Bacteriological Diagnosis of Gas Gangrene due to *Clostridium oedematiens*

IN gas gangrene infections it is important to determine the infecting organism as quickly as possible, but hitherto there has been no satisfactory method available for the rapid recognition of *Cl. oedematiens*. By means of the cultural reaction described below, it is hoped that it will usually be possible to make a provisional diagnosis in less than twenty-four hours. With heavily sown or overgrown plates, in which the reaction may be doubtful, it may be necessary to sub-culture without delay from a suspected colony to a fresh plate.

The medium employed is a modified Weinberg's V.F.¹ agar (2.25 per cent) to which 10 per cent defibrinated sheep's blood and 10 per cent egg yolk suspension is added. The egg yolk suspension consists of equal parts of normal saline and egg yolk freshly removed from an egg aseptically. In surface cultures on plates of this medium, colonies of *Cl. oedematiens* attain a diameter of 0.8–1.5 mm. after 16 hours incubation anaerobically. Colonies of *Cl. oedematiens* type A are surrounded by a hæmolytic zone outside which is a dark red 'reduction' zone. Covering the colonies and the hæmolytic zone is an opaque film with a mother-of-pearl lustre. All available species of Clostridia and a number of common aerobes have been tested, but none of them produces both the pearly layer and the reduction zone together, although some species show one or the other feature separately.

Although we have had the opportunity to test this method of diagnosis on actual clinical material in only five cases to date, our experience with these cases and with artificially mixed cultures suggests that it will be possible to detect *Cl. oedematiens* type A in material from lesions in which various other organisms are present. Swarming is avoided by opening and drying the plates for approximately five hours in the incubator before inoculation.

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¹ Described as "peptic digest" by Carne, H. R., *J. Path. and Bact.*, 51, 199 (addendum p. 212) (1940).

in a bacterial slime. It may be necessary first to put the fruits, etc., into the water, that is, to 'set bait', and this is best done in some container (within which the bait will not be reached and eaten by goldfish, snails, etc.).

Bait. (1) Fruits: hips, haws, windfall apples, small tomatoes and other berries. (2) Seeds, for example, hemp. (3) Thin twigs and leaf stalks,

cut into short lengths.

Container. (1) Galvanized tin with holes punctured in the top and bottom. (2) Glass jar with coarse muslin stretched over the mouth.

Treatment. Allow from a few days to a month or more to pass before gathering bait (leaves and soft fruits are attacked by fungi more quickly than are twigs and seeds). Wash the 'bait' well in tapwater, and keep it in the dark. It is possible that sporangia may not appear until after the water has been changed.

Identification. Hyphæ: coenocytic (no cross walls), of various widths, branched. Sporangia: ovoid with papilla, borne at the end of a slender hypha.

Transport. Drain the material collected and wrap in damp paper; enclose it in a tin and post it to me with the following particulars (and, if possible, drawings): (1) nature of substratum or 'bait'; (2) nature of water, (a) flowing or stagnant, (b) acid (alkaline or neutral), (c) depth; (3) date of collection, and, if bait set, date of setting bait; (4) any further treatment.

Material will be most appreciated this year in May, June, September, October, November. I will refund postage and, if asked, return the tin. I shall gratefully acknowledge, in any subsequent report, all help given.

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March 11.

Species of *Phytophthora* as Water Moulds

At the last meeting of the British Mycological Society I directed attention to the fact that (although omitted from published lists of water moulds), parasitic species of *Phytophthora* may live for long periods of time as water moulds. Masquerading thus as saprophytes, they may be dispersed in water and carried to fields where they may become parasitic upon crops. I suggested that with the co-operation of fellow mycologists, naturalists, microscopists and 'pond-dippers', it should be possible to determine the distribution of these species in the streams, ponds, ditches, even bird-baths, of Britain; and I appealed for helpers. I should be grateful for space in *NATURE* for the following instructions on how to find, and to send to me, anything that looks like a possible member of the Pythiaceæ, collected in this way.

Method. Collect from streams, ponds, etc., half-decayed fruits, twigs, stems of water weeds which are bearing a straggling, greyish mycelium probably

Variation in the Nitrogen-fixing Property of *Rhizobium trifolii*

WHILE testing a number of strains of *Rhizobium trifolii*, two cases have occurred (cultures 61 and 91, isolated from *Trifolium tomentosum* L. and *Trifolium glomeratum* L. respectively) in which a culture, from a single colony developed from a single nodule, has shown at the time of its first testing on white clover (*T. repens* L.) the presence of definite 'sub-strains' with distinctly different nitrogen-fixing ability. Culture 91 showed the same behaviour when tested against *T. glomeratum*. In each case, while some plants were scarcely better than those of the uninoculated control, other plants in the same pot were very much better. The former showed the usual characteristics associated with inefficiency, namely, early yellowing of cotyledons, poor yellow growth and the presence of many small nodules. The healthier plants carried typically large effective nodules, although other smaller nodules, similar to those on the poorly grown plants, also occurred.