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Cerebro-Spinal Fever and Meningococci Infection

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This contribution consists in a review of work done during the severe epidemic of cerebro-spinal meningitis which occurred in Belfast during 1907 and 1908, together with some additional observations that have been made in France during the Great War and also during the present year in Belfast.

During 1907 and 1908 we devised an application of Sir A. E. Wright's opsonic technique for the laboratory diagnosis of this infection. We found that this technique was not difficult and gave positive results in several hundreds of cases of the disease examined during the epidemic. By means of this test, a number of cases of meningitis not due to the meningococcus could be definitely excluded, and these negative findings were confirmed in a number of instances by post-mortem examination. Sera obtained from cases infected with other micro-organisms or sera from normal individuals never gave this reaction.

One of us, while in France during the Great War, further tested the accuracy of this method, and again during the present epidemic in Belfast has re-applied this test to the diagnosis of the disease and to determine the type of the infecting meningococcus. The points of interest in this serological test are—

(1) Its great value in the diagnosis of meningococcal infection—the first place in diagnosis must of course be given to the isolation of the meningococcus from the cerebro-spinal fluid or the blood, but in a number of cases such examinations may fail to give positive results, and again in so-called meningococcal septicæmia without meningitis blood-culture may be negative.

(2) The reaction to be described occurs in cases treated with Sulphapyridine, and as this reaction is no doubt a reaction of immunity, its presence in such treated cases seems to support the view of Fleming and others that the action of such drugs is bacteriostatic and that the defences of the tissues are also necessary for the cure of the disease.

(3) This method has only differentiated two types of meningococcus, and has not confirmed Gordon's classification into four types.

The details of this technique are as follows : An actively growing meningococcus of the required type is necessary for the test. Mr. Fred Burns, one of the laboratory assistants in the Institute of Pathology, Belfast, has found the following plan a reliable one for keeping the meningococcus alive.

To 1 per cent. agar in ordinary broth is added 20 per cent. serum, and the medium is allowed to set in test-tubes. Three or four stabs are made into this medium with a culture of the meningococcus, and the tubes put in the incubator overnight. The medium is then covered with sterile liquid paraffin and stored in the incubator. By slanting the tube, a small piece of agar can be fished out, and this gives a good growth of the meningococcus on a serum agar slant. In this way the culture remains viable for months.

The meningococcus usually makes a perfect emulsion in saline, but some cultures recently isolated may show some small clumps in saline. Such strains should be cultured daily for several times, when a more uniform emulsion free from clumps will result. In these cultures it is best to use a 3 per cent. agar with serum, as with 1 per cent. agar small pieces are apt to get into the emulsion.

(1) The test emulsion is made in saline or Ringer's fluid, and should be as uniform as possible. It should be freshly prepared, and not kept for more than an hour. It must be made from a rapidly growing meningococcus, and a six- or seven-hours growth should be used. If a twenty-four hours growth is used, the emulsion contains numerous degenerating cocci, which stain badly and act like particles of Indian ink and do not give the specific reaction.

(2) The washed corpuscles are prepared in the usual way for doing an opsonic index, i.e., a few drops of a Type IV (O) blood are put into a centrifuge tube containing citrate solution, and separated by the centrifuge, the supernatant fluid is pipetted off, and the deposit is washed with saline and again separated by the centrifuge and the supernatant saline pipetted off. The deposit furnishes the white cells for the test.

(3) The sera from the patient's blood and the controls are obtained in the usual way.

The test is done as follows :

The technique is that of the opsonic index. A capillary pipette is taken and a fiduciary mark made at a convenient distance from the distal end : Then equal parts of the washed corpuscles, the emulsion, and the serum are drawn into the pipette with a rubber teat, each separated by a bubble of air. The contents are thoroughly mixed on a slide and reaspirated into the pipette, and the distal end sealed in the flame. The pipettes are then incubated for fifteen or twenty minutes at 37°C., preferably in an opsonic incubator.

After incubation the contents are again mixed and films are made on glass slides. The making of suitable films requires a little practice, but one who has done opsonic indexes will find no difficulty. The films are stained by Leishman's stain and examined with an oil immersion lens.

The positive films show marked agglutination and high phagocytosis. The low opsonic effect always found with the control preparations made with normal or

non-infected sera, combined with the uniform distribution and absence of agglutination of the meningococcus, forms a striking contrast to the very high opsonic effect and marked agglutination of the positive films. Opsonic index determinations with other micro-organisms require most careful and accurate counts, but in the case of this reaction such laborious work is usually unnecessary. The picture is so characteristic that an examination of the films is all that is necessary to arrive at a definite diagnosis. The validity of this technique depends on the fact that normal sera or sera from cases infected with other organisms have very little opsonic and no agglutinative effect on the meningococcus.

Another interesting character of the meningococcus developed while we were working with a coccus isolated from one of the first cases of cerebro-spinal fever in Belfast. This coccus had been subcultured about 150 times, and was about five months old. We found that at this time the leucocytes in the preparations made with normal sera phagocytosed more cocci than they had formerly done. Thus whereas in using an emulsion of approximately the same density the earlier preparations showed from twenty-five to fifty cocci in fifty leucocytes—the later preparations showed from two hundred to three hundred cocci in fifty leucocytes. At first this coccus had agglutinated in a marked degree with sera from cases of cerebro-spinal meningitis, but shortly after we had noticed this rise in the ingested cocci in the control preparations we found that this coccus was no longer agglutinated with serum which in preparations containing a more recently isolated coccus gave a high opsonic power and marked agglutination. This observation is exceedingly interesting, as it shows that the meningococcus by prolonged growth may entirely lose these important properties. Various other experiments such as heating the emulsion, growth on unsuitable media, etc., made the meningococci unsuitable for doing opsonic determinations, as these altered cocci were phagocytosed in a non-specific manner. By the examination of sera from several hundreds of cases we found that this technique gave reliable and definite results.

The following table is a synopsis of the results obtained by the examination of 114 cases by this method :—

Day of Disease	Number of Determinations	Number Giving Positive Reaction	Number Giving Negative Reaction	Percentage of Positives
1st	1	0	1	0
2nd	12	3	9	25
3rd	11	3	8	27
4th	13	2	11	15
5th	5	3	2	60
6th to 133rd	130	125	5	96.1

Thus of 114 different cases of epidemic cerebro-spinal meningitis, 40 were examined during the first five days of the disease, 10 of these were positive and 30 were negative. Of the 74 cases examined from the 6th day onward, 4 were

convalescent for some time and 70 were suffering from the disease : of these 70, 68 gave a positive result and 2 gave a negative result, that is, 97.1 per cent. gave a positive result. As a rule this reaction quickly disappears when the disease subsides, but it persists longer in cases that have been ill for a prolonged period.

Having satisfied ourselves about the accuracy of this method as a means of diagnosis, the reaction was used—

(1) To contrast the opsonic and agglutinative power of the blood-serum and that of the cerebro-spinal fluid. It was found that the cerebro-spinal fluid had always a much lower opsonic and agglutinative power than that of the blood-serum of the same patient taken at the same time.

(2) To distinguish different cocci.
   (a) Were there different strains of meningococci?
   (b) Was the epidemic which we were investigating always due to the same strain of meningococcus?

The first question (a) was answered in the affirmative through the kindness of the staff of Great Ormond Street Children's Hospital, who sent us sera and meningococci from the cases of meningitis occurring in their hospital. Some of these cases were acute, and resembled clinically cases of epidemic cerebro-spinal fever, while others might be classed as cases of typical posterior basal meningitis.

It was evident from our results that—

(1) The meningococci of the Great Ormond Street cases react with the blood of these cases, but give no reaction with the blood of the epidemic cases in Belfast.

(2) The meningococci from the Belfast cases react with the blood of these cases, but do not react with the blood of the Great Ormond Street cases of meningitis.

These results were confirmed by Dr. Alice Taylor at Great Ormond Street.

The second question, viz., were the epidemic cases which occurred during the years 1907-1908 all due to the same strain of meningococcus? To answer this question we obtained menigococci and sera from the following centres :—

(1) Ruchill Fever Hospital, Glasgow.
(2) City Hospital for Infectious Diseases, Edinburgh.
(3) Hamburg—meningococci sent by Dr. Carnwath and Dr. Trautman.
(4) New York and Municipal Hospital, Philadelphia — through Dr. Simon Flexner.

The evidence derived from these examinations by this technique was sufficient to show that cases of epidemic cerebro-spinal fever which have occurred during 1907-1908 on the Continent, in America, in Glasgow, Edinburgh, and Belfast, were all due to a menigococcus having the same agglutinative and opsonic reactions. This meningococcus was called Type I, while the meningococcus obtained from the Great Ormond Street cases termed Type II.

While at Etaples in France, one of us with the help of Dr. John McCloy had an opportunity of verifying the value of this test on several cases of cerebro-spinal

fever. We also made a study of the meningococci obtained from the naso-pharynx of carriers occurring amongst the Forces stationed there, and we found that a number of these cocci belonged to Type I, while others were Type II, and also that some strains from carriers could not be classified in either type.

Having read the Medical Research Council's Report of the work of Dr. M. Gordon (No. 3) and his colleagues, we endeavoured to classify our strains of meningococci, using the sera and technique recommended in this report, into Gordon's four types. In our hands this investigation was far from satisfactory, often giving indefinite and irregular results. We think it possible that such unsatisfactory results may have been due to the fact that the emulsions for agglutination were prepared from an eighteen- or twenty-four-hour growth of the meningococci, and were always heated before use. We have indicated already that such procedures may destroy the specific properties of the cocci in such a way that they may not give the specific opsonic and agglutinative qualities described in this paper.

We found, however, that the serum of some of the persistent carriers gave the opsonic and agglutinative reaction, although they had no symptoms of meningitis.

As a number of cases of cerebro-spinal meningitis were occurring at the present time in Belfast and the neighbourhood, one of us with the help of Dr. McCoy has re-applied this test to the diagnosis of the disease and to determine the type of the infecting coccus.

We are indebted to Dr. Kane of the Purdysburn Fever Hospital for a number of specimens of blood from infected cases that had been treated with Sulphapyridine; and to Dr. Norman Graham for strains of the meningococcus recently isolated from the cerebro-spinal fluid in the laboratory at the Royal Victoria Hospital.

These recent results have fully confirmed our previous findings of 1907, and have shown that this reaction is almost invariably present in this form of meningitis, and does not seem to be prevented by treatment with Sulphapyridine. The cases examined up to the present have all belonged to Type I, and the meningococci isolated from cases of meningitis here have all belonged to this type.

Within the last month, Major F. B. Smith, B.E.F., has drawn our attention to the fact that cases of meningo-septicæmia are occurring in France. These cases are characterised by attacks of intermittent fever, no symptoms of meningitis, metastatic cutaneous nodes and rash—the intermittent attacks of fever are coincident with the rash. The patients are usually not severely ill.

The following memorandum was sent to pathologists in France :—"Meningo-septicæmia (chronic or subacute), e.g., eight cases from two hospitals: Fever 3-6 weeks, painful muscles and joints, painful raised small nodes in the skin, headache, leucocytosis, two positive blood-cultures of Type I and Type II meningococci. A mild illness easily missed, responds promptly to Sulphapyridine."

The serum from one of these cases that gave a negative blood-culture was sent to Belfast by Major Smith, and gave the characteristic reaction described in this article, with Type I meningococcus.

We are also indebted to Major J. M. Houston, who is pathologist to a military hospital in England, for an account of a similar case with bouts of temperature and rash but no symptoms of meningitis. The meningococcus (Type II) was isolated by blood-culture and the case responded to Sulphapyridine.

We are indebted to Dr. Norman C. Graham for the following observations, which may have some bearing on the preparation of the suspensions used in the test:

"During the recent outbreak of cerebro-spinal fever I was interested in the types of meningococci isolated from the cerebro-spinal fluid of cases admitted to the Royal Victoria Hospital. In the experiments I used recently isolated cultures and type sera supplied by the Medical Research Council. By the use of a dry, dark ground condenser, microscopic observation of the agglutination of the coccus was facilitated and the type rapidly determined. The majority of strains studied were Type I, and this was subsequently confirmed by the usual macroscopic technique.

"It was considered that this method of microscopic observation of agglutination might be applied in studying the agglutinins in the patient's blood during the early stages of the disease. For this purpose a Type I strain was used, and seven specimens of the sera of patients from the eighth to the thirty-ninth day of disease were investigated conjointly with Sir Thomas Houston. All gave a positive result in a dilution of 1 in 10, and no agglutination with the specimens of normal sera investigated. With the majority of specimens a much higher titre could be readily demonstrated, in one specimen in a dilution of 1 in 100. In using this technique it is essential to use a young actively growing culture, incubated for not more than six to eight hours. A small portion of the growth is taken directly from an agar slope and carefully emulsified in an appropriate dilution of the patient's serum in such a way that no visible microscopic clumps occur as shown by a control preparation. A satisfactory way is simply to emulsify the dilution of serum and culture on a cover-slip, and invert over a hollow ground slide, which is then sealed with vaseline. Agglutination usually occurs immediately or within ten minutes in a 1 in 10 dilution in a positive case.

"From preliminary experiments it has been found that suspensions of older cultures (fifteen to eighteen hours) in normal saline are inagglutinable and unsuitable. Similar suspensions prepared from a twelve- to fifteen-hour-old culture are also unsatisfactory unless used immediately. These observations support the view of Flexner (1907) that the salt solution has an injurious action on the meningococcus, but they also may result from antigenic changes in the micro-organism during the later stages of its growth on an artificial medium.

"It is suggested that further investigation of patient's sera by this simple technique may prove useful in the diagnosis of meningococcal infections."

Our original papers on this subject are to be found:—

Lancet, 4th May, 1907.

British Medical Journal, 16th November, 1907.

Journal of the Royal Sanitary Institute, 1911, 32, No. 9.