COMPARATIVE STUDY OF PROLIFERATIVE ACTIVITY OF CELLS FROM VESSELS OF DIFFERENT CALIBER

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In the past, opposite views have been expressed on the ability of cells of the vascular wall to undergo physiological regeneration. Sarkisov [6], for instance, emphasizes that physiological regeneration takes place continuously in all layers of the vascular wall and is accompanied by renewal of the cell composition and of the fibrous structures produced by them. On the other hand, it is stated that the main cellular component, the endothelium, possesses no proliferative activity [7, 19], and the sources and mechanisms of its physiological regeneration still remain unexplained [4]. The aim of this investigation was a comparative autoradiographic analysis of the proliferative activity of the endotheliocytes, smooth-muscle cells, fibroblasts, and adventitial cells of different caliber in intact adult rats.

EXPERIMENTAL METHOD

Experiments were carried out on seven male albino rats weighing 160-210 g, each of which received 12 intraperitoneal injections of ^{3}H-thymidine in a dose of 0.8 μCi/kg body weight at intervals of 8 h. Choice of the time between injections was based on the need for it to be shorter than the duration of the synthetic period of the life cycle of endotheliocytes. According to available data [1], the latter for endotheliocytes of the rat aorta is 13.5 h. The animals were killed with ether vapor 1 h after the last injection. The duration of the experiment (the time from the beginning of ^{3}H-thymidine injection until fixation of the material) was 89 h. Fragments of the thoracic and abdominal aorta and of the inferior vena cava with adjacent tissues were excised from the rats, fixed in Bouin's fluid, and embedded in paraffin wax. Sections 4-5 μm thick were covered with type M liquid emulsion, exposed for 22 days at 4°C, developed, and stained with hematoxylin and eosin. Indices of labeled nuclei (ILN) of the epitheliocytes (Ec), smooth-muscle cells (SMC) desmocytes (counted in 2000-4000 cells), and fibroblasts (Fb) of the tunica media (aorta) and tunica externa, and of adventitial cells (AC) in vessels of the microcirculatory bed of the paravasal tissues (in 8000-10,000 cells for each animal) were counted. Knowing the number of cells which passed through the reproductive cycle during 89 h, their corresponding number for 24 h (the 24-hourly proliferative pool) was calculated. On the basis of this calculation the renewal time of the population (doubling of the number of cells, under the condition that all 100% of cells passed through one reproductive cycle each) was determined. The significance of differences was estimated by Student's test.

EXPERIMENTAL RESULTS

The proliferative pools of the main cells of the vessel wall are shown in Fig. 1 and Table 1. The endothelium was found to have the greatest proliferative activity. Incidentally, in different parts of the vascular bed ILN of the endothelial cells varied considerably with a minimal value of this parameter in the inferior vena cava, namely under 2%; the renewal time of the EC population in this case was 192 ± 43 days. The highest value of ILN was found in EC of vessels of the microcirculatory bed, and also in EC of veins of small and medium caliber, with poorly developed muscle cells. For instance, in the capillary endothelium the label could be recorded in only 30% of cells and the size of the 24-hourly proliferative pool was 7.95 ± 1.67%; the renewal

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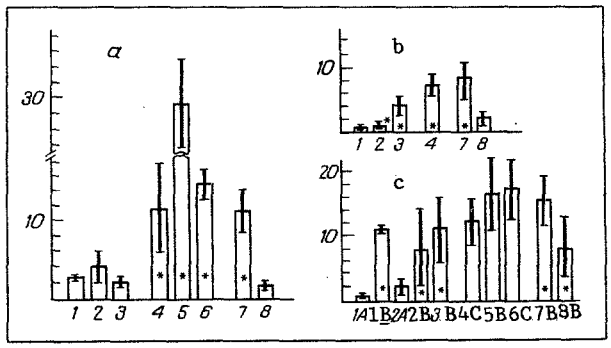

Fig. 1. ILN of principal cells observed in walls of blood vessels of different caliber in intact adult rats. Abscissa: 1) thoracic aorta, 2) abdominal aorta, 3) arteries of muscular type, 4) arterioles, 5) capillaries, 6) venules, 7) veins of medium and small caliber (with poorly developed muscle cells), 8) inferior vena cava; ordinate, ILN (%). a) Endothelium, b) smooth muscles, c) fibroblasts of tunica media (a) and externa (b) or adventitial cells (c). *$p < 0.05$ Compared with lowest value.

time of the EC population in the capillaries was 13 ± 3 days. Analysis of the literature also indicates that after injection of ^{3}H-thymidine, cells synthesizing DNA appeared in the endothelium of the blood vessels of the laboratory animals [1, 3]. Their distribution in the intima differed in different vessels. For instance, there is evidence that labeled nuclei are found more often in the endothelium of the arch and abdominal part of the aorta, less frequently in the thoracic aorta [8]. A difference has been observed between the number of labeled nuclei in the dorsal and visceral parts of the aorta [12]. ILN may reach 10% or more [12, 13]. In the course of 24 h, only one of 10^2-10^4 cells was labeled with ^{3}H-thymidine [11]. The duration of the mitotic cycle of EC of the thoracic and abdominal aorta of 5-day-old rats is 92 h [1], in adult rabbits it is 142 days, and in the vascular endothelium of the guinea pig the life cycle lasts 100-180 days [10]. In rat capillaries, similar calculations have shown the life span of EC to be 1000 days [15]. The endothelium of the microcirculatory and venous beds of the visceral vessels, unlike the arterial bed, is more active as regards its ability to incorporate labeled ^{3}H-thymidine [14]. In response to repeated injections of ^{3}H-thymidine for a period of 85 h ILN in the thoracic aorta of adults rats was 2.3%, in the abdominal aorta 2.7%, and in the posterior vena cava 2.5% [3].

The most active cells as regards proliferative potential were SMC (Table 1, Fig. 1). However, ILN of these cells showed considerable scatter. The minimal value was obtained in SMC from the tunica media of the thoracic aorta, namely 0.43%, and the renewal time of the SMC population in this case amounted to 833 ± 119 days. Maximal labeling was observed in SMC of veins of small and medium caliber, namely about 8%, and the renewal time of the SMC population here was 46 ± 32 days. It was shown previously that during long-term injection of the isotope SMC of the thoracic and abdominal aorta and the posterior vena cava of adult rats had ILN values of 0.7, 0.5, and 0.6%, respectively [3]. Calculations show that for complete replacement of SMC in a rat blood vessel, about 1000 days is required [2, 3].

During assessment of the proliferative activity of the connective-tissue component of different blood vessels it was found that the label was not detected in the nuclei of the desmocytes during analysis of 2000-4000 cells. ILN of Fb of the tunica media of the thoracic and abdominal aorta was lower than in the tunica externa. The renewal time of the Fb population in the tunica media of the thoracic aorta was 455 ± 85 days, whereas in the outer part of the wall it varied between 25 ± 6 and 48 ± 20 days. With respect to the property of their nuclei of incorporating the label, AC were closely similar to Fb of the arteries and veins. Minimal values of ILN were recorded in AC of the arterioles (12.0 ± 3.7%) and the renewal time of the AC population in this case was 31 ± 7 days. The maximal value of ILN in AC of the venules was 17.0 ± 4.9%, where the renewal time of the population varied between 22 ± 5 days. According to data in the literature [3], based on repeated injections of ^{3}H-thymidine, Fb of the tunica externa of the thoracic and abdominal aorta and the vena cava contain labeled nuclei in 1.9-2.4% of cases. Thus we cannot confirm the view that the intensity of proliferation of the principal tissue components of the vessel wall is identical.

TABLE 1. 24-Hourly Proliferative Pool (1, in %) and Population Renewal Time (2, in days) of Principal Cells in Wall of Vessels of Different Caliber of Intact Rats (12 injections of ^{3}H-thymidine at intervals of 8 h; $x \pm S_x$)

Vessels	Endothelium		Smooth muscles		Fibroblasts of tunica media (A) and externa (B) or adventitial cells (C)	
	1	2	1	2	1	2
Arteries of mixed type						
thoracic aorta	0,7±0,1	139±9*	0,1±0,02	833±119*	0,2±0,1 (A)	455±85
					3,0±0,2 (Б)	34±3
abdominal aorta	1,1±0,6	92±34*	0,2±0,01*	588±32*	0,6±0,4 (A)	169±68
					2,1±1,5 (Б)	48±20
Arteries of muscular type	0,6±0,2	172±40*	1,0±0,5*	99±31	3,0±1,5 (Б)	33±11
Arterioles	3,2±1,6*	32±11*	1,9±0,6*	54±13	3,3±1,0 (В)	31±7
Capillaries	8,0±1,7*	13±3			4,4±1,6 (В)	23±6
Venules	4,1±0,6*	25±4*			4,6±1,3(В)	22±5
Veins of medium and small caliber	3,1±0,9*	32±7*	2,2±0,9*	46±32	4,1±1,1(Б)	25±6
Inferior vena cava	0,5±0,2	192±43	0,5±0,4	217±102*	2,1±1,4(Б)	48±20

Legend. *$p < 0.05$ Compared with smallest value in vertical columns.

It can be concluded from the results of this investigation that with respect to proliferative activity the endothelium is the most labile cellular system of the vessel wall. In vessels of the microcirculatory bed and veins of small and medium caliber, with high concentrations of metabolites and a high level of metabolism, the shortest renewal time of the cell population was discovered in Ec. Fb of the tunica media have equal proliferative activity in all parts of the vascular bed studied. SMC of arterioles are distinguished by higher proliferative activity than these cells in other vessels. No difference was found in the proliferative activity of Ec and Fb in the thoracic and abdominal aorta, whereas ILN for SMC differs here. In terms of the three-component model of a proliferating cell system, suggested by Romanov [5], it can be postulated that the principal tissue components of the vessel wall are evidently similar to parenchymatous organs, where a low level of proliferation is observed, to correspond to the small size of the proliferative pool, the prolonged balloting of the cells and the considerable floating pool, cells of which perform their specific functions; the fixed pool in these tissues is unimportant.

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