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# MICROVASCULAR PERMEABILITY IN RATS PREDISPOSED AND NOT PREDISPOSED TO DEVELOP EXPERIMENTAL ALCOHOLISM

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Previous investigations [2] showed that ethanol, if injected intraperitoneally into rats predisposed to develop experimental alcoholism, enters the blood stream more slowly but is excreted from it more rapidly than in rats rejecting alcohol. It was suggested that these differences in the pharmacokinetics of ethanol in animals of these groups were connected with differences in the functioning of their microcirculation and, in particular, differences in permeability of the walls of the microvessels.

It was therefore decided to compare the state of permeability of the microvascular walls in rats predisposed and not predisposed to develop experimental alcoholism (PR and NPR) respectively.

## EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-250 g. The rats were tested for predisposition to form experimental alcoholism by determining the duration of ethanol anesthesia, judged by the time spent by the animal in the side position after intraperitoneal injection of a 25% solution of ethanol in a dose of 4.5 g/kg [1]. Rats remaining only a short time in the side position (under 60 min), which are predisposed to voluntary consumption of ethanol, formed group 1. Group 2 included animals staying for a long time (over 120 min) in the side position. Each group consisted of 9 rats. Under pentobarbital anesthesia (mean dose 50 mg/kg, intramuscularly) a cannula was introduced into the left carotid artery of the rat in the proximal direction, fixed with a ligature, and the distal end of the artery was ligated. The anterior abdominal wall was opened and a loop of small intestine brought out through the incision, and wrapped around a light guide on a constant-temperature stage. Biomicroscopy of the mesenteric vessels was carried out in the usual way [3] under an optical magnification of 31.

Permeability of the walls of the mesenteric microvessels was studied by a luminescence method. The contents of 2 ampules, each containing 0.5 ml of dried serum (rabbit luminescent serum, produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Sciences of the USSR), labeled with fluorescein isothiocyanate (FITC), were dissolved in 0.8 ml of physiological saline. Through a cannula 0.5 ml of serum was injected into the aorta, and its outflow from the microvessels was observed during illumination with UV rays through an opaque illuminator (exciting filter of BP type (390-480 nm), cutoff filter of LP type (515 nm)). The process of outflow of the indicator was recorded on RF-3 photographic film 1, 5, 10, 15, 20, and 30 min after its injection. Images obtained on negatives were estimated quantitatively by means of a TAC television analyzing system (Ernst Leitz, West Germany) [4]. In this way values of the area of spread of the indicator from a test microvessel were obtained during different periods after injection: $S_n$, $S_{n+1}$, $S_{n+2}$. The

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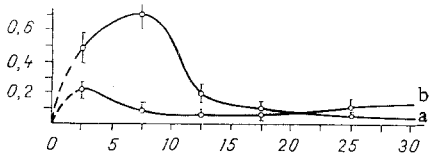


Fig. 1. Rate of outflow of indicator from mesenteric microvessels of PR (a) and NPR (b). Abscissa, time after injection of indicator (in min); ordinate, rate of outflow of indicator (in $min^{-1}$).

rate of outflow of the indicator through the vessel walls into surrounding tissues was determined by calculating the relative increase of area during the given time interval (t):

$$V_{ind}\ (min^{-1}) = \frac{S_{n+1} - S_n}{S_n \cdot t}.$$

## EXPERIMENTAL RESULTS

Outflow of indicator from the microvascular system was observed mainly at the capillary level, and most of all at the level of venular capillaries.

The character of the indicator outflow curve from microvessels in PR during the first 10 min after injection differed significantly from that in NPR (Fig. 1). The rate of outflow of the indicator during this period into the perivascular space of the animals of group 1 increased rapidly to reach a maximum between the 5th and 10th minutes. This was followed by a sharp fall in this parameter, and by the 15th minute no statistically significant difference could be found between the groups. By this time the blood level of the indicator also had fallen in these animals. By the 30th minute of observation the rate of outflow of the indicator in PR was close to zero. In NPR, this parameter had its peak value during the first 5 min after injection of the indicator, but later, toward the 10th and 15th minutes, it decreased gradually, and later still remained at virtually the same level. Thus in the animals of group 1 comparatively rapid evacuation of the indicator injected into the blood stream was observed, when most of it after 15 min was outside the blood vessels. Animals of group 2 were characterized by a relatively uniform and prolonged process of evacuation of the dye into the tissues.

These results are evidence that permeability of the microvascular walls is higher in PR than in NPR, and since they were obtained by the use of a high-molecular-weight indicator, permeability of the microvessels for ethanol, which has a lower molecular weight, accompanied by high lipophilicity, must be even higher still.

Permeability is associated with the structural and functional properties of the vessel wall and, in particular, of its endothelium. Since vessels with the same type of structure were studied in the animals of the two groups, the differences observed in their permeability may be the result of differences in the state of the enzyme systems affecting permeability. We know, for instance, that the rate of outflow of an indicator from microvessels largely depends on the state of the clotting system of the blood, which is intimately connected with the formation of the periendothelial layer of fibrin, whose barrier function is determined by dynamic equilibrium between the enzymes of that layer and fibrinolytic enzymes. It must also be recalled that ethanol itself influences the state of the clotting system, and its influence, moreover, is greater on the enzyme stage of that system and, in particular, on $Ca^{++}$-ATPase activity [6], than on the aggregating power of the platelets [5]. The level of permeability of the microvascular wall after entry of ethanol into the blood stream is thus determined both by the state of the blood enzyme systems and by the state of the endothelium of the animal concerned.

The facts thus established agree with the results of previous investigations, which showed that the elimination constant in rats predisposed to alcoholism is higher than that of rats rejecting alcohol [2].

When results obtained on mesenteric microvessels are assessed, the existence of a permeability gradient for the organ must be taken into account: The gradient is manifested by the fact that microvessels of skeletal muscles, heart, brain, lungs, and skin possess relatively low permeability, whereas vessels of the liver, spleen, and bone marrow possess high permeability [3]. The mesenteric microvascular system is a convenient object for investigations of this type, for it occupies an intermediate position between the organs of the two categories mentioned above.

The results of this investigation are evidence of the existence of functional differences between the microcirculation in animals predisposed and not predisposed to the development of experimental alcoholism, and they also provide a basis for the further study of the pharmacokinetics of ethanol in animals of these two groups, as a preliminary step to the elucidation of the mechanisms of alcohol addiction and the development of alcoholism.

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