RATE OF METABOLISM OF BONE MARROW PHOSPHOLIPIDS UNDER NORMAL CONDITIONS AND IN DISTURBANCES OF ERYTHROPOIESIS

A. V. Kargapolov

UDC 616.155.1-007.1-07:616.419-008.939.53

The rate of metabolism of the individual phospholipid fractions under normal conditions, in anemia produced by blood loss, and in the posthemorrhagic period was studied in rabbit bone marrow homogenates with the aid of P^{32}, using the method of thin-layer chromatography. Under normal conditions the phosphatidylcholines have the highest rate of renewal; the intensity of phosphatidylserine and phosphatidic acid metabolism is also high. In anemia the relative phosphatidylserine activity is increased. The intensity of phosphatidic acid and, in particular, of phosphatidylcholine metabolism falls sharply. In the posthemorrhagic period, with an increase in the hemoglobin concentration in the blood, the intensity of phosphatidic acid metabolism rises sharply. This evidently can be explained by increased synthesis of neutral lipids and phosphatidylcholines. The rate of renewal of the individual fractions returns closer to normal.

Phospholipid (PL) metabolism in the bone marrow under normal conditions and in various states of hematopoiesis and, in particular, during disturbances of erythropoiesis, has received insufficient study. Only individual communications on this problem are to be found in the literature.

In experiments on rabbits, cows, and sheep, Mulder et al. [9] found that phosphatidylcholines (PCs) possessed the highest specific activity (SA) in bone marrow. The intensity of incorporation of P^{32} into phosphatidylethanolamines (PEA) and phosphatidic acids (PA) plus phosphatidylinositols (PI) is much lower. Sphyngosine-containing phospholipids (SPM), according to these workers, do not incorporate P^{32}.

James, et al. [7] also found that during normal hematopoiesis the PCs also possess the highest rate of renewal. In phenylhydrazine anemia, phospholipid synthesis is reduced [4].

The object of the present investigation was to study the rate of metabolism of the total phospholipids and their fractions under normal conditions, in anemia caused by blood loss, and also in the posthemorrhagic period.

EXPERIMENTAL METHOD

Rabbits aged 4-5 months were used. The experiments consisted of 3 series: series I (control) – animals with a normal state of hematopoiesis (15), series II – animals with anemia (14), and series III – in the posthemorrhagic period (12).

Anemia was produced in the rabbits of series II by daily withdrawal of 15-20 ml blood for 6-7 days. The total value of blood removed from individual animals varied from 110 to 230 ml. With a decrease in the blood hemoglobin concentration to 3-4 g% and of the erythrocyte count to 1,400,000, the animals were sacrificed. Anemia was produced in the rabbits of series III, after which the bleeding was stopped. During the next 10-14 days of the posthemorrhagic period, the hemoglobin level rose to 9-10 g%, the erythrocyte

Department of Biochemistry, Kalinin Medical Institute. (Presented by Academician V. V. Parin.)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 10, pp. 44-47, October, 1971. Original article submitted January 8, 1971.

TABLE 1. SA of Total Phospholipids and Phospholipid Fractions in the Femoral Marrow of Rabbits in Different States of Hematopoiesis (M ± m)

Experimental conditions	Weight of animal (in kg)	Hemoglobin (in g%)	Erythrocytes (millions)	Leukocytes (in thousands)	Total lipids (in percent)	SA of phospholipids (in thousands of pulses/min/mg P)					
						total	PC	PEA	SPM	PS	PA	PGP
Normal	2,12±0,12	12,51±0,33	5,1±0,38	8,6±0,17	34,4±2,39	15,4±4,47	32,9±1,06	3,8±0,37	2,8±0,24	10,2±0,97	9,9±0,61	—
Anemia . . .	2,07±0,23	3,85±0,28	1,42±0,11	8,8±0,14	19,0±2,05	11,58±0,90	11,0±1,21	6,4±0,53	4,95±0,45	27,05±2,27	5,89±0,47	2,4±0,3
P	>0,05	<0,001	<0,001	<0,05	>0,01	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
Posthemorrhagic period	2,28±0,10	9,20±0,47	4,03±0,28	9,44±0,20	36,81±3,4	13,84±0,93	15,88±1,41	4,68±0,79	4,73±0,51	14,84±1,41	74,1±5,44	—
P	>0,05	<0,01	<0,01	>0,05	>0,05	>0,05	<0,001	>0,05	<0,001	<0,001	<0,001	

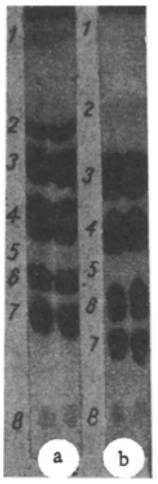

Fig. 1. Chromatography of rabbit bone marrow PL in a normal state of hematopoiesis (a) and during anemia following blood loss (b). Conditions of chromatography: activation for 25 min at 120°C, duration of chromatography 2 h 40 min; development with concentrated sulfuric acid; samples each contained 0.01-0.015 mg lipid P. 1) PGP; 2) PA; 3) PEA; 4) PC; 5) PI; 6) SPM; 7) PS; 8) Origin.

count rose to 4,000,000, and the animals were then decapitated. Homogenates of the femoral marrow (0.5-1 g) were placed in beakers containing 3 ml nutrient medium (Tyrode solution). Radioactive phosphorus was added in the form of $NaH_2P^{32}O_4$ at the rate of 500,000 pulses/min to 0.5 g tissue. After saturation with oxygen the samples were incubated at 37-38°C and shaken for 1 h 45 min. Preliminary tests showed that by the end of the 2nd hour the tissue respiration had begun to diminish.

The lipids were extracted with a mixture of chloroform and methanol. Non-lipid impurities were removed in separating funnels with 0.1 M KCl (once) and a mixture of chloroform, methanol, and 0.1 M KCl until disappearance of P^{32} from the washings. The resulting chloroform extract was evaporated in a current of nitrogen at 35-45°C and at reduced pressure, after which the dry residue was dissolved in petroleum ether and separated by filtration. Neutral lipids were separated from phospholipids (PL) by means of rubber membranes. Ordinary rubber tests, treated in a Soxhlet apparatus first with diethyl ether (3 h) and then with petroleum ether (3 h), were used as the membranes.

The PL were fractionated on layers of silica gel without plaster of paris by the continuous-flow horizontal method in a system of chloroform—methanol—ammonia (13 : 5 : 1) [2]. Individual fractions of bone marrow PL were identified with the aid of "witnesses" and by color tests [1].

The quantity of P^{32} incorporated into the PL was determined with a B-2 apparatus in conjunction with an MST-17 counter.

The SA of the total PL and their separate fractions was calculated per mg lipoid phosphorus (P) and expressed in pulses/min per

mg P. Lipid phosphorus in the mineralized samples was determined quantitatively by the method of Fiske and Subbarow.

EXPERIMENTAL RESULTS

The results are given in Table 1 and Fig. 1. They show that the qualitative composition of the PL was unchanged in the various states of erythropoiesis (Fig. 1), although definite changes were observed in the SA of the phospholipid fractions (Table 1).

In a state of normal hematopoiesis, renewal of PC took place most rapidly in the bone marrow, and a high SA was also found for phosphatidylserine (PS) and for PA. The intensity of metabolism of SPM and PEA was at a low level. Incorporation of the label into the PI fraction and into the polyglycerophosphatide (PGP) fraction was not observed. The total SA of the PL was 15,400 pulses/min per mg P.

In annemia produced by blood loss, when the hemoglobin concentration in the peripheral blood had fallen by 3.2 times and the erythrocyte count by 3.6 times below the normal level, a decrease in the rate of renewal of the total PL by 1.3 times was found in the bone marrow.

Unlike under normal conditions, the greatest intensity of metabolism in this period was observed with PS, the SA of which was increased by 2.7 times compared with normal. Whereas the rate of metabolism of PC was reduced by 3 times, and on PA by 1.5 times, the specific activity of SPM and PEA was increased by 1.8 and 1.7 times, respectively, over normal. Incorporation of P^{32} into PGP was observed, and their SA was 2400 pulses/min per mg P.

In the posthemorrhagic period the hemoglobin concentration and erythrocyte count in the peripheral blood were increased by 2.3 times compared with their values in anemia. An increase in the SA of the total PL was observed in the bone marrow and the intensity of P^{32} incorporation into most phospholipid fractions was almost the same as normally.

It is noteworthy that the rate of PA renewal in the posthemorrhagic period was high. Their SA was 74,000 pulses/min/mg P, which is almost 7.5 times higher than normal and 12 times higher than in anemia. The rate of incorporation of P^{32} into PA (15,900 pulses/min/mg P) was 1.4 times higher than in anemia, but it did not approach the normal level.

The intensity of PS metabolism (14,800 pulses/min per mg P) was reduced by 1.8 times relative to its intensity during anemia, and was almost indistinguishable from normal.

Similar changes were observed in the PEA fraction. The SA of the sphingosine-containing PL was almost unchanged compared with the SA in anemia. No appreciable incorporation of P^{32} into PGP was found.

This investigation showed that the bone marrow PL and, in particular, the PC, PS, and PA are active and rapidly metabolized compounds during normal hematopoiesis.

Synthesis of PL de novo is not observed in erythrocytes [11, 12, 14], and PS is absent in blood plasma or present in only very small quantities [3, 5]. The high SA of phosphatidylserine observed during the period of anemia thus suggests that this fraction, which is present in large quantities in the erythrocytes in blood clotting [2, 8] and in the transport of ions through the erythrocyte membrane [3, 6, 13], is synthesized more intensively in the bone marrow tissue.

The decrease in SA of the phosphatidic acids in anemia and its sharp increase in the phosthemorrhagic period deserve attention. Changes in the SA of the phosphatidic acids, precursors in triglyceride synthesis correlate with the content of neutral lipids in the bone marrow tissue, which is reduced in anemia and increased in the posthemorrhagic period.

The results thus indicate that changes in the rate of metabolism of the bone marrow PL are connected with the state of hematopoiesis.

LITERATURE CITED

1. A. V. Kargapolov, Abstracts of Proceedings of a Scientific Conference of Junior Research Workers at Kalinin Medical Institute [in Russian], Kalinin (1969), p. 27.
2. P. G. Barton, E. T. Yin, and S. J. Wessler, J. Lipid Res., 11, 87 (1970).
3. R. M. C. Dawson, N. Hemington, and D. B. Lindsay, Biochem. J., 77, 226 (1960).

4. G. T. Dimopoullos and A. T. James, Proc. Soc. Exp. Biol. (New York), 125, 569 (1967).
5. I. T. Dodge and G. B. Philips, J. Lipid Res., 8, 667 (1967).
6. D. J. Hanahan, R. M. Watts, and D. Pappagonh, J. Lipid Res., 1, 421 (1960).
7. K. J. James, C. P. Lim, and F. Snyder, Proc. Soc. Exp. Biol. (New York), 124, 1158 (1967).
8. A. J. Marcus, Advances Lipid Res., 4, 1 (1966).
9. E. Mulder, I. D. Gier, and L. L. M. Van Deenen, Biochim. Biophys. Acta, 59, 502 (1962).
10. G. I. Nelson, Biochim. Biophys. Acta, 144, 221 (1967).
11. H. I. Raderecht, Acta Biol. Med. Germ., 8, 199 (1962).
12. Z. B. Snohet and D. G. Nathan, Biochim. Biophys. Acta, 202, 202 (1970).
13. A. Solomon, F. Lionetti, and P. F. Curran, Nature, 178, 582 (1956).
14. L. L. M. Van Deenen, in: C. I. Bishop and D. M. Surgenor (Editors), The Red Blood Cell, New York (1964), p. 285.