MITOTIC ACTIVITY OF THE ENDOTHELIUM OF MESENTERIC MICROVESSELS OF VARIOUS DIAMETERS IN WISTAR RATS

N. E. Barbashina, A. S. Golub', and K. A. Shoshenko

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The percentage of endothelial cells (EC) actively taking up thymidine label into the nuclei (index of labeled nuclei — ILN) is used as an indicator of endothelial growth in cell cultures and in vessels [7, 9]. Experimental data concerning the important role of mechanical factors (the velocity of the blood flow in the vessel, the hydraulic pressure, and juxtamural shear stress) in the acceleration of capillary growth and differentiation into arterioles and venules has been published recently [7, 11]. The increase of pressure and of the blood flow in microvessels in inflammation due to burns [2], during training and pharmacologic dilatation of arterioles [7], in arterial hypertension [8, 9], and during tumor growth [7] stimulates proliferation of EC and incorporation of the label.

ILN in zones of disturbance of the developed flow [7] and in regions of branching is particularly high: up to 14% in the aorta [8] and up to 6.5% in the capillaries [2]. For instance, the calculated life span of EC in the unbranched segment of aorta is 100-180 days, compared with 60-120 days in the region of bifurcation [11].

Among mechanical factors capable of reducing the life span of EC and stimulating reparative growth, the pressure gradient $\Delta P/\Delta l$ and the juxtamural shear stress τ_w, demand consideration in the first place, and in a Poiseuille approximation they can be calculated by the equations:

$$\Delta P/\Delta l = 32\eta V/D^2;\ \tau_w = 8\eta V/D,$$

where η is the apparent viscosity of blood, V the mean linear velocity of the blood flow, D the internal diameter of the vessel, and l its length.

The pressure gradient and juxtamural shear stress are known to increase in the direction from the large arteries and veins toward the capillaries [1, 12]. The distribution of these parameters in microvessels of the rat mesentery, calculated on the basis of our own experimental data [1], is shown in Fig. 1.

Thus if the distribution of proliferative activity of EC in the mesenteric vascular bed reflects to some extent the distribution of the level of mechanical influence on the vessel wall, this will be an argument in support of the mechanical hypothesis of regulation of the duration of the mitotic cycle of EC.

EXPERIMENTAL METHOD

Experiments were carried out on the mesentery of rats aged 4 months (n = 9, average weight 177 g) and 3 weeks (n = 3, 40 g). For 3 days the animals were given intraperitoneal injections of ^{3}H-thymidine daily in a volume of 0.5 ml, with total injected specific radioactivity of 140 MBq/kg body weight. The rats were killed with ether 1 day after the last injection of label.

To study the distribution of proliferative activity of EC in the microvessels a method of injection autoradiography was developed, by means of which the investigation could be conducted on a total preparation. The killed animal was immediately autopsied, a cannula was tied in the abdominal aofta, and in complete darkness the vessels were filled with melted nuclear photographic emulsion (type M). At the end of infusion the mesentery was cooled for 20 min with ice, excised, placed in 2% formalin solution with thymol, and allowed to stand in the refrigerator (3-5°C) in a lightproof container. After exposure for 3-4

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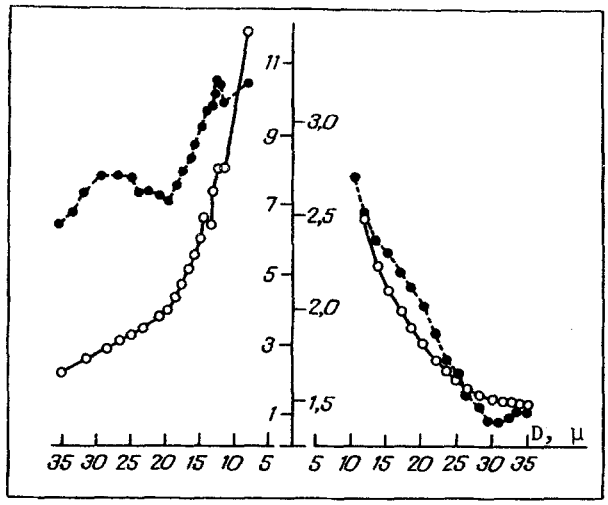

Fig. 1. Pressure gradient ($\Delta P/\Delta l$, mm Hg — empty circles) and shear stress (τ, Pa — filled circles) on the vessel wall in arterial (left) and venous (right) portions of mesenteric vascular bed of Wistar rats. Calculation from data in [1], for η = 2.35 cP.

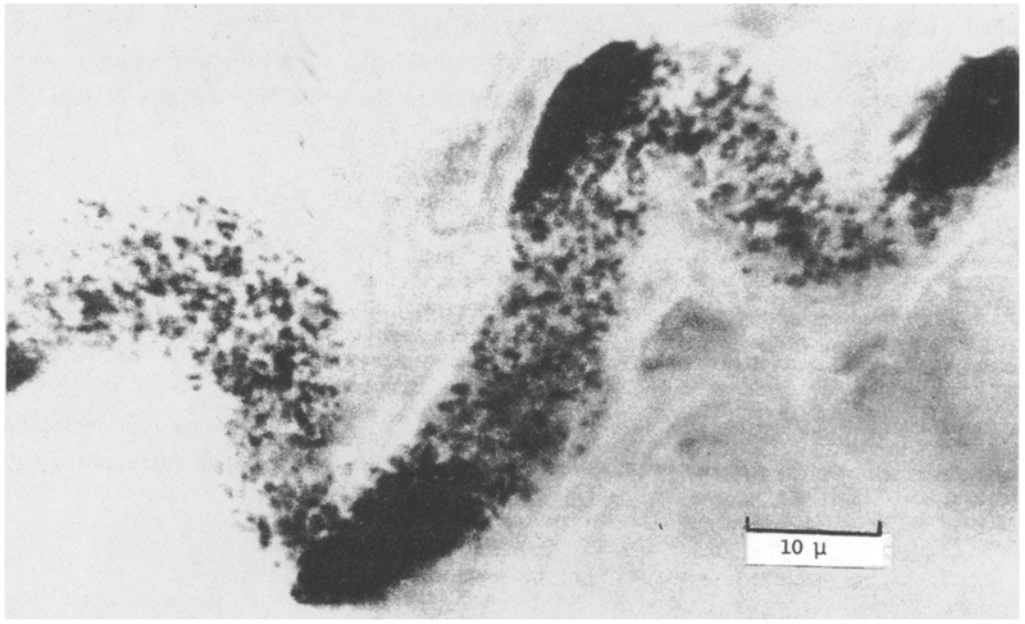

Fig. 2. Intravascular autoradiography of nuclei of EC labeled with ^{3}H-thymidine. Capillary from mesentery of a 4-month-old Wistar rat.

weeks the mesentery was treated as an autoradiographic preparation [3]. Pieces of mesentery were then placed on a slide, stained with hematoxylin and eosin, and cleared with glycerol. The result achieved by this method is illustrated in Fig. 2: because of the close apposition of the photographic emulsion to EC, the labeled nucleus could be reliably localized in a particular zone of the microcirculatory bed.

Under the microscope the internal diameter of the vessel, its length, the number of labeled nuclei in the vessel ($N_{l.n.}$) and the space period of EC ($D_{e.c.}$), calculated from the number of nuclei of EC present in the particular segment of the vessel in the direction of its axis, were measured. During calculation of $N_{l.n.}/mm^2$ it was assumed that the distance between the nuclei in the perpendicular direction also was equal to $D_{e.c.}$ and that the area of EC was $D_{e.c.}^2$.

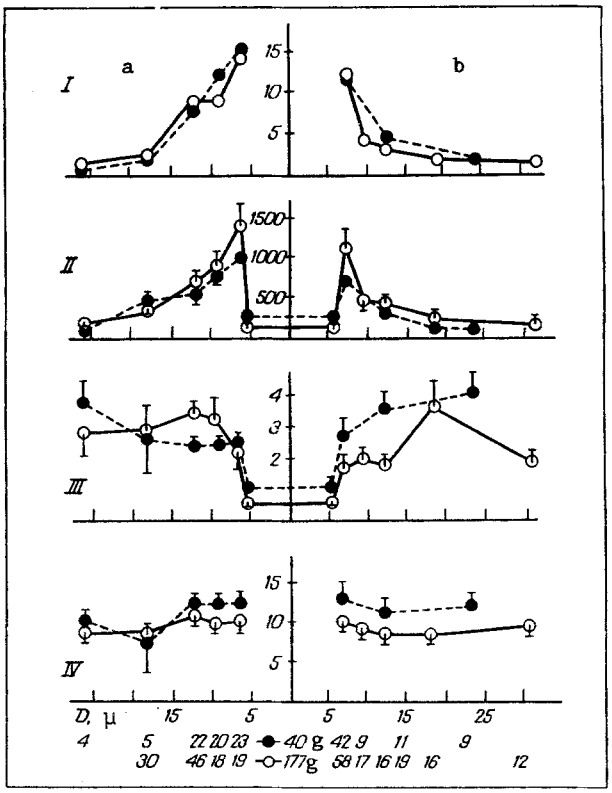

Fig. 3. ILN (I, in %), their density (II, calculated per mm^2 of inner surface of the vessel, III, for the whole vessel), and diameter of an endothelial cell (IV, in μ) in arterial (a) and venous (b) microvessels of Wistar rats aged 3 weeks and t months. $M \pm m$; number of vessels measured given below.

EXPERIMENTAL RESULTS

Data showing that $N_{l.n.}/mm^2$ and ILN increased from comparatively large microvessels toward capillaries, and did so similarly to changes in the pressure gradient and juxtamural shear stress (Fig. 1) are given in Fig. 3. The similarity of the relationships compared also was exhibited in the differences between arterial and venous regions: a reduced incorporation of label corresponded to low hemodynamic loads. It will also be noted that the hemodynamic characteristics of the microcirculation of young and of virtually adult rats differed only a little, and this same coincidence of the values of parameters of proliferative activity also follows from the data given in Fig. 3.

The number of labeled nuclei in the vessel was relatively constant in vessels of different caliber, except in capillaries, and its value was between 2 and 4, although the area of the inner surface of the vessel in our sample varied by a factor of 10-15 (for arteries of adult rats, by four times). EC of the capillaries were distinguished by the lowest values of ^{3}H-thymidine incorporation, especially in adult rats: 72% of capillaries did not contain labeled nuclei. In young rats the fraction of these capillaries was somewhat less, namely 45%. Cells with labeled nuclei were distributed as a rule at the beginning or end of the capillary, confirming the hypothesis [2] that a site of branching is a zone of growth. Mean values characterizing the geometric dimensions and distribution of labeled nuclei in vessels of different calibers in animals of the two age groups are given in Table 1. The age dynamics of proliferative activity of EC can be clearly seen by comparing ILN averaged for different regions: by the 4th month of life this parameter in the arterial portion fell from 5.7 ± 0.9 to 3.8 ± 0.5%, in the venous portion from 3.6 ± 0.9 to 2.2 ± 0.4%, and in the capillary portion from 3.1 ± 0.7 to 1.0 ± 0.4%. The value of ILN in young and mature rats was lower in the capillaries than in the veins, and lower still than in the arteries.

ILN in the capillaries of rats falls to 1% by the age of 3 months [6, 9, 10] irrespective of whether ^{3}H-thymidine is injected once [9] or twice [10] in the course of the 24-h period. Three injections, at intervals of 24 h, which was the procedure followed in the present investigation, gave closely similar values. Hence it can be concluded that if the life span of EC of a capillary is of the order of 1000 days [4, 5, 7, 8], the group of cells in the synthetic phase of the mitotic cycle shows little change

TABLE 1. $N_{l.n.}$ of Endotheliocytes in Vessels of Different Sizes in the Rat Mesentery ($M \pm m$)

D of vessel	Artery				Vein			
	n	D, μ	L, μ	$N_{l.n}$	n	D, μ	L, μ	$N_{l.n}$
				Rats weighing 40 g				
⩽10 μ	43	7,5±0,22	147±16	2,4±0,17	9	7,1±0,43	191±34	2,7±0,44
>10 μ	31	15±0,9	215±44	2,6±0,29	20	17±1,4	466±73	3,8±0,46
Total	74	11±0,6	175±21	2,5±0,16	29	14±1,3	301±57	3,5±0,35
Capillaries	42	5,8±0,26	240±14	0,95±0,17	—	—	—	—
				Rats weighing 177 g				
⩽10 μ	37	7,9±0,23	139±19	2,7±0,40	33	7,9±0,48	116±13	1,8±0,31
>10 μ	90	16±0,5	189±13	3,4±0,34	47	19±1,2	285±77	2,4±0,37
Total	127	14±0,7	174±11	3,2±0,27	80	14±1,0	215±30	2,2±0,25
Capillaries	58	6,8±0,23	220±17	0,52±0,16	—	—	—	—

in the course of 2 days, i.e., labeling of this duration may also be regarded as pulse labeling [3]. Under these circumstances ILN is determined by the relative duration of the synthetic phase of the mitotic cycle and the fraction of cells able to proliferate. The proliferative pool of EC has not been established yet, even for endothelium of large vessels, because of the impossibility of using the method of labeled mitoses: no mitoses whatever were observed in large groups of EC [4]. Consequently, ILN may be a characteristic of the proliferative pool if the duration of the synthetic phase is similar to the duration of the mitotic cycle, and conversely, if all cells proliferate, ILN will reflect the relative duration of DNA synthesis in the cycle. Despite the indeterminacy mentioned above, ILN is a reliable characteristic of the state of the EC population as a whole and makes comparative analysis possible.

There is a possible alternative explanation of the decrease in ILN with an increase in the caliber of the microvessels. Since EC in culture divide about 80 times, and ILN falls sharply in the last third of the generations [2], endothelium of large arterioles and venules is "older" in this sense than in the capillary-forming fraction of cells. However, this hypothesis assumes the presence of this fraction with cytologically "young" EC and it cannot explain the high rate of growth of EC of the aorta [4, 8, 9, 11].

The correlation established between the level of hemodynamic influences on the vessel wall and proliferative activity of the endothelium thus indicates a key role for EC in the formation of the definite spatial organization of the microvascular bed and its adaptive transformation in response to a change in hemodynamic parameters.

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