IDENTIFICATION OF THE GONOCOCCUS FROM CULTURES AND THE EFFECT OF CERTAIN ANIMAL SERA ON THE FERMENTATIONS OF THE GONOCOCCUS

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The value of identification of suspicious organisms when cultures are employed in the diagnosis of gonorrhea is still being debated by many. Some believe that fermentation tests are essential; others consider them necessary only in doubtful cases. The latter consider the source of infection, the fact that colonies are oxydase-positive and consist of bean-shaped gram-negative diplococci as sufficient proof of identity. It is the purpose of this paper to show that the isolation of suspicious organisms and their identification is of primary importance, especially in cultures taken from females. It is also desired to report on the effect on gonococcus fermentations of certain animal sera commonly used as enrichment in culture media.

In our work, the sum of the following cultural features is usually thought to be necessary in identifying the gonococcus:

1. Typical colony morphology, not only on primary culture, but in pure culture on known media.
2. Typical appearance of the organism in gram-stained films.
3. Absence of growth at room temperature on enriched agar.
4. Absence of growth of freshly isolated strains on plain agar.
5. Absence of hemolysis of 5 per cent washed horse blood cell agar (to differentiate from the meningococcus).
6. Clear-cut sugar fermentation tests.

These various cultural characteristics are considered because in cultures from females we found gram-negative, oxydase-positive staphylococcus-like and streptococcus-like organisms. There were also oxydase-positive gram-negative coccobacilli, some forms of which were microscopically indistinguishable from gonococci. On primary culture it was difficult to judge correctly

the colony morphology of these diverse oxydase-positive organisms. It was only after their isolation on plain and enriched agar and by the effect of room and incubator temperatures on their growth that their distinguishing characteristics appeared.

Various forms of *Neisseria* other than the gonococcus were seen very frequently in female cultures. Sometimes more than one specie of the Neisserian group was found in one culture. In the case of organisms belonging to this group, sugar fermentations are almost always necessary to eliminate the possibility of false diagnosis. In the literature there are numerous reports (Murray 1939, Schubert and Toenges 1931, Jadassohn 1930, Anderson and Schultz 1923, Clauberg 1930) on the presence of gram-negative bean-shaped diplococci in cultures and smears which are not gonococci.

Atypical organisms may appear in cultures. This further complicates the problem since smears of such colonies may show irregular, elongated forms, single round cells and giant diplococcus forms. On the other hand, *Neisseria catarrhalis* and even *Micrococcus flavus* can readily be mistaken for the gonococcus in smears and on primary cultures.

When the source of the culture is other than the genital tract, the necessity for identification of the suspected organism becomes even more apparent. Recently in our laboratory a meningococcus was identified from a knee fluid, a gonococcus from a blood culture. In such cases, since the fermentation of the meningococcus can be atypical (when it ferments glucose only), to avoid confusion a 5 per cent washed horse red-blood-cell agar plate is used along with the sugar fermentation. The meningococcus hemolyzes the red cells in this agar in from 24 to 48 hours. The gonococcus causes no hemolysis (Gordon, 1920; Cohn, 1939).

Among the cultural characteristics of the gonococcus, clear-cut sugar fermentations are the best criterion for identification. However, certain errors may arise due to the sugar medium. This is illustrated by the work of Lentz and Shaffer (1937), who in testing the effect of various sugars and alcohols on the fermentations of the gonococcus found that only glucose was fermented. However, when they added egg-white to improve growth, acid

was produced in the glucose, maltose and levulose tubes, because, as is known, egg-white contains minute amounts of glucose. Since animal serum is commonly used for enrichment of gonococcus cultures (Peizer, 1939; Cohn and Kruger, 1940) the following investigation was undertaken to study the effect of horse, beef, sheep, human, rabbit and guinea pig serum on the sugar fermentations of the gonococcus. Soluble starch was also tested.

Pure gonococcus strains were grown in 0.3 per cent semi-solid proteose peptone agar prepared in our laboratory (Peizer and Steffen, to be published). The semi-solid agar was enriched with 5 per cent horse, beef, sheep, rabbit, human or guinea pig serum respectively. The sugars tested were 0.5 per cent glucose, maltose and levulose with 0.001 per cent of Bacto-phenol-red as indicator. Ascitic fluid was employed in the control sugar medium for all fermentation tests.

With horse, beef, or sheep serum, the gonococcus always produced acid in the glucose, and maltose agar, although in the control medium with ascitic fluid the identical strains showed fermentation only in glucose agar. When, however, the sugar medium was enriched with either human, rabbit or guinea pig serum, the strains consistently produced acid only in the glucose agar. About 500 strains were tested with the various sera.

The effects of dilution and filtration, and heat on the fermentable substances in horse, beef and sheep bloods were then tested, using 25 known gonococcus strains.

When 0.5, 0.05 and 0.08 per cent amounts of the respective sera were used, acid production in the maltose tubes by the gonococcus strains was reduced as shown in table 1.

Filtration through "V" and "N" Berkefeld filters did not alter the fermentable substances in the sera.

The effect of heat was tested as follows:

Cool melted semi-solid agar was enriched with 5 per cent horse, beef or sheep serum respectively, and the mixture heated in a water bath at 55°C. for 30 minutes. Some of the serum-enriched agar was heated in a water bath at 68°C. for 30 minutes, and some at 100°C. in the Arnold for 10 minutes. After the serum agar cooled to about 45°C. in each case, 0.001 per cent phenol red and

0.5 per cent of glucose, maltose or levulose were added. The agar was tubed and inoculated with pure strains of gonococci.

In the tubes heated at 55°C. for one-half hour, the gonococci formed acid in both glucose and maltose agar. In the tubes heated at 68°C. for one-half hour, and in those heated at 100°C. for 10 minutes, as well as in the control tubes, the identical gonococcus strains produced acid in the glucose agar only. This

TABLE 1

SERUM (FILTERED) ENRICHMENT	SERUM USED	SUGAR REACTION			TIME NECESSARY FOR ACID PRODUCTION	
		Glucose	Maltose	Levulose	Glucose	Maltose
	per cent				*hrs.*	*hrs.*
Horse	0.5	S	S	0	18–24	24–48
	0.08	S	M	0	18–24	48–72
	0.05	S	M	0	18–24	48–72
Beef	0.5	S	S	0	18–24	24–48
	0.08	S	M	0	18–24	48–72
	0.05	M	W	0	18–24	48–72
Sheep	0.5	S	S	0	18–24	24–48
	0.08	M	W*	0	48–72	72
	0.05	M	0	0	24–48	72

S, strong fermentation; M, moderate fermentation; W, weak fermentation; 0, no fermentation.

* Six out of 25 strains showed weak acid production. The remaining 19 strains did not produce acids. Growth of the strains when small quantities of serum was used was stimulated by the addition of ascitic fluid.

seems to corroborate the earlier findings of Hendry (1938) and suggests the presence of a maltase in horse, beef and sheep sera.

Since soluble starch in combination with proteose peptone No. 3 agar or meat infusion agar is a colorless medium which satisfactorily supports the growth of isolated gonococcus strains, it was tested as a possible base for sugar fermentations. It was found that 25 per cent solution of soluble starch in distilled water, sterilized fractionally to avoid hydrolysis, and then added to sterile agar, gave a suitable fermentation agar base. On cooling, the 25 per cent starch solution produced a jell which dissolved

again on heating in a water bath or in the Arnold sterilizer. Enough of warm starch solution to make 5 per cent starch agar was added to melted 0.3 per cent semi-solid agar along with 0.5 per cent of the respective sugars, 0.001 per cent of Bacto-phenol-red and 0.038 per cent of dissodium phosphate to adjust the pH to 7.3. The mixture was then tubed in 2 ml. quantities, and heavily inoculated with pure strains of gonococci. In this starch semi-solid agar, gonococci fermented glucose more slowly than in agar enriched with either 20 per cent ascitic fluid or with 5 per cent human, rabbit or guinea pig serum.

CONCLUSIONS

It appears from the work of others and from our own experience that when cultures are used as an aid in diagnosis of gonorrhea, the identification of the gonococcus by sugar fermentation tests along with other cultural characteristics is essential for correct diagnosis.

Sugar fermentations of the gonococcus are accurate and clear-cut when suitable media are employed. Normal horse, beef and sheep bloods are not suitable enrichments for gonococcus sugar fermentation tests since in their presence the gonococcus produces acid in maltose agar as well as in glucose agar. Ascitic fluid, human, rabbit and guinea pig sera are good and stable enrichments for sugar fermentations of the gonococcus. Fractionally sterilized soluble starch may also be employed.

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