METHOD OF OBTAINING CHROMOSOME PREPARATIONS FROM EPITHELIAL CELLS OF THE RAT INTESTINE

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A method of obtaining chromosome preparations from epithelial cells of the rat intestine is described. The distinguishing feature of the method is that the epithelial cells are isolated after placing the intestine in toto in hypotonic solution and then fixing it in methanol and glacial acetic acid. The method has also proved suitable for obtaining preparations of metaphase plates from intestinal tumors.

KEY WORDS: intestinal epithelial cells; chromosome preparations.

No direct method of obtaining dried chromosome preparations from the epithelial cells of the intestine for cytogenetic analysis has yet been developed. The main difficulty in the way of preparing metaphase plates by the usual method [2] is that the cells form clumps because of the mucoproteins which they contain. When acetic acid, a component of the fixative used in cytogenetic investigations, is added it forms an insoluble complex with the mucoproteins, making it impossible to obtain a suspension of isolated cells. Attempts to remove the mucous by repeatedly washing the cells in physiological saline or with the aid of glycolytic and proteolytic enzymes (hyaluronidase, trypsin, pronase) or by changing the composition of the fixative did not yield the desired results. The number of metaphase plates prepared by the squash technique [2] did not prove satisfactory because the chromosomes in such preparations were not clearly outlined, evidently because of poor fixation, and this made the subsequent cytogenetic analysis more difficult.

The method of obtaining dried preparations as usually used in cytogenetic research was accordingly modified. The main feature of this modification is that the epithelial cells were isolated after immersing the intestine in toto in hypotonic solution and then fixing it in methanol and glacial acetic acid.

Colchicine was injected intraperitoneally into the rat in a dose of 0.1 mm/100 g body weight. The rat was decapitated 2.5 h later and a piece of the large intestine 3-5 cm long was removed. The intestine was opened by a longitudinal incision and stretched out and fixed on a wax slab placed in a Petri dish, into which hypotonic solution (50 ml of 0.25% aqueous solution of KCl), heated to 37°C, was poured. The hypotonic solution was poured off after 10 min and replaced by 8 ml of freshly prepared methanol–glacial acetic acid (3:1) fixative. After fixation for 5 min the cells were vigorously scraped off with a stiff brush and the suspension was poured into a conical tube, where it was allowed to stand for 5 min.

Histological study of the intestinal preparations after these manipulations showed that the epithelial cells were completely removed and the underlying layers of the intestinal wall remained intact.

The resulting cell suspension was centrifuged at 1000 rpm for 4 min, the supernatant was poured off, and the residue was resuspended in 8 ml fresh fixative. The suspension was again centrifuged for 8-10 sec to throw down the large clumps of cells. The supernatant was decanted into a clean tube and centrifuged after 10 min at 1000 rpm for 3 min. The residue was resuspended, 4 ml of fresh fixative of the same composition was added, and after 10 min the sample was again centrifuged under the same conditions; the cen-

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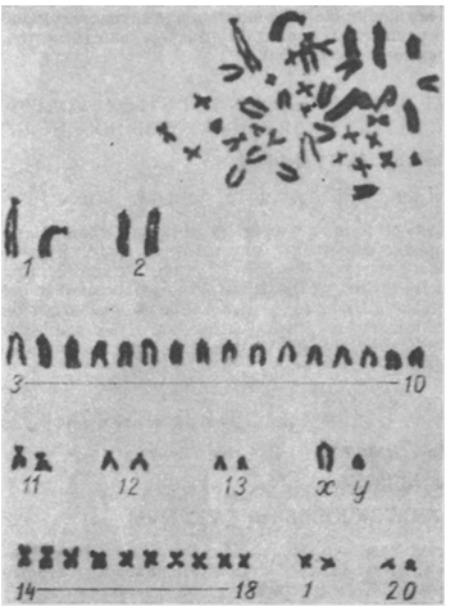

Fig. 1. Metaphase plate obtained from epithelial cell of male rat bred at the Rapolovo Nursery, Academy of Medical Sciences of the USSR (karyotyped in accordance with Dyban and Udalova's classification [1]).

trifugation was repeated once more 10 min after a change of fixative. The residue was resuspended and fresh fixative added in order to obtain moderate turbidity of the suspension. A drop of the suspension was then applied to a series of slides cooled in distilled water, and then flamed. The specimens were stained with azure-eosin.

By this method metaphase plates suitable for cytogenetic analysis have been obtained from unchanged intestinal epithelium (Fig. 1). The method has also been used to study chromosomes in intestinal tumors induced in rats by 1,2-dimethylhydrazine.

LITERATURE CITED

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