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STUDIES ON CHANGES IN SKIN SURFACE BACTERIA IN INDUCED MILIARIA AND ASSOCIATED HYPOHIDROSIS

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Abstract. Data on bacteria and on sweating were collected from 30 adult Caucasian soldiers on active duty with the US Army, before and after the experimental induction of miliaria rubra by polyethylene wrapping of large areas. The following alterations in surface flora were noted: (*a*) The numbers of aerobic bacteria (primarily *S. epidermidis* and diphtheroids) increased to levels greater than 500 000 per cm^2 of skin surface on the lower portion of the back. (*b*) Species of bacteria not found on the normal dry skin surface were isolated from the wrapped surfaces. (*c*) Return to normal flora had not occurred by 7 days after the wrap was removed.

No relationship appeared to exist between total numbers of bacteria present immediately after wrap removal and severity of the induced miliaria. However, a possible direct relationship may exist between magnitude of bacterial increase and severity of the induced miliaria.

The total number of bacteria found immediately after unwrapping, as well as their increase in number over that found before wrapping, did not appear related to the degree of hypohidrosis produced.

Gram-negative organisms do not appear to play a role in the pathogenesis of experimental miliaria. A striking finding was that 18 of 30 subjects developed these organisms in sites where they were not present (by standard culture methods) before occlusion.

A marked difference in the total number of organisms, and the magnitude of change in organisms during the wrapping period in different individuals, points to a natural resistance either to bacterial flora or to the condition (maceration) which encourages bacterial growth.

Maceration of the horny layer and occlusion of the sweat ducts and orifices are recognized as fundamental events in the pathogenesis of miliaria rubra. However, there is as yet no agreement as to the possible role of bacteria in the pathogenic process. O'Brien (1) and Lyons et al. (4) have presented data incriminating bacteria, especially *Staphylococcus aureus*. The development by Sulzberger et al. (5) of the occlusive plastic wrapping technique for the experimental production of miliaria in human volunteers made possible the systematic study of miliaria, anhidrosis, and changes in the bacterial population over large areas of the body's surface. The present study was initiated to ascertain whether changes in the bacterial flora which occur during the experimental induction of miliaria could be related to the severity of the induced miliaria and its associated hypohidrosis. In particular we attempted to discover possible correlations between the clinical severity of the miliaria and hypohidrosis, and: 1) the types of bacteria; 2) the density of bacteria per cm^2 of skin surface; and 3) the changes in numbers of bacteria per cm^2.

METHODS AND MATERIALS

Thirty Caucasian soldiers on active duty with the US Army served as volunteers for these experiments. All volunteers were given the same brand of non-germicidal soap (Ivory®) to use for regular bathing, beginning 7 days prior to the first bacterial sampling and extending throughout the experimental period. Each man was instructed to bathe the night preceding each bacterial sampling, except during the 48 hour wrapped period described below.

Each volunteer was wrapped by the following slight modification of the technique described by Sulzberger et al. (5). A double thickness of occlusive polyethylene wrapping (Saran Wrap®, Dow-Corning Chemicals, Midland, Michigan) was tailored to fit either the left side or the right side of the volunteer's trunk, completely covering the skin from the neck to the waist, and from the mid-line of the back to the mid-line of the chest and abdomen. The occlusion also included the shoulder, axilla, and the upper one-third of the arm. This latter modification eliminated leakage of air around the shoulder and resulted in a wider total surface area of induced miliaria. The polyethylene wrapping was reinforced with flexible plastic tape (Blenderm®, Minnesota Mining and Manufacturing Company, St. Paul, Minnesota). The wrapping

Table I. *Geometric means of total aerobic bacteria per cm² of skin surface on the lower back of volunteers having Grades 0–3 experimental miliaria rubra*

Grade of clinical severity of miliaria[a]	Total aerobic bacteria per cm² × 10^{-2}					
	Side occluded			Side not occluded		
	Initial	48 hrs[b]	9 days[c]	Initial	48 hrs[a]	9 days
1 (12)[d]	2.1	5 200	16.0	2.8	82.0	1.1
2 (17)	1.5	28 000	28.0	0.87	48.0	2.2
3 (1)	2.6	12 000	8.3	32.0	710.0	12.0
Mean of all 30 subjects	1.7	8 600	19.0	1.4	60.0	1.7

[a] See text under Methods and Materials: Hypohidrosis and Miliaria Rubra Determination.
[b] At 48 hours the wrap was removed from the occluded side and side sampled. Non-occluded side also sampled at this time
[c] At 9 days (7 days after wrap removal) the side experimentally occluded and the non-occluded side were sampled.
[d] Numbers in parentheses indicate the number of volunteers having that grade of clinical severity of miliaria.

was held in place by the volunteer's T-shirt, which was itself held firmly against the volunteer's body by the use of flexible plastic tape. The wrap was left in place for 48 consecutive hours and was then followed, just prior to unwrapping, by a 15 minute heat stress at 120°F and 40% relative humidity in a controlled environmental room.

Skin surface bacterial samples were obtained from the lower lateral back region immediately prior to wrapping, immediately after photographs were taken on the day of wrap removal, and again 7 days after removal of the wrapping. These samples were obtained by two 1-minute scrubs with 1 ml of 0.1% Tween-80 in pH 7.9 phosphate buffer inside a sterile glass ring applied to the skin surface. The area within the glass ring was 13 cm². This method is the Williamson technique (6) modified by substituting Tween-80 for Triton-X-100.

Culturing

Viable counts of aerobic bacteria were obtained by culturing serial dilutions of the skin surface samples. Sterile, predried tryptic soy agar (Baltimore Biological Laboratories, Baltimore, Maryland) plates containing 0.5% Tween-80 and eosin methylene blue agar plates were inoculated uniformly with 0.1 ml of the diluted sample. Plates were incubated at 37°C for 48 hours and then counted with the aid of a Quebec counter.

Hypohidrosis and Miliaria Rubra Determination

The degree of hypohidrosis was evaluated using the Gordon & Maibach modification of the Bullard-type sudorometer, as described by Griffin et al. (3). Eleven volunteers had been pre-tested with the sudorometer to ascertain that none had asymmetry of sweating greater than 10% between the two lateral portions of the back.

Clinical severity of miliaria rubra was graded arbitrarily from zero through 3: Zero = no rash; Grade 1 = scattered patches of microvesicles; Grade 2 = a diffuse confluent papulovesicular eruption; Grade 3 = Grade 2 rash with addition of non-follicular pustules.

RESULTS

Table I presents the geometric means of viable bacterial counts per cm² of skin surface on the lower back of the wrapped and control sides for aerobic bacteria. The geometric mean values are given throughout. These indicate that the mean bacterial count ranged from 140 to 170 per cm² skin surface prior to occlusion. After 48 hours, bacterial count of the skin surface covered only by a T-shirt (control side) rose to 6 000 per cm², while the skin surface count of the portion of the back occluded by polyethylene film (wrapped side) rose to 860 000 per cm². One week after the removal of occlusion, the population on the control side returned to 170 per cm², just slightly above the initial population. The population on the wrapped side had dropped to 1 900 during the week, but was still about 10 times higher than the initial population.

Differences in total bacterial counts among volunteers with different grades of experimental miliaria rubra were not striking. There was about a 5-fold higher population per cm² of skin surface on the wrapped portion of those volunteers with the Grade 2 rash over those with the Grade 1 rash. After 1 week there still existed about a 2-fold difference, the population being higher on those with Grade 2 rash. These count differences are less than one order of magnitude and are not statistically different.

In volunteers developing a Grade 1 miliaria, the increase of total aerobic bacteria under the wrapping was approximately 2 450-fold; in Grade 2 volunteers the increase under the wrapping was approximately 18 600. The increase in the Grade 2 group was thus 7.6 times greater than the increase in the Grade 1 group. The possible significance of such a difference is discussed below.

Since only one volunteer exhibited Grade 3 miliaria, the findings on this individual may reflect unusual circumstances and may not be comparable with the data from Grade 1 and Grade 2 groups of volunteers. The volunteer who developed a Grade 3 rash exhibited a total aerobic

Table II. *Gram-negative bacteria on the skin surface of the lower back of volunteers with Grades 0–3 experimental miliaria rubra*

Numbers in parentheses give total number of volunteers developing that grade of experimental miliaria

Grade of clinical severity of miliaria	Number of subjects yielding gram-negative bacteria	Time gram-negative bacteria isolated			Identity of organisms
		Prewrap	At wrap removal	7 days after wrap removal	
1 (12)	3		+	+	*Klebsiella–Enterobacter* group
	3	+	+	+	*Mimia–Herella* group
	2		+	+	*Escherichia coli*
	1		+		*Proteus vulgaris* and *Proteus rettgeri*
2 (17)	4		+		*Klebsiella–Enterobacter* group
	2	+	+	+	*Mimia–Herella* group
	1		+		*Escherichia coli*
	1		+		*Achromobacter* sp.
	1		+		*Neisseria* sp.
3 (1)	1		+	+	*Escherichia coli*

population intermediate between the values for the Grade 1 and Grade 2 groups. The increase in his total bacterial numbers was approximately 4 615-fold.

The findings relating to Gram-negative bacteria are summarized in Table II. Seven groups of Gram-negative bacteria were recovered from 18 of the 31 volunteers in this study. The most commonly isolated organisms belonged to the *Klebsiella-Enterobacter* group, but even these were found in only 7 of 18 volunteers. Thus, there was no one prevailing species in all the subjects. Gram-negative organisms were found in only 8 of 12 volunteers with Grade 1 miliaria, 9 of 17 volunteers with Grade 2 miliaria, and the single volunteer with Grade 4 miliaria. Thus, Gram-negative bacteria were found in only 18 of the 30 subjects with miliaria. Our findings do not indicate an obvious role of Gram-negative bacteria in either the etiology or the severity of experimental miliaria.

Table III lists the types of Gram-positive bacteria isolated from the lower back skin surface of the 30 volunteers during the course of the experiments. Both *Staphylococcus epidermidis* and diphtheroids were isolated from all 30 volunteers. These data suggest that *if* bacteria do play an etiological role in experimental miliaria, these are the most likely responsible organisms.

Table IV presents the data obtained from the 11 volunteers tested for hypohidrosis with the sudorometer following occlusion of the skin surface. Using the unwrapped (control) side of the back as the base, the percentage difference in sweating stimulated on the wrapped and control sides of the back by intradermal injection of 0.1 ml of a 1:1 000 solution of methacholine chloride was calculated for each man. The percentages were corrected for any slight asymmetry of sweating which had been noted in the pre-wrap sweating test. The range in percent of sweating increase induced by metacholine chloride on the control side over the wrapped side of the back 7 days after unwrapping was 28.1% to 106.9%. The mean percentage difference in the two sides was 76.3%. Simply stated, 7 days after removal of the wrapping the average volunteer delivered

Table III. *Gram-positive bacteria cultured from the lower back skin surface of volunteers with Grades 0–3 experimental miliaria rubra*

Organism	No. of volunteers from which organism was cultured	No. of volunteers within each grade		
		1	2	3
Staphylococcus epidermidis	30	12	17	1
Diphtheroids	30	12	17	1
Micrococcus tetragenus	9	5	4	0
Staphylococcus aureus	7	5	2	0
Bacillus sp.	7	3	4	0
Alpha-hemolytic *Streptococcus* sp.	1	1	0	0

Table IV. *Total aerobic bacteria count per cm², increase of bacteria during occlusion, percent difference of sweating increase of control sides over wrapped sides, and Gram-negative bacteria isolated from the lower back of 11 volunteers tested with the Bullard-type sudorometer*

Subject	Grade of clinical severity	Pre-occlusion aerobic bacterial per $cm^2 \times 10^{-2}$	Post-occlusion aerobic bacterial count per $cm^2 \times 10^{-2}$	Post-occlusion count − Pre-occlusion count	Gram-negative bacteria isolation	% difference of sweating increase of the control over the wrapped side
J. S.	1	1.40	8 200	5 800	No	40.9
R. G.	2	1.50	56 000	75 000	No	106.9
E. M.	2	0.13	19 000	150 000	No	104.0
L. M.	2	7.20	50 000	7 000	No	102.6
W. B.	2	0.60	20 000	33 000	No	106.5
E. K.	2	0.60	43 000	72 000	No	45.5
W. O.	2	8.60	17 000	2 000	Yes	84.9
J. G.	2	0.91	880	440	No	28.1
R. H.	2	5.50	11 000	2 000	Yes	84.8
J. ●.	2	14.00	110 000	8 500	Yes	45.2
W. S.	2	1.50	55 000	37 000	Yes	90.2

$$\frac{C_2 - W_2}{C_2} \times 100 - \frac{C_1 - W_1}{C_1} \times 100$$

where C_1 = % increase in sweating of control side (prewrap), W_1 = % increase in sweating of wrapped side (prewrap), C_2 = % increase in sweating of control side (48 hour occlusion), and W_2 = % increase in sweating of wrapped side (48 hour occlusion).

76.3 % more sweat from the control side of his back than from the miliaria side, i.e., the side that had been wrapped for 48 hours.

DISCUSSION

The present study provides information about bacterial flora of the skin surface when the technique of Sulzberger et al. (5) is used to produce experimental miliaria on large areas of skin. Changes in types and numbers of bacteria present on the skin during and following the miliaria induction period were determined.

Prewrap isolation of *Staphylococcus epidermidis* and diphtheroids was expected. The appearance of new types of bacteria on the skin surface following occlusion can be attributed to one or a combination of three factors: 1) the organism may not be present in sufficient numbers to be detected on normal dry skin surfaces; 2) the skin surface may be contaminated with the newly detected organism during the wrapping procedure (however, samples of the occlusive wrapping material taken from a freshly opened roll were examined in two experiments and found to be sterile, and the application of the wrap was done carefully to minimize contamination by the hands of the applier); and 3) the skin surface may be contaminated by the volunteer while the wrap is in place, the moist surface allowing the spread of contaminants under the wrap.

The bacterial population level was examined to determine if there was a threshold level necessary for initiation of pathological conditions. The possibility that a threshold level of bacteria is necessary is suggested by the fact that *S. aureus* usually produce infections in normal human subjects only when more than 10^6 viable cells are inoculated into skin (2). Our findings do not provide proof that a threshold level is needed for miliaria induction. Miliaria was produced in all volunteers, the lowest bacterial count under the wrap being 2 300 cm^2. It is noteworthy that counts on the control (unwrapped) side of the backs of the volunteers ranged from 75 to 2 000 000 per cm^2, but none of the volunteers developed miliaria on the control side.

The increase in bacterial numbers under the wrapping was examined to see whether magnitude of increase was related to severity of induced miliaria. Examination of geometric mean values indicates that the differences between the wrapped and control sides immediately after wrap removal were 63.4-fold in the Grade 1 miliaria group and 583.3-fold in the Grade 2 group. The increase in aerobic bacteria per cm^2 was 9.2 times greater

in Grade 2 volunteers than the Grade 1 group. The possibility exists that absolute numbers of bacteria on a volunteer's skin surface may not be as important as the magnitude of increase from his normal level. It is likely that individuals may vary greatly in their susceptibility to bacteria or their products; one individual's skin may support without harm a bacterial population a thousand or more times as great as that which would harm the skin of another individual.

Data obtained from the 11 volunteers studied with the sudorometer did not suggest any trends in the severity of hypohidrosis as related to the numbers of bacteria per cm^2 or the magnitude of bacterial increase under the wrapping. Gram-negative bacteria were isolated from 4 of the 11 volunteers and these 4 were intermediate in the group with respect to degree of hypohidrosis. One volunteer (J. G. see Table IV) appeared to be unique as he not only exhibited the lowest degree of hypohidrosis but also had the lowest bacterial count per cm^2 and the smallest increase in bacterial numbers of any of the volunteers studied with the sudorometer.

Although this series of experiments did not exclude the possibility that bacteria play a role in the pathogenesis of miliaria rubra, they established that, except, for *Staphylococcus epidermidis* and diphtheroid organisms, no particular Gram-positive or Gram-negative aerobic organism was common to all of the volunteers in our study. With the exception of a possible correlation between the magnitude of change in the numbers of bacterial organisms during the miliaria induction period, it is our opinion that miliaria is not related to bacteria multiplying in the sweat gland orifices with resulting occlusion.

Gram-negative bacteria are not commonly seen on the normal skin of the back. The findings in this study suggest that they are present in some subjects in presumably small numbers; occlusion increases their numbers to a measurable amount. The mechanisms controlling this require elaboration, for this may be of clinical significance in our general freedom from Gram-negative cutaneous infection.

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