AGE DIFFERENCES IN PLATELET PROTEIN BIOSYNTHESIS AND CATABOLISM

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The biosynthesis and catabolism of platelet proteins in adult and month-old rabbits were studied with the aid of lysine-^{14}C in vivo. It was found that free lysine-^{14}C does not pass through the membrane of platelets circulating in the blood stream and that it can appear in the platelet proteins only at the stage of thrombocytopoiesis. Protein formation in month-old rabbits takes place more slowly than in adult animals. The half-life of the total proteins of adult rabbit platelets averages 2 days, whereas in month-old rabbits it is lengthened to 3 days. The circulation of platelet proteins takes place more slowly in early postnatal development.

KEY WORDS: platelets; protein biosynthesis and catabolism; age differences.

The biosynthesis of platelet proteins takes place during formation of the platelets from the megakaryocytic series of bone marrow precursor cells [7, 10, 12]. Thrombocytopoiesis differs in character at different stages of development [1, 3-5], and the character of platelet protein metabolism reflects the level of the functional state of the megakaryocytic system of the bone marrow [6, 13].

It was therefore decided to study the biosynthesis and catabolism of platelet proteins from the ontogenetic standpoint.

EXPERIMENTAL METHOD

Protein biosynthesis was judged from the appearance of radioactivity in the proteins of circulating platelets after a single injection of labeled amino acids; catabolism was judged from excretion of the label from already tagged proteins. Lysine-^{14}C (specific activity 110 mCi/g) was injected into sexually mature rabbits and month-old rabbits in a dose of 330 μCi/kg. Blood was taken by cardiac puncture from different animals after 1, 3, and 6 h, and thereafter daily for 10 days, the platelets were separated, and the level of the radioactive label in the platelet proteins was determined. The blood was stabilized in tubes made of nonwettable material by 3.3% sodium citrate solution containing 0.5% Na_2EDTA. The platelets were separated from 5 ml blood by centrifugation at 250 and 750g. Contaminating erythrocytes were removed from the platelet suspension by differential lysis in hypotonic medium [11]. The platelets were washed four times with isotonic mixture (0.7% NaCl and 0.5% Na_2EDTA, pH 7.35). The proteins were precipitated with cold 10% trichloroacetic acid (TCA), washed with 5% TCA, heated with 5 ml 5% TCA for 20 min at 70°C, and dissolved in 0.4 N NaOH. Radioactivity in samples (0.01-0.02 ml) was counted in a mark VSP gas-flow counter and in parallel samples the protein concentration was determined by Lowry's method. The results were expressed in counts/min/mg protein.

EXPERIMENTAL RESULTS

Radioactivity was virtually absent in the nonprotein fractions of platelets from the adult animals and also from the month-old rabbits 1, 3, 6, and 24 h after injection of the label. This indicates that the free lysine of the blood does not pass through the platelet membrane and, consequently, it can enter the platelets only at the stage of thrombocytopoiesis. The appearance of a certain number of labeled platelets in the blood, gives

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TABLE 1. Specific Radioactivity (counts/min/mg) of Total Platelet Proteins in Month-Old and Adult Rabbits

Time after injection of lysine-^{14}C, days	Adult rabbits	Month-old rabbits
1/4	505±20	300±45
1	1810±68	1010±100
2	3050±46	1555±75
3	3940±294	1674±91
4	4207±130	1640±160
5	3724±388	1585±98
6	2300±153	1261±101
7	1492±40	970±68
8	980±86	670±38

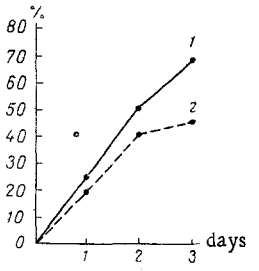

Fig. 1

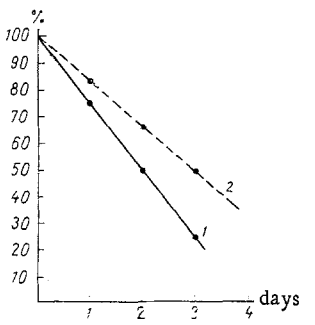

Fig. 2

Fig. 1. Rate of biosynthesis of platelet proteins in adult (1) and month-old (2) rabbits. Ordinate, increase in specific radioactivity (in %); abscissa, time (in days).

Fig. 2. Rate of catabolism of platelet proteins in adult (1) and month-old (2) rabbits. Ordinate, decrease in specific radioactivity (in %); abscissa, time (in days).

rise to a maximum and plateau, and the beginning of liberation of the label from the proteins were observed at the same times in the animals of both groups. For instance, the label was not found in the proteins of the circulating platelets until 6 h after injection of radiolysine (Table 1). On the subsequent days the specific activity of the proteins increased to reach a maximum after 3 days, followed by a plateau. On the sixth day the quantity of label was reduced by 38% in the adult animals and by 20% in the young rabbits. On the subsequent days the radioactivity of the platelet proteins in both groups of animals gradually fell, but the shape of the curve reflecting this process was less steep in the young rabbits, indicating slower liberation of the label in these animals. Comparison of the rates of platelet protein biosynthesis based on the increase in specific radioactivity per unit time (Fig. 1) showed that platelet proteins are formed in the adult animal faster than in the month-old rabbits. The lower level of specific activity in the platelet proteins of the young rabbits, as investigation of the rate of appearance of lysine-^{14}C showed, was evidently unconnected with a change in the reserves of free amino acids, but was due to differences in the genesis of the platelets at that age period and the character of their liberation into the blood stream.

Since, according to some workers [8, 9], one of the mechanisms regulating the liberation of platelets from the bone marrow into the blood stream is disintegration of some of the circulating platelets, and also since the life spans of platelets are to some degree predetermined by the character of their biosynthetic processes [14], it was important to determine the half-life of the platelet proteins in the adult and growing animals. The half-life of platelet proteins was calculated by the equation:

$$T_{1/2} = \frac{0.69}{K} \text{ [15]; } K = \frac{2.3}{t} \cdot \lg \frac{A_0}{A_t}.$$

The half-life of the total platelet proteins of the adult rabbits was between 1.7 and 2.2 days, whereas in month-old rabbits it varied from 2.5 to 3.2 days. The decrease in specific radioactivity in the platelet proteins is shown graphically in Fig. 2. The position of the curves indicates that the platelet proteins of adult rabbits are renewed faster than those of young rabbits. It can accordingly be concluded that the platelets of young rabbits live longer in the blood stream and, probably for that reason, their liberation into the blood stream is slower.

The lengthening of the period of circulation of the platelet proteins in young rabbits may be connected with the fact that the protein-synthesizing functions of the megakaryocytic system in early postnatal development and the mechanisms controlling it are still in the stage of formation. This suggestion is confirmed by data indicating that in the early stages of postnatal development the bone marrow contains large numbers of undifferentiated cells, which account for its functional hyperplasia [1, 2, 5].

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