PRODUCTION OF MONOLAYER CULTURES OF AMPHIBIAN TISSUES AND THEIR USE FOR CULTIVATION OF VACCINIA AND MEASLES VIRUSES

S. A. Demidova and G. Ya. Svet-Moldavskii

D. I. Ivanovskii Institute of Virology of the AMN SSSR
and L. A. Tarasevich Control Institute
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Little attempt has been made to study the problem of the viruses and virus infections of the lower vertebrates (fishes, amphibians, and reptiles), yet without such a study our knowledge of the ecology and evolution of viruses must be incomplete. A very important factor in the investigation of these problems is the creation of tissue cultures from the tissues of poikilothermic vertebrates.

Experiments to prepare tissue cultures from cold-blooded animals by Carrel's method were carried out many years ago [5] and such cultures have been used several times [2, 6]. Trypsinized monolayer cultures of the tissues of cold-blooded animals have received little or no study. Wolf and co-workers [8] obtained trypsinized cultures from tissues of fishes and frogs. Similar results are described by Auclair [3]. Little is known of the proliferation of viruses attacking mammals and birds in the tissues of cold-blooded animals. Poliomyletits virus does not proliferate in a culture of frogs' tissue [7], but the RNA of this virus is able to pass through one cycle of proliferation in such a culture. Vaccinia virus has been cultivated successfully in the embyos of reptiles [1].

In the present paper we describe the use of a modified method of obtaining cultures of tissues of axolotls and frogs (Rana temporaria) and their tadpoles.

EXPERIMENTAL METHOD

Explants and trypsinized skin-muscle, lung, and kidney tissues of the axolotl, skin-muscle, liver, kidney, and heart tissues of the frog (Rana temporaria) and tissues of the tadpoles of this species of frog were cultivated. The axolotls and frogs were kept at a temperature of 4° and were not fed. Before the experiment they were placed in a concentrated solution of antibiotics (50,000 units penicillin/ml and 50,000 units streptomycin/ml) at 4°. This time was long enough to give sterile tissues for culture. The nutrient medium consisted of Locke-Lewis solution ($NaCl$ – 0.7 g, KCl – 0.042 g, $CaCl_2$ – 0.025 g, $NaHCO_3$ – 0.02 g, dextrose – 0.25 g and 90 ml bidistilled water), 25% of chick embryonic extract, and 10% of inactivated calf serum. The medium was diluted with bidistilled water until an isotonic state for poikilothermic vertebrate animals was attained. The plasma from a cock fasted for several hours before its blood was taken was used. Trypsinization was carried out at 4° for 18 h in a magnetic mixer.

As a rule the cells obtained during the first hour gave poor results, and cells obtained during the following periods of trypsinization were therefore used. They were sedimented by centrifugation, and the suspension was diluted in a medium of: 10% inactivated calf serum, 20% chick embryonic extract, 30% medium 199, and 30% Earl's medium; the pH of the medium was adjusted to 7.2-7.4 with soda. Cells were cultivated at 20 and 37°. The amniotic fluid of a cow was added for cultivation of cells from the axolotls.

To study the cytopathogenic action of viruses in cell cultures of the tissues of cold-blooded vertebrates, measles virus strain Leningrad-4 and vaccinia virus were used.

EXPERIMENTAL RESULTS

Of the trypsinized frog's tissues, only the kidneys grew well. The cells attached themselves at once and the cell layer reached a satisfactory development after 5-6 days; during cultivation at 20° the cells started to degenerate after 2 weeks. The cells also grew at 37°, but their degeneration in this case started much earlier – after 6-7 days.

The tadpoles were trypsinized whole (after removal of the intestine). The cells adhered quickly and a continuous layer could be obtained on the fourth-sixth day (Fig. 1). On microscopic examination of the cell cultures it was difficult to make out from which organs the epithelial cells had been cultivated.

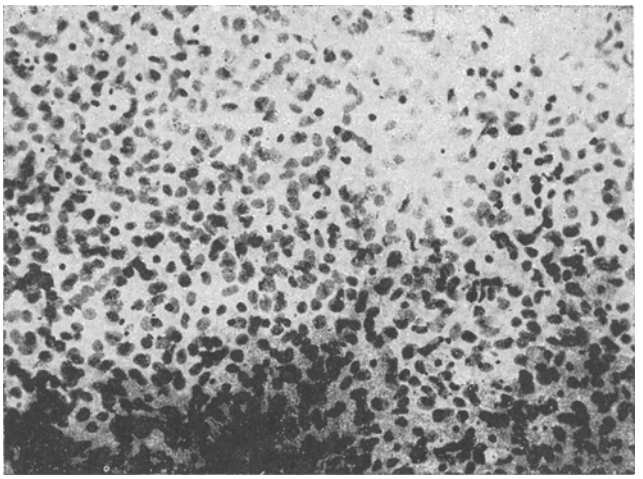

Fig. 1. Tissues of a tadpole. Hematoxylin-eosin. ×106.

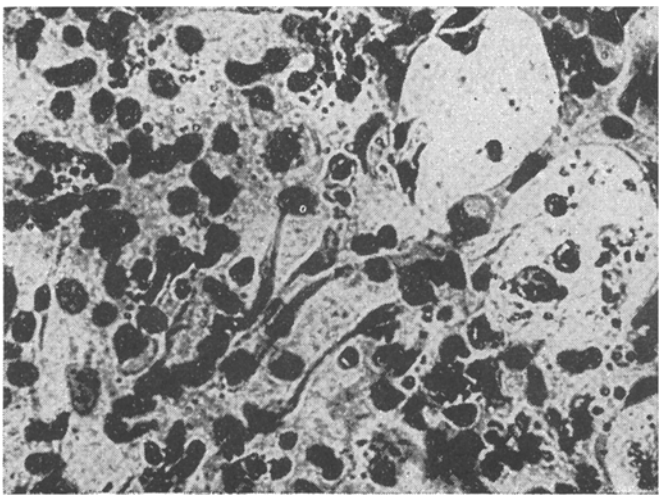

Fig. 2. Skin and muscle tissues of an axolotl. Hematoxylin-eosin. ×280.

During cultivation of explants of skin and muscle tissues of the axolotl at 20-25° growth of the cells was observed after 3 days. The cell layer reached its maximal development after 7-8 days (Fig. 2), and this was maintained for 3 weeks. After 3 weeks degeneration of the cells began. During cultivation of tissue fragments and explants at 37° growth of the cells was observed much sooner—after 2-3 days. However, the cells started to degenerate early also—after 10 days.

Various cells were encountered in the cell cultures: fibroblast-like, epithelial, pigment, and large round cells. In old cultures the fibroblasts had undergone some degree of degeneration. No monolayer culture of the liver cells of the axolotl could be obtained.

During the attempt to trypsinize the skin-muscle tissue of the axolotl the cells separated rapidly—after 3-5 min. Growth of the cells was observed after 3-5 days and reached its maximal development after 7-10 days. The medium was changed for the first time after 3 days, and thereafter once per week. Cell cultures grew better at 20° than at 37°. At 37° the tissues grew faster, but they also degenerated much faster.

Inoculation of the cell cultures from the frog's kidney and the skin and muscle tissue of the axolotl with measles and vaccinia viruses did not give a clearly defined cytopathogenic action, despite the fact that 16 passages were carried out. Observations were made on cells cultivated at 20 and 37°. When inoculated with massive doses of virus (vaccinia and measles) a toxic action on the tissue was observed—degeneration of the whole cell layer. Measles virus was detected in the first two passages in a titer of 0.5 $\log_{10}$ in the culture fluid taken from frog's kidney tissue. The virus was titrated in continuously growing human amniotic tissue. After the third passage, however, it was impossible to detect the virus in the undiluted culture fluid. No evidence of proliferation of these viruses was found.

SUMMARY

Methods were derived for obtaining trypsinized tissue cultures of cold-blooded animals, frogs and their tadpoles. After infecting these cellular structures with vaccinia and measles viruses no distinct cytopathic effect was obtained and no multiplication of these viruses was noted.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.