PATHOGENIC STAPHYLOCOCCI ON THE NORMAL HANDS
A GROUP OF MEDICAL WORKERS AS COMPARED
WITH A LAY GROUP

by

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PATHOGENIC STAPHYLOCOCCI ON THE NORMAL HANDS

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Introduction

"The real source of infection of a wound deliberately made by a careful surgeon whose materials are properly prepared and who uses them aseptically is to be found in most circumstances either in the skin of the patient or in the hands of those directly concerned in the operation." This statement was made by Dr. Taylor (27) in 1914. Although our methods of surgery have greatly improved since that time, and the number of surgical infections has greatly decreased, this statement still holds true to some extent.

With this thought in mind it is of interest to know what pathogenic organisms are on the skin and the relative numbers of these organisms as compared with the number of and types of non-pathogenic organisms.

The normal flora of the skin has been quite well established to be composed of staphylococci, sarcina, micrococci, and diphtheroids (34, 35, 31, 8). The white staphylococci constitute 90 per cent of the total colonies developing on blood agar plates pressed against the normal skin, the golden yellow

staphylococci compose 3 to 5 per cent. Price (25) has estimated the number of organisms on the normal skin of the hands and arms up to the elbows to be roughly five million. His estimates were made on the growth of the organisms on plain agar. Since this was a minimum number and since the more delicate organisms do not grow upon this medium very readily, it is quite likely that the normal skin flora is much greater than five million and if the staphylococci compose 95 per cent of the normal flora of the skin the importance of these organisms may readily be seen.

Welch (34) has said that the bacteria, which may be concerned in surgical infections are many, but the pyogenic cocci, far out rank in frequency, and therefore in importance all the other bacteria combined.

It is therefore of utmost importance to the surgeon, as well as to the practicing physician to know the relative occurrence of pathogenic staphylococci on his own hands as well as the skin of the patient.

The normal skin flora is considered to be relatively free from pathogenic organisms (31, 8). In view of this fact it is of interest to know the relative number of people that are normal carriers of pathogenic organisms and also how they obtained these organisms as part of their normal skin flora.

Smith (26) has said that among the frequenters of a general hospital in Glasglow, including a few doctors, students, and

nurses it is uncommon for pathogenic staphylococci to be found on the hands, unless the person is suffering from frank pyodermia, or else has been in contact with those so suffering.

The exceptions may be regarded as pathogenic staphylococcus carriers.

Price (25) believes that if the normal skin is in contact with certain bacteria over a period of time there is a tendency to establish a normal flora of those bacteria on the skin. While Price was in charge of a wardful of patients with virulent infections following gun shot and shell wounds although dressings were done with unusual care, though without gloves--after several weeks he noticed a large proportion of pathogenic organisms had been established on his hands as part of the normal flora. Scrubbing and application of germicides did not appear to reduce the proportion of pathogenic organisms any more rapidly than the non-pathogenic. In spite of frequent washing and daily use of hand disinfectants of various sorts, the last of these pathogenic organisms did not disappear from his hands for many weeks after he had ceased work in the septic ward. The importance of this to doctors and nurses in constant charge of infectious cases and doing surgical work can readily be seen.

Gillespie (11) however, believes the pathogenic staphylococcus carrier condition on the normal skin to be dependent upon the presence of pathogenic staphylococci in the nose, which in turn

is directly associated with a history of chronic nasal disease. In a recent experiment he determined the nasal and skin carrier rates in medical students and compared them with the incidence of nasal and skin infections. Of 159 students examined, he found a nasal carrier rate of 43.4 per cent, and a skin carrier rate of 19.5 per cent. Of the entire group, 13 per cent were found to be double carriers. He also states that there was no relation between the nasal and skin carrier rates and the length of the students sojourn in the hospital.

In view of the facts brought out above, that pathological organisms may remain for considerable periods of time on the normal skin, it was thought that a comparison of skin carrier rates between two groups differing with respect to their probable contents of pathogenic staphylococci would be of interest. Consequently, a group of medical people who are in constant contact with patients having various diseases and infections, were compared with a lay group.

In selecting the members of each group to be tested, special care was taken to select people free from infections of any sort, i.e., colds, sinusitis, pyoderma. The members of the medical profession consisted of practicing physicians and nurses. Farmers, housewives, high school students, stenographers, composed the lay group.

Cultures were taken from the hands of 50 members of each group. If present, three *Staphylococcus albus* and three *Staphylococcus aureus* strains from the hands of each person were cultured and tested for pathogenicity by the coagulase, fibrinolytic, alpha-hemolysin, mannitol fermentation and the gelatin liquefying tests.

Review of the Literature on Pathogenicity Tests for Staphylococci

The value of a pathogenicity test for staphylococci depends upon its ability to differentiate between the pathogenic and non-pathogenic strains. To determine the value of such a test, a large number of tests would be run on pathogenic staphylococci known to be pathogenic for human beings (isolated as the causative agent of osteomyelitis, furunculosis, septicemia) as compared with similar tests run on staphylococci isolated from non-pathogenic sources (such as air, dust and water). However, in the latter case there may be some doubt as to the absolute non-pathogenicity of the staphylococci as there may be staphylococci which were previously directly connected with pathological cases. A test which would give a high degree of accuracy on the pathogenic organisms with a negative or very small proportion of positives on the non-pathogenic would then be a good test for pathogenicity. That such a test should, if possible, be easily carried out, require readily available materials, and not be too time consuming, can readily be seen.

Many tests by different workers have been originated for the purpose of differentiating the staphylococci. One of the earliest methods was based on the formation of pigment (6). The golden yellow *Staphylococcus aureus* was regarded as pathogenic and the white *Staphylococcus albus* as non-pathogenic. While it is undoubtedly true that the majority of pathogenic staphylococci are of the golden yellow variety, the fact that there may be pathogenic *Staphylococcus albus* and non-pathogenic *Staphylococcus aureus*, plus the difficulty of a proper differentiation of the in-between pigments leads to uncertainty in using pigment formation as the criterion of pathogenicity (2, 9).

That the fermentation of mannitol was first used by Gordon in 1903 is mentioned by Dudgeon (6). Dudgeon also used this and other methods in differentiating the staphylococci. He found that of 14 strains of *Staphylococcus aureus* isolated from supperative lesions all fermented mannitol; 15 out of 16 strains isolated from subcutaneous lesions fermented mannitol, and 5 out of 6 isolated from the normal skin also gave positive mannitol fermentation. In contrast to the *Staphylococcus aureus* reaction on mannitol, of the *Staphylococcus albus* strains he obtained from healing wounds only two out of 16 strains isolated gave positive fermentation reactions. A great many workers have obtained similar results with the fermentation of mannitol (30, 9, 29, 24, 4, 5, 12). Although a very high degree of correlation with pathogenicity has been found, the fact that an appreciable

proportion of non-pathogenic staphylococci also ferment mannitol, from 11 to 53 per cent (2), would seem to limit the use of this test.

Dudgeon (6) was also one of the earliest users of gelatin as a medium for differentiating the staphylococci. He found that it was quite an exception for *Staphylococcus aureus* to fail to liquefy gelatin; but that the majority of *Staphylococcus albus* also liquefied it, although there were numerous exceptions. In 1928 using the gelatin, precipitin, and other tests for differentiating the staphylococci, Dudgeon and Simpson (7) found that out of 50 *Staphylococcus aureus* strains 32 liquefied gelatin at 37°C., the remaining 18 liquefied gelatin at 22°C. Thirty *Staphylococcus albus* strains were also tested, 13 of these liquefied gelatin at 37°C. and six at 22°C.; the remaining 11 failed to liquefy gelatin by either method. Other workers have obtained similar results (29, 24). This test appears to give similar results to the mannitol fermentation test. The number of the non-pathogenic organisms giving positive tests seems to be the limiting factor in the use of these tests.

Coagulase production was first observed by Loeb in 1903 (17). It was associated with pathogenicity by Much in 1908, and since then its value in identifying staphylococci of potential pathogenicity has been established (4, 5, 10). Chapman (4), Cruikshank (5) and Hallman (12) have found a correlation of better

than 96 per cent, with a corresponding lack of ability to coagulate plasma on the part of non-pathogenic staphylococci. Chapman tested 5,000 strains of staphylococci by the coagulase, hemolysin on blood agar, and agglutinin tests, using pathogenicity for rabbits as the basis of pathogenicity. He found that all of the plasma coagulating organisms were pathogenic for rabbits. In the summary of his work he states that regardless of its color and hemolytic ability a coagulating strain is usually pathogenic. Cruikshank (5) has made the following statement in regard to coagulase production--that although unrelated to the necrotoxin and alpha-hemolysin of the staphylococcus, staphylocoagulase, like them is present only in the pathogenic members of the species whether albus or aureus. Because of its constancy and ease of demonstration, coagulase production is recommended as a test for pathogenicity of the staphylococci. Blair (2) in a recent review of the literature on the pathogenic staphylococci suggests that while hemolysis and pigment formation frequently parallel the coagulase reaction, variations occur sufficiently often to render them inadequate, and only suggestive at best. It is Blair's opinion that the coagulase reaction alone is a sufficient invitro indication of the pathogenic potentiality of staphylococci. He believes that the fermentation of mannitol supplies a valuable confirmatory test, and that the use of numerous invitro tests serves only to multiply confusion, particularly if one is forced by disagreeing reactions to postulate varying degrees of patho-

genicity. Besides having the above qualities, the coagulase test is easily carried out and the results may be known after three to four hours incubation. The only necessary materials are an 18 to 24 hour culture of the organism and human or rabbit plasma. Fairbrother (19) has found human plasma to be utilizable for this test after six weeks of storage at 0 to 2°C. Only 0.5 c.c. of a 1:5 dilution of the plasma is necessary. Thus, the accuracy, combined with simplicity and readily available materials would seem to make this a very valuable test.

Regarding the fibrinolytic reaction, Madison (18) found that 90 per cent of the strains isolated from internal human lesions were fibrinolytic, while 77 per cent from superficial lesions were inactive against human fibrin. Fisher (10) and Tillet and Garner (28) have reported that relatively few strains of staphylococci from human pathogenic sources dissolve plasma clot. In testing 26 strains of pathogenic staphylococci, Fisher found that only seven could dissolve plasma clot. Due to the fact that fibrinolysis by staphylococci often requires several days to bring about dissolution of a plasma clot (10) and that when many hours of incubation are required to demonstrate fibrinolysis, complicating factors are introduced by the retraction of the clot from the sides of the tube, and by the tendency of fibrinogen clots to spontaneous lysis (2), this test also has a very limited value.

That certain strains of *Staphylococcus aureus* and *Staphylococcus albus* produce exotoxins is quite well known (36). Five

different toxic effects have been described as being produced by these toxins. They are the following: hemolysin, leukocidin, necrotizing toxin, lethal toxin, and gastro-enteric toxin.

Many of these toxins have been employed as tests for pathogenicity. Hemolysis was one of the earlier ones used. Julianelle (15) gives a brief report concerning the early use of hemolysin; Kraus, 1900 (16) brought out the fact that staphylococci lake blood on blood agar plates. In 1901 Neisser and Wechsberg (20) demonstrated a hemolytic substance in filtrates of broth cultures. They showed in a general way that aureus and virulent staphylococci produced greater quantities of hemolysis than did either albus or non-virulent strains. The work of Van durme 1918 (33) showed similar results in regard to virulent and avirulent strains. However, in his work Julianelle (15) draws the definite conclusion that although different strains of staphylococci will vary in degree of hemolysis, and in rapidity of hemolysis, but if sufficient time is given, all strains of staphylococci, pathogenic and non-pathogenic, will show hemolysis.

It is generally agreed that hemolysis on blood agar is not necessarily related to the production of soluble hemotoxin (2). Out of 262 strains of staphylococci, 62 aureus and 200 albus, Hudson (14) found 63 per cent positive for hemolysis on blood agar after 72 hours. L. Thompson (29) found 45 out of 50 strains isolated from pyogenic infections positive; 22 out of 23 strains from boils and osteomyelitis positive; 26 out of 30 from atypical

blood cultures, and 12 out of 30 from the normal skin. Dudgeon and Simpson (7) found that 68 per cent of Staphylococcus aureus, and 56.6 per cent of Staphylococcus albus gave positive hemolysis on blood agar. Chapman (4) found that only 51.7 per cent of 690 strains of Staphylococcus aureus were hemolytic. Hallman (12) found that 91.4 per cent of 480 strains hemolyzed blood agar, while only 67 per cent of the strains were pathogenic. Thus it may be seen that the ability of a staphylococcus to hemolyze blood agar is not necessarily an indication of pathogenicity. However, Chapman (4) believes that hemolysis on blood agar may be a very valuable index to pathogenicity through the following interpretation: that if a strain is aureus and hemolytic it is probably pathogenic, while if a strain is albus and non-hemolytic it is probably non-pathogenic, while aureus negative hemolytic, and albus positive hemolytic strains are doubtful.

Due to the various methods of obtaining toxic filtrates, the different erythrocytes used, and also the different techniques in preforming hemolysin tests there seems to be considerable disagreement in the literature as to the degree of correlation of hemotoxin production with pathogenicity (30). However, some workers have obtained a very good correlation with pathogenicity by this method. R. Thompson (30) found a correlation of 91 per cent, Cruickshank (5) 91 per cent and Gillespie (11) 100 per cent. Due to the time consuming procedure of preparing the toxin and the necessity of having freshly prepared rabbit erythrocytes on

hand, and also to the fact it is not possible to demonstrate hemolysin in a number of freshly isolated strains of staphylococci (1), this method would appear to have limited use.

Difficulty in the methods used for testing the production of leucocidin have also hampered the use of this test as an index of pathogenicity of the staphylococci (2). Recently Valentine (32) has employed a microscopic method, observing the destruction of leucocytes directly in a series of slide preparations stained with a suitable blood stain. He finds that strains of pathogenic staphylococci isolated from serous lesions are capable of producing leucocidin in considerable amounts. However, out of 36 pathogenic strains tested, only 27 were capable of producing leucocidin; a correlation of about 75 per cent

Agglutinins have in the past been used mainly as a method of classifying the staphylococci (15, 13), but not as a method of separating the pathogenic from the saprophytic forms. However, recently Thompson and Khorazo (30) using the original groupings of Julianelle have found a high degree of correlation with pathogenicity. They found that staphylococci from diseased sources tend to fall into a fairly uniform group possessing certain biochemical and antigenic properties differentiating them from non-pathogenic staphylococci. However, the time consuming technique and uncertainty would be factors leading us to look for a more perfect test.

The ability of staphylococci to form precipitins has been correlated with pathogenicity. Dudgeon and Simpson (7) in using 50 strains of aureus and 30 strains of albus obtained from pathogenic sources, concluded that cultures of *Staphylococcus aureus* produced efficient antisera more readily than *Staphylococcus albus* cultures, but when an active *Staphylococcus albus* antisera was produced, it appeared to be as effective as the *Staphylococcus aureus* antisera. A marked difference in efficiency between *Staphylococcus aureus* and *Staphylococcus albus* antigens was noted. *Staphylococcus aureus* antigens were almost three times more active than the *Staphylococcus albus* antigens. Pathogenic strains of *Staphylococcus aureus* and *Staphylococcus albus* produced more efficient precipitin antigens than non-pathogenic. Burky (3) found a very high degree of correlation between the precipitin reaction with rabbit pathogenicity. However, in a preliminary report Blair (2) had found no correlation with pathogenicity and the precipitin test. He suggested that the coagulase test may apparently serve for preliminary separation of staphylococci into pathogenic and non-pathogenic strains.

Many workers have used animal pathogenicity as the basis of virulence in carrying out experimental tests on staphylococci (4, 5, 6, 10, 3). Burky (3) has stated that while the correlation is not definitely proven there is a strong suggestion that only strains capable of producing human lesions produce any effects in

rabbits. In his experiments Durky has shown that in the majority of cases a lethal toxin is not produced, that cultures usually kill rabbits with definite abscess formation in the kidneys and heart muscle.

It seems that the ability of a staphylococcus to produce exotoxin is in no way related to the severity of the infection from which it was isolated (2). Parish (22) found no relationship between the potency of the toxin prepared and the clinical severity of the condition from which the strain had been isolated. Panton (21) also found no correspondence between the amount of toxin produced and the severity of the lesion from which the strain was isolated. That the dermonecrotoxin appears to parallel the lethal toxin in its production, properties, and antigenicity, has been brought out by Blair (2). Just as few pathogenic strains have been found to produce lethal toxins, so Parker (23) working with dermonecrotoxin found that out of 21 strains of Staphylococcus aureus isolated from boils, carbuncles, dermatitis, abscesses, septicemia, only four produced filtrates which were effective on the skin of rabbits.

It would appear from this brief review of the different tests which have been used as tests for pathogenicity of the staphylococci, that the coagulase reaction gives the highest degree of accuracy with pathogenic strains; with very few positive for non-pathogenic strains, that it is very easily carried out, and that it is not time consuming. All of these factors leads us to select this test

as the criterion for determining the pathogenicity of staphylococci isolated from non-pathogenic sources. Since we are very interested in the correlation of the other tests with the coagulase reaction we have also included pigment formation, hemolysis on blood agar, production of alpha hemolysin, gelatin liquefaction, mannitol fermentation and fibrinolysis.

Experimental Work

Procedure

For our work we obtained cultures from the hands of 50 members of the medical group and 51 members of the lay group. Care was taken to avoid using anyone that had infections of any kind. The hands of each member of each group were thoroughly washed with soap and water. Brushes were not used. The hands were not dried as the moist hands would facilitate in swabbing. Sterile swabs were rubbed over the entire surface of the hands, including backs, palms, between fingers and fingernails. The swab was then streaked over the surface of blood agar plates in a manner so as to have well isolated colonies on the last part of each plate. The plates were then incubated at 37°C. for 24 hours. Hemolytic colonies were encircled and the plates were placed in semi-daylight for 48 hours in order to facilitate the development of pigment. At the end of the 48 hours, if present three albus and three aureus colonies were picked and transferred to extract agar slants. In selecting the three aureus colonies, golden yellow colonies were picked if three of these were present, if three of these were not present, and staphylococcus colonies of varying shades from cream colored to other shades of yellow were present, enough of these were picked to make a total of three. The type of pigment was noted in each instance. All

hemolytic colonies were given preference to non-hemolytic colonies, however, if three golden yellow aureus colonies were present, although non-hemolytic, these were taken instead of hemolytic lemon yellow colonies.

The cultures made on extract agar slants were incubated at 37°C. for 24 hours; and used as the stock cultures, from which the different tests were carried out. Strains were made of each organism to insure its being a staphylococcus. All organisms that were gram positive cocci, with cells occurring singly, in pairs, and in grape-like clusters, not packets, were considered as staphylococci.

All six organisms, three albus and three aureus, from each person were tested for coagulase, alpha-hemolysin, fibrinolysis, mannitol fermentation and gelatin liquefying ability. The tests were carried out as follows:

For the coagulase test rabbit plasma was used. The plasma was usually not over 24 hours old, although in a few cases it had stood a week in the ice box. A 1:5 dilution of the plasma with physiological saline was made and 0.5 cc. of this dilution was used for each organism. A loopful of a 24 hour extract agar culture was emulsified in the diluted plasma and incubated at 37°C. Readings were made hourly for four hours; final readings were taken at the end of the four hours. Tubes which contained firm clots; which allowed the tube to be inverted, were considered as

four plus for the coagulase reaction, others were graded accordingly. A control organism was used which clotted plasma within one and one-half hours. If plasma was not clotted by this organism within this time all tests run at that time were repeated.

For the fibrinolysis test the tubes containing plasma clot--from the coagulase test--were kept for seven days and read daily to determine dissolution of the clot. Complete fibrinolysis was regarded as a four plus reaction.

For the alpha-hemolysin reaction a loopful of a 24 hour culture from extract agar was inoculated into petri plates containing 0.3 per cent infusion agar. The plates were incubated in an atmosphere containing 30 per cent carbon dioxide, and 70 per cent oxygen, for 48 hours. After incubation the agar was cut up with a sterile loop and poured into sterile funnels containing cotton gauze and allowed to drain over night in the refrigerator. The filtrates were then centrifuged at top speed for 30 minutes in order to completely sediment the bacterial cells. One-tenth of a c.c. of this supernatent fluid was used in the titration. A set up of seven tubes was used for each titration. Nine-tenths c.c. saline was placed in the first tube and 0.5 c.c. in the others. One tenth c.c. of the supernatent fluid, or exotoxin, was mixed with the saline in the first tube, 0.5 cc. of this dilution was transferred to the second tube, after thorough mixing 0.5 c.c. was transferred to the third tube, and so on with each succeeding tube, 0.5 c.c. being discarded from the last tube, leaving 0.5 c.c.

in all the tubes. Five tenths c.c. of a two per cent suspension of washed rabbit erythrocytes was added to each of the seven tubes. A control tube containing 0.5 c.c. saline and 0.5 c.c. of the cells was made for each five organisms, or 35 tubes. The tubes were set in a 37°C. water bath and readings taken after one hour. Complete hemolysis was regarded as four plus; other amounts of lysis were graded accordingly.

For the mannitol fermentation, 48 hour agar cultures were used. A loopful of the culture was inoculated into tubes containing mannitol broth. The tubes were incubated at 37°C. and observed daily for a period of seven days for the production of acid. As andrades indicator had been included in the medium, the intensity of the color change could be noted daily. The time taken to produce acid, and the degree of acid as shown by the color, was noted. A bright red color was considered as four plus. Faint pink and other shades were graded accordingly.

A 48 hour agar culture was also used for the gelatin liquefaction test. A stab inoculation was made into each gelatin tube and observed daily for seven days for liquefaction. Time taken for liquefaction, and amount of liquefaction was noted. Complete liquefaction was regarded as four plus.

Results

Discussion and Results of the Incidence of Pathogenic Staphylococci on the Hands of the Two Groups

Using the coagulase reaction as a criterion of pathogenicity for the staphylococci, we find that of the 50 medical people examined, nine were found to be potential pathogenic staphylococcus carriers, or 18 per cent. Of the 28 nurses examined, five were found positive or approximately 18 per cent, and of the 22 doctors examined, four were found to be positive or approximately 18 per cent. It is rather interesting to find that there was approximately the same percentage of positives from both doctors and nurses. Of the 51 people from the lay group examined, only one was found positive or approximately two per cent. The fact that one out of every six in the medical profession is a potential pathogenic staphylococcus carrier, brings three questions to our minds: Why does the medical group have more pathogenic staphylococci on their hands than the lay group, in spite of more frequent washings and the more general use of germicides? What is necessary to prevent this high carrier rate? What is the danger of spread of infection by the doctor to his patients?

In answering the first question, we can readily see that the great difference between the two groups is mainly in their occupations. One might bring up the question of whether or not

the medical group had more chronic infections (colds, sinusitis, boils) due to pathogenic staphylococci than the lay group; but this, if it were true, would lead us back again to the occupational differences. In looking at the occupational differences we can also readily see that the main difference which would have any bearing on pathogenic organisms is that the medical person has far more direct contact with infectious cases of various types than members of the lay group. This then, would seem to answer our first question; that constant contact with infectious cases tends to establish a flora of these pathogenic bacteria on the skin in contact. In spite of frequent washing and the daily use of hand disinfectants, Price (25) could not rid his hands of pathogenic organisms for many weeks after he had ceased work in a septic ward. How then would any doctor in constant charge of infectious cases either rid his hands of these pathogenic organisms, or prevent such a flora from establishing themselves on his hands. Since direct contact with the infected part, or infected dressing, would seem to be the only way of establishing such a flora, prevention would appear to be in preventing such a contact. The wearing of rubber gloves in the dressing of all wounds would seem to answer this question.

One may bring out the view of Gillespie (11) that the pathogenic staphylococcus carrier condition on the normal skin is dependent upon the presence of pathogenic staphylococci in

in the nose; which in turn is directly associated with a history of chronic nasal disease. Whether or not doctors and nurses suffer more from chronic nasal disease than lay people is questionable. That nurses and doctors may have more pathogenic staphylococci in the nasal cavity than the lay group may be true, but even assuming this to be the case, constant association with the organisms would still seem to be the only explanation for the difference between the two groups. The question of whether the organisms are first established in the nose and then transferred to the hands or whether the reverse is true is also quite debatable.

The question of the danger of spread of infection by doctors to patients would, of course, depend on whether sterile rubber gloves were worn, the technique used in handling and dressing the wound, the type of wound, the resistance of the patient. However, there is no question but that the danger of spread of infection in the dressing of wounds by doctors without the use of rubber gloves is greatly increased.

In the course of our work the hands of four maids of a hospital were also tested for pathogenic staphylococci. One of the girls who had been employed at the hospital for a period of more than four years, was a pathogenic staphylococcus carrier, while the others that had been employed within the last eight months were not pathogenic staphylococcus carriers. This may serve further to bring out the point of association with pathogenic organisms and the establishment of these organisms as part of the flora of the hands.

Discussion and Results of
Pathogenicity Tests

Table I shows the relationship between the staphylococci of the two groups. Although the lay group contained more aureus organisms according to chromogenesis; the aureus strains from the medical group gave more positive reactions in all of the pathogenicity tests; 54 per cent giving positive mannitol fermentation as compared with 29 per cent from the lay group; 67 per cent liquefied gelatin, as compared with 36 per cent from the lay group. Thirty-one per cent of the Staphylococcus aureus from the medical group showed hemolysis on blood agar, while only 18 per cent from the lay group gave positive hemolysis on blood agar. There was perfect correlation between coagulase and alpha-hemolysin production on all tests carried out. Twenty per cent of the aureus from the medical group were positive in both of these tests; while only 0.8 per cent gave this reaction from the lay group. However, in comparing the albus strains from both groups we find that practically the reverse is true of the organisms giving positive tests. Hemolysis on blood agar is the only exception, 37 per cent of the medical group giving positive hemolysis, and only 16 per cent of the albus from the lay group. Twenty-nine per cent of the albus from the medical group gave positive mannitol fermentation tests and 41 per cent from the lay group. Twenty-two per

Table I. Summary of All Reactions of All Organisms Tested.

Number of Strains Giving Positive Reactions and Per Cent of Total Strains.

Medical Group	Total number of strains tested	Mannitol		Gelatin		Hemolysis on agar		Alpha-hemolysis		Coagulase		Fibrinolysis	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
S. aureus	111	61	54	75	67	45	31	20	18	20	18	20	18
S. albus	145	43	29	32	22	54	37	0		0		0	
Lay Group													
S. aureus	122	36	29	44	36	22	18	1	.8	1	.8	1	.8
S. albus	117	49	41	54	46	19	16	0		0		0	

cent from the medical group gave positive gelatin liquefaction tests, while 46 per cent from the lay group gave positive gelatin tests.

The relation of pigment to pathogenicity is further brought out in Table II. That although the golden yellow (true aureus) strains in the medical group gave the highest percentage of pathogenicity, 41 per cent, 9 per cent of the lemon yellow strains were pathogenic, and the cream colored strains showed 19 per cent positive. In the lay group the golden yellow strains showed two per cent positive for the coagulase reaction, or pathogenicity and none of the other shades of aureus showed any positives.

Another instance showing the relationship of pigment to pathogenicity may be brought out here. While debating as to whether we should pick three definite aureus non-hemolytic strains or three cream colored hemolytic strains, we decided in this one case to take three of each. The cream colored strains proved to be potential pathogens, while the golden yellow strains were not.

Table III shows the interesting relationship with regard to chromogenesis, hemolysis on blood agar, and coagulase production of the three aureus strains isolated from each person.

Table IV shows the reactions with the coagulase, fibrinolysis, gelatin liquefaction, and mannitol fermentation tests of all the pathogenic organisms. While practically all of the organisms (with the exception of one that did not liquefy gelatin) gave

Table II. Relation of Pigment Production to Other Tests.

Medical Group	Total number of strains tested	Mannitol		Gelatin		Hemolysis on agar		Alpha-hemolysin		Coagulase		Fibrinolysis	
		No.+	%	No.+	%	No.+	%	No. +	%	No.+	%	No.+	%
G.Y.	29	16	55	20	68	12	41	12	41	12	41	12	41
L.Y.	42	28	66	31	73	11	26	4	9.5	4	9.5	4	9.5
C.C.	40	17	42	24	60	22	54	7	17	7	17	7	17
albus	145	43	29	32	22	54	37	0	0	0	0	0	0
Lay Group G.Y.	47	14	29	16	34	10	21	1	2	1	2	1	2
L.Y.	63	18	28	23	36	9	14	0	0	0	0	0	0
C.C.	12	4	33	5	41	3	25	0	0	0	0	0	0
albus	117	49	41	54	46	19	16	0	0	0	0	0	0

G.Y. = Golden Yellow L.Y. = Lemon Yellow C.C. = Cream Colored

Table III. Relationship of All Aureus Strains Isolated From Each Person Having Pathogenic Staphylococci.

Strain	Pigment	Hemolysis on agar	Pathogenicity (Coag. + hem. +)	Strain	Pigment	Hemolysis on agar	Pathogenicity (Coag. + hem. +)
JB* 1	L.Y.	-	Path.	NER 1	C.C.	+	Path.
JB 2	C.C.	+	Non-path.	NER 2	C.C.	+	Path.
JB 3	C.C.	+	Non-path.	NER 3	C.C.	+	Path.
BS 1	G.Y.	-	Non-path.	TRC 1	G.Y.	+	Path.
BS 2	L.Y.	+	Path.	TRC 2	G.Y.	+	Path.
BS 3	C.C.	+	Path.	TRC 3	G.Y.	+	Path.
DM 1	C.C.	-	Non-path.	MEB 1	L.Y.	+	Path.
DM 2	G.Y.	-	Path.	MEB 2	L.Y.	+	Non-path.
DM 3	G.Y.	-	Path.	MEB 3	L.Y.	-	Path.
LB 1	G.Y.	-	Path.	DEM 1	C.C.	+	Path.
LB 2	C.C.	-	Non-path.	DEM 2	C.C.	+	Path.
AR 1	G.Y.	+	Path.	DEM 3	C.C.	+	Path.
AR 2	G.Y.	+	Path.	BCS 1**	L.Y.	-	Non-path.
AR 3	G.Y.	+	Path.	BCS 2**	G.Y.	-	Non-path.
				BCS 3**	G.Y.	-	Path.

* All similar initials refer to same person

L.Y. = Lemon Yellow
G.Y. = Golden Yellow
C.C. = Cream Colored

** From lay group

Table IV. Time Taken For Positive Tests For All

Pathogenic Organisms

No.	Pigment	Coagulase No. of hrs. for 4+ reaction	Fibrinolysis No. of das. for 4+ reaction	Mannitol No. of das. for 4+ reaction	Gelatin No. of das. for 4+ reaction
1	L.Y.	2	5	3	7
2	L.Y.	2	2	3	*
3	C.C.	2	2	2	3
4	G.Y.	2	1	3	6
5	G.Y.	2	4	3	6
6	G.Y.	2	1	3	6
7	G.Y.	3	4	2	6
8	G.Y.	3	4	2	6
9	G.Y.	3	4	2	6
10	C.C.	2	5	4	6
11	C.C.	2	5	4	6
12	C.C.	2	5	3	6
13	G.Y.	2	2	3	5
14	G.Y.	2	3	3	5
15	G.Y.	2	3	3	5
16	L.Y.	2	4	3	5
17	L.Y.	2	4	3	5
18	C.C.	2	5	4	5
19	C.C.	2	4	3	6
20	C.C.	2	3	4	5
21**	G.Y.	2	4	3	6

G.Y. = Golden Yellow

L.Y. = Lemon Yellow

C.C. = Cream Colored

* = Incomplete liquefaction

** = From lay group

positive mannitol and gelatin liquefying tests the great number of non-pathogenic organisms also giving these tests would seem to rule out the efficiency of these two reactions as tests for pathogenicity.

Table IV also shows that all organisms showing positive coagulase reaction gave positive fibrinolysis; however, some of these took as long as five days.

Forty-five of the aureus strains from the medical group gave definite positive hemolysis on blood agar after 24 hours of incubation (Table V). However, not all of the pathogenic Staphylococcus aureus strains from this group gave positive hemolysis on blood agar, 16 out of the 20 showed a positive reaction. This is a smaller proportion of positives than in either the mannitol fermentation, or gelatin liquefying tests, but lack of correlation here, also, would seem to rule out this test as a pathogenicity test.

Table V shows the relative amounts of toxin produced by the pathogenic strains. Many showing lysis in dilutions as high as 1:1280. It will be seen from this that alpha-hemolysin production correlates quite closely with coagulase. However, in the course of our work we ran tests on staphylococci obtained from the hands of a person with an infected finger. We did not include these results in our experiments, but we did find in this one instance that all three of the Staphylococcus aureus gave complete hemolysis

Table V. Hemolytic Titrations of Toxic Filtrates

Prepared From All Coagulase Positive Cultures.

No.	Pigment	Hemolysis on agar	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
1	L.Y.	-	4+	4+	3+	2+	1+	Sl.	-
2	L.Y.	+	4+	4+	3+	2+	1+	-	-
3	C.C.	+	4+	4+	3+	2+	2+	1+	Sl.
4	G.Y.	-	4+	4+	3+	2+	1+	-	-
5	G.Y.	-	4+	3+	2+	1+	-	-	-
6	G.Y.	-	4+	4+	4+	3+	2+	2+	Sl.
7	G.Y.	+	4+	4+	4+	3+	2+	2+	1+
8	G.Y.	+	4+	4+	4+	2+	2+	1+	Sl.
9	G.Y.	+	4+	4+	4+	3+	2+	2+	1+
10	C.C.	+	4+	3+	2+	1+	-	-	-
11	C.C.	+	4+	3+	2+	1+	-	-	-
12	C.C.	+	4+	3+	2+	1+	-	-	-
13	G.Y.	+	4+	3+	2+	2+	1+	-	-
14	G.Y.	+	4+	3+	2+	2+	1+	-	-
15	G.Y.	+	4+	4+	3+	2+	1+	-	-
16	L.Y.	+	4+	4+	3+	2+	1+	-	-
17	L.Y.	+	4+	4+	4+	3+	2+	1+	-
18	C.C.	+	4+	3+	2+	1+	-	-	-
19	C.C.	+	4+	3+	2+	1+	-	-	-
20	C.C.	+	4+	3+	2+	1+	-	-	-
21*	G.Y.	-	4+	4+	4+	3+	2+	1+	-

* From lay group

G.Y. = Golden Yellow
L.Y. = Lemon Yellow
C.C. = Cream Colored

in a 1:20 dilution and partial hemolysis in dilutions as high as 1:320, with a negative coagulase reaction.

That the alpha-hemolysin reaction is not as stable as the coagulase reaction was brought out in the reactions of our control organism, No. 161. This may be seen in Table VI. When first tested this strain showed complete hemolysis in dilutions of 1:40 and partial hemolysis in dilutions as high as 1:640. After two months of weekly transferring on plain agar this strain did not show complete hemolysis in any tube, and the highest dilution showing partial hemolysis was 1:40, while the coagulase test, which was done at the same time with the same transfer, showed complete coagulation of the plasma within one and one-half hours.

Table VI. Hemolytic Titrations of the Toxic Filtrates Prepared at Different Dates With the Control Organism, No. 161.

Date	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
1/6/40	4+	4+	3+	2+	2+	1+	-
1/13/40	4+	2+	2+	1+	Sl.	-	-
1/20/40	3+	2+	1+	Sl.	-	-	-
1/27/40	3+	2+	2+	Sl.	-	-	-
2/3/40	3+	2+	1+	Sl.	-	-	-
2/10/40	3+	2+	Sl.	-	-	-	-
2/17/40	3+	2+	1+	Sl.	-	-	-
2/24/40	2+	1+	-	-	-	-	-

Summary and Conclusions

In order to determine the relative occurrence of pathogenic staphylococci on the normal skin, and whether constant association with pathogenic organisms has much influence on this flora, we compared the flora of the hands of a group of medical people with a lay group.

Cultures were taken from the hands of 50 members of each group. If present, three *Staphylococcus albus* and three *Staphylococcus aureus* strains from the hands of each person were cultured and tested for pathogenicity by the coagulase, fibrinolysis, hemolysis on blood agar, the production of alpha-hemolysin, mannitol fermentation, and the gelatin liquefaction tests.

The coagulase test was used as the criterion of pathogenicity. The production of alpha-hemolysin correlated perfectly with coagulase production; while the other tests used gave varying results with both pathogenic and non-pathogenic organisms. From our experience with the pathogenicity tests employed, we conclude that the accuracy of the tests used--other than the coagulase and alpha-hemolysin--cannot be relied upon.

From our work with the staphylococci isolated from the members of each group, we found that one out of every six members of the medical profession and one out of every 50 members of the lay group is a pathogenic staphylococcus carrier.

Since the main difference between the two groups is their

occupation, it is indicated that constant association with pathogenic organisms may have the effect of establishing a flora of these organisms on the skin.

Since washing, and the application of germicides does not seem to reduce the pathogenic organisms any more readily than the non-pathogenic, the wearing of rubber gloves in dressing all wounds, and in handling infectious cases would appear to be a very important factor in cutting down the high rate of pathogenic staphylococcus carriers among the medical profession.

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