METHOD OF TOTAL STAINING OF MONOLAYER CELL CULTURES WITH VANADIUM HEMATOXYLIN

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Besides existing methods of selective and specific histologic, histochemical, and immunocytochemical staining of cells in culture, methods of total staining also are needed for quantitative and qualitative estimation of monolayer cell cultures, capable of simultaneously revealing all the cells of the monolayer with a sufficiently high degree of cytologic reliability. Our experience has shown that among the widespread and readily available dyes, certain rapidly oxidized forms of hematoxylin may be the most suitable for these purposes. We know that within the group of hematoxylin dyes staining properties are possessed by hematein, which is formed during prolonged maturation, for in fact during slow oxidation of hematoxylin [2, 3, 7]. The introduction of salts of metals (iron, chromium, aluminum, etc.) into solutions of hematoxylin dyes, forming compounds called lakes with hematein, enhances their staining ability. The overwhelming majority of hematoxylin dyes belong to the selective class, i.e., those which enable definite cellular structures such as, for example, the cell nuclei (alum and iron hematoxylins), myelinated fibers (Weigert's iron and its many modifications suggested by Kulchitsky, Pal, etc.), to be stained [3, 4].

The process of oxidation of hematoxylin and of hematein formation can be considerable accelerated by adding vigorous oxidizing agents (potassium iodide, postassium permanganate by adding vigorous oxidizing agents (potassium iodide, potassium permanganate, hydrogen peroxide, etc.) to a solution of the dye, and this is the essence of the numerous quickly maturing hematoxylin dyes [2, 3]. In the suggested staining method, ammonium metavanadate (NH_4VO_3) was used as the oxidizing agent. We know that the compounds of pentavalent vanadium, in weakly acid solutions, are vigorous oxidizing agents, in which the oxidation process is unaccompanied by side reactions or by the formation of any intermediate products [5], so that it can be effectively used in histologic practice. Histological staining with solutions of vanadium hematoxylin was first suggested by Heidenhain [3, 6, 7], but this method did not find practical application because of difficulty in preparing and using the staining solution. The solution of vanadium hematoxylin and the method of staining which we suggest is free from these drawbacks.

PREPARATION OF THE DYE AND METHOD OF STAINING

Stock solutions: 10% solution of hematoxylin in 96° ethyl alcohol, 0.5% solution of ammonium metavanadate (a weighed sample is dissolved in distilled water on heating to boiling; the solution is then cooled), and glacial acetic acid. All the stock components of the dye can be kept indefinitely.

Composition of the dye: distilled water 98 ml, 10% alcoholic solution of hematoxylin 1-2 ml, 0.5% solution of ammonium vanadate 1 ml, glacial acetic acid 1 or 2 drops. The solution of the dye, on the addition of ammonium metavanadate, turns a dark blue-violet color, which indicates its readiness for use. The solution is not stable and is suitable for only a few hours. When its color changes to dark brown or to dark cherry red, the dye is no longer suitable.

For staining with vanadium hematoxylin, it is best to have cultures grown on coverslips, covered with one of the polyamino-acid substrates (polylysine, polyornithine), polyethylenimine, or dried collagen. Collagen gel, condensed with ammonia, stains intensively with hematoxylin, and this makes it difficult to study cells grown on it. Good results are obtained by staining a cell monolayer grown on the surface of plastic dishes and flasks. In this case, preparation of permanent histologic preparations is difficult, although microscopic study of stained cultures in an aqueous or glycerol medium is possible.

1. The cultures are washed once with balanced salt solution (Hanks', Earle's, Simms', etc.).

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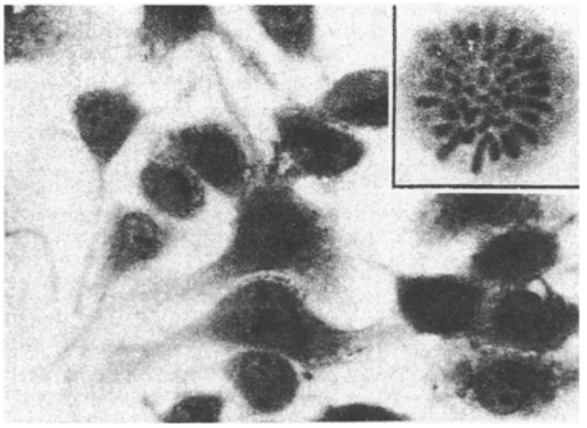

Fig. 1. Cells of monolayer culture of line Neuro-2a. 125×. Inset: mitosis in culture of NGUK cells. 250×. Here and in Fig. 2, staining with vanadium hematoxylin.

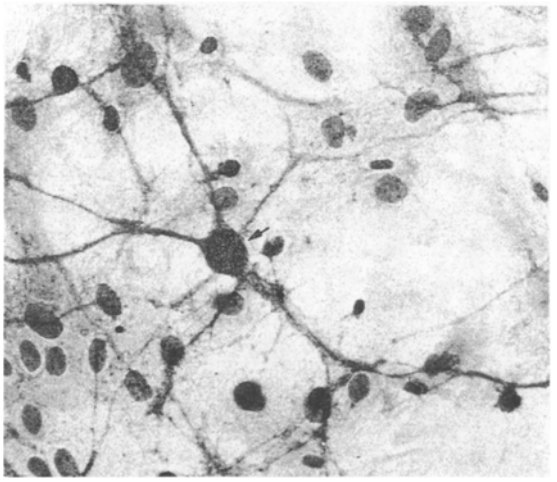

Fig. 2. Monolayer culture of dissociated cerebral cortex cells of a 17-day rat embryo. 24 days of culturing. A large neuron (arrow), lying on a monolayer of flattened glial and ependymal cells, is readily visible. Mag. 62.5×.

2. The cultures are fixed for 2-5 min in a fixing mixture consisting of 96 degrees alcohol (seven parts), undiluted formalin two parts), and glacial acetic acid (one part). It is possible to use other cytologic fixatives (10% neutral formalin for 10-15 min, Carnoy's mixture for 2-5 min, etc.). Carnoy's mixture can be used only to fix cultures grown on coverslips because this fixative, which contains chloroform, dissolves plastic (polystyrene) culture vessels.

3. The cultures are washed consecutively with 70 degrees alcohol and distilled water.

4. They are stained for 5-15 min with a solution of vanadium hematoxylin. The staining process can be monitored under the microscope.

5. The stained cultures are washed with distilled water.

6. They are dehydrated in alcohols of increasing concentration (70, 96, and 100 degrees), clarified in xylol, and mounted in Canada balsam.

RESULTS OF STAINING

The cells of the monolayer (including in the zone of growth of tissue explants) stained blue for bluish-violet. The density of staining depends on the hematoxylin concentration in the dye and the staining time. The staining of the cells is very firm: the dye is not eluted either by water or by alcohols.

As the photomicrographs (Figs. 1 and 2) show, staining with vanadium hematoxylin is total in character: all cells of the monolayer stain entirely, but this does not prevent differential, although unselected demonstration of nuclei and of intranuclear and cytoplasmic structures of cells. The dye also stains cell glomerations and aggregates, but the study of single cells in these cases may be difficult. Although the mechanisms of staining were not analyzed, in my opinion, the dye binds with proteins, glycoproteins, and ribonucleoproteins, and it is this which determines the total character of staining of the cells. Advantages of the suggested method of staining monolayer cell cultures are its simplicity, its universality, the possibility of obtaining qualitative cytologic preparations in a short time (20-30 min), its accessibility, low cost, and low consumption of reagents. This method can also be used to counterstain cells in culture during autoradiographic and certain histochemical and immunocytochemical investigations.

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LITERATURE CITED

1. I. V. Viktorov, Arkh. Anat., No. 12, 84 (1974).
2. R. Little, Histopathological Techniques and Practical Histochemistry [Russian translation], Moscow (1968).
3. B. Romeis, Microscopic Techniques [Russian translation], Moscow (1953).
4. G. I. Roskin and L. B. Levinson, Microscopic Techniques [in Russian], Moscow (1957).
5. V. S. Syrokomskii and Yu. V. Klimenko, Vanadatometry [in Russian], Sverdlovsk–Moscow (1950).
6. M. Heidenhain, Z. wiss. Mikrosk., **25**, 401 (1987).
7. R. Krause, Enzyklopädie der mikroskopischen Technik, Vol. 2, Berlin (1926).