AUTORADIOGRAPHIC STUDY OF CELL CULTURES

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KEY WORDS: autoradiography, cell cultures.

A wide range of reliable information about the morphology and function of cell cultures can be obtained by autoradiography. By the use of the radioactive precursor thymidine, the rate of cell proliferation can be determined. By electron-microscopic autoradiography with thymidine, the ultrastructural identification of proliferating cells and the most critical morphological study of their differentiation can be undertaken. If other radioactive precursors are used, many aspects of the metabolism of cells in culture can be studied. However, the advantages of autoradiography mentioned above are diminished by imperfections of the technique currently used to study cell cultures. For instance, for light-microscopic autoradiography, a layer of photographic emulsion is applied from above to the culture fixed in situ. Next, after exposure, development, and staining, the autoradiographic preparation obtained on the bottom of the dish is examined [1]. With this method it is impossible to study the same culture by electron-microscopic autoradiography. Another important disadvantage is that during work with stratified cultures, such as the epithelium of the skin, the photographic emulsion is applied to the top layer, consisting of differentiated cells which, in their metabolic behavior and, more especially, in their proliferative capacity, differ sharply from the lower cambial layer. Since only cells immediately beneath the layer of emulsion are involved in creation of the autoradiograph, i.e., the more highly differentiated cells, data on proliferation obtained by the use of such a method are worthless.

For electron-microscopic and electron-autoradiographic investigations the culture is fixed in situ, embedded in epoxide resin, and, from the dish-shaped block thus formed, fragments of plastic with the cell layer for ultramicrotomy are excised [2, 3]. The first disadvantage of this method is the impossibility of combining electron-microscopic and electron-autoradiographic investigations with the study of the entire cultural layer in the light microscope, or still less, by light-microscopic autoradiography. Another difficulty is that the area for ultramicrotomy is chosen randomly, and if the objects of interest to the investigator, such as thymidine-labeled cells, are rare in the culture, they will not be found in the majority of areas excised for ultramicrotomy. In other words, much of the complicated work, requiring a high degree of skill (ultramicrotomy of monolayers, preparation of electron-microscopic autoradiographs) is in vain. This paper describes a technique which is free from the above disadvantages.

In the proposed method the same culture, and even the same cells, are studied by both light-microscopic and electron-microscopic autoradiography, and accordingly, when the label is introduced, the doses used must be sufficient for preparation of an electron-microscopic autoradiograph (for example, 5-10 μCi/ml of ^{3}H-thymidine with incubation for 1-2 h). After incubation with the radioactive precursor, the medium is changed 3 times with an interval of 5 min between changes to wash out the unincorporated precursor. The culture is fixed with 1% glutaraldehyde solution in 0.1 M cacodylate buffer (pH 7.4) at 4°C for 12 h, then with 1% OsO_4 solution at 4°C for 2 h, after which the culture is dehydrated in ethanol solutions of increasing concentration. An Epon-Araldite mixture is used for embedding. Since plastic dishes are dissolved by propylene oxide or acetone, the epoxide resin can be saturated with a mixture of this resin with absolute alcohol. After polymerization of the Epon-Araldite mixture, the bottom of the plastic dish is separated from the plastic block thus formed. To do this, a circular incision is made in the side wall of the dish above the bottom, taken through the whole thickness of the wall as far as the block. To facilitate separation of the plastic bottom of the dish from the epoxide resin, the bottom is applied for a few seconds to a cold surface (the vaporizer of a refrigerator). After this the bottom can be separated from the lower surface of the epoxide block, catching on the edge of the incision with the knife. Type "M" photographic emulsion is poured on to the surface of the expoxide block exposed after separation of the bottom of the dish, after which the liquid emulsion is allowed to spread evenly over the surface,

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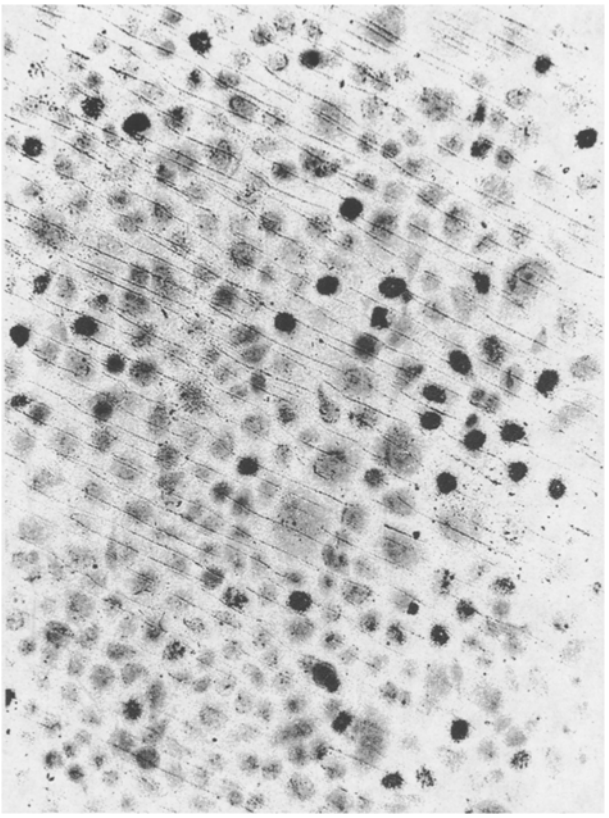

Fig. 1. Culture of epidermocytes incubated with ^{3}H-thymidine. Unstained cell layer embedded in epoxide block and converted into autoradiographic preparation. Single epidermocytes clearly visible. Labeled cells more clearly seen than in stained preparations. 200×.

the layer of emulsion is dried, and exposed for the required time in a refrigerator or at room temperature (when working with the above-mentioned dose of ^{3}H-thymidine the blocks are exposed for 2-4 days). Development and fixation of the blocks are undertaken just as with autoradiographs on slides.

On the completion of these manipulations an autoradiographic preparation is obtained on an epoxide block (Fig. 1). It is transparent and suitable for work with any objective of a light microscope. Cells, intracellular structures, and grains of metallic silver, constituting the label, are clearly distinguishable (Fig. 2). As a rule, in the course of an autoradiographic investigation it is not entirely necessary to use any staining method, but if necessary, the cells can also be stained with, for example, hematoxylin after fixation or before or in the course of dehydration.

The proposed method of preparing autoradiographs from an epoxide block significantly broadens the range of information which can be obtained by autoradiography on account of the new opportunities provided by it, as detailed below.

1. Studying the same cell culture and the same cells by methods of light-microscopic and electron-microscopic autoradiography increases the accuracy of the results.

2. Autoradiographic analysis of the cells of the lower layer characterizes the proliferative activity of the culture reliably.

3. Selection of the target area for ultramicrotomy. By examining the autoradiograph of the block it is possible to choose the required area, for example, a cell labeled with thymidine. In such an area, under low power of the microscope the block is trimmed to a point, after which a pyramid is cut out. This method eliminates mistakes and much unproductive work.

4. The method provides a unique opportunity of obtaining an electron-microscopic autoradiograph without the need for corresponding methods of application of the emulsion, long exposure times, or other complications of the technique of electron-microscopic autoradiography.

In the proposed method these manipulations are essential only for work with horizontal sections through the culture. Vertical electron-microscopic sections for autoradiographs can be obtained immediately by ultramicrotomy of the primary autoradiographic preparation on the block without the need for any additional photographic processing. Furthermore, unlike an

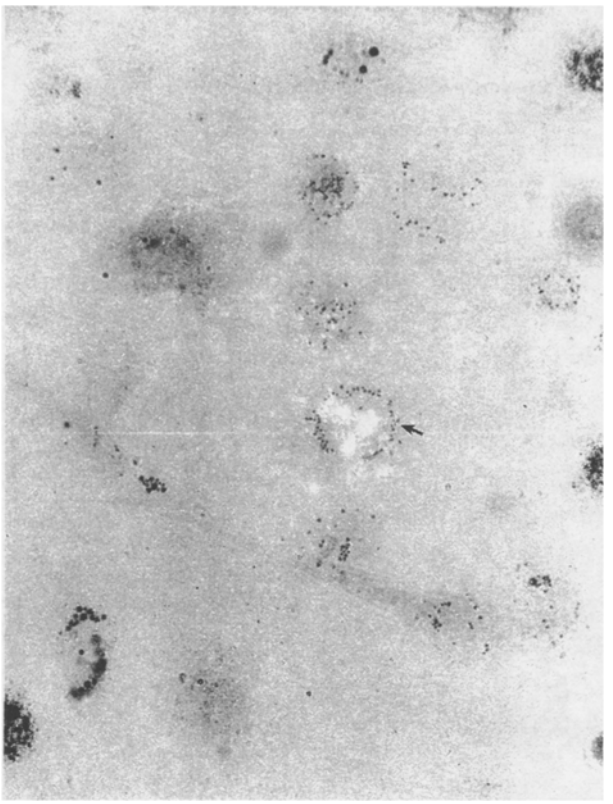

Fig. 2. Autoradiograph of unstained culture of epidermocytes. Cell labeled with ^{3}H-thymidine (arrow) photographed in reflected light, in which grains of silver appear as luminous points. Predominant site of label in nucleoli. 1000×.

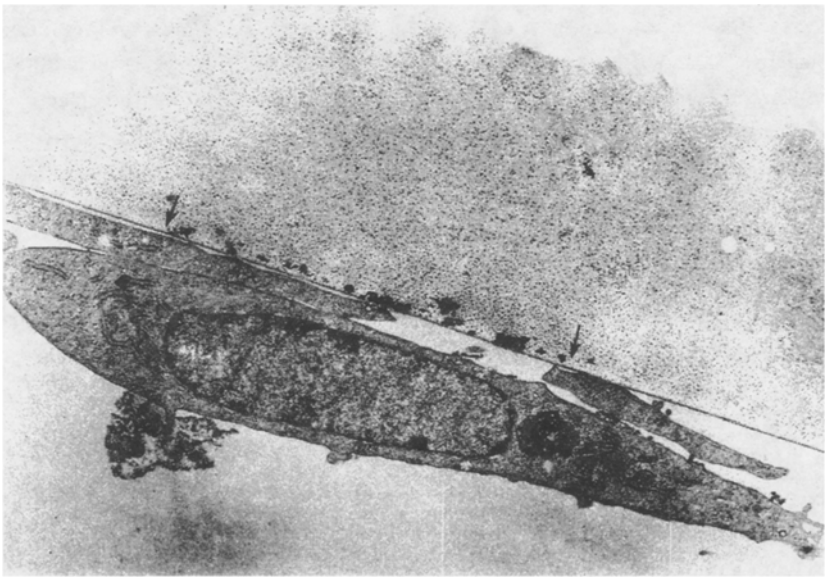

Fig. 3. Ultrathin section of autoradiograph prepared by the method described. Location of silver grains (arrow) indicates precisely the labeled nucleus. Layer of emulsion, at the side of the cell, does not reduce contrast of its image. 10,000×.

ordinary electron-microscopic autoradiograph, which is examined through the layer of emulsion, thus reducing the contrast of the image, the electron-microscopic autoradiograph obtained by the proposed method is not inferior in image quality to ordinary electron-micrographs.

The grains of silver in such an autoradiograph (Fig. 3) are distributed, not above the cell but at its side, but nevertheless this localization of the grains only at the side of the nucleus of the cell under examination and their absence elsewhere in the preparation are absolute evidence of DNA synthesis in the particular cell. This important indicator of cell function is reinforced by its characteristic ultrastructural morphology, as revealed by a thin electron-microscopic section, whose contrast is not reduced by the layer of emulsion as is the case with the ordinary electron-autoradiograph.

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ULTRASTRUCTURAL CHANGES IN AUTONOMIC NERVE GANGLIA IN EXPERIMENTAL BURN TRAUMA

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KEY WORDS: burn trauma, experiment, nerve ganglia, ultrastructure.

Definite difficulties arise in the evaluation of data obtained by the study of cadaveric material, especially if electron-microscopic and histochemical methods of investigation are used. These difficulties are associated primarily with postmortem changes, which may complicate the interpretation of the structural disturbances observed. Nerve cells of the vein are most sensitive to postmortem changes, and for that reason, it is recommended that material for electron-microscopic histochemical studies be collected soon after death [3, 8]. Investigations by a number of workers has shown that this period can be considerably increased [1, 2, 4-7, 9, 10]. However, no special investigations have been undertaken into the possibility of studying the state of the neuronal ultrastructure at different times after death.

In order to undertake a comparative ultrastructural analysis of intravital changes in neurons of autonomic ganglia in patients dying at different stages of burn trauma, with postmortem cadaveric changes in these ganglia, we made a special study of neural structures 4-6 h after death of animals in the control group, and also at various times after experimental burn trauma.

EXPERIMENTAL METHOD

Experiments were carried out on 15 noninbred male albino rats aged 5-7 months and weighing 230-280 g. The animals were fixed on a special table, anesthetized with ether and oxygen, and the hair cover was removed in the spinal region. Burn trauma was inflicted by applying a metal plate measuring 4 × 9 cm, heated to 80-100°C, to the depilated skin. The exposure was 5-6 sec, and as a result, a burn of the IIIa, b degree was produced on an area of 20-25% of the rats' body surface. The animals were decapitated 3, 7, and 11 days after thermal trauma, corresponding to the principal periods of burn healing. Material was taken from five control animals immediately after decapitation and 4-6 h after death. The superior cervical and stellate ganglia

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