EXPERIMENTAL BIOLOGY

SECONDARY DIFFERENTIATION OF EXPLANTED EMBRYONIC HEART MUSCLE

L. M. Grigoryev

From the Department of Pathological Anatomy (Director: L. M. Grigoryev), Velikie Luki Regional Hospital (Head Physician: A. K. Glushkov, Meritorious Physician of the Latvian SSR)

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We know of only one report on the secondary differentiation of explanted mammalian heart muscle, published in 1932 by Goss [2]. N. G. Khlopin [1], in his monograph "Biological and Experimental Principles of Histology," correctly pointed out that Goss's material represented persisting muscle fibers, and not secondary differentiation of explanted heart muscle. Goss published only one photomicrograph of a living, unstained culture of heart muscle, showing numerous differentiated muscle fibers, all oriented strictly in one direction, and firmly and uniformly bound to each other, filling the whole field. There can be no doubt that Goss was dealing with persisting differentiated muscle fibers, and not with secondary differentiation of elements of explanted heart muscle. It may thus be said that secondary differentiation of heart muscle outside the body has not yet been observed, and this may be ascribed largely to the difficult technique of culturing heart muscle.

The present paper presents the results obtained by us in the study of the process of secondary differentiation of explanted heart muscle.

EXPERIMENTAL METHODS

We used fragments of heart muscle of 10-day chick embryos, which were cultured in cells for 10-15 days. They were examined in the living condition, and after histologic treatment according to Bielschowsky and Bouquet.

Fig. 1. Differentiated heart muscle myoblast; 12-day culture of heart muscle of a 10-day chick embryo.

EXPERIMENTAL RESULTS

Myoblasts growing in tissue culture have served as experimental material for numerous researches on the most varied problems. The growth of myoblasts in tissue culture has been described in detail in many publications, for which reason we shall not enter into any detailed description of their undifferentiated extra-corporeal growth. As a general rule, the growth of embryonic heart tissue explants is characterized by proliferation of myoblasts, and only rarely by formation of symplasts, in which it is sometimes possible, with the greatest difficulty, to distinguish fibrils staining diffusely with silver by the Bielschowsky-Bouquet method. The myoblasts are little differentiated, whether after short or prolonged culture (10-15 days) outside the organism. This shows clearly that special conditions are required in order to achieve secondary differentiation of elements of embryonic cardiac muscle. We were successful, by prolonged culturing of heart muscle, in achieving secondary differentiation of cardiac myoblasts.

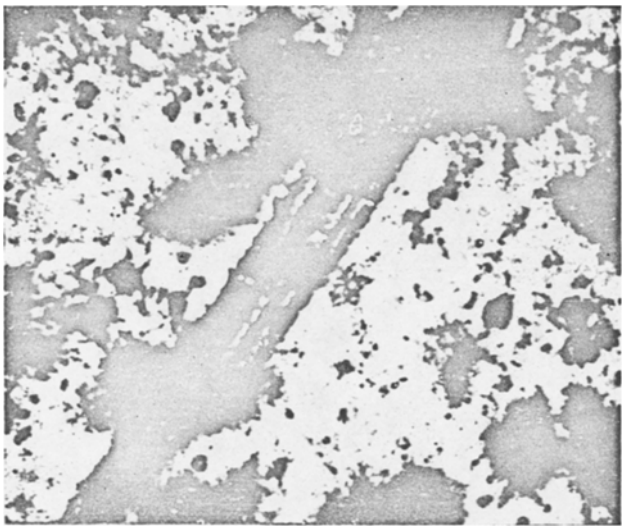

Fig. 2. Secondarily differentiated heart muscle fibers: 10-day culture of heart muscle of a 9-day chick embryo.

It will be seen from Figs. 1 and 2 that secondarily differentiated myoblasts are elongated bodies, sometimes fusiform; the nuclei are displaced towards one end of the cells. The myoblasts have a well-marked myofibrillar structure, with cross striations. These differentiated myoblasts are generally encountered in small numbers among the mass of proliferating undifferentiated cardiac myoblasts. This is evidence that some special conditions need to be satisfied in order to achieve differentiation of cardiac myoblasts: they appear only in very small regions of the myoblast culture, while the surrounding mass, including the myoblasts in direct contact with the differentiated ones, consists of undifferentiated cells of explanted myocardial tissue. All this shows that, as compared with achievement of secondary differentiation in tissue cultures of embryonic skeletal muscle, secondary differentiation of cardiac muscle explants remains a most complex and difficult process. Much painstaking and persevering work needs to be done in order to work out a method which would allow the ready initiation of the process of secondary differentiation of cardiac myoblasts.

SUMMARY

A 10-day chick embryo heart muscle was cultivated out of the organism. A secondary differentiation of myoblasts was obtained, which was characterized by development of myofibrillar structures with marked transverse striations.

LITERATURE CITED

[1] N. G. Khlopin, General Biological and Experimental Principles of Histology,* Moscow, 1946.

[2] C. M. Goss, Arch. exp. Zellforschung 12, 233-248 (1932).

* In Russian.