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EXPERIMENTAL *STAPHYLOCOCCUS AUREUS* INFECTIONS IN HUMANS*

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ABSTRACT

S. aureus infections have been consistently induced in normal human skin by applying large inocula to areas degermed with ethanol and kept moist under an occlusive dressing. The ED_{50} was approximately $10^3/cm^2$. With about 10^6 organisms/cm^2, an erythematous rash appeared in three days which evolved to an intense papulo-vesicular eruption by six days. Removal of the dressing was followed by swift death of almost all the organisms followed by immediate resolution of the lesion within a few days.

There was a great loss of viable cells in the first hour after application which continued for 24 hours; thereafter, the organisms proliferated to a peak density of about $2 \times 10^7/cm^2$. The resident cocci and diphtheroids began to re-enter the site by about the 3rd day and were completely restored by 6 days.

The organisms were confined to the surface and did not proliferate within the living portion of the skin. Histopathologically the lesion was a toxic dermatitis with epidermal necrosis, edema, hemorrhage and thrombosis. This clinical entity may be described as toxic pyoderma.

Clinicians never question the capacity of *Staphylococcus aureus* to produce human skin infections; they believe it is overwhelmingly the cause of primary and secondary pyodermas. It is otherwise with the experimentalists. The record of attempts to induce infections with cultures or lesional material is one of astonishing disappointment.

Creating experimental infections is not of course primarily to confirm the virulence of *S. aureus*; to induce an infection at will provides an opportunity to study pathogenesis. We know little about the factors which influence susceptibility to *S. aureus* infection and really nothing of the events which take place during its inauguration. An experimental model would be advantageous in appraising prophylactic and therapeutic measures as well as relative virulence.

S. aureus is probably universal in or about the human body (1). Its recoverability is proportional to the efforts made to culture it. Thus, while a single swabbing of one area may capture the organism in 10–20% of the cases, sampling of the same site weekly for eight weeks raises the take to 82% (2). Though *S. aureus* is not ordinarily a resident of human skin, there is no doubt that it can sometimes become established in certain areas of normal skin, particularly on the perineum, and also in the skin of the newborn. The few isolates that are usually recovered from different areas of normal adult skin are not true colonists but originate from a nearby pool, most often the anterior nares (1). Despite this ubiquitousness primary infection is not very common under ordinary circumstances. Usually it is not easy to explain why infection occurs in a particular case. Most statements to patients are more glib than truthful.

Oddly enough the first persons to attempt human infection had more success than they could fully enjoy. These were two auto-inoculators of the last Century, Garre and Bockhart (3, 4). They applied massive amounts of organisms to their own abraded skin. Pustules appeared which culminated in skin furuncles in the one and a carbuncle in the other. This experience evidently cooled off the curious for about half a century until a debate arose whether staphylococci or streptococci were the chief cause of impetigo (5, 6). Many tried to induce infections but takes were uncommon and unpredictable. The luck of the beginners had vanished!

The frustrating experiences of recent investigators vividly illustrate the surprising diffi-

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culties in creating infections with this highly virulent organism. Maibach made hundreds of human inoculations using broth cultures containing 100,000,000 organisms per ml (7). Usually nothing of note happened when cultures were applied to skin which had been sweated, blistered, irradiated with ultra-violet light, stripped, or depilated. The bacteria proliferated under cover slips but the skin remained indifferent.

Earlier, Elek, who knew the virulence of *S. aureus* better than most became so emboldened as to inject the organisms intradermally (8). With several different strains he found it impossible to produce pustules with less than a million organisms except when a contaminated suture was inserted into the skin.

Foster and Hutt applied over-night broth cultures of *S. aureus* and *S. albus* to small areas denuded of epidermis and sealed the sites under glass cover slips (9). Both organisms multiplied vigorously. Within 24 hours, *S. aureus* induced a suppurative exudate containing many degenerating polymorphonuclear leucocytes. Two of 3 subjects subsequently developed boils elsewhere. Neither organism multipled when the site was left uncovered.

Recently, Duncan *et al.* (10), following O'Brien (11), applied plastic cups containing a nutrient agar whose surface was streaked with a loopful of a 48-hour broth culture of *S. aureus*. The cups were fastened to the skin for several days. In this manner which is halfway between *in vitro* and *in vivo* circumstances they procured 10 pustules in 78 attempts. Prior traumatization did not improve the outcome. Stripping sometime enabled infection to take place but in one experiment the failure rate was 58 of 60 times. Their greatest success was by stabbing the skin through a droplet of a 48-hour broth culture; the site was immediately covered with an impermeable plastic tape (Blenderm 3M). A suppurative reaction occurred about 15% of the time on the arms and back whereas on the legs the rate rose to 37%. The significance of this result is somewhat marred by their having used a mixture of *S. aureus* and β-hemolytic streptococci. More recently, these same workers varied the technique by applying the organisms to thigh or leg skin from which the hair had been plucked immediately beforehand. Again an occlusive covering was required. The organisms proliferated in the follicular canals and incited the formation of pustules which transformed into ecthyma-like lesions. Takes were more frequent with a mixture than with either organism alone (personal communication).

We propose now to describe a method which regularly induces cutaneous lesions without requiring prior traumatization of the skin.

MATERIALS AND METHODS

Subjects. The subjects comprised about 150 male prisoner volunteers, age 25 to 40; most were black. A few were infected more than once.

Bacterial strains. Nearly all of the studies utilized a non-typable *S. aureus* isolated from infected skin. This was selected because of its sensitivity to all the common antibiotics. In preliminary studies with 5 different phage types including types 71 and 80/81 we did not observe strong differences in virulence. With about a million organisms, all were capable of producing similar lesions.

Inoculation procedure. A just hazy suspension in saline from a 24 hour surface colony on trypticase soy agar (TSA) was found to contain about 10^9 organisms per ml. This was appropriately diluted to give inocula of desired size using the surface drop technique (v.i.) to establish actual numbers.

The subjects washed with Ivory soap for one week prior to inoculation. This was done because bacteriostatic soaps perhaps may prevent staphylococcal pyoderma (12, 13). The volar forearm was used throughout, mainly for convenience. By encircling the arm with tape, occlusive dressings can be securely maintained for at least a week. We ascertained that the trunk and lower extremities were about equally susceptible. The site was pretreated for 2 minutes with a pad of gauze saturated with 70% alcohol. This brought about virtual sterilization of the surface. Usually not more than a few colonies of aerobic organisms survived this treatment. After drying, an inoculum droplet approximately 0.02 ml was applied with a capillary pipette. Immediately this was covered with a 3 cm. square of polyethylene film (Saran Wrap) which uniformly distributed the organisms within the square. The preparation was securely fastened to the skin by overlapping strips of cloth-backed adhesive tape (Johnson and Johnson Zonas). We had previously demonstrated that this tape will not permit bacterial multiplication on skin (14). Usually the dressing remained in place for 6 days.

Bacteriologic methods. The detergent-scrub technique was utilized to quantify the number of organisms present at any time after inoculation (15). Plating out of the organisms on appropriate media was accomplished by our standard techniques including tenfold dilution in half strength wash fluid (15) and drop inoculation of appropriate media from 0.2 ml pipettes (16). Because of the detergent such drops are repeatable in size (0.025

ml) yet spread adequately on the surface of the plate.

Histology. Five mm punch biopsies were taken from 41 infections at different times. After formalin fixation, 5 μ sections were prepared and stained with haematoxylin and eosin and by the Brown-Brenn modification of Grams' stain for bacteria.

Precautions. Though we applied the organisms to unbroken skin, the rights and safety of the volunteers had to be safeguarded. The risks involved were explained to each subject. We used only fit persons with a satisfactory history of personal health in whom the following laboratory tests were normal: urinalysis, SGOT, CBC and FBS. The subjects were seen daily. At the end of the study the site was treated twice daily for two days with neomycin-polymyxin-bacitracin cream (Neosporin Cream®, (Burroughs-Wellcome). After biopsy each subject received either one gram of erythromycin or tetracycline daily for 3 days. These antibiotics were also immediately given to any subject who developed a fever exceeding 101°F whatever the reason. Before these precautions were adopted we encountered three subjects in whom hot, tender, furunculoid nodules appeared around the infection site a few days after removing the dressings. Two of these had lymphangitis, axillary adenopathy, and modest temperature elevations. These signs abated rapidly under penicillin therapy. On the whole, the discomfort caused by the lesions was cheerfully borne by the volunteers. Usually we did not produce more than two infections per forearm.

Clinical grading. The clinical severity of the infection was graded using a 5 point scale from 0− no lesions through erythema, papules and pustules to 4+ ulceration.

Influence of the resident flora. Ricketts *et al.* demonstrated that *S. aureus* would die fairly rapidly on skin unless covered with a moisture proof dressing (17). When protected from dehydration, some of the organisms survived for days but no infection occurred.

The mean density of the aerobic microflora of the forearm of adult males in our population is about 10^3 organisms/cm². These are mainly staphylocci and diphtheroids. The skin hydrates under polyethylene film and within 24 hours an explosive multiplication occurs to levels ranging between 1 to 10 million/cm² (18). We wished first to learn how *S. aureus* would fare against a rapidly expanding population.

Procedure. In the first study, both forearms of 5 subjects were occluded for 24 hours under polyethylene film after the technique of Marples (18). One side was then pretreated for 2 minutes with ethanol-saturated gauze. Immediately, the density of resident organisms on both sides was determined by the detergent-scrub sampling technique. Both sides were inoculated with 3.7×10^4 organisms/cm² and occlusively covered for 4 days. The quantity of *S. aureus* was then determined by the scrubbing method and the presence of lesions noted. With this inoculum, most subjects will develop a mild erythemato-papular reaction by 4 days.

In the second study, one side was degermed with alcohol as above but there was no 24 hour period of occlusion prior to inoculation. Three sites on each side received respectively 3×10^2, 3×10^3 and 3×10^4 organisms/cm² followed by occlusion for 6 days at which time the severity of the infection was appraised clinically.

RESULTS

Pretreatment

Table I shows that pretreating a bacteria-rich, hydrated site with alcohol enables an inoculum of 3.7×10^4 *S. aureus* organisms to reach a mean density of nearly 3×10^6/cm² in a 4 day period whereas only a mean of 3.45×10^4 organisms/cm² could be recovered from the control side. It should be noted that 2 minutes of ethanol treatment did not completely degerm the hydrated site but left a mean of 4.5×10^3 cells/cm². Though the descendants of these organisms became overwhelmingly dominant, *S. aureus* multiplied

TABLE I

Effect of competition by normal skin organisms (Bacteria per sq cm) inoculated with 37,000/cm² S. aureus

Subject	Alcohol degermed			Not degermed		
	Pre-inoculation skin organisms	Post inoculation (4 days)		Pre-inoculation skin organisms	Post inoculation (4 days)	
		All organisms	*S. aureus*		All organisms	*S. aureus*
A	10	5,368,000	5,000,000	6,320	3,368,000	10,500
B	12,000	41,470,000	789,000	1,180,000	12,930,000	31,600
C	21	14,740,000	6,053,000	1,050	9,779,000	122,000
D	10	36,630,000	368,000	2,737,000	11,440,000	4,200
E	10,800	26,100,000	2,105,000	1,347,000	20,210,000	4,200
Mean	4,568	24,860,000	1,054,000	1,054,000	11,545,000	34,500

TABLE II

Lesion severity, 4 days

Subject	Alcohol treated	Not degermed
1	++	0
2	+++	+
3	+	0
4	0	0
5	+	0

TABLE III

Effect of prior degerming with ethanol

Subject	Alcohol treated			Not degermed		
	Organisms/cm²			Organisms/cm²		
	30	300	3000	30	300	3000
	Lesion severity			Lesion severity		
1	−	−	++	−	−	−
2	+	++	++	−	+	+
3	−	++	++++	−	−	++
4	−	+	+++	−	−	+
5	−	−	++	−	−	−

to reach a level of millions/cm² and lesions eventuated in 4 of 5 instances (Table II). On the side not degermed *S. aureus* failed to propagate in competition with an initial resident population of 10^6 residents/cm². The colonies recovered may safely be regarded as mere survivors of the original inoculum. A mild skin reaction of unknown cause occurred in one case.

The second study (Table III) brings out vividly the importance of eliminating the resident microflora when inoculating unhydrated normal skin followed by 6 days of occlusion. Each of 5 cases pretreated with ethanol and inoculated with 3×10^3/cm² developed moderate to severe lesions while only 3 reactions occurred on the control side and two of these were minimal. With 3×10^2 organisms 3 of 5 alcohol treated sites developed moderate lesions compared to one mild lesion on the control side.

Dosage Responses

Method. It became evident early that the severity of the infection was dose dependent. To gain an approximate idea of the quantity of organisms that could produce lesions in half the sites inoculated (ED_{50}), twenty subjects were inoculated at six sites with numbers ranging from forty organisms to 10^7. Occlusion was maintained for 6 days and the percentage of takes appraised clinically. One site was sampled each day.

Results. Table IV shows that as little as 40 organisms could induce a lesion in about a fifth of the subjects while one million practically always produced a reaction. There was an approximately linear relationship between the log of the dose and the percentage of takes. The ED_{50} was estimated to be a little more than 10^3/cm². With a million organisms redness was sometimes evident within 24 hours, a marked erythemato-papular reaction by 3 days, and exudative lesions by 6. With the lowest doses, little was seen before 5 days and the rash was invariably mild.

When the dressing was taken down on the 6th day, resolution followed quickly and usually at the same rate whether or not antibiotics were administered orally or topically. Crusting and scaling became conspicuous within 24 hours of uncovering. The lesions lost their highly inflammatory quality by 8 days and slowly dried up leaving a scaling, inactive patch by about the 12th to 14th day. This was usually followed by hyperpigmentation although hypopigmentation was sometimes a sequel when the rash was unusually severe. *S. aureus* could still be recovered after two weeks but in vastly reduced numbers.

In a number of instances a fresh occlusive dressing was reapplied after 6 days and maintained for another 8 days. The suppurative reaction increased up to about the 10th day but, on the whole, the intensity and character of the lesion was not very different in the second week. Ulceration was not observed microscopically and the anticipated steady worsening did not materialize. These lesions too regressed rapidly when the dressing was removed. The speed of involution of the lesions when opened to the air

TABLE IV

Inoculum/cm²	Number of takes
40	4 (20%)
220	8 (41%)
2,000	13 (67%)
105,000	14 (72%)
1,000,000	19 (95%)
10,000,000	20 (100%)

eloquently expressed the cardinal importance of a thoroughly wet environment for maintaining the infection.

The decline in the number of organisms after uncovering was only examined in a limited way. Our initial fear that scrub-sampling might produce an ugly local aggravation or even a systemic reaction proved groundless. Topical application of an antibiotic cream after scrubbing may have helped prevent this. Despite sporadic sampling we could perceive that the *S. aureus* population was reduced by about 90% within 24 hours after uncovering. For the next few days, it was not common to recover more than a few hundred organisms/cm^2. The residual scaling reaction at 14 days generally yielded only a few colonies, though this was a consistent finding.

Population Kinetics

We were concerned with determining the fate of inocula of various sizes as the infection evolved.

Methods. Five sites were inoculated in each of 3 groups; the first (3 subjects) received 4×10^2/cm^2 per site, the second (5 subjects) 1.5×10^4/cm^2 and the third (16 subjects) 2×10^7/cm^2. In each group, one site was sampled on 1, 2, 3, 4 and 6 days after application. In addition 3 subjects received 10^7 organisms/cm^2 and the numbers of *S. aureus* determined only at the end of 7 and 13 days.

In a second study concerned with the immediate fate of the inoculum, 4 subjects received 4×10^6 organisms/cm^2 in each of 4 sites; these were respectively sampled at 1, 6, 24 and 48 hours.

Results. Figure 1 depicts the quantities of organisms at various times for each of the 3 dosage levels for those persons who developed a lesion. In those who did not the bacterial population declined steadily and only a few colonies could be recovered by 4 days. With the highest dose (2×10^7/cm^2) takes occurred in 15 of 16 subjects. At the first sampling at twenty-four hours, the mean density had fallen by about 10 fold; however the original level was approximately restored by 48 hours. Thereafter the population remained stable at about 2×10^7/cm^2 *S. aureus* cells. In the three individuals whose occlusive dressings were maintained for 14 days, samples on the 7th and 14th also yielded mean values of approximately 2×10^7/cm^2. It must be noted that these figures apply only to the number of *S. aureus* and not other organisms. (see below)

Of the 5 subjects who received 1.5×10^4/cm^2, three developed lesions. These showed the characteristic 24 hour dip, then a steady climb to attain the 2×10^7/cm^2 maximum level by the 4th day. In contrast to the first group which showed papulo-vesicular lesions by 3 days, the rash did not make its first appearance till the 6th day, about 2 days after attaining the maximum.

Only one of the three subjects inoculated with 4×10^2/cm^2 developed a rash. In this case, it took 6 days for the organisms to build up to the maximum level.

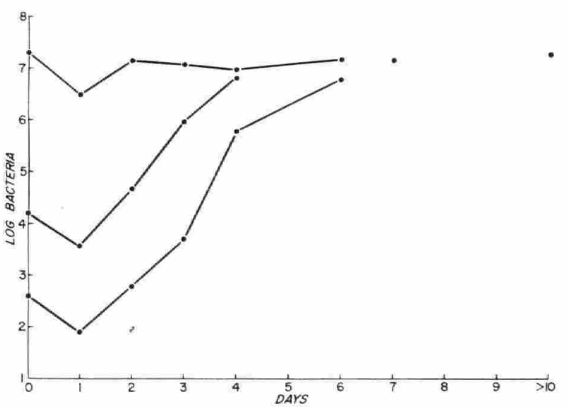

FIG. 1. *Population kinetics:* Quantities of *S. aureus* for 3 dose levels for those subjects who developed lesions. Initial values are inoculum sizes.

TABLE V

S. aureus remaining after inoculation of 4,000,000/cm²

Subject	1 hour	6 hours	24 hours	48 hours
1	970,000	830,000	970,000	2,900,000
2	930,000	790,000	450,000	3,200,000
3	790,000	240,000	30,000	190,000
4	550,000	130,000	50,000	400,000

Thus, at all 3 dose levels, there emerged a specific pattern of bacterial growth. In successful inoculations there were fewer organisms at 24 hours than had been applied; from then on, however, the *S. aureus* population increased to a rather fixed upper level of about $2 \times 10^7/cm^2$. The time required to attain this maximum was inversely proportional to the quantity of organisms remaining at 24 hours. Somewhat less than a million residual organisms at 24 hours would expand to a maximum in the next 24 hours. With a residual population of only $10^2/cm^2$ at 24 hours, the few hardy survivors had to compete against a returning native flora and did not reach the maximum for almost a week. At that time, the lesion was only an erythematous patch with a few scattered papules.

Having witnessed this pattern, we inoculated 4 individuals with $4 \times 10^6/cm^2$ and sampled at 1, 6, 24 and 48 hours to gain more exact knowledge of the early decremental phase. Table V shows that within the first hour there was in every instance a fall to somewhat less than a million/cm². This decline continued in 3 of the four reaching a nadir at about 24 hours. In 2 of these the 24 hour count was very much lower than the original inoculum, 3×10^4 and 5×10^4 organism/cm² respectively. Thereafter a growth phase began and by 48 hours a substantial increase had occurred. We explain these events by assuming an immediate loss in viability by the very act of transferring the organisms to the skin (transplantation shock) and thereafter, the chance happening of the organisms being able to find suitable niches for reproduction in the lacunae of the desquamating portion of the horny layer.

Restoration of the Resident Microflora

Despite alcohol degerming, organisms from the surrounding area have access to the inoculation field and can recolonize the infection site. We made only cursory observations regarding the re-establishment of the native microflora. At 24 and 48 hours, scrub samples revealed a virtual absence of resident cocci and diphtheroids. No more than a few colonies could be recovered in most instances. By 3 days, however, some cocci were regularly present, the numbers usually not exceeding 50. By the 4th day diphtheroids had begun to return and the cocci now commonly numbered thousands. By the 6th day, the resident flora had rather completely recovered. Both in numbers and composition, the population structure except for *S. aureus* was like that of a normal, uninoculated site covered occlusively. Ordinarily, an impermeable dressing will bring about an expansion to about $10^7/cm^2$ within 48 hours (18). Since there were only modest numbers of cocci and diphtheroids by 3 days in degermed, inoculated sites it would appear that the presence of a burgeoning *S. aureus* community does not appreciably hinder the multiplication potentialities of the native population which is entirely restored qualitatively and quantitatively by the 6th day. The combined numbers of residents and *S. aureus* was considerably greater than the levels attained by the residents after simple occlusion.

Histopathology

The following description applies to rapidly evolving lesions produced with large inocula, i.e., greater than $10^6/cm^2$. The tissue was essentially normal 24 hours after inoculation. The earliest definite effects were observed by 48 hours and consisted of decreased staining and blurring of the outermost epidermal cells, attenuation of the granular layer, and slight emigration of lymphocytes and polymorphonuclear leucocytes (PMN's) from small blood vessels. By three days, many PMN's were wandering through the epidermis which was by then frankly spongiotic. The granular layer was totally eliminated. Tiny sub-corneal vesicles had formed in foci containing aggregates of PMN's. Leucocytes had moderately infiltrated the dermis with round cells congregating peri-vascularly and PMN's dispersed throughout the tissue. Eosinophils tended to be strongly represented whenever the infiltrate was intense. In strong reactions PMN's and eosinophils predominated while in weaker ones lymphocytes were in the majority. By the fourth day, the outer epidermis was necrobiotic and intra-epidermal vesicles had formed. These were usually filled with PMN's but a surprising number

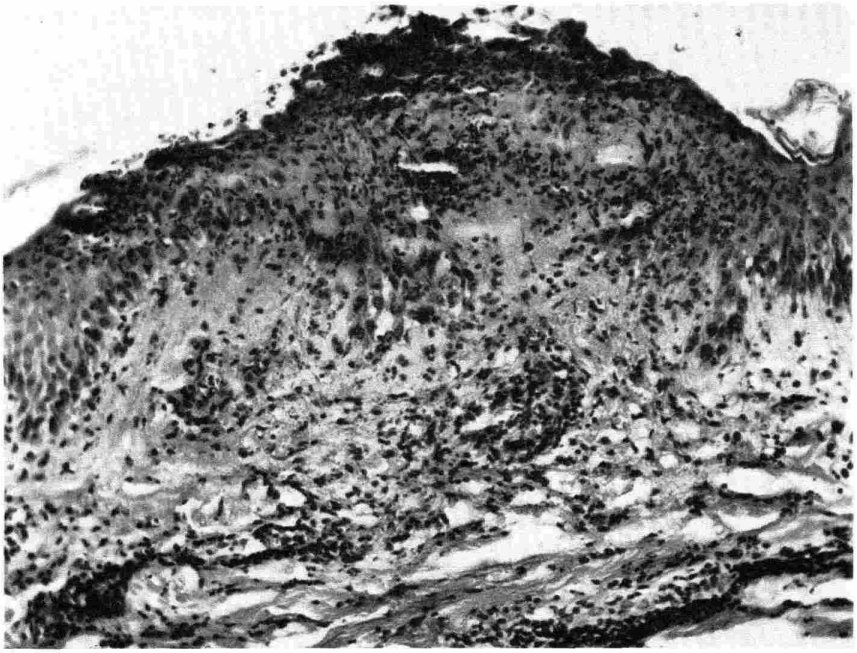

FIG. 2. Six day infection showing complete destruction of epidermis centrally, dermal necrosis, and diffuse infiltrate.

of the smaller ones contained mainly large lymphocytes. There was marked vascular dilation, dermal and epidermal edema, large intra-epidermal PMN filled vesicles and vivid lymphocytic and granulocytic infiltration of the dermis. By the 5th and 6th day, the severest lesion sometimes showed focal destruction of the entire epidermis to produce an erosion under which the connective tissue was necrotic with masses of disintegrated PMN's and basophilic collagen (Fig. 2). In such areas, the papillary capillaries were not infrequently thrombosed or hemorrhagic. Occasionally, epidermal lacunae formed which did not contain PMN's but serum (Fig. 3). This was further testimony of intense vascular damage. For the most part the original stratum corneum remained intact up to the 6th day, and formed the roof of the vesicles and bullae (Fig. 4). After removing the dressings on the 6th day, the healing phase (9th day) showed substantial acanthosis and parakeratosis, moderate perivascular infiltration with lymphocytes and no vasodilatation. By the 12th to 14th day, a new stratum corneum was being formed and increased epidermal regeneration was evidenced by strong basophilia and modest acanthosis.

When occlusive dressings were maintained for 14 days, the picture tended to progress only slightly beyond the peak reaction at 6 days except that the vesicles occassionally burst through the horny layer. There was little evidence of healing; the epidermis was extremely spongiotic or necrotic, with great edema and lymphocytic infiltration of the dermis. Hemorrhage was common from the dilated superficial vessels which frequently showed marked endothelial damage.

The epidermal changes showed no tendency to localize around sweat ducts and hair follicles. Neither was an appendageal distribution observed clinically. Vesicles formed at random in the epidermis and for the most part between the outlets of the follicles and sweat ducts. Correspondingly, the dermal infiltrate did not tend to

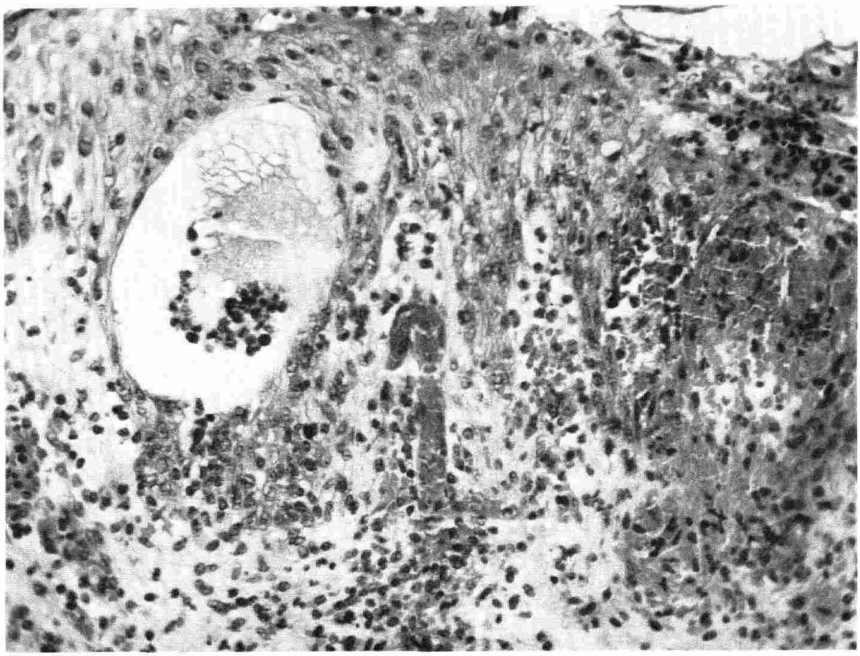

FIG. 3. Five day infection with serum filled intra-epidermal vesicle, necrobiotic epidermis, thrombotic capillary and many neutrophils.

localize around appendageal structures. However, we did occasionally find miliaria-like lesions with PMN's in the intra-epidermal portion of the eccrine sweat duct (Fig. 5). Likewise, vesicles crowded with PMN's occasionally formed perifollicularly in the infundibular region (Fig. 6) but such pustules were in fact rare. Thus, it appeared to be only a matter of chance whether epidermal destruction occurred in relation to the presence of appendages.

We were at pains to determine whether bacteria could be identified within the tissue, especially in intact vesicles. This was never observed histologically in Brown-Brenn stained sections. Even more conclusive was our failure to find cocci in spreads made from aspiration of intact vesicles and pustules. Such studies also included attempts to cultivate the organisms. The surface of 5 and 6 day old lesions were bathed for several minutes in 70% ethanol, then the vesicle contents removed for smears and culture. An occasional positive culture was interpreted as contamination from residual surface organisms. Bacteria were never found within PMN's from aspirated pustules nor were clusters seen in smears. This contrasted sharply with smears made from the surface where huge numbers of cocci could regularly be seen with no effort whatever.

After rupture of the pustules in lesions kept covered for another 8 days, we did occasionally observe a limited number of organisms in the necrotic epidermis.

In summary, the findings point to severe damage produced by potent tissue toxins elaborated by *S. aureus*. This begins with epidermal necrosis from the surface downwards and is soon accompanied by severe injury to the vessels as reflected by a huge outpouring of leucocytes intense edema, and even hemorrhage and thrombosis.

DISCUSSION

The question that immediately arises is why we were regularly able to induce lesions by surface inoculation of normal skin when capable

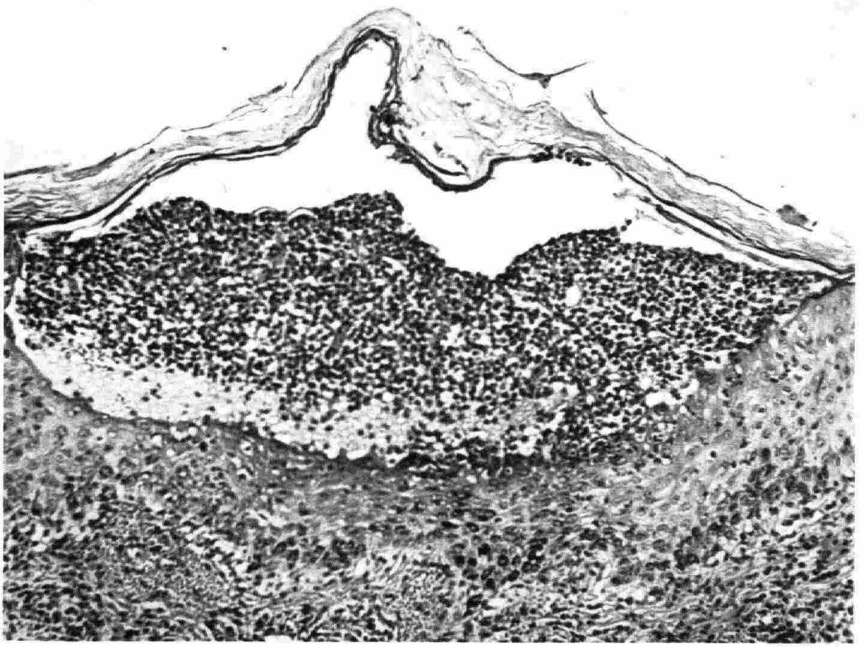

FIG. 4. Intra-epidermal pustule, 6 days. The original horny layer forms an intact roof. Serum is prominent in the pustule.

predecessors experienced mainly frustration (7). The explanation, we think, has to do with a better appreciation of the factors which promote bacterial growth the knowledge of which has been much enlarged by researches emphasizing ecologic relationships (19). Some of the ideas that embattled investigators a few decades ago now seem curiously immaterial. One cannot obtain good answers to bad questions.

An example was the dreadful din over the "self-disinfecting" powers of the skin which was thought to be endowed with some marvelous properties that quickly destroyed foreign organisms (20). These degerming powers were thought to be specifically designed to prevent infection. The "acid mantle" theory prevented progress for decades until it was realized that the slightly acid pH of the skin was really not hostile to alien or to resident species (21). Then, fatty acids in the surface lipids, especially oleic, emerged as the great protectant (22). A truer picture began to form when it was realized by several groups than non-specific factors such as desiccation could largely account for the rapid loss of viability when bacterial suspensions were sprayed on the skin (21). Except in the hairy and intertrigenous areas the surface of the skin is too dry to support more than a paltry population of aerobic cocci and diphtheroids, usually less than a thousand organisms per sq cm. Merely hydrating the surface by an impermeable plastic dressing could increase the population from hundreds to millions in twenty-four hours (18). Such densities are normal for the wet axilla (23). There is no question that moisture is the chief factor limiting the growth surface organisms.

Ricketts *et al.* were among the first to realize that *S. aureus* could be recovered for days if the site was merely kept covered by an occlusive wrapping (17). The inability to isolate β-hemolytic streptococci under like circumstances was still attributed to certain fatty acids in the surface lipids. Even for this fragile organism chemical disinfection is not an adequate explanation as witness the recent success rate of 33% by Duncan *et al.* who induced follicular

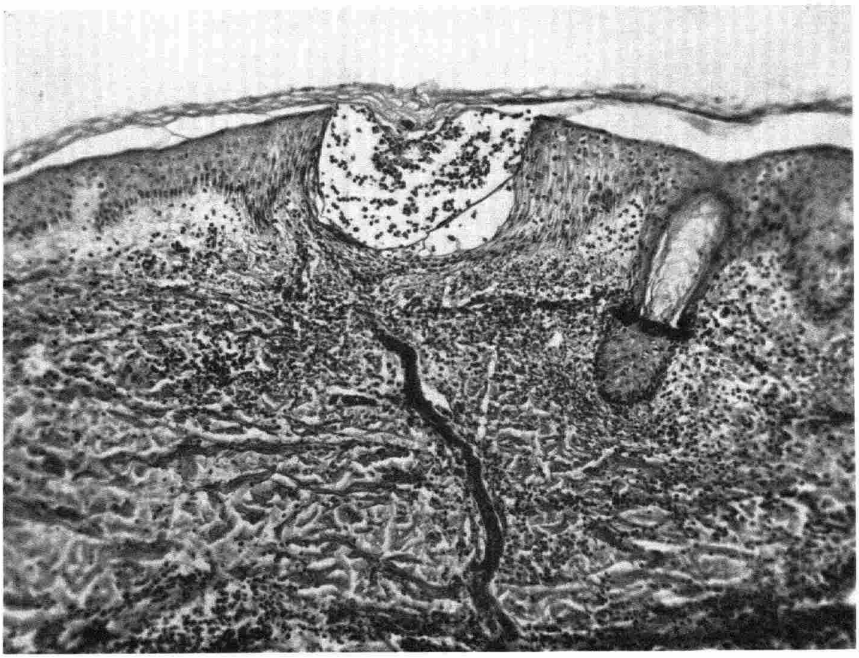

FIG. 5. Four day infection showing intra-epidermal vesicle forming in relation to a sweat duct (miliaria).

mpetigo by applying broth cultures to epilated skin (10). We found (unpublished studies) that streptococci could indeed multiply on hydrated skin under occlusion although lesions did not result. It now seems evident that many kinds of organisms can proliferate on hydrated skin *provided that competitors are curbed*. The insight to be gleaned from such observations is that ecologic interactions are strongly operative in controlling the composition of the microflora. The resident organisms are those best adapted to normal skin. By occupying most of the suitable niches their very presence is a deterrent to the introduction of alien bacteria. A healthy normal flora is a principal factor in preventing colonization by potential pathogens. On eczematous skin, however, pathogens are often just as successful in forming stable communities.

Returning to our success in inducing *S. aureus* infections regularly, we see at work several key agencies which were largely neglected by previous researchers. First is the necessity of hydrating the horny layer by sealing the site occlusively. Without this stimulus staphylococci, no matter how frequently applied, cannot gain a foothold. The mere act of transplantation from the test tube to the skin (or any surface) results in a drastic loss of viability within the first few minutes. The initial inoculum must be large enough to withstand this early trauma and leave a sufficient residuum from which a new population can be generated. A million *S. aureus* per sq cm will almost always satisfy this requirement. Failures will be frequent with a few thousand per sq cm owing to this early damage to viability. The odds are strongly against takes with a few hundred cells.

Another key factor is elimination of bacterial competitors during the initial phase. Pretreatment of the skin with 70% ethanol for two minutes virtually abolished the aerobic microflora and opened up the site for foreigners to multiply without interference. By creating an ecologic vacuum, even a few hundred *S. aureus*

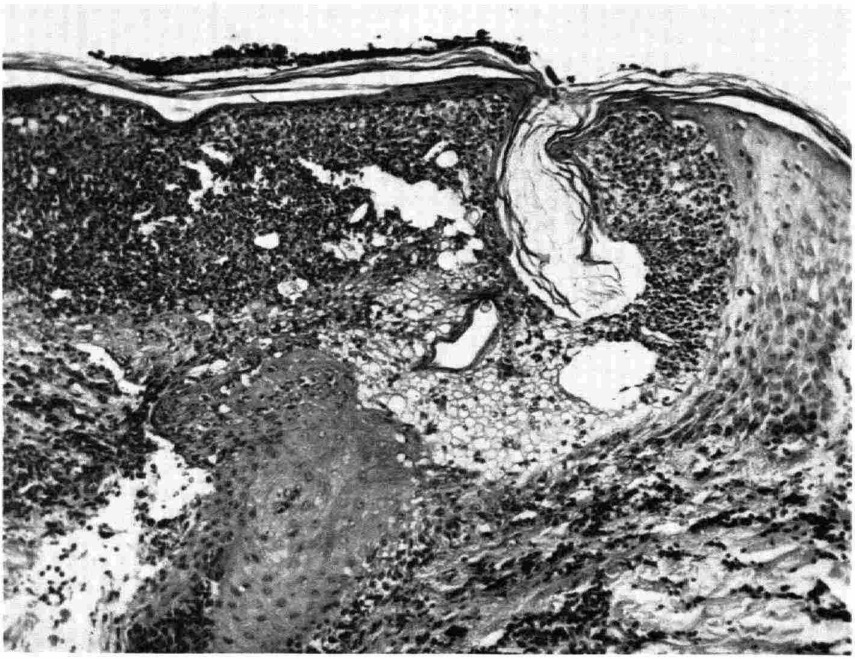

FIG. 6. Five day infection showing intense infiltration of necrotic epidermis by neutrophils enveloping nearby hair follicle and marked dermal edema.

can sometimes take hold and produce lesions. When alcohol degerming is omitted one can procure lesions only by using huge inocula. The normal flora can thus be overwhelmed by sheer force of numbers, a circumstance limited to the experimental situation.

Another important factor in our success was maintaining the occlusion for six days. By often removing the dressings in 48 hours or less previous investigators did not allow enough time for the enlarging population to incite skin damage. When wetness is not artificially maintained, the great majority of cells die within a few hours.

These events are explicable in terms of the kinetics of bacterial growth. When lesions occurred, the *S. aureus* population attained a maximum of ten to twenty million organisms per sq cm, a level which had to be sustained for about another two days to cause significant damage. Huge numbers of *S. aureus* must be present to produce a visible lesion. The numbers of *S. aureus* could not be increased by merely prolonging the occlusion for another 8 days. The habitat imposes definite limitations on the quantity of organisms it can support. Interestingly, the expanding population of *S. aureus*, virtually a pure culture for the first 48 hours, did not succeed in preventing the reentry of normal skin organisms. In fact, by the sixth day these were about as numerous as *S. aureus* and were qualitatively the same as on normal skin, chiefly cocci and diphtheroids. However, it should be appreciated that the skin was no longer normal by that time. The ability of the resident bacteria to re-occupy the site in the face of hearty growth of *S. aureus* illustrates again the obvious fact that these are highly adapted to the integument even when altered by disease.

It is very important to note that the lesions we have induced are not typical of the familar pyodermas. They certainly do not resemble ecthyma or impetigo contagiosa. As far as these two rather characteristic disorders are concerned we need not dilate on the matter for these

are certainly due to group A streptococci, though *S. aureus* may enter later (24). On the other hand, *S. aureus* is the specific etiologic agent of bullous impetigo (25) which in no way resembles the experimental lesion. Likewise furuncles were not mimicked. Indeed, the appearance on the sixth day might easily pass for a contact dermatitis of the allergic or irritant type. The classical signs of infection, suppuration, edema and heat were not marked. One might think of miliaria or folliculitis but with close inspection it was clear that most of the lesions were not situated in sweat ducts or hair follicles (Fig. 7). The eruption was, in short, fairly non-descript. Earlier we described the unexpected development of a similar pyoderma at skin sites to which neomycin had been occlusively applied (26). The initial diagnosis was contact allergy to neomycin. It turned out, however, that the subjects bore neomycin-resistant *S. aureus* which could then multiply explosively without interference from the resident microflora; the latter, of course, was held in check by the neomycin. This was an ecologic maneuver like ethanol "degerming".

We may rightly call these lesions infections but certain qualifications must be added. The host-parasite relationship is not of the conventional type in which the organism wages a battle within the living tissues. Instead the bacteria remain external, separated from the viable epidermis by an intact horny layer; so long as the horny layer fails to rupture the organisms are denied access. In no instance at 6 day infections were we able unequivocally to detect these in smears, cultures or biopsies of vesicles or pustules. By contrast surface smears showed great numbers of cocci. Removal of the dressing after 6 days was followed by swift death of *S. aureus* and prompt clinical regression. On the few occasions when occlusion was maintained for 2 weeks, the lesion became more exudative though not greatly aggravated. However, this was enough time for the original horny layer to have exfoliated so that some cocci were in contact with living epidermal tissue. Still the organisms were not numerous and did not breach the basement membrane.

It is necessary to conclude that the tissue reaction was provoked by soluble products which permeated the horny layer. Such products include

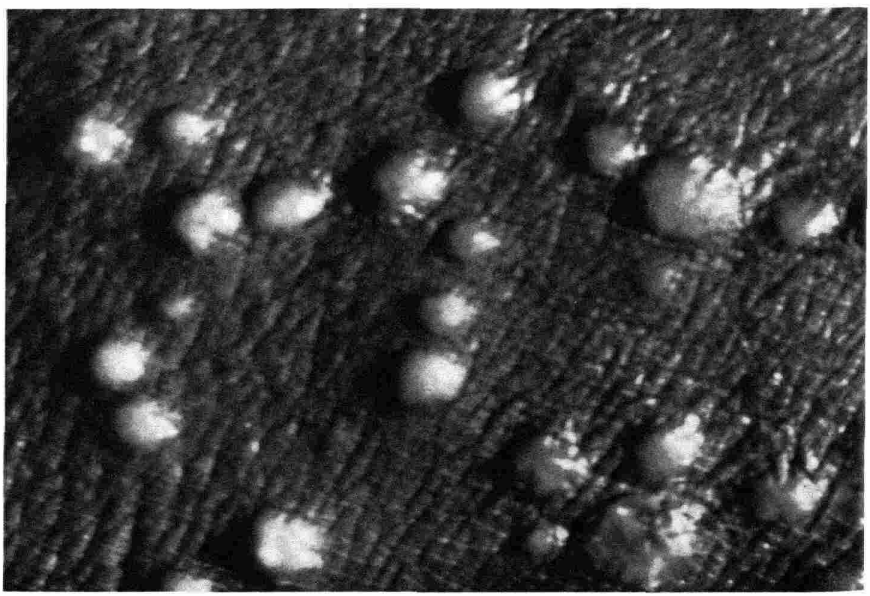

FIG. 7. Pustular eruption on the 6th day. The lesions are not situated in relation to follicles or sweat ducts.

the well known *S. aureus* toxins and doubtless less well characterized enzymes. We observed a similar host-parasite relationship in experimental moniliasis and used the expression "biologic type of contact dermatitis" to describe the interaction (27). The sequence of anatomical damage and the histologic picture permit the identification of a toxic (primary irritant) rather than an allergic type of contact dermatitis. Allergy has never been inculpated in the pathogenesis of *S. aureus* infection, the histologic manifestations in no way resemble delayed type allergy. Instead, the process begins with death of the outer epidermis, followed shortly by an outpouring of PMN's. At peak the lesion vividly expresses severe toxic damage: focal necrosis of the epidermis and dermis, hemorrhage, thrombosis, and edema.

Our experiments have been limited to surface inoculation of normal skin. Elek's intradermal injection of millions of organisms (8) and Duncan's (10) recent implantation of organisms through puncture wounds or depilated follicles may seem hazardous to the uninitiated. It should be recalled, however, that these procedures climaxed a formidable series of exaggerated infection trials (7). Apparently these techniques are safe, for a large number of experiments have been performed without serious mishap. Lest complacency or even carelessness be encouraged by such data, we hasten to record other experiments which remind us that *S. aureus* is not a friendly organism. Foster and Hutt (9) scraped off the epidermis and sealed the inoculation site under a cover slip. Two of the three subjects developed boils at the site and weeks later at distant sites. While we were casting about for an experimental model we placed suspensions on scotch tape stripped skin which was then occluded. We soon had reason for regret. Within hours the subjects had fever, malaise, pain, and swelling at the inoculation site as well as regional adenopathy a little later. These signs of bacteremia caused us to terminate the study post-haste by parenteral penicillin. The lesson was clear enough: *S. aureus* can grow luxuriantly in a site deprived of its barrier. Stripping practically eliminates the resident microflora and also provokes a serous exudate which is evidently a splendid culture medium for *S. aureus*. The removal of the horny layer barrier enables bacterial products to penetrate with little hindrance. We have thus been compelled to modify the stripping model (28). Our current technique is to wait 24 hours until a temporary barrier has formed and then inoculate with a few thousands rather than millions of organisms. A suppurative infection which is purely local occurs within 48 hours; we usually terminate the study at that time by giving an appropriate antibiotic. It is easy to understand why surface inoculation of stripped skin is so much more dangerous than introducing the organisms by injection or punctures. With the latter the organisms are concentrated in a small pocket in a hostile environment where they can be both walled off destroyed by phagocytes. On the surface they occupy a relatively large area and are not immediately accessible to tissue defences.

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