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Studies In Blood Preservation

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Fate of Cellular Elements in Relation to Potassium Diffusion

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IN A PREVIOUS publication[1] we reported a tenfold rise in plasma potassium of preserved blood. This was not due to bacterial contamination. It was increased by trauma, such as shaking, and was definitely modified by the area of the interface between the sedimented cells and the overlying plasma.

The question was raised as to whether this increase in plasma potassium was a pure diffusion process or whether it was due to actual cell destruction. This aspect of the problem is reported here.

METHOD

Five cubic centimeters of freely flowing venous blood were collected in each of 35 sterile round-bottomed test tubes containing 5.0 mg. of heparin as an anticoagulant. After inverting each tube three times, they were plugged with cotton and kept in a refrigerator at approximately 4° C. throughout the period of the experiment.

Each day one tube was taken from the refrigerator, and centrifuged for one hour. After 0.5 c.c. of plasma had been removed for potassium analysis,[2] and after the blood had been thoroughly mixed by inverting the tube fifteen times, the following determinations were made:

(1) Red cell count.
(2) Hemoglobin (Hellige).
(3) White cell count.
(4) Differential white cell count.
(5) Platelet count.
(6) Mean cell diameter of red blood cells (Halometer method).

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RESULTS

The donor, A. J., was a professional man of International Group 0.

	Venous Blood		
	At start of phlebotomy	At end of phlebotomy (590 c.c.)	
Hematocrit	46.0	44.7	per cent cells
Plasma specific gravity	1.0286	1.0266	
Plasma proteins	7.38	6.70	gm. per cent
Whole blood potassium	212.0		mg. per cent
Plasma potassium	21.5		mg. per cent
Cell potassium (calculated)	434.0		mg. per cent
pH		7.51	

Values on Initial 5.0 c.c. Sample*		
Red blood cell count	5,500,000.0	
Hemoglobin	14.2	gm. per cent
White blood count	7,000.0	
Differential white cell count		
Polymorphonuclear leucocytes	52.0	per cent
Eosinophilic leucocytes	2.0	per cent
Basophilic leucocytes	1.0	per cent
Lymphocytes	42.0	per cent
Monocytes	3.0	per cent
Platelets	140,000.0	
Mean cell diameter	7.4	
Plasma potassium	20.6	mg. per cent

The results are graphically represented in Charts 1 to 4.

*Corrected for 0.5 c.c. plasma removed.

DISCUSSION

Red Blood Cells.—The maintenance of the red blood cell counts at approximately a constant level over a period of a month suggests that, in the steady increase of the potassium content of the plasma, cell destruction plays at most only a small part.

Durán Jordá and his co-workers[3] reported there was a loss of 1,500,000 erythrocytes over a period of sixteen to twenty days in blood conserved in citrate. They suggested the stroma of these destroyed cells might play a part in some of the slight posttransfusion reactions. We have not found changes as great as they report. The fact that all of our tubes were centrifuged for an hour before each count may play some part in this discrepancy.

Yudin,[4] from Russia, has reported that there is no loss in ability of cells so stored to carry oxygen, hence the chief biological function of the red blood cells seems unimpaired.

Hemoglobin.—The hemoglobin content remains constant. This confirms the findings of previous writers. The work of Amberson[5] has indicated that the part of the hemoglobin which escapes from the cells to give the usual picture of hemolysis is still capable of functioning normally.

Volume Index and Diameter of Red Blood Cells.—The volume index of the cells decreases in successive specimens. There is a diminution in white blood cells, but the greater part of the decrease seems due to the diminution of the size of the red blood cells. At the end of thirty days these cells may lose as much as 25 per cent of their chief base, 20 per cent of their hemoglobin, and decrease 20 per cent in diameter. Ponder[6] has shown that red blood cells do not

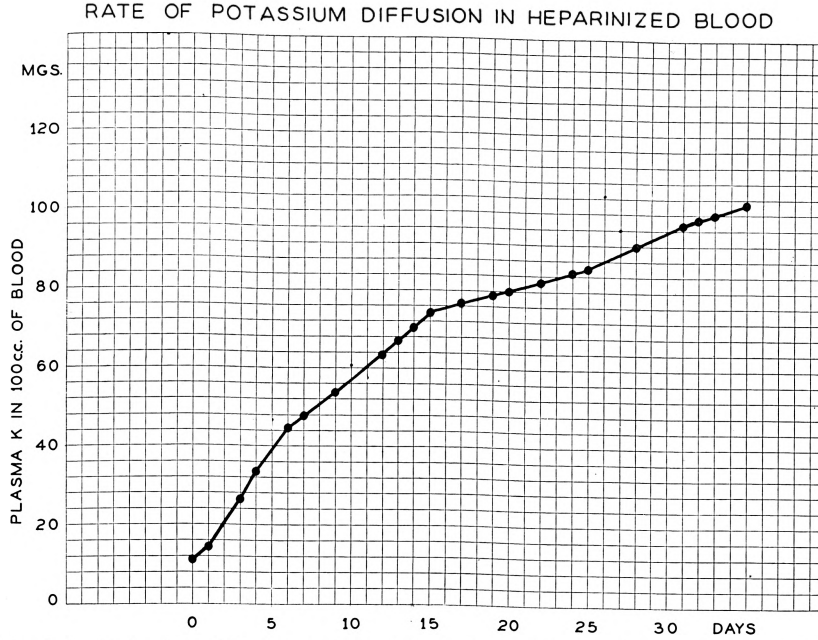

Chart 1.—Potassium diffusion in heparinized blood. Each point on the chart represents a determination done on different test tube samples each day and is calculated to represent the actual amount of potassium in milligrams in the plasma of 100 c.c. of blood. The values are approximately the same as in previous experiments where daily determinations were done on the same blood with the final curve representing a summation of the increments.

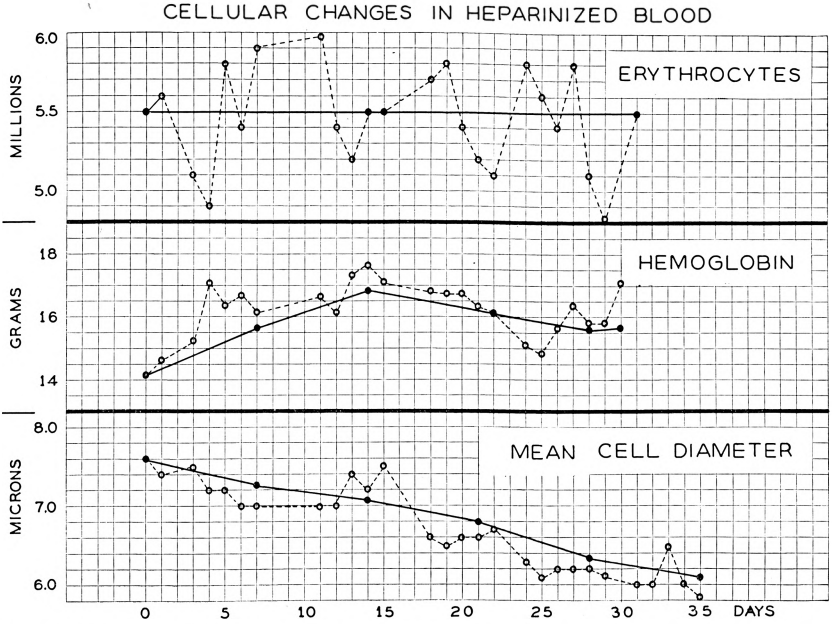

Chart 2.—*Upper third.* Red cell counts varied between 6,200,000 and 4,800,000, the mean being 5,500,000. No actual loss in number of red blood cells.

Middle third. Hemoglobin values varied between 14.2 and 17.6 gm. per cent. The curve, allowing for slight concentration due to evaporation, represents a constant hemoglobin content. The drop after the fifteenth day may be accounted for by the gradually increasing amounts of hemogrobin removed in the plasma taken for potassium determinations.

Lower third. There was a gradual decrease in the size of the red blood cells from an initial 7.6 microns to a final 5.8 microns as the cells lost both hemoglobin and potassium.

always act as perfect osmometers, a fact which he attributes to the possible loss of their salt content.

White Blood Cells.—The total white blood cell count rapidly diminishes with the polymorphonuclear leucocytes disintegrating most rapidly. The reported loss of half of the complement of the blood by the sixth or seventh day, and complete destruction in fifteen to twenty days, may be associated with this loss of leucocytes.[3]

The lymphocytes are more resistant. At the end of thirty days they are easily recognizable when found, but there are so few that counts are uncertain. This holds true, likewise, for monocytes.

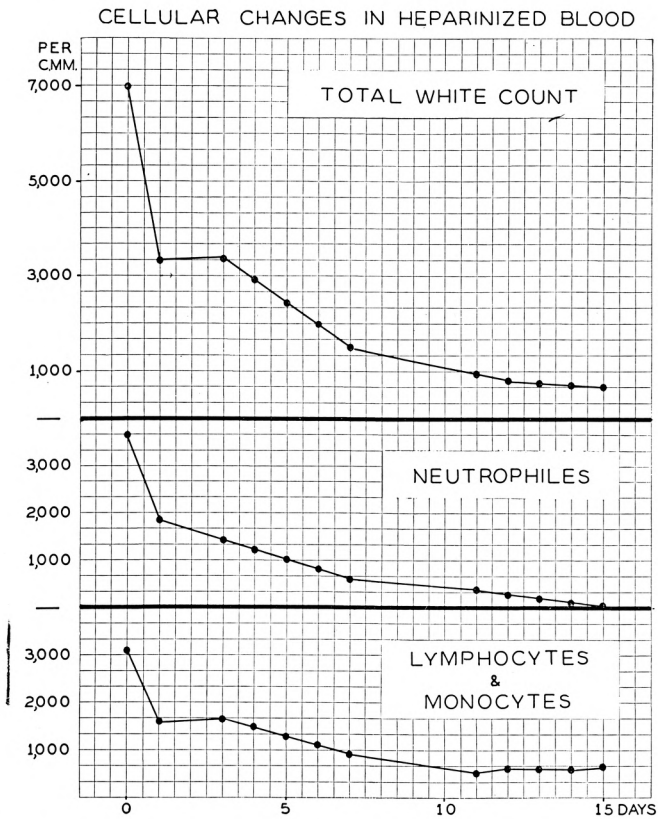

Chart 3.—*Upper third.* The total white blood cell count fell 50 per cent in twenty-four hours, the cells lost shape and gradually disappeared by the sixteenth day; those remaining appearing only as gelatinous-like clumps. Range 7,000 to 675 cells.

Middle third. The polymorphonuclear leucocytes showed earliest and most rapid changes; their nuclei soon losing shape and disintegrating. Range, 3,600 to 14 cells.

Lower third. Lymphocytes and monocytes disappeared at a slower rate and retained their shape, size, and staining better than the leucocytes, being quite distinct when found on the thirtieth day. Basophilic and eosinophilic cells, while too few to be charted, retained their shape, size, and staining qualities. The latter were least changed and very distinct on the thirtieth day. Range, 3,100 to 654 cells.

The eosinophiles appear most resistant. The nucleus breaks up late and the granules remain unusually well defined.

Durán Jordá[3] has reported similar findings.

Thrombocytes.—The platelets in the initial venous blood sample numbered 140,000. These rapidly diminished in the first three days and remained at about

30,000 for fifteen days. Centrifugation may have some part in these counts. This suggests, however, that syndromes presenting thrombocytopenia would not be benefited by blood which had been stored too long.

Potassium.—The rate of potassium loss from the cells in the individual test tubes is of the same order as that found in the flasks from which daily samples were taken. The potassium content of white blood cells (those obtained from the buffy coat in case of lymphatic leucemia) is greater than the red blood cells. In normal blood their relative number is insignificant. However, their early rapid destruction may account in part for the steep slope of the curve during the first few days.

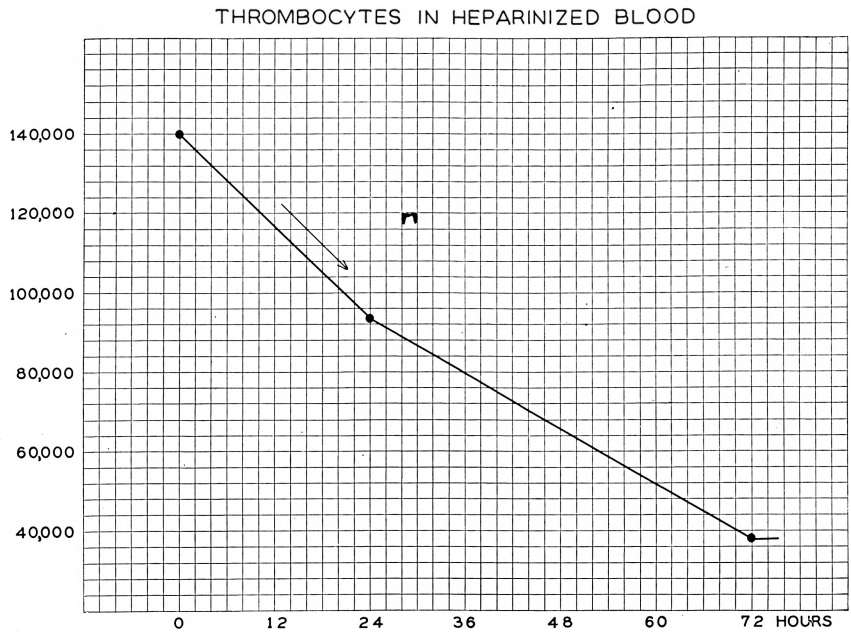

Chart 4.—Thrombocytes rapidly fell to about 40,000 and for thirty days remained approximately at this figure. Only the first three days are charted.

CONCLUSIONS

1. In heparinized preserved blood there is little or no actual loss in the number of red blood cells over a period of thirty days.

2. The hemoglobin content remains constant in the total sample, though 15 to 25 per cent may be found in the plasma.

3. The mean cell diameter of the red blood cells is reduced approximately 20 per cent in thirty days.

4. The polymorphonuclear leucocytes are diminished by 50 per cent in forty-eight hours and are amorphous masses in fifteen days.

5. The lymphocytes, monocytes, and eosinophiles do not disintegrate so rapidly, the latter are particularly well preserved.

6. The thrombocytes rapidly fall to a low level, then remain constant at about 30,000 for about fifteen days.

7. Potassium in the plasma of heparinized blood reaches a level ten times normal in thirty days.

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