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STREPTOCOCCI: THEIR TOXINS AND ANTITOXINS.

Lecture delivered at St. Mary's Hospital, May, 1927,

BY

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STREPTOCOCCI:

THEIR TOXINS AND ANTITOXINS.

Our time will be spent mainly in considering the streptococci, and chiefly the pathogenic ones, under the headings of classification and pathology —i.e., what the streptococci are and what they do. It is probably fair to say that the attention of bacteriologists has recently, and more fruitfully, become more centred on what these organisms do than on what they are.

Classification.

In all scientific work accurate classification is of fundamental importance, and many attempts have been made in the past to classify these streptococci by the careful investigations of eminent workers in bacteriology; light has gradually dawned, and we are to-day almost in sight of a rational view of the group.

We may for the sake of clearness take some historical liberties and consider each of these attempts in sequence.

The first attempts were naturally based on morphology from the time when Pasteur drew his historical chain of cocci on the blackboard at a meeting in Paris, and soon such names as *longus* and *brevis* arose. Throughout all this work attempts were constantly being made to link these various groupings with the various diseases produced by the cocci. Thus, *Streptococcus longus* was thought by von Lingelsheim, 1899, to be pathogenic, and the short-chained variety, *brevis*, much less so.

Next, cultural characters were carefully explored and groupings were arranged on the basis of fermentation of carbohydrates, &c; now arose the names *pyogenes*, *fæcalis*, &c. This work is

largely due to English bacteriologists—Gordon, Andrewes, and Horder (1902–1906).

Blood plates were next (Schottmüller, 1903, Th. Smith and Brown, 1915) extensively used and a division was made into: (1) the non-hæmolytic group; (2) those giving a green ring, the *viridans* group; and finally (3) the hæmolytic group, which produce a clear ring around the colony, a so-called "hæmolysis," though the appearance suggests that there has been also decolorisation. This latter group gives hæmolysis of red blood cells in suspension in a test-tube. It was clearly pointed out by these workers that there was a transition from cocci giving rapid and complete hæmolysis to those with feeble hæmolytic power, but the grouping "hæmolytic" and "non-hæmolytic" is convenient.

If one requires a bird's-eye view of the results up to this period, that given by Park and Williams [1] is perhaps one of the most convenient, with its division into hæmolytic and non-hæmolytic groups. The *beta* group of Smith and Brown includes *S. pyogenes*, *anginosus*, and *equi*; the *alpha* group is divided into two groups: those producing methæmoglobin—e.g., *fæcalis* and *salivarius*—and those not producing methæmoglobin—e.g., the *gamma* type, *anhæmolyticus*, &c.

The next attack during the immediate past has been by agglutination and absorption methods (Dochez, 1919, Gordon, &c., and recently by Smith, Griffith, and James, &c.). The technical difficulties were great, and only within the past few years has it proved practicable by various methods of agglutination in buffered broth, rapid subculturing, or rapid microscopic testing, to work with sufficiently stable emulsions to give reasonably consistent results. It was at first thought that by agglutination we might be able to separate clearly the pathologically defined groups—e.g., scarlet fever, erysipelas, puerperal fever, &c., but later work has shown that by careful agglutination and absorption work the streptococci can be subdivided into a number of main groups and probably a considerable number of subgroups.

[1] Pathogenic Micro-organisms, 1925, p. 303.

Overlapping of Groups.

The groups definitely overlap. Thus, the scarlet fever strains can be fairly clearly divided into three main groups and an unknown number of smaller groups, but some cultures derived from puerperal fever and erysipelas are indistinguishable from those in the main scarlet fever groups.

Finally, since the use by the Dicks (1924) of the intradermic method in human beings, the attempts have been mainly directed to seeing what the pathogenic actions of the various streptococci are, and whether any classification can be based thereon. But here again we are faced with great confusion. At first it was thought that the scarlet fever streptococcus would produce a scarlet fever toxin and would on injection into animals cause the production of a scarlet fever antitoxin; similarly it was thought that the puerperal streptococcus would give a characteristic toxin and antitoxin, that "cellulitis" and "septicæmic" strains would similarly give clearly distinguishable toxins and antitoxins.

Amoss and Birkhaug concluded as the result of their work that the erysipelas streptococcus had clearly specific toxin-antitoxin relationships quite independent of the scarlet fever streptococcus. It is not certain that further experience will confirm this clear specificity.

It is already clear that there is some overlap amongst the main groups. How much is not yet determined. Some workers go so far as to conjecture that there is one antigen only—i.e., that the toxins of the streptococci causing scarlet fever "septic sore-throat," erysipelas, puerperal fever, cellulitis, &c., are all one and the same toxin, and that whatever toxin be used the same antitoxin is obtained.

Methods of Investigation.

There are several methods of investigating this problem, and they are all being actively pursued at the present time. If we take the *S. scarlatinæ* of Dochez and the Dicks and make a toxin from it, we find that when the toxin is injected in suitable

doses intradermally into human beings, some give a positive red reaction, and others give no response —the so-called negative reaction. A group of people who have had scarlet fever will have a much higher percentage of negative reactions than those who have never had scarlet fever or been in contact with it.

(1) If we can obtain large groups of people who have had, e.g., "septic sore-throat" or puerperal fever, we can test them with the scarlet fever toxin and with a culture filtrate or "toxin" made from cultures of streptococcus obtained from puerperal fever or tonsillitis. If we end by finding that all the people who give negative reactions to the scarlet fever toxin also give negative reactions to the tonsillitis and puerperal fever toxins, and those giving a positive reaction to one toxin give a positive to the other two, we shall be justified in assuming that there is a close antigenic relationship between the three toxins and presumably between the three diseases. The results from this line of research are at present too meagre to justify any confident conclusion, but apparently there is a considerable degree of agreement in the groups—i.e., the majority of people who give a positive response to the Dick scarlet fever test will also probably give a positive response to the injection of the other toxins, and "Dick negative reactors" will usually be "negative" to the other toxins.

(2) We may test the hypothesis in another way—make an antitoxin to one of the streptococci—e.g., scarlet fever antitoxin, and test its effect on streptococci obtained from the other diseases and on their toxins. Parish and Okell have shown that, in the rabbit test, scarlet fever antitoxin has a significant protective action against a number of hæmolytic streptococci obtained from other diseases.

(3) We may mix the scarlet fever antitoxin with the toxin of, e.g., puerperal fever, and inject the mixture into the skin of subjects who give a positive response to the injection of "puerperal toxin." It is found in practice (Eagles, McLaughlin) that a considerable degree of overlap occurs; thus scarlet fever antitoxin will neutralise tonsillitis

toxin or puerperal toxin in a considerable number of instances.

(4) We may use one antitoxin for the treatment of the other diseases—e.g., scarlet fever antitoxin for tonsillitis, puerperal fever, &c. Here again the results are too few to justify any definite conclusion, but evidence giving some support to the "unitarian" hypothesis is beginning to accumulate.

(5) We may find a dose of the culture of—e.g., scarlet fever streptococcus that will kill laboratory animals—e.g., rabbits, and see if the other antitoxins give any protection. The table which my colleagues Parish and Okell have kindly allowed me to use shows that these other antitoxins have considerable protective effect against the scarlet fever streptococcus and other heterologous hæmolytic streptococci.

(6) We may inject intradermally into a scarlet fever rash, antitoxins made from cultures of—e.g., puerperal or cellulitis strains, to see if the Schultz-Charlton blanching results. We have reports showing that in a few recent observations definite blanching was so produced.

(7) We may inquire whether people who are immunised with scarlet fever toxin until they give a negative reaction to the Dick test will be immune against tonsillitis or puerperal fevers, &c.

The "Unitarian" View.

Where the story will lead us we shall not know for a year or two. But it is of extraordinary interest that at present an amount (though not all—cf. Park, Blake, and co-workers, &c.) of recent immunological evidence seems to be almost leaning towards the hypothesis that all pathogenic streptococci are identical in their pathogenic and "toxic" action. But if the implications of this "unitarian" hypothesis conflict with the very large body of evidence enshrined in clinical medicine, we must be very hesitant to adopt the hypothesis. If immunology hints that follicular tonsillitis and puerperal fever, for example, are caused by the same organism as scarlet fever, we must inquire what clinical evidence there is in support of

this view. With regard to tonsillitis, various clinicians, after a lifelong experience of the exanthemata, have inclined to the view that one may have scarlet-fever-without-rash—i.e., "septic sore-throat" or follicular tonsillitis; and others have maintained that there is a close relationship between puerperal fever and scarlet fever. Goodall and Washburn [2] wrote thus: "Nurses suffering from erysipelas have conveyed puerperal fever to lying-in women; and medical men and nurses have contracted erysipelas when in attendance upon cases of puerperal fever."

So far we may account the clinicians on the side of the hypothesis. But if these diseases are essentially identical, one disease should give rise to the other. Can epidemiology help us here? Can we on the one hand find communities in which one of these diseases was common and yet never gave rise to the other diseases?

In the old military epidemics of "septic sore-throat" in former times, did the introduction of this disease into a new batch of troops lead to the outbreak of sore-throat or *also of scarlet fever and erysipelas*, &c.? When puerperal fever raged a century ago, so that midwifery hospitals had to be closed, did erysipelas and scarlet fever rage simultaneously in those hospitals? I have been unable to discover the evidence dealing with these points which almost certainly exists in medical literature. Can we find clear instances in which an isolated community free of these diseases became infected with, say, scarlet fever through the introduction of one case of tonsillitis, puerperal fever, erysipelas, or cellulitis into its midst?

I lived for some years in a rather isolated small town overseas, in which I saw puerperal fever and follicular tonsillitis from time to time, but never a case of scarlet fever, nor had one been recorded in the history of the town so far as I could find.

It may be that a streptococcal disease of any kind requires the streptococcus plus another factor. It will be remembered that for years the *B. suipestifer* was thought to be the cause of

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[2] Infectious Disease, 1896, p. 330.

swine fever in pigs, for by the injection of this organism a disease apparently identical with swine fever can be produced. Dorsett in his masterly work showed that only one feature was lacking—i.e., infectivity. The pigs injected with the culture developed what appeared to be the typical disease, but his great discovery was that these pigs did not infect others, whereas the natural disease was highly infectious. Further investigation showed that a filterable virus was the cause of the naturally occurring highly infectious swine fever, and that the other bacillus was so common in pigs that, when the real swine fever disease occurred, the bacillus infected the animal's body and produced the characteristic intestinal lesions. It is possible to have a herd of pigs infected with the virus—i.e., true highly infectious swine fever, and another herd infected only with the *B. suipestifer*, showing fever and intestinal lesions. So close is the alliance and so universal is the bacillus that it is to-day safe in England for administrative purposes to diagnose the presence of swine fever because intestinal lesions produced by another infecting agent—the *B. suipestifer*—are present.

The Dicks have described in human volunteers the production of true clinical scarlet fever following the application of their culture of streptococcus. The crucial experiment is lacking. Would the patient have infected other people? In other words, had he the natural *infectious* disease? It is doubtful if this experiment will ever be done. In its absence we must depend on the collateral lines of evidence above referred to.

We may sum up by saying that some of the lines of investigation at present being pursued point to the suggestion that all the ordinary diseases caused by hæmolytic streptococci are different manifestations of the same disease. It is by no means certain that clinical and epidemiological evidence will support this hypothesis. For the moment, however, we must keep an open mind, and, in the treatment of the diseases caused by the hæmolytic streptococci, be prepared to use whatever serum is on reasonable clinical evidence found to cure the given disease.

Present Knowledge.

It would perhaps be useful at this stage to take a general view of our knowledge in this field. Where the ground is shifting under our feet from day to day, it is of some service to consider which landmarks are so firmly established as to be probably permanent, which have been set up or are in process of being erected, and which may probably not stand the stress of time.

Streptococci mainly of the hæmolytic variety, and with various fermentation reactions, are constantly present in various "septic" conditions. It is practically certain that they are the real cause, for we know from experiment on human volunteers that with cultures of *S. erysipelatis* characteristic erysipelas has been produced. The production of experimental erysipelas with cultures of streptococci obtained from lesions of erysipelas, or from septic ones (presumably *S. pyogenes*), was for some time a method of "treatment" of inoperable cancer. (In this connexion an old experiment by Koch and Petruschky records that in a volunteer erysipelas was produced by the injection of streptococci isolated from erysipelas. After recovery, experimental erysipelas was produced again on the same area. The experiment was repeated ten times.)

The many unfortunate infections of pathologists, after accidental finger-pricks while doing autopsies or while handling cultures of streptococci, leave little doubt that the streptococci are the real cause of these various manifestations of lymphangitis, cellulitis, and even septicæmia. Similarly, we are entitled to believe that streptococci are the cause of severe puerperal fever with septicæmia.

With regard to the *viridans* group of hæmolytic streptococci, they are so frequently found in certain pathological conditions, notably subacute endocarditis with bacteriæmia, that it is reasonable to believe that they are the cause thereof.

With regard to scarlet fever, the Dicks and Nicolle believe that they produced typical scarlet fever in human subjects by the inoculation of cultures of the *S. scarlatinæ*. They have not shown, what from a coldly scientific point of view would be

interesting and valuable, that the disease so produced is infectious to others and therefore in every respect resembles natural scarlet fever.

It is further reasonable to believe that most of the symptoms at least of uncomplicated scarlet fever are due to the "toxin" of the *S. scarlatinæ*, for, by the injection of sterile filtrate made from a culture of the streptococcus, subcultured perhaps hundreds of times since its isolation from the human subject, one can produce pyrexia, headache, "strawberry tongue," the characteristic throat condition, albuminuria and rash followed by peeling—in other words, a pathological condition indistinguishable from naturally ocurring scarlet fever. It is practically certain that this so-called "scarlatinoid syndrome" is non-infectious.

It appears to be established beyond doubt that the response to the Dick test is related to immunity against scarlet fever, and that scarlet fever antitoxin is effective in the treatment of uncomplicated scarlet fever; it is probably without effect on the late septic or pyogenic conditions occurring in scarlet fever. Further, by immunising with Dick toxin one can make positive reactors negative to the Dick test, and this negative reaction indicates a reasonably high immunity against scarlet fever.

When we consider the more recent work, we find a distinct suggestion that the toxins and antitoxins of all the hæmolytic streptococci are so closely related as almost to amount to identity. But whether this view will be entitled to a "land mark," time and further experience alone can tell. Current clinical views may be summarised under some such headings as the following:—

(*a*) *Experimental evidence.*—(1) *S. pyogenes* when injected has caused at least local septic conditions. (2) *S. erysipelatis* when injected has caused erysipelas. (3) *S. scarlatinæ* when rubbed on the tonsils has caused scarlet fever.

(*b*) *Conclusions reasonably based on much clinical observation.*—Streptococci, usually hæmolytic, are the cause of puerperal fever, mastoiditis, septic pneumonia, &c. *S. viridans* is the usual cause of subacute infectious endocarditis.

(*c*) *Current hypotheses.*—Truth dependent on experience in the future. Because streptococci occur in dental septic

conditions, they cause constitutional symptoms. Because streptococci (enterococci, &c.), occur in the bowel in large numbers they cause diarrhœa, &c.

Although the title of our prescribed subject deals with the toxins and antitoxins of the streptococci, I may be permitted to refer to the other aspect of streptococcal attack, the so-called "septic" manifestations. A hypothesis which we might use for the moment for the sake of clearness is that the *S. scarlatinæ* has two modes of attack —the "toxic" and the "septic." In violent epidemics, it is the early acute toxic attack which kills most patients; in mild epidemics it is the later "pyogenic" or "septic" manifestations, in which the living organisms settle down locally in the glands, joints, mastoid, &c., that cause the greatest harm.

It is a curious thing that in the Parish-Okell rabbit method, scarlet fever antitoxin gives complete protection against the first "toxic" attack of the streptococcus, which is rapidly fatal to unprotected rabbits, but that this antitoxin apparently gives but small or no protection against the development of the later septic lesions in joints, &c. Similarly, it is probable that no scarlet fever antiserum now available has any direct effect on the late septic complications of scarlet fever. It is probable that the solutions of this difficult part of the problem will be found in the investigation of cellular immunity with which the Medical School of St. Mary's Hospital has been for so long identified, and that future work may lead to a convergence of the "cellular" and "humoral" aspects of immunological research of the activities of the streptococci.

Summary.

The examination of agglutination, toxin and antitoxin relationships of the various "pathogenic" streptococci suggests that there is a very close relationship between all the members of the group. The further analysis of this close relationship is the problem before us.

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