ANTIBACTERIAL ACTION OF HUMAN SKIN

IN VIVO EFFECT OF ACETONE, ALCOHOL AND SOAP ON BEHAVIOUR OF *STAPHYLOCOCCUS AUREUS*

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NORMAL skin is an effective barrier to pathogens entering the human body. There has been considerable interest about the mechanisms whereby pathogenic organisms are destroyed on the skin surface. Ricketts, Squire and Topley (1951) extracted lipid from the forearms of volunteers with acetone and showed that the extract rapidly killed a variety of micro-organisms *in vitro*. The *in vitro* killing curve of this extract resembled pure oleic acid which therefore could be an important protective agent against skin pathogens. Pillsbury and Rebell (1952) showed that many fatty acids present in sebaceous secretions, especially caproic and caprylic acids were lethal *in vitro* for several organisms. This paper describes an investigation of the antibacterial action of skin lipid *in vivo* by testing the effect of treating human skin with defatting agents.

MATERIALS AND METHODS

Principle.—The principle of the method was to treat 1 forearm with a defatting agent, the other arm being left untreated as a control. Small numbers of *Staphylococcus aureus*, suspended in distilled water were then placed on standard areas on each forearm, and after drying, were covered with a self-adhesive label for 5 hr. The numbers of staphylococci on the skin were then estimated by a standard sampling method.

Detail.—Healthy male and female volunteers were provided from members of the medical, technical and secretarial staff of a clinical laboratory. They did not use antiseptic soap during the span of the experiments. Prior to each 5 hr. experiment they did not wash their forearms for 12 hr. before inoculation. In practice this meant not washing after 10 p.m. the night before as the experiments were commenced at 10 a.m. the following day. Either immediately before, or at various times preceding inoculation, the right or left forearm was treated with an agent under test. The time of 5 hr. for the experiment was chosen because: (1) it was safe with the inoculum size used—indeed no lesion was produced and when the skin was sampled the following day, the inoculated strain was never recovered. (2) It was an easy time to standardise. (3) With use of a small inoculum the resultant colonies were generally distinct even if some multiplication had occurred.

The flexor aspects of prepared and control arms were then marked with ballpoint pen into 4 rectangles 24 × 40 mm.—the size of the adhesive label. Each rectangle was then sampled with an impression of a label which was then pressed onto nutrient agar and removed. This initial sampling was done to exclude the presence of existing *Staph. aureus* on the forearm skin. In fact such organisms were very rarely found. An overnight broth culture of a recently isolated strain of *Staph. aureus* was diluted in sterile distilled water so that 0·02 ml. contained about 4×10^2–4×10^3 organisms. The organisms in this volume of distilled water were then dropped onto the centre of each marked rectangle; the number of viable organisms applied was counted (Miles and Misra, 1938). With the arm horizontal, flexor aspect uppermost, a sterile wire loop was used to spread the drop evenly within the rectangle. The areas were allowed to dry and then covered with a " Presson " label, adhesive side in contact with the skin. The label was left in position for 5 hr.

Thomas (1961) described the sticky film method for detecting skin staphylococci. In qualitative surveys she found self-adhesive films to be simple, sterile and rarely inhibitory. " Presson " labels have been used both as a dressing and as a sampling agent, possessing the following advantages: they have no anti-staphylococcal activity on *in vitro* testing, are non-irritant yet adhere well to the skin, are water impervious, arrive sterile from the manufacturer and are inexpensive.

Whilst the label was in position volunteers continued their normal activities. After 5 hr. each label was manually peeled off the arm and the adhesive side applied to the surface of plain nutrient agar. Using sterile forceps each label was repositioned 8 times onto fresh agar surfaces so that one label on removal from the arm produced 9 successive maps of the skin. A fresh label sampled the underlying skin and this was inoculated similarly twice more. Thus each rectangle produced a total of 12 impressions. The plates were incubated aerobically at 37° for 24 hr. Colonies of *Staph. aureus* were identified by colonial appearance, gram-stain, coagulase production and a proportion from each experiment were phage typed in order to show whether the strain recovered was likely to be the same as that applied.

After incubation the sum of the colonies identified from each impression represented the count for each rectangular area. Generally, of the total area count 95 per cent of the colonies were derived from the covering label and 5 per cent from the skin sampling. The figures obtained show some spot to spot variation but are amenable to analysis.

RESULTS

The results could conveniently be expressed as a ratio of the sum of the square root of counts from the areas of 1 arm over the other arm. To establish the normal variation between arms 39 experiments on volunteers were performed when neither arm was treated and the sum of the square roots of counts from the right and left arms were compared. The distribution of these ratios was normal (mean 0·998, standard deviation 0·206). The actual counts obtained showed considerable variation between individuals, between strains, and with day to day differences. The following example illustrates the individual variation, and the derivation of the ratios.

	Organisms applied = 629 Subject R.L.		Organisms applied = 629 Subject M.S.	
	Actual Counts Obtained			
	R. arm	L. arm	R. arm	L. arm
Area 1	1	5	104	232
Area 2	3	2	292	168
Area 3	1	0	240	207
Area 4	4	0	276	329

$$\frac{\text{sum of } \sqrt{\text{counts}} \text{ R. arm}}{\text{sum of } \sqrt{\text{counts}} \text{ L. arm}} = \frac{1{\cdot}14}{1} \qquad \frac{\text{sum of } \sqrt{\text{counts}} \text{ R. arm}}{\text{sum of } \sqrt{\text{counts}} \text{ L. arm}} = \frac{0{\cdot}98}{1}$$

Treatment with 100 *per cent acetone and* 74 *per cent ethanol*

The standard procedure for preparing 1 forearm with either of these agents was as follows. Immediately before inoculation, a cotton wool swab soaked in solvent was stroked firmly from wrist to elbow along the flexor aspect for 60 sec., to be followed by a thorough rinsing in fresh solvent. The treated arm was allowed to dry, and then both arms were inoculated. Tables I and II show the results of 5 hr. experiments after such treatment. The area counts are shown

in full in Table I to illustrate area to area variation. The right and left arms were treated a similar number of times. It can be seen that there is a large and significant increase in counts from forearms treated with 100 per cent acetone or 74 per cent ethanol.

TABLE I.—*One Arm Washed in* 100 *per cent Acetone for* 60 *sec. Identical Inoculation Afterwards of Strains of* Staph. aureus

Subject	Strain	Number applied	Numbers recovered from areas on untreated arm (sum of 12 impressions)	Number recovered from areas on acetone washed arm (sum of 12 impressions)	Ratio of sum of $\sqrt{\text{counts}}$ of treated arm / untreated arm
			0	9	
R.L.	1059	450	0	25	12·1 : 1
			0	21	
			2	20	
			6	300	
M.S	1059	450	19	947	5·1 : 1
			16	1031	
			32	234	
			3	125	
A.E.	1737	440	0	122	12·4 : 1
			2	97	
			0	40	
			8	113	
A.C.	1737	440	4	122	5·5 : 1
			2	76	
			0	16	
			3	18	
R.L.	1054	630	8	125	5·9 : 1
			2	62	
			0	131	
			4	148	
M.S.	1054	630	31	832	7·4 : 1
			11	411	
			7	970	

Mean ratio = 8·06 : 1 $t = 5{\cdot}7$ $P < 0{\cdot}001$
(Normal population ratio = 0·988 S.D. = 0·206).

TABLE II.—*One Arm Washed in* 74 *per cent Ethanol for* 60 *sec. Identical Inoculation Afterwards of Strains of* Staph. aureus

Subject	Strain	Ratio of sum of $\sqrt{\text{counts}}$ from ethanol treated arm/untreated arm
R.L.	1059	2·86 : 1
A.E.	1059	1·95 : 1
M.S.	1182	2·91 : 1
A.C.	1182	1·55 : 1
H.B.	1182	2·11 : 1
K.W.	1182	3·03 : 1
R.H.	1054	5·91 : 1
J.L.	1054	3·10 : 1
M.S.	1054	1·95 : 1
R.L.	1054	1·51 : 1
J.L.	1054	3·85 : 1
R.L.	1054	12·1 : 1
H.B.	1059	2·11 : 1
M.S.	1059	2·90 : 1

Mean ratio = 2·64 : 1 $t = 5{\cdot}3$ $P < 0{\cdot}001$

Treatment with soap

A cake of chemically pure sodium stearate was used to wash the forearm under test. For 60 sec. either the right or left forearm was washed in warm soap and water using regular strokes between elbow and wrist. This was followed by a thorough rinsing in water after which the arm was dried with sterile paper towels. Table III shows the results of comparisons between the soap treated and control arms. As a group the results show significantly higher ratios following soap treatment, although with subject A.C. on 3 occasions the ratio is near unity.

TABLE III.—*One Arm Washed in Sodium Stearate (soap) for 60 sec. Identical Inoculation Afterwards of Strains of* Staph. aureus

Subject	Strain	Ratio of $\sqrt{\text{counts}}$ from washed arm/control arm
R.L.	1059	3·30 : 1
M.S.	1059	3·73 : 1
A.E.	1054	2·04 : 1
R.L.	1054	2·38 : 1
M.S.	1054	3·08 : 1
J.L.	1059	5·10 : 1
K.W.	1182	1·70 : 1
H.B.	1182	1·95 : 1
J.L.	1182	2·12 : 1
R.H.	1182	2·01 : 1
A.E.	1054	1·11 : 1
A.C.	1059	1·10 : 1
A.C.	1054	1·04 : 1
J.P.	1054	1·40 : 1
A.C.	1182	1·12 : 1

Mean ratio = 2·21 : 1 $t = 4·0$ $P = < 0·001$.

For how long does acetone treatment of the skin encourage persistence of staphylococci?

This problem has been investigated by washing the forearms of volunteers with acetone at 1, 2, 12 and 24 hr. before application of the organisms. Apart from the interval between washing with acetone and inoculation, the experimental procedure was as above, the arms not being washed during this interval. Results are set out in Table IV. When acetone has been applied up to 2 hr. before there was a significant increase in the counts from the acetone treated arms. But when acetone was applied 12 or 24 hr. previously the results as a group were less significant ($P > 0·05$); although on 3 occasions with an interval of 24 hr. very high ratios were obtained.

DISCUSSION

The results show that either 100 per cent acetone, 74 per cent ethanol, or soap applied to the forearm skin cause higher numbers of surface inoculated staphylococci to be recovered 5 hr. later. As the experimental method was to spread about 10^3 organisms over 960 sq. mm. and the sampling was done when the counts were usually still small, it was not therefore thought that clumping accounted for the difference in ratios. The actual numbers of organisms recovered from the skin at any moment must be related to 2 processes—rate of death of

TABLE IV.—*Acetone (100 per cent) Treatment of One Arm Before Inoculation*

Treatment 1 hr. before Inoculation

Subject	Strain	Ratio of sum of $\sqrt{\text{counts}}$ of acetone treated arm/control arm
P.B.	1788	4·7 : 1
A.R.	1788	4·1 : 1
S.C.	1737	1·82 : 1
R.H.	1737	4·8 : 1
P.F.	1788	1·18 : 1
S.C.	1788	1·81 : 1
A.R.	1737	1·36 : 1
P.B.	1737	1·55 : 1

Mean = 2·66 $t = 2·98$ $P = < 0·01$.

Treatment 2 hr. before inoculation

Subject	Strain	Ratio
A.R.	1788	1·65 : 1
P.B.	1788	1·70 : 1
M.S.	1737	1·66 : 1
P.F.	1737	1·67 : 1

Mean = 1·67 : 1 $t = 14·3$ $P = < 0·001$

Treatment 12 hr. before inoculation

Subject	Strain	Ratio
M.S.	1737	2·80 : 1
S.C.	1788	1·11 : 1
J.L.	1737	1·30 : 1
M.S.	1788	2·40 : 1

Mean = 1·90 : 1 $t = 2·2$ $P = < 0·05$.

Treatment 24 hr. before inoculation

Subject	Strain	Ratio
R.L.	1910	1·01 : 1
M.S.	716	0·94 : 1
R.L.	716	1·12 : 1
M.S.	1910	3·38 : 1
S.C.	1884	1·25 : 1
R.H.	1910	1·16 : 1
R.H.	1884	2·33 : 1
P.F.	1910	1·06 : 1
M.S.	1059	1·21 : 1
R.L.	1059	5·40 : 1

Mean = 1·88 : 1 $t = 1·93$ $0·05 > P > 0·1$.

the initial population and rate of multiplication of the whole or part of the inoculum. The technique used here does not separate these processes. The increased counts obtained after use of organic solvents could possibly be due to either retardation of destruction of the organisms or an acceleration of the growth of the inoculum or a combination of the 2. Treatment of the skin with these agents would remove organic substances from the skin and be unlikely to supply a specific growth activator. It would appear then that the most likely explanation for the increased counts obtained is the removal of antibacterial organic matter.

This would be *in vivo* evidence consistent with the *in vitro* testing of Ricketts *et al.* (1951) and Pillsbury and Rebell (1952). Ricketts *et al.* (1951) found that skin lipid following extraction reaccumulated quite quickly and was largely replenished after 24 hr. In the above experiments the effect of acetone 24 hr. previously applied was probably still effective. The findings on 3 out of 10 occasions of markedly increased counts can be explained on several grounds. The reaccumulation of skin lipid may not occur uniformly—the most bactericidal fraction could take longer to reform. Or there may be a difference between individual subjects, or indeed between the skin of the same subject on different occasions. Factors such as sweating or diet could be important.

The question as to whether interference with the normal flora is related to staphylococcal survival is difficult to answer. The resident flora would be unlikely to be significantly altered by washing with soap yet in many instances this procedure favours persistence of *Staph. aureus*. But both acetone and alcohol would probably affect the normal flora during the period of the experiment and some of the facilitation of staphylococcal survival could thus be explained.

The mechanisms for the significantly increased counts obtained from the treated arms are not therefore fully clear. Removal of skin lipid is the likeliest explanation. Whatever the mechanism, the results may have a bearing on antiseptic procedures currently used. The practice of treating the skin with ethanol as a pre-operative procedure is well established. It would appear that such treatment will decrease the natural antiseptic property of the skin. Sterilisation of an operating site can be considered in 2 phases: (1) immediate elimination of pathogens (2) conditioning of the skin to discourage subsequent contamination. Use of organic solvents as antiseptics would not seem ideal, unless combined with other agents.

Washing with soap is a well established social habit of the human species. Results presented above suggest that far from discouraging pathogenic invasion of the skin, washing can enhance bacterial growth on the skin surface. In the condition of acne vulgaris, long established treatments include frequent washing and use of ether on the skin. The basic pathology in acne is probably an oversecretion of sebum. This is then colonised by *Staph. albus* and the acne bacillus in symbiotic association, the latter organism being dependent on fats for nourishment. If the basic lesion is an over production of skin lipid which is secondarily infected then the clinical lesions would be expected to improve when defatting agents are employed. Otherwise washing the skin could be harmful in certain circumstances in that the skin is rendered less harmful to micro-organisms.

SUMMARY

A method is described for studying the behaviour of *Staph. aureus* on the human skin over 5 hr. Skin previously treated with 100 per cent acetone, 74 percent ethanol, or soap, favours the persistence of surface inoculated staphylococci. The possible mechanisms and implications of this are discussed.

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