, 5 and 6									
	B, P and V	7,936	6,422	5,205	1,197	833	539	2,205	1,864	1,504	1,429
		Normal Procedure									
4	A, H and P	4,693	3,901	3,143	2,575	2,239	2,035	1,853	1,664	1,479	1,402
		1 percent Rosin Soap in Water Used in Basins 4, 5 and 6									
	A, H and P	4,931	4,224	3,285	17	20	19	1,387	1,374	1,323	1,055
		Normal Procedure									
5	P, W and Y	7,431	5,963	4,839	4,113	3,605	2,823	2,592	2,283	1,877	1,681
		1 percent Rosin Soap + 0.1 percent Palm Oil Soap in Water Used in Basins 4, 5 and 6									
	P, W and Y	6,379	4,693	3,819	0	0	0	749	640	564	466
		Normal Procedure									
6	D, K, L and S	7,401	5,408	4,670	3,875	3,354	2,626	2,429	2,236	1,921	1,808
		20 ml. of 10 percent Rosin Soap Substituted for the Granular Soap at Basins 4, 5 and 6									
	D, K, L and S	8,193	6,016	4,563	63	27	20	350	385	414	398
		Normal Procedure									
7	B, G and R	6,276	5,058	3,851	3,435	2,837	2,603	2,098	1,782	1,734	1,582
		2 gm. of Brown Bar Soap[1] Used in Place of Regular Soap at Basins 4, 5 and 6									
	B, G and R	5,888	4,416	3,605	571	518	423	1,468	1,235	1,157	1,116

[1] The brown bar soap is a commercial soap that contains rosin.

soap solution is germicidally active. The activity on the bacteria remaining on the hands is not as great as that observed with 70-percent alcohol but it is definitely measurable. This is illustrated in Plate 2, figure 1.

To compare the germicidal activity of coconut oil soap and rosin soap, the procedure used for testing coconut oil soap was repeated, using 20 ml. of a 10-per cent rosin soap solution (Table 2, test 6). The data are plotted in Plate 2, figure 2. This test shows that rosin soap was superior to coconut oil soap, in the lather state, as a germicide. Also it was equally as effective against the resident flora as washing two minutes in 70-percent alcohol.

A commercial brown bar soap[1] containing rosin soap was tested by substituting an amount equivalent to

[1] A 1 percent solution of this soap had a pH of 10.2 (glass electrode measurement). Also, this soap was designed for general household purposes and, of course, is not to be considered a toilet soap. After using this soap a week, the subjects stated that their hands were beginning to chap.

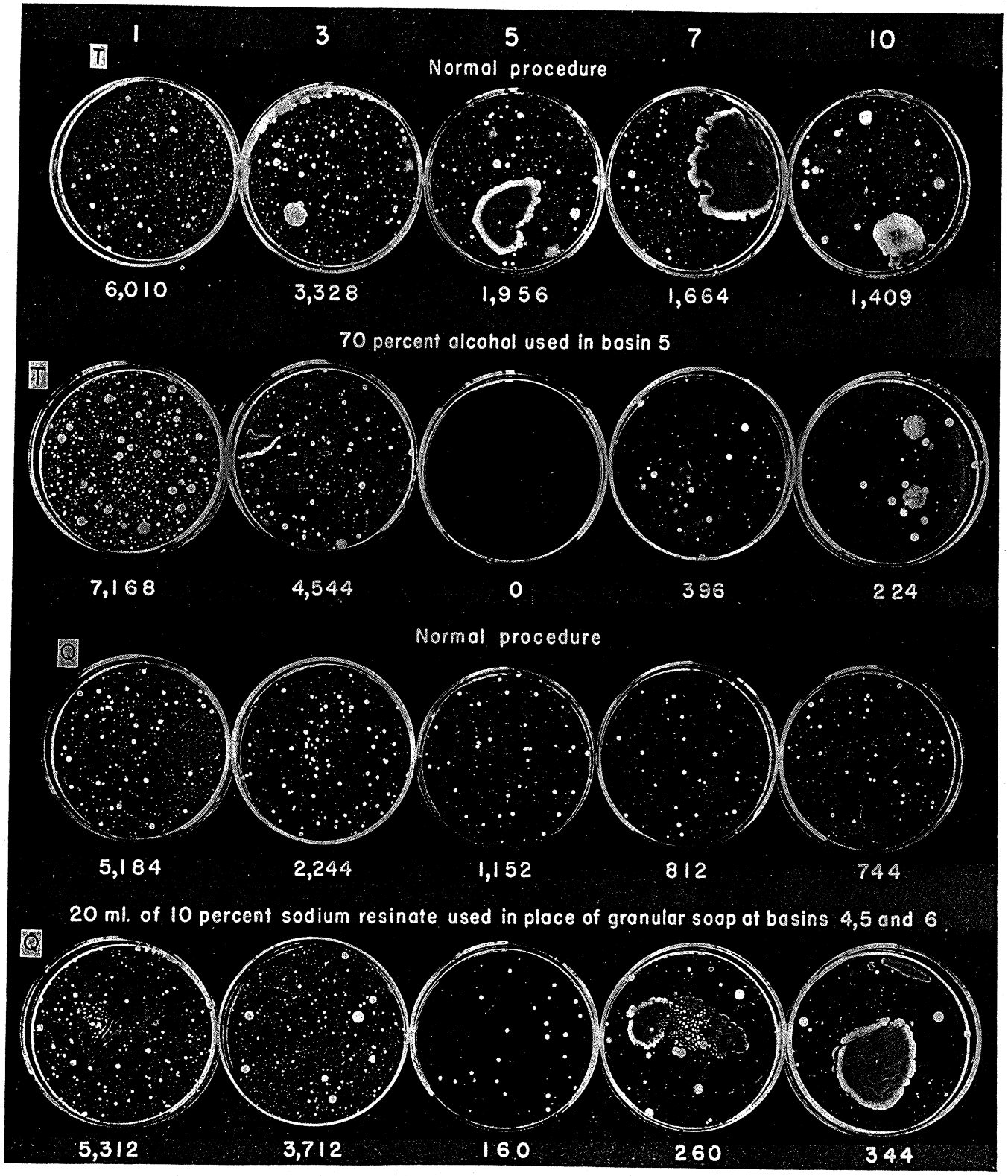

Plate 3.—Bacterial plate counts as obtained in typical tests

TABLE 3.—EFFECT OF CONTINUOUS USE OF A COMMERCIAL SOAP CONTAINING ROSIN SOAP ON THE TRANSIENT AND RESIDENT FLORA OF THE HANDS

Individuals	Bacterial Count per Basin, in Thousands									
	1	2	3	4	5	6	7	8	9	10
NORMAL PROCEDURE										
F	6,784	5,440	4.416	3,904	2,496	2,560	2,112	1,984	1,470	1,408
L	10,560	8,768	6,446	5,640	5,492	4,160	3,840	3,464	3,190	3,116
O	10,500	7,552	6,272	5,632	5,668	5,656	4,672	4,608	4,204	4,188
P	8,256	6,784	5,120	4,158	3,580	3,062	2,816	2,560	2,112	1,984
S	4,324	2,048	1,920	1,492	1,281	1,172	1,269	1,072	752	634
Y	7,488	6,594	5,760	5,174	4,288	3,328	3,200	2,624	2,176	1,778
Averages	7,985	6,196	4,989	4,334	3,801	3,323	2,985	2,719	2,317	2,185
NORMAL PROCEDURE AFTER USING BROWN BAR SOAP[1] FREQUENTLY FOR ONE WEEK										
F	3,728	2.422	960	327	252	256	174	188	172	184
L	4,480	3,968	3,136	2,318	2,112	1,920	1,856	1,856	1,732	1,672
O	2,314	1,984	1,472	1,216	1,152	1,152	1,152	1,024	896	896
P	4,288	3,008	2,752	2,368	2,500	1,728	1,472	1,216	1,216	1,024
S	1,728	1.344	476	356	284	280	259	204	198	180
Y	3,200	2,688	2,240	1,984	1,856	1,340	940	800	670	654
Averages	3,290	2,569	1,839	1,428	1,359	1,113	976	881	814	768

[1] The brown bar soap is a commercial soap that contains rosin soap.

2 gm. of anhydrous soap in place of the granular soap at basins 4, 5 and 6 (Table 2, test 7). This test showed that the commercial soap containing rosin soap had about the same germicidal properties as the coconut oil soap on the flora of the hands, but was slightly more active in the lather state. The average counts given in Table 2 are plotted in Plate 2, figure 3.

The greater germicidal activity of the lather of the commercial soap containing rosin soap suggested that continued use of such a soap might have an effect on the resident flora. To test this, six subjects were given two bars of the commercial brown bar soap and asked to wash their hands with it at home and in the laboratory for one week at their convenience. At the end of the week they washed according to normal procedure, using granular soap at all basins. The counts for each subject and the averages of the counts obtained with the normal procedure before and after using the brown bar soap for one week as prescribed are given in Table 3.

The curves obtained by plotting the average counts for the normal procedure before and after using the commercial soap containing rosin soap for one week (Plate 2, figure 4) show that both the transient and resident flora of the hands were materially reduced.

Photographs of plates from a normal series, a series in which 70-percent alcohol was used in the fifth basin, and a series in which 20 ml. of 10-percent sodium resinate was used in place of granular soap at basins 4, 5 and 6, are shown in Plate 3.

The bacterial count for the basins shown in Plate 3 are given in thousands. It must be remembered that the smallest colonies on the plate do not show in the photograph, so that plates having the same count may not look exactly alike. The transient and resident flora of different individuals vary in size, type of organisms, or both. Subject T shown in Plate 3 has in the resident flora an aerobic spore forming bacteria that appear in all plates. Subject Q shown in Plate 3 has many very small colonies on each plate. This makes accurate counting tedious.

Summary and Conclusions

Price's procedure as slightly modified (4) was used for studying the germicidal action of cleaning agents on the hands and showed that rosin soap and a commercial soap containing rosin were more active germicidally on the bacteria normally found on human skin than the usual commercial fatty acid soaps free from rosin soap.

The experiments indicate that the lather of a 10-per cent coconut oil soap solution and of a 10-percent coconut oil-rosin soap solution are germicidally active against the organisms removed. However, three 2-minute washes with these soap solutions had little effect on the bacteria not removed from the hands. The use of a 10-percent rosin soap solution in the same manner had a marked germicidal effect against both the organisms removed and those remaining.

The lather of a commercial soap containing rosin soap was shown to be germicidally active, but three 2-minute washes had only a slight effect on the resident flora. However, the regular use of this soap daily for one week led to substantial reduction of both the transient and resident flora of the hands.

The evidence of germicidal activity of rosin soaps and soaps containing rosin, both with regard to these hand-washing experiements and the "in vitro" (7) experiments, indicates that wider consideration might well be given this type of soap where germicidal activity is of importance.

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