RABBITS' CORNEAL CELLS STUDIED IN TISSUE CULTURES

II. ENZYME REACTIONS

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Received August 3, 1965

Summary. Enzymatic reactions of corneal epithelial cells are, by and large, stronger than reactions of corneal fibroblasts. The cytoplasm of corneal epithelial cells and fibroblasts has stronger enzymatic activity than the nuclei of the two types of cells. The nuclei of epithelial cells react stronger than the nuclei of fibroblasts. Certain enzymes have a fairly characteristic cytoplasmic distribution in corneal epithelial cells but not in fibroblasts. The intensity of some enzyme reactions changes with aging of the cells.

Introduction

Having studied (part I) the basic aspects of rabbits' corneal cells in vitro (such as life span, morphology, and reproduction) we turned our attention to the enzymatic activities of corneal epithelial cells and fibroblasts.

Materials and Methods

Reference is made to the pertinent paragraph in the first part (I) of our report on rabbits' corneal cells studied in tissue cultures. Except for the cells pictured in Fig. 1, which were two weeks old, the photomicrographs presented here were from three weeks old cultures. The special staining methods used to demonstrate nine different enzyme reactions were as follows:

1) Acid Phosphatase. The lead phosphate method of Gomori (1950) was used, with neutral buffered formalin fixation for 10 minutes at 4°C, with a 3 hour incubation period at 37°C, and with sodium beta-glycerophosphate as the substrate.

2) Alkaline Phosphatase. The calcium-cobalt method of Gomori (1951) was applied after fixation in acetone for 5 minutes at 4°C. The incubation lasted for 3 hours at 37°C.

3) Adenosine Triphosphatase. The technique of Naidoo and Pratt (1951) was employed. No fixation was used. Incubation was for 90 minutes at a pH of 6.5 and at a temperature of 37°C.

4) Lipase. This enzyme was studied by the Tween 80 method of Gomori (1949). The slides were fixed in cold formalin and incubated over night at 37°C.

5) Monoamine Oxidase. The method of Gellner et al. (1957) was employed. Slides were not fixed. Incubation lasted 4 hours at 37°C.

6) Lactic Dehydrogenase. The method used was that of Nachlas et al. (1960). Acetone was used as a fixative for 5 minutes at 4°C. Incubation was for 4 hours at 37°C.

7) Malic Dehydrogenase. The technique developed by Nachlas et al. (1960) for lactic dehydrogenase was adapted, with DL-malic acid substituted for lactic acid.

8) Succinic Dehydrogenase. Ogawa and Okamoto (1961) described a method based on 1 hour incubation at 37°C in a mixture of nitro-pectenazolium chloride, phenazine methosulfate, and sodium succinate in a phosphate buffer at pH 7.6.

9) Cytochrome Oxidase. The technique of Buestone (1960) was used to demonstrate this enzyme. The substrate consisted of a mixture of p-amino-diphenyldalanine and 3-amino-9-ethylcarbazole. Cytochrome C was added to enhance the reaction. The reaction product was chelated with cobalt.
Observations

The results of enzyme stains of the two types of cells recovered regularly from cultures of corneal explants were as follows:

1) Acid Phosphatase. Brown to black lead sulfide precipitate covered the entire cytoplasm of epithelial cells, except for a narrow, lucid perinuclear zone. A portion of the nucleus close to the nuclear membrane also stained dark. Fibroblasts reacted weakly and only their nucleoli stained dark; even this reaction decreased with aging of the cells.