Clinical and other Notes.

CONCENTRATION OF COMPLEMENT AND AGGLUTINATING SERUM.

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AND

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Through the courtesy of Dr. A. S. Plant and the Editor of the British Medical Journal we are permitted to publish the following note which appeared in the British Medical Journal of March 11, 1933:

"COMPLEMENT OF HIGH TITRE.

"If one freezes solid a test tube of guinea-pig serum in a mixture of ice and freezing salt and then thaws it in either a water bath or incubator, and looks at it from time to time, a change will be seen taking place. On gently rocking the tube there is a movement of oily-looking streaks between the top and bottom portions, and ultimately, when the thaw is completed, a darker-coloured lower and a lighter-coloured upper portion will be observed. If, without shaking or inverting the tube, the freezing and thawing is repeated a few times the differences between the two portions are more marked, until the lower portion becomes highly-coloured and heavy-looking, and the top portion colourless. If the complement titres of the extreme upper portion and extreme lower portion are tested with sensitized sheep cells a vast difference in the two will be noted. The top may give a titre of 1 in 5 and the bottom 1 in 150. Evidently that portion of serum responsible for haemolysing sensitized cells has largely migrated to the bottom of the tube. On inversion of the tube a few times the two portions blend together, and an apparent return to normal serum occurs.

"I found this out about a year ago, when, for economic reasons, I began to freeze solid and preserve in a freezing mixture in a vacuum flask in the ice chest the guinea-pig serum left over from the day's Wassermann tests.

"By applying similar methods, the greater part of the serum can be recovered from the saline dilutions employed in the Wassermann tests, and can be used again on another day. There did not appear to be much of practical value gained except the possibility of being able to preserve the high titre portion of the complement in liquid or powder form. In the frozen solid state I have kept it for six months, and obtained quite a good titre."
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"I communicated my observations to Dr. B. J. Wyler, the Ministry of Health pathologist for serum tests for syphilis, who confirmed my findings with undiluted serum. Experimenting with the inactivated serum of rabbits immunized with sheep's red cells, he found he could get an increased haemolytic titre of the lower portion. I am publishing this note after reading Dr. Gordon's account of the part played by complement fractions in combating disease, as it occurs to me that the freezing and thawing of serum which causes the separation of certain components may be of value to those who are working on this subject."

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The Meerut District Laboratory is one of five laboratories in India which carry out the Wassermann tests. It undertakes the Wassermann tests for the Eastern Command. The laboratory remains in Meerut during the cold weather, and before the commencement of the hot weather it moves to Ranikhet in the Kumaon hills for a period of six to seven months. The laboratory animals move with it.

Our experience with regard to complement is as follows:—

In Meerut complement of good working titre can usually be obtained although some falling off is noticed in February and March. As a rule the move to the hills takes place in March. On arrival the complement approximates to the titre of that in Meerut, but a gradual falling off takes place which appears to reach its maximum after three or four weeks' residence in the hills. At this period it becomes difficult to obtain a workable complement and rebleeding in the hope of obtaining such a serum is of frequent occurrence. We have attempted to prevent this deterioration in complement in various ways, but so far without success. Stoves were installed in the animal house to minimize the effect of the sudden change of temperature. Lucerne cannot be obtained in Ranikhet, but a liberal supply of carrots, lettuce and vegetable tops has always been available. We feel certain that the question of feeding does not account for the low titre of the complement. A portable run was made so that the animals should have fresh air and sunshine in plenty.

At the time of the arrival of the British Medical Journal containing Dr. Plant's note we had been one month in Ranikhet and were experiencing our usual trouble and anxiety with regard to complement. The method described appeared to offer a way out of the difficulty, and we hastened to confirm it. A complement was titred and found to be 1 in 40 (complement row only). About five cubic centimetres of this serum were then placed in a centrifuge tube and lowered into a vacuum flask which contained a mixture of ice and common salt. About five minutes was sufficient to freeze the complement completely. It was then thawed in a water bath at 37°C. This method of thawing did not prove satisfactory owing to the
sudden loosening of the frozen serum from the sides of the tube and resulting mixing of the complement. Subsequent thawings were allowed to take place at room temperature. Freezing and thawing were repeated five times. The two layers described by Dr. Plant were well marked. On gently agitating the tube, an effect similar to the mixing of glycerine and water could be noted. A titration of the extreme lower portion was carried out with sensitized sheep’s cells. Within five minutes complete hæmolysis in a dilution of 1 in 100 (complement row only) had taken place. This was the highest dilution put up, but we feel certain that the limit was not less than 1 in 150. A titration of the extreme upper portion was then carried out, and no appreciable hæmolysis was present even in 1 in 10 after half an hour in the water bath. About 1½ cubic centimetres of the bottom layer were then pipetted off and added to 4½ cubic centimetres of a complement having the useless titre of just less than 1 in 30 (complement row only). The two sera were thoroughly mixed and the resulting titre of the mixture ascertained. It proved to be over 1 in 50 (complement row only).

Further experiments have proved that by taking, say, 10 cubic centimetres of a complement, which when titred with the serum and antigen control rows will not give a workable complement, i.e. at least 1 in 30, it is possible by the freezing and thawing method to produce 4 or 5 cubic centimetres of complement with an average titre of 1 in 50 to 1 in 60. On trying out such a complement in the actual Wassermann tests with numerous controls it was found to work admirably and no anomalous results were experienced.

One has the belief that centrifuging at a very high speed would result in a similar concentration of complement. Perhaps someone with a high speed electrical centrifuge will try this out.

A minor but important point noted is that thorough mixing of the concentrated complement with the saline in the dilution tubes is necessary. The concentrated complement is rather viscid and tends to settle to the bottom of the saline if mixing is not thorough, but this presents no difficulty. A point which also emerges is that if one is ever using complement which has been frozen, thorough mixing is necessary to ensure an even titre throughout.

CONCENTRATION OF AGGLUTININS.

As an extension of the principle described it was decided to carry out experiments to determine if agglutinins could be concentrated in a similar manner.

As all soldiers in India are inoculated with T.A.B. at definite intervals, their sera contain “H” agglutinins against T.A. and B. Patients’ sera left over from the Wassermann tests were thoroughly mixed together, and the titre of the mixture against a standard emulsion of B. typhosus was ascertained. It was found to be a trace in 1 in 500. Freezing and thawing of the mixture was carried out four times. Separation into two layers was
well marked. The titre of the extreme upper and lower portions against the standard T emulsion was then ascertained. That of the former was 1 in 50, while that of the latter was a trace in 1 in 1000. A further experiment with a similar mixture of sera gave similar results.

CONCLUSIONS.

(1) That it is possible by alternate freezing and thawing to concentrate complement in the lower layer of the serum.

(2) That agglutinins may be concentrated in a similar fashion.

PRACTICAL APPLICATIONS.

(1) During periods in which a workable complement cannot be obtained by direct bleeding, complement suitable for the Wassermann reaction may be obtained by the method described above.

(2) The practical application of the concentration of agglutinins is not so obvious. It is known, however, that it is difficult to obtain a high titre serum against some organisms. By the concentration method described it should be possible to obtain a small quantity of serum of high titre from a larger quantity with a low titre.

Our thanks are due to Dr. Plant and the Editor of the *British Medical Journal* for permission to publish Dr. Plant’s note. They are also due to Jemadar Sarju Prasad, I.M.D., of this laboratory, who carried out the tests on agglutinins at our request and to the late Colonel J. A. Turnbull, D.S.O., A.D.M.S., Meerut District, for permission to forward this article for publication.

AN INTERESTING CASE OF CYSTICERCOSIS.

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The following description of the clinical history and post-mortem findings of the late Pte. J., who died at the Royal Victoria Hospital, Netley, on January 24, 1932, aged 28, as a result of cysticercosis of the brain, is published with the object of drawing attention to the varied symptoms and signs cysticercosis infection may produce. During the last eighteen months I have seen ten cases of cysticercosis all invalided home definitely diagnosed epilepsy major or petit mal, and all with varying types of symptoms referable to some pathological condition of the brain.

Pte. J. enlisted on February 2, 1924, and remained in England until November 12, 1926, when he went to Gibraltar; on February 7, 1927, he went to India; on November 9, 1930, he was transferred to Burma where he remained until May 28, 1931, when he was moved to the Andaman Islands, returning to Burma on August 14, 1931. During the first six years of his