Clinical and other Notes.

A NEW SOLID MEDIUM FOR THE ISOLATION OF THE CHOLERA VIBRIO.

By Captain H. Gräme Gibson.
Royal Army Medical Corps.

Based on the fact that the cholera vibrio alone of all the intestinal organisms acidifies starch,1 the following alkaline medium has been devised for the rapid isolation of this vibrio. Owing to the medium possessing differentiating properties it should be especially useful in the detection of "cholera carriers," as the feces emulsified in broth can be plated directly on to it. In the case of water examination, after enrichment in peptone water for a few hours, if a drop or two of the peptone water is plated a tentative diagnosis can be arrived at in eighteen hours' time, owing to the allied vibrios taking a longer time to bring about acid production than does the true cholera vibrio. The medium consists of the following:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
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</thead>
<tbody>
<tr>
<td>Agar</td>
<td>30 grammes</td>
</tr>
<tr>
<td>Peptone</td>
<td>20 grammes</td>
</tr>
<tr>
<td>Starch</td>
<td>10 grammes</td>
</tr>
<tr>
<td>Soda bicarb.</td>
<td>1.5 grammes</td>
</tr>
<tr>
<td>Litmus</td>
<td>Sufficient to colour medium</td>
</tr>
<tr>
<td>Water</td>
<td>1,000 c.c.</td>
</tr>
</tbody>
</table>

The medium is prepared in the following manner:

Weigh out thirty grammes of powdered agar and emulsify with 250 cubic centimetres of cold water. Then weigh out twenty grammes of peptone (Chapoteaut) and 1.5 grammes of sodium bicarbonate. Mix together and emulsify in another 250 cubic centimetres of cold water. The two emulsions are then mixed in a two-litre flask and another 500 cubic centimetres of water added. The solution is completed in the steamer. When dissolved the medium is clarified with white of egg and filtered in the steamer. The next step is to add the starch. If this is added direct to the peptone agar it forms a mass which it is almost impossible to dissolve. It is easily added if an emulsion is first made. Weigh out ten grammes of potato starch and emulsify it with some of the filtered agar. The emulsion is then added to the remainder of the medium. The whole is sterilized by the fractional method, after which enough sterile watery solution of litmus is added to bring about a blue colour of the medium.

ERRATA.

Page 354, line 18, for "Peptone 20 grammes" read "10 grammes."

line 25, for "twenty grammes" read "ten grammes."
Clinical and other Notes

The final reaction of the medium will be found to be -2 to phenolphthalein. I tried several degrees of alkalinity and found that 0.15 per cent sodium bicarbonate gave quite the best results.

If the plates are examined eighteen hours after inoculation, by looking obliquely through them with a dark background behind, the plate being held parallel to the window, the cholera colonies will be seen to have acquired a faint pink colour, while the colonies of the other intestinal organisms are blue or of a whitish colour. The examination is facilitated by the use of a hand lens. At this time the allied vibrios also produce blue colonies, but at the end of about thirty-six hours they also acidify the medium, though to a less extent than cholera. At the end of twenty-four to twenty-six hours the cholera colonies have attained a delicate pink colour with a faint pink halo round them, while the other colonies still remain blue; also the colonies are of a good workable size to pick off and proceed with the serological tests.

After forty-eight hours, if the cholera colonies are in excess and the plate spread somewhat thickly, the medium itself becomes distinctly acid, and colonies other than those of cholera take on the pink tinge. However, the cholera colony even at this time can still be distinguished by the deeper red centre which the other colonies lack. The only other organisms which are known to acidify starch are some of the diphtheroid group and some of the non-pathogenic water vibrios. These should not present any great difficulty, as Gram’s stain on the one hand, and the serological test on the other, dispose of these organisms. The following experiments have been carried out:

Experiment 1.—Ten cubic centimetres of broth were inoculated by emulsifying some faeces in it. To this was added a very small quantity of a culture of V. cholerae. The tube was well shaken and 0.25 cubic centimetre of the broth transferred to a second tube of broth. Two drops of this broth were immediately plated on to the medium; the same rod being successively used for three plates. The first plate was too crowded to be of any use, but the second and third plates gave good discrete colonies, and on these plates the cholera colonies could be recognized in eighteen hours. Every colony on these plates was picked off, and all the pink colonies were proved to be cholera, whilst the blue colonies in every case proved otherwise.

Experiment 2.—The first broth tube from the previous experiment was kept at room temperature for two days. At the end of that time 0.25 cubic centimetre of this broth was added to ten cubic centimetres of fresh broth, and a drop of this was immediately plated out as in the first experiment. The plates were rather too thickly spread to give good discrete colonies, but the cholera colonies could be easily detected.

These colonies were again tested with cholera immune serum and the differentiation proved correct.

Experiment 3.—This experiment was undertaken to see whether any
Clinical and other Notes

of the normal water vibrios were capable of acidifying starch. The water was taken from a pond after heavy rain, and was taken straight to the laboratory.

Some of the water was first enriched by incubating in peptone water for a few hours. One drop of this peptone water was then plated and at the end of eighteen hours a few pinkish colonies were present.

Some more of the water was plated direct and in this case it took twenty-four hours before any pinkish colonies appeared. In both cases the pink colonies that were present were of a lighter shade than that which is produced by the cholera vibrio, and I do not think that they are very likely to be confounded with it. In addition the red centre to the colony that is produced by the cholera vibrio in forty-eight hours was not present in these cases. This organism proved to be a normal vibrio of water.

The following organisms were also plated out:

- B. typhosus.
- B. paratyphosus A.
- B. paratyphosus B.
- Coliform organisms.
- B. dysenteriae (Shiga and Flexner).
- B. enteritidis (Gaertner).
- Streptococci.
- V. Finckler Prior.
- V. Metchnikovi.

In no case did the above organisms acidify the medium except in the case of the two vibrios which produce a slight pink halo, but the colonies themselves, when viewed obliquely, do not become pink until a very much longer time has elapsed than that required to recognize the vibrio of cholera.

A SIMPLE AND EASILY MADE ACTIVE SERVICE ALCOHOL LAMP—A SUBSTITUTE FOR THE BUNSEN BURNER.

By Captain Lawrence J. Rhea.

Canadian Army Medical Corps.

When hospitals in the field occupy tents, huts, or permanent buildings without gas connexions, some form of lamp for laboratory work has to be substituted for the ordinary Bunsen burner.

The most common types of lamps used are the ordinary alcohol lamp, and the automatic Bunsen burner for methylated spirits. Both are useful, though both have certain disadvantages. The chief disadvantages of the alcohol lamp are the comparatively small amount of heat it produces and the unsteadiness of the flame. The principal objections to the automatic Bunsen burner for ordinary laboratory work are: that it is not easily