ENERGY METABOLISM OF PLATELETS

N. B. Chernyak, M. B. Sventsitskaya, and Ch. S. Guseinov

From the Order of Lenin Central Institute of Hematology and Blood Transfusion (Director – Active Member, Acad. Med. Sci. USSR, A. A. Bagdasarov) of the USSR Ministry of Public Health, Moscow

(Presented by Active Member, Acad. Med. Sci. USSR, S. E. Severin)

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Among the formed elements of the blood, the structure, composition, and metabolism of the thrombocytes (blood platelets) have been least studied. We need only point out that up to now it has not been conclusively established whether the platelets are cells or fragments of the cytoplasm of megakaryocytes, and that the question of whether a nucleus is present in these elements also has not been entirely settled. Negative results obtained in searching for desoxyribonucleic acid in platelets by various chemical and histochemical methods caused Maupin [2] to classify them as "incomplete" cells.

It is well known, however, that these formed elements are characterized by the presence of several enzymes [2], and play a substantial role in the process of blood coagulation.

As noted above, the metabolism of platelets has not been extensively studied. Tullis [3], who found only insignificant glucose and oxygen consumption and lactic acid accumulation, believes that platelets have little or no metabolic activity. Other authors, however, hold the opposite view [1].

METHOD AND RESULTS

Experiments were conducted on platelets removed from the blood of donors on the same day it was drawn, by the method of two-step centrifugation in a refrigerated centrifuge at +4°. For the prevention of agglutination and traumatization of platelets, a solution of Trilon B (the disodium salt of ethylenediaminetetraacetic acid) was added to the suspension. From 800,000 to 3,000,000 platelets were contained in 1 mm of suspension. The platelet suspension in plasma was of a high degree of purity.

The platelet suspension was studied on the day it was prepared, or the day following. Respiration was determined manometrically in a Warburg apparatus with the flasks saturated with oxygen; glycolysis, from the accumulation of lactic acid, the amount of which was determined colorimetrically with p-hydroxyphenyl; sugar, by the method of Hagedorn and Jensen, with cadmium reagent; and ATP, after an 8-minute hydrolysis of a trichloroacetic filtrate in 1N HCl, for inorganic phosphate, by the method of Fiske and Subbarow.

RESULTS

It was found that platelets consume oxygen and have the capacity for glycolysis. It is characteristic that, like leukocytes (granulocytes), platelets carry on glycolysis under both aerobic and anaerobic conditions, anaerobic glycolysis being considerably more intense than aerobic (Table 1).

The values cited are for 1 ml of densely packed platelets incubated for two hours. It is likewise characteristic that the addition of glucose caused a depression of oxygen consumption by the platelets (see figure).

A depression of respiration was observed following the addition of various amounts of glucose (Table 2).

The concentration of sugar in the platelets, and the consumption of sugar during incubation were small. When glucose was added to the platelet suspension, its consumption increased, especially under anaerobic conditions, in comparison with samples to which glucose was not added although not all the glucose in the latter samples was utilized.

TABLE 1. Respiration and Glycolysis by Platelets

Experiment No.	Composition of sample*	Incubation conditions	Respiration ($\mu l\ O_2$)	Glycolysis (increase in lactic acid in mg)	Sugar consumption (in mg)
1	A	O_2	1 345	3,9	3
	B	O_2	872	3,6	29
2	A	O_2	—	0,60	2,1
	B	O_2	—	0,85	19,2
3	A	O_2	—	3,5	3,4
	B	O_2	—	5,9	12,8
	B	N_2	—	11,1	50,6
4	A	O_2	1 080	2,5	1,3
	B	O_2	976	2,7	9,5
	B	N_2	—	6,2	26
5	A	O_2	1 832	3,2	7,0
	B	O_2	1 758	2,6	8,4
	B	N_2	—	11,4	47

*(A - without additional glucose; B - with additional glucose.

The data presented show that platelets possess rather vigorous energy metabolism.

TABLE 2. Effect of Various Concentrations of Glucose on Respiration and Glycolysis by Platelets

Respiration and glycolysis	Composition of samples			
	no glucose added	glucose added		
		200 mg%	600 mg%	4,000 mg%
O_2 consumption in μl per hour	1,836	1,532	1,668	1,338
Glycolysis (increase in lactic acid per mg in 2 hr).	6.4	7.2	6.0	9.6

TABLE 3. Respiration and Glycolysis by Platelets During Storage (series no. 8)

Indices	Days of storage					
	first		fifth		tenth	
	no glucose added	glucose added	no glucose added	glucose added	no glucose added	glucose added
O_2 consumption, in μl	704	564	566	600	352	330
Content, in mg, of:						
lactic acid	9.0	9.0	20	21.6	23.8	28.2
sugar.	42	381	22	381	18	359
ATP	0.30	0.36	0.40	0.42	0.29	0.22
inorganic P.	1.80	1.54	1.87	1.71	1.75	1.70
Retraction, in %. . .	77	81	45	63	–	–

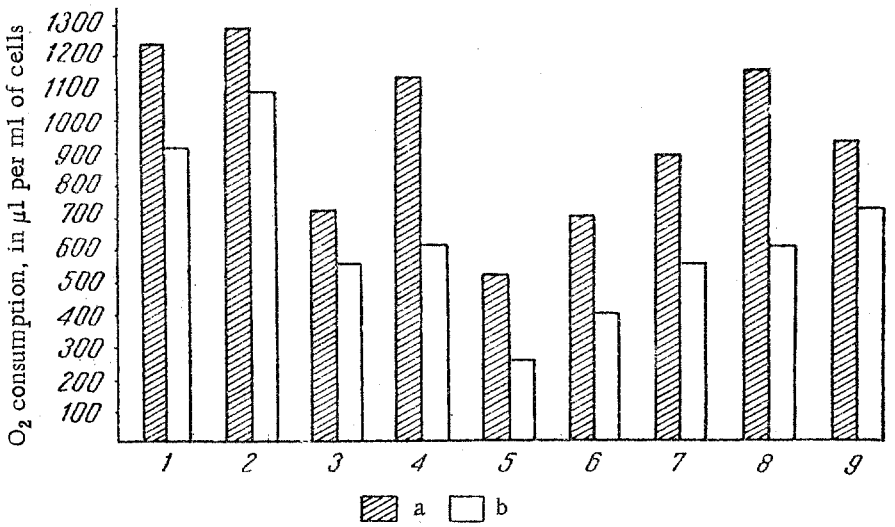

Figure. Reduction in 0_2 consumption by a platelet suspension following addition of glucose. a – Samples without additional glucose; b – Samples with additional glucose.

Subsequent experiments were for the purpose of determining whether it is possible to keep platelets in a viable condition. To this end, our thrombocyte suspension was divided up into individual samples, which were stored in the refrigerator at +4° – +6° for ten days, and examined on the first, fifth, and tenth day of storage. During storage, the analyses mentioned earlier were carried out, and in addition, clot-retraction capacity was determined as an indication of the viability of these formed elements. The amount of retraction was noted after a one-hour incubation of plasma together with platelet suspension diluted with a solution of calcium chloride. As the retraction capacity is reduced, the density of the clot formed decreases, more fluid being

retained in it. The amount of fluid remaining was determined in the graduated portion of a special flask in which the determination was made and was compared with the initial value, which was taken as 100%.

Study of the platelet suspension during the storage process showed that the respiration rate gradually falls, but remains at a sufficiently high level during the storage intervals investigated. The increase in lactic acid sometimes stops in the first few days of storage, or continues until the end of the observation period. The inorganic phosphate content does not show any substantial changes. The quantity of ATP (or rather, of readily hydrolyzable P) is reduced somewhat, but this fraction does not disappear entirely (Table 3).

The results are given on the basis of 1 ml of densely packed platelets. Sometimes an increase in the content of readily hydrolyzable P was observed during the final periods of storage, as in the case of storage of erythrocytes. It should be noted that the stability of this phosphorus fraction in platelets can be regarded as one of the characteristic features of these formed elements. The retraction capacity of the platelets is largely preserved for several days, being more pronounced in samples to which glucose was added. Maintenance of energy metabolism at a rather high level for a prolonged time argues in favor of retention of the physiological capacities of the platelets.

Our data do not conform to the generally accepted view that platelets are extremely unstable. The preservation of respiration for a long period, as well as the relative constancy of the content of readily hydrolyzable phosphate, does not support this view.

On the basis of the observed stability of platelet metabolism in storage, we may conclude that preservation of these elements largely depends on storage conditions. The duration of platelet storage in a physiologically normal condition can apparently be increased by the selection of factors that will promote the preservation of their metabolism.

SUMMARY

Platelets isolated from the blood of donors vigorously consume oxygen and carry on glycolysis, under both anaerobic and aerobic conditions. Addition of glucose brings about a reduction in the oxygen consumption rate. During storage of a platelet suspension under sterile conditions at 4° C., respiration and the ability to accumulate lactic acid are preserved. The level of the readily hydrolyzable phosphorus fraction remains relatively constant.

LITERATURE CITED

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