OBSERVATIONS ON THE NATURE OF STAPHYLOCOCCAL INFECTIONS*

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DURING the past decade, staphylococcal infections in hospitalized patients have gradually emerged as one of our more distressing therapeutic problems. Recurrent outbreaks of staphylococcal disease are now common in nursery units where newborn infants are congregated. Important surgical procedures are occasionally compromised by serious staphylococcal sepsis. Staphylococcal infections are a common terminal event in the course of debilitating medical illnesses which require hospital care[1].

It appears progressively clear that our present problems with this microbe reflect a shift in the nature of infections which can produce life-threatening disease in medically sophisticated communities today. Before the development of antimicrobial drugs, acute bacterial infections were a major cause of human illness and death. Here the contest was explosive and the outcome was determined in a relatively short span of time—either the patient died or the infection was controlled and eradicated by the host.

As these infections have come under the control of antimicrobial agents, certain infections caused by microorganisms commonly residing within most normal human beings have emerged to prominence and may be increasing in incidence. Infections caused by staphylococci, certain enteric bacilli, and fungi—all normal inhabitants of normal humans—are now common in our hospitalized patients.

These microbes and the infections they produce differ in certain important characteristics from the acute bacterial problems of yesterday. All commonly produce less decisive, focalizing disease in which

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both the human host and the bacterial parasite can survive together for long periods of time. Classic humoral immunity does not arise, or if present, does not appear to contribute materially to recovery. Perhaps of most significance, these microorganisms *appear to require local or systemic alterations in host resistance before progressive infection ensues.* Indeed, these microbes might be considered the jackals of the microbial parasite world: unable to mount an attack against normal humans, they commonly invade in other disease states where host resistance appears crippled or compromised.

There is much evidence to suggest that host factors play a more important and more complex role in such endogenous infections than in acute bacterial disease. In the case of staphylococcal infections, the status of the skin, the reticuloendothelial system, the bone marrow, or other host tissues has seemed of more importance than the *virulence* of staphylococcal strains in determining whether serious clinical infection will occur. Our increasing intrahospital difficulties with staphylococci appear, in certain measure, to be caused by the disease states which require hospital care, or by the things which we do to patients in modern hospitals which alter human resistance.

Operating on this thesis, we have focused our experimental attention on the ways in which host tissues deal with experimentally induced staphylococcal infections. This evening I should like to present certain observations derived from these studies which tend to set the staphylococcus apart from other gram-positive cocci with which it is frequently grouped. Recognizing the pitfalls of extrapolating from animal experiment to human disease, these experimental facts allow some tentative guesses about certain peculiar features of staphylococcal disease in man, and can perhaps form a background for the problems surrounding treatment to be presented by Dr. Walter*.

The behavior of staphylococci within phagocytic cells. Some years ago we noted that pathogenic staphylococci were steadily ingested by human leukocytes in systems in which other virulent microbes failed to be phagocytosed. Despite the fact that virtually all staphylococci were ingested by leukocytes, little or no killing of staphylococci could be detected. Subsequent studies indicated that certain staphylococci could survive for long periods within both human and rabbit polymorpho-

* Paper on *The Control of Staphylococcal Sepsis,* presented later at this same session by Carl W. Walter, Associate Clinical Professor of Surgery, Harvard Medical School.

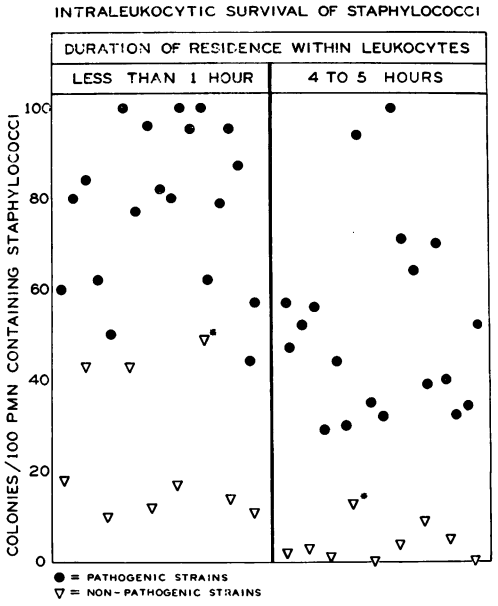

Fig. 1—Strains of coagulase-positive staphylococci isolated from infection survive in leukocytes for long periods. Strains of coagulase-negative staphylococci obtained from the body surfaces do not.

nuclear leukocytes and that this ability correlated well with human or animal pathogenicity[2].

As shown in Figure 1, strains of coagulase-positive staphylococci isolated from human infection invariably survived for four to five hours within the interior of polymorphonuclear leukocytes. In contrast, coagulase negative strains isolated from the skin, air, or anterior nares were almost uniformly killed within leukocytes during this time period. A sharp differentiation could thus be made between these two broad species of staphylococci.

Subsequent studies by Tompsett[3] showed that such pathogenic staphylococci could also survive within mononuclear phagocytes obtained from rabbits while non-pathogenic strains did not. These studies have since been confirmed by others[4].

Recent studies have attempted to determine whether such intracellular survival modifies the nature or course of experimental staphylococcal infection. We now believe that it can and does.

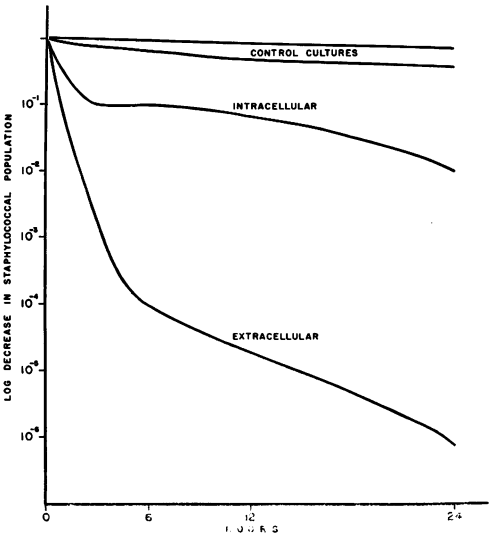

Fig. 2—The protection of intracellular staphylococci from penicillin (redrawn from Tompsett, reference 5). Intra- and extracellular staphylococci survive in *in vitro* systems. Extracellular staphylococci are 50 to 200 times more susceptible to destruction by penicillin.

First, intracellular residence may protect staphylococci from substances in extracellular fluids which ordinarily destroy them. The studies of Tompsett indicate that intracellular staphylococci are protected from antimicrobial drugs present in the surrounding milieu. From 50 to 200 times the amounts of penicillin or streptomycin necessary to kill extracellular staphylococci are required to appreciably affect intracellular staphylococci[5].

Such an experiment is shown in Figure 2. Control cultures of staphylococci residing extracellulary or intracellulary survive for long-time periods. However, when antimicrobial, in this case penicillin, is added in amounts which produce rapid and significant killing of extracellular staphylococci, little change is noted in staphylococcal populations residing within human leukocytes. Thus, as is the case with Brucella[6] or tubercle bacilli[7], incorporation within cells appears to offer the staphylococcus a protective cocoon in which it can survive in unfavorable climates.

It appears clear that this protection cannot be explained by simple

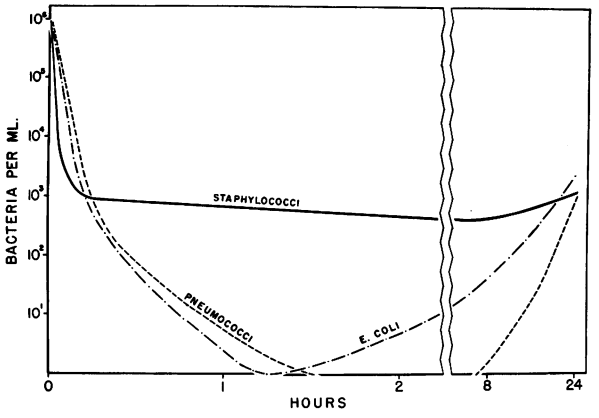

Fig. 3—The blood stream clearance of three different bacteria. Initial disappearance rates are similar. Only staphylococci persist in the circulation for long periods (see text). Figures 3-6, inclusive, from Rogers, D. E., Cellular Management of Bacterial Parasites, Pasteur Fermentation Centennial 1857-1957; a Scientific Symposium, pp. 61-73, 1958, reprinted by permission of Chas. Pfizer & Co., Inc.

failure of antimicrobials to reach the interior of leukocytes. What seems more probable is that staphylococci are metabolically less active within cells and that this sluggishness conditions their susceptibility to antimicrobial drugs. We will have more to say about this later.

Intracellular survival may also modify the way staphylococci behave within the vascular system of experimental animals[8]. When large numbers of staphylococci are injected into the rabbit blood stream, the majority of microorganisms are removed during the first 10 to 15 minutes by the liver and spleen. Then something happens abruptly. The splanchnic tissues no longer trap staphylococci, and a prolonged bacteremia lasting for hours, indeed for many days, results. As shown in Figure 3, this is not the case with bacteria commonly destroyed within phagocytes. Pneumococci or *E. coli* initially disappear from the circulation even in fatal infections in which bacteremia subsequently returns.

It is our current belief that staphylococci surviving within polymorphonuclear leukocytes may play a role in this surprising persistence of microorganisms within the blood stream.

During the initial period of rapid blood stream clearance of staphylococci or pneumococci, polymorphonuclear leukocytes almost disappear from the circulation[9]. As shown in Figure 4, leukocytes then re-

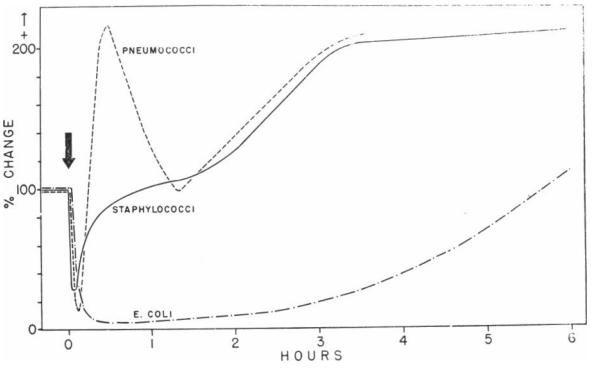

Fig. 4—Changes in the number of circulating granulocytes following intravenous injection of three different bacteria. Injection of staphylococci or pneumococci results in rapid disappearance and reappearance of circulating leukocytes. Injection of *E. coli* produces a prolonged granulocytopenia. (Reprinted by permission, see Fig. 3.)

appear in the blood stream at about the time when bacteremia becomes constant. In both these infections there is a subsequent increasing leukocytosis. This transient granulocytopenia seen following injection of staphylococci or pneumococci differs sharply from the prolonged leukopenia which follows injections of gram-negative bacilli like *E. coli*[10].

By sampling the in-flow and out-flow blood of various organs it can be shown that this sharp decline in circulating leukocytes is produced by their trapping in the pulmonary vascular bed. Ten to 20 minutes later they appear to reemerge from this site[9].

Serial histologic preparations suggest that these leukocytes transiently contained in lung capillaries avidly ingest staphylococci. As leukocytes reappear in the circulation it can be demonstrated that the majority of staphylococci in the circulating blood are intracellular and that such intraleukocytic transport is associated with the failure of the liver and the spleen to remove circulating staphylococci (Figure 5).

That the intracellular site of staphylococci may be responsible for the failure of the reticuloendothelial system to clear them from the circulation is further supported by the fact that animals rendered agranulocytic tend to remove more staphylococci from their circulating blood than do animals with normal leukocyte counts. Furthermore, if staphylococci are placed within rabbit leukocytes *in vitro* and these phagocytes containing bacteria are reinjected into the circulation, such

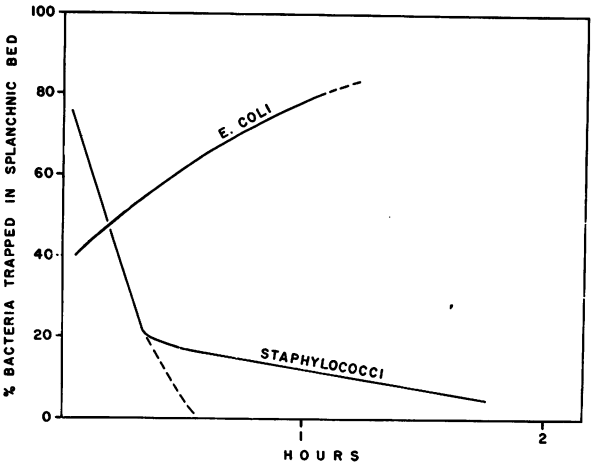

Fig. 5—The characteristics of splanchnic bed trapping of staphylococci and *E. coli* following their intravenous injection.

Splanchnic trapping of staphylococci rapidly declines and generally ceases 20 to 30 minutes after injection of bacteria. In contrast splanchnic sequestration of *E. coli* is unaltered or improves during the course of bacteremia. (Reprinted by permission, see Fig. 3.)

intracellular bacteria appear to remain in the blood stream for longer periods of time than do unphagocytosed staphylococci[8].

Thus the ability of staphylococci to survive phagocytosis may have bearing on the nature and persistence of staphylococcal infection. It may protect staphylococci from antimicrobial drugs present in extracellular fluids. It may allow microorganisms in the blood stream to escape removal within the liver and the spleen where circulating bacteria are usually trapped. It is possible, though unproven, that cells containing staphylococci may lodge in other organ sites and that the phagocytic cell may serve as a focus in which initial multiplication can take place with subsequent focal abscess formation.

The organ selectivity of staphylococci. When injected into the blood stream of experimental animals, staphylococci have a very predictable and intriguing organ selectivity. Although the bulk of microorganisms is rapidly sequestered within the liver and spleen, subsequent multiplication occurs primarily within the tissue of the kidney. Thus the paradox shown in Figure 6 takes place. While staphylococci slowly disappear from the livers and the spleens of infected animals, they multiply rapidly to high titers within the kidneys of the same

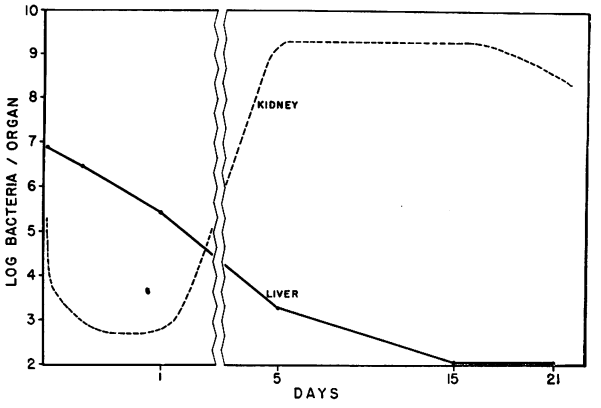

Fig. 6—The behavior of staphylococci in mouse livers and kidneys following intravenous injection of bacteria. Liver titers are initially high, suggesting sequestration in reticuloendothelial cells. Titers fall gradually over 21 days. In contrast, kidney titers rise rapidly after the first 24 hours and remain constant as progressive renal abscess formation takes place. (Reprinted by permission, see Fig. 3.)

animals, resulting in progressive renal abscess formation. The ability to produce progressive renal involvement appears to determine the outcome of the infection.

Why are there such striking differences in the host management of staphylococci which lodge in different organs? This is currently a fascinating unanswered question. It would appear that these differences are probably associated with differences in living cellular processes within the organ rather than limiting growth substances or inhibitors present in certain tissues. For example, when livers and kidneys are removed from the animal body and cell suspensions prepared, staphylococci grow promptly to high titers in preparations of both tissues. These differences in organ susceptibility may offer important clues as to just what biochemical conditions constitute local susceptibility or encourage vigorous staphylococcal multiplication within the host.

The persistence of staphylococci within animal tissues. Most acute bacterial infections either kill the experimental animal in short periods of time or disappear rapidly from organs and tissues. In contrast, staphylococci persist at detectable levels for long periods of time in animals which survive their initial infection. Indeed, staphylococcal infections more closely resemble tuberculous infections than other coccal

disease in the experimental animal. Staphylococci can be demonstrated within the renal parenchyma of infected mice for many months. Bacteremia can be manifest in rabbits for weeks following infection, despite apparent good health. Thus experimental staphylococcal infections are often less decisive than acute bacterial infections, and both the animal host and the microorganism may survive together for prolonged periods of time.

This persistence of staphylococci in host tissues appears to represent an ever present threat to animals with experimental staphylococcal infections. Schaedler and Dubos[11] have shown that non-specific stimuli such as the administration of products of mycobacteria or endotoxin can cause prompt and explosive multiplication of small numbers of staphylococci chronically residing in host tissues to produce progressive disease and death.

It seems probable that this experimental finding has its human counterpart. Job's biblical difficulties with recurrent boils appeared under stressful circumstances. Relapses in chronic staphylococcal osteomyelitis may follow local stress. Patients with apparently healed staphylococcal pulmonary infections may suddenly develop pulmonary abscesses following a non-specific respiratory illness.

The factors which set the stage for vigorous multiplication of staphylococci residing chronically and comfortably within host tissues are completely unknown and have been difficult to approach experimentally. Nevertheless, work in this area offers possibilities for future control of certain problems in staphylococcal disease.

The staphylococcal abscess and staphylococcal infection. Certain facts suggest that the hallmark of staphylococcal disease, the focal abscess lesion, may of itself play an important role in the nature of staphylococcal infection. It has been demonstrated that enormous populations of staphylococci, from 10 million to one billion microorganisms, reside within any abscess lesion[12]. Clinical and experimental experience indicates that mircoorganisms residing in abscesses are not destroyed by antibiotic drugs regardless of their *in vitro* sensitivity[12-14]. It is clear that this is not a problem of inadequate drug penetration into the abscess cavity. Some years ago Robert McCune[12] of our institution showed rather beautifully that penicillin and streptomycin appear promptly within the interior of avascular fibrous wall cavities which mimic the typical staphylococcal lesion. Our own *in vitro* studies suggest that

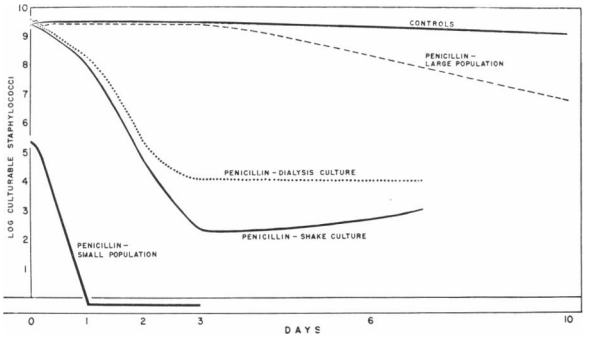

Fig. 7—The effect of penicillin on large and small staphylococcal populations. Despite rapid killing of small populations of staphylococci, large amounts of penicillin do not significantly affect large populations of the same strain unless the microorganisms are stimulated by shaking or suspension in large amounts of media (see text).

populations of staphylococci at the levels found in abscess cavities may be insusceptible because of the crowding and sluggish metabolic activity. When large populations of penicillin-sensitive staphylococci similar to the numbers found in abscesses are exposed to penicillin in test tubes, little or no killing of the microorganisms occurs, despite the fact that smaller microbial populations of the same staphylococcal strain are rapidly destroyed (Figure 7).

If, however, similar large staphylococcal populations are placed on a shaker or enclosed in a dialysis bag suspended in large amounts of media—both situations in which some growth of large numbers of bacteria can occur—the addition of penicillin results in significant killing not seen in ordinary stationary cultures.

These *in vitro* studies appear to have application in experimental infections. The numbers of staphylococci present within certain tissues at the time of antibiotic treatment appear to make a profound difference in the outcome. McCune[14] and Louria[15] have shown that if penicillin or streptomycin treatment is initiated when kidney titers of staphylococci are low, killing of microorganisms and eradication of kidney populations can be demonstrated. If, however, treatment is withheld one to two days, the numbers of staphylococci in kidneys have reached one million or more microorganisms per cc. of tissue. If treatment is started at this point, prolonged therapy results in only a sluggish re-

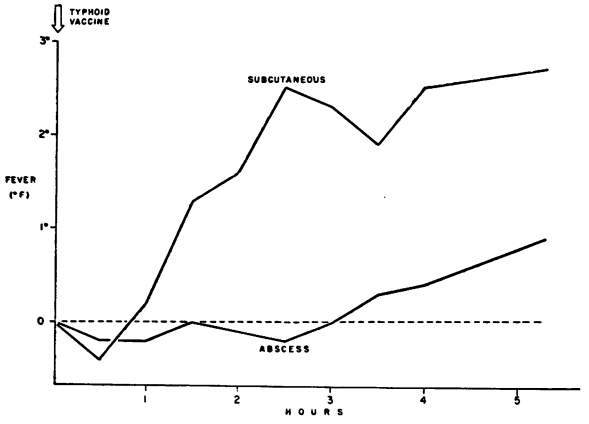

Fig. 8—The effect of the abscess on the febrile response to typhoid vaccine. An amount of subcutaneous vaccine which produces prompt fever in control animals has little effect when given into an experimental subcutaneous abscess.

duction in the number of bacteria and staphylococci persist at high concentrations for many weeks.

Thus staphylococci growing in abscesses are physiologically insusceptible to antibiotics present in the tissues regardless of their *in vitro* susceptibility to the drugs in use, and the sluggish nature of such crowded populations may contribute to this problem.

The abscess and immunity. Staphylococcal infections fail to behave according to the rules of classic immunologic dogma. Antistaphylococcal antibodies are often not demonstrable during infection and humoral immunity is believed of little importance in recovery from staphylococcal disease. These puzzling immunologic peculiarities need re-examination.

We have recently designed studies to test the possibility that the focal abscess does not represent a good antigenic stimulus. This thesis derives from two sets of facts: First, patients with chronic focal staphylococcal disease often fail to develop appropriate antibody to their infecting microorganism, but a prompt rise in antibody is noted when such infections disseminate[16-18]. Secondly, Hughes[19] has demonstrated that fibrin membranes similar to the membrane surrounding the early abscess may operate as a selective filter which will pass only certain proteins and other substances. Thus it appears possible that important

antigenic products fail to get to systemic antibody forming centers from focal staphylococcal lesions.

Although our studies are in their infancy, we already have definite evidence that certain substances placed within an experimentally produced sterile abscess-like cavity do not pass through the abscess wall. For example, the injection of Evans blue or trypan blue dye into such abscess cavities does not result in staining of the surrounding tissues. Further, injections of typhoid vaccine which give prompt and typical fever in simultaneously injected control animals produce little or no fever when placed in the abscess, suggesting that endotoxin transfer across the abscess wall is significantly modified (Figure 8).

Thus we have experimental evidence to suggest that the focalizing nature of staphylococcal infection may operate in multiple ways. The abscess lesion surely plays an important role in the chronic relapsing nature of staphylococcal disease. The poor response of staphylococcal infection to appropriate antimicrobial drugs may be conditioned by the physiologic insusceptibility of large populations of staphylococci residing in abscesses. Indirect and tentative evidence suggests that perhaps the abscess *per se* may influence the immune response to staphylococcal disease by preventing antigenic substances from reaching antibody production centers.

How do these studies relate to the problem of human staphylococcal infection? Extrapolation from animal work to human disease is always dangerous. This is perhaps particularly true of staphylococcal animal research, for to date no experimental animal has been found susceptible to infection by the small numbers of staphylococci believed to initiate human disease. As with poliomyelitis virus, it seems increasingly clear that certain humans represent the most susceptible hosts for staphylococcal infection, and experimental animal systems are gross and cannot be utilized to study many questions of pressing importance.

Despite these deficiencies, certain experimental findings allow tentative explanation of some features of human staphylococcal infection.

First, the work in animals reinforces the clinical impression that major alterations in local or systemic resistance are necessary before staphylococci can invade and actively multiply within human tissues. Although information in human disease is empiric, our own experience indicates that life-threatening staphylococcal infections arise in patients under rather predictable circumstances which affect resistance to all

TABLE I—INTRAHOSPITAL SITUATIONS IN WHICH STAPHYLOCOCCAL INFECTIONS COMMONLY ARISE

1. Patients with probable systemic alterations in resistance.
   - A. Newborn infants.
   - B. Patients with leukemia, Hodgkins disease, lupus erythematosis, diabetes, renal failure, chronic pulmonary disease.
2. Patients with local alterations in resistance.
   - A. Surgical procedures—major and minor (including tracheotomy, urinary catheters, venous cutdowns, multiple irritating injections.)
   - B. Influenza, exfoliative dermatitis, burns.
3. Patients receiving certain types of therapy. (ACTH, steroids, nitrogen mustards, purine or folic acid antagonists, possibly broad-spectrum antibiotics.)

types of infection. At The New York Hospital, over 95 per cent of serious progressive staphylococcal infections have arisen in clinical situations summarized in Table I. Procedures designed to minimize contact with potentially dangerous strains of hospital staphylococci are now an important aspect of the care of these patients.

Secondly, animal studies have shown that staphylococci survive and persist within tissues for prolonged periods. As in tuberculosis, it is clear that ill-defined stresses applied to the host can allow inapparent infections to flare to overt active disease. Staphylococci survive within phagocytic cells and persist in abscess lesions. These characteristics help explain our clinical difficulties in controlling infection with antimicrobial drugs and suggest that therapy should be intensive, prolonged, and combined with surgical drainage of local foci whenever possible.

Lastly, it is apparent that the immunologic peculiarities of staphylococcal infection need more investigation. Although current evidence suggests that classic immune mechanisms do not play an effective role in controlling human infection, perhaps we have not yet asked the crucial immunologic questions. That resistance to staphylococcal disease increases with age is clinically well documented. If it could be shown that immune mechanisms play a role in this process, perhaps we could design better means for preventing disease in certain groups now known to be susceptible to staphylococcal infection.

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