

STARCH AGAR, A USEFUL CULTURE MEDIUM *

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The medium about to be described was devised in the course of experiments to obtain a more suitable medium for the cultivation of the gonococcus. We needed to keep in stock a number of cultures of gonococci for the preparation of vaccine and antigen for the gonococcus fixation test. We were using salt-free veal agar prepared according to the method used by the New York Board of Health. This medium possesses many advantages, but the transfers must be made every two or three days in order to keep the cultures alive, and we desired a medium on which gonococci could remain alive for a longer period in order to avoid the necessity for such frequent transfers.

The actual work in these experiments was performed chiefly by one of our laboratory assistants, Sergeant Frederick G. Abner, and it is only fair to state that the results here presented are due to the interest he has taken in this work.

The medium on which we have had the best success in cultivating the gonococcus consists of beef infusion agar to which 1 percent of starch has been added.

METHOD OF PREPARATION

Beef infusion is prepared according to the usual method employed for making broth. It is essential to use a beef infusion because commercial meat extracts do not give as good results. To this beef infusion sufficient agar is added to make 1.5-1.75 percent agar. No peptone or salt is added, since the gonococcus appears to grow better on media without peptone or salt. If much more than this amount of agar is added, the medium will be too dry.

This mixture is cooked, clarified, and filtered according to the method of preparing ordinary agar. It is then neutralized so that the final reaction will be from 0.2-0.5 percent acid to phenolphthalein. Ten grams of cornstarch to each liter are now added to this medium. It is well to grind up the cornstarch in a mortar with a little of the

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agar in order to avoid lumps. We have tested this medium using varying amounts of cornstarch and have found that about 1 percent is the optimum amount. After the addition of the cornstarch, the mixture is boiled for a few minutes and this concludes the preparation of the medium, which is then tubed and sterilized at not more than fifteen pounds pressure to avoid breaking down the starch. The medium so prepared will be slightly clouded owing to the presence of the cornstarch.

We have tested other kinds of starch in this culture medium in order to determine whether starch from any source was equally suitable. For this purpose we have used tapioca, potato starch, and wheat starch. The gonococci grew on all of these starch agars better than on the ordinary media employed for the cultivation of these organisms, but the growth appeared to be less luxuriant than when cornstarch was used. For the most part, cornstarch appeared to be more suitable for other organisms also. Kahlbaum's cornstarch for medicinal use was first tried, but ordinary commercial cornstarch appeared to give as good results and is of course much cheaper. The tapioca and the potato starch media, while not producing so luxuriant a growth, have not the slight clouding that appears unavoidable when cornstarch is used.

ADVANTAGES OF THIS MEDIUM

Starch agar medium has proved to be the best medium that we have tried for the cultivation of the gonococcus. The growth of this organism is so profuse that it would lead us to suspect that the organism is not really the gonococcus were it not for the fact that our work has been done with the original Torrey strains of this organism, which were kindly furnished us by the New York Board of Health. It also might be supposed that this profuse growth is obtained because the gonococci used are old strains that have been under cultivation for a long time, but we have also been successful in isolating gonococci from several fresh cases of gonorrhea on this medium, and the strains so isolated also grow freely. There is free growth after twenty-four hours' incubation and this increases up to three or four days, after which there is no apparent increase in the density of the growth. Cultures on this medium are suitable for the preparation of antigen, as the starch appears to have no bad effect and the dense growth is a distinct advantage.

Gonococci remain alive upon this medium for a long time. It has been our custom to remove the cultures from the incubator after several days and to keep them thereafter at room temperature. Experiments have been made to determine how long the gonococci will remain alive under these circumstances. In one of these experiments, out of seven Torrey strains that were kept for forty days, five of the seven were alive at the end of this time. We have found that they remain alive with great regularity for twenty days, and that we can safely make transfers of our stock cultures every two weeks, instead of every two or three days as is necessary when salt-free veal agar is used.

This medium may be melted and used in plates to plate out cultures of gonococci, or the pus from a fresh case of gonorrhea may be smeared on the surface of a plate and the gonococcus isolated from the colonies that develop.

Some other organisms that are usually cultivated with some difficulty grow readily on this medium. We have tried a number of strains of tubercle bacilli and one of the possible lepra bacilli isolated by Duval, and all of these organisms grew freely on this medium. Freshly isolated streptococci and pneumococci also grow more freely than on the other media.

The medium is suitable also for routine use. Practically all of our stock cultures grew either as well or more luxuriantly on this starch medium than on plain agar.

The great simplicity of this medium and its easy preparation are strong recommendations. It is even easier to prepare than salt-free veal agar because it is easier to obtain beef than veal at a certain time and no peptone is required.

A starch agar, prepared by a different method with potato starch, has been used by several workers¹ in the study of certain soil bacteria, and starch has also been used in various ways by general bacteriologists, but, so far as we are aware, no one has heretofore used a starch agar prepared as described here for the cultivation of the gonococcus. It has been known for some time that a considerable number of bacteria produce amylases, or ferments capable of breaking down starch, and that by cultivating certain bacteria upon starch agar plates the amylase can be demonstrated by the clearing of the medium immediately surrounding the colonies. This effect is seen very clearly on plates on

1. McBeth and Seales: U. S. Dept. of Agriculture, Bureau of Plant Industry, Bull. 266, 1913; Kellerman and McBeth: *Centralbl. f. Bakteriol.*, I, O., 1912, 34, p. 487.

this starch medium sown with cholera spirilli, but colonies of the gonococcus rarely present this appearance. In spite of this fact, it seems evident that the gonococcus is able to utilize the starch.

Why should starch serve better as a culture medium than the various sugars? It might be assumed, since starch must be broken down into sugars before it is available for absorption by an organism that, therefore, nothing could be expected of starch in a culture medium which would not be obtained equally well from the use of sugars. This appears not to be the case however. Gonococci grow better on this starch medium than upon any sugar medium that we have tried. Just why starch should produce this effect remains a subject for further study. It may be that when starch is acted upon by the amylases peculiar to the organism, or in other words is subject to the normal process of digestion, that the resultant products of decomposition are more suitable for the growth of the organism than is the case when certain sugars are furnished. But whatever the explanation, it appears that this starch agar is a simple culture medium specially suitable for the cultivation of a number of bacteria that have hitherto required rather complicated culture media.