EFFECT OF PROSTAGLANDIN $F_{2\alpha}$ ON MICROVESSELS IN A TRANSPARENT CHAMBER IMPLANTED IN THE RABBIT EAR

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Interest of research workers in the study of a new class of natural chemical compounds, the prostaglandins (PG), which play an important role in intracellular control processes, has increased in recent years. PG of the E and B groups are known to stimulate proliferative processes and to influence DNA and collagen biosynthesis and epithelial proliferation. Their effect on repair processes, including on the mechanisms of wound healing, likewise cannot be ruled out [1, 2]. Activity of PG of the F group in relation to repair processes have not been adequately studied. In the investigation described below quantitative parameters of formation of the regenerating microvascular network under the influence of $PGF_{2\alpha}$ were studied by means of intravital observations. The method of implanted chambers combined with biomicroscopy was used for this purpose. The implanted chamber was modernized so that the method of automatic image analysis by means of the Leitz-TAS television analyzing system [4] could be used to quantify the process of regeneration of the microvessels.

EXPERIMENTAL METHOD

In experiments on chinchilla rabbits weighing 2-2.5 kg a transparent chamber [3] was implanted in the ear tissue. The main structural part of the chamber was a narrow closed space (25 μ) between two parallel glass disks, into which 8 radial holes opened. Through these holes the tissue could grow into the space between the disks. A new element in the design was a positioning frame, fixed on the glass disk before assembly of the chamber, so that long-term observations could be made on strictly definite areas of the regenerating microvascular network (Fig. 1a). By means of this positioning frame, there was no need to use complicated transducers to monitor displacement (coordinates chosen to observe areas of the microvascular network) [6], the accuracy of discovery of areas chosen for observation was increased, and measurements of the microvessel could be made over a wider area of the field, up to 44% of the total area of the chamber. An important step in the operation of implantation was reliable hemostasis. This is particularly important because if even a very small quantity of blood should enter the space between the disks, it would make quantitative assessment of regeneration of the microvessels by automatic analysis difficult. Experiments were carried out on 13 chambers. In two series of experiments the dynamics of formation of the microvascular network in the chamber was evaluated quantitatively under normal conditions (7 chambers) and under the influence of $PGF_{2\alpha}$ (6 chambers). Starting two weeks after implantation of the chambers, the animals received daily injections of 0.5 ml $PGF_{2\alpha}$ (from Upjohn Ltd., Canada), in a concentration of 10^{-8} g/ml for 7 days into the internal auricular vein. The chambers were photographed on the 35th and 49th days after implantation, under magnification of 10 and 50. The area of the chamber to be observed, in which the microvessels were measured, was divided by the positioning frame into six standard fragments (magnification 50), each of which corresponded to one photographic frame (Fig. 1b). The total area of projection of the microvessels A_i (in μ^2), the area of the region in which the measurements were made S (in μ^2), the vascularization of the part of the chamber observed A_i/S (in %), the total length of the microvessels L_i (in μ), the relative length of the microvessels LS_i (in μ), the mean diameter of the microvessels D_i (in μ), and the percentage distribution of length and areas of microvessels of different diameters (histograms), were determined from the negatives thus obtained by automatic image analysis.

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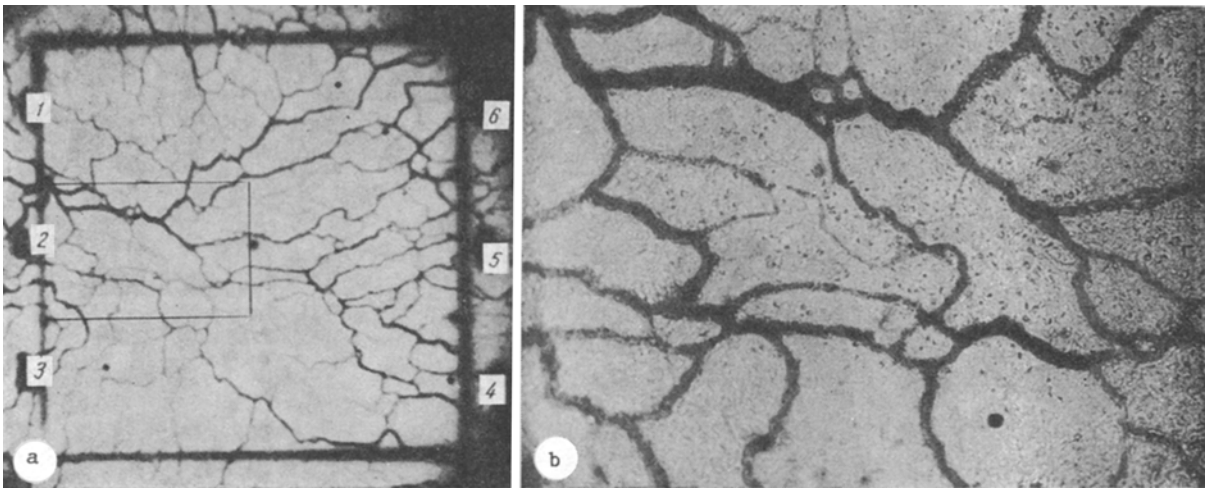

Fig. 1. Microvessels of subcutaneous aureolar tissue of rabbit ear on 35th day after implantation of chamber (intravital microscopy). a) Chamber with positioning frame (objective 2.5, ocular 4); b) fragment No. 2, of positioning frame (objective 10, ocular 5).

TABLE 1. Dynamics of Parameters of Regenerating Microvascular Network under the Influence of $PGF_{2\alpha}$

Experimental conditions	Time of observation, days	Number of changes	Total area of projection of microvessels, μ^2	Vascularization (A_i/S), per cent	Total length of microvessels (L_i), μ	Relative length of microvessels (LS_i), μ/mm^2	Mean diameter of microvessels (D_i), μ
1. Control	35	42	12 631±568	21,5±1,0	3,790±84	6,46±0,15	4,2±0,36
2. Control	49	42	13 186±1 457	22,4±2,5	3,967±288	6,76±0,49	4,13±0,37
P_{1-2}			H	H	H	H	H
3. $PGF_{2\alpha}$	35	36	13 033±693	23,6±1,2	3,228±85	5,50±0,15	5,84±0,47
P_{1-3}			H	H	<0,001	<0,001	<0,001
4. $PGF_{2\alpha}$	49	36	9 347±130	15,9±0,23	2,742±76	4,62±0,13	4,29±0,43
P_{2-4}			<0,05	<0,05	<0,01	<0,01	H
P_{3-4}			<0,001	<0,001	<0,01	<0,01	<0,05

Legend. N) Differences not statistically significant.

The image of the microvascular bed was projected by means of an enlarger from the negative to the Plumbicon of a television camera and transformed into a television signal, which was analyzed by means of programs specially written in TEZIK language (LSI-11 microprocessor). Images of microvessels were isolated automatically at the corresponding gray level in the television signal emitted by the discriminator. The image was automatically "freed" from artefacts by successive application of erosion and reconstruction operations. The area of projection of microvessels of different diameters in the plane of the chamber was measured by means of an erosion operation followed by dilatation.

EXPERIMENTAL RESULTS

Measurements were made on microvessels up to 25 μ in diameter. In control groups of animals at times of observation between the 35th and 49th days no significant differences were found with respect to the above parameters (Table 1). Injection of $PGF_{2\alpha}$ on the 35th day of observation was accompanied by a decrease in the total length of the microvessels by 19% and an increase in their mean diameter by 28%. With these changes in mind, and also the fact that this increase in the total area of the microvessels was not statistically significant, it can be concluded that this reduction in length of the microvascular bed took place on account of microvessels with small diameters. This conclusion also is confirmed by histograms of distributions of length of microvessels depending on their diameter. By the 49th day of observation the total length of the microvascular bed formed after injectionof $PGF_{2\alpha}$ was reduced, both compared with the control at the same time (by 31%) and compared with development of the network in the experimental animals on the 35th day (19%). These changes also were accompa-

nied by a significant decrease in the mean diameter of the microvessels (by 26%) at this same time relative to their value on the 35th day, and this naturally led to a decrease in the total area of the microvascular bed studied (by 33%) and to a decrease in the vascularization index. The mean diameter under these circumstances was virtually indistinguishable from that in animals of the two control groups. Thus by the 49th day of observation, after injection of $PGF_{2\alpha}$ into the animals, not only was the formation of new microvessels delayed, but existing microvessels with large diameters were constricted.

The results on the whole indicate that toward the end of the first month after implantation of the chamber formation of the microvascular network in it, under the experimental conditions specified, was complete. Chronic intravenous injection of $PGF_{2\alpha}$ in the doses used delayed growth of new microvessels, mainly capillaries. Moreover, in the later stages (about 2 months) after implantation of the chambers treatment with $PGF_{2\alpha}$ affected the state of the larger microvessels, leading to constriction of the arterioles and venules. This reaction may be both a manifestation of the direct vasoconstrictor action of the PG studied and the result of the action of mechanisms controlling the inflow of blood to the capillary bed, the volume of which, in the case under consideration, was reduced through the action of $PGF_{2\alpha}$. These results showing a constrictor effect of $PGF_{2\alpha}$ agree with those obtained by other workers [5, 7] who observed a similar effect after local application of $PGF_{2\alpha}$ to microvessels of the rat urinary bladder and meseometrium.

LITERATURE CITED

1. I. S. Azhgikhin (ed.), Prostaglandins [in Russian], Moscow (1978).
2. A. V. Nikolaev, A. B. Shekhter, L. A. Mamedov, et al., in: Prostaglandins in Experimental and Clinical Medicine [in Russian], Moscow (1978), p. 155.
3. M. I. Reutov and A. M. Chernukh, Byull. Éksp. Biol. Med., No. 7, 116 (1977).
4. V. S. Shinkarenko and A. M. Chernukh, Byull. Éksp. Biol. Med., No. 5, 497 (1979).
5. G. S. Dimitrievich, K. Fischer-Dzoga, R. M. Lee, et al., Microvasc. Res., 18, 18 (1979).
6. W. F. Young, R. D. Dey, and R. Echt, Microvasc. Res., 17, 1 (1979).

A BIOPOTENTIAL AMPLIFIER WITH NONLINEAR CURRENT-VOLTAGE CHARACTERISTIC CURVE FOR RECORDING LOW-AMPLITUDE SPIKES

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During electrophysiological research it is sometimes necessary to quantify the frequency of spikes transmitted along nerve trunks or generated by single neurons. For convenience of processing of the results and evaluation of the data in the course of the experiment, specialized instruments are used: frequency meters, integrators, and threshold discriminators [1-5].

Furthermore, if a computer is used in the experiments, it is necessary not only to distinguish spikes of a certain amplitude, but also to form standard spikes with parameters required for leading into the computer. As a rule, the first element of these instruments is a Schmitt trigger. If the useful signal is considerably stronger than noise, tuning the trigger to pick out spikes of a certain amplitude present no difficulty. The task is made difficult if the signal exceeds the noise level of the amplifier or electrodes only a little, for in that case distinguishing spikes above noise by means of the trigger becomes not only difficult, but often impossible. Improvement of the signal to noise ratio by reducing internal noise of the biopotentials amplifier (BPA) and electrodes is not enough. It is essential to obtain a

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