BACTERIAL ASPECTS OF THE PROBLEM OF STAPHYLOCOCCAL INFECTION AND DISEASE*

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DURING the past two decades there has been an abundant increase in knowledge of the biology of *Staphylococcus aureus*. Nevertheless, we remain in virtual ignorance of the bacterial factors responsible for the occurrence and persistence of staphylococcal infection and disease. However, it is possible to frame the relevant questions more precisely than before by utilizing current information on the structural, metabolic, and toxic properties of *Staph. aureus*.

It is generally conceded that staphylococcal disease is frequently preceded by asymptomatic infection with the offending organism. Hence the carrier state is a risk not only to contacts but to the carrier himself. However, it is of particular significance that although asymptomatic carriers are common, serious staphylococcal disease is rare in the normal population. Understanding the dynamics and critical balance found in the carrier state may ultimately be as important as delineating the host and bacterial factors at work in overt disease.

Of all the bacteria with which man has an early and continuing association, *Staph. aureus* is near the top of the list. After the first week or 10 days of life, 60 to 90 per cent of infants will be colonized in the anterior nares by *Staph. aureus*. In urban areas this is apparently true whether delivery occurs at home or in a hospital. The carrier rate decreases during the first few years of life, and then gradually increases to the adult rate of 40 to 60 per cent. Certain adults never harbor *Staph. aureus*, others are always carriers, and the largest group are intermittent carriers, often of the same strain. There are no known reasons for these variations in susceptibility to staphylococcal infection, but it is assumed that they are related to the host rather than the microorganism.

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One central question that remains unanswered is: Why is man so readily colonized by *Staph. aureus*? It is notoriously difficult to induce a persistent carrier state in most animal species. Obviously, either the human nasal mucosa is intrinsically more "hospitable" or factors extrinsic to the mucosa proper may be at work. Studies on the role of bacterial interference in the nasal carriage of staphylococci in man suggest that the microflora play a significant role in determining whether persistent colonization will take place. The broad biologic implications of the interference phenomenon are far-reaching. Understanding precisely how one species or strain of bacterium can influence the acquisition by the host of another organism will be particularly relevant to the staphylococcal problem.

There are many unanswered questions regarding the status and physiology of the organism in the carrier state. For example it is proper to ask whether the staphylococci are extracellular or whether they remain viable within nasal epithelial cells or long-lived phagocytic cells. Conceivably the titer of organisms could determine whether the charge of bacterial cells delivered to a vulnerable area, such as a wound, would be sufficient to overcome the rather solid host defense mechanisms of normal man. Yet there are no reliable quantitative data on the numbers of staphylococci normally resident in or on human tissue. It would also be useful to know, for the same reasons, whether staphylococci are metabolically active and multiplying or if they are dormant. Are there differences in this regard between the epidemiologically virulent and community strains of *Staph. aureus*? It is perhaps worth noting that the recent development of a multitude of sophisticated experimental techniques in many spheres of biology has not provided useful tools for the study of these deceptively "simple" kinds of problems in microbial ecology.

The possible role of aberrant bacterial forms in both the carrier state and disease has attracted a great deal of interest. L-forms and the small colonial variants (G-forms) can be induced by a variety of natural products and appropriate antibiotics. The L-forms have variable deficiencies in the content of cell-wall material and are therefore usually structurally unstable in an environment of low osmolarity. G-forms appear to have an adequate cell wall.[1]

Interest in these forms derives from two properties found most particularly in the L-forms. The first is their resistance to certain anti-

microbial agents, especially those that function by inhibiting synthesis of the cell-wall. The second is the difficulty in culturing these variants on standard media. Thus it is conceivable that an L-form might be induced *in vivo* by an antimicrobial agent such as penicillin and then, when the agent is withdrawn, reversion to parental type might occur. Alternatively, a stable "L" might be produced, i.e., one which would survive in this state in the absence of the inducing agent. Subsequent reversion could occur by a variety of mechanisms as yet unknown.

Invocation of L-forms and other abnormal forms in the concept of staphylococcal disease and infection provides numerous easy answers to questions thus far unsolved. They could explain recrudescence of the carrier state or disease with the original organisms despite what appears to be bacteriologic eradication of culturable organisms.

Nevertheless it must be borne in mind that there are certain restrictions that might make it difficult for these variants to survive within the host, especially the requirement for an environment in which the osmolarity is greater than normal. This theoretical objection is readily met by enumerating the instances in which L-forms have been shown to occur, persist, and revert within animal tissues.

A more important objection is simply the fact that the isolation of staphylococcal L-forms from human tissues has proved difficult. The evidence for their existence has often been based on the less than satisfactory method of demonstrating an increased efficacy of treatment of disease with both an antibiotic which affects the cell wall and one which affects L-forms such as tetracycline. Especially important is the need for identification of an isolated L-form with respect to its origin. If the L-form spontaneously reverts or can be encouraged to revert by laboratory manipulations, there is no problem in determining the parental species of the organism. Other methods, which thus far have not been applied very often in relation to staphylococci, are to determine whether a known property of the parental form can be demonstrated in the L-form. Antigenic analysis is one method that could be employed since some of the antigens and toxins of the parental organism are often produced by the wall-less variants and species-specific properties of *Staph. aureus* could be identified. A more cumbersome and time-consuming method of identification could be developed by utilizing some of the bacteriophages for *Staph. aureus* which attack a variety of strains and are thus not utilized in the International Typing Set. Identification by

means of bacteriophage lysis or growth would be dependent upon the existence of sufficient cell-wall material to provide receptor sites for the phage or, alternatively, the use of infectious nucleic acid from the phage. The scheme for antigenic analysis seems more hopeful of being realized.

It is certain that understanding the ecology of staphylococci is of paramount importance in ultimately determining the dynamics of staphylococcal disease. This is true of many of the other bacterial diseases which are now prevalent, particularly within hospital environments where the host is subjected to a variety of surgical and medical treatments unheard of years ago. These manipulations of the host make him prey to all infectious diseases, but bacterial diseases caused by such organisms as pneumococci and streptococci are less common, primarily because of the relative efficacy of antimicrobial agents. Bacterial diseases afflicting these susceptible populations are generally due to organisms which normal humans carry with few if any ill effects and to organisms which are resistant to a variety of antimicrobial agents. Often both properties coexist as in the case of *Staph. aureus* and the enterobacteria.

It is relatively easy to develop a thesis in which the essential element is that the organisms in question are really avirulent in the classical sense but that in the face of a breakdown in local or general host-defense mechanisms—humoral or cellular—they do have the ability to multiply, invade, and produce tissue damage. Such a thesis implies that one recognize orders of pathogenicity and virulence. It implies also that under normal circumstances serious disease generally does not occur. When the host is abnormal, certain organisms such as *Staph. aureus* are then more likely to cause disease, and even bacteria such as *Staph. epidermidis (Staph. albus)*, which have even less apparent propensity to cause disease, can produce serious and even fatal illness.

On the other hand, present evidence suggests that this thesis is not sufficient. In addition to the reasonable question of finding the bacterial factors that are responsible for even these lesser orders of pathogenicity and virulence, there is unequivocable epidemiological evidence in the case of *Staph. aureus* that there is a hierarchy of disease-inducing properties among the strains.

In nosocomial disease certain strains of *Staph. aureus*, as determined by bacteriophage typing, are more commonly found than others; e.g.,

strains in phage groups I and III including the 80/81 complex. These organisms as compared with others must be considered to be epidemiologically virulent. Often the elimination of carriers of these strains from contact with susceptible populations will reduce the incidence of hospital-acquired staphylococcal disease, even though other staphylococci remain within the setting.

It is perfectly possible that there are no unique properties of these epidemiologically virulent strains in terms of the production of "toxic" factors or other determinants which would make them relatively more pathogenic. As indicated previously this may be a manifestation of a quantitative difference in the carrier state such that the dose or charge of organisms delivered is greater than can be handled when host defenses break down.

No matter how the question is ultimately answered it remains pertinent to inquire into the known properties of *Staph. aureus* which might play a role in the development of disease.

The classical method of examining bacterial pathogenicity deals essentially with three types of question. Does the organism have structural components which make it resistant to phagocytosis by cellular defense mechanisms of the host? Is it capable of being killed by the host's phagocytic cells or humoral factors? Does it produce toxins or enzymes which are injurious to the tissues and cells of the host?

Structural inhibitors of phagocytosis are exemplified by the type-specific capsular polysaccharides of the pneumococci and by the surface type-specific M-protein of the group A streptococci. In the absence of antibody to these substances the organisms are not ingested by phagocytic cells and multiplication of the organisms *in vivo* proceeds without restriction. The surface of conventional *Staph. aureus* strains does not reveal any capsular material when viewed under the light or electron microscope. There are, however, a certain few strains which do have a well-defined capsule. The capsular material has been isolated and is primarily composed of the acidic aminosugar glucosaminuronic acid. By means of normal rabbit serum and rabbit polymorphonuclear leukocytes used in *in vitro* test systems it has been shown that this material is clearly antiphagocytic and that ingestion of the strains is promoted by a specific antiserum directed against the capsular polysaccharide.

There are three important observations which make it unlikely that

this capsular polysaccharide plays a significant part in human staphylococcal disease. In test systems in which human granulocytes and serum are employed the capsule does not render these strains resistant to phagocytosis, and ingestion occurs efficiently. The distribution of these strains bears no relation to the occurrence of disease, for they have been isolated from healthy tissue as well as from diseased areas. Finally, in any collection of staphylococcal isolates from lesions, encapsulated strains are only rarely found.

Other surface antigens of *Staph. aureus* are probably numerous, since many subtypes have been recognized by agglutination reactions. One of these antigens, found in virtually all strains, is attached to the cell wall and is a protein designated Antigen A or Protein A. Protein A when first isolated was found to react with all normal human adult sera, it was believed that this was further evidence for the ubiquity of staphylococci within the environment of man. Recently it has turned out that the reaction of Protein A with human sera is in part a nonspecific phenomenon.[2] To be sure the protein also behaves as a true antigen but in large measure the observed interaction is due to the nonimmune precipitation of immunoglobulins by Antigen A. Indeed the majority of gamma globulin from normal sera can be precipitated by the protein and it has come to be a useful reagent for the isolation of γG-globulin. Thus far, however, there is no evidence that the presence of Protein A plays a role in the pathogenicity of staphylococcal disease although injection of gamma-globulin-Protein A complexes produces an Arthuslike reaction.[3]

The species-specific antigen of *Staph. aureus* is also a cell-wall antigen termed "Polysaccharide A." It is one of the group of carbohydrate compounds called "teichoic acids," which are composed of polymers of either glycerol or of ribitol phosphate. In the case of Polysaccharide A the polymeric unit is ribitol phosphate. Appended to this backbone are *N*-acetylglucosamine residues as well as ester-linked *D*-alanine. The antigenic determinant of the compound is the glucosamine moiety and variations in reactions seen with antigens isolated from different *Staph. aureus* strains is dependent upon the glycosidic linkage of the determinant to the backbone. In some strains either the α- or the β-linkage predominates; in others both bonds are present in roughly equal proportion. All human sera have antibody against *Staph. aureus* teichoic acids. It has been suggested that the

addition of purified teichoic acid to *in vitro* phagocytic test systems inhibits phagocytosis to a certain extent but the relation of this finding to human staphylococcal disease remains uncertain, particularly since all human sera do contain antibodies.

Over-all there is little evidence that *Staph. aureus* strains are resistant to ingestion by human granulocytes in the presence of normal human serum in the same way that group A streptococci are resistant in the absence of antiserum against the type-specific M-protein. Killing of *Staph. aureus* within phagocytic cells also appears to proceed at a relatively normal rate with the exception, of course, of the cells from children with granulomatous disease.[4] This does not mean that antiphagocytic surface substances are not produced *in vivo* which are quantitatively or qualitatively more effective. It may also be true that the slightly slower rate of ingestion and killing of *Staph. aureus* by phagocytic cells when compared to that of *Staph. epidermidis* is critical within the host.

There are numerous extracellular products of *Staph. aureus* which are potentially damaging to tissues and cells. Among them are the hemolysins, leukocidins, coagulase, hyaluronidase, staphylokinase, nucelease, and lipase. Almost all of them have been implicated at one time or another as primary virulence factors. Alpha hemolysin, which also has necrotic activity (alpha-toxin), and coagulase have previously been the major suspects. In recent times attention has focused upon the leukocidins active against human leukocytes. These comprise delta-hemolysin and the Panton-Valentine leukocidin, which has no hemolytic activity. Yet the fact remains that no conclusive evidence has been adduced which would make one believe that any one of the factors is of paramount importance in the production of staphylococcal disease. Thus there have been many observations that have demonstrated that isolates from human disease may lack one or several of these products. In addition, normal human sera usually contain antibodies against most of the extracellular substances. Finally, results from animal experimentation—always a hazardous extrapolation since there is no satisfactory animal model of human staphylococcal disease—have not pointed clearly to any one particular factor.

As in the case of phagocytosis and intracellular killing of staphylococci, it would be unwise to dismiss the possibility that the toxins and enzymes of *Staph. aureus* play a role in the induction and per-

sistence of staphylococcal disease. Given the epidemiology of staphylococcal disease and the ecologic setting, it is reasonable to suppose that serious staphylococcal disease is the result of a multicomponent interaction. If there is a break in local or systemic host defense mechanisms which maintain the staphylococci in the carrier state, any or many of the structural, metabolic, and toxic features of *Staph. aureus* may serve to give the organism the advantage with resultant invasive disease. It remains uncertain which of the bacterial factors is the key. It is also unclear whether epidemiologic virulence is dependent upon quantitative or qualitative differences between organisms and their products or, as suggested above, by differences in the extent of the carrier state such that a greater inoculum is present. Also there is still the possibility that an unknown pathogenicity factor is produced *in vivo* which has yet not been demonstrated or characterized.

Studies on active immunity with staphylococcal products have shed little light upon the role in disease of antigenic factors of *Staph. aureus.* There are adherents as well as detractors of such procedures as autogenous vaccination and other forms of immunization with staphylococci and staphylococcal products. Certainly the successes generally have not been dramatic and are usually subject to the criticism that staphylococcal disease may improve as a result of nonspecific immunization. Proper controls consisting of subjects injected with heterologous vaccines are not often used. However, as Louis Greenberg's studies have shown, some of the failures in immunization may not have been the result of random effects but of the lack of essential protective antigens in the preparations. It is pertinent to note in this regard that there are several antigenic types of staphylococcal products such as coagulase. Further information on effects of immunization in man is needed but because of the nature of the disease pattern it may be difficult to obtain.

One of the aspects of *Staph. aureus* that has relevance to the topic of this symposium concerns the reasons for the most troublesome aspect of staphylococcal disease—the high frequency of antibiotic-resistant strains.

In recent years another dimension has been added to the biology of antibiotic-resistant staphylococci. It now appears that the genetic determinants which result in the production of penicillinase which determines the state of penicillin resistance are extrachromosomal

genes. The extrachromosomal units carrying these genes are termed plasmids and are similar in most respects to the episomes of the enterobacteria that carry the R or resistance factors. In *Staph. aureus* it now appears that in addition to penicillin resistance, resistance to heavy metals and in certain instances insensitivity to the macrolide antibiotics, tetracyclines, and chloramphenicol may also be determined by plasmid genes.[5]

Like R factors, plasmids can be transferred from one organism to another. Unlike episomal transfer, transfer of plasmids from one strain to another is not accomplished by the process of conjugation but by transduction. Most strains of *Staph. aureus* are lysogenic. In any growing lysogenic population a certain percentage of the organisms undergo spontaneous induction with resulting lysis of the organism and liberation of mature bacteriophage. Part or all of the plasmid may be incorporated into the staphylococcal bacteriophage and, upon injection into a competent recipient, transduction of the plasmid and hence of the resistance-determining genes can occur. Such transfer has not only been demonstrated in the test tube but has also been shown to occur *in vivo* in mixed infections produced in mice.[6]

It does not seem likely that the chances for transduction of genes determining antibiotic resistance are as great as in the case of *in vivo* episomal transfer. Nevertheless the lysogenic character of the organisms usually isolated as well as the occasional natural occurrence of infection or disease with two strains makes it possible that genetic transfer could play a significant role in the occurrence and spread of antibiotic-resistant staphylococci.

Irrespectively of the origin of the antibiotic resistant strains, whether by mutation or gene transfer, it is clear that the prevalence of these organisms is essentially dependent upon the use of antibiotics. Therefore a primary concern for all physicians must be to guard against the indiscriminate use of these agents.

The observations on the potential role of staphylococcal bacteriophage in the epidemiology of antibiotic resistant strains make it wise to proceed cautiously in an attempt to reintroduce phage therapy as a nonchemotherapeutic approach to staphylococcal disease. In addition to plasmid genes other staphylococcal genes can be transduced, and it is well known that the lysogenic state itself has profound effects on the biology of the organism. However, bacteriophage therapy at

one time had its supporters, and it is known that lytic phage will infect and destroy staphylococci *in vivo*.[7]

An injunction to the fictional discoverer of staphylococcal bacteriophage in Sinclair Lewis' *Arrowsmith*[8] still applies to all unproved methods of treatment of staphylococcal disease. The investigator had given "some of the original principle to a doctor in the Lower Manhattan Hospital for the treatment of boils and from this had excited reports of cures, more excited inquiries as to what this mystery might be." Reporting these results, his superior replied, "You want fake reports of cures to get into newspapers and have everybody in the world that has a pimple come tumbling in to be cured?"

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