wound during treatment under dressings is sharply retarded and not until 21-24 days after the beginning of treatment do polymorphonuclear neutrophils predominate in the wound exudate. Correspondingly, skin autografting is carried out at a later stage than in patients treated in a germfree environment.

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METHODS

QUANTITATIVE ESTIMATION OF AORTIC WALL PERMEABILITY

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KEY WORDS: permeability; aorta; fluorescence microscopy; photometry; experimental hypertension and atherosclerosis.

Permeability of the walls of large blood vessels, including the aorta, is studied experimentally on quite a wide scale in models of various pathological states, notably atherosclerosis, hypertension, and so on [3, 5, 10-12]. Substances labeled with radioactive isotopes, or vital dyes are used for this purpose [2, 6, 8, 9]. Adams and Bayliss [4] proposed and tested a fluorescence-microscopic method of studying permeability of the aortic wall, using the dye trypan blue. The method is based on the ability of trypan blue to give red fluorescence in monochromatic green light (570 nm). When injected into the blood stream of an animal the dye forms stable complexes with plasma albumin, and in the composition of these complexes it penetrates into the vascular wall. Entry of the dye into the aortic wall is judged from the intensity and distribution of fluorescence, and on that basis the permeability of its membranes is determined. The state of permeability is assessed on the basis of a purely descriptive characteristic of luminescence in the aortic wall.

The object of this investigation was to develop a method of quantitative estimation of permeability of the aortic wall using the dye trypan blue. The technique included photometry and a method of quantitative analysis of scanograms obtained by means of it, developed by the present writers. Minor changes were introduced into the method of preparing the material for investigation as suggested by Adams and Bayliss [4].

EXPERIMENTAL METHOD

Chinchilla rabbits were used. Trypan blue was injected into the marginal vein of the animals' ear in a dose of 20 mg/kg body weight as a 1% solution made up in physiological saline. The animals were killed by air embolism 90 min after injection of the dye. The aorta was removed, washed with ice-cold physiological saline, and freed from loose connective tissue. Tubular specimens about 5 mm high were cut from different parts of the vessel.

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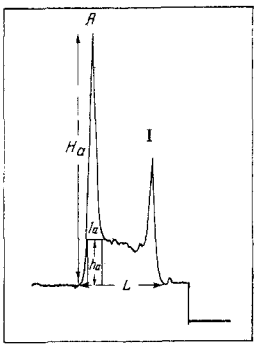

Fig. 1. Graphic representation of luminescence in wall of rabbit aortic arch (normal, 90 min after injection of trypan blue). Explanation in text.

The specimens were quickly cooled, and frozen sections 15 μ thick through the whole circumference of the aorta were cut on a freezing microtome. The sections were placed on slides, fixed for 10 min in 5% TCA solution in 10% formalin, mounted in 50% glycerol, and covered with coverslips. The sections were examined under the "Lyuman-I2" microscope in reflected light. The source of radiation was a DRSh-250-3 mercury-quartz lamp. Luminescence of the dye was excited by green radiation with a wavelength of 570-600 nm, using 3S11-3 filters. To select the red region of the spectrum, S3S24-4 and S3S21-2 filters weme used (red light-dividing plate; red KS-11 blanking filter). The intensity of luminescence was determined by means of the FMÉL-1 photometric attachment with FÉU-39A photoelectronic multiplier, with a 0.5 mm probe (size of area subjected to photometry 0.01 mm); objective 40, ocular 5. The current induced by the photic flux of luminescence in the photoelectronic multiplier was led to a U5-7 amplifier and recorded on an EPP-09 M3 automatic electronic potentiometer. Photometry was carried out on the region of the aortic wall for study throughout its depth from adventitia to intima; for this purpose the preparation was moved under the microscopic objective at constant speed by means of a special automatic traction system. A graphic representation of the intensity of luminescence throughout the depth of the aortic wall was obtained on the paper tape of the potentiometer. At least 20 measurements were made on each preparation, the regions for which were chosen by a random step method, using tables of random numbers [7]. Luminescence recorded in each region of the aortic wall was represented graphically in the form of a curve (Fig. 1). One peak of it corresponded to luminescence of the intima and adjacent part of the media (I peak), the other to luminescence of the adventitia and outer part of the media (A peak). Several quantitative parameters were calculated on the basis of these curves.

1. The intensity of penetration of the dye from the adventitia (IP_a), expressed as the area of luminescence bounded by the curve forming the corresponding peak (A peak). It was determined as the sum of the areas of the triangle (top part of the peak) and rectangle (base of the peak): $IP_a = S_{\Delta a} + S_{\square a}$, where $S_{\Delta a} = l_a(H_a - h_a)/2$, $S_{\square a} = l_a \cdot h_a$; l_a denotes the base of the triangle in the upper part of the peak and the side of the rectangle of the base of the peak; H_a the height of the peak; h_a the height of the rectangle.

2. The intensity of penetration from the intima (IP_i) was determined in a similar way: $IP_i = S_{\Delta i} + S_{\square i}$.

3. The index of depth of diffusion from the adventitia (IDD_a); $IDD_a = l_a/L$, where L is the total thickness of the aortic wall (according to the scanogram).

4. The index of depth of diffusion from the intima (IDD_i):

$$IDD_i = l_i/L.$$

TABLE 1. Parameters of Permability of Wall of Aortic Arch of Normal Rabbits and Rabbits with Experimental Atherosclerosis and Hypertension

Experimental conditions	Statistical parameter	IP_i	IP_a	IAR	IDD_i	IDD_a
1. Normal	M	102	198	0,59	0,19	0,24
	$\pm m$	6	23	0,05	0,02	0,01
	n	5	5	5	5	5
2. Hypertension	M	126	183	0,79	0,18	0,22
	$\pm m$	16	23	0,10	0,10	0,02
	n	5	5	5	5	5
3. Atherosclerosis	M	299	288	0,96	0,22	0,25
	$\pm m$	58	25	0,14	0,02	0,01
	n	6	6	6	6	6
	$P_{1-3}<0,01<0,05<0,05$					
	$P_{3-4}<0,02<0,05$					
4. Atherosclerosis and hypertension	M	357	279	1,25	0,20	0,21
	$\pm m$	65	25	0,19	0,02	0,03
	n	5	5	5	5	5
	$P_{1-4}<0,01<0,05<0,02$					
	$P_{2-4}<0,01<0,05>0.05$					

5. The intimal–adventitial penetration ratio (IAR):

$$IAR = IP_i/IP_a.$$

An example of calculation of IP_a and IDD_a from the scanogram illustrated in Fig. 1, in which according to the scale of the figure H_a = 85 mm, h_a = 16 mm, l_a = 6 mm, and L = 27 mm, is given below:

$$IP_a = 66\ \text{mm} \cdot (85\ \text{mm} - 16\ \text{mm})/2 + 6\ \text{mm} \cdot 16\ \text{mm} = 303\ \text{mm}^2;$$

$$IDD_a = 6\ \text{mm}/27\ \text{mm} = 0.22.$$

This quantitative method of estimating permeability of the aortic wall, studied by the fluorescence-microscopic method with the aid of trypan blue, was used to investigate animals with experimental hypertension (renal form) and cholesterol atherosclerosis induced against the background of hypertension [1]. In addition, observations were made on normal rabbits, rabbits with experimental hypertension (with a duration of 4 months) not receiving cholesterol, and animals with atherosclerosis but with a normal blood pressure. The ages of the animals were 6-10 months.

EXPERIMENTAL RESULTS

Mean values of the intensity of intimal and adventitial penetration of dye–plasma albumin complexes into the wall of the aortic arch, and also the depth of their diffusion in the wall in normal rabbits and rabbits with experimental pathology, are given in Table 1 by way of example.

In hypertensive rabbits kept on an ordinary cholesterol-free diet (Table 1) no significant differences were found in the parameters tested or, consequently, in permeability of the wall of the aortic arch compared with normal. Consequently, the disturbances of permeability of the inner and outer layers of the wall of the aortic arch discovered in the present experiments in rabbits with hypertension combined with atherosclerosis were probably due mainly to the action of the developing atherosclerosis.

By the method used it was thus possible to discover differences in permeability of the aortic wall under normal conditions and in the forms of experimental pathology described above, and to undertake the comparative evaluation of this process under different experimental conditions.

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PHOSPHOLIPID HYDROLYSIS AND Fe^{++}-INDUCED CHEMILUMINESCENCE OF RAT LIVER MITOCHONDRIA DURING SURVIVAL *IN SITU*

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Anoxia of any origin is accompanied by disturbance of the structure and function of cell organelles as a result of hydrolysis of the membranes by endogenous phospholipases [8, 13, 15]. This applies chiefly to mitochondria, the degree of integrity of which will be the main factor predetermining the reparative potential of the cell. The effectiveness of restoration of structural and functional integrity of the cell after reoxygenation will depend not only on the degree of preceding hydrolysis of membrane lipids, but also on many other factors, including the level of lipid peroxidation (LPO). Investigations have shown accumulation of primary and secondary products of LPO in lipids extracted from the mitochondrial and microsomal fractions of liver in ischemia, even without reoxygenation; at the same time the level of antioxidative activity (AOA) was observed to fall [1, 2, 5, 10, 11]. However, in these experiments the oxygen balance of the tissues was not monitored and it is not clear whether conditions of anoxia were reached. Moreover, activation of LPO may have taken place during extraction of the lipids. Nevertheless, these results may be evidence if not of activation of LPO in the tissues during ischemia, at least of an increase in the activity of pro-oxidant factors in cell membrane lipids. At the same time there is evidence that AOA may increase in the cell during anoxia. For instance, lengthening of the latent period and a decrease in the intensity of Fe^{++}-induced chemiluminescence (Fe^{++}-CL) have been observed in homogenates or mitochondrial fraction during long periods (3-24 h) of survival of the rat liver in situ [6], and ascorbate-dependent LPO in liver homogenates has been found to be reduced in ischemia [11]. Estimation of the LPO capacity of the mitochondria at the time of onset of irreversibility of anoxic liver damage is particularly interesting. For

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