METHODS

A DEVICE FOR NERVE TISSUE CULTIVATION

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A construction and method of preparing a Petri dish for long-term culture of nerve tissue enabling organotypical explants of nerve tissue to be obtained for subsequent histological and electron-microscopic investigation are described. Methods of working with the suggested dish are also described.

KEY WORDS: *nerve tissue culture; methods of tissue culture.*

The principal method of organotypical culture of nerve tissue at the present time is on double coverslips in Maximow's chambers [1-4]. This method has many undoubted advantages over nerve tissue culture on "flying coverslips," in rotating tubes, or in Leighton's tubes [5]. However it requires considerable effort on the part of laboratory assistants when changing the medium and washing the large numbers of coverslips and Maximow's chambers. Furthermore, the use of this method is restricted because of the shortness of supply and the high cost of manufacture of Maximow's chambers. A simple calculation shows that a laboratory must keep a reserve supply of at least three or four times as many Maximow's chambers as the number of cultures being grown at any particular moment.

The object of this methodological investigation was to devise a system for long-term culture of large series of explants under conditions closely similar to those created in Maximow's chambers (culture in a "resting drop" with a minimal volume of nutrient medium, good aeration of the cultures).

The suggested modification to the Petri dish for culture is simple (Fig. 1). The device is made from two identical (top or bottom) halves of ordinary Petri dishes. On the base of one of them, at about 1 cm from the wall, octagonal* coverslip supports made of mirror glass 5-6 mm thick are secured with epoxy resin in a circle. The coverslip supports must be slightly smaller in size than the diameter of the coverslips on which the explant is placed, to prevent the nutrient medium from flowing away (coverslips measuring 22 X 22 mm are used and the maximal diameter of the coverslip support is 18 mm).

The dishes are washed like the remaining glassware for tissue culture, but without bichromate treatment. They are then dried, wrapped in paper, and autoclaved. During culture, the coverslip coated with collagen is taken from the dish containing solution for keeping coverslips and placed on the coverslip support (Fig. 1C). The small volume of fluid that remains on the coverslip is sufficient to ensure that the coverslip is firmly held by surface tension on the support. If dry coverslips are used, a small drop of sterile distilled water must first be placed on the support and

*We found coverslip supports of this shape more convenient and easier to make. They could certainly be of different shapes.

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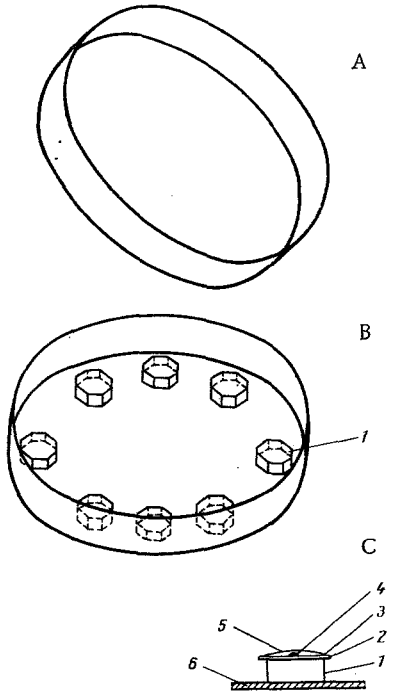

Fig. 1. Petri dish with device for tissue culture: A) lid of dish; B) base of dish with coverslip supports glued to it (1); C) diagram showing arrangement of coverslip with culture on support: 1) coverslip support, 2) coverslip, 3) layer of collagen, 4) explant, 5) drop of nutrient medium ("resting drop"), 6) base of dish.

the coverslip is then placed on it (if too much fluid is used the slide may "float" and this must be avoided). The explants are placed on the surface of the collagen and one or two drops of nutrient fluid added. Next, 3-5 ml of sterile bidistilled water is poured into the bottom of the dish, the lid is applied, and the chamber rendered airtight with a strip of adhesive tape. When the medium is changed, which is done twice or three times a week, the coverslip is taken from the support with curved forceps, and the old medium is poured off and replaced by fresh. As a rule tissue can be grown in culture in the same dish for several weeks. If necessary, an atmosphere of a particular composition can be created easily in the dish simply by blowing the sterile gas mixture through the dish.

Series of control experiments, during which explants of spinal cord and spinal ganglia from 16-18-day mouse embryos were cultured for 3-4 weeks in Maximow's chambers and in the suggested dishes, showed that the explants developed synchronously in the two cases and attained a high degree of organotypical differentiation. There is no doubt that other tissues can be grown equally well in culture in the suggested dishes.

A fundamental defect of the suggested method is the impossibility of intravital microscopic observation on development of the cultures; if this must be done, the slides with the cultures have to be transferred into Maximow's chambers. At the same time, as experience shows, the suggested method proved very convenient for long-term culture of large batches of explants for subsequent histological, histochemical, and electron-microscopic investigation, for it greatly reduces the time spent by the laboratory assistants and leads to substantial economy in articles of glassware that are in short supply (large coverslips and Maximow's chambers).

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