RECENT ADVANCES IN CLINICAL PATHOLOGY

SERIES III

By Various Authors

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PREFACE

THE vast expansion of the application of the methods of the laboratory in all branches of medicine is one of the major features of medical practice in recent years; the position of the clinical, or if the term be preferred, the hospital pathologist and of his instrument, the laboratory, is far more important now than it was in 1947 when the first volume of this series appeared, and is likely to become ever increasingly so.

This growth in importance of the laboratory is a result of the prodigious expansion of knowledge in all fields of medicine within the last two decades. Much of this new knowledge has been obtained through the use of laboratory methods applied to clinical medicine—both in diagnosis and treatment—the practice of which has come to depend increasingly upon these same methods. The laboratory in 1947 could rightly be described as a complex instrument; in the intervening years the demands upon it and upon those who govern it have multiplied and become ever more intricate. This increasing intricacy of certain laboratory investigations makes it advisable on many grounds that some of them should be performed in specially equipped and staffed central reference laboratories. Where such instances occur in this volume they are given specific mention together with, so far as concerns the United Kingdom, the name of the institution of reference. The Editor regrets his inability to do the same for other countries in which this book may find readers. These instances are not numerous; by far the greater part of the procedures described will be performed by pathologists in their own laboratories working in close contact with their colleagues in other fields of medicine.

How close this contact must be is made clear again and again in the ensuing pages; in some chapters it is the major theme, in others it is not so evident, but from no chapter is it entirely lacking. This close co-operation makes it essential that the governance of the clinical laboratory should be in the hands of a clinical pathologist so trained that he is in a position fully to understand the problems presented to him by his colleagues in other branches of medicine, and to determine the plan of campaign most likely to lead to their solution. It must also enable him to instruct his colleagues in the potentialities—and likewise in the limitations and fallibilities—of his instrument and further to assist them in interpreting the evidence

obtained through its use. Such a training demands more than a schooling in the discipline of the laboratory, essential though this may be; it demands first and foremost a sound knowledge of clinical medicine such as can only be achieved through a full course of medical studies leading to the attainment of a recognized qualification to practise medicine; only on the basis of such grounding can the clinical pathologist know how best to place at the disposal of his colleagues the resources of his powerful but delicate instrument.

This volume consists entirely of new matter; the contents of the previous volumes of this title are by no means out of date; in fact, reference back to earlier issues will be found in the present one. The rate of growth of clinical pathology is such that it is impossible to keep in touch with it by mere re-editing. Thus in 1947 nuclear physics found no mention in the first volume of the series; in the second, appearing in 1951, one chapter was devoted to it; in the present volume it demands repeated reference. This development seems likely to continue, even to accelerate; it is for this reason that the present volume is designated not the Third Edition but the *Third Series* of " Recent Advances in Clinical Pathology."

As General Editor my thanks are due first to the Section Editors; it was with great regret that the Editorial Board learned from Professor R. Cruickshank and Dr. R. G. Macfarlane that pressure of other work made it impossible for them to continue to edit the Sections of Bacteriology and Hæmatology respectively. The Board is fortunate in having found successors in the persons of Dr. Mary Barber for the Section of Bacteriology and Dr. Rosemary Biggs for that of Hæmatology. As General Editor I am indebted to the Section Editors for their help, patience and courtesy. I am further deeply grateful to all those who so willingly acceded to the request for contributions.

Owing to numerous and vexatious delays, culminating in a protracted dispute in the printing trade, publication of this volume is long past the scheduled date. As General Editor my apologies are due to all contributors and in particular to the many who submitted their chapters in accordance with the original timetable, but in whose subjects developments requiring notice had since taken place; I am grateful to all these for so willingly undertaking the extra labour of bringing their contributions up to date.

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SECTION 1
BACTERIOLOGY

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CHAPTER 1

SOURCE AND CONTROL OF ANTIBIOTIC-RESISTANT STAPHYLOCOCCAL INFECTION IN HOSPITALS

THE staphylococcus is so familiar to us that we are tempted to think we understand its mode of attack. In fact there are few subjects more baffling to medical science. Since the great Aberdonian surgeon, Sir Alexander Ogston (1881), first demonstrated the organism in pus from operation wounds in 1880, we have learned much about its potentialities, but are still unable to forecast its effect on a given individual or community.

SOURCE, PATHOGENICITY AND VIRULENCE OF STAPHYLOCOCCI IN THE HOSPITAL ENVIRONMENT

Sources

Staphylococcal Sepsis. The most dangerous source of pathogenic staphylococci in the hospital environment is staphylococcal sepsis. Staphylococci in open pathological lesions are more readily disseminated than carrier strains and, as indicated below, tend to be of increased virulence.

Carriers. More than a third of the normal population of this island carry pathogenic staphylococci in the anterior nares (Hallman, 1937; McFarlan, 1938; Gillespie, Devenish and Cowan, 1939) and the carriage rate among nurses is considerably higher (Miles, Williams and Clayton-Cooper, 1944; Allison and Hobbs, 1947; Barber, Hayhoe and Whitehead, 1949).

Skin carriage of pathogenic staphylococci is also quite common (Gillespie *et al.*, 1939; Miles *et al.*, 1944). Early work suggested that the anterior nares was the essential reservoir and that skin carriage was secondary to this (Gillespie *et al.*, 1939; Williams, 1946), and could often be eliminated by treatment of the anterior nares (Moss, Squire, Topley and Johnson, 1948). Staphylococci may, however, be persistently carried on skin sites in the absence of nasal carriage (Hare and Ridley, 1958).

Secondary Sources. *Staph. aureus* is probably spread from person to person by an indirect route rather than by droplet infection (Hare and Mackenzie, 1946; Hare and Thomas, 1956). Infected

patients readily contaminate their bedding, especially blankets, and from this source the staphylococci may be widely disseminated throughout the ward during bed-making. Members of the staff who have lesions or are carriers invariably contaminate their clothing, their handkerchiefs and their hands, and certain carriers appear to be particularly liable to disperse staphylococci into the surrounding atmosphere (Hare and Thomas, 1956).

Aerial contamination of wards or operating theatres may arise from any of these sources.

Laboratory Tests of Pathogenicity and Virulence

Staphylococci live and multiply in the anterior nares and on the skin of healthy people, as well as in septic lesions, and since many of the carrier strains are relatively harmless, identification of pathogenicity is of prime importance. Most bacteriologists accept coagulase-production as the criterion of pathogenicity, and use the term *Staph. aureus* to denote any coagulase-positive staphylococcus regardless of pigment production. This terminology is adopted here, but a positive coagulase test is only a rough guide which does not necessarily imply full pathogenicity, and gives no indication of virulence.

Alpha-toxin, leucocidin, fibrinolysin and pigment production probably all play an important part, but not one of these can be regarded as essential for pathogenicity nor do quantitative estimations of any one of these or of coagulase give much indication of virulence. There is evidence to suggest, however, that a broad spectrum of toxins is of more significance than high production of any one (Lack and Wailling, 1954).

Animal pathogenicity tests are not much more satisfactory, because humans and laboratory animals react differently to staphylococcal infection. Selbie and Simon (1952) recommended intramuscular injection of strains into the thighs of mice. They found that this led to the production of a local lesion which increased in size up to about the fourth day. They estimated virulence by measuring the increase in girth and found that this was correlated with alpha-lysin, coagulase and fibrinolysin production.

Bacteriophage Typing. It is now recognized that *Staph. aureus* can be divided into three principal groups (I, II and III) by means of their susceptibility to various bacteriophages (Williams, Rippon and Dowsett, 1953, and Anderson and Williams, 1956). These groups have a fundamental basis in the constitution of the staphylococci concerned, since they correspond to the three serological

groups identified by slide agglutination (Cowan, 1939; Hobbs, 1948) and the coagulase produced by members of each of the three groups is antigenically distinct (Barber and Wildy, 1958).

Strains of phage group III yield antibiotic-resistant variants more readily than do strains of other phage groups and are frequently associated with severe infection in surgical wards (Barber and Whitehead, 1949; Rountree and Thomson, 1949; Jackson *et al.*, 1954; Barber and Burston, 1955). It cannot be assumed from this that strains of phage group III are necessarily of greater virulence than those of other groups, but they may be so in a given institution.

Recently there has been evidence to suggest that staphylococci of phage-type 80 are likely to be of enhanced virulence. The strain was first isolated as a new type by Rountree and Freeman in 1955 from an epidemic of unusual severity and extent, affecting several maternity hospitals in Australia. Since then, outbreaks of severe staphylococcal infection due to strains of this type have been recorded in many parts of England (Gillespie and Alder, 1957; Duthie, 1957; Barber and Dutton, 1958).[1]

Conclusion

From a practical point of view it must be concluded that there is no simple means of determining the virulence of a strain of staphylococcus and that all coagulase positive strains must be regarded as potentially dangerous. Nevertheless, it may be taken as a general rule that strains from pyogenic infection are more likely to be virulent than those from carriers, and with the help of bacteriophage typing and antibiotic resistant patterns it may be possible to determine which carrier strains are associated with clinical infection in a particular institution.

SUSCEPTIBILITY OF THE HOST

Non-specific Factors

The resistance of the host probably plays at least as important a rôle in determining staphylococcal disease as does the virulence of the microbe, and many modern forms of therapy render patients peculiarly susceptible to infection. Indeed, in the words of McDermott (1956), "an unfortunate by-product of our life-saving and life-prolonging procedures is to create a favourable market for

[1] Phage-typing of staphylococci is outside the scope of most routine laboratories, but in Great Britain representative strains can be typed by the Central Public Health Laboratory Service.

aphylococci ". The most important factors are probably prolylactic administration of antibiotics, pre-operative starvation (f. Smith and Dubos, 1956) and corticosteroid therapy (Coste *et al.*, 951: Shaper and Dyson, 1955; Smith and Cleve, 1957).

Specific Immunity

Specific immunity to staphylococcal infection is a complex problem. The staphylococcus is both highly invasive and toxigenic, and antibodies have been demonstrated to the cocci themselves and to alpha-lysin, leucocidin and coagulase. All these, as well as other factors, probably play a part in the development of immunity to staphylococcal infection, and it is unfortunate that most investigators have concentrated on the protective value of one agent to the exclusion of others. This has caused some confusion which has been further increased by the great variety of experimental methods employed.

At present it must be admitted that we do not know how to protect patients against staphylococcal infection. Although from time to time good results are obtained with autogenous vaccines or preparations of toxoid, the protective agent remains obscure. Attention is once more being drawn to this subject now that antibiotics have failed to control staphylococcal infection in hospitals. An active vaccine to protect the more vulnerable patients, such as those undergoing massive surgery and newborn infants, might save many lives.

EMERGENCE OF ANTIBIOTIC-RESISTANT STAPHYLOCOCCI

After the report of the first clinical trial of penicillin at the Radcliffe Infirmary at Oxford in 1941 (Abraham *et al.*, 1941) many thought that the answer to the staphylococcus had been discovered, and for a time this appeared to be the case. The effect of this agent on staphylococcal infections of all grades of severity was truly dramatic, and it was not unreasonable to hope that if the staphylococci in septic processes could be promptly and efficiently eradicated, spread of infection to other members of the community would become negligible.

Unfortunately the staphylococcus did not take the attack lying down, and a powerful defence mechanism in the form of the penicillin-destroying enzyme, penicillinase, was soon forthcoming. Within a few years of the widespread use of penicillin in hospitals an ever-increasing proportion of staphylococcal infections in these

institutions were found to be due to penicillinase-producing staphylococci (cf. Barber, 1947a and b, and Barber and Rozwadowska-Dowzenko, 1948).

Chemists and bacteriologists have countered with new weapons, and six antibiotics are now freely available for the treatment of staphylococcal infection. Penicillin was followed by the discovery of streptomycin in 1944, chloramphenicol in 1947, aureomycin, the first of the tetracycline antibiotics, in 1948, erythromycin in 1952, novobiocin in 1955. But, as each new antibiotic was introduced, strains of staphylococci resistant to it began to make their appearance, and staphylococci resistant to one or more of all these agents have now been encountered in clinical infections.

Three new antibiotics with powerful antistaphylococcal properties must be mentioned, vancomycin, ristocetin and kanamycin. Unfortunately all three are toxic. Vancomycin and ristocetin can, at present, only be administered by intravenous injection.

Penicillin-destroying Staphylococci. Penicillin-resistant staphylococci isolated from clinical cases owe their resistance to the production of penicillinase, which inactivates penicillin. This property is a relatively permanent characteristic, but many strains tend to yield a proportion of cells which have completely lost the capacity to produce the enzyme (Barber, 1949; Bondi, Kornblum and De Saint Phalle, 1953; Fairbrother, Parker and Eaton, 1954). The change appears to be sudden and complete, suggesting spontaneous mutation. It is probable, therefore, that the gain in enzyme production also occurs by spontaneous mutation from penicillin-sensitive staphylococci, but in view of the failure to demonstrate such an occurrence *in vivo* or *in vitro* the mutation must be a rare one.

Staphylococcal penicillinase is an adaptive enzyme (Geronimus and Cohen, 1957) and the effect of the antibiotic on the emergence of penicillinase-producing strains cannot be overlooked. Barber (1957) found that certain penicillin-sensitive strains of *Staph. aureus* gave rise to variants with weak penicillin-destroying activity when subcultured for many months in very low (0·005–0·01 μ/ml.) concentrations of penicillin *in vitro*. Similar concentrations of penicillin probably remain in the tissues of patients for some time after the cessation of penicillin treatment and, as Gould (1958) has shown, may be present in the air of wards where penicillin injections are being given without special precautions. It may well be that in hospital wards where cross-infection is common, strains of staphylococci are in fact being serially transferred in the presence of subbacteriostatic concentrations of penicillin.

Antibiotic-tolerant Staphylococci. With all the other antibiotics the resistant variant is capable of growing in the presence of an increased concentration of the antibiotic, although the latter is unchanged. These antibiotic-tolerant strains almost certainly arise by single or multi-step mutation (cf. Luria and Delbruck, 1943; Demerec, 1945, 1948; Lederberg and Lederberg, 1952). With streptomycin, erythromycin and novobiocin the rate with which resistant strains emerge is so rapid that a gross increase in the resistance of the infecting strain frequently occurs during the treatment of a single patient, whereas with chloramphenicol and tetracycline this is unusual unless treatment is prolonged.

Streptomycin-resistance. Variants of staphylococci resistant to streptomycin develop with such rapidity that a few days' treatment is often sufficient to cause a hundred-fold or more increase in the resistance of the infecting strain.

Chloramphenicol-resistance. Staphylococci resistant to chloramphenicol emerge more slowly and in most hospitals are relatively uncommon, since the use of this antibiotic has been kept low because of its toxicity to the bone marrow. Slight or moderate increase in resistance to chloramphenicol has been demonstrated in association with the development of resistance to tetracycline (Monnier and Schoenbach, 1951) and erythromycin (Barber, Csillag and Medway, 1958).

Tetracycline-resistance. Staphylococci resistant to tetracycline also appear slowly (Paine, Collins and Finland, 1948), but, owing to the extensive use of the tetracycline antibiotics, are now quite common in hospitals. Cross-resistance between chlortetracycline, oxytetracycline and tetracycline is almost complete.

Erythromycin-resistance. Staphylococci appear to develop resistance to erythromycin more rapidly than to any other antibiotic except streptomycin (Hobson, 1954; Garrod and Waterworth, 1956). Since the discovery of erythromycin in 1952 three other closely related antibiotics have been described, namely carbomycin, spiramycin and oleandomycin. Cross-resistance between erythromycin and carbomycin and between spiramycin and oleandomycin appears to be complete, but between one pair and the other pair there may be dissociation. Thus Garrod (1957) showed that while strains habituated to erythromycin *in vitro* always showed cross-resistance to spiramycin and oleandomycin, this was not always the case with erythromycin-resistant strains isolated from patients. Strains showing the dissociated type of resistance appeared to be resistant to the other two antibiotics if erythromycin was added to the

medium. Another related antibiotic, E 129, shows only a minor degree of cross-resistance (Garrod and Waterworth, 1956).

Novobiocin-resistance. Staphylococci readily yield novobiocin-resistant strains *in vitro* (Lin and Corriell, 1956) although such strains appear rather more slowly than with erythromycin (Garrod and Waterworth, 1956). Nichols and Finland (1956) recorded a significant increase in the resistance of the infecting strain in three cases of staphylococcal infection treated with novobiocin.

Vancomycin-resistance. Preliminary *in vitro* studies indicate that vancomycin-resistant strains of staphylococci do not readily occur (Garrod and Waterworth, 1956). Since early experience with chloramphenicol and tetracycline was similar it cannot be assumed from this that such strains will not appear in clinical infections.

Hospital Selection of Multiple-resistant Strains

Whatever the mode or origin of antibiotic-resistant staphylococci, their increasing incidence in hospital communities is undoubtedly due to a process of selection of such strains by the widespread and haphazard use of antibiotics, and their spread from patient to patient by cross infection (Barber, 1947b; Barber and Rozwadowska-Dowzenko. 1948; Rountree and Thomson, 1949). The story of the rapidly increasing incidence of penicillin-resistant staphylococcal infection in hospitals all over the world, a few years after penicillin became freely available, has now been repeated, first with streptomycin and then with the teracyclines (Clarke, Dalgleish and Gillespie, 1952; Rountree and Thomson, 1952; Lowbury, Topley and Hood, 1952; Kirby and Ahern, 1953). At the time these antibiotics were introduced the staphylococcus prevalent in hospitals was already penicillin-resistant and it was this type that yielded strains resistant to the latter antibiotics. Thus what is now spoken of as the "hospital staphylococcus" was first penicillin-resistant, then penicillin and streptomycin-resistant and now penicillin, streptomycin and tetracycline resistant. Penicillin-sensitive strains resistant to tetracycline, streptomycin or both are, in fact, of very rare occurrence.

By the time erythromycin and novobiocin were discovered bacteriologists and clinicians were alike aware of the problem, so that they have been used sparingly and, in many hospitals, only for infections resistant to other forms of treatment. The incidence of strains of staphylococci resistant to erythromycin and novobiocin has, therefore, remained low, but when they do occur they are likely to be resistant also to penicillin, streptomycin and tetracycline.

Resistant Strains in Surgical Wards. The prevalent staphylococci responsible for infection in the general surgical wards of most hospitals to-day are strains resistant to penicillin, streptomycin and tetracycline and belonging to phage group III or type 80. There is much evidence to suggest that such strains are of enhanced virulence. Certainly they appear to be responsible for the majority of cases of post-operative infection, although they are relatively uncommon among staff carriers (Alder, Gillespie and Thomson, 1955; Barber and Burston, 1955; Shooter *et al.*, 1958 and Barber, 1958).

Shooter and his colleagues (1958) studied a surgical ward over a period of eight months, during which 311 patients were admitted to the ward and 17 (5·5 per cent) developed staphylococcal wound sepsis. Although 186 different types of *Staph. aureus* were encountered, only 13 caused disease; only 10 gave rise to ward infections and only 3 were responsible for sepsis in more than one patient. All the last 3 were resistant to penicillin, streptomycin and tetracycline and belonged to phage group III.

It does not, of course, follow from this, that increased virulence is necessarily associated with multiple resistance or vice versa. The virulence may be related to bacteriophage type, since most of the multiple-resistant staphylococci either belong to phage group III (Alder *et al.*, 1955; Barber and Burston, 1955; Anderson and Williams, 1956) or are of phage type 80 (Rountree and Freeman, 1955; Gillespie and Alder, 1957; Duthie, 1957).

An alternative explanation is that the few strains of *Staph. aureus* which have undergone selection in hospital communities because of their capacity to resist antibiotics, have been left a clear field and, freed from competition with other strains, have more readily been transferred from patient to patient. It is probable that the virulence of any strain of *Staph. aureus* becomes enhanced in a septic lesion and there is much evidence to suggest that passage from patient to patient may lead to outbreaks of infection of unusual severity (Rountree and Freeman, 1955; Beavan and Burry, 1956).

Another characteristic of strains showing multiple resistance is that they appear to yield variants resistant to yet other antibiotics more readily than do penicillin-sensitive strains (cf. Barber and Dutton, 1958; Barber, Csillag and Medway, 1958). It is possible that in the selection of antibiotic-resistant staphylococci, strains with a high mutation rate to drug resistance and other characteristics occur (cf. Chadduck, Alexander and Trefers, 1954).