PREPARATION OF PARAFFIN SECTIONS FOR IMMUNOFLUORESCENCE ANALYSIS OF ANTIGENS DIFFERING IN THEIR CHEMICAL NATURE

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A technique is suggested for preparing paraffin sections from tissues fixed with acetone which can be used for the immunohistochemical detection of antigens which differ in their chemical nature, including α-fetoprotein, antigens of mouse leukemia viruses, alcohol-soluble antigens of hepatocyte membranes, and certain phospholipids.

KEY WORDS: *Immunofluorescence; localization of antigens.*

A decisive factor in the immunofluorescence study of antigens is the correct choice of the method of fixation and processing of the material.

For a long time investigations of this sort were carried out on sections obtained in a cryostat from unfixed tissue and subsequently fixed with acetone or alcohol [8]. A method that is increasingly finding favor nowadays consists of the preparation of paraffin sections with fixation, dehydration, and clearing of small blocks of tissue in the cold followed by rapid soaking of the blocks with petrolatum at 52-56°C [10].

The advantages of the paraffin method over the frozen section method are well known. Material embedded in paraffin can be preserved much longer, serial sections necessary for the study of the localization of several antigens in the same cells can more easily be obtained with it, and the paraffin method is technically more readily available. However, the spectrum of antigens detectable in paraffin sections is substantially narrower than in frozen sections.

The object of this investigation was to select a technique for fixation and embedding in paraffin whereby antigens with different physicochemical characteristics, including α-fetoprotein (α-FP), a water-soluble protein readily precipitated by alcohol, and alcohol-soluble antigens of the cell membranes of hepatocytes, can be detected in neighboring serial sections.

Antigens. α-FP was detected in sections of the liver regenerating after CCl_4 poisoning in SWR mice [1], and bile capillary antigen (BCA) and cardiolipin were detected in liver sections from healthy SWR mice [4, 7]. Group-specific antigen (GSA) of mouse leukemia viruses and type-specific antigen of Gross leukemia virus (TSA) were detected in sections through the spleen of healthy AKR mice [2, 9]. Antigens were detected by the indirect immunofluorescence method [11]. The sections were treated with immune antisera in the usual way [5].

Antisera. Monospecific antibodies against α-FP were obtained from the corresponding antiserum on glutaraldehyde immunosorbent [6]. Monospecific antibodies against GSA [9] and BCA [4] were obtained in a similar way. Antibodies against TSA were obtained by decomposition of the specific precipitate [2], and monospecific antibodies against cardiolipin were obtained from antisera against lipid fractions of hepatocyte membranes on cholesterol–lecithin immunosorbent [7]. Luminescent donkey antisera against rabbit γ-globulin produced by

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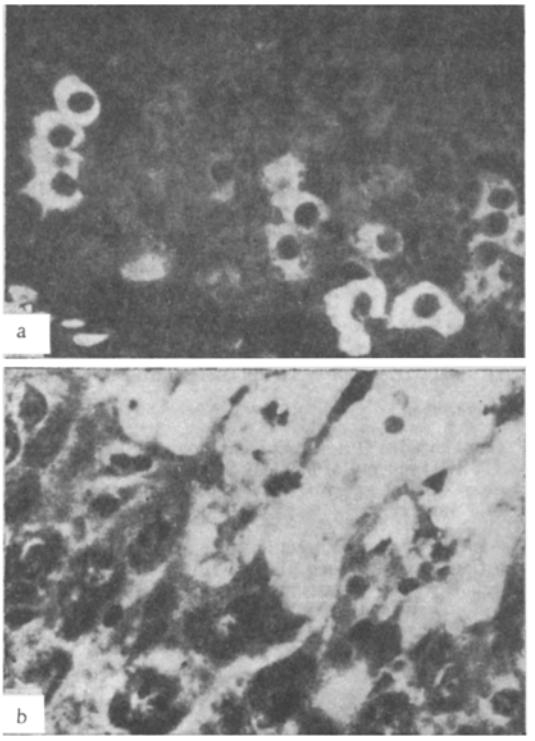

Fig. 1

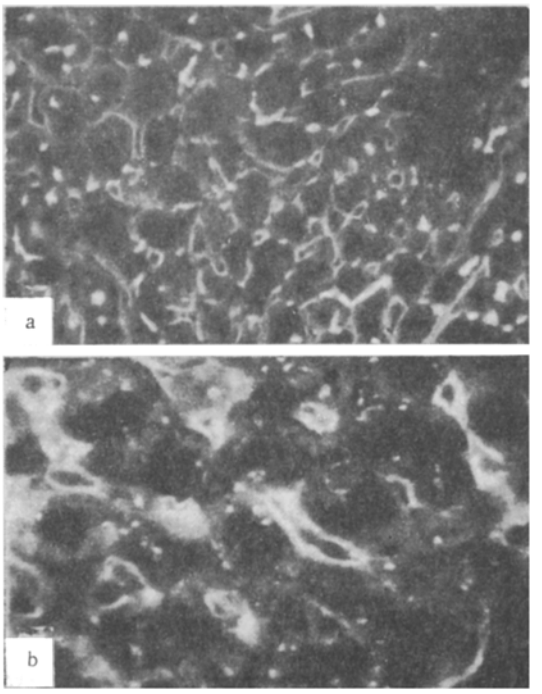

Fig. 2

Fig. 1. Liver of SWR mouse regenerating after CCl_4 poisoning: a) fluorescence of α-FP in individual hepatocytes; 66 h after poisoning (objective 40×, ocular 3×); b) morphology of regenerating liver, hepatocytes in region of necrosis; 48 h after poisoning (Toluidine Blue, objective 60×, ocular 10×).

Fig. 2. Antigens in liver of healthy SWR mice: a) BCA (objective 40×, ocular 3×); b) cardiolipin (objective 70×, Homal ocular 3×).

the N. F. Gamaleya Institue of Epidemiology and Microbiology (batches Nos. 184, 272, and 903) were used.

Technique of Fixation and Embedding. Acetone was chosen as the fixative and the degree of its dehydration was important. The paraffin solvents usually used, namely benzene, toluene, xylene, and chloroform, damaged certain antigens, especially α-FP. It was decided to use petroleum ether, which preserved the activity of all antigens examined.

Pieces of the test organs 3-5 mm thick were fixed in anhydrous acetone (analytically pure acetone to which culinary gelatin was added in the proportion of 100 g per liter) at 0°C for 18-24 h in 20-50 volumes of fixative. The material was then transferred to the same volume of petroleum ether for 40-60 min at room temperature. It was soaked with a mixture of petrolatum and wax at 52°C for 40-60 min, after which it was embedded in paraffin wax. The paraffin blocks were kept at 4°C.

Preparation of Sections. Series of sections 3-5 μ thick were cut on a Reichert rotating microtome. The paraffin strips can be kept in a refrigerator for several months without loss of activity. Sections from material fixed in acetone are extremely brittle when the wax is melted. Melting must therefore be carried out with special care, best of all after the paraffin strips have been cooled in a refrigerator in distilled water at a temperature not higher than 20-23°C. For dewaxing the slides are placed in petroleum ether for 1-2 min, in acetone for 1-2 min, and in physiological saline buffered to pH 7.2-7.4.

Localization of Antigens in Sections. The localization of α-FP in the cytoplasm of individual hepatocytes in sections of the regenerating liver after fixation with acetone agreed completely with its localization after fixation with ethanol and acetic acid [6]. The background luminescence of the tissue was a little higher, but the brightness of the specific luminescence was sharply increased (Fig. 1a). In quality the acetone sections were a little inferior to alcohol, but they were perfectly suitable for histological analysis (Fig. 1b).

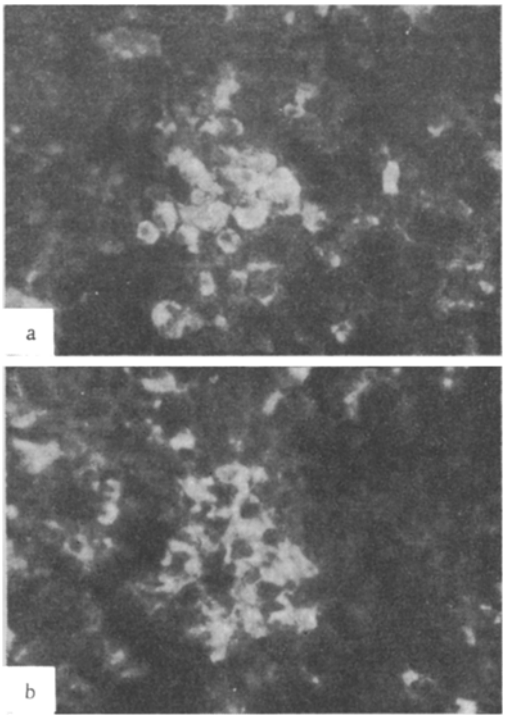

Fig. 3. Antigens of Gross leukemia virus in spleen of AKR mice in the same group of cells: a) GSA, b) TSA. Objective 40×, Homal ocular 3×.

The remaining antigens studied had previously been investigated only in frozen sections fixed with acetone. The results now obtained agreed completely with existing data and the brightness of luminescence of the antigens in the paraffin sections was not less and sometimes was even greater than in frozen sections.

BCA was localized on the outer membrane of the hepatocytes. The brightest luminescence was observed in the bile capillaries (Fig. 2a). Cardiolipin was mainly concentrated near the hepatic sinuses. It was also found as separate granules in the cytoplasm of the hepatocytes (Fig. 2b).

GSA and TSA were found in the red and white pulp of the spleen of AKR mice. The intensity of their luminescence varied in different cells. Cells containing large quantities of GSA also gave the brightest luminescence for TSA (Fig. 3).

Fixation of histological material with acetone followed by treatment with petroleum ether is thus evidently the gentlest method of processing as regards preserving the activity of most protein and some phospholipid antigens. This method has also proved suitable for preserving phosphamidase activity in sections [3].

The suggested method provides great opportunities for the study of localization of antigens of different chemical nature in the same cells.

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DESIGN OF A BATH FOR PERFUSING ISOLATED LYMPH VESSELS

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The design of a bath for perfusing the isolated thoracid duct is described. The method enables changes in the lumen of the duct resulting from the action of various agents *in vitro* to be assessed.

KEY WORDS: *Isolated thoracic duct; perfusion.*

Perfusion of isolated lymph vessels can conveniently be carried out in a bath of the suggested design (Fig. 1). The bath is made of transparent plastic. It measures 30×8×4 cm and the capacity of its central part is 150 ml.

The inlet cannula (1) is inserted into the thoracic duct (TD) below the arch of the aorta and the outlet cannula (2) into the mouth of the duct in the neck. The TD is carefully isolated from the surrounding tissues and placed in the bath.

The cannulas are connected by short pieces of rubber tube (3) to glass tubes (4, 5), on which are fitted rubber sleeves (6) which fit snugly in grooves on the walls of the bath. These sleeves prevent the physiological saline from spilling when the bath is filled and they serve to arrange the glass tubes (4, 5) at a small angle to the floor of the bath so that the TD is immersed in the physiological saline. The tubes can be moved along their long axis in order to apply known tension to the duct, which can be verified by the length of a ligature which is equal to the length of the segment of TD *in situ*.

The bath is filled with Tyrode solution through tube (7) and the solution is drained through tube (8). To maintain a constant temperature, a polyethylene tube is provided on the floor of the bath and its ends protrude outside the bath (9, 10). Warm (37°C) water circulates through this tube.

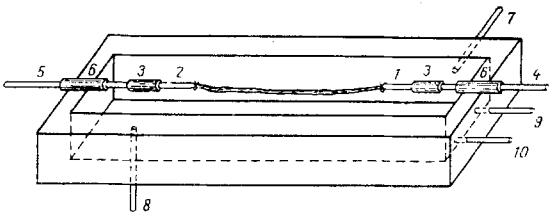

Fig. 1. Bath for perfusing isolated lymph vessels (explanation in text).

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