## REFERENCES

- A. A. Pal'tsyn, E. G. Kolokol'chikova, I. A. Grishina, et al., Arkh. Pat., No. 5, 15-19 (1994).
- A. A. Pal'tsyn, E. G. Kolokol'chikova, N. V. Chervonskaya, et al., Byull. Eksp. Biol. Med., 119, No. 6, 665-668 (1995).
- M. Brown and P. Williams, Annu. Rev. Microbiol., 39, 527-556 (1985).
- 4. W. R. Clark, The Experimental Foundations of Modern Im-
- munology, New York (1980).
- P. Cornelis, D. Hohnadel, and J. Meyer, *Infect. Immun.*, 57, 3491-3497 (1989).
- E. Griffiths, H. Chart, and P. Stevenson, in: Virulence Mechanisms of Bacterial Pathogens, J. A. Roth (Ed.), Washington (1988), pp. 121-137.
- 7. D. Law and J. Kelly, *Infect. Immun.*, **63**, 700-702 (1995).
- 8. M. Rosok, M. Stebbiins, K. Connelly, et al., Ibid., 58, 3819-3828 (1990).

# **Effect of Direct Cardiac Massage** on Peripheral Blood Erythrocytes

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The effect of direct cardiac massage on peripheral blood erythrocytes is examined. A significant increase in the count of transitory reversible forms of erythrocytes is observed after 1 h of direct cardiac massage without no significant changes in the hemoglobin content, hematocrit, and erythrocyte and reticulocyte counts. It is concluded that direct cardiac massage produces less severe damage to erythrocytes than other means of assisted circulation.

Key Words: direct cardiac massage; erythrocyte morphology; scanning electron microscopy

A relationship between erythrocyte damage and progressive microcirculatory disorders upon artificial [1,5,7] and assisted circulation [6] has been established. However, the information regarding the effect of direct cardiac massage (DCM) on peripheral blood erythrocytes is scanty.

Our goal was to examine the state of erythrocytes and their count upon DCM.

# MATERIALS AND METHODS

Experiments were performed on 10 mongrel dogs (body weight 8-10 kg) maintained under the standard vivarium conditions.

After droperidol premedication, pentobarbital (30-40 mg/kg) and intubation anesthesia were provided, and neuroleptic analgesia was used during experiment.

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Routine thoraco- and pericardiotomy were performed, and the heat was exposed. Direct cardiac massage with an appropriate assistor was started after a 10-min heart arrest. The massage lasted 4 h. Hemodynamic parameters were monitored throughout the entire experimental period.

Blood was collected from the femoral vein immediately before, 1, 2, and 4 h after the start of DCM. Hematocrit, hemoglobin content, and erythrocyte and reticulocyte counts were determined by conventional methods. For identification of the lipoprotein complex blood smears were stained by the method of Barenbaum (1956), and light absorbance was measured in a LYuMAM-I2 microscope by one-beam photometry at 590 nm. Preparations for electron microscopy were processed by routine methods and studied in a REM-200 electron microscope [3,4].

The results were analyzed using Student's t test.

TABLE 1. Effect of Direct Cardiac Massage on Blood Parameters

Parameter	Before DCM	DCM, h		
		1st	2nd	4th
Hematocrit	0.41±0.03	0.39±0.07	0.40±0.03	0.38±0.07
Erythrocyte count, ×1012/liter	6.65±0.38	6.08±0.93	6.10±0.58	5.81±0.47
Hemoglobin, g/liter	159.2±10.4	149.6±15.4	137.6±11.4	145.6±12.5
Reticulocytes, ‰	2.75±0.75	5.50±1.89	4.20±0.74	4.60±1.08

### RESULTS

There were no statistically significant changes in hematocrit, hemoglobin content, and erythrocyte and reticulocyte counts during DCM (Table 1), although these parameters tended to decrease throughout the experiment. By the first hour, erythrocyte count decreased by 8.7%, hemoglobin content by 6.0%, and hematocrit by 5.3%. During the second hour, erythrocyte count and hematocrit remained stable and slightly dropped by the fourth hour. The hemoglobin content was minimal after 2 h of DCM, then it slightly increased to the level of 91.5% of the initial value. It was reported that artificial circulation devices [1,5,7] and some assisted circulation techniques (central and intra-aortic counterpulsation and intraventricular pulsation [6]) induce much more pronounced changes in these parameters.

Scanning electron microscopy showed that in anesthetized dogs the bulk of the peripheral blood erythrocyte population is made by biconcave diskocytes ( $85.72\pm1.26\%$ ). Cells capable of reverse transformation amounted for  $6.87\pm1.66\%$ . The count of irreversibly changed and hemolytic erythrocytes was  $6.99\pm16.95\%$ , while that of erythrocyte with degenerative changes was  $0.46\pm0.14\%$  (Table 2).

Direct cardiac massage induced a statistically significant (p<0.001) decrease in diskocyte count by 17, 32, and 50%, respectively, after 1, 2, and 4 h DCM compared with the initial value. This resulted predominantly from an increase in the proportion of reversible transitory forms of erythrocytes (ellipses, flat disks, diskocytes with a crista, one or several processes, and mulberry-like cells). The total count of these forms was respectively,  $13.69\pm5.03$ ,  $28.26\pm8.29$ , and  $47.7\pm3.97\%$  after 1, 2, and 4 h of DCM (Fig. 1, b).

The total count of irreversible prehemolytic forms (spherical, domed, and flat-ball) reached the maximum by the first hour of DCM ( $14.43\pm3.24~\nu s$ .  $6.99\pm1.95\%$  before DCM). Then it gradually decreased, being equal to  $7.12\pm1.27\%$  after 4 h of DCM, i.e., slightly higher compared with the initial value. This increase was due predominantly to a rise in the number of spherical erythrocytes (Fig. 1, b, c).

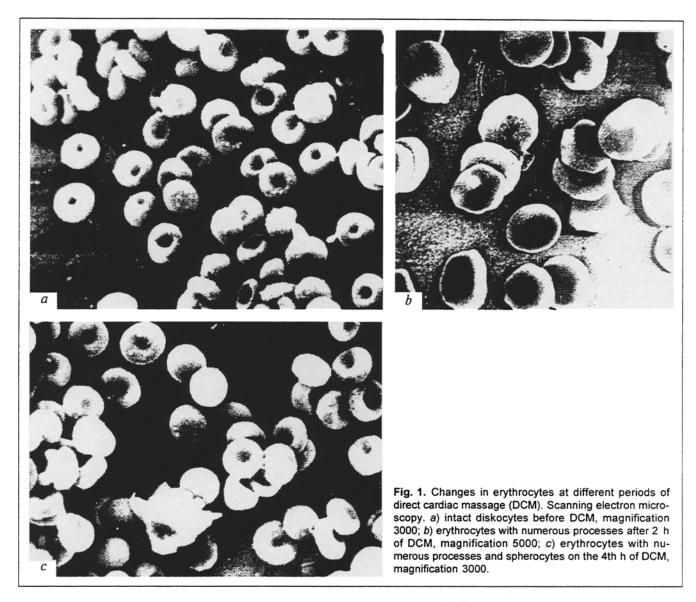
Direct cardiac massage induced no statistically significant differences in the percent of degenerative forms, although a tendency toward an increase in their count was observed.

Our data on the surface architectonics of erythrocytes are consistent with changes in the lipoprotein content of these cells. A decrease in the lipoprotein content is associated with structural perturbations in

TABLE 2. Morphological Types of Erythrocytes Before and During Direct Cardiac Massage (M±m, %)

Morphological type	Before DCM	DCM, h		
		1st	2nd	4th
Diskocyte	85.72±1.26	70.90±1.99***	57.98±6.38**	42.69±3.93***
Flat disk	0.95±0.26	1.81±0.23***	0.86±0.30***	1.57±0.28***
Diskocytes with a process	1.41±0.24	1.85±0.25	2.84±0.40*	2.81±0.26**
Diskocytes with a crista	2.27±0.39	3.69±0.54	5.65±1.66*	2.73±0.67
Processed diskocytes	2.12±0.69	5.76±3.64	17.23±5.38*	38.02±2.16***
Mulberry-like	0.12±0.08	0.58±0.37	1.68±0.55*	2.57±0.60***
Domed	1.74±0.54	2.25±0.26	0.98±0.13	0.91±0.15
Spherical	4.38±1.10	11.45±2.74*	10.28±1.22**	5.71±0.94
Flat-ball	0.87±0.31	0.73±0.24	0.69±0.25	0.50±0.18
Degenerative	0.46±0.14	0.99±0.50	1.81±1.06	2.49±1.39

Note. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 compared with the initial value.



the erythrocyte plasma membrane, which increases the membrane permeability for sodium ions and changes erythrocyte shape [2]. A 21% decrease in lipoprotein content of the erythrocyte plasma membrane was observed after 1 h of DCM. Then it gradually increased, being equal to 96.5% of the initial value by the 4th hour of DCM, which is consistent with the spherocyte content after this time period.

After comparing our findings with the data on the damaging effects of other means of assisted circulation on erythrocytes [1,5,7], we have concluded that DCM produces a smaller damaging effect on these cells. We did not observe any increase in the number of erythrocytes with degenerative changes during 4 h of DCM.

Thus, a 4-h DCM after a 10-min heart arrest produces a moderate damaging effect on peripheral blood erythrocytes compared with other means of artificial and assisted circulation.

### REFERENCES

- G. A. Boyarinov, V. P. Smirnov, L. B. Snopova, et al., Byull. Eksp. Biol. Med., 103, No. 3, 281-283 (1987).
- Yu. I. Grinshtein, "Mechanism of progression of renal insufficiency and endovascular therapy in renal disorders," Author's Synopsis of Doct. Med. Sci. Dissertation [in Russian], Krasnoyarsk (1994), pp. 76-90.
- 3. G. M. Kozinets, I. V. Ryapolova, Z. G. Shishkanova, et al., Probl. Hematol., No. 7, 19-21 (1977).
- 4. G. I. Kozinets and Ya. A. Samivart, Surface Architectonics of Peripheral Blood Cells in Health and Blood Diseases [in Russian], Tallin (1984).
- L. D. Krymskii, A. A. Mozhina, and S. N. Tereshchenko, Arkh. Pat., No. 7, 46-54 (1985).
- T. V. Chechelashvili, "Effect of artificial circulation on hemostasis," Author's Synopsis of PhD Dissertation [in Russian], Moscow (1982).
- D. V. Shporova, "Changes in blood cells upon artificial heart implantation," Author's Synopsis of PhD Dissertation [in Russian], Moscow (1982).