THE OXIDASE REACTION IN THE LABORATORY DIAGNOSIS OF GONORRHOEA.

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The oxidase reaction is due to the presence of oxidizing ferments in certain organisms, and is characterized by a colour reaction produced when a reagent, such as dimethyl-para-phenylene-diamine hydrochloride, is poured on to the colonies of these organisms. The reaction is not specific for any organism, but is given, amongst others, by *B. anthracis*, *B. pyocyaneus*, *B. subtilis*, *B. cholerae*, and the Neisserian group.

Dietrich and Liebermeister¹ in 1902, whilst studying the detailed structure of the anthrax bacillus, made use of the oxidizing ferments present. In 1910 Schultze published a paper² on methods of observing oxidation and reduction in bacteria, and noted the organisms giving rise to this oxidase reaction. Kramer³ in 1912 confirmed this work. Little further progress was made until 1928, when Gordon and McLeod published a paper⁴ confirming most of Schultze's work, but they modified the latter's method so that it could be used as a means of differential diagnosis in mixed cultures and not be limited to pure subcultures. Their method was used throughout the present investigation, and consists in pouring on to the colonies of the organisms either phenylene-diamine hydrochloride or dimethyl-para-phenylene-diamine hydrochloride. Those colonies containing the oxidizing ferments present a colour change, whilst those that do not, remain unaffected.

The objective of the present investigation was to ascertain the value of this method in the routine examination for the presence of the gonococcus in cultures taken from the genital tract of patients who, clinically, had, or were suspected of having, gonorrhoea. The inoculation was made on to a modification of Thomson's medium (pH 7.5), as it became evident that the colour change shows up much more readily on this transparent medium than on the opaque brownish-red surface of blood agar. The test was applied after forty-eight hours' incubation at 37° C., the reagent used being a 1 per cent. solution of dimethyl-para-phenylene-diamine hydrochloride (supplied by the British Drug Houses) in distilled water. The solution should not be over seven days old, since with older solutions the colour reaction is less well defined.

The colonies were first examined with a $\times 6$ hand lens, and samples of any having the appearance of being gonococci were picked off, stained by Jensen's modification of Gram's stain, and examined microscopically. If the organisms had the morphological appearances of gonococci this was noted. The surface of the medium was very gently washed with about 0.5 c.cm. of the reagent, thus avoiding contamination of colonies of gonococci by other organisms from other colonies. This is important when pure subcultures are required for vaccines, etc. The cultures were then observed by the naked eye and with a $\times 6$ lens at intervals of 1, 3, 5, 10, 15, 20, and 30 minutes, and any colour change in a colony carefully noted. The results were recorded as positive, "pseudo," and negative reactions.

A positive reaction is shown by the development, almost immediately, of a very faint fine pink, which at this stage is only to be seen with the aid of a $\times 6$ lens. In three minutes this deepens to a cerise shade, and the colonies are easily seen by the naked eye amongst those which are unaffected. Gradually the colour changes to red and then to reddish purple, finally ending, in about thirty minutes, in an intense jet black. This may be regarded as a normal reaction, but variations in the speed of the reaction do occur. They appear to depend on the age of the culture and the reagent; the older these are, the quicker, but less well defined, the reaction, whilst, within limits, the converse holds good.

^a The term "pseudo-reaction" was applied to a change of colour which was distinguishable from that seen in the

truly positive reaction by being brownish pink rather than pure pink, and becoming in the end brownish black instead of jet black. It was afforded by some non-gonococcal colonies. A negative reaction indicates no change in a colony.

Two thousand cultures were examined, the first 250 of which were selected as a "trial run." These contained obvious gonococci colonies, doubtful gonococci colonies, and cultures obviously not containing any gonococci. The following 1,750 were routine cultures sent into the laboratory for examination. Of these 1,750 cultures there were 117 (6.7 per cent.) positive reactions, and all of these were morphologically gonococci. Many of these organisms were cultured and gave the typical glucose fermentation, other sugars, such as maltose, saccharose, levulose, etc., being unaffected. They were also soluble in caustic soda.

Other members of the Neisserian group also gave positive reactions, but the only one likely to confuse the issue in a case of doubtful gonorrhoea is the *M. catarrhalis*, and most authorities⁵ are agreed that its presence in the genital tract is rare. The intensity and celerity of the reaction given by a few of the members of this group appear to depend on their ability to ferment the sugars. Thus, *M. catarrhalis* gives an extremely quick and vivid colour reaction, whilst *M. flavus* (Type 1 only tested) gives a slow response. Between these two extremes comes the gonococcus.

The "pseudo"-reactions (2.2 per cent.) were almost invariably given by one of the protean varieties of coliform bacilli. Gordon and McLeod report negative reactions with *B. coli*, whilst Schultze and Kramer found that it gave a definite response. From this investigation it would appear that some varieties of coliform bacilli give an oxidase reaction, but that the great majority remain unaffected. *B. subtilis*, when present, always gave a response, and in two instances this was noted with a variety of streptothrix.

Amongst the mixed cultures which gave negative oxidase reactions (91.1 per cent.) the gonococcus was not encountered, but on four occasions a very small Gram-negative coccus was noted, which it was possible to isolate in a pure culture on only one occasion, and then with great difficulty. It had the following characteristics: (1) Gram-negative, about one-quarter the size of the gonococcus, not arranged in pairs, and giving a negative oxidase reaction; (2) ferments glucose, lactose, maltose, and mannite with the production of acid but no gas; (3) no action on inulin or dulcitol; (4) clots milk; (5) very poor growth on agar, but somewhat better on hydrocele agar, producing pin-point colonies, which were dull and sticky and required frequent subculture (every two or three days) in order to keep the strain alive. This organism appears to belong to the Neisserian group in spite of its negative oxidase reaction.

From these results it is evident that this method of diagnosis of the presence of gonococci colonies in a culture tube tends to increase accuracy, particularly in cultures where the colonies of gonococci have grown feebly or have been almost smothered by the growth of such secondary organisms as *B. coli* or staphylococci. A definite positive reaction removes the necessity of staining, whilst colonies giving doubtful results can always be stained and subcultured provided this is done prior to the colony assuming a blackish hue. When this occurs it is an indication that the organisms have been killed. Pseudo-reactions practically never give rise to any doubt except in cases where the reagent is not fresh or the cultures have been incubated for more than forty-eight hours. The strepto-pneumococcal colonies, which are so frequently a cause of anxiety owing to the similarity of their appearance to those of the gonococcus, never give a positive reaction, hence this potential error is removed. Finally, where large numbers of cultures are examined by this method there is a great saving of time and labour.

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REFERENCES.

- ¹ Dietrich and Liebermeister: *Centralbl. f. Bakt.*, Orig., 1902, xxxii, 858.
- ² Schultze: *Ibid.*, 1910, lvi, 544.
- ³ Kramer: *Ibid.*, 1912, lxi, 394.
- ⁴ McLeod and Gordon: *Journ. Path. and Bact.*, vol. xxxi, No. 2, 185.
- ⁵ Pelouze: *Gonococcal Urethritis in the Male*, p. 40. Morton: *Genito-Urinary Diseases and Syphilis*, p. 128.