The relation of streptococci to scarlet fever and its complications / [R.A. O'Brien, C.C. Okell].

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THE RELATION OF STREPTOCOCCI TO SCARLET FEVER AND ITS COMPLICATIONS.*

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THE title of our discussion assumes that a relationship exists between streptococci and scarlet fever. It may be of service to sum up the grounds for the acceptance of this assumption and for our personal belief that streptococci are the cause of scarlet fever. Haemolytic streptococci have been found practically always present in true scarlet fever. Clinical scarlet fever has been produced (according to the statements of the Drs. Dick) by the swabbing of cultures of these streptococci on to the tonsils of susceptible people; a culture filtrate or "toxin" prepared from the streptococci gives reactions which are specifically related to scarlet fever, for practically all patients, in the earliest stage of the disease, give a positive response to the intracutaneous injection of the toxin, and during convalescence, when their immunity is developed, the great majority of patients give a negative response; the serum of animals immunized with these streptococci or their toxins, when injected intracutaneously, produces the specific blanching of a scarlet fever rash; finally, the serum of immunized animals has what, in the opinion of medical men with long training in the observation and treatment of infectious fevers, amounts to a specific curative action. This "confession of faith" will cause a certain repetition in the logical consideration of our subject, but it has appeared advisable to define at the outset what is believed to be the ground already won.

When the subject of our discussion was chosen, some months ago, it was fervently hoped that before this meeting a test applicable in the laboratory for the measurement of scarlet fever toxin and antitoxin would have been discovered, and that with such a test we should have been able to clear up many uncertainties. That hope proved vain, for no satisfactory test has yet been found. In consequence we shall be obliged to some extent rather to formulate questions than to add much to our common stock of knowledge.

We are to inquire, What is the relation of the *Strepto-*

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coccus haemolyticus to scarlet fever and its complications? There seems little doubt that haemolytic streptococci are closely related to scarlet fever, and we are justified, for the purpose of discussion, in postulating a "*Streptococcus scarlatinae*" (or possibly a group of streptococci) which causes infectious scarlet fever. But as soon as we seek to define this *S. scarlatinae* our difficulties begin. If we isolate from the throat of a patient suffering from scarlet fever a streptococcus which we think may be the characteristic organism, we must determine that it is haemolytic, and that on the ordinary media it grows like the haemolytic streptococci obtained from any other pathological conditions; it has, unfortunately, no characteristic of growth sufficiently distinctive to help us materially in identification. Its fermentation reactions must agree with those of *Streptococcus pyogenes;* lactose, salicin, and saccharose must be fermented, but not raffinose or inulin; mannite is fermented by some strains and not by others, yet in both cases we must accept these strains as true *Streptococcus scarlatinae.* (In passing we may note that this character appears to be not very deeply implanted, for a strain of the mannite-fermenting variety, which Dr. Dick kindly sent us, has in our laboratory, in the course of some months, apparently lost its power to ferment mannite.)

The organism, when grown in ordinary peptone or tryptic digest broth, must produce in the filtrate a "toxin" having the same property as that described by G. F. and G. H. Dick. It must, in a reasonably high dilution, give a positive "Dick" reaction in almost all cases of scarlet fever tested during the first two or three days of the disease; it must, when injected into positive Dick reactors in a quantity containing about 1,000 times the minimal skin reacting dose, produce in the majority of instances "miniature non-infectious scarlet fever" or the "scarlatinoid syndrome"—that is, sore throat, fever, vomiting, and characteristic rash; it must be neutralized by the specific antiserum, so that when a mixture of toxin and antitoxin is injected intradermally into a positive reactor to the Dick test, no reaction is produced. Before our scientific scepticism is satisfied we may ask more—that is, that our living culture will, when applied to the throat of a positive Dick reactor, produce unmistakable scarlet fever. It is probable that only three strains in existence have had their authenticity tested thus far—the mannite-fermenting and also the non-fermenting strains of the Dicks, and one strain in Park's laboratory, which was accidentally sucked into the mouth of a worker and apparently caused typical scarlet fever.

We may inhumanly still press our demands and say that we are not yet satisfied, for natural scarlet fever is infectious, and we have yet to prove that the disease we have produced will infect susceptible contacts as the natural disease does. This test has not been carried out, nor is it probable that our common humanity will ever allow it. It is only the interesting history of swine fever that makes

us even contemplate such a demand. Until Dorsett's classic investigation it was everywhere accepted that the *B. suipestifer* was the cause of swine fever, for with the bacillus can be produced a fatal disease clinically resembling swine fever and showing *post mortem* certain characteristic ulcers in the intestine. Dorsett noted that the disease this bacillus produced was not infectious, and it was only when he obtained his " filterable virus " free from bacilli that he was able to produce a disease clinically resembling natural swine fever and also resembling it in its intense infectiousness. The *B. suipestifer* is apparently a " secondary invader " which produces the ulcers, and is so commonly present in pigs in most countries where true swine fever occurs that in the opinion of those charged with the control of animal diseases it is safe to found a diagnosis of swine fever, justifying administrative measures, on the finding of the ulcers really caused by *B. suipestifer.*

With regard to septic complications, there seems little doubt that the majority of the organisms causing otitis, septic adenitis, etc., belong to the *Streptococcus scarlatinae* group, for a number of workers (Stevens and Dochez, 1926; J. Smith, 1926; etc.) have isolated strains from such complications which agreed with the Dochez-Dick strains in every test which was applied.

There remain for discussion at least three important questions:

(*a*) Given a haemolytic streptococcus of unknown origin, can we decide quickly and certainly whether it is a *Streptococcus scarlatinae* or not?

(*b*) Are there different types of scarlet fever caused by different strains of the streptococcus, or is there but one clinical scarlet fever caused by serologically differentiable streptococci?

(*c*) Does the *Streptococcus scarlatinae* attack only as a toxigenic organism, or has it another mode of attack which we may call the septic or pyogenic method?

Our best hope of getting answers to questions (*a*) and (*b*) lies at present in the agglutination and intracutaneous tests.

Agglutination.—There seems little doubt, from the investigations of the past few years in which both American and English workers have played an important part, that the agglutination and agglutinin-absorption methods are of service. If one handed to any of these workers two groups of some twelve strains of haemolytic streptococci, one group taken from the throats of scarlet fever patients, the other from patients suffering from erysipelas, puerperal fever, or septicaemia, etc., it is probable that in every such experiment the scarlet fever group would be identified with certainty. But if one wishes to identify individual cultures difficulty arises Our own limited experience suggests that a small number of individual strains fail to give satisfactory results, and it is interesting to note in the American literature the occasional reservations of the more critical workers that a certain small proportion of strains will not form stable suspensions or do not

fall clearly into the scarlet fever agglutination group or subgroups. Stevens and Dochez (1926) stated their position thus:

> "Erysipelas strains form a closely related group of haemolytic streptococci. Scarlatina strains form an equally compact group. The two groups are related antigenically, but less closely related than the strains within the groups. These groups are related to pathogenic strains, but less closely than they are related to each other."

Had these authors added that there were apparently subgroups, as indicated by agglutination and absorption reactions, inside the scarlet fever group, etc.,[1] their position would have corresponded almost completely to that of most American workers and of Gordon, Smith, Eagles, and F. Griffith in England.

The answer to our first question is that, unfortunately, we cannot quickly and certainly identify a given haemolytic streptococcus as *Streptococcus scarlatinae*, for though the group is fairly clearly defined serologically, occasional scarlet fever strains fail to agglutinate with the specific scarlet fever serum, and some strains derived from other diseases agglutinate and absorb with the specific serum. The difficulty in dealing with suspected "carriers" is unfortunately obvious.

If we attempt to answer question (*b*) by the agglutination method we cannot give a clear reply. From various published reports (notably that of J. Smith[2]) it seems fairly certain that inside the scarlet fever group of streptococci it is possible by agglutination to define more or less closely several subgroups. The biological significance of these subgroups we cannot at present determine. So far as I know, no worker has suggested that these different subgroups are associated with different varieties of scarlet fever. Our own results in diphtheria, where we can take a group of true virulent *C. diphtheriae* excreting a toxin neutralizable by one specific antitoxin, and proceed by agglutination and absorption methods to differentiate a large number of subgroups, make us disinclined to believe that this serological subgrouping has any pathological significance.

Toxigenic Power.—There remains another method of attempting to answer questions (*a*) and (*b*). The specificity of the intradermic toxin reaction is high, but it is probably not as absolute as it certainly appears to be in connexion with *C. diphtheriae*. If a haemolytic streptococcus which grows normally and is specifically agglutinated by anti-streptococcus agglutinating serum produces a toxin which is neutralized only by scarlet fever antitoxin, the evidence that the organism is a *Streptococcus scarlatinae* becomes almost convincing. Yet in the light of the available evidence it cannot be said that the absence of this toxigenic power is sufficient *absolutely* to exclude a given organism from the scarlet fever group, or that its presence warrants a dogmatic assertion that the organism is undoubtedly the *Streptococcus scarlatinae*.

The idea that there may be varieties in the toxins pro-

duced by the group of scarlet fever organisms has suggested itself, and Park[3] has, from his observations on specific neutralization with scarlet fever serum, recently put forward the conception that there may be groups of streptococci which produce toxins not identical but having an antigenic overlap.

Schultz-Charlton Blanching.—This test may carry us a little further. If an antitoxin made by injecting animals with the toxin of a given streptococcus blanches the rash of scarlet fever and not of measles, rubella, enema rashes, etc., there is strong justification for considering the organism as the specific *Streptococcus scarlatinae*. Yet even here we must make a slight reservation, for, though we know that the great majority, some 80 to 95 per cent., of scarlet fever patients, if tested within seventy hours of the appearance of the rash, will show the specific blanching, we cannot be certain that we can produce the phenomenon on 100 per cent. of patients tested. Since scarlet fever antitoxin produced in the horse, or the serum of a patient convalescent after scarlet fever, will " blanch " a very high percentage of scarlet fever rashes, we are justified in considering all strains of *Streptococcus scarlatinae* as toxigenically identical. It is true that few clinicians obtain 100 per cent. of positive blanching results when testing scarlet fever patients, but so far as one can judge from the very meagre observations available, the serum of these "non-blanching" patients, taken during convalescence, will in its turn blanch ordinary scarlet fever rashes, which also blanch with the antitoxin serum produced in the horse. There is here no clear evidence that there are two types of scarlet fever.

Our answer to the second question, then, is that there is probably only one type of clinical scarlet fever, which may be caused by a group of haemolytic streptococci containing subgroups that are differentiable by agglutination methods, but not at present clearly separable by intracutaneous injection of toxin or the Schultz-Charlton method of test. " Puerperal scarlet fever " and " surgical and burns scarlet fever " are puzzling syndromes, and their relation to ordinary infectious scarlet fever is not at present clear. Many authorities consider these attacks as true infectious scarlet fever occurring in patients whose illness may possibly have increased their liability to scarlet fever; others think that such patients develop " scarlet fever "—that is, fever with scarlatiniform rash but with little or no inflammation of the throat—caused by streptococci derived from the vagina or wound, which may not be identical with the ordinary *Streptococcus scarlatinae*; further, that such attacks are not infectious, probably because the cocci are not liable to be spread as droplets in coughing and talking as they are in an ordinary attack of scarlet fever with sore throat. It should be easy, by a careful study of such cases by means of the Dick and Schultz-Charlton reactions, and of the cocci isolated therefrom, to establish clearly what is the relation between these curious attacks and true scarlet fever.

In attempting to answer our third question the available data at present are insufficient to give much help. The large amount of evidence available has convinced practically all observers that scarlet fever antitoxin is a specific curative serum, and that, given early in the disease, its beneficent action approaches that of the specific antitoxin used in the treatment of diphtheria.

In addition, considerable evidence is also available suggesting that the giving of antitoxin early in the disease decreases the liability to complications. Some clinicians state that scarlet fever antitoxin will benefit late septic cases also; most physicians have had no such good results. If " late septic cases " gave invariably or usually a " Dick-positive " response this would be fairly clear proof that they possessed little or no antitoxin, and one could believe that the antitoxic serum might be useful. Unfortunately, no long series of Dick tests on late septic cases have been published, but apparently these patients more often than not show a negative response to the test. We would not, therefore, expect antitoxic serum to benefit these patients. If further experience were to prove that the giving of purely antitoxic serum to " septic " cases is beneficial we should almost be compelled to believe that the " toxic " mode of attack of the streptococcus is by far the most important and almost the only one—that is, that the toxin causes local damage in the ear or in the tissues, and that in this damaged area the streptococci, which must be circulating in the patient's blood at some stage of his illness, can settle down and produce further damage.

The laboratory evidence is at present against the extreme view that the toxic attack is all-important and the attack by the living streptococcus but of secondary import. The rabbit is almost completely resistant to the toxin of the *Streptococcus scarlatinae*, whether given intracutaneously, subcutaneously, or intravenously, whereas the living culture injected intravenously kills, and, given intracutaneously, causes a large lesion often followed by septicaemia and death.

Summary.

Haemolytic streptococci cause infectious scarlet fever. These streptococci tend to form a group which can be differentiated by agglutination methods from the haemolytic streptococci of erysipelas, septicaemia, puerperal fever, etc. There is some agglutination relationship between the more or less sharply defined scarlet fever group of haemolytic streptococci and the groups associated with other diseases. The streptococci causing the " septic " complications of scarlet fever probably usually belong to the group of scarlet fever haemolytic streptococci.

References.

[1] See *Journ. Exper. Med.*, xliii, 1926, Table IV, p. 387. [2] *Journ. of Hygiene*, xxv, 2. [3] *Journ. of Immunol.*, x, 1925, p. 829.

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With regard to the validity of the streptococcal theory of scarlet fever there seems little to add to the general statement of the opener of this discussion. It cannot, of course, be maintained that the streptococcus is the cause of scarlet fever with the same certainty that the Klebs-Loeffler bacillus is the cause of diphtheria. At the same time the work of the Dicks and Dochez has shown how profitable a working hypothesis the streptococcal theory of scarlet fever has proved. It would be unwise, however, to overlook the possibility of other agents being involved in the etiology of the disease. All who have worked in this field have met with facts which appear to be discordant with the simple statement of the theory. That this is the usual state of affairs in attacking any new problem is admitted, but it must also be confessed that in this subject the difficulties and discrepancies become more numerous with the progress of observation and experiment.

The solidly established facts are: (1) The Dick test indicates—though by no means with precision—the state of susceptibility or non-susceptibility to the disease. (2) Streptococcal "antitoxin" has a curative value in suitable cases of the disease. There seems little doubt that both the toxigenic and the pyogenic activities of the scarlet fever coccus are responsible for most of the clinical manifestations of the disease, and that the injection of sterile toxin alone can produce a syndrome bearing the closest resemblance to scarlet fever.

With regard to most other aspects of the problem there seems much to be said on both sides. It has been frequently pointed out that one great obstacle to the advance of knowledge on this subject is due to the difficulties in titrating both scarlet fever toxin and antitoxin. To begin with, the only animal which has been conclusively shown to be susceptible to the toxin is the human being, and such progress as has been made is entirely due to experiments on human volunteers. The activity of toxin is estimated from its activity on susceptible persons, but it must be remembered that probably comparatively few people, especially in the older age groups, can be taken as being virgin soil so far as scarlet fever infection is concerned, and still fewer so far as streptococcal infections in general are concerned. This may or may not be the explanation of the difficulty met

with in trying to compare the activity of two toxins. One can imagine the uncertainty that would have arisen in connexion with the similar problem in diphtheria had the only experimental animals been men or horses, who have so frequently acquired some degree of immunity to that infection. As it is, the comparison can only be made after a large series of observations and with great caution.

The difficulties of finding the neutralizing power of an antitoxin in terms of a known toxin dose are still greater. One of the difficulties is that reactions due to the serum may be misleading, but even apart from this it has proved very difficult to evaluate either serum or toxin in terms of the only phenomenon which is at present available for the purpose—namely, the delicate skin reaction due to the toxin. The toxin reaction, indeed, appears to be almost entirely a vasomotor effect, and can be easily disturbed by non-specific factors. It tends, moreover, to be an all or nothing phenomenon. It may happen that injections containing very different amounts of toxin produce almost indistinguishable reactions. In the case of diphtheria toxin the size of the reactions bears a definite relation to the amount of toxin used.

Most of those who have experience in titrating streptococcal antitoxin in terms of skin doses of toxin admit that very wide discrepancies may occur even when the greatest pains have been taken to ensure standardized conditions of test. In some cases the neutralizing value of a sample of serum, as determined by different observers in terms of skin doses of toxin, has varied as much as tenfold. Under such conditions it is obviously premature to lay down standards of either toxin or antitoxin. Most of the competent authorities in America seem to be in agreement that such standards as are chosen should be of a purely provisional character.

If accurate standardization is aimed at it is important that the fundamental standard should be that of a serum rather than a toxin. Drs. McCoy and Dyer of the Hygienic Laboratory in Washington have prepared a serum which they propose to issue as a provisional standard, and there seems no reason why this should not be accepted in this country also. It is a serum of apparently high antitoxin content, and will serve as a constant for comparison until more definite standards can be laid down. The method at present customary in America of giving the titre of antitoxin in terms of units is liable to be misleading, and in this country we have not as yet seen any sound reason for adopting it. The present position in regard to the standardization of scarlet fever serum may be summarized as follows: The distinction of good from bad serum depends ultimately on its therapeutic effect. By means of toxin neutralization tests, blanching tests, or the passive immunity method, the therapeutic value of the serum can be predicted, but at the moment anything like close quantitative comparison of serums is impossible. On the evidence available it is not even clear which of the methods

customarily used in evaluation is the best. The important thing for the time being is that serum of reasonable clinical efficiency be issued, and that what titration experiments indicate as the effective dosage should be stated.

Before leaving the question of toxin and its titration reference should be made to the methods of test which do not involve the use of human subjects. The *in vitro* methods on the lines of the Ramon test gave no results in our hands. Like Dyer we found that flocculation occurred in mixtures of scarlet fever toxin and antitoxin, but we could establish no numerical relationship between the two. This is confirmed by Dyer's latest and as yet unpublished work. No animal we have tried has reacted at all promisingly to the toxin except the goat. This animal has been used by Kirkbride and Wheeler for the titration of toxin and antitoxin. We have found goats which reacted to 0.2 c.cm. of very dilute (1 in 4,000) toxin, but here again the numerical difficulty occurred. As much as 80 times the minimum reacting dose produced a reaction of very much the same size as the minimum reacting dose. One goat also, which gave a good reaction intradermically with 0.2 c.cm. in 1 in 1,000 dilution, failed to show the least abnormality when 5 c.cm. of pure toxin was given subcutaneously. If the skin reaction in goats is therefore a true toxin effect it is strange that 25,000 skin doses should produce no effect, general or local, when given subcutaneously. It would be unwise to say that the goat cannot function as a test animal, but until such discrepancies as we have mentioned can be explained away it would be as well to be cautious

It must not be forgotten that it is one of the most characteristic properties of a true toxin that the toxic effect produced is proportional to the amount of toxin given. Dick toxin, as regards its effect on human beings, falls into line with other toxins. As far as the work of Parish and myself has been concerned this has not been the case with goats or other animals, and we are disposed to look upon such reactions as are produced in these animals by filtrates as due to some form of allergy rather than to a true toxin effect.

Another point to which I wish to refer is of both clinical and immunological importance. The streptococcus plays a dual role in scarlet fever: (1) it produces a toxin, and (2) it may invade tissues like any other pyogenic organism. Uncomplicated scarlet fever appears to be almost purely a toxaemia, which is rapidly combated by the production of streptococcal antitoxin; but the streptococcus can, and often does, take a further role as an invasive organism, producing effects similar to those produced by any other pyogenic streptococcus. The pyogenic function of the streptococcus appears to be more or less separate from the toxaemia function from the immunological and even from the clinical point of view.

The most important fact bearing on this is that the patient who has a pyogenic complication of scarlet fever

is " immune " to the toxin—that is, he almost always has a negative Dick reaction. Once pyogenic complications are established antitoxic serum has no effect on them. This dual nature of the streptococcus is paralleled by animal experiments. Mice and rabbits are not susceptible to the streptococcal toxin, but they may be killed with a septicaemia by the scarlet fever coccus. Rabbits may also be given a typical pyogenic infection of the skin with the scarlet fever streptococcus. It is not clear that any amount of scarlet fever antitoxin *per se* can be depended upon to protect against such infections. H. J. Parish, working with me on this point, has been unable to show that serums prepared either against toxin or bacterial bodies are able consistently to prevent the septicaemia of mice or the pyogenic skin reactions in rabbits. On the other hand, rabbits may be killed with 5 c.cm. of a young broth culture of the scarlet fever streptococcus, given intravenously. Scarlet fever antitoxin which was prepared entirely by immunization with sterile filtrates (toxin) when given five hours beforehand will prevent the death of the rabbit. Under certain conditions of experiment this effect is quite constant. It is not clear to us if this preventive property of the " antitoxin " is purely an antitoxic effect, but if it proves to be such it gives us hope that an animal test of an antitoxic activity might be evolved on the basis of Parish's experiments. If, on the other hand, the effect is due to the accidental presence of an antibody other than antitoxin it still seems worthy of close investigation, since a serum rich in such antibody might be useful in treating human patients in the pyogenic stages of the disease.

Though scarlet fever antitoxin does not deal with all the pathogenic activities of the streptococcus it is very important as a therapeutic agent. Experience has been uniformly in favour of the view that if it is given sufficiently early it tends to prevent the septic complications of the disease. The aggressive action of the toxin seems to be an important factor in the invasive power of the streptococcus. Antitoxin appears, therefore, to be an invaluable therapeutic agent in the toxic phase of scarlet fever, while it is quite ineffective in the purely pyogenic phase of the disease. Indeed, in the pyogenic phase there is generally in the patient's serum more than enough antitoxin to do all that antitoxin can do.

With regard to the value of agglutination as a means of identifying scarlet fever streptococci I might refer to the results obtained by Miss G. I. Steed and myself. We examined thirty-four strains of haemolytic streptococci from cases of scarlet fever by the technique described by Mervyn Gordon in his work on the subject. Twenty-seven strains absorbed the agglutinin from a serum made by injecting a rabbit with the killed Dochez streptococcus. The titre of the serum was 1 in 800. No detectable agglutinin was left when 1 in 100 was tested after absorption. Two strains completely failed to absorb agglutinin from this serum, but absorbed it from serum prepared from

S. F. 18, a toxigenic strain isolated by ourselves. The two Dick strains, and also one from Dr. Anna Williams, only absorbed Dochez serum to 3/4 titre; S.F. 18 serum was completely absorbed by these three strains. Eleven strains of haemolytic streptococci from sources other than scarlet fever—for example, puerperal fever, pyorrhoea, etc.—were also tested for absorption; the great majority of these did not absorb the agglutinin, but five strains which seemed to have no connexion with scarlet fever did absorb it. These were respectively a strain isolated from strangles in a horse, one from a case of pyorrhoea alveolaris, and two from cases of pyogenic infection. It was thus possible arbitrarily to separate by absorption the bulk of scarlet fever strains from non-scarlet fever strains, but not with sufficient certainty to allow the diagnosis of an individual strain. These results confirm the work of Stevens and Dochez, and other recent investigators. At the best positive agglutination or absorption results are but presumptive evidence that a given streptococcus is a scarlet fever organism.

Dr. O'Brien, in closing the discussion and in answer to questions, said that the work of Dr. Parish seemed to show that a good scarlet fever antitoxin would consistently protect a rabbit against a lethal dose of culture of *Streptococcus scarlatinae*, both being given intravenously, while a poor serum would not. The immunization of nurses was already being practised in England with, so far, very promising results. It was at present premature for the bacteriologists to claim to speak with authority to the public health administrator and tell him which people were potential carriers—that is, distributors of scarlet fever. Dr. Trevan had tried the effect of scarlet fever toxin on the capillary loops of the intestine, but could not find any consistent effect. He agreed with Professor Douglas that the absence of research in infectious fever hospitals was lamentable, but hoped that the appointment of the pathologists to the Metropolitan Asylums Board and also, probably, to the fever hospital in Manchester, would mark the commencement of a time when the amount of clinical pathology in infectious fever hospitals would be comparable with that in large teaching general hospitals. He felt that if the antibacterial serum made by the injection of streptococci could be of any use, that fact would have been discovered during the many years that antistreptococcal serums had been used for the treatment of scarlet fever. He was, however, at present disinclined to hope much from antibacterial scarlet fever serum. He had concentrated scarlet fever serum on a large scale; apparently considerable losses occurred if the routine processes were used, but it was very difficult to check the loss, since the present methods of titration were so difficult to command and so liable to fallacy. The speaker now fully accepted the *Streptococcus scarlatinae* as the cause of the

disease. If belief was retained in a filterable virus it was necessary to show that "subcultures" through many generations of Dick "toxin" must still produce the "Dick reaction," and, further, it must be assumed that the virus survived and grew *pari passu* with the streptococcus through many generations in artificial culture, for antitoxin made by injecting the toxin of the streptococcus subcultured for several years was specific.