Effects of Systemic Demethylchlortetracycline on Human Cutaneous Microflora

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The aerobic flora of the axilla and forehead of 20 normal human subjects was studied through a 3-week course of 600 mg of demethylchlortetracycline daily and a 3-week follow-up. In general in the axilla, an initial fall in bacterial density was followed by recovery in numbers due to the proliferation of resistant coagulase-negative cocci and diminution of the lipophilic diphtheroid group of bacteria within 1 week of treatment. During the remainder of the treatment period, the flora was essentially stable in both density and composition. In the follow-up phase, the initial flora was slowly re-established but resistant cocci persisted for more than 3 weeks. On the forehead the fall in density was slower and recovery during treatment was incomplete. Resistant coagulase-negative cocci became dominant and persisted through the follow-up phase. In two individuals, temporary colonization of the axilla by *Staphylococcus aureus* was seen.

The customary way of comparing the absorption of different antibiotics and of estimating therapeutic activity is to use serum levels as a main parameter. The assumptions made include a belief that the serum level indicates the amount of antibiotic in various tissue compartments and that prolonged elevated serum levels indicate prolonged activity in the tissues. It has been long recognized that the serum level does not always give a good estimate of the amounts of antibiotic crossing the blood brain barrier and, similarly, activity in the urinary tract may differ markedly without change in the serum level. When the distribution of an antibiotic in the skin is studied, the amounts of antibiotic in the compartments of this complex tissue cannot be determined from the level in the serum. It is probable that excretion of different antibiotics by the skin varies from antibiotic to antibiotic and from skin area to skin area. An antibiotic may be carried to the surface by incorporation into keratinizing cells or by excretion in sweat or sebum. Alternatively, the antibiotic may not enter the secretory structures or be inactivated, metabolized, or resorbed in the process of delivery of cellular, watery, or lipid secretions to the skin surface.

The human aerobic cutaneous microflora exists only in the very superficial layers of the stratum corneum. Because of this location the microflora is a test system for the arrival of an antibiotic on the skin surface. Changes in the ecological balance of the microflora, in density, and variety of species present are of importance not only in the study of cutaneous ecology but also in their effects on the ability of the skin to resist invasion by more pathogenic species.

Ehrenkranz et al. (2) reported that the intensive use of an antibacterial soap on one foot suppressed the gram-positive species permitting colonization with enterobacteria. *Pseudomonas* was then able to colonize the skin, producing lesions which later became infected with *Candida albicans*. Shehadeh and Kligman (5) reported that almost complete suppression of the gram-positive flora of the axilla was necessary before gram-negative rods were able to dominate the flora. A more specific relationship between viridans streptococci and enterobacteria in the throat which can be upset by antibiotics was reported by Sprunt and Redman (6).

Little attention has been paid to the effects of systemic antibiotics on the cutaneous microflora. Goltz and Kjartansson (3) studied the effect of systemic tetracycline in acne patients. They found a lowering of the density of both aerobic and anaerobic species on the forehead. A reduction in free fatty acids in sebum after tetracyclines but not penicillin was reported by Strauss and Pochi (7), presumably through reduction in bacterial density.

The purpose of this study was to determine the effect of demethylchlortetracycline on the aerobic

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flora of the forehead, an oily dry region, and the axilla, a moist, nonoily region. The parameters studied were firstly the total density of aerobes, the percentage composition of the microflora, and the proportion of resistant organisms. These parameters were followed in healthy adult males during a 3-week course of treatment and a 3-week follow-up.

MATERIALS AND METHODS

Subjects. Twenty volunteers, inmates of the Philadelphia House of Correction, were studied in four groups over a period of 14 months. Antibacterial soaps were avoided.

Treatment. Demethylchlortetracycline (Declomycin, Lederle Laboratories, Pearl River, N. Y., 600 mg) was given daily in four divided doses for 21 days.

Sampling times and sites. At least one and usually two pretreatment samples were obtained from each site. All subjects were sampled at least once in the first 4 days of treatment, at the end of the first week of treatment, on day 14 and day 21, and at various times in the follow-up period. The sites sampled were both sides of the forehead and the apex of each axilla.

Sampling methods. The sampling method of Williamson and Kligman (8) was followed. This method is a localized scrub technique with the use of a nonionic detergent, 0.1% Triton X-100 (Rohm and Haas), in 0.075 M phosphate buffer (pH 7.9) as the wash fluid.

Bacteriological methods. A portion of each sample was diluted in 10-fold steps in half strength wash fluid; 0.25 ml was plated in molten Trypticase Soy Agar (Difco, TSA), in TSA with the addition of 0.5% Tween-80 (Atlas Chemical, Wilmington, Del.) as an oleate source, and in TSA containing 10 μg/ml of tetracycline. Anaerobic plates were also made in Brain Heart Infusion agar supplemented to 1% glucose. Surface inoculation of these media and MacConkey Agar was also performed. The plates were incubated for 48 hr at 37 C before counting and for a minimum of 2 days at room temperature before identification of the bacterial groups. Anaerobic plates were incubated for 7 days at 37 C in an atmosphere of 90% N_2 and 10% CO_2. Colonies were counted with a "Quebec" colony counter, and an estimate of the total number and the number of each bacterial group in terms of bacteria per cm^2 of skin was calculated, assuming fluid losses in the technique to be unimportant.

Colonial, morphological, and biochemical characteristics were studied to separate the bacteria into groups.

The cocci present consisted of: *Staphylococcus aureus*, (coagulase-positive, catalase-positive cocci); coagulase-negative cocci, a broad group including strains falling into both *Staphylococcus* and *Micrococcus* [in our experience the dominant type is *S. epidermidis* Baird Parker type S II (1)]; and *Micrococcus luteus*. [Because these organisms can be readily recognized on microscopic and cultural morphology, this part of Baird Parker Micrococcus type 7 (1) could be separately enumerated.]

The diphtheroids present consisted of: lipophilic diphtheroids, diphtheroids growing poorly on TSA but luxuriantly with added Tween 80 (4); miscellaneous diphtheroids, several pigmented and nonpigmented species growing well on TSA within 48 to 60 hr aerobically, including *Corynebacterium xerosis* and *C. minutissimum*, and other strains. Aerobic sporeformers, several species of *Bacillus*; yeast-like forms, genus *Candida*; and gram-negative rods, mainly *Enterobacteriaceae* but also including members of the *Mima-Herellea-Moraxella* complex, were also present.

Calculations. Because individuals exist in the population who consistently carry much higher bacterial densities than is usually found, the distribution of values is not the normal bell-shaped curve but is markedly skewed. To correct this to a normal distribution, the densities must be converted to logarithms before statistical analysis is possible. The arithmetic mean of the logarithms of the counts when retransformed is the geometric mean of the original values. This value is the one used in the graphs of total numbers.

In characterizing the floral composition, however, the percentages of each bacterial group in each sample were calculated and combined arithmetically to give equal weight to each sample irrespective of total density. It was clear from the crude data that each sample should be included separately since bilaterally symmetrical sites often differed in composition of the flora or in the time course of changes.

Resistance. Antibiotic resistance was referred to a level of 10 μg of tetracycline per ml in incorporation plates. This is the accepted level inhibiting all clinically sensitive strains. Incorporation media were used to expose the maximum number of cells to the antibiotic to estimate accurately the proportion of "resistant" to "susceptible" cells.

RESULTS

Normal flora. The samples taken before treatment were studied to determine the normal flora of each site. There were 64 samples from the axilla and 68 from the forehead available for this analysis.

Normal flora of the axilla. The commonest organisms in the axilla were coagulase-negative cocci and lipophilic diphtheroids. These two groups made up more than 85% of the total flora. Miscellaneous diphtheroids and gram-negative rods were also commonly present but in lower densities. Eleven individuals carried gram-negative rods. Carriage was bilateral in seven and unilateral in four. The species isolated were *Enterobacter aerogenes*, 6 individuals; *Enterobacter cloacae*, 3; *Proteus mirabilis*, 2; *Herellea vaginicola*, 2; *Escherichia coli*, 1. One subject carried three species and two others carried two species. *S. aureus*, yeasts, and aerobic spore formers were rare.

We soon learned that there were two types of individual axillary flora dominated either by diphtheroids or cocci. Arbitrarily we defined one group as those individuals in whom the cocci

TABLE 1. *Percentage incidence of bacterial groups in normal flora*

Bacteria	Axilla			Forehead		
	Group I	Group II	Total	Group I	Group II	Total
S. aureus	2.3	0	1.6	2.1	0	1.5
Coagulase negative cocci	100.0	100.0	100	100.0	100.0	100
M. luteus	2.3	15.0	6.3	14.6	35	20.6
Lipophilic diphtheroids	100.0	90.0	96.9	79.2	90	82.4
Miscellaneous diphtheroids	61.4	25.0	50.0	8.3	20	11.8
A.S.F.[a]	0	0	0	16.7	0	11.8
Yeasts	4.5	0	3.1	8.3	10	8.8
Gram-negative rods	40.9	20	32.8	6.3	5	5.9
Samples	44	20	64	48	20	68

[a] Aerobic spore formers.

made up less than 50% of the total aerobic count (group I, 14 individuals) and the other in whom cocci made up greater than 50% of the aerobic count (group II, 6 individuals).

The bacterial density in group I was higher. The geometric mean (GM) density was 3.2×10^6 bacteria per cm^2 (log GM = 6.5054, standard deviation = 0.45).

The flora was more diverse (Table 1). Miscellaneous diphtheroids and gram-negative rods were more often recovered, and the minor isolations of yeasts and *S. aureus* came from this group. It should be noted, however, that coagulase-negative cocci were isolated from every sample with an average representation of 7% of the total. Lipophilic diphtheroids were isolated from every sample and dominated the flora in all but four samples in which dominance was shared between the two diphtheroid groups. The average representation was 80% of the total.

In group II, the geometric mean bacterial density was significantly lower ($P < 0.01$) at 4.2×10^5 bacteria per cm^2 (log GM = 5.6236, standard deviation = 0.5).

The flora showed less diversity than group I, though *Micrococcus luteus* was more often isolated from these subjects (Table 1). Lipophilic diphtheroids were recovered from 18 of the 20 samples with an average representation of 17%. Coagulase-negative cocci averaged 80% of the total.

The normal aerobic flora of the forehead was predominantly composed of coagulase-negative cocci. This group had an average representation of 93% of the total flora. Lipophilic diphtheroids were isolated in low numbers from all subjects but not in all samples. *Micrococcus luteus* was third in incidence. The density was usually low, but in one individual more than 1,000 per cm^2 were recovered in all four samples.

The geometric mean density of aerobes was 15,700 organisms per cm^2 (log GM = 4.1950, standard deviation = 0.957). The large standard deviation shows that the range of forehead counts from individual to individual is much greater
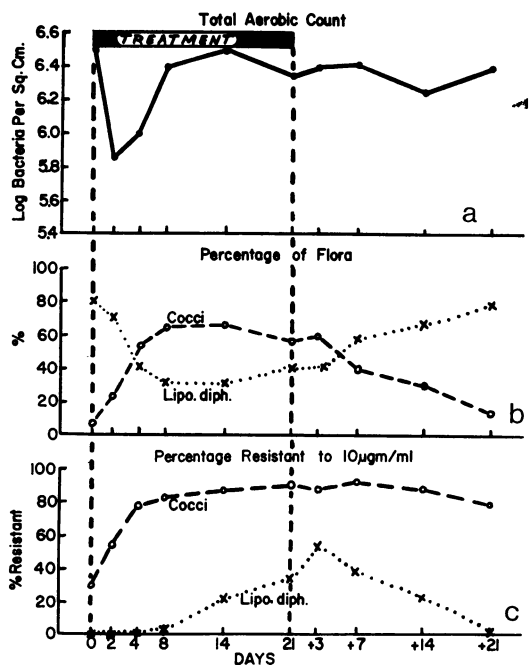

FIG. 1. *Effects of 3 weeks of treatment with demethylchlortetracycline on the axillary flora in diphtheroid-dominated subjects (Group I). (a) Geometric mean bacterial density. Note recovery of density during treatment. (b) Average percentage of the total for coagulase-negative cocci and lipophilic diphtheroids. Note the induction of a coccal flora by treatment and return to pretreatment diphtheroid dominance in the follow-up. (c) Percentage of coccal cells and lipophilic diphtheroid cells resistant to 10 μg of tetracycline per ml.*
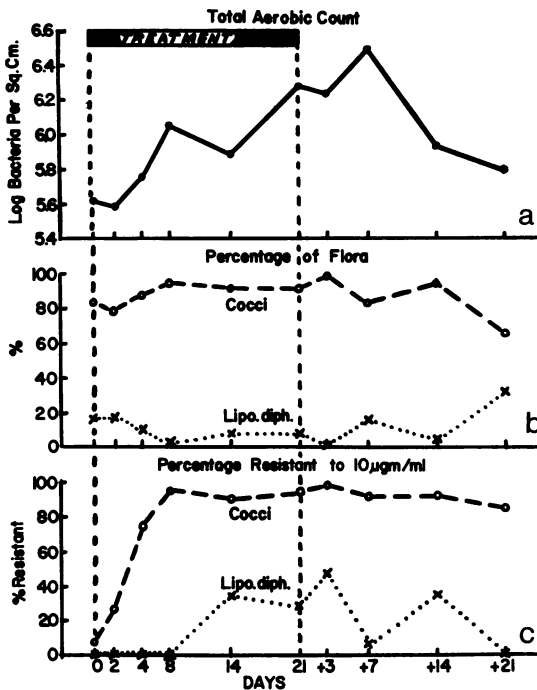
FIG. 2. *Effects of 3 weeks of treatment with demethylchlortetracycline on the axillary flora in group II. (a) Geometric mean density. (b) Composition of the flora. (c) Percentage of cells resistant to 10 μg of tetracycline per ml.*

than for the axilla, although there is good correlation (r = 0.755) between the left and right sides of the same individual.

It was of interest to examine the data in terms of the two groups as defined by the axillary flora. Group I carried a lower bacterial density than group II. The geometric mean density was only 7,880 (log GM = 3.8964, standard deviation = 0.86) in group I, whereas, in group II, the geometric mean density was higher at 81,600 (log GM = 4.9115, standard deviation = 0.80). This difference is statistically significant (*P* < 0.01). No differences in the flora were detected.

Effect of treatment on bacterial density. The geometric mean density was calculated for each site for each group at each sample time. Because the changes and the time of change differed in the symmetrical sites, particularly in the axilla, each site is considered separately.

For axilla group I, the geometric mean density fell within the first 2 days of treatment and then recovered to pretreatment levels within a week (Fig. 1a). Thereafter, the total count remained relatively constant. There was some variation from axilla to axilla in the rapidity of recovery but, in all but three axillae, recovery of numbers

was complete by the 14th day of treatment. In the follow-up period the counts changed very little.

The changes in axilla group II were profoundly different from those in group I (Fig. 2a). Inexplicably the geometric mean bacterial density increased from day 2 throughout the treatment period and reached a peak 7 days after the end of treatment before returning towards the pretreatment level. This increase in density above pretreatment density was seen in five of the six subjects, but in one subject the density remained constant.

In the forehead, both groups showed the same response to treatment (Fig. 3a). Little change in density was seen in the first few days of treatment but a clear reduction in density was apparent by day 8. The density increased slightly to about 50% of the pre-treatment level during the remainder of the treatment period. The rate of increase accelerated in the first two weeks of the follow-up period but appeared to fall again in the final week. This fall may be explained by the fact that only 8 of the 14 subjects in group I and 4 of the 6 subjects in group II were sampled on this day, and these happened by chance to carry a low bacterial density.
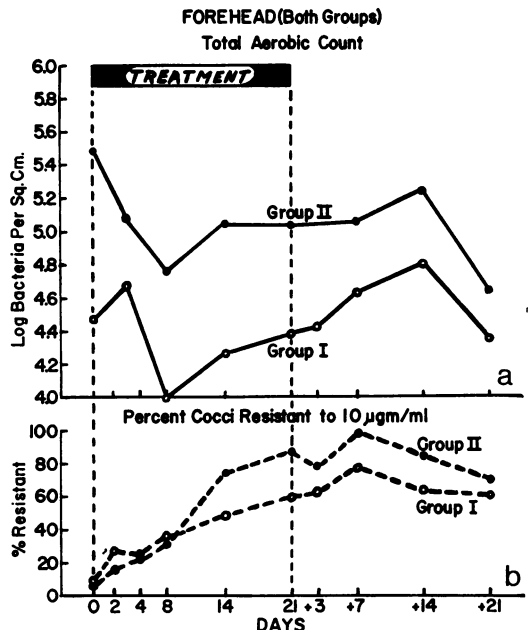

FIG. 3. *Effects of 3 weeks of treatment with demethylchlortetracycline on the flora of the forehead of both Group I and Group II. (a) Geometric mean bacterial density. Note partial recovery in density during treatment. (b) Percentage of coccal cells resistant to 10 μg of tetracycline per ml.*

Effect of treatment on population composition. The major effect of treatment in the diphtheroid-dominated axilla group I was a reversal in the proportions of coagulase-negative cocci and lipophilic diphtheroids within the first 8 days of treatment (Fig. 1b). The coccal-dominated flora reverted to diphtheroid dominance during the follow-up phase after a lag of 3 days. Miscellaneous diphtheroids comprised 12.2% of the pretreatment flora. These fell to 4.2% by day 4 and remained low thereafter. The other bacterial groups also showed a decline in percentage representation and a slight fall in carriage of gram-negative rods from eight carriers to six carriers was detected. In the follow-up period gram-negative rod densities increased.

Treatment had little effect on the population composition in axilla group II, in which coagulase-negative cocci were dominant. The lipophilic diphtheroids decreased in representation and the cocci reached a higher level of dominance during treatment, with reversal in the follow-up phase, but the changes were minor in comparison to group I (Fig. 2b). Gram-negative rod carriage fell from three to one subject. In this subject, however, *E. aerogenes* was isolated from all but one treatment sample.

Treatment had little effect on the composition of the flora of the forehead. Coagulase-negative cocci dominated every sample. The isolation of *M. luteus* and aerobic spore formers continued sporadically throughout the treatment and follow-up phases, even though these groups never displayed resistance to 10 μg of tetracycline per ml.

Effect of treatment on resistance to tetracycline. The major effect of treatment in the axilla was to initiate an increase in the proportion of coagulase-negative coccal cells resistant to 10 μg of tetracycline per ml (Fig. 1c and 2c). In both group I and group II, this process was detectable within 2 days, and the coccal strains were practically completely resistant after 1 week of treatment. It is not necessary to postulate mutation or induced enzymatic activity to explain the rapidity of this change, since 25% of the coccal cells in group I and 8% in group II were resistant to the test level before treatment was instituted, and resistant cells were recovered from all but one subject.

The proportion of resistant cells to the total coccal cells remained essentially constant throughout the remainder of the treatment period, whereas the coccal density was either steady (group I) or increasing (group II), and also throughout the follow-up period when the coccal density was decreasing in both groups.

In contrast, among the lipophilic diphtheroids,

resistant cells were rare before treatment. Only 0.2% of the cells tested before treatment could grow on media containing 10 μg of tetracycline per ml, and these were isolated from only 7 of the 57 samples so tested. With treatment, there was no increase in the proportion of resistant cells in the first week, during which time the density of lipophilic diphtheroids was falling in both groups. In the second and third weeks of treatment, the proportion of cells resistant to 10 μg of tetracycline per ml rose to around 30% of the total. In the follow-up period, there was an increase in the day +3 sample, but thereafter the proportion of resistant cells declined to a negligible degree by day+21, although the lipophilic diphtheroid density was increasing.

Forehead. The effect of treatment on the forehead was again to cause an increase in the proportion of resistant coagulase-negative coccal cells but much more slowly than in the axilla. The rate of increase appears to intensify at the end of 1 week of treatment and shows a further increase in the early follow-up period. Some diminution in the level of resistance of the coccal population occurred in the later follow-up samples (Fig. 3b).

Because of the low numerical representation of lipophilic diphtheroids, no average proportionate change can be shown. The incidence, however, rose through the treatment period from 12 of 50 samples to 7 of 19 samples at the end of the treatment period.

Effect of treatment on colonization by S. aureus. In one subject, *S. aureus* resistant to 10 μg tetracycline was found in one pretreatment sample from the right axilla and not recovered from the forehead or anterior nares. By day 2, the strain was present in both axillae, reaching a peak in the left axilla at 2.37 × 10⁶ cells per cm² on day 7. At this time, the subject complained of irritation, and a mild papular rash on an erythematous base was noted. Treatment was continued and the reaction resolved spontaneously as coagulase-negative resistant cocci multiplied. The level of *S. aureus* cells fell from 52% of the cocci on day 7 to 2.7% by day 14. *S. aureus* was thereafter recovered only from the day +3 and +7 samples in low numbers. In the right axilla, *S. aureus* was recovered from every treatment and follow-up sample, and, though the count rose to 300,000 cells per cm² by day 21, no clinical effect was seen.

In another subject carrying resistant *S. aureus* in the nose, temporary colonization of both axillae occurred from day 4 but produced a level of only 150,000 by day +3 and no signs or symptoms.

Anaerobes. Initially we planned to include anaerobes in this study. The earlier phases of the

study, however, were technically inadequate, and the densities of *C. acnes*, the only anaerobe of numerical importance, are therefore somewhat dubious. It was clear that the anaerobic flora of the forehead exceeded the aerobic flora in practically every sample (48 of 49 acceptable samples). In the axilla, *C. acnes* was not numerically dominant but was recovered from 70% of the pretreatment samples. The effect of treatment on the density of anaerobic diphtheroids in the forehead was in general to cause a decrease which was not detectable before the second week of treatment and which persisted at least a week into the follow-up period. No decision can be made on the effect of treatment on *C. acnes* in the axilla.

DISCUSSION

It is clear from the results of this study that the systemic administration of 600 mg daily of demethylchlortetracycline to normal subjects is associated with profound changes in the density and composition of the cutaneous microflora of both the axilla and forehead.

Because of differences in the initial flora, not all subjects reacted in the same way. The group of subjects in whom the initial flora of the axilla was dominated by lipophilic diphtheroids showed the most dramatic changes. In this group the first detectable effect was a fall in total density of bacteria within the first 2 days of treatment, with survival of pre-existing resistant coagulase-negative coccal cells. This must be due to direct antibacterial action of the drug. Multiplication of these resistant cells resulted first in a change of dominance from diphtheroid to coccal dominance and then to a restoration of density to close to the original level. These changes were essentially complete by the end of 1 week of treatment. Thereafter the flora remained stable in density and composition for the rest of the treatment period. Resistant lipophilic diphtheroid cells became somewhat more common in subsequent samples but this group did not greatly increase in density while treatment continued. In another experiment the treatment period was lengthened to 6 weeks without the occurrence of any further change in floral composition or density.

In the follow-up period, there was an early increase in resistant lipophilic diphtheroids without change in the percentage composition of the flora, but, after 3 days, nonresistant lipophilic diphtheroids replaced resistant diphtheroids and cocci without detectable change in overall density. Particular attention is called to the inverse relationship shown by the densities of lipophilic diphtheroids and coagulase-negative cocci. When the diphtheroid density fell as a result of treatment, the cocci were able to multiply, and, as the

lipophilic diphtheroids returned, the coccal density fell without loss of resistance. Our interpretation is that lipophilic diphtheroids are able to suppress the growth of cocci and are the group controlling the composition of the flora of the axilla.

In the remaining subjects, the bacterial density appeared to rise through the treatment period. This finding needs to be repeated before it can be taken at face value. What appears to be a rise may be due to falsely low initial values perhaps brought about by undetected use of medicated soaps or deodorants. The difference in floral composition may be ascribed to similar causes. The behavior of the flora in terms of composition and resistance is otherwise similar to the main group.

On the forehead, both groups showed the same changes. The total density of bacteria fell to a minimum by the end of 1 week of treatment, whereas resistant coagulase-negative coccal cells increased slightly in proportionate representation. In the second week of treatment, multiplication of resistant cocci caused some restitution of density but this was never complete. These results are very similar to the findings of Goltz and Kjartansson in acne patients receiving tetracycline (3).

There are several possible ways in which the antibiotic could reach the skin surface to cause these changes in the cutaneous microflora. Because of the rapidity with which changes were detectable in the axilla, it is likely that the antibiotic was delivered via the sweat; the slower more prolonged changes on the forehead suggest that incorporation into keratinocytes may be more important at this site. Preliminary evidence from microbiological assay shows that appreciable amounts of demethylchlortetracycline can be detected in normal epidermis and stratum corneum scrapings as well as in the sweat of subjects treated for three weeks with this drug.

Individual differences in the bacteria present in the normal flora were very evident in this study. Whereas coagulase negative cocci and lipophilic diphtheroids were almost universal at both sites at all times, individuals differed markedly in the occurrence of miscellaneous diphtheroids and gram-negative rods but tended to maintain the flora found initially. Of the 11 subjects carrying gram-negative rods in the initial samples, 7 still showed carriage at the end of the treatment period, and, in 4 subjects, all but one sample of the whole series taken yielded the colonizing organism. None of the samples from initial noncarriers of gram-negative rods were positive at any time throughout the treatment and follow-up phases. Similarly, treatment reduced the den-

sity of miscellaneous diphtheroids but had little effect upon incidence of this group.

In one subject, *Micrococcus luteus* clearly colonized the forehead; treatment eradicated this colonization.

Colonization of the axilla by resistant *S. aureus* occurred on two occasions. In one, the source was prior minor colonization of one axilla, and, in the other, the organism was present in the nose. In both cases, colonization occurred while the total density was low, and, although the strains persisted, the numbers of *S. aureus* were reduced as coagulase-negative resistant cocci increased.

The clinical implications of this study include the realization that: (i) the excretion of some antibiotics occurs through the skin by differing routes, (ii) systemic administration of some antibiotics may lead to changes in the normal flora with possible colonization and infection by pathogens at sites not directly under clinical appraisal, (iii) the presence of a cutaneous microflora is part of the protective mechanisms of the individual against infection and (iv) an originally susceptible bacterial population can become largely resistant to the antibiotic given.

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