BACTERIAL FLORA OF NORMAL SKIN

A Study of the Effect of Sulfathiazole and Some Ointment Bases[1, 2]

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The growing importance of local sulfonamide therapy in dermatology (1, 2, 3, 4) and the relative paucity of literature dealing with the effects of ointment bases on skin bacteria has prompted the present investigation.

The latter problem was partially studied by Pillsbury, Livingood and Nichols (5) in a previous report. These workers used the method of Price (6) as modified by Pohle and Stuart (7) in determining the effect of various cutaneous applications in changing the rate at which bacteria were removed from the skin during a standard scrubbing technic.

They found, in brief, that certain ointments, when applied to the skin for periods varying between several minutes and one hour, resulted in a rise in bacteria recovered as compared to scrubbing experiments where no applications were used. The ointments used included petrolatum, lanolin, 3% and 5% ammoniated mercury in ointment, U.S.P., 3% and 5% ammoniated mercury in an emulsion type base, phenylmercuric nitrate (1:1,500) in an emulsion type base, and lastly ointment U.S.P.

Similar effects were noted with distilled water, 0.02% aqueous solution of potassium permanganate, 0.1% aerosol in water, 0.5% aqueous solution of aluminum acetate and the simple wearing of rubber gloves.

A suggestion was made that this rise in bacterial count might be due to the softening action of the various applications allowing better removal of bacteria, surface debris and superficial stratum corneum.

The opposite action was observed following the local use of 70% ethyl alcohol and Castellani's Carbolfuchsin paint.

Of interest, in connection with these findings, is a study by Cromwell and Leffler (8) using a technic similar to the above workers. They found that astringent substances (ethyl alcohol and potassium alum) have a hardening action on the epidermis and, thereby, prevent bacteria from being scrubbed off the skin. By contrast, other substances (sodium sulphide and ammonium sulphide) which tend to soften the epidermis result in a rise in bacterial count.

[1] This study was aided by a grant from the Smith, Kline, and French Laboratories.

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From the Department of Dermatology and Syphilology, Dr. John H. Stokes, Director, and the Pepper Laboratory, Dr. J. H. Austin, Director, of the Hospital of the University of Pennsylvania.

[2] Read at the Fifth Annual Meeting of The Society for Investigative Dermatology, Atlantic City, N. J., June 9, 1942.

They correlated these observations with electrical turbidity measurements of the washings and concluded that the number of bacteria removed had little to do with the antiseptic qualities of the preparations but was greatly influenced by the action of the substances in hindering or facilitating the removal of surface material containing bacteria.

METHOD OF STUDY

The scrubbing technic followed here was essentially identical to the method employed by Pillsbury, Livingood and Nichols (5). Only a brief summary of this procedure will be repeated at this time. An exception to the original technic was the omission of the preliminary three-minute scrubbing period. Instead the arms and forearms were simply washed with soap and water before beginning the scrubbing procedure.

A trained subject using nine grams of soapy solution scrubbed his hands and forearms for a period of one minute. The suds then were rinsed into two separate basins using one liter of distilled water for each arm. This procedure was repeated fifteen times. A standard sample of fluid obtained from each basin was cultured in petri dishes with bacterial infusion agar.[3] From colony counts the number of bacteria removed with each minute scrub was calculated. Averages of these figures for each minute scrub from each experimental group were plotted on a curve using as ordinates a logarithmic scale for bacterial counts and showing a zone corresponding to the standard error of the mean.[4]

The subjects used in the study were two physicians and four medical students.

There were a total of 100 scrubbing experiments performed at weekly intervals. Each subject began with three control scrubs in order to indicate the number of bacteria normally present on his hands and forearms. Subsequently, control scrubs were alternated at weekly intervals with scrubs performed after the application of various ointments twice daily for six days.

These preparations were 2% and 5% sulfathiazole in an emulsion base,[5] an emulsion base,[5] yellow petrolatum U.S.P., and lanolin U.S.P.

[3] All culture media used in this experiment contained para-aminobenzoic acid in a concentration of 5 mgm./100 cc.

[4] These computations (as well as many valuable suggestions regarding the interpretation of our data) were made by Dr. J. H. Austin. A description of the method used is quoted below.

"The standard error of the mean has been computed at the beginning, at the eighth washing and at the end of each curve.

By interpolation the standard error times the square root of two has been drawn about the smoothed curve. This has the effect that where the zones overlap, there is no significant difference between the levels of the curves.

The counts are plotted on logarithmic scale, both for convenience and because the linear relation approached has a meaning."

[5] The formula of this emulsion base is:

	per cent
Sulfathiazole	5.0
Sodium lauryl sulphate	1.0
Stearyl alcohol	10.0
Cetyl alcohol	3.0
Spermaceti	10.0
Glycerine	10.0
Water	61.0

In addition, qualitative studies of bacterial types were made on eighteen successive scrubs on one subject (W. M.) according to the following technic.

Two tubes containing 15 cc. of beef infusion broth at pH 7.4 were inoculated with 1 cc. of the soapy washing of the first minute scrub and likewise of the second, seventh, eighth, fourteenth and fifteenth. One tube was incubated aerobically and the other anaerobically for forty-eight hours. Subcultures were then made from the broth to blood (human) agar plates which likewise were incubated for forty-eight hours with observations at twenty-four hours. Many of the staphylococci showed slight if any hemolysis until forty-eight hours. Hemolysis was often retarded for staphylococci under anaerobic conditions and for this reason the anaerobic plates were further incubated over night aerobically and again observed.

RESULTS

Chart 1 summarizes our experience with 100 scrubbing experiments divided as 50 control scrubs, 14 scrubs after applying 5% sulfathiazole in emulsion base, 8 scrubs after 2% sulfathiazole in emulsion base, 7 scrubs after applying lanolin, 10 after petrolatum, and 11 following the use of the emulsion base.

The curve for the control experiments with its corresponding zone of standard error is shown at the upper left. The other five graphs represent data on scrubs following the various applications and are drawn superimposed on a control curve for ready comparison.

It will be noted that no significant difference exists between the experimental and control curves where 2% sulfathiazole in emulsion base, lanolin, and petrolatum were used. The curve for 5% sulfathiazole in emulsion base shows a significant reduction in bacteria removed from the skin and the reduction is quite parallel throughout the length of the curve. But the curve for the emulsion base shows an appreciable rise in bacteria removed. This increase is not apparent in the first scrub basin but becomes more obvious with successive scrubs.

The data in regard to qualitative studies are found on Tables 1 and 2. The organisms recovered can be divided roughly into three groupings; those that are found constantly, those that are inconstant in their appearance, and lastly organisms rarely recovered.

In the first group are included micrococcus albus and hemolytic staphylococcus albus in both aerobic as well as anaerobic cultures. The former is found with great constancy throughout the whole of the scrubbing periods, while the hemolytic staphylococcus albus tends to be less persistent. The aerobic form is often absent in the initial basins while the anaerobic variety sometimes is absent in the later scrubs.

Inconstant but persisting organisms were also found. These include aerobic micrococcus aureus and anaerobic grey-white micrococcus. Inconstant organisms, at times persistent, at other times rapidly eliminated, were hemolytic staphylococcus aureus (both aerobic and anaerobic), while inconstant organisms that tend to be eliminated early by scrubbing include anaerobic micrococcus aureus, aerobic micrococcus grey-white, aerobic and anaerobic indifferent streptococcus,

aerobic and anaerobic gram positive spore bearing rod and aerobic diphtheroid bacillus. Among the rare organisms were grouped anaerobic streptococcus viridans, anaerobic bacillus diphtheroid, aerobic sarcina lutea, anaerobic and aerobic gram negative colonoid bacillus.

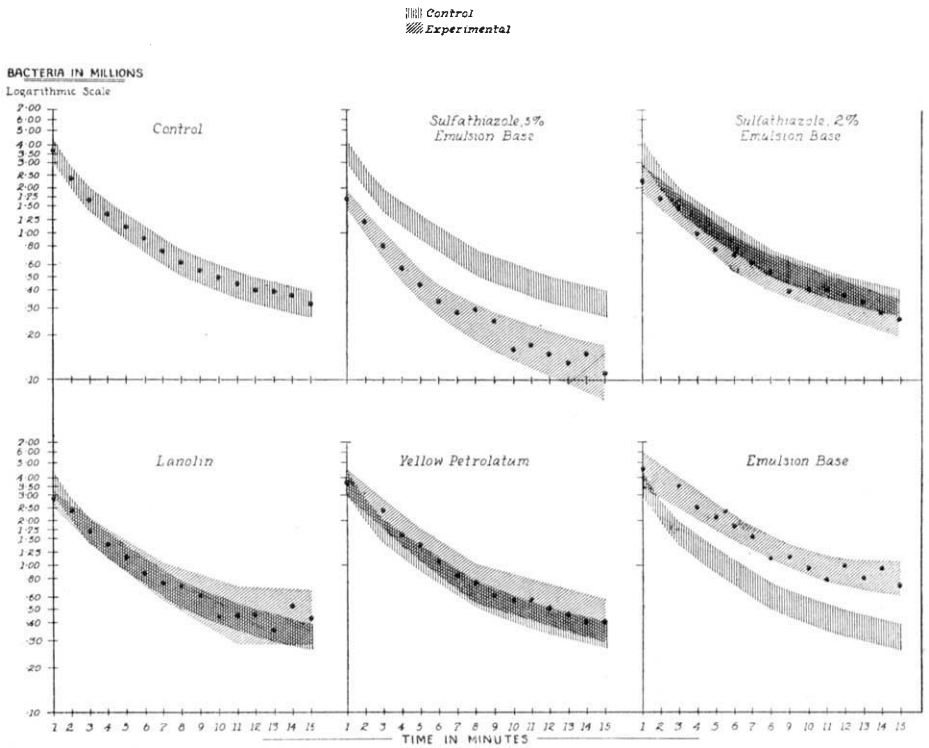

CHART 1. BACTERIA REMOVED BY STANDARD SCRUBBING TECHNIC: SUMMARY OF RESULTS OF 100 SCRUBBING EXPERIMENTS

50 control scrubs, 14 scrubs after applying 5% sulfathiazole in emulsion base, 8 scrubs after 2% sulfathiazole in emulsion base, 7 scrubs after applying lanolin, 10 after petrolatum, and 11 following the use of the emulsion base.

Each group was averaged for each minute scrub and these points were recorded in curves as shown above. The standard error of the mean was then computed and drawn as a band about these points.

Differences between the experimental and control curves are not significant when the bands overlap.

The number of experiments is small, but an examination of the data fails to show a relationship between the use of any of the ointments and the recovery of certain types of bacteria.

DISCUSSION

The increase in bacteria removed from the skin after the application of the emulsion base may, we believe, be adequately explained by its softening and detergent effect on the epidermis. Other bases not having this property to so great an extent fail to show a significant rise in bacteria removed.

The shape of the curve is of some importance. If the same proportion of bacteria were removed from a fixed available source with each minute scrub, the logarithmic relationship is such that the graph would be a straight line. This situation, however, does not pertain in any of the curves shown (chart 1), indicating that relatively greater additions to the number of available bacteria are made with each minute scrub as deeper parts of the epidermal layers are removed.

The emulsion base curve begins at the same level as the control curve, but by the time the third minute scrub is reached it is significantly higher than the control, and it continues thus throughout the remainder of the curve. This indicates that although the original source of bacteria (with the first minute basin) was the same as the control, subsequent scrubs resulted in an increased bacterial count because further sources of bacteria were uncovered.

The sulfathiazole used in this experiment was incorporated in the emulsion base. The 5% sulfathiazole ointment showed a significant reduction in bacteria removed throughout the entire length of the curve. It appears, therefore, that 5% sulfathiazole has a definite bacteriostatic effect on the superficial bacterial flora of the skin.

The curve for the 2% sulfathiazole emulsion base ointment shows no significant variation from the control. When it is appreciated, however, that the emulsion base of itself ordinarily results in an increased removal of bacteria, it seems justifiable to presume that the 2% sulfathiazole ointment was relatively effective in its bacteriostatic action.

Much work has been done on the mode of action of the sulfonamides (9), but it is now generally agreed that these drugs affect the bacteria directly by interfering with their internal metabolism and by reducing their rate of growth. Most experimenters agree that the sulfonamides play very little role in modifying the immunologic mechanism of the host.

The present experiment seems to justify this point of view. The superficial bacteria of the skin are probably subjected to relatively little systemic immunologic influence. Yet sulfathiazole, locally applied, was quite active in its bacteriostatic action.

Although various experimenters (9) have pointed out that protein substances interfere with the action of the sulfonamides, this does not appear to be the case in the stratum corneum. Perhaps the high concentration of sulfathiazole used here was able to overcome this inhibiting effect.

We have used both the 5% and the 2% sulfathiazole ointment on patients with various bacterial dermatitides. Our clinical impression parallels the findings experimentally observed here on normal subjects, in that the 5% preparation appears to possess much greater therapeutic activity than the 2% ointment.

The studies dealing with the qualitative aspects of the bacterial flora of the normal skin (Tables I and II) are small in number. They do not show any characteristic change in bacterial types following the applications of the various ointments. Further studies are needed to elucidate this aspect of the problem.

TABLE I

Qualitative analysis of 18 scrubbing experiments

The large figures represent the last basin in which an organism was recovered. The small experimental figures record the first basin in which an organism was found.

TYPE OF SCRUB	DATE	TYPES OF ORGANISMS																					
		M. albus		*M. aureus*		*M. grey-white*		*Hem. s. albus*		*Hem. s. aureus*		Indif. strep.		*Strep. viridans*		Spore bearing rod, G+		*B. diphtheroid*		*Sarcina lutea*		*B. colonoid, G—*	
		ae.	an.	ae.	an.	ae.	an.	ae.	an.	ae.	an.	ae.	an.	ae.	an.	ae.	an.	ae.	an.	ae.	an.	ae.	an.
Control	1/6	14^{1}				2^{1}		14^{7}		14^{1}													
Sulfathiazole, 5%	1/13	15^{1}	15^{1}		15^{8}																		
Control	1/20	15^{1}	15^{1}		15^{15}		15^{15}	15^{7}	15^{15}							15^{15}	2^{2}						
Emulsion base	1/27	15^{1}	15^{1}		7^{2}				7^{7}	2^{1}	2^{1}		2^{2}		1			1		1			
Control	2/3	15^{1}	15^{1}		7^{7}			15^{1}	15^{1}		2^{2}												
Yellow petrolat	2/10	15^{1}	15^{1}			7^{7}		15^{1}	15^{14}			15^{7}				2^{1}	2^{1}						1
Control	2/17	15^{1}	15^{1}				15^{15}		15^{2}							15^{1}	1						
Sulfathiazole, 2%	2/24	15^{1}	15^{1}					15^{1}	15^{1}			2^{1}				1				15^{15}			
Control	3/3	15^{1}	15^{1}					14^{7}	15^{8}			1				2^{1}	7^{1}						
Lanolin	3/17	15^{1}	15^{7}			7^{7}		15^{1}								14^{1}	2^{1}	7^{1}					
Control	3/24	15^{1}	14^{7}		15^{1}			15^{1}	8^{8}		15^{1}	15^{1}				2^{1}	2^{1}						
Sulfathiazole, 2%	3/31	15^{1}	15^{1}	14^{14}	8^{8}		15^{14}	15^{7}	7^{7}	2^{2}		1				1	2^{2}						
Control	4/7	15^{1}	14^{1}		14^{2}				14^{1}	15^{2}	14^{1}	2^{1}	14^{1}			1	7^{7}					15^{15}	
Emulsion base	4/14	15^{1}	15^{1}			2^{2}		15^{1}	15^{1}			1	1			2^{2}		2^{2}					
Control	4/21	15^{1}	15^{1}					15^{1}	15^{1}			2^{1}	2^{1}							1			
Sulfathiazole, 2%	4/28		15^{1}	15^{1}					15^{1}	15^{1}							14^{7}		1				
Control	5/5	15^{1}	15^{1}					15^{1}								1	2^{1}		2^{2}				
Yellow petrolat	5/12	15^{1}	15^{1}					15^{2}	15^{1}													1	1

In 208 separate cultural experiments performed, in which qualitative studies were made, B. hemolytic streptococcus was never found in spite of the fact that the subject was a physician working in an active dermatologic clinic.

TABLE II

Grouping of data from Table I

Categories of relative frequency with which organisms are recovered

CONSTANT		INCONSTANT		RARE
Persisting	Eliminated	Persisting	Eliminated	
M. albus, ae.	*H. staph. albus*, ae.	*M. aureus*, ae.		*Strep. viridans*, an
M. albus, an.	*H. staph. albus*, an.	*M. grey-white*, an.		*B. diphtheroid*, an.
		H. staph. aureus, ae.		*Sarcina lutea*, ae.
		H. staph. aureus, an.		*B. colonoid* G−, ae.
			M. aureus, an.	*B. colonoid* G−, an.
			M. grey-white, ae.	
			Indif. strep, ae.	
			Indif. strep, an.	
			Spore rod G+, ae.	
			Spore rod G+, an.	
			B. diphtheroid, ae.	

CONCLUSIONS

1. Sulfathiazole in 5% concentration in an emulsion base reduces the bacterial flora of the normal skin.

2. Yellow petrolatum and lanolin have no apparent effect in modifying the rate at which bacteria are removed from the normal skin by a standard scrubbing technic.

3. The emulsion base increases the rate at which bacteria are removed from the normal skin. This action is probably dependent on the ability of the emulsion base to soften the superficial epidermis, thereby facilitating its removal by scrubbing.

4. Qualitative studies fail to reveal a constant effect of any of these preparations in modifying the types of bacteria found in the normal skin.

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DISCUSSION

DR. ADOLPH ROSTENBERG, JR., *Washington, D. C.:* I can only offer praise to Doctors Shaffer and Pillsbury for their excellent work in this paper, as revealed by the careful statistical analysis and the segregation of the various factors which might be considered as variables.

I think dermatologists should consider vehicles more attentively than they have done heretofore. Doctors Pillsbury and Shaffer have shown the importance of vehicles in the detergency of a preparation. The vehicle may, to some extent, determine the ability of an active agent to get at an organism and exercise bactericidal effect.

Another point raised is the question of sensitization, and I think that part of the answer as to whether an individual becomes sensitized to one of these agents is the vehicle involved. As an illustration: recently, in my capacity as dermatologist to the Food and Drug Administration, I interrogated a dozen physicians on the question of sulfonamide ointments. Some never used more than 5 per cent of sulfathiazole, yet equally competent dermatologists never use less than 25 per cent. One man states sulfadiazine ointments are worthless; others state they act the same as sulfathiazole does. Obviously there must be a question of difference in the vehicle used in these instances.

DR. WILEY SAMS, *Miami, Florida:* One factor which I feel is important and which should be emphasized in this study is the size of the particles which make up the preparation in use. Some preparations of sulfathiazole I have found are quite coarse; others are very fine, and since solubility of the preparation is low, a powder with very fine particles should be more effective.

DR. J. G. DOWNING, *Boston:* The factor of sensitization is important, and may vary according to the vehicle or base used in the preparation of an ointment of sulfathiazole. In a series of 10 cases of industrial dermatoses with secondary infections, an ointment of sulfathiazole made with a base containing triethanolamine was found to be useful; however, two patients suffered a dermatitis of the face not from contact, but from sensitization precipitated by sunlight.

DR. J. LAMAR CALLAWAY, *Durham, N. C.:* I think we all recognize that certain types of patients who have identical clinical pictures with the same organisms present respond well to sulfathiazole ointment while others do not. In my experience patients have done best using an emulsion base and have responded with a 5 per cent concentration as well as they do with 10 per cent or 25 per cent sulfathiazole concentration. In my experience if they are going to respond at all they will respond to the lower concentration and this also has an advantage in that fewer reactions are seen.

Miss Mary Poston in the Bacteriology Department of Duke Hospital has been studying in vitro the reaction of the various concentrations of the sulfonamides on the various pyogenic organisms. In some instances the organisms are inhibited by a small concentration of sulfadiazine or other sulfonamides whereas they won't respond at all to high concentrations of sulfathiazole and visa versa. When we have patients who are recalcitrant to treatment with sulfathiazole, cultures are made and studied in vitro with some of the other sulfonamides and the sulfonamide radical that appears to do best in vitro is then used.

DR. DONALD M. PILLSBURY, *Philadelphia:* Dr. Rostenberg mentioned the importance of the vehicle as the agent for the application of sulfathiazole. We have now treated about

1500 patients with sulfathiazole locally, and I believe we can state quite definitely that there is no essential difference between an emulsion or water type base. I think that both are more effective than an all-grease base.

Both Drs. Rostenberg and Downing mentioned sensitivity. We looked for sensitivity when applying sulfathiazole to normal skin. We did not observe any such reactions. There were no reactions to the sulfathiazole ointment in primary infection, such as impetigo or acutely infected eczema. However, reactions to eczematous processes, in which infection seems to be the rule, may take place, and they may flare up suddenly. This occurs frequently in occupational eczemas, in which there is some element of infection, and here sulfathiazole is to be used very cautiously.