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A SIMPLE METHOD FOR ISOLATION AND PHOTOGRAPHY OF CLOSTRIDIUM BOTULINUM COLONIES FROM SURFACE CULTURES ON AGAR PLATES

By

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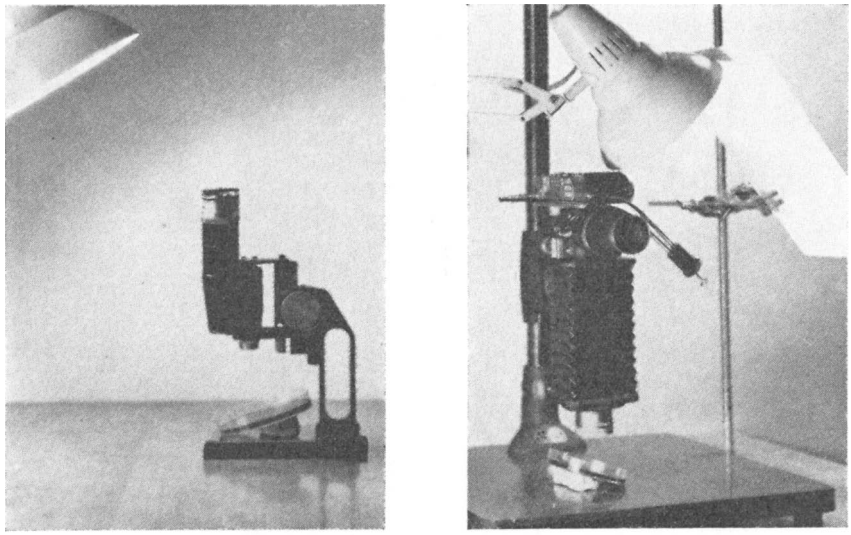
The isolation of motile Clostridium species from surface cultures on agar plates may sometimes cause considerable difficulties, especially when plating from heavily contaminated primary enrichment cultures. One preventive measure against swarming is to isolate the colonies after only 24 hours incubation. Methods in isolating toxigenic colonies of Clostridium botulinum type E and their varying appearence on solid media are described by *Dolman et al.* (1, 2 and 3).

Observations with Clostridium botulinum type E indicate that it is advantageous to inoculate a relatively large number of blood agar plates (12—24) from each enrichment culture. Working with Cl. botulinum type E the enrichment cultures should not be incubated for more than 4 days before plating. The chances of isolating the organism are best in cultures with relatively high toxicity. Series of 3—4 plates using decreasing inoculates may be preferable.

ISOLATION PROCEDURES

Cl. botulinum type E surface colonies incubated for 24 hours on blood agar plates are generally quite minute. Most type E colonies grow with a narrow and rather faint zone of haemolysis. In type E a correlation seems to exist between toxigenecity and haemolysis; non haemolytic colonies in some cases giving rise to nontoxic subcultures. The preliminary procedure therefore is

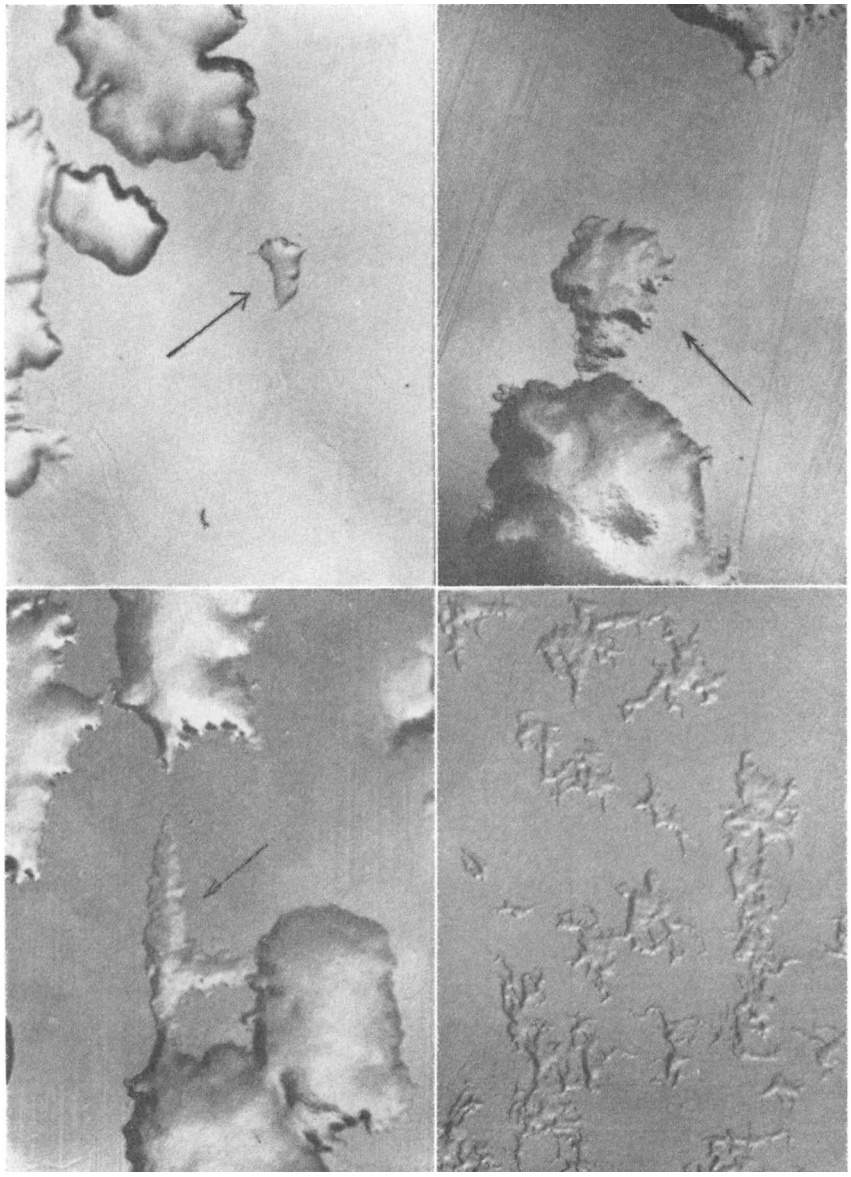


A B

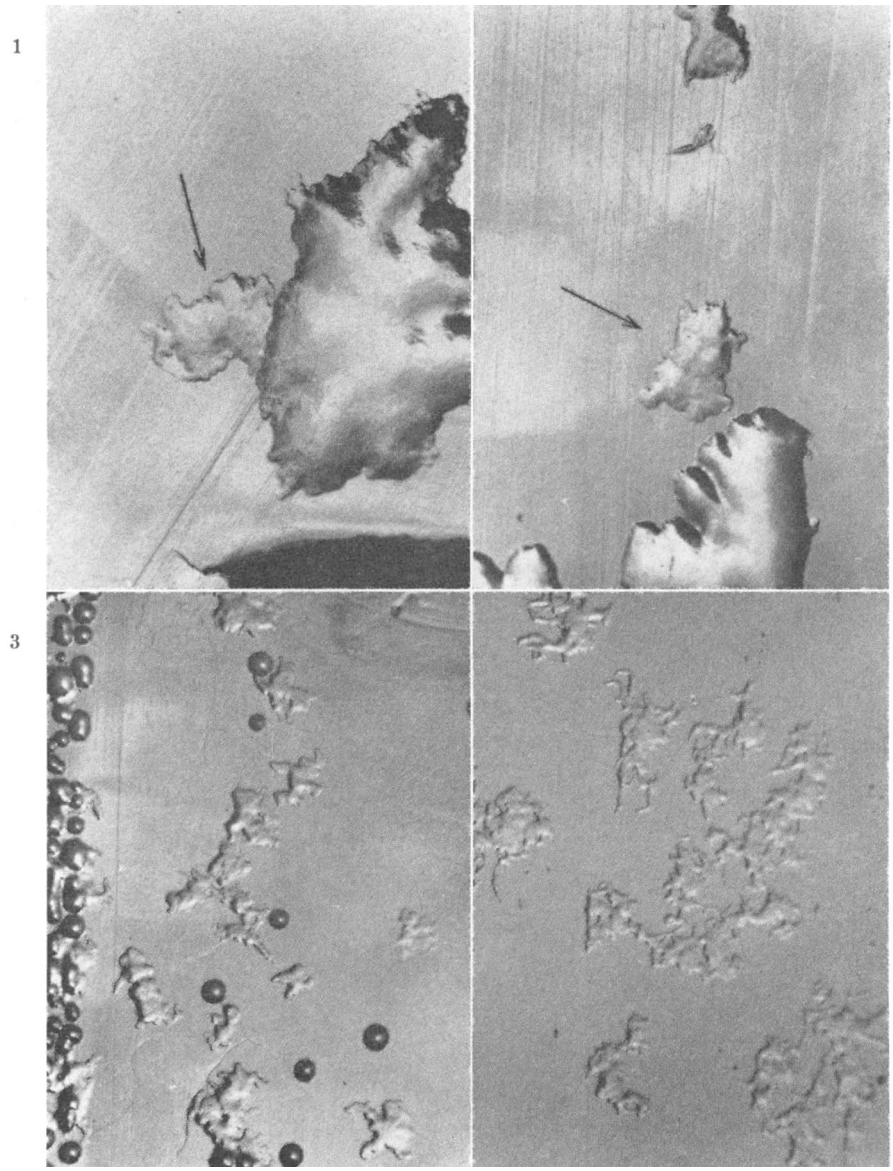
Fig. 1. Simple arrangements for examining, isolating and photographing surface colonies on blood agar plates using reflected light.

to select the faintly haemolytic colonies in transmitted light, using a low magnification and marking the appropriate colonies with a platinum needle.

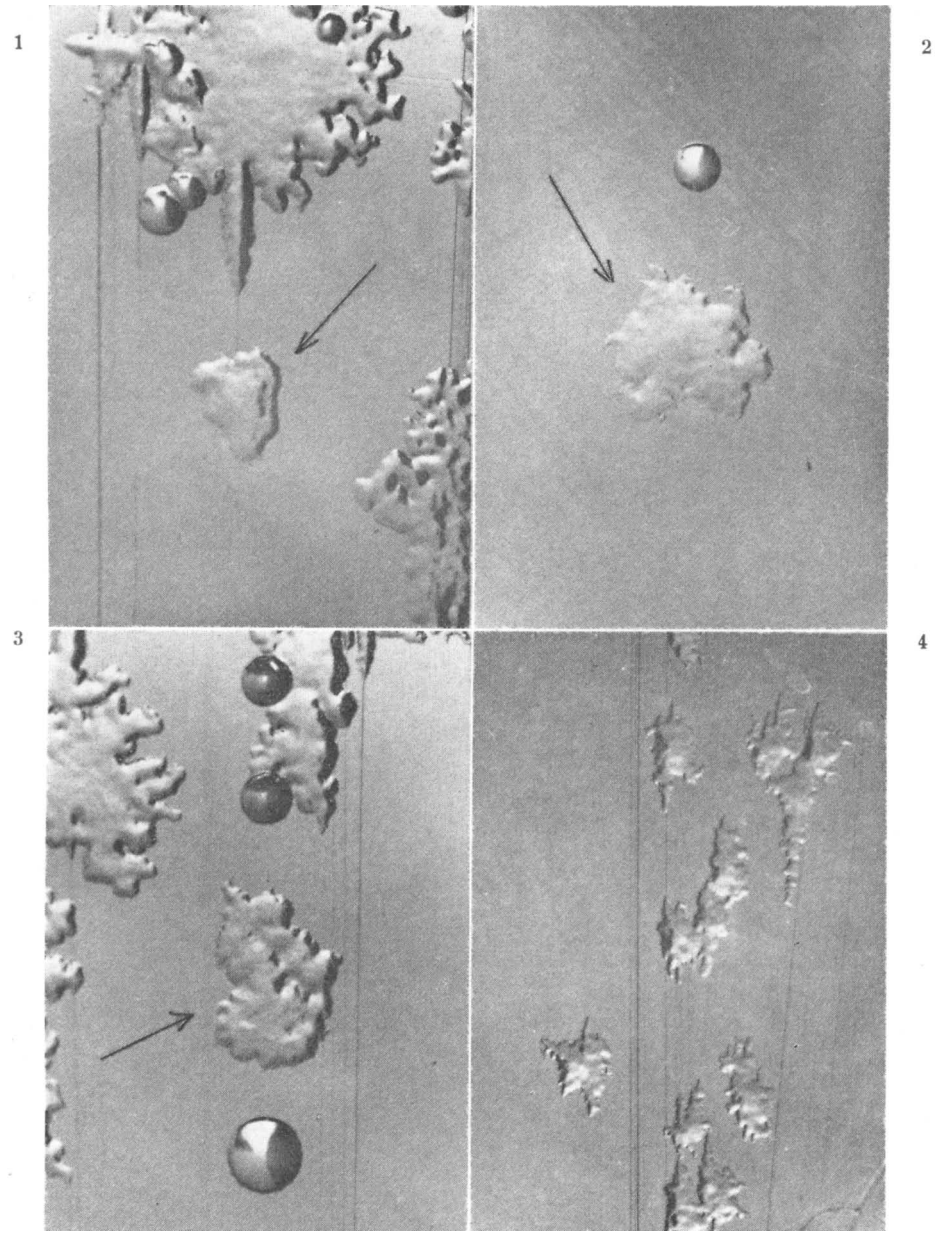
It is preferable to carry out the isolation procedure with a magnification of about 20 χ using reflected light. A stereoscopic microscope or similar equipment may be used. Suitable reflected light may be obtained by placing the plate in an oblique position. The light is thus reflected by the blood agar surface into the microscope and a good contrast is obtained. By changing the position of the light and the inclination of the plate the contrast may be varied as desired. If the microscope is not equipped with a special arrangement to put the plate in an oblique position this may be done simply by means of a piece of plasticine (Fig. 1 A). The oblique position of the plate is essential. If the plate is left horizontally the colonies generally have a misty appearance in the microscope with hardly contrast to the background. By means of the mark made previously the particular colony may easily be focused and isolated. In this way pure cultures are often obtained from primary plates. By means of the procedure described pure cultures of more han 100 strains of Cl. botulinum type E have been obtained.



F i g. 2. Surface cultures of Cl. botulinum type B on blood agar plates. 1, 2, 3 in mixed cultures — 4 in pure culture.



F i g. 3. Surface cultures of Cl. botulinum type C on blood agar plates. 1, 2, 3 in mixed cultures — 4 in pure culture.



F i g. 4. Surface cultures of Cl. botulinum type E on blood agar plates. 1, 2, 3 in mixed cultures — 4 in pure culture.

PHOTOGRAPHIC PROCEDURE

A corresponding arrangement has been used for photographing surface colonies on agar plates (Fig. 1 B). As source of light a sheet of white cardboard illuminated by a 60 W lamp is used. By adjusting the inclination of the plate and the cardboard the light is reflected from the agar surface through the lens on to the film. The plates are placed in an oblique position and in order to obtain a satisfactory depth of focus a small diaphragm aperture should be used. Because the lighting arrangement described gives very high contrast, film of soft gradation is best. It should also have a high speed to permit comparatively short exposures at the small apertures which are necessary. Kodak Tri X Pan film has proved suitable. Other data: Lens f = 5 cm.

Bellow extension corresponding to a 2,5 χ magnification increases the exposure time 12 χ giving exposure at f = 16 for 15 sec.

REFERENCES

1. *Dolman, C. E.:* Canad. J. Publ. Health, 1957, *48*, 187.
2. *Dolman, C. E., M. Tomsich, C. C. R. Campbell & W. B. Laing:* J. Infect. Dis. 1960, *106*, 5.
3. *Dolman, C. E. & L. Murakami:* J. Infect. Dis. 1961, *109*, 107.

SUMMARY

Reflected light gives a very good contrast using blood agar plates in an obliquous position under a stereoscopic microscope. Thus it is possible to isolate pure colonies of Cl. botulinum in the early stage of the growth on blood agar plates.

ZUSAMMENFASSUNG

Eine einfache Methode um die Kolonien von Cl. botulinum auf der Oberfläche der Blutagarplatten zu isolieren und photographieren.

Durch Verdrehung der Agarplatte unter dem Mikroskop damit das Licht in der Agaroberfläche reflektiert wird, erhält man einen sehr guten Kontrast. Dadurch ist es möglich aus der Mishflora der Anreicherungkultur in den frühen Wachstum auf den Agarplatten reine Kolonien von Cl. botulinum zu isolieren.

SAMMANFATTNING

Användning av reflekterat ljus för isolering och fotografering av Cl. botulinum kolonier å blodagar.

Genom snedställning av agarplattan under mikroskopet så att ljuset reflekteras i agarytan erhålles en synnerligen god kontrast. Det är härigenom möjligt att från blandflora från anrikningskultur isolera rena kolonier av Cl. botulinum på ett tidigt stadium av växten på blodagar.

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