also noted that satisfactory results were obtained by irradiating preparations with ultraviolet light while in acridine orange solution. The Na_2HPO_4 solution also can be replaced by isotonic sodium chloride solution. However, better contrast between staining of the sister chromatids and better quality of staining were obtained by the method suggested above. Instead of acridine orange it is possible to use an aqueous solution of Hoechst 33258 (1 μg/ml) with the same effect. If barium hydroxide treatment was prolonged or if the temperature of the solution rose to 37°C, both chromatids were palely stained and the phenomenon of differential staining did not occur.

If 5-bromodeoxyuridine was added to a culture of Chinese hamster cells under the conditions described above an effect of differential staining of the sister chromatids was observed in 95-98% of cells, and if added to a culture of human lymphocytes it was observed in 75-90% of cells depending on the individual. These variations can evidently be attributed to differences in the duration of the cell cycle in different individuals and also with the more rapid asynchronization of the human lymphocyte culture than the culture of Chinese hamster cells.

The level of sister chromatid exchanges in Chinese hamster cells was determined in the course of the experiments. On analysis of 500 cells the mean number of sister exchanges per cell was 6.004 with a dispersion of 7.376 and a 95% confidence interval of the mean from 4.768 to 7.557 exchanges per cell. The number of exchanges per cell varied from 0 to 15. The level of sister chromatid exchanges in human lymphocytes was determined after different methods of fixation and with the use of cultures from the same donor. In each case 50 metaphases were analyzed. With fixation at 72 h the mean number of exchanges per cell was 7.00, and after 96 h it was 6.92, or virtually the same. Later fixation can thus be used in order to obtain a larger number of mitoses in cultures of human lymphocytes, for the time of fixation does not affect the number of sister chromatid exchanges.

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METHOD OF COMBINED HISTOLOGICAL STAINING OF THE LIVER WITH ALCIAN BLUE AND CARBOL FUCHSIN

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A modification of Novelli's combined histological staining method whereby the functional state of hepatocytes can be determined is suggested.

KEY WORDS: liver; hepatocytes; alcian blue.

The differentiation of functional states of cells during the study of native and fixed tissues continues to attract the attention of experimental workers and pathomorphologists. There are indications in the literature of differences in the functional state of cells and the lability of cellular structures in the organs under normal and pathological conditions [1, 5, 6].

Novelli [7] suggested a method of double staining – with alcian blue and carbol fuchsin – of different organs of experimental animals, including the liver. Novelli concluded that this method can be used to differentiate cells on the basis of their functional state. Parallel experiments on isolated nuclei and with staining by the

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Brachet and Feulgen methods (Novelli [7]) have shown that this method reveals the total DNA and RNA content and that mitotic figures and connective tissue are well stained. A formula for the method, whereby differences in the functional state of the hepatocytes can be detected, is given in [7]. The hepatocytes are divided into the following six types: type 1) cells with blue cytoplasm, nuclear chromatin red against a blue background, nucleoli bright red; type 2) cells with blue cytoplasm, nucleus completely stained red; type 3) cells with blue cytoplasm, perinuclear space red, nucleus blue, and chromatin granules purplish-red; type 4) cells with red cytoplasm, nucleus deep blue, nucleoli and chromatin red; type 5) cells with yellow cytoplasm, sometimes with a narrow red rim around the nucleus, the nucleus itself deep blue; type 6) cells in a state of karyokinesis, cytoplasm blue, chromosomes purplish-red. Intermediate types also exist.

Cells of types 1 and 2 reflect different degrees of weakening of the protein-synthesizing activity of the hepatocytes. Cells of types 3 and 4 reflect stimulation of protein biosynthesis in the liver cells. Cells of type 5 are in a state of necrobiosis.

However, by using the existing formula it is very difficult to obtain clear results: the first phase of staining with alcian blue does not work, and it is difficult to obtain rapid differentiation. Novelli likewise does not state whether other fixatives can be used or whether sections can be cut with a freezing microtome.

We have therefore modified the above method in order to obtain clear and permanent staining of the preparation. The modification is as follows:

1. Carnoy's fixative can be used together with formalin and alcohol.
2. After staining of the preparation in 1% $KMnO_4$ solution the sections must be kept in air at room temperature for 30 sec to 1 min.

The $KMnO_4$ solution is best made up ex tempore. If the solution is used on the second day it can be warmed for 4-5 min in an incubator at 37°C.

3. The preparation is stained in the 1% solution of alcian blue for 20 sec to 1 min, and not for 5-10 min as stated in the formula. It should be noted that the solution of the dye must be warmed in an incubator at 37°C for 35-40 min before use, and in the room where the staining is carried out the temperature must be between 20 and 22°C. In this case not only the dye made in England – alcian blue 8GN150 – but also the Soviet preparation can be used.
4. After differentiation in 90° alcohol, acidified with acetic acid, dehydration in absolute alcohol is immediately carried out.
5. Not only paraffin sections but also tissue sections cut with a freezing microtome can be used.
6. If staining is carried out in alcian blue solution for 15-25 sec and the sections are then at once rinsed in tap water, and after staining in carbol fuchsin careful (gentle) differentiation is carried out, only the Kupffer cells will be stained.

Novelli states that alcian blue (experiments in vitro) exhibits affinity "for some insoluble carbohydrates, glycoproteins, and certain phosphorous compounds." We carried out a series of experiments to stain the liver of experimental animals with alcian blue and in parallel experiments used Shabadash's method to stain for glycogen (with Schiff's reagent). The glycogen content in the hepatocytes was found to be directly proportional to the intensity of staining of the cytoplasm of the cells with alcian blue. If sections were stained by Shabadash's method and then counterstained with alcian blue, very weak and not always distinct staining of the cytoplasm of the cells a bluish-green color could be observed, but in this case the nuclei often did not stain.

When parallel series of sections were stained for RNA by Brachet's method and by Novelli's method, if RNA accumulated in the cytoplasm staining with alcian blue and carbol fuchsin appeared as crimson granules, scattered over the cytoplasm of the hepatocytes, with some increase in concentration around the nucleus. In sections with little or no RNA (with Brachet's reaction for control), on staining by Novelli's method the granules described above could not be seen or were only solitary.

Solutions of alcian blue and carbol fuchsin are suitable for use for 1.5 months provided that they are kept in darkness at room temperature. The stained films likewise should be kept in darkness. Their stain lasts for 2-2.5 years. It should be noted that the sections obtained by this staining method are convenient for morphometry, if a measuring grid for cytohistostereometric investigations is used [2-4].

Considering that the total time taken to stain the preparations is very short (of the order of 2-3 min), this method can also be recommended as a special rapid method. It can also be used as a method of prelimi-

nary assessment of the state of the protein, nucleic acid, and carbohydrate metabolism and of the elastic membranes of the blood vessels of the liver, when a large quantity of material has to be processed in a short time.

The method is suitable for use by experimental morphologists when studying the effect of chemicals on the liver and other organs and when determining the functional state of the tissues.

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INTRAVITAL MICROSCOPY OF THE LUNG TISSUE IN SMALL LABORATORY ANIMALS

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A method of biomicroscopy of the lungs using a modified experimental lung fixing device is suggested. It enables intravital investigation of the microcirculation of the lung tissue to be carried out not only during artificial respiration, but also during spontaneous breathing of the animals.

KEY WORDS: microcirculation; lungs; lung fixing device.

A difficult stage of intravital investigation of the pulmonary microcirculation is fixation of the lung tissue. Various technical approaches to the solution of this problem are known [1, 2, 6]. However, all have certain shortcomings: considerable trauma during fixation of the lobe of the lung, the impossibility of using high magnifications during visual observation, the use of complex optical systems, etc.

The method of fixation of a superficial part of a lobe of the lung by means of a special fixing device [7] is less traumatic and enables the investigation to be undertaken under high power of the microscope. The immobility of the region under observation is achieved by the use of a negative pressure created in the tubing of the fixing device.

This particular design of the device has been suggested for large animals (dogs), so that it naturally cannot be used to study the microcirculation in small laboratory animals.

In this paper a design for a fixing device for the lobe of the lung is suggested [3] which not only allows the microcirculation to be studied in small laboratory animals (albino rats), but if necessary the animals can be switched to spontaneous breathing in the course of the observations [4].

The lung fixing device (Fig. 1) consists of the body 1, the lid 2, a glass 3, the floor of the inner chamber 4, and the tubes 5 and 6. The body contains two chambers (inner 7 and outer 8), isolated from each other by the partition 9. Holes 10 are made in the base of each chamber. The ML-2 luminescent microscope, working in reflected light [5], was used for biomicroscopy.

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