ADDITIONAL NOTES ON THE POTASSIUM-SULPHOCYANIDE NEUTRAL-RED GLUCOSE BLOOD SERUM MEDIUM.

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Since the publication of my paper "A Medium for *Bacillus diphtheriae*" (This *Journal*, Vol. xi. p. 271), I have had further experience of the medium and have tried various modifications.

No medium that would be favourable for the diphtheria bacillus and unfavourable for all others has been evolved so one must still be content with potassium-sulphocyanide-neutral-red-glucose-serum.

It has been found that the composition of the medium can be altered without interfering with its efficiency. The original medium contains three parts of blood serum and one of broth. This means, of course, that broth must be prepared beforehand. Now, it is possible to use water in place of the broth or a mixture of water and broth can be used. This effects a saving of time and also, to a certain extent, of money. The medium prepared in this way gives as good results as, if not better than, the original medium. The colour produced by the acid-producing organisms appears more rapidly and is more distinct. The only apparent defects are that a slight coloration due to cocci is sometimes very like the red produced by the diphtheria bacillus and that the change of colour due to some diplococci is also like that due to *B. diphtheriae*. These effects may be more apparent than real and do not interfere with the accuracy of the results. One very noticeable feature is that there is no red colour when there is no diphtheria bacillus. This, as has been pointed out before, is one of the chief features of the original medium, but it is more emphatic in this modification. All cocci do not produce acid, but the majority of them do and most of the diplococci do the same. Rarely does one find a diplococcus in the culture without a red

colour. Naturally, this refers to the organisms found in the respiratory passages.

I have noticed and my attention has been drawn to the fact that some organisms, having the same morphological characters as the diphtheria bacillus, do not produce a red colour. This is possibly due to one of two reasons, first, that these organisms are not *B. diphtheriae*, or secondly, that they are not virulent diphtheria organisms. It is probably due to both. Many of them are undoubtedly modified Hofmann bacilli, while some are probably non-virulent diphtheria bacilli. A modified Hofmann bacillus is one that does not conform morphologically to the typical Hofmann bacillus. It is well known that in broth cultures this bacillus assumes an appearance very like that of *B. diphtheriae*. This modification may occur on this medium as well. As regards the other point, it has been shown that non-pathogenic diphtheria bacilli produce as much acid as the pathogenic; but may some non-virulent varieties not fail to produce acid? In no case, where such were found in patients discharged, was there any untoward result. No recurrence of the disease occurred in the patient and no case seemed to have been developed from them. The only way to prove the point would be to inoculate guinea pigs. This I must leave to others. It would be very instructive if someone undertook this and published the results. If the evidence proved the theory correct, it would enhance the value of the medium considerably.

Cultures from the nose showing a bacillus that might be that of diphtheria or the *B. coryzae* have a red colour similar to that produced by diphtheria bacilli. Of course, the medium is not of any use in differentiating these: other methods must be employed.

The addition of sodium chloride to the medium has been tried but it has not proved of much value. It seems to inhibit many organisms, but it does not enhance the value of the medium in any way. In some cases, it interfered with the early production of the distinctive red colour, while in others it did not allow a colour to appear when there were undoubted diphtheria bacilli present in the culture. Another modification that also proved ineffective was the addition of a weak solution of crystal violet. The results obtained from this modification were of no value whatever.

The experience I have had with the medium since last summer serves to emphasise the points originally brought forward in favour of it. No better medium has come to my knowledge and no other has been found as good. An interesting point may be brought forward here.

Some weeks ago this medium was not made and some ordinary Loeffler's blood serum was used. The growth of the ordinary organisms was remarkable and it was only with great care that *B. diphtheriae* could be found. I had not worked with this medium for some time and was surprised at the difference between it and the medium under consideration. It was ample proof of the fact that the growth of the common throat organisms was inhibited by the potassium sulphocyanide.

The value of the medium has chiefly been demonstrated in discharging cases. Three clear cultures are required in this hospital before a convalescent diphtheria patient is discharged. In this work, the medium has proved of great value and has considerably lessened one's labours. When there is no red colour, it may be safely assumed that there are no diphtheria bacilli present in the culture. If there is a red colour, one can, by experience, pick out the colour due to the presence of the diphtheria bacillus.

The points I have emphasised before are now emphasised more than ever.

(1) The serum must be good. This is a very important point in connection with the manufacture of the medium. At times, I have had to use bad serum and the resulting medium has simply been a dirty mass which did not give any differentiating features. Perhaps others have obtained this result and no other and so condemned the medium. I would advise them to try again and get a medium of a more or less flesh tint. The colour varies at different times, but this is of no importance. The serum used in this hospital is that of the sheep: only on rare occasions has ox serum been used. It is stated that ox serum is much superior to that of the sheep for media. As far as this medium is concerned, nothing has been done in the way of comparison and no statement can be made.

I have also noticed a red coloured deposit at the bottom of the tube when fresh. This may be due to some red corpuscles and neutral-red falling to the bottom when the tubes are being sterilized. At any rate, it often disappears in a short time and, if it persists, does not cause any trouble.

(2) All solutions used should be freshly made. This point is not to be overlooked as the results may be unsatisfactory if old solutions are used. In addition to this, the materials employed ought to be as pure as possible. Sometimes the glucose is not pure and causes trouble.

(3) One can use a 2 % of a 0·5 % watery solution of neutral-red or 1 % of a 1 % solution.

Method of Preparation :—

Recently clotted sheep's blood is obtained and the supernatant exuded serum is removed with great care. This may be kept in a cold place for a day or two or it may be used at once. In practice, I use it within a few hours. To three parts of this are added one part of broth, water, or a mixture of broth and water. The mixture of broth and water does not require to be of any particular strength. Water alone has been used for some time in this hospital. Then 1 % glucose, 1 % potassium sulphocyanide, and 1 % of a 1 % watery solution of neutral-red are added. The resulting mixture is then placed in small sterilized test tubes and sterilized in the steamer for an hour on three successive days at a temperature of from 180° to 190° F.

The following quantity is commonly prepared in this hospital :— To 300 c.c. of blood serum add 100 c.c. of a glucose solution made by adding 20 grammes of glucose to 500 c.c. of water and sterilizing. Then 4 c.c. of a 1 % watery solution of neutral-red and 8 c.c. of a 50 % solution of potassium-sulphocyanide are added. Prepared in this way and due care being taken, a good medium of uniform colour is obtained.