AN EASILY PREPARED SELECTIVE MEDIUM FOR THE CULTIVATION OF *NEISSERIA GONORRHOEAE**

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Public health and clinical laboratories will in future be called upon to play an increasingly important part in the diagnosis and control of gonorrhoea. This forecast is based on the fact that bacteriological examination of genital discharges from both sexes has been considerably improved during recent years, so that there is now good prospect of isolating the causal organism from the specimen submitted. This in itself is not so important as the fact that successful isolation makes it possible to determine the sensitivity of the strain to penicillin and other antibiotics, thus providing essential information on which a rational plan of treatment can be formed.

In this field of bacteriology the choice of a suitable culture medium is of paramount importance. All the formulae recommended in earlier times have been rendered obsolete by the introduction of highly selective antibiotic-containing media capable of sustaining good growth of the gonococcus while at the same time inhibiting other bacteria commonly present in genital discharges. The first attempt to use an antibiotic for this purpose was made by Stokinger, Ackerman, and Carpenter (1943). They employed tyrothricin, the active component of which was gramicidin. This had a broad inhibitory effect on Gram-positive organisms but failed to control the growth of coliforms which are the most common contaminants. This line of investigation seems to have been abandoned until Crookes and Stuart (1959) recommended the addition of polymyxin B (Aerosporin) to Peizer's medium as a means of inhibiting Gram-negative contaminants. A further considerable advance was made by Thayer and Martin (1964), who incorporated two antibiotics, polymyxin B and ristocetin, in a chocolate agar base (Bacto G.C. Medium Base with added haemoglobin and yeast supplement). Ristocetin in a concentration of 10 µg./ml. chocolate agar medium effectively inhibits the growth of Gram-positive organisms.

Recently Thayer and Martin (1966) have recommended a modification of this medium; Polymyxin B and ristocetin are replaced by sodium colistimethate and vancomycin respectively; and nystatin is added in order to prevent overgrowth of the culture plate by yeasts.

In this laboratory, where Peizer's medium (Trowbridge and McConkey, 1944) had been in use for many years, we preferred to add the two antibiotics originally recommended by Thayer and Martin to that medium because it is transparent and gives a firm gel on which colonies of *N. gonorrhoeae* stand up well and are easily recognized when examined under the dissecting microscope. This selective medium was used for a period of one year, during which about 20,000 specimens were cultured on it. The results were highly satisfactory. Unfortunately, however, the preparation of Peizer medium is difficult and time-consuming and is, therefore, not suitable for a small laboratory. An alternative antibiotic-containing transparent medium which can be prepared from readily available constituents is here described. It has been in routine use for more than 12 months with results that compare very favourably with those obtained by using the more complicated media hitherto employed.

Preparation of Medium

The following formula was developed as the result of a long investigation in which many factors such as iron concentration, presence of protein (denatured or native), heat lability of yeast extracts, and "toxic substances" were separately studied. It will be convenient to reserve the presentation and discussion of these experiments for a separate communication.

(A) Basal Medium

Proteose peptone No. 3 (Difco or Polypeptone (BBL))	15.0 g.
Corn starch	1.0 g.
Dipotassium phosphate	4.0 g.
Monopotassium phosphate	1.0 g.
Sodium chloride	5.0 g.
Agar	18.0 g.
Water, distilled	800.0 ml.

* Received for publication October 21, 1966.

This may be compounded in the laboratory or it may be obtained ready mixed under the designation *G.C. Medium Base* from Difco Laboratories or Baltimore Biological Laboratory. If the commercial preparations are used the agar must be increased from 10 to 18 g. per litre.

The solids are brought into solution by bringing to the boil. Sterilization is effected by autoclaving at 121°C. for 15 minutes. This basal medium is then cooled to 50°C. before adding the supplement.

(B) Sterile Supplement

Dextrose	7.0 g.
Yeast extract (Difco or BBL)	10.0 g.
Water, distilled	95.0 ml.
Serum (ox, sheep, or horse)	100.0 ml.

Dissolve the solids without the use of heat. Sterilize by Seitz filtration.

(C) Antibiotics

Polymyxin B, 25,000 units/ml.	1.0 ml.
Ristocetin, 1.0 per cent. solution	0.5 ml.

It is now recommended that these two antibiotics should be replaced by sodium colistimethate and vancomycin, as follows:

Sodium colistimethate (Warner Chilcott)	
1.0 per cent. solution	0.75 ml.
Vancomycin (Eli Lilly) 1.0 per cent.	0.30 ml.

The antibiotic solutions are prepared at 6-monthly intervals, dispensed into small tubes, and maintained in the frozen state. A fresh tube is used for each batch of medium.

To prepare the complete medium add the antibiotics (C) to the sterile supplement (B). Then thoroughly but carefully mix A and B so as to avoid froth formation and pour into sterile culture plates allowing a depth of about 4.0 mm. of agar.

Sodium colistimethate and vancomycin, recommended for the Thayer-Martin chocolate agar medium (Thayer and Martin, 1966) proved to be equal to polymyxin B and ristocetin as selective antibiotics in the medium here described. Yeast extract, as specified, must be employed: commercial dried yeast is not a satisfactory substitute. It is permissible, however, to use a fluid yeast extract prepared in the laboratory from fresh brewers' yeast according to the method described by Cohen and Wheeler (1946). The amount required is 50 ml. per litre of medium, replacing an equal volume of water in the formula given above. Nystatin is not included in this medium because the recognition of yeasts is considered to be an important part of the general investigation for pathogens.

The medium should be stored in the refrigerator and used within 10 days after its preparation.

Evaluation of the Medium

The first large-scale trial of the new medium was carried out by direct comparison with Peizer medium

containing polymyxin B and ristocetin as described above. 500 specimens of cervical or vaginal exudate were cultured on both media under identical conditions. These specimens were taken either at a hospital venereal diseases clinic or at a clinic for female prisoners and were, therefore, expected to include a fairly high proportion of Neisserian infections. They were forwarded in transport medium and arrived at the laboratory within 24 hours.

The results of culturing these 500 specimens in parallel on the two media are given below.

Number of Specimens Examined	..	..	500
<i>N. gonorrhoeae</i> isolated:			
On Peizer medium	..	85 strains (17.0 per cent.)	
On new medium	..	79 strains (15.8 per cent.)	

The difference between the results obtained in these two series is not significant.

In the routine bacteriological diagnosis of gonococcal infection much attention must be paid to colonial appearance. In this respect the two media were equally satisfactory. It is well known that different strains of gonococci vary considerably in their size, consistency, and other physical properties of the colonies; but whatever colonial form was found the appearance on the two media was very similar, except for the slightly blue colour of the growth on Peizer medium.

Experience of the New Medium in the Routine Diagnosis of Gonorrhoea

In view of the results presented above it was decided to abandon Peizer medium in favour of the more simple new formula. This was done in order to reduce the work load on the media preparation staff who were being called upon to prepare 7 to 8 litres of medium for this purpose alone each week. An analysis of the results obtained during a 6-month period following the introduction of the new medium is compared below with the same 6-month period of the previous year when Peizer medium with antibiotics was employed.

Year	March-August, 1965	March-August, 1966
Total Number of Specimens examined by Culture ..	9,775	12,842
Number of Isolates of <i>N. gonorrhoeae</i>	616	798
Percentage of Cultures yielding <i>N. gonorrhoeae</i>	6.3	6.2

Most of these specimens (86 per cent.) were obtained from female patients seen in private practice who were not expected to have a gonococcal infection. This explains the low percentage of positive isolations in comparison with the first series cited.

Comment

On the evidence presented, it seems justifiable to claim that the new medium is as satisfactory as the older and more complicated medium of Peizer. Compared with a chocolate agar, the new medium has the considerable advantage of transparency and firmness; and a technician who has once become accustomed to these advantages will certainly not wish to return to an opaque medium. Provided that a dissecting microscope is used to examine each culture plate, the recognition of *N. gonorrhoeae* colonies becomes a rapid and certain procedure well within the scope of most clinical bacteriology laboratories.

Summary

The medium here described combines the highly selective properties of Thayer-Martin chocolate agar with the transparency and firmness of the modified Peizer medium previously employed in this laboratory. It is simple to prepare in large or small amounts; and all the constituents except the blood serum are available commercially in powder form.

The results of evaluation studies, carried out on a large scale, are presented. The medium is highly acceptable to the technical staff responsible for its preparation and use.

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Un milieu de culture sélectif préparé facilement pour la culture du *Neisseria gonorrhoeae*

RÉSUMÉ

Le milieu décrit ici combine les propriétés hautement sélectives de la gélose chocolat de Thayer-Martin à celles de la transparence et de la fermeté du milieu modifié de Peizer jusqu'ici employé dans ce laboratoire. Il est facile de le préparer en grande ou en petite quantité; et tous les constituants excepté le sérum sanguin sont disponibles dans le commerce sous forme de poudres. Les résultats des études d'évaluation entreprises sur une grande échelle sont présentés. Ce milieu de culture est très prisé par le personnel technique responsable de sa préparation et de son usage.

BOOK REVIEW

***Treponema pallidum*. A Bibliographical Review of the Morphology, Culture and Survival of *T. pallidum* and Associated Organisms.** By R. R. WILLCOX and T. GUTHE. 1966. Supplement to Vol. 35 of the *Bulletin of the World Health Organization*, Geneva.

Many workers have tried to grow *Treponema pallidum* in artificial media and although success was claimed by some of the early workers, such as Noguchi and by Schereschewsky, their results have not been confirmed by others. Inability to grow the organism except in an animal host is a bar to obtaining sufficient quantities of treponemes for immunological and chemical studies and is hindering progress in many fields of research on treponemal disease, especially attempts to produce an artificial immunity by vaccination. The growth requirements of non-pathogenic treponemes have been widely studied in the hope that these might give clues to the needs of *T. pallidum* itself, so far without success.

A very large literature has accumulated round these and other aspects of the biology of treponemes and it is becoming difficult to see the wood for the trees. The authors of this review have done a very real service to all those interested in treponemes in providing a guide to the most important papers which have been published on the morphology, taxonomy, growth requirements, and behaviour in experimental animals of the disease-producing treponemes and of their saprophytic relatives. Over a thousand papers are cited by title and reference, ranging in time from that of Schaudinn and Hoffmann (1905) to some published in 1965. The review is of necessity bibliographical rather than critical and does not attempt to cover the serological response of the host to treponemal infection. It is a pity that such a useful compilation was not published in hard covers as it is a reference book which many venereologists will consult frequently. A.E.W.