A CYTOLOGICAL ANALYSIS OF THE TESTICULAR TISSUE OF THE MONKEY GROWN IN VITRO AS A TRYPSINIZED MONOLAYER CULTURE

L. B. Tsypkin

Institute of Poliomyelitis and Virus Encephalitis (Director, Active Member AMN SSSR M. P. Chumakov) of the AMN SSSR, Moscow
(Presented by Active Member AMN SSSR M. P. Chumakov)
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Although much information has been published on the morphology of cells growing in monolayer cultures, the question of the derivation of these cells (from connective tissue or epithelium) has remained unanswered in most cases. It is not by accident, therefore, that many workers, when describing the elements of monolayer cultures, have preferred to use terms such as "epithelioid," "fibroblast-like," "round," "flattened," "fusiform," and so on.

The difficulty in the identification of cells in monolayer cultures seems to be due largely to the fact that trypsinization creates conditions specially favoring the growth of cells, in contradistinction to the conditions of the old methods of tissue culture. At the same time, it should be noted that most workers have usually confined their attention to the study of cells after only a single period of cultivation, and as a rule have used only general histological methods.

The present research was an attempt to make a cytological analysis of the testicular tissue of monkeys using monolayer trypsinized cultures, on the basis of prolonged observation of the growth of this tissue, employing various histological techniques.

EXPERIMENTAL METHOD

The original material consisted of the testicles of healthy monkeys (Macacus rhesus) aged from 1 to 1½ years. The monkeys were sacrificed by exsanguination. The testicles were removed aseptically, placed in Hanks's solution and, after removal of the tunicae, cut up finely with scissors. Trypsinization and cultivation were carried out in the usual manner. The minced tissue was trypsinized in a 0.25% solution of trypsin for 40-50 min at 37°. The resulting suspension was centrifuged and the solid cell residue was diluted with nutrient medium consisting of lactalbumin and ox serum (10%) in a proportion of 200,000 cells to 1 ml of nutrient medium. The cells were grown on narrow glass slides, placed in test tubes, for 25 days at 37° without reseeding. The nutrient medium was changed once in 5-7 days. The slides on which the cells were growing were taken daily from the test tubes and immersed in a 10% solution of formalin in 70% alcohol and in a 10% solution of neutral formalin. The fixed cultures were stained with hematoxylin-eosin, by Van Gieson's method, with Heidenhain's iron-hematoxylin, and with Weigert's hematoxylin, and impregnated with silver by Snesarev's method.

EXPERIMENTAL RESULTS

During the first few days of explantation the culture grew in the form of progressively enlarging islets, separated by loosely arranged "interislet" cells.

The "islet" cells, greatly in the majority at this stage, were polygonal or slightly rounded in shape, and frequently were sharply outlined when stained by Heidenhain's method. The nuclei of these cells were regular, and round or slightly oval in shape, translucent, vesicular, and usually containing a single nucleolus-like structure. From the character of these cells, there was no doubt that they were cells of the spermatogenic epithelium.

The "interislet" elements were seen at this stage in much smaller numbers than the epithelial cells, and consisted mainly of fusiform, elongated connective-tissue cells with chromatin-rich nuclei, and sometimes with fibrillary cytoplasmic processes branching from the poles of the cell.

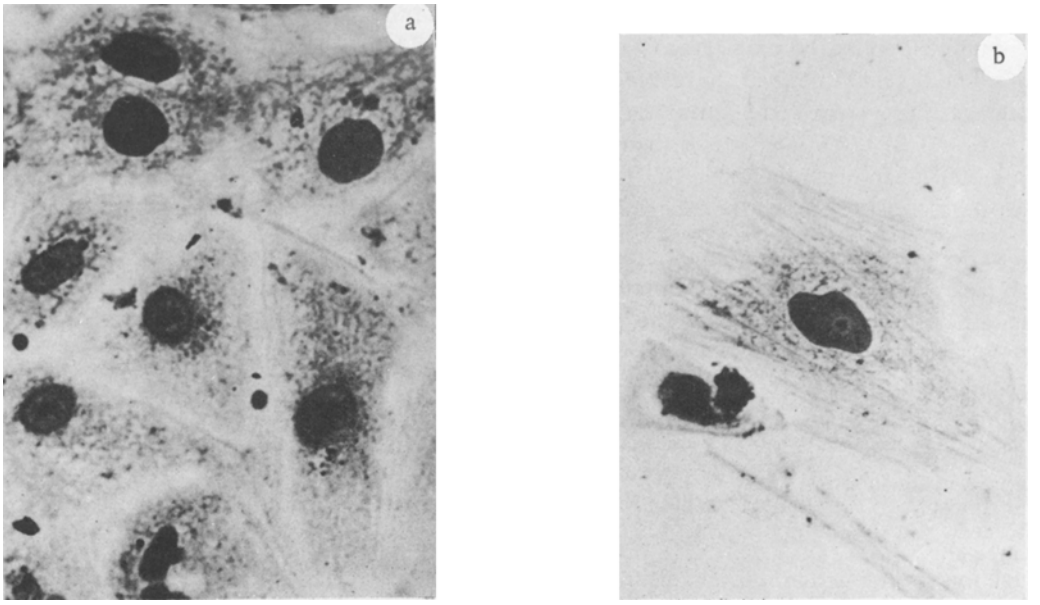

Fig. 1. Appearance of epithelioid cells in a culture of testicular tissue (on the 10th day). a) epithelioid cells. Stained by Van Gieson's method. Magnification 250; b) epithelioid cell containing thin fibrils in its ectoplasm. Stained by Heidenhain's method. Magnification 250.

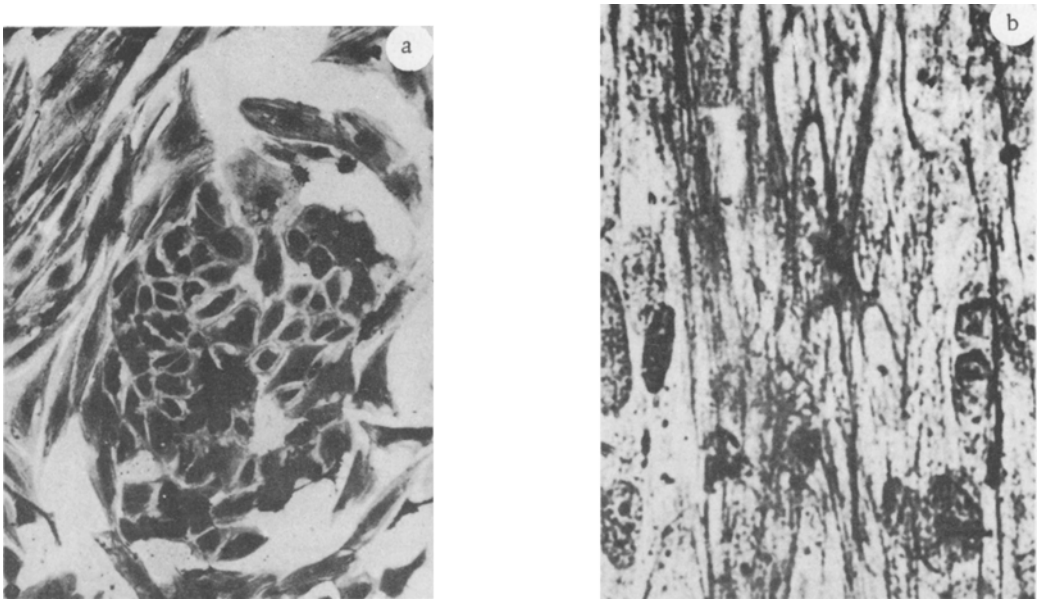

Fig. 2. Proliferating connective-tissue elements among which are groups of epithelial cells (on the 21st day). a) islet of degenerating epithelial cells, situated among elongated connective-tissue elements. Stained by Heidenhain's method. Magnification 70; b) extracellular argyrophilic fibers. Impregnation by Snesarev's method. Immersion.

As a rule the connective-tissue elements were loosely arranged, parallel to each other, and sometimes showed a tendency to form fascicular structures.

Subsequently there were no marked signs of proliferation of the epithelial cells and the size of the islets was stabilized. Meanwhile the connective-tissue elements proliferated energetically, and the cells already described were joined by others of a different type. These had a triangular or polygonal body of cytoplasm, and a translucent

nucleus; they were arranged in a mosaic, so that in sections stained with hematoxylin-eosin or by Van Gieson's method they closely resembled epithelial cells (Fig. 1, a). Sections stained by Heidenhain's method, however, showed that these epithelioid cells sometimes had uneven edges, and their cytoplasm was frequently divided into a compact, granular endoplasm and a clear ectoplasm. The latter contained very slender fibrils, which could also be seen after silver impregnation (Fig. 1, b). Sometimes these fibrils were apparently situated on the surface of the cell and spread over the territory of several neighboring cells, to form distinctive fibrillary bands. It should be emphasized that intermediate forms were present between the elongated and epithelioid cells, including flattened, rod-like cells.

During this period the number of epithelioid cells progressively increased, so that epithelial and epithelioid cells began to participate equally in the formation of the monolayer. At the periphery of the islets these cells frequently intermingled; they could be distinguished only in sections stained by Heidenhain's method or impregnated with silver.

The final stage of cultivation was characterized by further proliferation of the connective-tissue cells, and the small groups of epithelial cells situated among them underwent degeneration (Fig. 2, a). At this stage comparatively few epithelioid cells were seen. The connective-tissue cells were mainly flattened fusiform and rhomboidal cells, rich in fibers, with long oval nuclei containing a moderate amount of chromatin. Intermediate forms were observed between these cells and the epithelioid cells, long triangular cells being especially numerous. In this late period of cultivation, the connective-tissue cells everywhere showed fibril formation. Powerful bundles of argyrophilic fibers were situated not only within the cells, but also extracellularly, frequently forming a particularly dense network around the microexplants (Fig. 2, b).

This investigation showed that the testicular tissue of monkeys, when cultivated in standard conditions, produced both parenchyma and stroma in monolayer cultures. In this respect, our results agreed with those of other workers [3].

Changes took place in the connective-tissue elements of the testicles during cultivation: the elongated cells of the cambial or adventitial type were replaced by epithelioid cells, and these in turn gave way to elements of the fibrocyte type with intensive formation of fibers. This change in the cells is an expression of their differentiation, associated with the prolonged stay of the cells in culture without reseeding [4, 5].

From the point of view of the problem of the identification of cells in monolayer cultures, in our opinion the epithelioid cells are of the greatest interest. Some features of these cells, revealed when stained by Heidenhain's method or impregnated with silver (the presence of an endo- and ectoplasm, moderate fibril formation, the character of the markings of the cytoplasm), together with the intermediate position which they occupy in the process of conversion of cambial cells into fibrocytes, suggest that these elements are flattened fibroblasts. The ability of connective-tissue cells in vitro to become flattened and to become epithelioid in appearance is not, in principle, a new fact. Such a possibility was mentioned in the past by workers using old methods [6, 12]. In monolayer cultures the ability of connective-tissue cells to assume an epithelioid appearance has been demonstrated by the cultivation of the brain tissue of the monkey [7].

Many writers have found epithelioid cells in both primary monolayer cultures and transplanted cultures obtained from various parenchymatous organs and also from tumors [1, 2, 9, 10, 11, 13, 14, 17]. Because of the presence of such cells, often constituting the majority of the cells growing in culture, some workers also consider that epithelial cell cultures or epithelial lines are a possibility [2, 10, 17].

In lieu of our findings, we consider that in order to discover or clarify the nature of the epithelioid cells, they should be stained with Heidenhain's iron hematoxylin and impregnated with silver by Snesarev's method, and it is unfortunate that these methods have not been used by researchers working with monolayer cultures. To obtain reliable results, it is also desirable to lengthen the period of cultivation of the cells without reseeding as much as possible, in order to facilitate the differentiation of the connective tissue and the formation of fibrous structures.

The progressive growth of connective-tissue elements in culture, with the gradual supplanting and degeneration of the epithelial cells which we observed in every case, was of particular interest. Our findings did not support the conclusion [3] that the predominance of connective-tissue elements in testicular tissue culture is due to the advanced age of the animal: all the monkeys used in our experiments were sexually immature. The energetic proliferation of the connective-tissue cells in monolayer cultures is thus not always associated with the age factor [8, 16]. One investigation [8], in which a cell line obtained from the renal cortex of a new-born mouse was found to be connective-tissue in nature, was especially demonstrative in this respect: when the culture cells were implanted into mice, a sarcoma developed. In all probability the cause of the energetic proliferation of the stromal elements must be sought

in the intrinsic properties of the monolayer trypsinized culture, providing conditions in which the mesodermal elements can realize their high proliferative potential. It must be emphasized, here, that some writers [15] have suggested that the transplanted cell lines obtained from parenchymatous organs are connective-tissue in nature. This demonstrates yet again the desirability of using methods capable of revealing connective tissue for the study of monolayer cultures apparently composed of epithelial cells.

SUMMARY

The investigations show that in the monolayer trypsinized culture of the monkey testicular tissue there is growth of epithelial and connective tissue elements. At first there is a prevalence of epithelial, and later – of connective tissue elements which, proliferating, actively depress the epithelial growth. Connective tissue cells undergo changes and form fibrous structures during the cultivation; they transform into flattened fibroblasts which are difficult to differentiate from epithelial cells. True (connective tissue) origin of these epithelium-like cells may be authentically established only with the aid of Heidenhain's strain, as well as by silver impregnation after Snesarev. These methods are recommended for cellular identification in monolayer cultures.

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