A MEDIUM FOR *BACILLUS DIPHTHERIAE* (POTASSIUM-SULPHOCYANIDE NEUTRAL-RED GLUCOSE SERUM).

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The preparation of a medium that would allow the bacillus of diphtheria to grow and exclude all other organisms would be an ideal medium for those whose work is concerned with that organism, but, as such a medium has not yet been devised, we must be content with the modifications of the ordinary media which we possess. Various varieties of media have been prepared for the purpose of favouring the growth of *B. diphtheriae*, but very little seems to have been done with regard to selective media.

Dr Myer Coplans, of Leeds, has been experimenting with numerous varieties of selective media for some time and, of late, I have been using his various media both in their modified and original forms. The following notes are concerned with the results obtained by the use of such media and, as over 6000 cultures were examined in this hospital during the past half-year, the results may be of some value and may influence others likewise to experiment with such media. On an average the number of cultures examined yearly in this institution is between 11,000 and 12,000. Dr Coplans has done most of his work with agar and plates, while I have used mainly blood serum in tubes. Agar tubes have been used in this hospital, but the results were not so satisfactory as those obtained with blood serum. The medium originally used in this institution consisted of three parts of blood serum, one part of bouillon, and glucose 0·5 %.

This medium was very good, but it did not present any selective properties. The first modification was the addition of neutral-red. A

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0·5 % watery solution of neutral-red was prepared and various quantities of this were added. After many experiments it was found that 2 % of this solution gave the best results. Solutions of 0·5 %: 1 %: 1·5 %: 2 %: 3 % and 5 % were added and gave more or less satisfaction. The 2 % solution, however, gave the best coloration and enabled one to distinguish the varieties in colour produced by the various organisms. The use of neutral-red as an indicator is by no means new and has been used by many bacteriologists. The neutral-red becomes pink in the presence of acid and yellow in the presence of alkalies. *B. diphtheriae* is an acid-producing organism when glucose is present and renders the neutral-red pink, but, unfortunately, this organism is not the only one of those found in the respiratory passages that produces acid. Consequently, the production of acid is not diagnostic of *B. diphtheriae.* The next step in the scheme was to find a substance which would allow the bacillus to grow and inhibit the other acid-producing organisms. Unfortunately, this substance was not found, but it was found possible to inhibit the growth of some of the organisms and render the detection of bacillus of diphtheria more easy. The salts of potassium were added to the medium in rotation *e.g.* potassium cyanide, potassium sulphocyanide, potassium ferrocyanide, and potassium ferricyanide, in various proportions. The potassium sulphocyanide acted best and the amount introduced was 1 %. I have come to the conclusion that this is the best proportion and ought not to be departed from. The introduction of larger or smaller quantities has not been a success and any apparent failures, I have had, have been due to proportions of this salt other than 1 %. The medium containing the neutral-red and potassium sulphocyanide has proved to be the best medium. It allows the bacillus to grow and seems to inhibit the growth of many of the other organisms. Cocci grow well and some of them produce a red colour somewhat similar to that produced by *B. diphtheriae.* Experience soon enables one to distinguish the colour produced by the cocci and that produced by the diphtheria bacillus. *B. megatherium* and *B. subtilis* are not uncommon in cultures and it has been found that the above medium inhibits their growth for some hours. It must not be thought that their growth is prevented; such is not the case. The point is that they are inhibited for a sufficient time to enable us to diagnose the presence of *B. diphtheriae,* and this only applies to the number present in ordinary cultures and not to pure cultures of the organisms. Out of the thousands of tubes examined here only in a few cases was the medium contaminated sufficiently to render it useless.

A further series of experiments was conducted with the addition of bile salt to the medium containing neutral-red and potassium salts. Then the combination of various potassium salts with and without bile salt was tried. The results of these experiments were valueless except to indicate that the addition of bile salts was a useless proceeding.

The various constituents of these media are of use for certain specific purposes, but did not further the investigation with regard to diphtheria.

The sum total of all these experiments is that the coagulated and sterilized medium consisting of

Blood serum of the sheep	3 parts
Bouillon	1 part
Glucose	0·5 %
Potassium sulphocyanide	1 %
·5 % sol. (watery) of neutral-red	2 %

is the best medium for the bacillus of diphtheria.

The value of this medium is that it can enable one to say when the bacillus is present or absent without the aid of a microscope. When the bacillus is present a pink colour is produced. A pink colour is produced by cocci, but, by experience, this can be distinguished from that produced by *B. diphtheriae.* When diphtheria is absent there is no pink colour; this is the main point of the medium. One can definitely say when *B. diphtheriae* is absent. This is of importance when many tubes have to be examined as it is possible to dispense with the microscope and save valuable time. I have used over 3000 tubes of this medium and have not had a case in which the non-pink medium showed *B. diphtheriae.* Up to the present, I have not dispensed with the microscope, but have verified the result in every case. Of course, infallibility is not claimed for it, but considering the results I have obtained until now I think it is worth a trial.

There are a few points to be mentioned in conclusion:

(1) Use good serum. The quality of the serum influences the medium greatly.

(2) Always add glucose.

(3) Use 1 % potassium sulphocyanide and make it fresh each time.

(4) Use 2 % of a 0·5 % watery solution of neutral-red.

(5) Do not keep the medium too long. I have not had any medium kept for more than six weeks.