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TURNOVER OF ERYTHROCYTE AND PLATELET GLYCOPROTEIN AMINO SUGARS IN CARBOHYDRATE-FED RATS

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Previous investigations have shown that the chemical composition of diets has a significant effect on the half-renewal time of proteins and lipids of subcellular fractions of the rat liver [3, 5-9]. These investigations not only have extended our knowledge on the plastic function of food, but have also provided new opportunities for the quest for adequate methods of assessing foodstuffs at the intracellular strucutral level. However, isolation of subcellular structures and obtaining isolated membranes from tissues, such as the liver, require the use of complex and time-consuming physicochemical methods. As the model, we therefore chose erythrocyte ghosts and platelets, which can be isolated without any such difficulty. In addition, tests of renewal of blood components can be used in clinical practice.

The content of glycoproteins in erythrocyte membranes and in platelets is relatively small compared with the important role which they perform in the functions of these cells. The qualitative contribution of the carbohydrate component in biomembranes is not determined by their quantitative composition. For instance, if sialic acid is removed by the use of enzymes from a number of serum glycoproteins, the half-life of these asialoglycoproteins is shortened from several tens of hours to a few minutes, i.e., the presence of sialic acids determines the circulation time of glycoproteins in the blood stream. Sialic acid determines the lifetime not only of glycoproteins, but also of certain cells, and in particular, of erythrocytes. Other monosaccharides of carbohydrate chains, fucose for example [2], also plays the same role.

The aim of this investigation was to study the rate of degradation of amino sugars of platelets and erythrocyte membranes of rats kept on carbohydrate diets, using starch and sucrose.

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EXPERIMETNAL METHOD

Male Wistar rats weighing initially 200-250 g were used. The animals (10 in a group) were kept for 14 and 28 days on two diets of equal calorific value. The first diet contained protein (casein) 12%, fat 18% (9% of lard and 9% of sunflower oil), and corn starch 70%. The second diet contained the same components, but starch equivalent to 40% of the calorific value of the total diet was replaced by sucrose. Both diets contained the normal physiological quantities of essential vitamins and minerals.

^{3}H-Glucosamine hydrochloride (from Amersham Corporation, England), injected intraperitoneally in a dose of 200 μCi per rat, 1, 2, and 3 days before sacrifice, was used as the label.

Blood was taken from the rats into test tubes with 3 ml of stabilizer (trisodium citrate 6.6 g, citric acid 2.4 g, glucose 12.5 g to 500 ml of distilled water). Platelets were isolated by the writers' own method. The blood obtained was layered above 7 ml of a solution of Ficoll (46 g Ficoll, 1.7 g sodium citrate to 200 ml of 0.14 M NaCl). The density of the Ficoll solution must be 1.077 g/ml. After layering, the samples were centrifuged for 15 min at 200g in a bucket rotor. Platelets remained in the Ficoll solution, lymphocytes on the boundary, and erythrocytes in the residue. The supernatant was withdrawn and centrifuged for 20 min at 5000g to sediment the platelets. The residue thus obtained was washed twice with solution containing 1 mM EDTA, 10 mM Tris-HCl, and 0.15 M NaCl, pH 7.4, at 4°C. The platelets thus obtained were suspended in 2 ml of the same solution. This method of isolating platelets has several advantages over those described in the literature: Rapidity of isolation and, what is very important, pure platelets, uncontaminated by lymphocytes, are obtained.

The erythrocyte residue obtained after sedimentation in Ficoll was hemolyzed in phosphate buffer, 20 milliosmoles, pH 8.0. For 2 ml of erythrocytes 28 ml of buffer was taken. Erythrocyte ghosts were sedimented at 17,000g for 30 min. The erythrocyte membranes thus obtained were washed and suspended in the same solution as the platelets.

Amino sugars were obtained by hydrolysis in 4 N HCl at 100°C for 4 h in sealed ampuls under nitrogen. The protein concentration during hydrolysis must not exceed 0.5%. Protein was determined by the Lowry–Ciocalteu method [1], using bovine serum albumin to plot the calibration curve. The digest was evaporated to dryness on a rotary evaporator. Hexosamines were adsorbed on Dowex 50 × 4 (200-400 mesh, H^+-form). Elution was carried out with 2N HCl, which was removed by evaporation. The samples were dissolved in water, 1 ml was taken for colorimetric determination [4], and parts of the rest for counting in RiA Luma scintillation fluid (Lumac, The Netherlands). The samples were counted on a "RackBeta" 1215 counter (LKB, Sweden). Calibration curves were plotted beforehand by the hat-trick method. The final specific radioactivity was thus expressed in cpm. The half-renewal time of the amino sugars was determined by the equation [11]:

$$P_t = P_0 \cdot e^{-K_g \cdot t},$$

where P_t is the specific radioactivity of the amino sugar at time t; P_0 the initial specific radioactivity of the amino sugars; K_d the velocity constant of degradation; t the time elapsing after injection of the isotope until sacrifice of the animal. Between semilogarithmic coordinates this relationship is expressed by a straight line; the angle of slope is determined by the value of K_d, which is related to the half-renewal time $T_{1/2}$ by the equation; $K_d = 0.693/T_{1/2}$ [11], where K_d stands for the fraction of hexosamines which is degraded in unit time. The numerical results were subjected to statistical analysis by Student's test. To estimate K_d, characterizing the slope of the straight line, the method of least squares was used.

EXPERIMENTAL RESULTS

As Table 1 shows, replacement of starch equivalent to 40% of the calorific value of the diet by sucrose significantly changed the half-renewal time of the amino sugars of rat erythrocyte membranes. After 14 days the half-renewal time was increased almost fourfold. If renewal of amino sugars is compared after 28 days of the same diets, the picture is drastically changed. The half-renewal time on the diet with sucrose was restored just as after 14 days on the diet with 70% of starch, and was about 1 day. After 28 days on the diet with starch an increase in the half-renewal time to 2 days was observed. Replacement of starch in the diet by sucrose increased the half-renewal time of the amino sugars in platelets also (Table 1), but

TABLE 1. Effect of Readily Assimilable Carbohydrates on Half-Renewal Time of Amino Sugars of Erythrocyte Membranes and Platelets

Test object	Diet	14 Days on diet			28 days on diet		
		K_d, days^{-1}	$T_{1/2}$ days	% of renewal per diem	K_d, day^{-1}	$T_{1/2}$ days	% of renewal per diem
Erythrocytes	70% Starch	0,65±0,06	1,1	65	0,33±0,06	2,1	33
	30% Starch + 40% sucrose	0,16±0,06	4,3	16	0,67±0,097	1,03	67
Platelets	70% Starch	0,20±0,06	3,5	20	0,24±0,02	2,9	24
	30% Starch + 40% sucrose	0,12±0,06	5,8	12	0,08±0,035	9,1	8

this increase was much smaller than in erythrocyte membranes. After 28 days the difference was even greater, namely 9 days. The life span of platelets, according to data in the literature, varied between 2.7 and 11 days. We also know that the platelet count undergoes significant fluctuations depending on the nature of the diet [10]. In the present experiments, replacement of most of the starch by sucrose led to a threefold increase in the life span of the platelet amino sugars by the 28th day of the experiment.

Lengthening of the life span of the amino sugars of the erythrocyte membrane glycoproteins on replacement of starch by sucrose could be due to at least three factors. First, replacement by readily assimilated carbohydrates could affect the hormone level which, in turn, could affect erythropoiesis. Second, although mature erythrocytes have no nuclei and no protein-synthesizing system, nevertheless the erythrocyte membrane plays an active part in several physiological processes, which are accompanied by active glycolysis. Third, it is well known that death of an erythrocyte may take place also during agglutination, the activity of which depends on the glycoprotein layer on the surface of the erythrocyte membrane [12].

Replacement of part of the starch in the diet by sucrose thus has a marked effect on the half-renewal time of amino sugars of blood cell membrane glycoproteins. Investigation of the dynamic characteristics of the amino sugars of the blood cells can be used to assess the effect of food on metabolic and adaptive processes in man.

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