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ULTRASTRUCTURAL CHANGES IN ERYTHROCYTES AFTER IMPLANTATION OF AN ARTIFICIAL HEART

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UDC 616.12-089.28-07:616.155.1-091.8-076.5

KEY WORDS: erythrocytes; artificial circulation apparatus; artificial heart.

Whether or not satisfactory results are obtained with implantation of an artificial heart is largely determined by the adequacy of the artificial circulation. Under unsatisfactory conditions of artificial circulation, serious injury may take place to the erythrocytes, leading to massive hemolysis. These changes may be observed to a lesser degree even if perfusion is adequate [2, 3, 6-8]. Data have recently been published on changes in erythrocytes in animals with an implanted artificial heart, which can also be regarded to some extent as an intracorporeal artificial circulation apparatus [5, 6, 9]. However, no information could be found in the current literature on the sequence of development of ultrastructural changes in the blood cells after implantation of an artificial heart. This paper is devoted to a study of this problem.

EXPERIMENTAL METHOD

Ten experiments were carried out (Professor V. I. Shumakov) on calves of the Kholmogorok breed weighing 80-120 kg and aged 3-4 months. In the course of the experiments an artificial circulation apparatus (ACA) was connected and continued to function until an artificial heart (AH) of the "Poisk" type was connected to the blood flow.

Blood samples were taken 5 min and 1 and 2 h after the ACA began to work, at the time of its disconnection, and also 1, 3, 6, 9, and 12 h after the beginning of functioning of the AH. A cell count was carried out on the blood samples: Blood films were fixed with methanol, stained by the Romanovsky–Giemsa method, and examined under the microscope in the usual way to determine structural changes in the erythrocytes. For electron-microscopic examination, the blood cells were fixed with glutaraldehyde, postfixed with osmium

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 4, pp. 406-408, April, 1980. Original article submitted May 16, 1979.

0007-4888/80/8904-0420$07.50

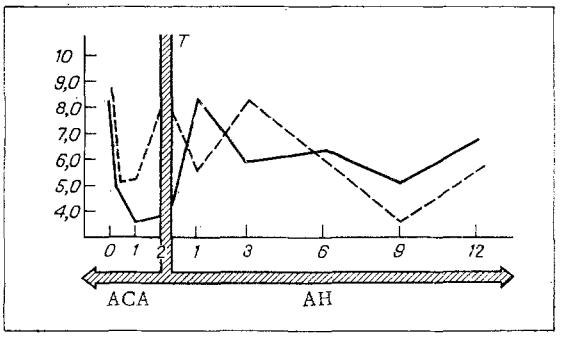

Fig. 1. Fluctuations in total erythrocyte count during functioning of ACA and AH. T) Time of connecting AH. Abscissa, duration of ACA and AH (in h); ordinate, erythrocyte count (in millions/mm^3). Continuous line – expt. No. 79, broken line – expt. No. 86.

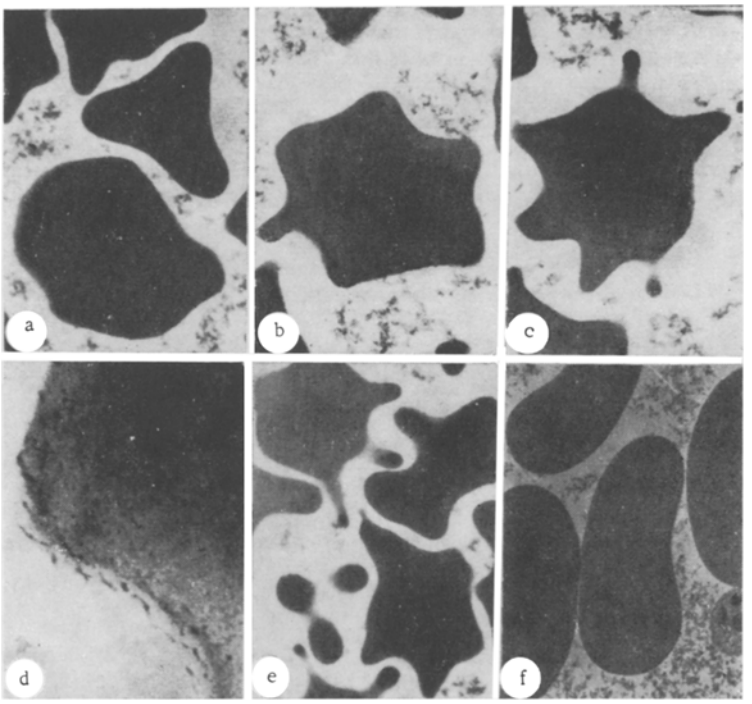

Fig. 2. Calf erythrocytes after functioning of ACA for different times: a) 5 min, b) 1 h, c) 2 h; d) separation of cell membrane of erythrocyte, after functioning of ACA for 2 h; e) fragmentation of erythrocytes after functioning of AH for 1 h; f) calf erythrocytes after functioning of AH for 3 h. Magnification: a) 6000; b, c, e, f) 10,000; d) 30,000.

tetroxide, embedded in Epon-Araldite, stained by Reynolds' method, and examined in the UEM-100V microscope.

EXPERIMENTAL RESULTS

After the ACA had been in operation for 5 min the erythrocyte count was 20-40% lower than initially (Fig. 1). Anisocytosis and poikilocytosis of the erythrocytes were found. Further light on these findings was shed by electron-microscopic investigation. More than half of the erythrocytes were not of the characteristic dumbbell-shape. Many erythrocytes had wide processes. Their cell membrane was indistinct in some parts. The distribution of the hemostromatin complex was relatively uniform (Fig. 2a).

After functioning of the ACA for 1 h, the stained blood films often showed erythrocytes of "mulberry" appearance. Furthermore, throughout the period of functioning of ACA numerous fragments of erythrocytes of hyperchromic character were observed in the blood films. Electron-microscopic investigation at this stage revealed the presence of erythrocytes with digitate, spinous, and club-shaped processes, with high osmiophilia (Fig. 2b).

Starting from the 2nd hour of operation of ACA, many reticulocytes and also a few normoblasts appeared in the blood of the experimental animals. The proportion of normoblasts to erythrocytes varied in different cases from 2 to 4%. After disconnection of the ACA the number of normoblasts in the peripheral blood fell gradually to 1%. All erythrocytes appeared considerably deformed on the electron-micrographs at this period of functioning of the ACA (Fig. 2c). The membrane of these cells was separated over quite wide areas (Fig. 2d). The distribution of the hemoglobin was in discrete clumps and, in some cells, there was a tendency toward its concentration in the center. Fragmentation of the erythrocytes was marked. Destructive forms of erythrocytes with escape of the intracellular osmiophilic substance into the plasma (erythrocyte ghosts) also appeared.

After disconnection of the ACA and connection of the AH the structural changes in the erythrocytes remained the same for 1 h as after functioning of the ACA for 2 h (Fig. 2e). Later, however, if the AH functioned adequately, a gradual increase in the number of erythrocytes with the normal structure was observed. For instance, after functioning of the AH for 3 h the proportion of normocytes in the calf's peripheral blood was about 50% and the distribution of hemoglobin was uniform (Fig. 2f). Meanwhile, in some erythrocytes concentration of the hemoglobin in the center of the cell in the form of a "pseudonucleus" was observed. Later these cells disappeared (on average, 6 h after connection of the AH).

The results described above indicate that connection of the ACA is associated with considerable trauma to erythrocytes circulating in the animal's (calf's) blood stream. Destructive changes in the erythrocytes increased, as the results of light and electron microscopy indicate, gradually in the course of the experiment. After 5 min of functioning of the ACA the first signs of lysis of the erythrocyte cytolemma appear, and it seems likely that these can be connected with mechanical influences on the erythrocyte surface. Later, structural changes in the erythrocytes increase. A picture resembling ultrastructural changes during hemolysis following sharp disturbances of the osmotic conditions and disturbance of the chemical structure of the erythrocyte cytolemma, especially its lipid component, begin to be observed under these circumstances. During connection of the AH, if functioning adequately, a compensatory reaction of the body begins to take place. As a result of stimulation of the calf's bone marrow, young cells of the erythrocyte series (up to normoblasts) are liberated into the blood stream. Observations thus show that after connection of the AH conditions are created in the body for replacement of the changed erythrocytes in the peripheral blood. This process evidently depends on increased liberation of young forms of erythrocytes from the recipient's hematopoietic organs and elimination of erythrocytes changed in the course of the artificial circulation.

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