A MEDIUM FOR THE NAGLER PLATE REACTIONS FOR THE IDENTIFICATION OF CERTAIN CLOSTRIDIA

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Hayward (1941, 1943) developed a plate reaction for the rapid identification of *Clostridium perfringens* (*C. welchii*) based upon the so-called Nagler reaction. This reaction involves the splitting by certain toxins of insoluble fatty material from human blood serum or egg yolk, and the appearance of a zone of precipitation around positive colonies in plate cultures. The medium recommended by Hayward (1943) was nutrient agar containing 20 per cent human serum and 5 per cent peptic digest of sheep's blood. For the identification of *Clostridium oedematiens* (*C. novyi*) Nagler (1944*a*, 1944*b*) used Weinberg's V. F. agar (peptic digest of beef liver, etc.) with 10 per cent defibrinated sheep's blood and 10 per cent egg yolk suspension.

We have investigated these reactions, using a large number of each of these species and several others. One of our objectives has been to devise a medium on which the reaction could be demonstrated that could be prepared from easily available materials. Several media which appear to give promise will be discussed in later publications. The following is recommended as a satisfactory substitute for the media suggested by Hayward and by Nagler:

Proteose peptone no. 2	40 g
Na_2HPO_4	5
KH_2PO_4	1
NaCl	2
$MgSO_4$	0.1
Glucose	2
Agar	25
Distilled water	1,000 ml
pH	7.6

Sterilize: 240 F for 20 minutes

After autoclaving, add 10 ml of sterile egg yolk suspension to 100 ml of warm medium and pour approximately 15 ml in plates of 100-mm diameter. The addition of the blood to the medium, as suggested by Nagler, is unnecessary. To prepare egg yolk suspension aseptically, withdraw to a sterile rubber-stoppered tube by aspiration the yolk from a fresh hen's egg after first removing the white. Add an equal amount of sterile 0.85 per cent NaCl to the yolk and invert the tube to mix the contents.

We shall describe in subsequent papers the characteristic differentiating reactions, which may be inhibited by appropriate specific antiserum, obtained with streaked plate colonies of each of the following members of the genus *Clostridium: C. perfringens* (*C. welchii*), *C. oedematiens* (*C. novyi*) types A and B, the *C.*

oedematoides-C. sordelli-C. bifermentans group, *C. parabotulinum* types A and B, *C. botulinum* types B, C, D, and E, and *C. sporogenes*. The following species have failed to give reactions: *C. tetani*, *C. histolyticum*, *C. tertium*, *C. septicum*, *C. capitovalis*, *C. chauvoei*, and *C. cochlearium*.

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