Section of Pathology.

President—Dr. J. A. MURRAY, F.R.S.

A Method of Isolating and Handling Individual Spores and Bacteria.

By SYDNEY DICKINSON.

(*Dept. of Mycology, Rothamsted Experimental Station, Harpenden, Herts.*)

THE difficulty in the past of isolating a single organism of microscopic size and the uncertainty of the result when the dilution method has been used, have been a great hindrance to the progress of microscopical science. Barber, in 1904, overcame these difficulties by picking up single spores in glass capillaries, while recently Chambers has used a modification of this method with conspicuous success in "micrurgy." Other methods have been attempted, such as Barnard's mercury drop method, Dunn's microloop, Edgerton's closed capillary, but none have reached the stage of everyday use, and nearly all have been but modifications of the dilution method. Hanna, however, has evolved a dry-needle method which is of great utility where large spores are concerned. The method to be described, a preliminary account of which has been published,[1] is thought to be of sufficient value and reliability, combined with ease of manipulation, to become an everyday method.

It is known that on the surface of an agar gel, when first solidified, there is a film of liquid which is a dilute solution of agar. This film, under ordinary conditions, is very thin, its depth being considerably less than the diameter of a bacterium. That this is so is evident if some half a dozen motile bacteria, lying singly in this film, are observed. They appear to be non-motile, but if a relatively large object, such as a fragment of glass, is placed gently among them, those which are in the shadow, so to speak, of the glass, immediately begin to move about in the liquid between the glass fragment, the film surface and the agar. Owing to the thinness of the film it follows that when a small number of bacteria are on an agar surface, they will lie side by side, and not superimposed one upon the other, unless agglutination has taken place. This being the case, any method by which a local thickening in the surface film can be obtained will not only allow motile bacteria to show their motility but will also provide, where the angle between the side of the thickened area and the surface film is sufficient, a means of moving motile and non-motile bacteria from place to place on the agar surface.

In the method to be described this local thickening is the cone of liquid formed between the cleanly broken end of a fine glass rod or "needle" and an agar surface, when the rod has been withdrawn slightly from the agar. This rod is mounted in balsam on the arm of a machine, to which the name "isolator" (fig. 1) has been given. This instrument, which can also be used for micro-dissection, gives a fine movement in all directions by means of three rack and pinion elements. With

[1] *Annals of Botany*, January, 1926, xl, clvii.

square stage microscopes the "isolator" is fastened to the stage, but with circular stage microscopes it is mounted on a separate stand. The needles can be made by hand by means of a micro-burner, but are also supplied by the makers of the instrument.

The actual technique is as follows. A thin layer of agar is placed on a sterile coverslip, either of glass No. 1 type or of mica, and a small amount of the material, from which it is desired to isolate a bacterium, is placed in the centre of the agar. This coverslip is then inverted over a Van Tieghem drop cell, which is fastened to a microscope slide, and this slide is placed on the stage of the microscope. Part of the wall of the Van Tieghem drop cell (preferably of vulcanite) is cut away in order that the arm of the "isolator," carrying the glass needle, may be inserted and have free access to all parts of the agar film. The essential working distance for the needle between the coverslip and the substage condenser is 2 mm. When using an oil immersion lens it is necessary to fasten the coverslip to the Van Tieghem drop cell with vaseline.

The material is focused under a low-power lens and the edge of it centred by means of a mechanical stage, while the needle is brought into position under the edge of the material by means of the "isolator." For bacteria and spores of like size mica coverslips are more suitable though not essential. A higher power lens is brought into position and focused on the edge of the material, the needle being in position just below. The needle is raised into contact with the film of liquid on the agar surface (fig. 2, *A*). When contact has been made, it is lowered slightly so as to form a cone of liquid as in fig. 2, *B*. The material is now moved up to this cone by means of the mechanical stage, and as soon as the cone is reached, some of the bacteria will be seen to float out into it. The cone is then drawn away from the material along the surface, leaving some of the bacteria in its path. While the material and the needle are still in the field of view, the needle is lowered, leaving all the bacteria on the agar. The particular bacterium which it is wished to isolate is now noted, a cone formed as before, and this bacterium is brought up to the cone and then carried in it away from its neighbours. The needle is again withdrawn, and the bacterium examined in order to make quite certain of its isolation and identity. It is quite possible with a little practice to take away one of a group of three or more bacteria, and leave the remainder untouched.

When it has been decided that the bacterium now lying alone is the one required, the cone is re-made and the bacterium is carried in it, under observation the whole time, to another part of the agar surface distant 2 mm. or more from the bacterial mass. Here again the needle is withdrawn, and the bacterium examined, and, if satisfactory, its position on the agar is marked by two scratches, one on either side of it, made in the agar by the needle, so as to be visible to the naked eye. Next, if the coverslip is made of mica, after the oil has been wiped off by a brush dipped in ether, the upper surface of the coverslip is sterilized with absolute alcohol while still on the Van Tieghem cell. It is then lifted off, and the piece of mica and agar around the isolated bacterium cut out with sterile scissors, and dropped into a test-tube containing nutrient solution. If, however, a glass coverslip is used, the agar around the bacterium, marked off by the two scratches, is cut off as a slab and transferred with a sterile scalpel to a test-tube containing nutrient agar or solution. It is interesting to note that in a series of some 500 single spore isolations made in this way only 1 per cent. of air contamination occurred, showing that the risk of contamination during the process of cutting out the agar slab and transferring it to a test-tube is negligible.

The reason why the bacterium is carried along in the cone, fig. 2, *B*, is that the direction of the applied force is nearly parallel to the plane of motion. In fig. 2, *A* the direction of this force is at an angle to the plane of motion, and the surface

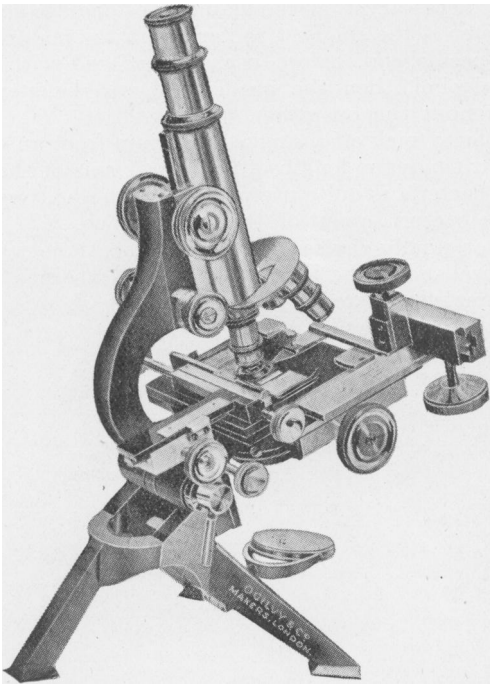

FIG. 1.

Square stage type of "Isolator." (By courtesy of Messrs. Ogilvy and Co.)

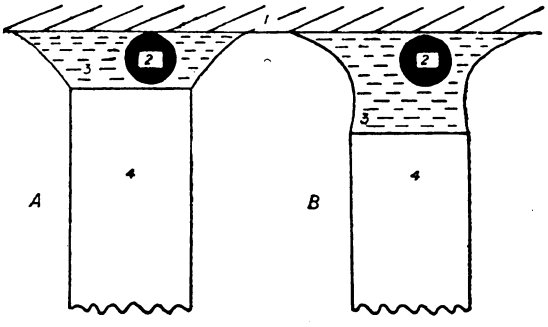

FIG. 2.

(By courtesy of the Editor, *Annals of Botany*.)

tension is then not sufficient to overcome the friction between the bacterium and the agar. In order to ensure that the needle, when withdrawn, does not carry away any of the bacteria, it is necessary that the diameter of the needle should be less than three times the diameter of the smallest bacterium in the material. If this condition is fulfilled, and the needle end, before use, is sterilized with a camel-hair brush dipped in absolute alcohol, then the needle end will be sterile, except from air contamination, throughout the operation.

It is quite possible to pick off a single bacterium from an agar film, using a rod whose diameter is four times the length of the longer axis of the bacterium, and this method of handling bacteria is quite useful, but the method preferred by the author is that of carrying the bacteria about on the agar surface.

The method was originally devised at the Phytopathological Laboratory of the Ministry of Agriculture and Fisheries, but has been modified and tested out very thoroughly at Rothamsted, where in the department of mycology it has been in constant use during the past year.