Some Methods for the Isolation of Amoebae[1]).

By

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Probably the most sucessful method for the isolation of Protozoa is that which involves washing the animal in sterile nutrient medium or water in order to free it from contaminating organisms. The animal is then isolated in a hanging drop of culture medium or in a nutrient medium on a depression slide. The culture medium is usually inoculated with Bacteria or Protozoa which serve as food organisms. At times it is desirable that a few contaminating organisms be carried through the wash waters with the animal. These organisms serve as food.

The foregoing method, which has been used by the author regularly, has not always been successful. In attempts to establish pedigreed stock, success or failure may depend on a number of factors. Some of these are the condition of the animal, the concentration of the food organisms and their metabolites, the resistance of the animal to a change of medium, and the extent of injury of the animal due to transfer by pipette. The number of amoebae which must be isolated to secure a pedigreed culture makes the method unsatisfactory. Accordingly, the methods described in this paper were devised in order to obtain a higher percentage of successful cultures.

Some amoebae culture well on agar, others do not seem to be able to adjust themselves to solid media. On the whole, the larger species grow better in liquid media, the smaller species on solid media.

Method 1: A drop of medium containing amoebae from a wild culture is placed on the surface of an agar plate which is poor in dissolved nutrients. After absorption or evaporation of the drop, observation will show whether the amoebae will adjust themselves

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to the new environment. If the animals migrate from the point of inoculation in such a way as to become separated widely from each other, then either of the following procedures may be adopted: a) A well isolated amoeba is selected. A small drop of a light suspension of Bacteria is placed over the amoeba. After absorption or evaporation of the drop, the amoeba may be observed through the bottom of the Petri dish. With a fine needle a piece of agar bearing all other amoebae is removed from the plate. The isolated amoeba with its contaminating Bacteria is left behind to grow and multiply. In a few days a pedigreed stock should result. b) In cases where the amoebae do not migrate extensively, an amoeba may be removed on a small bit of agar with a small, fine-pointed needle and placed on a sterile agar plate. A drop of a light suspension of Bacteria is placed over the amoeba to serve as food.

Method 2: An amoeba is washed and isolated into culture medium or sterile water. Then it is placed in a small drop of a light suspension of Bacteria on a sterile agar surface. This method usually gives results with amoebae which will culture on solid media. Several amoebae are isolated to insure success.

Method 3: An amoeba is washed and isolated to a drop of sterile culture medium or water on a slide which has been flamed previously. After the amoeba has attached itself to the slide, a mark is made underneath this point on the slide, the slide is submerged in sterile medium or water in a Petri dish, and three grains of sterile wheat are placed on the slide at the spot where the amoeba is located. Bacteria which accompany the amoeba in the manipulations will be drawn to the wheat and thus serve as an immediate source of food. Since the location of the animal is known by the mark on the slide, daily observation of growth and reproduction is possible.

Method 4: This method serves only when the amoebae are fairly large. A wild culture of the amoebae is subcultured into a Petri dish containing culture medium (sterile water and several grains of sterile wheat). The medium is adjusted to a depth of 4 mm. prior to subculture. The amount of inoculum is determined by trial. Just enough is used to give a scattered population of amoebae in 24 hours. Observation shows whether the amoebae are growing and multiplying. A glass plate is fastened to the stage of the microscope in such a way that a ring 6 mm. in diameter, marked on the lower side of the glass, is optically centered under the low power objective. The culture is placed under the micro-

scope, and an amoeba is sought which is separated from other amoebae by a distance of more than 4 mm. The amoeba is centered in the field. A micro-ring (made by grinding down short sections of 6 mm. glass tubing to a length of 5 mm.) is greased on one end with a thick vaseline, placed directly under the temporarily raised objective, lowered into the medium so that its wall coincides with the ring marked on the glass plate, and by a slight pressure of the finger made to seal with the bottom of the Petri dish. The amoeba is enclosed by the micro-ring and isolated from other amoebae by the procedure. The small volume of the medium within the ring is throroughly examined in order to be certain that other amoebae are not present on the substrate, sides of the ring, or surface film. After 24 hours observation generally shows numerous amoebae inside the ring. These are removed with accompanying food organisms by a micro-pipette and placed in sterile culture medium in a Petri dish. A pedigreed stock culture usually results after several days.

The faults of these methods will be apparent with experience. The advantages are as follows: The first and fourth methods eliminate mechanical injury due to handling. The second and third insure the presence of sufficient food, and the elimination of toxic products by absorption and diffusion into the agar plate. In the fourth method the medium is shown to be adequate by the presence of healthy, normal amoebae. The amoebae do not have to be handled. The environment is disturbed in no way, except by the introduction of a micro-ring. This method is especially useful for the isolation of sensitive amoebae, which tend to encyst, or react unfavorably, when handled very much by pipette.

The author has used all of these methods with success. Small and large species of limax amoebae from salt water may be isolated easily by the first and second methods. *Rugipes vivax* SCHAEFFER, also a marine amoeba, is adapted to the same methods. Small and large species of limax amoebae from fresh water are best adapted to the third method. Fresh water radiosa-like species may be isolated by the third and fourth methods.

None of the methods herein described is satisfactory for all species of amoebae; they do not supplant the older method of transfering an amoeba from one wash to another and subsequently isolating it in a hanging drop, but rather serve as alternatives.

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