THE STUDY OF PRIMARILY PANCREATIZED TISSUE CULTURES FROM THE HEART OF THE CHICK EMBRYO USING PHOTOMICROGRAPHY

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In virological research, at the present time the method of tissue cultures has assumed a leading position. In addition to the use of cell strains for studying the interaction between viruses and cells, extensive use is made of cultures primarily isolated from various organisms.

It is of considerable interest to examine the properties of tissue cultures obtained from individual organs of animals or embryos. To study the myocardium of chick embryos, some writers have used primarily trypsinized tissue [1], others have prepared hanging-drop cultures and made investigations with the electron microscope [2]. We considered that cardiac muscle might also be used successfully for these purposes.

EXPERIMENTAL METHOD

In our experiments we used the hearts of 11-12 day chick embryos, which were washed free from blood with Hank's solution and placed in a 50 ml flask.

As proteolytic enzyme we used a 0.5% solution of pancreatin. Pancreatization was carried out in a magnetic mixer at room temperature, the magnet revolving at the rate of 200 rpm. After 30 min the resulting cell suspension was poured into centrifuge tubes and centrifuged for 10 min at 900 rpm. After centrifugation, the residue was resuspended in a nutrient medium consisting of medium No. 199 and 20% ox serum. The cell suspension was filtered through two layers of gauze.

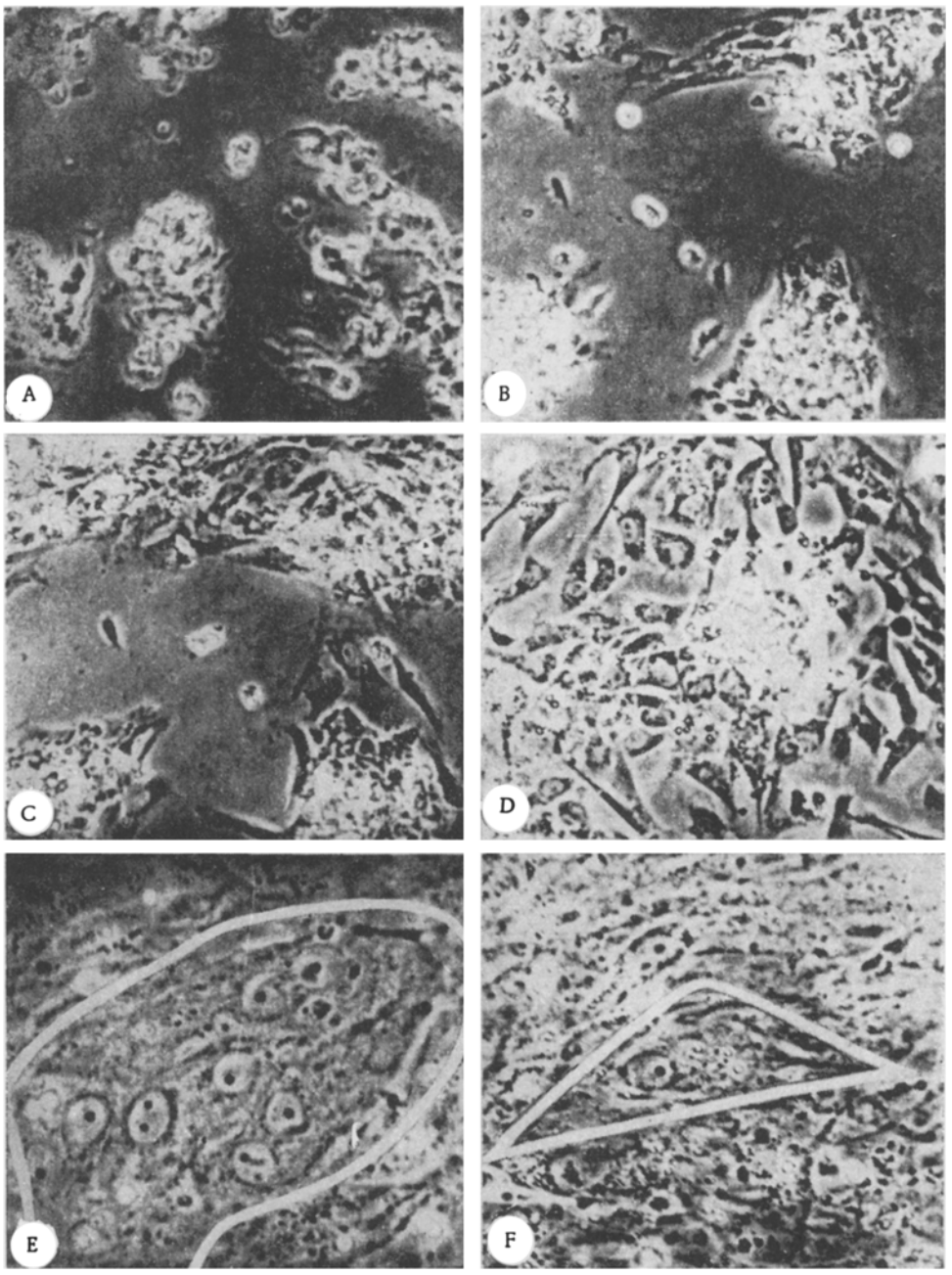
The cells were counted in a hemocytometer after preliminary staining with crystal violet solution and citric acid. Into each tube was placed 2 ml of a cell suspension containing 1,000,000-1,500,000 cells. Cultivation proceeded at 37°. Microscopic examination of the elements obtained immediately after pancreatization revealed the presence of clearly distinguishable large and small cells.

EXPERIMENTAL RESULTS

Examination of the tubes at various periods of cultivation showed that after 24 hr the cells were adherent to the glass and formed a monolayer culture with a continuous sheet of cells. By their morphological signs, two types of cells were detected: fibroblastoid and epithelioid. The latter were arranged in separate islets, increasing in size on subsequent days. From the 1st to the 8th day individual rhythmic contractions of the areas of epithelioid islets could be observed. On the 5th-7th day bands containing large numbers of nuclei appeared at the margin of these contracting islets.

The pattern of development of a culture of cardiac muscle cells of a chick embryo was studied by photomicrography. Experiments using slow-motion photomicrography showed that 3 hr after seeding the tissue suspension, individual conglomerates became adherent to the glass, although their cell composition could not be determined, probably because of their many layers and the presence of large numbers of injured cells (see figure, A). In the course of time, in some areas of these individual conglomerates processes of cytoplasm developed, and this was followed by the release of separate cells (see figure, B and C).

As a result of the active undulating movements of their cytoplasm, the cells migrated toward each other. A marked mitotic activity of the cells was observed. Because of the increase in their number and of their migration, a monolayer was formed (see figure, D, E, and F). Some of the primarily ejected conglomerates remained in the composition of the cell layer, and some developed rhythmic contractions after the second day. On the third day, in the newly formed layer individual cells were seen, the cytoplasm of which performed pulsating movements; rhythmic contractions of whole groups of cells were also observed. Motion picture photomicrography at a speed of 24



Growth and development of a culture of pancreatized cardiac muscle tissue from a chick embryo. Phase-contrast microscopy. Glass chamber. Magnification 20× 7. A, B, C) Frames taken after every 9 hr of growth of the culture; D) after 36 hr; E, F) frames of a continuous photograph (24 per second) of the contractions of a group of cells taken 74 hr after seeding of a culture in the chamber. The borders of the contracting cells are marked in the figures by a white line.

frames per second enabled this pulsation to be recorded against the background of the immobile cells surrounding this area. The frequency of the rhythmic contractions was 90-130 impulses per minute. In the newly formed layer the cells were polypoid, polygonal, or sometimes elongated in shape, with a round nucleus and one or two nucleoli.

The cytoplasm of the rhythmically contracting cells, in contrast to that of the others, appeared fibrous and optically denser. Despite the obvious morphological similarity between the cells composing the monolayer, their functional differentiation was clearly visible.

Further investigations will shed light on the character of the cells composing the primarily pancreatized cardiac tissue of the chick embryo, and will determine their functional characteristics and their relationships with viruses and rickettsias.

SUMMARY

A single-layer culture was obtained from the heart of 11-12 day chick embryos under the action of a 0.5% solution of pancreatin.

Microscopy of these cultures (10 x 10) demonstrated the presence of two types of cells in the composition of the cellular stratum (fibroblastoid and epithelioid). Rhythmical contractions of groups were revealed in the areas containing epithelioid elements.

Employment of photomicrography has made it possible to study the development of the primarily pancreatized cells of the cardiac muscle.

LITERATURE CITED

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