The various media were prepared as follows:

A 1% suspension of liver extract powder was made in 100 ml of distilled water, thoroughly mixed, filtered through absorbent cotton and then through filter paper. The clear brownish filtrate was sterilized by passage through a Seitz filter and stored in the ice chest until used. Sterilized citrated horse blood was hemolized by freezing. The proteose agar base was autoclaved in 100 ml amounts (4.5 g Difco Bacto Proteose No. 3 Agar in 100 ml of distilled water), after which sterile glucose solution was added to a concentration of 1%. The agar was cooled to 45 C and liver extract and citrated hemolized horse blood were mixed into the agar base and plates were poured. Five strains of Neisseria gonorrhoeae were examined. Table 1 shows the results concerning the size of the colonies grown in the liver extract and in hemolized horse blood. The two enrichments were used alone and combined in various amounts with the base media and are compared with colonies grown in the base sugar agar alone, with the addition of 2 mg % of glutamine and with rabbit-blood chocolate agar.

Depending on the amount of hemolized horse blood or liver extract added to the base agar media the cultures grow more or less luxuriantly.

Two mg % glutamine and 5% rabbit chocolate blood give equal results but are less effective than horse blood and liver extract. The best result in the growth for all strains of Neisseria gonorrhoeae are obtained when 25 ml of 1% aqueous liver extract and 5 ml citrated hemolized horse blood are added to 100 ml of the proteose sugar agar base.

REFERENCES


SUBSTITUTES IN CULTURE MEDIA

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Three stable, economical culture media which can be duplicated successfully and which contain substitutes for products difficult to get are described. Two contain substitutes for meat; the third, a substitute for tomatoes. The media have been tested in teaching, clinical, and research laboratories.

A medium, superior to meat in nutritive properties, has been made from soy beans. The formula adopted follows:
1. Mammoth Yellow Soy beans, 500 g, well washed and soaked in 2000 ml of distilled water overnight at room temperature. They are not mashed.

2. Boil one hour, make up to 1 L, and filter, if necessary.

3. Add sodium chloride, 3 g, and dibasic potassium phosphate, 2 g, and adjust to pH 7.4 with about 5 ml of N/1 NaOH.

4. Sterilize in the autoclave by the usual method. The medium will tolerate 30 minutes at 121°C.

5. Add agar, 15 g, and dissolve, if desired.

Beans were secured locally at about $0.08 a pound. Of the varieties tested the Mammoth Yellow Soy bean was the most uniform. Poor results were secured with bean sprouts and the process for producing sprouts has proved laborious and often resulted in souring. Stainless steel or enamel buckets should be used; there is inhibition of growth when the medium is prepared in copper or aluminum containers.

Bacteria grow luxuriantly on or in this medium; not only those for which nutrient agar or broth is usually chosen but pathogenic species, like streptococci, for which blood is usually added. The medium is unsuitable for hemophilies. Cultures were sometimes secured from clinical specimens when other media failed. Chromogenic organisms produce good pigment. The survival of organisms on soy bean medium is good. On the other hand, when blood is added, the hemolytic reactions of streptococci are not typical. Fermentation tests are unsatisfactory due to the fermentable carbohydrate already present. The medium is not satisfactory for differential media using carbohydrates and bacteriostatic, tinctorial, or indicator dyes. Its virtues are in its nutrient qualities. The approximate analysis of the soy bean, dry weight, is: Protein, 42%; oil, 20%; sugar (calculated as sucrose), 8%; K, 1.7%, Ca, 0.3%, P, 0.7% and ash, 5% (Tech. Bull. 787, U. S. D. A., May 1942). This is not a measure of the extractives used. The total nitrogen of a standard meat "hormone broth" base was found to be 0.26% against 0.75% for soy bean broth.

A second medium devised uses a substitute less easily secured and subject to a commercial variation, but it lacks the high carbohydrate content of soy bean media. It is better adapted to use in differential media and retains most of the virtues of soy bean media. Hemolysis by bacteria with blood agar made from this medium is typical. It is a fish liquor known as B. G. Concentrate, put out by Philip R. Park, Inc., San Pedro California, at a cost of about $2.50 for five gallons.

The substance, a vacuum-concentrated extract, is a by-product of the production of fish meal. It has an approximate analysis of crude protein, 32%, crude fat, 2%, crude fiber, 0.1%, and ash, 12%, with a total solid content of at least 50%. The total nitrogen content of the finished broth was 0.23%, comparable to that of standard meat broth. It contains riboflavin, panthenolic acid, nicotinic acid, choline, and vitamins B₁ and B₆. The formula adopted follows:

1. Mix B.G. Concentrate, 50 g, with 1000 ml distilled water, and adjust to pH 7.4 with N/1 NaOH.

2. Boil for 15 minutes and filter through paper.
3. Add sodium chloride, 3 g, and dissolve. Add agar, 15 g, if desired.
4. Autoclave as usual.

A third medium was designed to meet the shortage in tomatoes used for media for *Lactobacillus acidophilus*. Colonies are similar to those developing on tomato or whey agar. The formula adopted is based on diastatic malt secured from a brewery:

1. To 1000 ml distilled water add diastatic malt, 40 g, Bacto Yeast Extract, 5 g, sodium chloride, 5 g, and agar, 0 g, and dissolve by boiling with constant stirring.
2. Fill in 1000 ml bottles, autoclave 30 minutes at 121°C, and store.
3. For use, add to melted medium concentrated acetic acid, 0.3 ml per bottle, mix well, and pour in plates.

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**A SIMPLIFIED TECHNIQUE FOR THE AGAR CUP ASSAY OF PENICILLIN**

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Submission of this paper was prompted by the interest in the agar cup method for the assay of penicillin. A simplified method for this procedure has been used in the Department of Pathology and Bacteriology, The Chicago Medical School, for some time with completely satisfactory results. The method is intended primarily for laboratories that do not have the extensive apparatus necessary for the usual cup assay procedures, and where mass production methods are not essential. (Foster and Woodruff)

**TEST ORGANISM**

The organism which has been found to be most satisfactory is a *Staphylococcus aureus* (Norsenski). This organism gives a sharply defined zone of inhibition in 18 hours. However, any strain of *S. aureus* can be used which is found not to give zones of partial inhibition and secondary zones of stimulation. The FDA 209 strain is unsatisfactory for this reason. The sensitivities of the different strains of *S. aureus* to the bacteriostatic action of penicillin vary considerably. *S. aureus* has been the organism of choice rather than a spore former, such as *Bacillus subtilis* (Foster and Woodruff), since we are primarily interested in the action of the penicillin against non-spore-forming organisms. The mode of inhibition may not be the same in organisms with such great differences in metabolism. The prime requisites for the organism that is chosen, are that it must give a clearly delineated outline to the zone of inhibition and that it should be consistent in its growth characteristics.

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