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Effect of Prolonged Occlusion on the Microbial Flora, pH, Carbon Dioxide and Transepidermal Water Loss on Human Skin

RAZA ALY, PH.D., CHARLENE SHIRLEY, M.P.H., BOB CUNICO, M.S., AND HOWARD I. MAIBACH, M.D.

Department of Dermatology, University of California, San Francisco, California, U.S.A.

The effects of prolonged occlusion on the normal microbial skin flora, pH, transepidermal water loss (TEWL) and carbon dioxide emission rate (CDER) were studied. The total average counts before occlusion were 1.8 × 10^2/cm^2 and increased to 4.5 × 10^6 on day 5. The highest counts were noted on day 4 (9.8 × 10^7/cm^2). The composition changed: controls comprised of 63% coagulase negative staphylococci, 6% micrococci, 17% diphtheroids and 6% bacilli. After 5 days of occlusion, the percent composition was: 63% coagulase negative staphylococci, 11% micrococci, 4% diphtheroids, 19% lipophilic diphtheroids and <0.003% gram negative rods. The pH of the skin before occlusion was 4.38 and increased to 7.05 on day 5. After 5 days of occlusion TEWL increased from 0.56 mg/cm^2/hr to 1.87 mg/cm^2/hr and CO_2 emission increased from 25 nl/cm^2/min to 118 nl/cm^2/min.

The ability of skin to control its microbial flora may be due to factors such as bacterial antagonism [1,2], skin lipids and desiccation [3–5]. Organisms, when artificially applied, survive longer on wet skin than on dry [6]. The normal flora is denser in moist intertrigenous regions [7]. When skin is treated with acetone or other lipid solvents, the antimicrobial activity of skin for certain applied microorganisms is decreased [3].

Marples demonstrated the effect of hydration on the bacterial flora of the skin by using several occlusive devices [8]. All methods produced a great increase in the number of organisms. Other factors such as transepidermal water loss [TEWL], pH and CO_2 emission rates were not quantitatively measured. In this investigation we measured the effect of complete occlusion of the skin on various parameters such as: the aerobic microbial flora, pH, transepidermal water loss and CO_2 emission rate.

MATERIALS AND METHODS

Ten healthy male and female volunteers, working in similar laboratory environments were studied. They did not use germicidal soap for 7 days before and during the investigation.

Occlusion

The arms were wrapped with 3 layers of vinylidene polymer plastic film (Saran Wrap). This film was tightly secured at the wrist and just below the elbow with paper adhesive tape (Micropore). Wrappings were replaced every 2 days to minimize the possibility of puncturing or tearing. Each day, a succeeding portion of the wrap was removed to take measurements and microbial samples. The same site was not used twice to avoid any effect from previous tests. Control values for all the parameters studied were made before the subjects' arms were initially wrapped.

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Reprint requests to: Dr. Raza Aly, Department of Dermatology 342A, University of California, San Francisco CA 94143.

Abbreviations:
CDER: carbon dioxide emission rate
TEWL: transepidermal water loss

Sampling of Microbial Flora

Samples were taken immediately after removal of a portion of the plastic film. One ml of wash solution (0.075 M phosphate buffer with 0.1% detergent-Triton-X100) was pipetted into a sterile aluminum cylinder (6.15 cm^2 area of skin) while it was held firmly against the forearm with one hand [9]. The skin was gently rubbed with a Teflon "policeman" for 1 min. The sample fluid was aspirated with a pipette and serially diluted in a 0.037 M phosphate buffer containing 0.05% detergent. Samples (0.1 ml) of varying dilutions were cultured on selective and differential media.

Media

Appropriate dilutions were plated within 10 min after taking the samples. Five percent sheep blood agar, eosin methylene blue agar, trypticase soy agar with Tween 80, crystal violet agar (Difco), and Sabouraud dextrose agar containing antibiotics (0.05 mg/ml chloramphenicol and 0.5 mg/ml cycloheximide) were used. Blood agar plates were used for total counts, trypticase soy agar + Tween 80 was used for lipophilic diphtheroids. Lipophilic diphtheroids grow poorly on blood agar plates, but luxuriantly in the presence of Tween 80. Sabouraud dextrose agar plates were used to estimate the total number of yeast colonies. Bacterial plates were incubated at 37°C for 48 hr, and the Sabouraud dextrose agar plates at room temperature. After incubation, colonies of different morphological types were counted and selected for identification as to the bacterial type. Gram stains were done on these selected colonies, and appropriate biochemical tests were performed for identification [10,11].

pH Measurements

A pH meter (Beckman Electromate) with flat surface electrode (model #39182) was used to measure the skin's pH. Measurements were performed daily on the occluded skin (following removal of wrapping); the control measurements had been done before occlusion. The meter was calibrated before use on each person each day using standards at pH 4, 7 and 10. The electrode was dipped in pH 7.0 buffer and applied to the arm. The measurement was taken after the pH reading stabilized. Four measurements were taken on each person's arm daily. The arithmetic rather than geometric mean was recorded.

Transepidermal Water Loss

Microgram quantities of water were measured with an electrolytic water analyzer (MEECO, Warrington, Penn) [12]. A 0.64 cm^2 cup was placed on the skin, the high purity nitrogen gas (100 cc/min) passed from a molecular sieve to remove residual water into the measuring cup. The effluent was directed into the water analyzer. TEWL was recorded 20 min after the cup was in place to remove the surface water trapped by plastic film.

Carbon Dioxide Emission Rate

The carbon dioxide emission rate (CDER) was measured with an infrared analyzer (Mine Safety Applications). Nitrogen gas (50 cc/min) was passed through a 9.6 cm^2 plexiglas cup collecting CO_2 from the skin which was measured in the analyzer. CDER was reported as the reading 20 min after the measuring cup was placed over the test site (immediately after removal of the occlusive wrapping).

RESULTS

Bacteria under Occlusion: Total Counts

The total average geometric microbial counts for the controls (before occlusion) were 1.8 × 10^2 organisms/cm^2 (Fig 1). The bacterial counts after occlusion increased dramatically after 1

day of occlusion ($1.4 \times 10^6/cm^2$), and continued on an upward trend to day 4, with average counts of $9.8 \times 10^7/cm^2$. This difference was statistically significant ($p < 0.02$). The average counts after 5 days of occlusion were $7.5 \times 10^6/cm^2$.

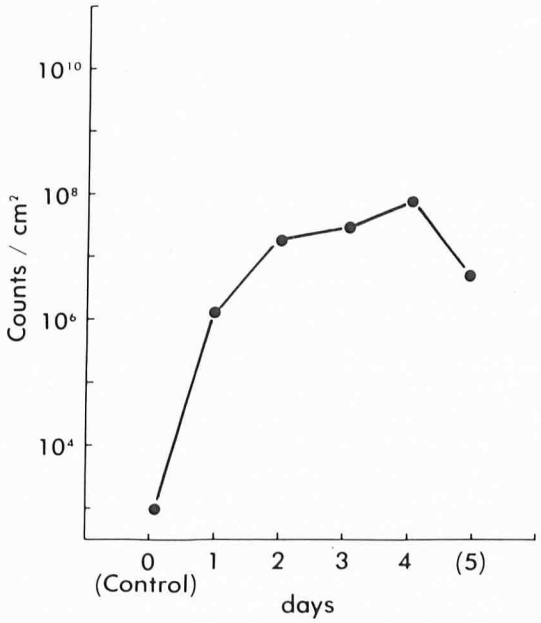

FIG 1. Total geometric microbial counts/cm² under the occlusion. The skin was occluded with plastic film for 5 days. The counts before occlusion were considered as control.

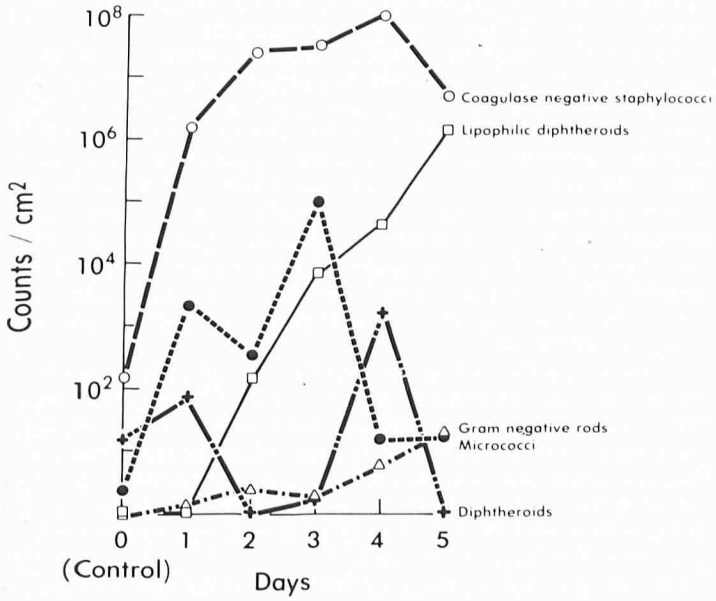

FIG 2. Geometric counts/cm² of microorganisms under the occlusion. The skin was occluded with plastic film for 5 days. The counts before occlusion were considered as control.

Quantitative Composition

The types of organisms comprising the total counts also changed (Fig 2). Coagulase negative staphylococci were present before occlusion ($1.5 \times 10^2/cm^2$), their number increased after the first day of occlusion and peaked on day 4 ($9.8 \times 10^7/cm^2$). Micrococci increased more slowly, peaking on day 3 at $9.9 \times 10^4/cm^2$. Diphtheroids fluctuated greatly day-to-day, not demonstrating a definite trend. Lipophilic diphtheroids were not detected before occlusion but were noted on day 2. The maximum counts ($1.5 \times 10^6/cm^2$) were noted on day 5 of the occlusion. Gram negative rods were not present before occlusion. After occlusion, the counts fluctuated from day to day, and were highest on day 5 ($2.7 \times 10^1/cm^2$). Yeasts were noted only on 1 person in low numbers ($10^1/cm^2$) on the last day of the study.

Percent Composition

A change in the relative proportions of organisms occurred under occlusion (Table I). Before occlusion the flora comprised 63% coagulase negative staphylococci, 6% micrococci, 17% diphtheroids and 6% gram positive bacilli. Lipophilic diphtheroids, gram negative rods and yeasts were not detected. The composition on the 4th day of occlusion was 67% coagulase negative staphylococci, 10% micrococci, 4% diphtheroids, 19% lipophilic diphtheroids and <0.003% gram negative rods. Coagulase negative staphylococci predominated throughout the study, varying somewhat in relative percentage from day-to-day. Lipophilic diphtheroids appeared on day 2 and remained the second highest flora on days 4 and 5.

Incidence

The percent incidence of countable organisms during the study is shown in Table II. The incidence of coagulase negative staphylococci was 90% (9 of 10 subjects) before occlusion, increased briefly to 100% incidence and dropped to 80% by day 5. The changes in the incidence of micrococci were greater. There was a dramatic increase from 30% (day 0) to 70% on day 1. Subsequently, the incidence dropped and remained at 20% on days 4 and 5. Diphtheroids fluctuated in percent incidence. The most striking changes in incidence were noted with lipophilic diphtheroids and gram negative rods. Neither was present before the occlusion, and by day 5, the incidence of lipophilic diphtheroids had gradually risen to 80% (8 of 10 persons). Gram negative rods appeared on one person on day 1, and increased to a 60% incidence on day 5.

pH of the Skin

The pH of the skin shifted gradually from the acidic range (4.38) before the occlusion to neutral (7.05) on day 4 (Fig 3).

Transepidermal Water Loss

TEWL (20 min after positioning the measuring cup) was 0.56 $mg/cm^2/hr$ before occlusion, and increased to 1.87 $mg/cm^2/hr$ on day 5 (Fig 3).

TEWL readings represent water diffusing through a moist membrane and water being lost as part of a dehydration process. It is impossible to separately quantify these 2 components.

TABLE I. *Percent composition of microbial flora*[a]

Organisms	Days					
	0	1	2	3	4	5
Coagulase negative staphylococci	62.8	72.7	64.1	60.4	66.52	63.2
Micrococci	5.8	8.8	17.6	23.1	10.43	10.8
Diphtheroids (nonlipophilic)	17.3	18.5	0	<0.001	4.1	0
Lipophilic diphtheroids	0	0	9.9	13.8	18.9	26.0
Bacillus	6.4	0	8.4	2.7	0	0
Gram negative rods	0	<0.001	<0.001	<0.001	<0.003	<0.01

[a] Average of 10 subjects. The percent of each organism for each individual was calculated and the average for the group was calculated by averaging the individual percentages.

TABLE II. *Percent incidence of microorganisms in 10 subjects before and after occlusion*

Days	Staphylococci[a]	Micrococci	Nonlipophilic diphtheroids	Lipophilic diphtheroids	Bacilli	Gram negative rods	Yeasts
Before occlusion	(90%)	(30%)	(50%)	(0)	(30%)	(0)	(0)
After occlusion							
1	(100%)	(70%)	(50%)	(0)	(0)	(10%)	(0)
2	(100%)	(40%)	(0)	(30%)	(40%)	(20%)	(0)
3	(90%)	(60%)	(10%)	(60%)	(10%)	(30%)	(0)
4	(90%)	(20%)	(50%)	(70%)	(0)	(30%)	(10%)
5	(80%)	(20%)	(0)	(80%)	(0)	(60%)	(10%)

[a] Coagulase negative.

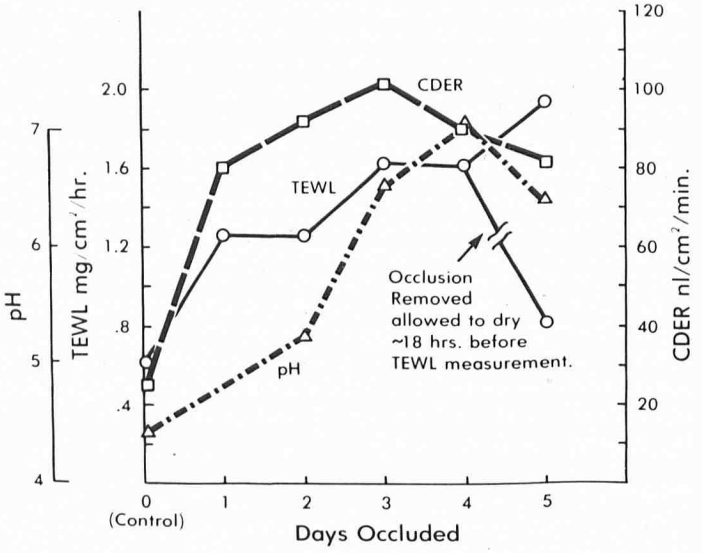

FIG 3. Transepidermal water loss (*TEWL*), carbon dioxide emission rate (*CDER*) and pH of skin under occlusion. The skin was occluded for 5 days. The unoccluded skin served as control.

Nevertheless, increased TEWL readings reflect increase in water content of skin and its surface, provided no damage has occurred.

TEWL levels fluctuated from day to day; the amount of water loss indicates that the skin was essentially saturated after 2 days. TEWL was measured 18 hr after removal of the occlusive dressing to determine if there was any structural damage to the skin. These values, although higher for previously occluded skin, were not significantly different than the controls.

Carbon Dioxide Emission Rate

The rate of carbon dioxide emission increased from 25 nl/cm^2/min before occlusion to 118 nl/cm^2/min on day 5. (Fig 3). This may reflect an increased diffusion rate (due to a moist membrane) for carbon dioxide and/or bicarbonate ion. Both effects lead to increased CDER. More important, perhaps, than the measured CDER is the concentration of carbon dioxide under occlusion. Normally carbon dioxide passes through the skin and into the atmosphere with little chance of building up local concentrations. Previous investigations have shown that after a few hours, the concentration of carbon dioxide under an occlusive dressing (such as Saran Wrap) approaches equilibrium with underlying tissues. The resulting atmosphere contains 5–7% carbon dioxide.

DISCUSSION

Several factors affect bacterial survival on skin; skin surface moisture is one of the most important. Occlusion not only hydrates the skin but alters pH, CO_2 emission rate, bacterial antagonisms and skin surface lipids.

Superhydrated skin supports an increased bacterial population [8,13]. In addition to quantitative changes in the microbial flora, qualitative changes also occur [8,13]. In previous studies the roles of hydration, pH, CO_2 emission and bacterial metabolites under occlusion were speculated on but not investigated. We measured TEWL, CO_2 emission and pH of skin daily for 5 days.

The counts under occlusion increased dramatically within 24 hr of occlusion. This increase was several thousand-fold (from 1.8×10^2 to 1.4×10^6/cm^2). The upward trend was noted for 4 days when the count reached 9.8×10^7/cm^2; on day 5 the total microbial counts declined about one log. Although not reaching statistical significance, this reduction might be related to the accumulation of toxic bacterial metabolites, and/or depletion of nutrients.

The composition of the microbial flora changed after occlusion. The major flora of unoccluded skin was comprised of 63% coagulase negative staphylococci, 17% diphtheroids, 6% micrococci and 6% gram positive bacilli. After 5 days of occlusion, coagulase negative staphylococci still remained the dominant flora (63%). Lipophilic diphtherhoid, not detected before occlusion, became the second major flora (26%). Gram negative rods formed a small portion of the total flora (<.01%).

Our results cannot be compared with the previous findings [8], as Marples did not differentiate between micrococci and staphylococci. The major flora in his investigation before the occlusion comprised of *Sarcina* (*Micrococcus luteus*), which is unusually high. He noted that coagulase negative staphylococci were reduced on the 4th day and gram negative rods increased to 10% of the total flora. In our study, coagulase negative staphylococci were not reduced and gram negative rods were less than .01% of the total flora. The increase in lipophilic diphtheroids corresponded with the previous study (although not in the same proportion) and occurred perhaps at the expense of diphtheroids.

Neither coagulase positive staphylococci nor beta hemolytic streptococci appeared under the occlusion. Although the incidence of gram negative rods increased, it was not alarming in a quantitative sense. No occluded areas demonstrated *Pseudomonas aeruginosa*. The increase in subjects (incidence) with gram negative rods was noteworthy. Before occlusion they were not detected and after 5 days of occlusion the incidence increased to 6 of 10 subjects (60%).

We interpret this data as showing that normal skin of its flora has profound capacity to minimize gram negative overgrowth even when provided with a luxuriant supply of moisture. We suspect that the gram negative rods may not have been acquired from the environment during the study but were present in nondetectable amounts before occlusion. Henning, Griffin and Maibach [14] made similar observations on skin of the back that had been occluded. We believe that normal skin's ability to limit gram negative growth is fortunate, as large numbers would presumably be destructive. Without this limitation of growth, occlusive therapy might be hazardous.

The absence of lipophilic diphtheroids on the forearms before occlusion was unexpected. Their presence on the skin has been reported previously by us and others [7,15]. Lipophilics are more abundant on the moist skin areas than on the dry skin. Our speculation for their absence was that their number might

be too small to be detected by the present methods. Despite the apparent absence of lipophilic diphtheroids on the forearms before occlusion, they became the second dominant flora on the 5th day of occlusion.

The change in pH (after occlusion) was gradual as opposed to the quick rise of TEWL and CO_2 emission within 24 hr of occlusion. The peak (pH 7.05) was noted on the 4th day and declined on the 5th day. The pH of the skin may have reached an equilibrium with the interstitial fluid, as the final pH was close to the body pH. In general, skin pH is higher in humid intertrigenous areas. Although Rebell et al [6] reported that most skin bacteria can grow under all pH conditions found on the skin, a slight change might cause an ecological advantage in growth rate for a particular organism with regard to its optimum hydrogen ion concentration. At present we do not know what aspect, if any, of the observed microbial shifts were due to the change in pH.

The changes measured in TEWL and CDER probably reflect increased permeability of the stratum corneum as it becomes hydrated. The fact that TEWL measured 18 hr after the occlusive dressing was removed was not significantly different from the initial readings (before occlusion), suggests that extended occlusion does not necessarily result in a damaged stratum corneum. Placing a covering over the skin does result in significant changes in the composition of the atmosphere directly above the skin. Carbon dioxide levels increase from less than 0.1% to over 5% and oxygen levels probably decrease somewhat. Changes in CO_2 concentrations have been shown to have effects on the type of microbial flora if 2 types are grown in competition [16].

In summary, skin occlusion with plastic film influences several skin factors. Some of these were measured in this investigation. Other factors such as nutrients, bacterial metabolites, bacterial antagonisms, skin surface lipids, and salt concentration may have been changed upon occlusion, exerting an accumulative pressure on the skin biota. These data are complex and not easily interpreted. This is the longest-term occlusion data presently available in which a broad attempt was made to profile the physiological changes involved.

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