6. A. N. Klimov and V. A. Nagornev, Atherosclerosis Rev., 11, 107 (1983).
7. M. B. Stemerman, Arteriosclerosis, 1, 25 (1981).

EFFECT OF INTRA-AORTIC BALLOON COUNTERPULSATION ON PLATELET ULTRASTRUCTURE AND FUNCTION

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KEY WORDS: counterpulsation; balloon pump; platelets; ultrastructure; functional properties

The intra-aortic counterpulsation method of assisted circulation is widely used in the treatment of acute heart failure. It has been found to be particularly effective in the treatment of cardiogenic shock complicating the course of acute myocardial infarction, and also during the intra- and postoperative periods in patients undergoing aortocoronary bypass surgery [1, 4].

The long-term use of counterpulsation may lead to trauma to the blood cells on account of mechanical action, and also as a result of contact between the blood and the material used in the construction of the intra-aortic balloon pump. Damage to platelets, which is connected with disturbances of clotting, rheology, vascular tone, and other important parameters of homeostasis, assumes particular importance under these conditions. Contact between blood and a foreign surface induces adsorption of plasma proteins and rapid (in the course of a few minutes) adhesion of circulating platelets to it. The resulting activation, under these circumstances, in the main factor in thrombus formation. When new models of balloon pumps are developed, attention must be paid not only to the hemodynamic efficiency of the design, but also the contact properties of polymer materials. Nowadays electron microscopy plays a leading role in the study of the mechanisms of thrombus formation and of surface-induced thrombogenesis, in order to assess the quality of polymers highly resistant to the development of thrombosis and of materials with a modified surface, that in the pure form do not possess this property.

We studied the effect of various materials (polyurethane, "Biomer") used in the intra-aortic balloon pumps developed in collaboration with the "Sever" LPTGO*, on the ultrastructure and function of platelets during experimental counterpulsation.

EXPERIMENTAL METHOD

Experiments were carried out on nine male mongrel dogs weighing 15-20 kg. The animals were anesthetized by intravenous injection of hexobarbital in a dose of 10-15 mg/kg body weight every 60 min. Adequacy of anesthesia was estimated by the usual clinical signs. Artificial ventilation of the lungs with moderate hyperventilation was used during the experiments, so that muscle relaxants were unnecessary. A balloon pump with a volume of 10 cm^3, made of polyurethane or "Biomer" was introduced through the common femoral artery and positioned in the descending part of the arch of the aorta and connected to an assisted circulation apparatus (AVK-5M) working on a 1:1 program. The duration of intra-aortic balloon counterpulsation was 5 h. Blood from the common femoral vein of the anesthetized dogs was collected in siliconized tubes before the experiment began and 5 h later, at its end. Aggregation of the platelets was determined [13] and their number counted. Platelets fixed with glutaraldehyde were separated from blood for electron-microscopic investigation by double centrifugation, post-fixed with OsO_4, dehydrated in increasing concentrations of alcohols, and embedded in Araldite

*Unidentified Russian abbreviation — Publisher.

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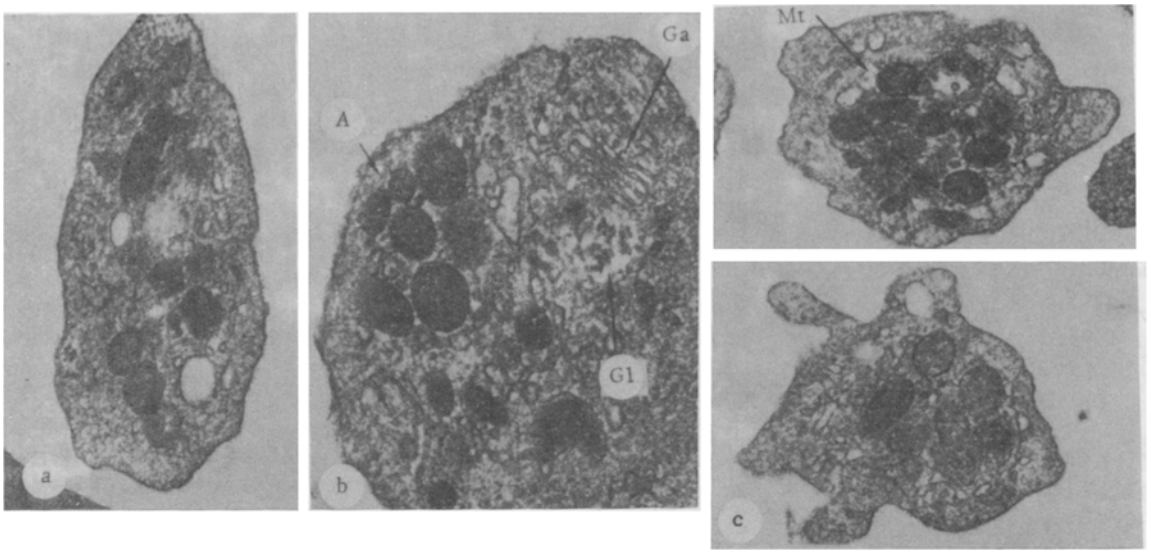

Fig. 1. Ultrastructure of dog's platelets after intra-aortic balloon counterpulsation for 5 h (balloon pump made of "Biomer"): a) platelet with intact structure. 24,300 ×; b) details of structure: A) α-granules; Gl) glycogen; Ga) Golgi apparatus. 75,000 ×; c) structure of activated platelets: deformation, granules shifted toward center by a ring of peripheral microtubules (Mt). 19,500 ×.

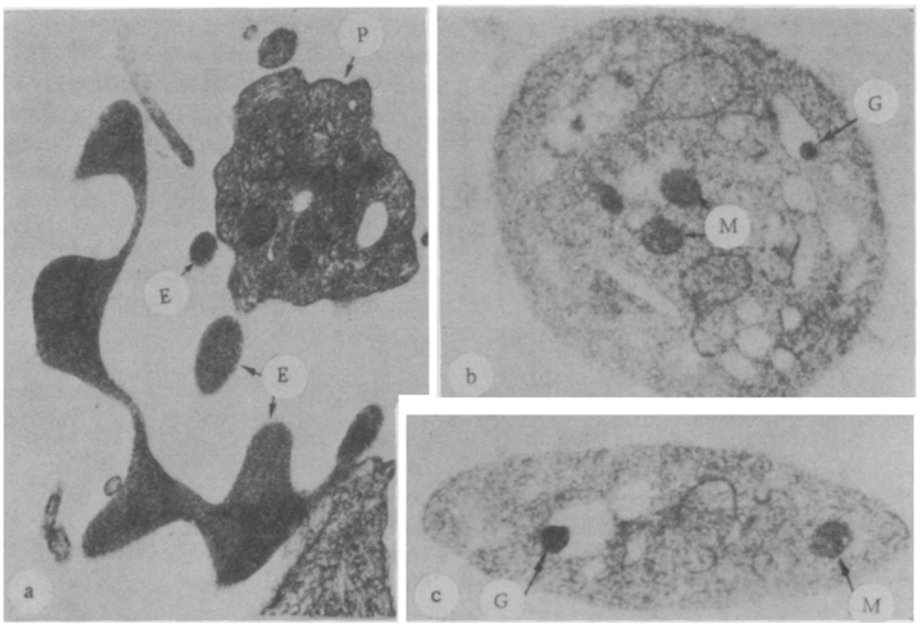

Fig. 2. Ultrastructure of dog's platelets after intra-aortic balloon counterpulsation for 5 h (balloon pump made of polyurethane). a) Activated platelet (P) and destroyed erythrocyte (E); fragments of erythrocytes are visible in field of vision. 14,200 ×; b, c) degranulated platelets; membranes of emptied granules and of granule in vacuole of surface-bound system (G) and mitochondria with high electron density (M) can be seen. Plasma membrane absent, matrix of platelets has reticular structure. 25,000 ×.

[5]. Ultrathin sections were cut on a LKB-III Ultrotome, stained with uranyl acetate and lead citrate, and examined under the JEM-7A electron microscope with accelerating voltage of 80 kV.

EXPERIMENTAL RESULTS

No changes were found in the structure of the dogs' platelets before the experiment.

In version I of the experiment, in which the balloon pump was made of "Biomer," evidence of surface activation of the platlets was observed (Fig. 1): deformation of the platelets, shifting of the granules toward the center by a ring of peripheral microtubules, the formation of small, round pseudopodia, microforms, and reduced electron density of the α-granules in individual platelets. The platelet count varied from 255 · 10^9/liter before the experiment to 200·10^9/liter after its end. The platelet count in the aggregates after the experimet was 19.7%.

In version II of the experiment, in which a polyurethane balloon pump was used, besides the structural changes described above, many degranulated platelets were observed, with discharge of α-granules into the vacuoles of the surface-bound system, destruction of the plasma membrane of the platelets, changes in the structure of the matrix, high electron density of the mitochondria, and numerous microforms. The platelet count varied from 250·10^9/liter to 195·10^9/liter after the experiment. The platelet count in the aggregates was 21.3% after counterpulsation for 5 h (Fig. 2).

The ultrastructure of the dogs' platelets is similar to that of human platelets [6]. They circulate in the blood stream in the form of biconvex disks, the shape of which is maintained by a ring of peripheral microtubules, which constitute the cytoskeleton of the cell. Acid mucopolysaccharides, calcium ions, growth factor, platelet factors 3 and 4, fibrinogen, β-thromboglobulin, adenine nucleotides, hydrolytic enzymes, serotonin, and other α-granules of depots of biologically active substances, which determine the quality of primary hemostasis [3], are located in the α-granules of platelets. Interaction of platelets with thrombin, collagen, and ADP, and their contact with an artificial surface, such as blood, are known to be accompanied by activation and changes in their ultrastructure [8, 12].

A comparative study of the structure of the platelets in the two versions of the investigation revealed a degree of activation of the platelets, due to the following factors: 1) the duration of the assisted circulation; 2) partial destruction of blood cells, raising the blood ADP level from the erythrocytes; 3) the wound surface; 4) the quality of the polymer material from which the intra-aortic balloon pump was made. Changes in platelet ultrastructure are the combined effect of all the factors mentioned above, but, other conditions being the same, any differences observed could be attributed to the polymer material from which the balloon pump was made. Ultrastructural changes in the platelets in version I of the experiment can be assessed as evidence of reversible aggregation, but with preservation of their function [11]. The appearance of microforms in the blood stream is connected with mechanical injury of the blood cells, for fragmentation was more marked in version II of the experiment, and also with utilization of large young, functionally more active, forms on the wound and foreign surfaces. Due to an increase in the number of microforms in the blood stream a comparatively small decrease in the total number of platelets was observed after the experiment. Large platelets are known to be functionally more active and to be utilized first under the influence of various activating factors [9]. It was shown previously that the α-granules of platelets contain serotonin [12]. The reduction of their electron density in the experiment may be connected with utilization of this biogenic amine on the wound surface.

A greater degree of activation of and injury to the platelets was observed in the experiment with the polyurethane balloon pump. Polyurethane is less resistant to thrombus formation than "Biomer." Contact with it leads to damage to the membranes of the platelets, with their fragmentation and degranulation. Changes of this type increased adhesion to the artificial surface and formed biological contact between structures [7, 10].

LITERATURE CITED

1. V. I. Burakovskii and V. G. Barvyn', Assisted Circulation [in Russian], Tashkent (1980) pp. 9-11.
2. V. K. Vashkinel', M. N. Petrov, and G. F. Oksenkrug, Probl. Gematol., No. 10, 41 (1975).
3. V. K. Vashkinel' and M. N. Petrov, Ultrastructure and Function of Human Platelets [in Russian], Moscow (1982), pp. 29-31.
4. L. S. Komarov, Problems of the Artificial Heart and Assisted Circulation [in Russian], Moscow (1970), p. 11.

5. M. N. Petrov, V. K. Vashkinel', and V. A. Almazov, Lab. Delo, No. 1, 22 (1975).
6. S. A. Simbirtsev, N. P. Belyakov, V. K. Vashkinel', and I. N. Zhuravleva, The Artificial Heart and Assisted Circulation [in Russian], Moscow (1981), pp. 15-18.
7. F. G. Uglov, V. N. Zubtsovskii, P. I. Orlovskii, et al., The Use of New Biopolymer Materials in Medicine [in Russian], Moscow (1979), pp. 74-79.
8. R. Allen, L. Zacharski, S. Widirstky, et al., J. Cell Biol., 83, 126 (1979).
9. S. Karpatkin and S. Garg, Br. J. Haematol., 26, 307 (1974).
10. D. Lerche, Ann. N.Y. Acad. Sci., 416, 66 (1983).
11. G. White, Progress in Hemostasis and Thrombosis (T. H. Spaet, ed.), Vol. 2, New York (1974), pp. 49-98.
12. G. C. White, D. E. Scarborough, and K. M. Brinkbous, Ann. N.Y. Acad. Sci., 416, 351 (1983).
13. K. K. Wu and Y. C. Hoak, Lancet, 2, 924 (1974).

EFFECT OF REFLEX THERAPY ON TIME COURSE OF ULTRASTRUCTURAL CHANGES IN MUSCULAR BRANCHES OF THE BRACHIAL PLEXUS

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Reflex therapy, the effectivensss of which has been proved by centuries of practical experience, has become widely used in medicine in recent years. However, the general mechanisms of this phenomenon have not yet been studied. There have been only isolated investigations of nerve fibers at acupuncture points [5] and data have been published on the composition of receptors at the Ximen, Jianshi, and Neiguan points of reflex therapy [4]. The fact that if a needle is inserted into man at an acpuncture point a wide range of somatic sensations arises (a feeling of bursting burning pressure, pain, and heat) indicates activation of the nervous system. The sensations mentioned above can be induced in man by stimulation of well known somatic receptors [3]. According to data in the literature [7] during acupuncture the needle penetrates to a depth of not more than 2.5 cm, i.e., it is often in muscle tissue. Meanwhile, no publications could be found in the accessible literature dealing with the study of the time course of changes in nerves of skeletal muscles at the ultrastructural level under the influence of reflex therapy.

The aim of this investigation was to study the trend of changes in myelinated nerve fibers during reflex therapy at the ultrastructural level.

EXPERIMENTAL METHOD

Experiments were carried out on mature male albino rats weighing 150-170 g. Active points on the forelimb were located by means of a small searching instrument, designed and made by engineers in the Research Department of Appliances and Methods of Measurement, Ustinov Mechanical Institute. Determination of acupuncture points by means of this apparatus is based on the principle of a lower resistance at the point to a direct current than in the zones immediately surrounding it. During the search a direct current with a voltage of 1-10 V was applied for not more than 500 μsec. By means of the instrument it was possible to look for points with different levels of sensitivity (10 different levels). The active point was recorded acoustically by changes in the sound heard in earphones. Muscular branches of the biceps and triceps muscles and also of the flexor digitorum sublimis and extensor digitorum of the forelimb, taken on the 1st, 3rd, 5th, and 10th days of stimulation and 10 days after the end of acupuncture, were used as the test objects. Acupuncture needles up to 5 mm long were inserted under ether anesthesia at points of the forelimb determined with the instru-

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