BACTERIAL FLORA OF THE NORMAL HUMAN SKIN*

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INTRODUCTION

The normal bacterial flora of the human skin has been a problem of interest to investigators for many years, yet it is difficult to find a satisfactory description of the usual bacertial population of ordinary human skin. Textbooks of bacteriology usually list staphylococci, diphtheroids, and a variety of saprophytic rods as occurring on skin and are vague as to the relative numbers of these organisms.

A review of the pertinent literature reveals a remarkable diversity of results of studies of the cutaneous flora, in terms of kinds of bacteria present and their numbers. One gains the impression that generalization is very difficult and that perhaps such diversity is a characteristic of the bacterial flora of the normal human skin.

Quantitative studies have frequently been reported by investigators whose interests did not encompass identification of the bacteria being counted. In other studies, identification of the bacteria present on the skin has been carried out, but only a rough estimate of their numbers has been given. Studies of diseased skin or of skin with unusual characteristics (scalp, axilla, etc.) have been favored by many over studies on ordinary healthy glabrous skin without special appendages. Few investigators have employed anaerobic methods, although it will be shown that on the skin of most persons, the anaerobic bacteria outnumber the aerobes.

The diverse results of quantitative studies of bacteria of skin is well illustrated by the following 3 instances. Canuto (1) reported an approximate value of 253 bacteria per square centimeter of skin in a group of 23 subjects. Price (2) stated that the total resident flora of his hands and arms maintained an equilibrium of 8,000,000 organisms over a 9 year period. Assuming that this skin region has an area of about 2500 square centimeters, his results would indicate about 3200 organisms per square centimeter of skin, a figure considerably higher than that of Canuto. Arnold (3) determined that an average of 1622 organisms could be cultured from a skin area of 1.5 square inches from both the palmar hand surface and the forearm. This is equivalent to about 170 organisms per square centimeter.

Studies concerning the identity of the bacterial inhabitants of the skin have yielded a multiplicity of results, as may be gathered from reading such early statements as that in Sternberg's Textbook of Bacteriology (4) as well as the more recent papers of such workers as Meyer and Spector (5) and Pillsbury and Nichols (6).

Price (2) distinguished between the "transient" flora, which include a large variety of organisms which may at one time or another be found on the skin surface, and the "resident" flora, consisting of a relatively few types of bacteria which apparently multiply freely in the skin—especially in the sebaceous glands.

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Pillsbury, Schaffer and Nichols (7) classified the skin organisms into 3 groups: (1) Organisms constantly found, such as *Staphylococcus albus*; (2) Organisms such as *Staphylococcus aureus*, which are found on some skins; and (3) Bacteria rarely found, such as, *Streptococcus viridans*, *Sarcina lutea*, anaerobic diphtheroids, and certain gram negative rods.

According to Wilson and Miles (8), the most common organism on the skin is the white micrococcus named *Staphylococcus epidermidis albus* by Welch (9). In a test utilizing 159 students to determine the presence of a potentially pathogenic bacterium, *Staphylococcus pyogenes*, Gillespie et. al. (10) found the nasal carrier rate to be 43.4 per cent, the carrier rate on the skin of the wrist was 19.5 per cent, and the carrier rate for both of these sites was 12.5 per cent.

Lovejoy and Hastings (11) first noted the presence of anaerobic diphtheroids in the normal skin. They were present in sebum expressed from sebaceous glands around the nasal folds. This organism was found to be the same as the "acne bacillus", which had been previously observed by Unna (12) in smears from comedones, and cultured from acne pustules by Sabouraud (13). In studying the etiology of acne vulgaris, Gilchrist (14), Hallé and Civatte (15), Hartwell and Streeter (16), Fleming (17), Sudmerson and Thompson (18), and Molesworth (19) had also described the bacterium. Gilchrist (14), named the organism *Bacillus acnes*, but it was later placed in the genus *Corynebacterium* in Bergey's Manual of Determinative Bacteriology (20). Douglas and Gunter (21) proposed that on the basis of its catabolic processes, this bacterium should be named *Propionibacterium acnes*. They were able to demonstrate that *P. acnes* must be considered to be a common skin resident. Their terminology is followed in this paper.

In examining the skin for fungi, investigators have usually chosen some area of the body which is unusual in that its pH is high (i.e. the axilla and toes), its exposure is great (i.e. the fingers), or it is a ready storage space for accumulated dirt (i.e. the nails and ears). There is little in published reports to indicate that protected skin areas which possess no unusual characteristics contain a constant population of fungi. Whether this statement is pertinent to the yeast, *Pityrosporum ovale*, is difficult to ascertain. Certainly, most adults harbor this organism in abundant numbers on their scalps and in the sebaceous glands around the nose. In a series of smears of sebaceous material from the nasal folds of 15 persons, we were able to observe in every instance yeast-like organisms which conformed in appearance to the description of *P. ovale*. Subsequent difficulty in culturing prevented our positive identification of these organisms. Doubtless *P. ovale* resides in the skin of other parts of the body as well. The small numbers of this organism and its relative resistance to culture render quantitative measurements difficult.

METHODS

Skin areas overlying the scapula and the deltoid muscle were chosen for the purpose of this study. The presence of moderate numbers of both sweat and sebaceous glands, the absence of any special appendages and the location in a clothed space rendered these areas likely to be representative of the average "non-special" skin.

Considerable preliminary work was carried out in an attempt to determine those methods which would yield maximal numbers of bacteria. For example, several experiments were performed for the purpose of finding the best abrasive and the optimum time for grinding skin scrapings. On other occasions, distilled water, saline, broth, and Ringer's solution were

tested to find the most satisfactory diluent. These and other procedures resulted in the following "standard technic" which was followed in the actual determination of normal skin flora.

The area to be tested, not prepared in any way, was scraped with a sterile scalpel with a 3.5 cm. blade. Twenty-five upward strokes were made, using even pressure. The area scraped was carefully measured, and the scrapings were placed in a sterile mortar, the adherent skin being washed off the scalpel with 0.5 ml. of sterile distilled water. One gram of sterile alundum, mesh #90, was added and the mixture was ground with a pestle for 3 minutes. The contents of the mortar were then diluted with 9.5 ml. of sterile distilled water, mixed thoroughly, and allowed to stand for about 10 minutes. Dilutions of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} were prepared and plated in triplicate for both aerobic and anaerobic cultures.

The medium used for culturing aerobically was proteose peptone #3 agar with a pH of 7.0, containing:

Proteose peptone #3	2.0 per cent
Dextrose	0.05 per cent
Sodium Chloride	0.5 per cent
Disodium phosphate	0.5 per cent
Agar	1.5 per cent

TABLE 1

Characteristics utilized in identification of the species of the genus Micrococcus

ORGANISM	NITRATE REDUCTION	GROWTH ON $NH_4H_2PO_4$ AGAR	MANNITOL BROTH	GELATIN LIQUEFACTION	PIGMENT ON MILK AGAR
Staph. albus*	Positive	Negative	Acid; no gas	Positive	White
Staph. aureus	Positive	Negative	Acid; no gas	Positive	Golden
M. flavus	Negative	Negative	No acid, no gas	Negative or slight	Yellow
M. epidermidis	Positive	Negative	No acid, no gas	Positive; slow	White
M. candidus	Negative	Negative	Variable	Negative	White

These characteristics are taken from *Bergey's Manual of Determinative Bacteriology* (20).
* About half of the isolated strains did not liquify gelatin.

The following medium, adjusted to pH 6.8, was used for the preparation of anaerobic cultures

Peptone, Difco	2.0 per cent
Dextrose	1.0 per cent
Dipotassium phosphate	0.25 per cent
Yeast extract, Difco	1.0 per cent
Sodium thioglycollate	0.10 per cent
Agar	1.5 per cent

Anaerobic plates were examined after incubation for one week at 37°C. in a Brewer jar containing a hydrogen atmosphere with 5 per cent CO_2. The aerobic plates were examined after 5 days at 37°C.

Following incubation, the colonies were counted with the Quebec Colony Counter, and the number of organisms per square centimeter of skin area was calculated. From 10 to 30 representative colonies were picked, and the morphology of the bacteria was determined by the use of negative stains.

For purposes of identification, several representative organisms were selected from the plates of each subject tested. *P. acnes* was considered to be identified if the organism was a gram positive diphtheroid with morphology varying from a short, thick rod to a longer branched rod; showed microaerophilic growth after 48 hours; liquefied gelatin in 10 days;

formed a rennet curd; peptonized and reduced litmus milk in 30 days; and was catalase positive. This was in accordance with the findings of Douglas and Gunter (21).

The micrococci were identified on the basis of their reactions in nitrate broth, $NH_4H_2PO_4$ agar, gelatin stabs, mannitol broth, and milk agar. Table 1 indicates the characteristics of the species of micrococci, as outlined in Bergey's Manual (20), which were utilized in classification.

For purposes of expediency, we have used the older names, *Staphylococcus aureus* and *Staphylococcus albus*, for the 2 organisms designated *Micrococcus pyogenes, var. aureus* and *Micrococcus pyogenes, var. albus* in the most recent edition of Bergey's Manual of Determinative Bacteriology (20).

In a few instances cultures were made by applying aluminum dishes containing agar to the surface of the skin and then incubating aerobically or anaerobically.

RESULTS

Kinds of Bacteria Isolated

During the course of this study, essentially all of the bacteria isolated from aerobic plates proved to be micrococci; whereas the bacteria from the anaerobic plates were either propionibacteria or micrococci. The aerobic micrococci could be placed in 5 species. During August and September, 1948, 130 cultures of micrococci were isolated from aerobic plates and identified. Over half of these were found to be *M. epidermidis*; more than 25 per cent were *Staphylococcus albus*; and a smaller number were *M. candidus*. *M. flavus* was cultured from one individual on 4 separate occasions. Strains of *Staphylococcus aureus* producing a frankly orange color occurred twice on 2 subjects. However, occasional tests on blood agar showed that an appreciable minority of the cultures designated *Staphylococcus albus* produced beta hemolysis. Undoubtedly many of the strains were closely related to the typical *Staphylococcus aureus*. The close relationship indicated by the current designation of albus and aureus as 2 varieties of the same species, should be emphasized.

We concluded that the aerobic flora of the skin tested consisted mainly of *M. epidermidis* and *Staphylococcus albus*; *M. candidus* being a less common inhabitant. *M. flavus* could not be considered to be a part of the usual resident flora. It is noteworthy that *M. citreus* did not occur among the 130 strains of micrococci selected for identification.

Strictly anaerobic micrococci of the species *M. saccharolyticus* were occasionally encountered. In a paper concerning these organisms, Foubert and Douglas (22) reported that in comparison to the total number of skin residents, the anaerobic micrococci occur infrequently. Our records tend to bear out this statement, for the majority of micrococci which grew on anaerobic plates from most individuals were found to be facultatively anaerobic. In a few instances, anaerobic micrococci were present in large numbers, far exceeding the aerobic or facultative micrococci, but these cases were rare.

Organisms with the morphology of diphtheroids proved to be a major component of the normal flora of the skin. All of the 158 cultures of anaerobic diphtheroids which were isolated proved to be *P. acnes*. Their relative numbers per square centimeter of skin are shown in the tables relating to individual experiments.

TABLE 2

Numbers of organisms obtained from skin scrapings of scapular and deltoid areas of twelve young men and women

SUBJECT	AGE	SEX	DATE TESTED	TIME SINCE BATH	AREA	ORGANISMS PER SQUARE CENTIMETER					
						Right			Left		
						Aerobic	Anaerobic		Aerobic	Anaerobic	
							Number	% Prop.*		Number	% Prop.
Organisms obtained from four different areas											
A. B.	29	M	8/26/48	Few hours	Deltoid	127	40	95	70	77	95
					Scapula	93	147,000	100	264	28,000	100
T. W.	28	M	8/26/48	2 Days	Deltoid	20	316	100	23	380	100
					Scapula	13	13,700	100	70	11,000	100
P. G.	34	M	8/25/48	3 Days	Deltoid	350	90	25	1,570	45	75
					Scapula	50	85	50	165	250	50
D. M.	32	M	7/6/48	4 Days	Deltoid	70	37	50	27	8	50
					Scapula	47	10	50	131	116	50
J. H.	16	M	8/25/48	1 Day	Deltoid	67	217,000	100	1,200	264,000	100
					Scapula	3,050	210,000	100	3,540	216,000	100
Organisms obtained from three adjacent areas											
E. L.	22	F	9/1/48	1 Day	Deltoid 1†	35	51	90			
					Deltoid 2	54	192	90			
					Deltoid 3	11	24	95			
E. J.	25	F	9/8/48	1 Day	Deltoid 1	370	276,000	55			
					Deltoid 2	1,430	277,000	60			
					Deltoid 3	236	277,000	60			
M. Y.	19	F	9/10/48	1 Day	Deltoid 1	6	30	0			
					Deltoid 2	9	24	0			
					Deltoid 3	3	3	0			
Q. M.	26	M	9/15/48	1 Day	Scapula 1	97	270	0			
					Scapula 2	262	300	0			
					Scapula 3	230	197	0			
D. P.	30	M	8/22/48	Few hours	Scapula 1	130	51,600	100			
					Scapula 2	190	196,000	100			
					Scapula 3	107	37,400	100			
W. S.	26	M	8/31/48	2 Days	Scapula 1	30	9,900	70			
					Scapula 2	23	35,700	65			
					Scapula 3	33	53	70			
K. S.	27	M	9/15/48	2 Days	Scapula 1	75	272	80			
					Scapula 2	80	410	75			
					Scapula 3	90	185	80			

* Prop. = Propionibacterium acnes.

† Numbers 1, 2, and 3 indicate adjacent skin areas.

Numbers of Bacteria on Skin

As has been pointed out, quantitative studies are essential to an analysis of the bacterial flora of the skin. For this reason, the routine procedure used in

TABLE 3

Number of organisms obtained from repetitions of skin scrapings from five subjects at different times

SUBJECT	AGE	SEX	DATE TESTED	TIME SINCE BATH	AREA	ORGANISMS PER SQUARE CENTIMETER					
						Right			Left		
						Aerobic	Anaerobic		Aerobic	Anaerobic	
							Number	% Prop.		Number	% Prop
C. E.	37	M	8/27/48	1 Day	Scapula 1	Less than 5	60	50			
					Scapula 2	20	25	50			
			9/21/48	Few hours	Scapula	50	30	20	Less than 3	81	25
			12/6/48	2 Days	Scapula	30	56	80	15	8	95
E. J.	25	F	9/8/48	1 Day	Deltoid 1	370	276,000	55			
					Deltoid 2	1,430	277,000	60			
					Deltoid 3	236	276,000	60			
			9/28/48	1 Day	Deltoid 1				20	74,000	100
					Deltoid 2				110	17,600	100
					Deltoid 3				3	48,500	100
			10/11/48	1 Day	Deltoid	300	7,430		47	3,330	100
			10/18/48	1 Day	Deltoid	2	10,300	85	25	20,800	100
			2/26/49		Deltoid 1		4,060	100		33,100	100
					Deltoid 2		4,700	90		17,900	100
J. L.	37	M	9/9/48	Few hours	Scapula	13	31,800	100	11	65,000	100
			10/12/48	1 Day	Scapula	42	95,000	100	45	865,000	100
			10/19/48	1 Day	Scapula	383	101,000	93	0	419,000	92
			3/4/49	Few hours	Scapula 1		78,000	100		101,000	100
					Scapula 2		15,100	93		276,000	100

D. P.	30	M	8/27/49	2 hours	Scapula	130	51,600	100			
					Scapula	190	196,000	100			
					Scapula	107	37,400	100			
			10/13/48	1 Day	Scapula	32	4,770	100	16	41,500	90
			10/20/48	Few hours	Scapula	2	3,220	100	0	1,660	100
			10/26/48	Few hours	Scapula	2	1,320	97	3	362	97
			3/14/49	Few hours	Scapula 1		5,240	100		14,300	100
					Scapula 2		70	93		14,400	95
T. W.	28	M	8/26/48	2 Days	Scapula	13	13,700	100		11,000	100
			10/13/48	1 Day	Scapula	33	57,200	100		132	62
			10/21/48	1 Day	Scapula	20	1,780	93		1,500	92
			10/27/48	1 Day	Scapula	3	3,760	95		31,400	93
			3/10/49	1 Day	Scapula 1		10,300	97		190	90
					Scapula 2		90,000	100		230	100

this study involved the plating of serial dilutions of suspensions of skin scrapings with calculation of the numbers of bacteria per square centimeter of skin. It is well known that this method of counting bacteria has certain limitations to its accuracy, and special factors operative in these experiments will be presented in the section entitled "Discussion". However, the results of these experiments have considerable significance and serve to establish certain points satisfactorily.

In Tables 2 and 3, the essential results of these quantitative studies are recorded. It will be noted that, in each instance, the results of both aerobic and anaerobic cultivation are given. In view of the limited number of kinds of bacteria found with significant frequency, it was possible to determine the relative numbers of bacteria of different genera on the basis of simple smears made with a negative stain. Virtually all bacteria growing on aerobic plates were micrococci. On anaerobic plates, propionibacteria and micrococci were encountered. The percentage of propionibacteria is recorded in each instance.

A striking feature of the data in Table 2 and 3 is the great range in numbers of bacteria found on skin. One specimen yielded only 6 bacteria to the square centimeter; another contained 865,000.

Inspection of Tables 2 and 3 will show that the skin of some individuals, J. H., E. J., and D. P., for example, usually harbored large numbers of bacteria; whereas others had relatively few bacteria. However, striking variation from these results may be noted.

When skin scrapings were cultured from the same area of a given individual on different dates, results of about the same magnitude were obtained in some instances; in other instances great variation was observed. Table 3 summarizes the data from these experiments. Of the 5 subjects tested, D. P. showed the most variation, his anaerobic count being as low as 70 and as high as 196,000; whereas his aerobic count varied from 0 to 190. T. W. and E. J. showed a similar tendency to inconstancy. Data are sufficient to establish certain differences between the usual numbers of bacteria on different individuals. C. E., for example, had a much lower count in every instance than did the other subjects tested in this series. Propionibacteria constituted a lesser proportion of bacteria on his skin than on the skin of other subjects.

In order to avoid variations in bacterial flora due to differences in structure of skin in different areas all specimens were obtained from skin over the deltoid or scapular areas. Usually two or more specimens were taken from the right and left sides at one time.

When it became apparent that even with simultaneous tests of skin of the right and left deltoid and scapular areas, great variation in number of bacteria were encountered, we further standardized conditions by taking at one time two or three speicmens from areas of skin within a few centimeters of each other.

Results of these tests indicated in Table 3 by the designation of "Area" as deltoid 1, 2, and 3, or scapula 1, 2, and 3, tended to show somewhat greater conformity than was observed with single tests from more distant deltoid and scapular areas. However, in one instance specimens from W. S. gave results of less than 100 and more than 35,000 in tests of two adjacent areas.

In the face of the marked variation in total numbers of bacteria on skin, it

was of obvious importance to determine which kinds of bacteria were principally involved, and if possible, exactly where the bacteria were multiplying.

The most striking fact with respect to the kinds of bacteria found on skin was the predominance of anaerobic bacteria. The average number of aerobic organisms present on the skin areas listed in Table 2 was 352 per square centimeter. A similar calculation for anaerobes shows an average count of 55,384 per square centimeter. These figures illustrate the preponderance of anaerobes over aerobes upon the skin of our subjects. Of the 41 specimens included in this table 16 were found to have more than 5000 anaerobes to the square centimeter. In no instance

TABLE 4

The influence of the interval following bathing upon the skin flora of the scapular area of four men

SUBJECT	AGE	SEX	TIME SINCE BATH	pH	NaCl	ORGANISMS PER SQUARE CENTIMETER					
						Right			Left		
						Aerobic	Anaerobic		Aerobic	Anaerobic	
							Number	% Prop.		Number	% Prop.
					mgm/cm²						
C. A. E.	37	M	Few hours	6.6	0.019	50	30	20	Less than 3	81	25
			2 Days	7.1	0.021	33	30	50	13	13	60
			5 Days	6.9	0.04	23	73	60	27	110	50
			7 Days	7.0	0.09	21	15		6	15	
P. G.	34	M	Few hours	7.0	0.005	93	20	50	37	24	50
			2 Days	7.0	0.005	10	27	40	7	20	65
			4 Days	7.0	0.019	17	15	50	7	9	50
W. K.	35	M	Few hours	6.8	0.011	30	845	40	130	60	10
			2 Days	6.9	0.023	30	200	10	287	153	10
			5 Days	7.0	0.025	17	13	25	20	25	10
G. M.	28	M	Few hours	6.9	0.007	67	3,000	90	10	2,240	90
			2 Days	6.9	0.011	47	67	85	43	134	90
			4 Days	6.9	0.022	40	12	100	13	9	100

did the number of aerobes reach this level. It is important to note, however, that in approximately one-third of the specimens, the anaerobic bacteria did not exceed 100 per square centimeter. In nearly half of the 41 specimens, there were more than 10 times as many anaerobes as aerobes. In 14 specimens, the ratio exceeded 100 to 1.

Influence of Bathing on Bacterial Flora

As one step in localizing the site of growth of bacteria of skin, the effect of bathing on the cutaneous flora was determined. It was reasoned that bacteria growing on the surface of skin or in or under surface dirt would be removed in considerable part by bathing, whereas bacteria growing in sweat glands, sebaceous glands or hair follicles would not be removed by this procedure.

An experiment was performed in which the time interval between bathing and tests of cutneous flora varied from a few hours to 7 days. Table 4 gives the

results of this work, in which the right and left scapular skin areas were scraped. It becomes obvious that an increasing period of time since the last bath, from 1 to 7 days, has no effect in increasing the numbers of bacteria on the skin. Also included in the table are simultaneous determinations of pH and of NaCl in mgms/cm^2 of areas immediately adjacent to the scraped region. These tests were conducted by inverting a tube 22 x 175 mm., containing 5 ml. of distilled water, over the skin and agitating for one minute. The pH was determined on 2 ml. of this specimen, using a Beckman pH meter, and for the NaCl determination, two 1 ml. samples were titrated with standardized $AgNO_3$, using K_2CrO_4 as indicator (according to the standard method (23)). When there was variation of pH or salt content, it appeared, within the limits of our experimental method, to play no role in enhancing or decreasing skin flora.

Influence of Sweating on Skin Flora

One obvious method of getting evidence on the question of whether bacteria of the skin grow largely in sweat glands was to determine the numbers of bacteria on skin before, during and after induced sweating.

Table 5 records the results of an experiment in which the effect of sweating upon skin flora was tested in the following manner. Each subject bathed the evening previous to or upon the morning of the experiment. The right and left scapular regions were scraped and cultured at sometime between 7:30 and 10:00 a.m. The test subject then performed some type of physical labor out-of-doors in the sun for the remainder of the day retaining his shirt covering over the scapular areas. A second set of scrapings was obtained while work was going on, between 10:00 a.m. and 1:45 p.m. A third scraping was done late in the afternoon following work, and a fourth on the next morning prior to bathing. The pH and NaCl content were simultaneously determined.

In each instance, the pH dropped during work and returned to normal on the following day. The NaCl content increased during sweating, as would be expected. Its failure to return to normal may be explained on the basis that no attempt was made to cleanse the area during the test period, so that salt could readily accumulate. A similar, although not so great increase in NaCl was seen in Table 4 where the individual had a constant accumulation of NaCl from a much smaller quantity of sweat. The counts of aerobic bacteria recorded in Table 5 are consistently low and show marked fluctuation only in the case of J. L. His skin showed considerable increases in anaerobic organisms, all *P. acnes*, during work and a marked decrease on the following day. R. B. had a similar but less pronounced increase and subsequent decrease in cutaneous flora. W. S., whose counts were regularly low, failed to respond to the stimulus.

Correlation of Numbers of Bacteria with Presence of Sweat Glands and Sebaceous Glands in Different Skin Areas

In order to obtain further evidence on possible sites of growth of skin bacteria several observations were made on selected areas of skin other than the scapular and deltoid regions.

TABLE 5

The influence of sweating upon the skin flora of the scapular areas of three men

SUBJECT	AGE	TIME OF SAMPLING	pH	NaCl	ORGANISMS PER SQUARE CENTIMETER					
					Right			Left		
					Aerobic	Anaerobic		Aerobic	Anaerobic	
						Number	% Prop.		Number	% Prop.
				mgm/cm²						
R. B.	26	Before work	6.9	0.017	20	6,040	100	34	9,780	100
		During work	6.2	0.027	7	14,600	100	27	14,600	100
		After work	6.2	0.037	Less than 10	4,900	100	10	3,640	100
		Following morn.	6.6	0.03	Less than 10	1,770	100	Less than 10	1,520	100
J. L.	37	Before work	6.7	0.007	13	31,800	100	11	65,000	100
		During work	6.1	0.016	654	2,000,000	100	307	1,210,000	100
		After work	5.6	0.020	21	635,000	100	43	356,000	100
		Following morn.	6.8	0.027	63	5,800	100	57	6,400	100
W. S.	26	Before work	6.5	0.005	35	60	70	28	70	100
		During work	6.0	0.080	146	54	100	22	29	100
		After work	6.2	0.11	50	74	100	9	20	100
		Following morn.	6.5	0.24	29	86	100	4	45	100

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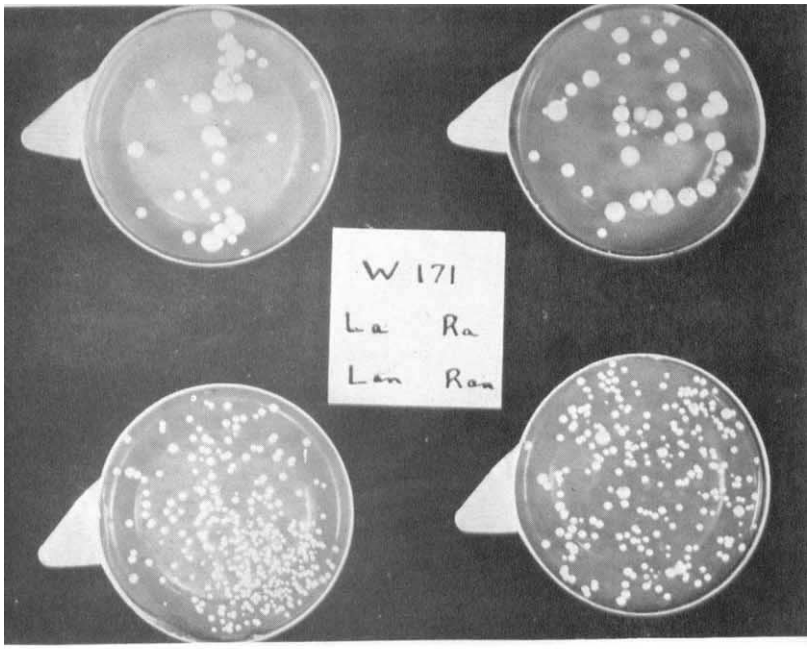

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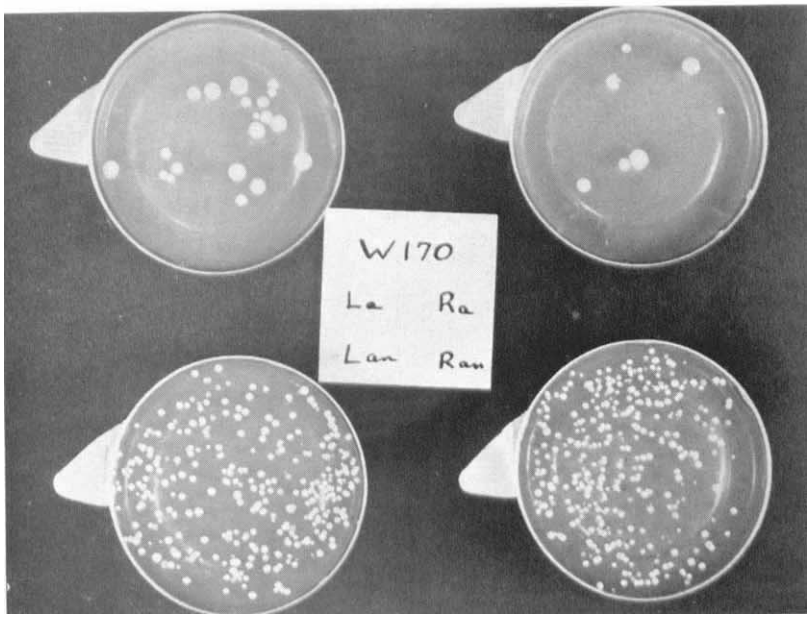

FIGS. 1 and 2. Aluminum plates containing an agar medium were placed in contact with the skin and subsequently incubated aerobically or anaerobically. Note the marked predominance of anaerobes. W170 and W171 refer to the persons designated as J. L. and T. W. in the tables.

La—Left arm, aerobic incubation
Ra—Right arm, aerobic incubation
Lan—Left arm, anaerobic incubation
Ran—Right arm, anaerobic incubation

It is well known that *P. acnes* may be found in sebaceous glands in acne and in the abundant sebaceous secretions expressed from glands on and beside the nose. We found that smears of sebum obtained from the sternum, face, neck and ear concha showed large numbers of gram positive rods which conformed to the morphology of that organism. Gram positive cocci arranged irregularly were seen in material from some of the sebaceous glands.

To obtain further evidence concerning the location of the majority of skin organisms, the following experiment was devised. The hypothenar eminence of

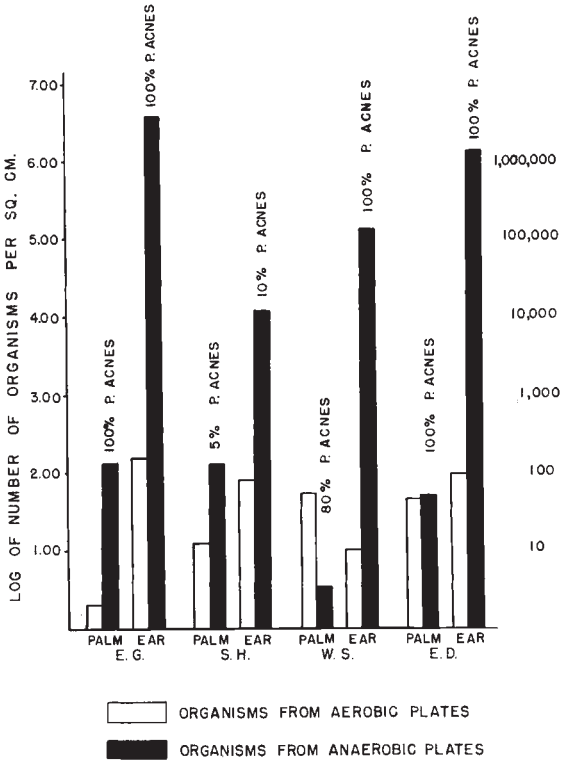

CHART 1. Number of organisms per square centimeter on the palmar area (devoid of sebaceous glands) and the ventral ear concha (devoid of sweat glands) of four subjects.

the palm, known to be free of sebaceous glands but rich in sweat glands, and the concave surface of the ear concha, devoid of sweat glands but well supplied with sebaceous glands, were scraped and cultured according to the standard procedure. Figure 1 shows the results of this work, representing 4 individuals between the ages of 28 and 36. It was necessary to plot the numbers of organisms per square centimeter in logarithms, because in 2 of the 4 instances, the counts from the ear concha exceeded a million, while the palmar counts were confined to numbers less than 150. This marked difference in bacterial population agrees well with the less conclusive data from experiments on the effects of bathing and sweating on

cutaneous flora and strongly suggests that the sebaceous glands and not the sweat glands are the primary habitat of most of the bacteria growing in the skin.

With this fact in mind, one may readily see that the number of sebaceous glands present and the amount of sebum obtained for any given skin scraping might well influence the bacterial counts. The significance of this point will be elaborated later in the paper.

Influence of Sex and Age upon Skin Flora

Data in Tables 2 and 3 indicate that sex is not a significant factor in influencing either numbers or kinds of skin organisms.

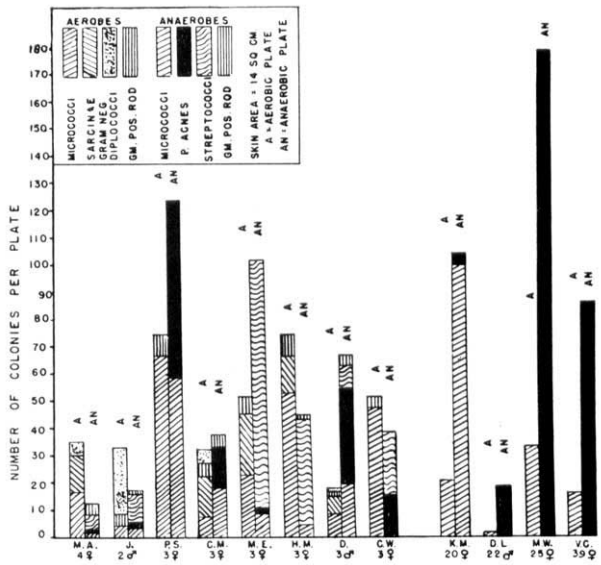

CHART 2. Skin flora of the ventral forearm of 8 subjects aged 2 to 4 years and 4 subjects aged 20 to 40 years. It should be emphasized that the designation "anaerobes" refers to all bacteria that grew anaerobically including those that were facultative.

That age might play a prominent role in determining both kinds and numbers of skin organisms was considered likely, particularly because of the changes which occur in the sebaceous glands at puberty. Accordingly, 8 children between ages of 2 and 4 were tested to determine numbers and types of bacteria present. Our standard technic was subjected to considerable revision to suit the conditions of this work. Aluminum contact plates containing agar with an area of 14 square centimeters were utilized in this experiment. The plates were placed upon the ventral surface of the forearm for a period of about 10 seconds. Incubation was carried out aerobically and anaerobically. Control plates from the same area were prepared using four adults. Figure 2 contains the results of this test, indicating both numbers and general types of organisms present. It must be emphasized that these may have been the result, in part, of contamination of the exposed skin by dust etc. since the children were at play and were not wearing sleeves.

Also, the limitations imposed by the use of contact plates prevented any dispersal of clumps of bacteria.

Several distinct differences in flora are immediately apparent. *Sarcina*, an organism rarely encountered in adults, was a prominent part of the flora of four of the children. There was, in addition, a small number of gram positive facultatively anaerobic rods, mainly spore formers, present on 12 of the 16 plates counted. Small numbers of gram negative diplococci, probably *Neisseria*, were encountered on the skin of 3 children; and one child had this organism as 75 per cent of his aerobic flora.

The occurrence of the streptococci on the anaerobic plates of 6 of the children was especially interesting, as these organisms were completely absent from all cultures of adults prepared during the course of our work. Of the 10 colonies of streptococci picked for identification, all proved to be non-hemolytic with the exception of 1 alpha hemolytic organism from H. M. Another strain from H. M. was found to be a strict anaerobe, whereas the remaining streptococci were facultative. Their failure to appear on aerobic plates may well have been due to antagonistic effects produced by the gram positive rods and *Sarcina*.

The 4 adults tested at the same time possessed only *P. acnes* and micrococci, most of which were facultative. The extreme variability in numbers both in the older age group and children is a typical observation and has been previously discussed.

Antibiotics Produced by Bacteria of Skin as a Factor in Limiting Cutaneous Flora

The possible role of antibacterial effects of cutaneous secretions in limiting the bacterial flora of skin has been studied by a number of investigators (26–30). The possibility that substances antagonistic to one species of skin bacteria may be produced by another skin organism was called to our attention by the following experience. In counting colonies on the plates of a culture of the right deltoid area of E. J., it was noted that the 10^{-1} dilution had an average count of 60 with 90 per cent micrococci; whereas, the 10^{-2} dilution had an average of 341 colonies, 93 per cent which were *P. acnes*. The left deltoid cultures showed similar results. Pure cultures of 2 of the micrococci, designated A.M. 54 and A.M. 55 were isolated from the 10^{-1} dilution plates. Both were found to be facultative anaerobes.

The following procedure was then carried out. A portion of agar about 1 cm. square was cut from the 10^{-2} dilution plates of the right and left deltoid regions which showed a predominance of propionibacteria. Each block of agar was ground in a mortar with 10 cc. of water. One cc. of the resultant suspension was then plated out in triplicate using thioglycollate agar medium. A.M. 54, from the right deltoid, was streaked on the appropriate set of seeded plates as was A.M. 55, the left deltoid area. The plates were incubated anaerobically at 37°C. for 4 days. As shown in Figure 3, wide zones of inhibition of *P. acnes* were present on either side of the streaks of micrococci.

When attempts were made to demonstrate the inhibitory activity of A.M. 54 and A.M. 55 against other strains of *P. acnes* isolated from the same individual,

no inhibition was noted. The exact nature of the inhibitory action is not known to us, and may be due to an antibiotic substance, incompatible pH, or other antagonistic activity. Murray (24) recently found 4 different types of antibiotics isolated from micrococci which were able to inhibit growth of gram positive organisms. *Staphylococcus aureus* has been shown by Gardner (25) to produce an antibiotic substance active against *Corynebacterium xeroeis*, *C. diphtheriae*, *M. lysodeikticus*, *Mycobacterium phlei*, *Staph. aureus* and *Bacillus anthracis*. It is

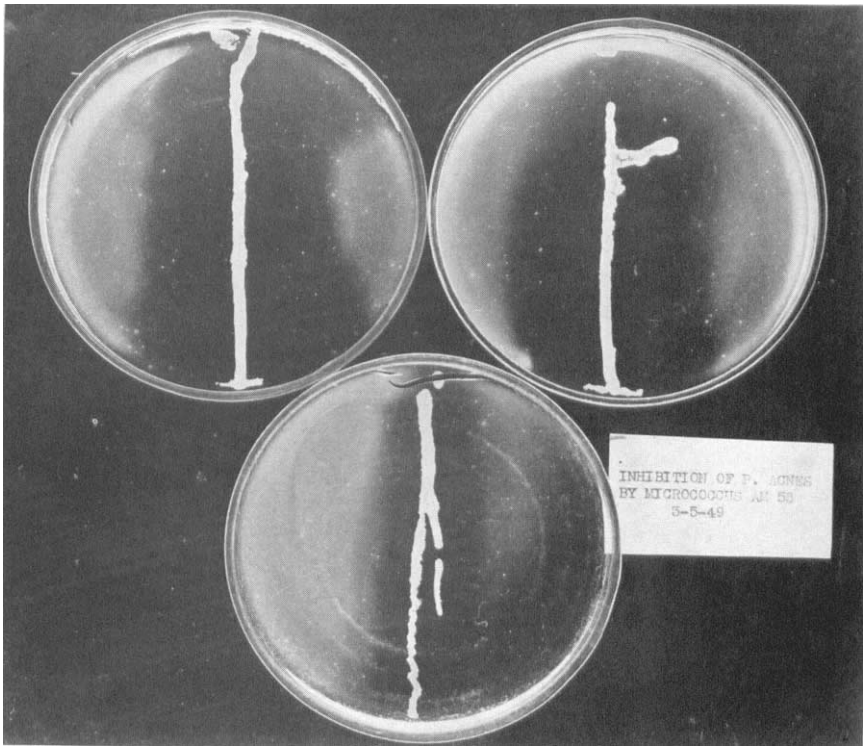

FIG. 3. Petri plates containing agar heavily seeded with P. acnes were inoculated on the surface with a streak of Staphylococci. The clear zones in which growth of P. acnes was inhibited are attributed to an antibiotic effect. Presumably such antibiotic effects are significant in limiting the bacterial flora of the skin.

conceivable that A.M. 54 and A.M. 55 elaborated some similar substance. The potential significance of such antibiotics as a factor in reducing the flora of the skin is apparent.

DISCUSSION

It is a remarkable fact that on an organ as exposed to external contamination as the human skin, only 2 genera of bacteria were found in the great majority of tests. The number of species was also remarkably small. Anaerobic organisms clearly predominated. The most abundant organism in nearly all instances was

the "acne bacillus," *P. acnes*. It was found that the second most common organism was usually *M. epidermidis*. However, in an occasional instance, strictly anaerobic micrococci of the species *M. saccharolyticus* were present in very large numbers. *Staph. albus* was very frequently encountered, and *M. candidus* was found in smaller numbers. In those areas of skin tested, *M. flavus* was distinctly unusual. *M. citreus* was not found. While frankly orange cultures of *Staph. aureus* were not observed often, an appreciable number of the strains of *Staph. albus* were hemolytic. It is probable that under appropriate conditions some of the strains designated albus would have developed orange pigment.

Cultures prepared during the summer and fall of 1948 yielded higher percentages of anaerobic micrococci than did subsequent studies. The earlier work was performed by W. S., and the later cultures were made largely by E. J. A careful comparison of technic used and methods of identification failed to produce a possible explanation for the difference in incidence of anaerobic micrococci.

Persons familiar with the difficulties encountered in counting lactobacilli in saliva or other bacteria in a variety of kinds of specimens will realize that the results of our procedures in making plate counts must not be interpreted as having a high degree of exactitude. Considerable caution should be exercised in evaluating these data. Clumping of bacteria, and failure to get an even dispersion is certainly an important difficulty that has not been successfully eliminated. Some absorption onto the abrasive apparently occurred.

The points to which significance is attached in this paper are supported by data which would still be valid if the results obtained represented only one-half, one-third, or even one-tenth of the total bacteria in a large proportion of the specimens.

There seems no reason to doubt the validity of the enumerations showing high counts.

Realizing the major limitations to the accuracy of our methods, a number of points not generally recognized appear to be established by our data.

The total numbers of organisms per square centimeter ranged from fewer than 10 to more than 800,000. Great variability in individual tests from any given person was the rule. In spite of this, there were clear-cut differences between individuals. Some showed many counts of fewer than 100 and nearly all under 500; others usually had more than 10,000 organisms per square centimeter and occasionally had several hundred thousand.

It appears that the principal location of growth of bacteria is the sebaceous gland. When this is realized, it is not surprising that the flora is predominantly anaerobic, inasmuch as the penetration of oxygen into the center of a mass of sebaceous secretion must be very low indeed.

The puzzling irregularity of results of our enumerations of bacteria of the normal human skin may be resolved to a considerable degree by a realization of the habitat of these bacteria. We must think of them as growing in minute colonies in sebaceous glands. It appears that sebaceous glands colonized by the bacteria may be distributed at appreciable distances from one another.

Our practice of scraping skin, and the other common procedures used by other

investigators such as rubbing the skin with a moist swab or applying a contact plate to the skin, could be expected to give a true picture of the bacterial population only if there were a relatively even distribution of the organisms over the general area under study. Actually, the conditions appear to be quite different, and it becomes apparent that scraping or swabbing the skin to determine the total number of organisms in a given area may be somewhat analogous to scraping an area of a petri plate on which a scattering of small colonies of bacteria are growing. One may obtain very high counts if he happens to scrape several colonies or may find very few organisms if he merely gets the margin of a colony or misses all colonies. This analogy is somewhat fallacious in that the skin is subjected to a constant rubbing and to the release of sebaceous secretion on its surface. Therefore, in addition to the major areas of bacterial contamination at the orifices of sebaceous glands, there is presumably a sparse population of bacteria distributed over the intervening skin. The single count of 70 bacteria to the square centimeter on D. P., who ordinarily had counts well over a thousand, probably represented this population between colonies.

Limited studies of the bacteria present on the forearms of children indicated that facultatively anaerobic spore forming rods were present in considerable numbers. One might interpret these as organisms from the environment, inasmuch as the children were playing out-of-doors with no sleeves covering the area of the forearm tested. The presence of streptococci and gram negative diplococci, presumably of the genus *Neisseria*, is also of interest.

SUMMARY

Aerobic and anaerobic cultures were made from the scapular and deltoid regions of 17 adults to determine the kinds and numbers of bacteria present at different times and on different areas of the skin. A total of 146 specimens taken over a period of 8 months was included in this study. Results indicated that the bacterial flora is considerably different from that described in most textbooks.

In a representative series of 41 tests on 12 subjects, anaerobic bacteria outnumbered aerobes by a ratio of at least 10 to 1 in 46 per cent and at least 100 to 1 in 34 per cent of the tests.

Propionibacterium acnes was by far the most numerous organism in most individuals. *Micrococcus epidermidis*, *Staph. albus*, and *M. candidus* were regularly present, the first two in appreciable numbers. Frankly orange strains of *Staph. aureus* were not encountered frequently. However, the well known intergrading between strains of aureus and albus was noted. *M. flavus* was relatively infrequent. *M. citreus* was never isolated. In a few specimens, *M. saccharolyticus*, an obligate anaerobe, was present in large numbers and was the predominant organism.

In considering the habitat of bacteria of the skin and factors favoring their multiplication, the following observations were made:

(a) Sebaceous glands appear to be the major site of growth of bacteria of the skin. This conclusion is consistent with the predominantly anaerobic nature of the bacteria on skin, and is supported by the occurrence of numerous bacteria

with the morphology of propionibacteria and micrococci in smears of sebaceous secretions from the sternum, neck, and face. Comparative studies of numbers of bacteria on the palms of the hands where sweat glands are numerous and sebaceous glands are lacking and the ear concha where there are many sebaceous glands, but no sweat glands, support the supposition that the sebaceous glands are the chief site of bacterial growth.

(b) Certain bacteria of the skin were active in inhibiting other skin organisms in some of our cultures. It seems fair to assume that such antibiotic activity may be a factor in the bacterial ecology of the skin, in limiting the flora to a few types.

(c) Going without a bath for periods of 5 to 7 days did not significantly increase the bacterial population of the skin.

(d) Exercise with sweating caused a transient minor increase in skin flora.

Limited studies of bacteria on the skins of the forearms of 8 children, aged 2 to 4 years, demonstrated considerable differences from the flora of adults. To what extent these differences were due to greater contamination of skin from the environment was not determined.

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