WHAT IS A STAPHYLOCOCCUS?[1]

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I. Introduction

Without doubt the staphylococci have produced disease and have been a continuing source of trouble for man since long before the days of recorded history. As an illustration of the probable antiquity of staphylococcal disease, I shall refrain from the temptation to refer to the Biblical patriarch, Job, whose distressing adversities included an attack of boils, for it is at least questionable whether this phase of his vexations actually was due to staphylococcal infection. Be that as it may, there is no question of the present wide distribution of staphylococci, or of the capacity of many of them to cause disease. Probably most individuals at some time have had personal experience with the staphylococci, at least in the form of an infected cut or other wound or a bout of food poisoning, which fortunately always is transient but often seems to be never-ending while it lasts. Other less fortunate individuals may suffer the insults of repeated attacks of boils, the distress of chronic osteomyelitis, the inconvenience of a hospital acquired infection, or certain other forms of staphylococcal disease.

The title of this brief essay, "What is a staphylococcus?", can be rephrased and expanded to ask "Can pathogenic staphylococci be described in terms of specific factors which have been shown beyond reasonable doubt to contribute to the establishment of infection?" It is my purpose to review some of the more prominent attributes of pathogenic staphylococci from this point of view.

It can be demonstrated readily in the laboratory that pathogenic staphylococci elaborate a variety of toxins and enzymes, and these substances can be shown experimentally to produce certain definite effects in living or in vitro systems. These cellular or metabolic products are encountered frequently in strains newly isolated from lesions and in strains capable of infecting experimental animals. It is easy to assume that they must play significant roles in the initiation of infection and the production of disease. Interesting and suggestive hypotheses have been proposed concerning the roles of these substances, which often are referred to as the "virulence factors." If it were possible to demonstrate conclusively that some of them play an active part in the establishment of infection, it should then be possible to characterize a pathogenic staphylococcus more exactly.

It is possible that the so-called virulence factors are no more than convenient markers which serve to identify strains that have the potential capacity to produce disease. It is even conceivable that staphylococci produce an as yet unidentified toxin, enzyme, or other metabolite which plays the major role. On the other hand, the common occurrence of some virulence factors in strains of known pathogenicity suggests that they contribute significantly to the economy of the cocci. It should be remembered that the pathogenic staphylococcus, normally and by preference, is a parasite. To become established in the living host, its economy requires that it possess mechanisms which enhance its chances

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of survival. The overt clinical manifestations of staphylococcal disease are no more and no less than a reflection of the operation of these mechanisms and, equally important, the responses of the host to them.

For the purpose of this discussion, these factors can be divided into two categories: those which enable the cocci to colonize the host and set up an infection, and those which damage the tissues or affect their normal functions. In the first category, assisting primarily in the establishment of infection, I shall mention coagulase, leukocidin, and hyaluronidase; in the second, the toxins and hemolysins.

II. Coagulase

The close correlation between the ability of staphylococci to clot blood plasma and their capacity to produce disease, and the corresponding absence of this property in nonpathogenic strains, have led to the assumption that the enzyme, coagulase, plays some role in the pathogenesis of disease. The assumption is supported by the fact that the plasma of species that are susceptible to natural or experimental infection contains the accessory "coagulase-reacting factor," a reagent which is not found in the blood of insusceptible animals.

It is easy to assume that coagulase may act either to produce intravascular clotting or thrombosis, or to lay down a fibrin barrier which contributes to the development of the lesion. However, there is no good evidence that in vivo clotting or thrombosis is exceptionally more prevalent in staphylococcal than in other microbial diseases, or that the fibrin network is related directly to the action of the enzyme.

It is conceivable that coagulase is of primary importance to the staphylococci only in the very early stages of infection, that is, during the time they are attempting to establish a foothold in the tissues; once this goal has been attained, the role of coagulase becomes less important. Upon gaining access to the tissues the cocci come in contact first with the tissue fluids and plasma, and only a little later with the leukocytes. The detailed experiments of Hale and Smith (9) indicate that at this early stage a fibrin coat may be deposited around the cocci which protects them from phagocytosis, at least temporarily or until such time as other virulence factors come into play to assist in the establishment of the lesion. An initial delay in phagocytosis has been observed by others (4); even this effect must be only transient. As was demonstrated by Rogers and Tompsett (18), coagulase-positive staphylococci ultimately are ingested by the leukocytes and are capable of survival within the leukocytes for relatively long periods.

It might be appropriate at this point to suggest that other surface components of the staphylococci may possess antiphagocytic properties. To draw an analogy with the pneumococci and the group A streptococci, it is possible that differences in the surface antigens of coagulase-positive and coagulase-negative staphylococci may help to explain the known differences in their resistance to phagocytosis. Very recently a surface antigen has been described by Morse which appears to be related to the resistance of pathogenic staphylococci to ingestion by the leukocytes (16).

It has been reported by Smith and Johnstone (20) and by Blobel and Berman (2) that a rapid depletion of blood fibrinogen and of total protein follows the intravenous injection of appropriate amounts of purified coagulase in rabbits. This occurs within 2 hr after injection and is followed by a return to normal values. In animals receiving larger doses the effect is intensified and the pulmonary capillaries and venules are found to be blocked by an unorganized coagulum; Blobel and Berman (2) interpret the coagulum as precipitated protein but not as true fibrin clot. Coagulase can be demonstrated in the precipitated material by fluorescein-antibody techniques, using labeled anticoagulase (2).

It is well known that coagulase-negative staphylococci are destroyed rapidly by normal human blood serum, whereas coagulase-positive strains resist this bactericidal action effectively. Studying these effects, Ekstedt and Yotis (5, 22) have demonstrated an antibacterial serum factor, localized in a water-soluble globulin fraction, which has a direct lethal and a partially lytic action on coagulase-negative cocci. In a concentration closely like that of gamma globulin in normal human serum, it markedly inhibits the oxidation of glucose by the coagulase-negative strains. Coagulase-positive staphylococci grow well and respire actively in normal serum; to exert a lethal action or to inhibit their oxygen consumption, a concentration of the serum factor is required which is eight times greater than that

necessary to inhibit the coagulase-negative strains. In the presence of appropriate amounts of purified coagulase, the coagulase-negative strains grow in serum and respire equally as well as coagulase-positive organisms. A quantitative relationship was found between coagulase and its protective effect on the cocci. Other staphylococcal metabolites such as polysaccharides and α- or β-toxin had no such effect. Yotis and Ekstedt (22) have suggested that coagulase may set up a physical barrier which prevents the reaction of the serum factor with its substrate in the cells.

Evidence in support of a role of coagulase is suggested by in vivo experiments. Thus, it has been shown that the virulence of staphylococci is enhanced significantly when the cocci are suspended in purified coagulase or in plasma before intracerebral or intraperitoneal injection (5); and a lesion may be produced in the skin of a normally insusceptible animal such as the guinea pig when the site is prepared by the injection of a coagulable plasma (21). Blobel and Berman (2) found that rabbits hyperimmunized to purified coagulase were fully protected against fibrinogen depletion and pulmonary vascular blockage, even when they used doses of coagulase that were lethal for normal animals. Although Boake (3) was able to demonstrate some degree of active or passive protection by anticoagulase, the antibody did not appear decisively to influence the ultimate course of experimental infection.

Various observations have been reported which seem to throw doubt on the relation of coagulase to pathogenicity. For example, a coagulase-negative, but toxigenic strain has been described that was fully infective for coagulase-immune and for normal rabbits (3), and a strain possessing both free and bound coagulase was found to be avirulent for rabbits (12). Though such observations cannot be ignored, they do not appear to constitute especially convincing arguments against the pathogenic role of coagulase. More important, such observations emphasize the fact that virulence results from the sum of activity of all factors at the immediate disposal of the cocci, aided by the particular circumstances of the infection. In both of the instances cited, the aberrant strains were variants of parents which possessed a full complement of virulence factors. Most, if not all, strains of staphylococci have the potential for variation and may give rise to forms whose biological and pathogenic patterns differ from those of the parent; changes from the norm must be expected both in the laboratory and in nature.

Regardless of the actual role of coagulase, the fact remains that the coagulase test is a valuable laboratory procedure. The reaction accompanies demonstrated pathogenicity so consistently that one cannot escape the implication that strains which give a positive test at least are inherently capable of disease production. The bulk of the evidence favors this view; if it is wrong, the error is on the side of safety for the patient.

III. Leukocidin

One may suppose that the staphylococci would be served importantly in the establishment of an infection by the elaboration of a substance which counteracts some defense mechanism of the host. Such an agent is leukocidin, which may be regarded in a sense as a defense mechanism of the cocci.

Three leukocidins have been described. The so-called "Neisser-Wechsberg leukocidin" probably is identical with α-hemolysin; it is active only on rabbit leukocytes, and its demonstration by the "bioscopic" method would seem to reflect merely the ability of α-hemolysin to inhibit the respiration of the leukocytes. In contrast, the two other leukocidins, the well-known "Panton-Valentine leukocidin" and "leukolysin" (8), are active on human leukocytes as well as those of the rabbit and some other species. The two could be referred to appropriately as "human" leukocidins. They differ from each other in certain significant properties and in the manner of their action on the leukocytes, the leukolysin appearing to have an effect directly on the nucleus of the cell. Although the Panton-Valentine leukocidin often occurs together with α-hemolysin in cultures, it is produced independently and is antigenically distinct. The leukolysin is formed in direct quantitative relationship with δ-hemolysin, with which it shares many of the same properties. Because of the closely similar properties of the two substances, it was suggested by Gladstone and van Heyningen (8) and by Jackson and Little (10) that leukolysin and δ-hemolysin are identical. Confirmation of this relationship must await their adequate purification.

The role of leukocidin is quite obvious. Be-

cause phagocytosis is an important primary defense mechanism of the host, an agent directed toward destruction of the white cells or inhibition of their activity would enhance the opportunities of the cocci for survival. The demonstrated fact that pathogenic staphylococci may survive within human leukocytes for some time after they have been ingested suggests that leukocidin may inhibit the metabolic properties of the leukocytes that are responsible for the digestion or destruction of the cocci.

IV. Hyaluronidase

In a sense comparable to the effect of leukocidin in counteracting a defense mechanism of the host, the action of the mucolytic enzyme, hyaluronidase, would be expected to enable the cocci or their products to pass through the barrier set up by the intercellular ground substance of the body tissues. Though this hypothesis is intriguing, a spreading factor hardly is compatible with the characteristic tendency of staphylococcal lesions to localize. During the initiation of a naturally acquired infection, the number of cocci that come in contact with the tissues probably is not great. Even if they should produce considerable hyaluronidase, it is probable that the potential infectivity of the cocci would be reduced by the simple fact of their dilution by the spreading factor. It is possible also that the dispersed cocci would be more effectively disposed of by the host's protective mechanisms.

Work by Rogers (19) indicates that extracellular enzymes which are formed at or near the cell surface of the staphylococci may be inhibited by certain negatively charged synthetic polymers, which he terms "macroanions." He suggests that "natural" macroanions such as chondroitin sulfate, hyaluronic acid, heparin, or nucleic acids may inhibit the formation or action of staphylococcal enzymes in the body.

It is conveivable that staphylococcal hyaluronidase may contribute to the severity of mixed infections with other bacterial or viral agents, by spreading both the organisms and their products. Experimental tuberculous infection is said to be enhanced in the presence of hyaluronidase-producing staphylococci. A synergistic action between vaccinia virus and staphylococci has been described, and staphylococcal complications of influenza are well known. This raises the further question whether a virus may potentiate a staphylococcal infection.

V. Toxin

The intense necrosis that follows the intradermal injection of many staphylococcal culture filtrates in experimental animals, and the rapidly fatal result of intravenous injection, give rise to the assumption that toxin may play a part either in the evolution of an infection or in the clinical manifestations of staphylococcal disease.

As bacterial toxins go, staphylococcal toxin (or α-toxin) is far less potent than those, for example, of the diphtheria bacillus or the clostridia. Nevertheless, when it is restricted to a limited area, it is possible that staphylococcal toxin exerts a direct effect on the tissues. It is probable that the products of tissue damage resulting from local necrosis may help to evoke the inflammatory response. Not all strains of staphylococci produce a potent dermonecrotic, lethal toxin. In our experience, not over about one-half of the strains isolated from active lesions are capable of doing so.

That toxin can be produced in vivo was demonstrated in our laboratory by the intravenous injection of washed, toxigenic strains in rabbits (15). Except for some elevation of temperature, the animals so treated appear to be clinically normal for 18 to 24 hr after injection. Then, during a brief agonal period of only 20 to 30 min just preceding death, they exhibit symptoms identical with those shown by rabbits receiving toxin; this is accompanied by electrocardiographic changes indicative of myocardial damage. Antitoxin protects against these effects, including damage to the myocardium, but it does not prevent death from infection.

One must use caution in applying these experimental observations to an interpretation of the role of toxin in staphylococcal disease in man. However, it is conceivable that toxin may contribute to the severity of the disease as it is encountered, for example, in acute hematogenous osteomyelitis in children, or as was graphically illustrated by the now classic Bundaberg disaster in 1928. We have observed a close similarity between the manifestations of toxicity in experimental animals and the clinical symptoms of acute toxemia in children whose blood cultures yielded highly toxigenic staphylococci (13). The

fact that acute toxemia in children was controlled by adequate therapeutic doses of staphylococcal antitoxin, often with dramatic amelioration of the symptoms, suggests that toxin may play a not inconsiderable role in this form of staphylococcal disease (14). Age seems to be a function in susceptibility to the toxin, for adults rarely show the acute symptoms exhibited by children, in spite of invasion of the blood stream by a highly toxigenic strain.

The recital of these effects in experimental animals and in man does not identify the specific metabolic product responsible for them. Based upon rather strong circumstantial evidence derived from their parallel occurrence in culture filtrates, their reactions to physical and chemical agents, and their neutralization by antisera, it is generally held that the necrotic, lethal, and hemolytic effects of staphylococcal toxin are due to a single entity. Some of these observations have been made with preparations of relatively high purity. However, the question is reopened by the examination of culture filtrates by gel diffusion techniques; these studies reveal a mutiplicity of antigens which deserve further investigation (7).

VI. Hemolysins

A characteristic of staphylococcal culture filtrates showing lethal and dermonecrotic properties is their ability also to hemolyze rabbit erythrocytes. The intravascular hemolysis, serosanguinous exudate into body cavities, or hemorrhages in the tissues that are found in rabbits given toxin intravenously are readily explained by the special sensitivity of the red blood cells of this species to α-hemolysin. In man, however, the situation is quite different, for α-hemolysin has little or no effect on human erythrocytes. Particularly for this reason, it appears to be unwise to attribute a role to α-hemolysin in the production of human disease.

The δ-hemolysin, which is produced by the majority of strains, does hemolyze human red blood cells. In experimental animals it evokes a relatively mild reaction in the skin, with no necrosis, and it is not lethal when injected intravenously. No evidence has yet been offered to suggest that the lysin is involved in toxic or other manifestations of disease in man. The possible relationship and identity of δ-hemolysin and leukocidin has already been mentioned.

VII. Enterotoxin

Although inferences as to the role of α-toxin are based largely upon circumstantial evidence, there can be little doubt of the relation of staphylococcal enterotoxin to food poisoning. This toxin is distinct from all other staphylococcal products antigenically and, of course, in its clinical effects. Staphylococcal food poisoning appears to be more common than any other type of microbial food poisoning. Probably very few individuals have escaped an attack. The mode of action of enterotoxin—whether on peripheral sensory structures, on smooth muscle of the alimentary tract, or directly on the emetic receptor site—is controversial and remains to be determined. There is some evidence that at least part of the symptoms in man may result from a direct action of the toxin on the gastric mucosa (17).

Very few clinical forms of staphylococcal disease have been shown to be related to specific types of staphylococci. Food poisoning is one of the few, for the capacity to produce enterotoxin is restricted to a relatively small number of strains. It is significant that the great majority, if not all, of the strains incriminated in staphylococcal food poisoning have been found to belong to phage type 42D or to show typing patterns in phage group III.

VIII. Host-Parasite Relationships

A predominating role in the production of staphylococcal disease cannot be assigned to any single virulence factor. The capacity of a strain to induce infection is derived from the sum total of the properties at its command.

Infection does not result from the mere presence of the cocci in the tissues. When one considers the wide distribution of staphylococci and the numerous opportunities for exposure to them, the incidence of staphylococcal disease, by comparison, actually is not great. To produce an infection experimentally in the human skin or to infect a laboratory animal requires an inoculum of the order of one million or more cocci. Under natural conditions it is very likely that only a relatively few cocci are involved at the beginning of colonization. Once they have gained access to the tissues, the cocci must find there a

favorable environment; the opportunity to grow and multiply is of immediate importance. The success or failure of the cocci is a function of the host fully as much as it depends on the activity of the cocci, for the two are closely interrelated.

Circumstances favorable to the cocci are contributed by the local conditions in the tissues where they lodge. Among numerous examples may be mentioned the devitalized tissues of a wound, temporary shelter from the body's natural defenses such as might result from lodgement on a burned area, and local pressure or friction which interfere with physiological function. Foreign bodies in the tissue serve very effectively to enhance the establishment of the cocci. Elek (6) demonstrated convincingly in human volunteers, and James and MacLeod (11) in mice, that as few as 100 to a few thousand staphylococci will produce a local abscess when they are introduced into the skin on a silk suture and the suture is left in place. Metabolic products elaborated by the host may contribute favorably or adversely, either by supplying acceptable nutrients to the cocci or by inhibiting their growth processes. It is well known that the kind and degree of the host's reaction to infection are influenced by such factors as the age of the individual, his state of nutrition, and metabolic or endocrine imbalances.

IX. Conclusion

Twenty-five years ago, when discussing the then recent advances in knowledge of the staphylococci, Dr. Joseph Bigger (1) commented: "Twenty years ago most bacteriologists believed that everything of importance in connexion with staphylococci was known, and yet the intervening years have been fruitful in fresh discoveries." During the two and a half decades since he made that comment, many significant observations have been reported; but surely he would be rash indeed who would conclude that even now the final chapter on the staphylococci has been written. In spite of, and even because of, recent advances in knowledge, this much is quite apparent: the staphylococci still are a challenge.

The challenge presented by the staphylococci takes several forms. First, it has become important to identify exactly those factors that are responsible for infection. The implication of their roles is evident, but the final proof is lacking. This requires that they be isolated, purified, and characterized. Fortunately there is a promising trend in this direction; a number of investigators in several laboratories are now applying modern biochemical and biophysical techniques to the isolation and study of staphylococcal toxins and enzymes.

A challenge is presented by the very evident ability of staphylococci to persist in the body for long periods of time. The persistent nasal carrier, the tendency for boils to recur in successive crops, and the case of osteomyelitis which lights up after months of dormancy, all indicate that staphylococci can remain viable and latent in the tissues. That a person may harbor the same strain for months or years has been demonstrated repeatedly by phage typing. A fruitful field of investigation is found in the study of the mechanisms by which the cocci and the host are able to strike a balance of coexistence, which does not appear to be especially detrimental to the cocci and is tolerated by the host. What is the nature of the imbalance which provokes a relighting of the infection or permits a state of subclinical infection just short of overt disease? Many staphylococcal lesions, especially superficial ones, tend to be self-sterilizing; some insight into the nature of staphylococcal virulence would be gained by investigation of the mechanism responsible for this spontaneous healing. Valuable information should be derived from a study of the local metabolic processes at these sites.

The problems presented by antibiotic-resistant strains, particularly those with multiple resistance, suggest that possibly it is time to think in terms of supplementing the antibiotics by increasing the body defenses. Admittedly, past attempts at immunization have been rather disappointing. Undoubtedly this has been due, at least in part, to lack of precise knowledge of the staphylococcal factors involved in infection. Should it become possible to identify their roles more exactly, it does not seem beyond the realm of possibility that enhancement of the body defenses directed specifically toward these factors might be feasible. If this is idealistic and utopian, my only rebuttal is to refer to the prophet, Joel, who said, "Your old men shall dream dreams, your young men shall see visions."

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