PRIMARY CULTURE OF TRYPSINIZED CHOROID PLEXUS TISSUE FROM THE LATERAL VENTRICLE OF THE PIG EMBRYO

B. L. Krikun and A. M. Amchenkova

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Tissue culture of the choroid plexus has been used by many investigators for various purposes. Studies have concentrated in particular on the structure and function of the choroid plexus [1, 10, 12], its embryogenesis [1, 2], and the functional properties of the villi and their role in the production of cerebrospinal fluid [1, 3, 5, 8, 9]. Plasma cultures of the choroid plexus of man and animals have been used for this purpose.

The embryogenetic properties of the choroid plexus, indicating its neuroectodermal origin, suggested that a tissue culture of this organ might be used for the cultivation of neurotropic visures. Another no less important fact here was that the preparation of a culture of choroid plexus cells and their cultivation on ordinary nutrient media present no special difficulty and do not require special conditions or a complicated technique, as is the case with nerve tissue cultures.

In virological investigations choroid plexus tissue cultures were first used for cultivating and studying the virus of the demyelinating disease of sheep—Visna virus [11]. Another interesting report [4] described the isolation of a virus from the spinal cord of a patient with multiple sclerosis in a culture of sheep's choroid plexus. It therefore appeared worthwhile to study the cultivation of the tissues of this organ.

In 1962 the authors obtained a primary trypsinized culture of cells of the choroid plexus of a human embryo and a cow embryo. It was subsequently discovered, however, that the most convenient source of this tissue for cultivation is the pig embryo.

The method of preparing the culture which was used as as follows.

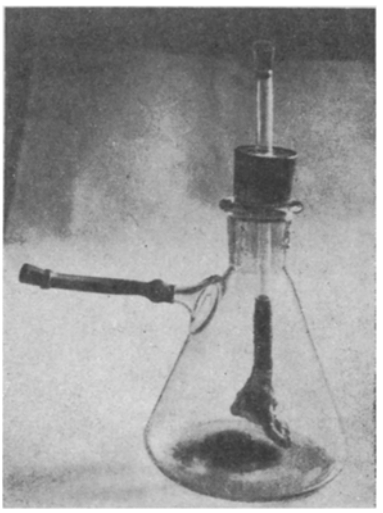

Fig. 1. Flask for trypsinization.

The choroid plexus was extracted in sterile conditions from the lateral ventricle of a pig embryo and washed several time in Hank's solution with antibiotics. The choroid plexuses from 30-50 embryos in medium No. 199 with antibiotics were left in a flask for 18 h at 6-8°.

The tissue was then washed out with a 0.1% solution of Difco trypsin in Hank's solution (pH 7.2-7.4) and trypsinized by Lahelle's mild method [7] modified by the authors.

A bag made of Kapron gauze, with a capacity of 25-30 ml, fixed to the end of a glass tube 250-300 mm long, was placed inside a 150 ml glass flask (Fig. 1), the tube being passed through a rubber stopper fitting in the neck of the flask. The system was sterilized by a current of steam in an autoclave.

Through the free end of the glass tube a 0.1% solution of Difco trypsin in Hank's solution)pH 7.2-7.4) was introduced from a Moore's pipet along with the tissue. The trypsin level in the flask must be 2-3 cm above the bag. The flask was placed in a water bath at 30-31° for 3 h.

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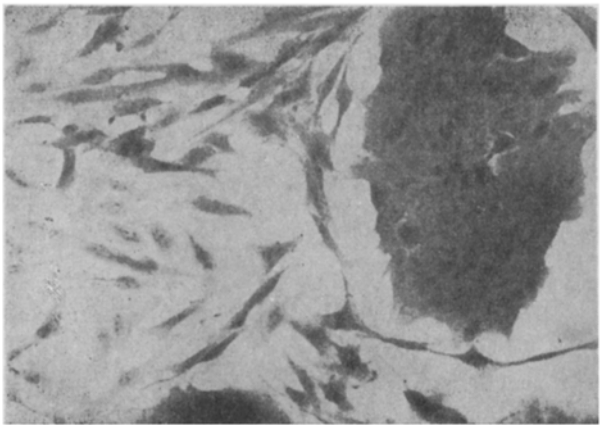

Fig. 2. General appearance of the culture on the 6th day of growth. Hematoxylincosin plexus. 860×.

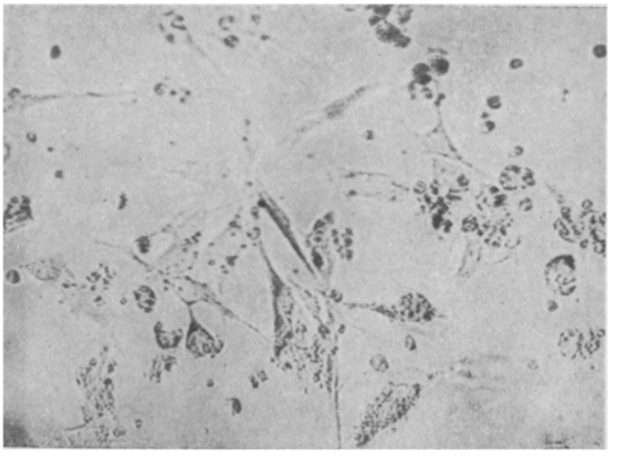

Fig. 3. Cytopathogenic action of Coxsackie B-3 virus in a tissue culture of pig's chorionic plexus. 860×.

The trypsin was then poured off through the side tube and 25-50 ml of medium No. 199 was added through the glass tube. The flask was replaced in the water bath for 15-20 min, after which it was agitated for several minutes to wash out the Kapron bag containing the tissue with medium No. 199. To obtain more complete dispersion of the cells the liquid was aspirated from the flask and returned through the glass tube into the Kapron bag with the tissue. As a result, a filtered suspension of trypsinized cells in medium No. 199 remained in the flask, and 10% beef serum was added to it.

The cells were counted in a Goryaev's chamber and their viability was determined by testing with methylene blue or eosin. The cells were seeded in penicillin flasks or Rous and Povitskaya flasks. The growth medium was medium No. 199 with the addition of 10% beef serum, a 50:50 mixture of medium No. 199 with 0.5% lactalbumin hydrolysate, and 10% beef serum.

As a result of the mild method of dispersion the mean yield of viable cells from 1 g tissue was 88.8 million, with only a negligible number of blood cells.

When the cells were cultivated in penicillin flasks with glass slides, on the 4th-5th day a continuous layer of cells was formed. The layer consisted of islands of epithelioid cells, the space between which was filled with fibroblast-like cells (Fig. 2).

The epithelioid cells had clearly defined borders, and their finely granular cytoplasm often contained phagocytosed particles of degenerated cells. The cell nuclei were clearly outlined and, as a rule, contained 2-3 nucleoli. The fibroblast-like cells were indistinctly outlined; their cytoplasmic processes gradually narrowed or formed syncytial connections with other elements. The nuclei of the fibroblast-like cells were long and oval in shape and were filled with finely granular chromatin.

In ordinary conditions the culture was grown until the 14th-17th day, and after a change of medium (medium No. 199 +5% or 10% aminopeptide) it was kept for up to 28-30 days.

Histochemical investigations showed that the epithelioid and fibroblast-like cells possesed a comparatively high content of RNA, protein, and SH-groups and high succinate dehydrogenase activity, presumably indicating their marked metabolic activity.

Because of the peculiar structure of the choroid plexus, the villi of which are covered by a single layer of epithelial cells, a particularly gentle method of dispersing the cells with trypsin had to be used. The ordinary method of trypsinization (Dulbecco and Youngner) in 0.25% trypsin solution on a magnetic mixer followed by centrifugation gave a low yield of viable cells and an abundance of erythrocytes. The trypsin concentration had therefore to be lowered to 0.1% and the incubation temperature to 30-31°. In this way the contact time of the trypsin with the tissue could be increased to 3 h, and there was no need to use the magnetic mixer or the centrifuge, thus elimiating the risk of mechanical injury to the superficial epithelium and the small blood vessels of the plexus.

When describing this culture as mixed the intention is to stress that the presence of epithelioid cells was evidently attributable to the use of a gentle method of trypsinization for dispersing the cells. When a different method was used to obtain choroid plexus cultures, growth of fibroblast-like cells only was observed [11].

In pilot experiments this culture proved to be sensitive to Coxsackie B-3 virus which propagated in its cells to a titer of 10^{-6}, producing the characteristic picture of cytopathic changes (Fig. 3). By itself, the fact of the propagation and cytopathogenic effect of the Coxsackie B-3 virus in a primary trypsinized culture of cow and pig embryos is of considerable interest. Hsiung [6], for example, after investigating the virus spectrum of various tissues, concluded that primary trypsinized tissues of nonprimates are insensitive to the cytopathogenic action of viruses of the Coxsackie B group.

We are therefore compelled to ask what caused the overcoming of the species-specific resistance of the primary trypsinized nonprimate tissues to the Coxsackie B virus when the tissue of the choroid plexus from an embryo of the same species of animal was used. The investigation of the virus spectrum of this tissue in different species of mammals is therefore of definite interest also for the study of the mechanisms of tissue and species resistance to virus infection.

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