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I wish to present to you an overview of my research work done on microbiology of skin during the last decade.

My interest in microbiology started when I was given thesis for my M. D. in 1961 on the 'Bacteriology of Pyodermas and Bacterial Flora of Normal skin'. The work fascinated me as it involved rather less equipment and had a very practical application, since microbial infections are very common in our country. I am still pursuing the work.

Throughout experiments that we have conducted, subjects were asked to use only bland soap for at least a week prior to the bacteriological studies. They were also prohibited the use of any medicated soap, powder or toiletry. Williamson and Kligman's technique[1] (1965) for sampling the microflora was used. It is a good, simple technique for quantitative work and is reproducible. The studies have been confined to aerobic bacterial flora.

Normal Bacterial Flora on Indian Population

Basically it consists of aerobic coagulase negative cocci (mostly belonging to type SII of Baird-Parker classification). These will be referred to as Staphylococcus epidermidis. The other organism found was diphtheroid. The aerobic diphtheroids belonged to a group which needs lipids for better growth. Tween 80 helped in the growth of these organisms and so were more easily identified on the plates containing this substance. The other group of diphtheroids isolated were not dependent on lipids and formed large colonies (referred to as Large colony diphtheroids). The predominent ones were those belonging to large colony group.

The ratio of number of cocci to diphtheroids varied at different sites and in different individuals as for e. g. on scalp and axillae where the staphylococci constituted 80 to 95%. On any area of the skin it was always a predominent organism numbering more than 50% of total bacterial population.

Twentyfive subjects were studied every month round the year from 5 different sites. The average total count of aerobic bacteria was 16,500 per sq. cm. of the skin. Staphylococcus aureus (S-I of Baird-Parker Classification) was seen only on one occasion in 2 subjects and on 3 separate occasions in another subject.

Bacterial flora of skin under diseased conditions

In association with Dr. D. J. Rao[2], we studied the bacterial flora of skin in cases of uncomplicated diabetes in whom diabetes has just been detected and it being their initial out patient visit.

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The cutaneous bacterial population from two sites of thirty-nine patients with diabetes mellitus who were not on treatment was compared with an equal number of matched control subjects using cup-scrub technique. No significant differences were observed in the type or quantum of population. The nasal carrier rate for staphylococcus aureus was also studied both in the diabetics and the controls. No significant differences were noted.

Lack of difference in the carriage rate of staphylococcus aureus and other pathogenic organisms, both on skin and anterior nares, shows that there is no increased frequency of skin infections in the true bacteriological sense. The reported higher incidence of the skin infections in fact refers to manifiest clinical infection of sufficient severity that the patient reports for medical attention.

We feel that infections from the bacteriological point of view are as frequent in diabetics as in healthy non-diabetics but in the former, once clinical infection occurs, it establishes itself and produces relatively severe forms of disease, which in healthy individuals could be very mild and would limit itself due to normal defence mechanisms.

Ecology of skin has no role to play in higher incidence of clinical skin infections in diabetics.

Qualitative and quantitative studies of cutaneous bacterial flora were carried out in psoriatic patients and normal healthy controls. In psoriatics the flora isolated from the affected skin was compared with the flora of adjacent normal skin. No significant qualitative difference was observed. The total number of bacteria isolated from the psoriatic plaque was significantly higher than the adjacent normal skin.

This shows that the psoriatic plaque is a good habitat for the growth of S. aureus or it may be assumed that psoriatic skin does not have the inhibitary capacity on the growth of S.aureus. That the psoriatic skin has a higher count of S. aureus could also be due to the larger number and size of scales that are washed off with the detergent solution used in the experiment. The organism may be lying in the crevices between the scales in psoriatic plaques while such a hiding place does not exist on the normal skin. This fact may be responsible for yielding higher counts. Other explanation for the increased number of bacteria on the psoriatic plaque may be the increased insensible perspiration through the psoriatic lesions[3] which helps the growth of organisms.

Despite the heavy colonisation of S. aureus the psoriatic plaque seldom gets clinically infected and it may be that some other unknown property of the psoriatic skin is responsible for the lesion not getting clinically infected.

Degerming Potentiality

Various theories have been put forward to explain the disappearance of staphylococcus aureus when brought in contact with intact skin. I have tried to study some of these factors experimentally[4].

1. Antibacterial Lipids

It has been observed that skin of cadavers have less disinfecting potentialities than living individuals. Is this due to some chemical processes peculiar to living skin? Burtenshaw[5] prepared ether extracts from skin, hair and nail. These extracts were found to be bacteriostatic for some strains of S. aureus and S. epidermidis, and bactericidal for E. coli. He attributed this to the presence of fatty acids in it. Pillsbury and Kilgman[6] think that rapid disappearance of streptococcus

pyogenes is probably due at least in part to the chemical sterilizing effect of fatty acids contained in sebum, particularly the unsaturated fraction. Pillsbury and Rebell[7] found that sebum had a mild inhibitory effect on normal skin micrococci in Vitro.

I studied the role of fatty acids and sebum[4]. There is clinical evidence that acne face is more prone to staphylococcal infection, which is contradictory to Pillsbury and Rebell's view[7]. In five normal subjects, I removed fatty acid from skin by using acetone and then inoculated the sites with staphylococcus aureus and occluded the area. On the opposite forearm the site was treated with 70% alcohol and then a thin layer of sebum (collected from other normal humans) was applied and the site occluded with the same amount of inoculum of staphylococcus aureus. No difference in growth rate of organisms was observed.

2. Dessidation

Staphylococcus aureus when applied on skin of forearm disappears very quickly but it persists much longer or even may permanently stay in vestibule of nose or on perineal skin. This may be because these areas remain moist even in winter months and therefore allow this organism to grow.

I found a marked increase in the count of S. aureus on the skin when it is hydrated by occlusion with a plastic film. The multiplication of staphylococcus aureus was serially followed during the period.

There was a clear cut pattern of multiplication. At 24 hours, the number of organisms recovered were less than those applied. Actually this decline in viable organisms really occurs shortly after inoculation and the population may already be increasing at 24 hours. Some organisms may die by the simple process of being transferred to a relatively dry state; some hours being required before the horny layer hydrates under occlusion. In any event after 24 hours, the number of organisms rose to a maximum of about 10 million per square centimeter of skin and did not increase above that even when the occlusion was maintained for 13 days. The peak level was reached in about 4 days with an inoculum size of 10,000/sq. cm. and in 6 days for 100/sq. cm.

The effect of humidity and atmospheric heat on the incidence of pyodermas was studied[8] by recording the out-patient department attendance of these patients and general skin attendance and correlating the percentage with the climatic variations. There was a definite increase in incidence of pyodermas during the months of June, July and August. During this period atmospheric temperature and relative humidity were also fairly high. The hydration of stratum corneum, which in turn is influenced by heat and humidity, is responsible for excessive growth of bacteria and higher frequency of pyogenic skin infections.

The effect of microenviornments was further studied on 12 subjects in whom different sites were chosen for bacteriological studies. One was a completely hidden unexposed area i. e. axilla. Two other sites were back and leg, which are always covered; the latter being loosely clothed and the former relatively heavily clothed. Another site chosen was the forehead which is always exposed. This was aimed to study how semipermeable or partial occlusion due to clothes affect the bacterial population. It was observed that in axilla, where there is practically no evaporation the highest counts were obtained, being significantly more than on the back or leg, both of which places are covered with cloths. Clothings are not impermeable. There were

significantly more bacteria on back than on legs. This could be explained by the fact that back is usually heavily clothed and during sitting position with back resting, there is always more perspiration. It is thus evident that humidity which in turn leads to skin hydration is the most important factor for the proliferation of resident organisms on the surface of skin.

3. Role of resident flora

To what extent the disappearance of S. aureus from intact skin is due to coactions of other organisms? The staphylococcus aureus as it reaches the intact skin could either die or survive, but not multiply.

Staphylococcus aureus when applied experimentally or occurring naturally on human skin enters a habitat already occupied by a microbial population or community and it is probable that under ordinary conditions is unable to compete with this resident flora. They have to face larger communities of both aerobic and anaerobic organisms, and it may be possible that it is unable to compete successfully for nutrients, site of attachment or other requirements. There is some clinical evidence to this effect. There are some situations when competition is lacking i. e. the sterile skin surface of new born infant. Staphylococcus aureus applied or introduced artificially or naturally establishes itself. Hurst[9] states that it actively multiplies in these habitats.

I made some experimental observations in this regard. 70 percent ethanol was applied to skin for five minutes and the net result was the virtual elimination of the aerobic resident flora and most anaerobic ones. One forearm was treated with alcohol and the other left as such and then equal amounts of same fresh S. aureus suspension was applied on both sides, covered with polythene patch and subsequently studied both qualitatively and quantitatively. S. aureus grew much better and had a very early rise in its count, almost reaching its peak in two days on alcohol treated sites. This indicates that there is a competition between S. aureus and resident flora. Virulent organisms were applied on a site on which resident cocci were virtually destroyed by alcohol pretreatment. Sampling the sites at various intervals revealed that those reoccupied the site and were capable of multiplying despite the greater number of S. aureus. This further indicates that the competition is between a favourite organism and a non-favourite one, the former being resident organisms, the latter being S. aureus.

This means that there is some factor which prevents the establishment of new invaders to a classical ecosystem, but the mechanism is not clearly understood.

Role of Antibiotics on Resistant Staphylococcal Infections

It has often been observed that administration of an antibiotic leads to great increase in number of organisms resistant to it. It has now been documented by many reports that systemic administration of antibiotics increases the susceptibility of an individual to colonization or overt infection by organisms resistant to the antibiotic used. Many views explaining the dynamics of this phenomenon have been presented, each lacking documentation.

I undertook the work by studying the effects, if any, of the topically applied neomycin on the growth of neomycin resistant staphylococci on intact human skin and the outcome of manifest skin infection[10]. I used the method of producing skin infection of human skin which I had developed earlier[11], and is described in the next chapter.

Interplay of resident flora and inoculated staphylococci was studied by prehydration of inoculated sites which resulted in heavy growth of resident flora on control sites only. Inoculated neomycin resistant staphylococci could hardly grow on control sites, producing no clinical lesions, when the bacteria grew wild on neomycin treated sites with clinical lesions in every subject. This work supports the simple competition between different species of organisms. Elimination of resident flora by antibiotics produces an ecologic vaccum thus permitting new entrants resistant to the antibiotic to take hold and grow. Elaboration of bactericidal substances by some species of organisms[12] however, cannot be ruled out.

Experimental Staphylococcal Infection of Human Skin[11]

Most of the common pyodermas stem solely from the rampages of this one organism. Its violence is not open to question to clinicians. The experimenter's experience on the other hand is baffling and inexplicable. The history of experimental staphylococcal infection is predominantly a record of failure. No investigator who has surface inoculated more than a few subjects can claim even modest consistency regardless of strain and quantity of organisms used or type of skin traumatization. The successful reproduction of typical lesions such as impetigo and furuncles have been even rarer. The extraordinary difficulties in producing experimental infections are best exemplified by Maibach's[13] extensive study on human volunteers. He performed more than 1,000 inoculations and achieved but a handful of convincing 'takes'. His technique encompassed every conceivable manoeuvre that might predispose to infection i. e. occlusion under different tapes, scotch tape stripping, denudation of epidermis, blisters, U.V. burns, sweating, epilation of hairs, epinephrine vasoconstriction etc. It is thus no little satisfaction to me that I have described a stunningly simple method of inducing experimental staphylococcal infection of intact human skin and postulated a hypothesis of its genesis[14].

S. aureus infections have been consistently indued in normal human skin by applying large inocula to areas degermed with ethanol and kept moist under occlusive dressing. ID50 (Dose that infects 50% of the subjects) was approximately $10^3/cm^2$. With about 10^6 organisms/cm^2, an erythematous lesion appeared in three days which evolved to an intense papulo vesicular lesion by six days. Removal of dressing was followed by swift death of almost all bacteria followed by immediate resolution of the lesions within a few 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, oedema, haemorrhage and thrombosis. This clinical entity may be described as toxic pyoderma. In fact it is being postulated that clinical pyodermas (impetigo, folliculitis) start as irritant dermatitis and invasion of organisms and the development of classical picture is a secondary process.

Experimental T. rubrum and Pseudomonas infections

A simple new method of producing experimental T. rubrum infection in man was developed[15]. A suspension of fresh culture using a granular colony was applied under occlusion on areas of skin. Occlusion was removed after 5 days and a semiocclusive dressing applied. It was possible to induce typical ringed or discoid lesions, which could persist for a long time if semiocclusive dressings were continuously applied.

An attempt at producing cutaneous lesions by surface inoculation with varying amounts of Pseudomonas aeroginosa under occlusion failed[16]. The only lesion produced was maceration of skin. No other local lesions or systemic symptoms were produced. Organisms grew uninhibited. Intradermal inoculations produced papules or nodules, which formed abscesses. Two more abscesses appeared proximal to the initial lesion in one subject[16].

Topical Steroid-Antibiotic combination in pyodermas

Systemic corticosteroids are notorious for spreading bacterial and fungal infections and even infestations like strongyoloides. Skin infections are known to occur more frequently under systemic steroid treatment. There has however, been a controversy as to the effect of local corticosteroids in exacerbation or precipitation of bacterial skin infections.

The experimental model of producing skin infections described earlier was used to sudy the effect of corticosteroids on experimental skin infections with S. aureus[17]. It was observed that corticosteroid when applied topically do not stimulate the growth of S. aureus. Skin lesions are either not produced or the intensity is much less on steroid treated sites as compared to control sites. These have a definite role to suppress the lesions in experimental staphylococcal infections on intact human skin. It may be derived from these experiments that fear from the use of topical steroids in infected lesions is unwarranted, particularly when it is used in association with specific antibacterial medicaments. The steroids will certainly expedite and hasten the symptomatic relief; during which time the specific medicine will start working. This does not mean that I am advocating combinations. These, however, could be used without fear in selected cases where urgency is demanded and the exorbitant cost of effective topical steroids is no bar.

Effect of Microflora on Cutaneous Physiology

Individual susceptibility to become anhidrotic on occlusion is very varied, It was observed[18] that an individual's skin which favours the growth of resident flora on occlusion is more prone to get anhidrosis by the same process. It was also observed that sites where growth of resident organisms was suppressed by antibacterial agents did not show the same degree of anhidrosis as on control sites. This indicates a causal relationship between the bacterial growth and anhidrosis.

Prickly heat and anhidrosis are closely related. It is generally believed that initially in the process of development of miliaria, there is occlusion of sweat pores or the terminal parts of sweat ducts, while the sweat is being continuously produced. This leads to diminished or non-pouring of sweat on the skin surface sweat escaping into the periductal tissues and producing accumulation of fluid and subsequent inflammation. This is prickly heat (miliaria). It is not known what leads to this chain of events. The author's data give support to the hypothesis that bacteria in some way initiate the process of anhidrosis. It is postulated on this basis that the number of bacteria on the surface of skin and/or the capability of the skin to markedly enhance the growth of resident bacteria on the skin may be the reason for individual susceptibility to anhidrosis and miliaria.

Virulence and Phage-types of staphylococci

Certain phage-types of S. aureus are said to be more virulent than others; some are said to have a special predilection for hair follicles and others

for glaborous skin. The human model of skin infection to study the capacity of different phage-type strains including popular 502A (type 7/47/53/81) to produce infections under similar conditions, using the same inoculum size was used[19]. No large differences in virulence were observed between the strains and these, if any, were not related to phage-types. Types 80/81, 71, 50/50A, 29/50/50A/70 and an untypable strain produced similar lesions. No predilection for hair follicles was found with any of the strains used.

In another study[20] the differences in virulence of 21 strains of S. aureus belonging to different phage-types and groups were compared using the Noble's technique[21]. Subcutaneous infection was produced on depilated albino mice weighing 20 to 25 g. each. Two 10-fold dilutions of S. aureus were injected in pairs of two different strains to be compared on each half of the body. The organisms used were in the order of 10^6 to 10^7 per ml. suspension. Variation in capacity of individual strains to produce reaction in the injected mice was clearly demonstrated which, however, was unrelated to phage-types of the strains. The different strains of same phage-type may have widely varying degree of virulence. On the contrary, strains of two different phage-types were found to have insignificant difference in thier virulence in man.

Germicidal Soaps and Skin Infections

An attempt was made to develop a model for the study of the efficacy of different medicated soaps in preventing skin infections[22]. Use of medicated soaps prevented the development of skin lesions when challenged with S. aureus. Bar soaps containing hexachlorophene were as effective as those containing halogenated salicylanilides. The effect of soaps gradually diminishes and does not last more than 48 hours, if it is not reinforced by further use of the soap. Germicidal soaps have no specific therapeutic role in suppressing the lesions over and above the bland soaps[23].

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TRUE or FALSE?

Patients with active Systemic Lupus Erythematosus (SLE) have impaired delayed skin hypersensitivity.

(Answer Page No. 144)