

5. E. B. Giricheva, "The action of local vibration on the human peripheral circulation," Author's Abstract of Candidate's Dissertation, Moscow (1975).
6. Yu. E. Moskalenko, in: Problems in Space Biology [in Russian], Vol. 5, Moscow (1967).
7. G. I. Mchedlishvili and L. S. Nikolaishvili, in: Mechanisms of Neurohumoral Regulation of Autonomic Functions [in Russian], Leningrad (1970), pp. 52-57.
8. A. I. Naumenko and V. V. Skotnikov, Fundamentals of Electroplethysmography [in Russian], Leningrad (1975).
9. N. M. Ryzhova, Byull. Éksp. Biol. Med., No. 7, 9 (1967).
10. Kh. Kh. Yarullin, Clinical Rheoencephalography [in Russian], Leningrad (1967).
11. K. Aukland, Acta Neurol. Scand., 41, 42 (1965).
12. H. Betz and H. Hensel, Pflüg. Arch. Ges. Physiol., 274, 605 (1962).
13. K. Hoedt-Rasmussen and E. Skinhoj, Acta Neurol. Scand., 40, 41 (1964).
14. D. H. Ingvar and P. Sourander, Arch. Neurol. (Chicago), 23, 1 (1970).
15. H. Jasper, Electroenceph. Clin. Neurophysiol., 10, 371 (1958).
16. E. Kanzow et al., Pflüg. Arch. Ges. Physiol., 273, 199 (1961).
17. F. Plum, J. Posner and B. Troy, Arch. Neurol. (Chicago), 18, 1 (1968).

A RAPID TEST OF LIVER MORPHOLOGY AND FUNCTION

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For the rapid and simultaneous determination of several vitally important parameters of the state of liver tissue the vital dye neutral red was injected into the portal vein. The morphological state of the tissue, the adsorptive power of the liver cells, the efficiency of the microcirculation of the blood, and the pH shift were determined in histological sections cut from part of the piece of liver taken for investigation. From the other part of the piece of liver tissue the dye was extracted and the total quantity of dye adsorbed was determined.

KEY WORDS: *Adsorptive power of the liver; microcirculation of blood; intravital staining.*

In some cases it is important to obtain rapid information on various parameters of the state of the liver tissue. For example, if the patient is on the operating table and the extent of further surgical procedures has to be determined, when faced with the problem of suitability of cadaveric or preserved liver for transplantation, and in various other clinical situations. Combined investigation of a number of different indices of the state of the liver is also important in experimental investigations of the physiology and pathology of the organ and its responses to various exogenous and endogenous factors. This is particularly true of ischemia of the liver [2].

There is no description in the literature of a method whereby indices of the state of the liver such as morphological changes, adsorptive power, efficiency of the microcirculation of the blood, and the pH shift can be estimated simultaneously. As a rule to study each of these parameters separate methods are used [1, 7, 8], and this is naturally time consuming and not always practicable.

The technique devised by the writers enables all the indices mentioned above to be estimated by the use of a small piece of liver tissue after preliminary injection of the vital

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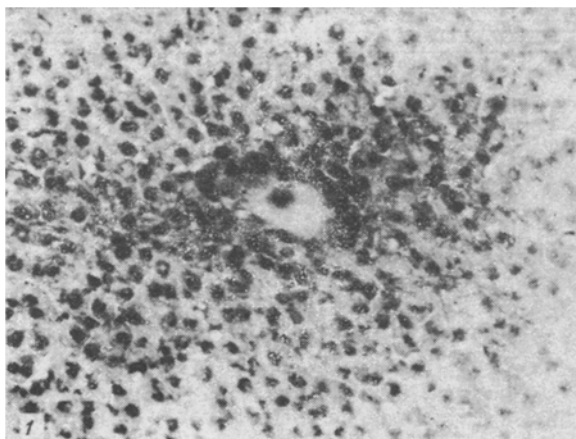


Fig. 1

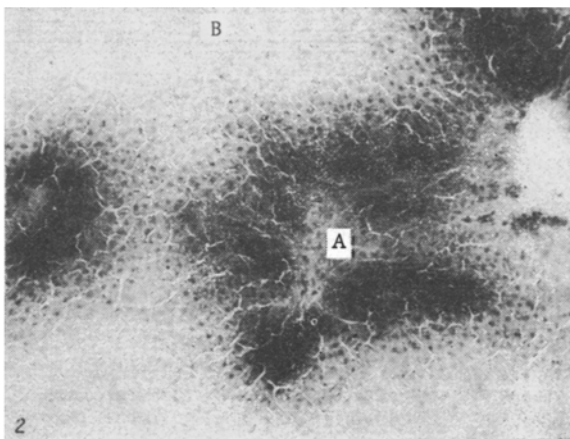


Fig. 2

Fig. 1. Adsorption of vital dye by liver cells after ischemia for 30 min. Neutral red, 280 \times .

Fig. 2. Functioning zones of liver and zones excluded from circulation after ischemia for 40 min: A) functioning, B) blocked. Neutral red, 80 \times .

dye neutral red into the portal vein. Investigations have shown that these parameters enable an integral index such as the viability of the liver tissue to be determined.

In experiments on animals a 0.5% solution of neutral red is injected into the portal vein in a dose of 1 ml/5 g weight of liver. A piece of liver measuring under 1 g is taken 2-2.5 min later and divided into two parts: One part is frozen in liquid nitrogen and the other placed in a mixture extracting the dye. Sections are cut from the frozen piece and can be subjected without further treatment to histomorphological and cytophotometric analysis and they can also be used for determination of pH and of the activity of the microcirculation of blood. The mixture with the extracted dye is examined colorimetrically in order to estimate the adsorptive power of the liver cells quantitatively.

Histomorphological Analysis. Stained liver cells and intra- and extracellular structures can be seen on the preparation, so that the morphological characteristics of the liver tissue can be obtained (Fig. 1). As a result of the vital or supravital injection of the dye the preparation contains no artefacts such as are found if an excised fragment is stained and, in particular, there is no inequality of staining of the central and peripheral parts. The results show that liver cells injured by hypoxia stain more intensely than intact cells. The method thus provides not only qualitative but also quantitative estimates of the degree of injury to the cells.

Adsorptive Power of the Liver Cells. In recent years considerable importance has been attached to this parameter [3-6]. It has been shown that it is one of the first to respond to a change in the physiological conditions of existence of the liver. For quantitative estimation of adsorption of the injected dye, as has already been said, part of the piece of liver is minced and kept for 2 h in the following extracting solution: 70° alcohol, acidified with sulfuric acid (2 ml/100). The extract is examined on the FÉK-56M photoelectric colorimeter using the No. 6 filter (wavelength 540 ± 10 nm), with distilled water as the control. The quantity of dye taken up is calculated per gram dry weight of liver.

State of the Microcirculation. By injection of vital dye it is possible to determine the efficiency of the microcirculation. Together with the morphological characteristics of the degree of injury to the hepatic blood vessels this index provides valuable information on the state of the microcirculation as a whole. To judge these parameters in histological sections, the ratio between the stained and unstained areas is determined by planimetry (Fig. 2). Control experiments showed that the healthy liver is virtually completely and uniformly stained. If the microcirculation is disturbed, through ischemia for example, areas are found into which the dye does not penetrate. The distribution of these areas increases with an increase in the severity of ischemia.

Determination of the pH Shift. This index is important for assessing the state of tissue metabolism. Following intravital injection of neutral red the pH shift of the tissue

is determined from the displacement of the position of the maximum in the absorption spectrum of the dye in the preparation. The method can be used to determine a pH shift in the cells and in the extracellular formations located in different parts of the liver, so that it is important for the comparative study of local injuries.

Adsorptive Power of Individual Cells. If the injury to the liver cells is not total in character, it will be necessary to discover the degree of damage to individual liver cells. For this purpose the adsorptive power of individual cells and, consequently, the degree of their injury are established cytospectrophotometrically.

Fairly frequently a disturbance of the adsorptive power of the liver cells develops simultaneously with a block of the microcirculation. The degree of retention of dye in the liver depends on two factors: the adsorptive power of the cells and the volume of zones of the liver perfused with blood. If no allowance is made for this state of affairs, a consistent mistake will arise. For example, when the disturbance of the microcirculation of the blood is severe, it may mask an increase in the adsorptive power of the liver cells because of a decrease in the number of cells in contact with the dye and, consequently, unable to adsorb it. For that reason, when adsorption of the dye is being assessed, the relative proportions of zones of the liver perfused and excluded from perfusion must be taken into account. To do this, the following calculation (conventional) is carried out: Suppose, for example, the planimetric measurement showed that 60% of the liver tissue was stained and the quantity of dye extracted per gram dry weight was 0.7. If all the tissue had been perfused with blood, the amount of dye extracted would have been

$$X = \frac{100 \cdot 0.7}{60} = 1.17.$$

Spectrophotometric determination of the adsorptive power of individual cells is used as the control.

LITERATURE CITED

1. Ya. I. Azhípa, Pat. Fiziól., No. 3, 77 (1960).
2. Yu. M. Lopukhin and É. M. Kogan, Criteria of Viability of Organs and Tissues before Transplantation [in Russian], Moscow (1975).
3. D. L. Rõžental', Tsitologiya, No. 4, 454 (1971).
4. G. E. Sviderskaya and G. I. Grigor'eva, Tsitologiya, No. 9, 1172 (1969).
5. I. P. Suzdal'skaya, Tsitologiya, No. 6, 702 (1973).
6. Z. I. Taraskina, Sud. Med. Ékspert., No. 1, 26 (1972).
7. A. M. Chernukh and N. Ya. Kavalenko, Pat. Fiziól., No. 6, 21 (1971).
8. U. Macmillan and B. K. Siesjö, Scand. J. Clin. Lab. Invest., 30, 117 (1972).