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The content of *N*-acetylneuraminic acid in glycoproteins of erythrocyte membranes in Morris hepatoma 5123 bearing rats

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Changes in the content of *N*-acetylneuraminic acid in rat erythrocyte membranes at different stages of experimental tumour (Morris hepatoma 5123) development were examined. Its content was lowered on the 30th and 40th day after transplantation of the tumour cells, as compared to the results for normal healthy rats. As a result of the tumour growth, the content of *N*-acetylgalactosamine, galactose and mannose in rat erythrocyte membranes became lowered, whereas that of glucose remained unchanged. The content of fucose was raised at early stage of tumour growth, and remained at this high level till the 40th day of experiment.

In animals with transplanted Morris hepatoma 5123, changes in the activity of several enzymes catalyzing the main metabolic pathways in erythrocytes were observed [1, 2]. The transplanted neoplastic tissue affects the permeability of erythrocyte membranes to sodium and potassium ions, and acts also on some structural components of these membranes, e.g. phospholipids and cholesterol [3].

An essential role in the control of cellular metabolism is played by carbohydrate components, mainly those of erythrocyte membrane glycoproteins. They determine antigenic properties of the cells, their susceptibility to phagocytosis, and formation of receptor binding sites for viruses and plant agglutinins [4, 5]. Among the carbohydrates of erythrocyte membranes, *N*-acetylneuraminic acid deserves special attention as it is decisive for the negative charge of erythrocytes, their life span, and group specificity [6, 7]. The content of this acid in human erythrocyte membranes was found to be lowered in β-talassemia [8] and in diabetes [9, 10]. The lowered *N*-acetylneuraminic acid content caused also an increased tendency to erythrocyte aggregation and their increased viscosity [9].

In the present work, we have followed the effect of Morris hepatoma 5123 on the content of *N*-acetylneuraminic acid in rat erythrocyte glycoproteins, as well as that of aminosugars, hexoses and fucose, in the course of development of neoplastic disease.

MATERIALS AND METHODS

Buffalo rats aged about 10 weeks were used for the experiments. Homogenized neoplastic tissue — Morris hepatoma 5123 — was injected bilaterally into thigh muscles. Determinations were carried out on the 10th, 20th, 30th and 40th day after transplantation, and parallelly on control healthy animals.

Isolation of erythrocyte membrane glycoproteins. Blood samples were collected into heparinized tubes and centrifuged at 3000 × *g* for 10 min at 5°C. Plasma and the upper layer of

erythrocytes (about 10%) containing blood platelets, leukocytes and reticulocytes, was discarded. Erythrocytes were suspended in 0.9% NaCl solution and washed three times with the same solution, each time followed by centrifugation at 1500 × *g*. Then the erythrocytes were haemolysed in 9 vol. of 10 mM Tris/0.1 M EDTA buffer, pH 7.4. Erythrocyte membranes were separated by centrifugation at 20000 × *g* for 20 min, then washed four times with 20 vol. of the same buffer, each time being followed by centrifugation under the same conditions.

Glycoproteins of the membranes were obtained according to Kornfeld & Kornfeld [11]. The membranes were suspended in 10 mM Tris/0.1 M EDTA buffer, pH 7.4, at 4°C, and homogenized. To one volume of the membrane suspension 9 volumes of chloroform-methanol mixture (2:2, v/v) were added. The whole mixture was shaken for 30 min at room temperature and centrifuged at 1500 × *g* for 10 min. The aqueous phase was carefully separated and centrifuged again under the same conditions to remove the interphase sediment. The aqueous phase was condensed to 1/5 of its volume in vacuum evaporator at 37°C. Then ethanol (9:1, v/v) was added and the whole mixture was centrifuged at 3000 × *g* for 20 min at room temperature. The ethanolic sediment obtained was freeze-dried and used for chemical analysis.

Chemical assays. Protein was determined according to Lowry *et al.* [12] with crystalline bovine plasma albumin as a standard.

N-Acetylneuraminic acid was determined by the method of Warren [13] with thiobarbituric acid, in the samples hydrolysed in 0.1 M H_2SO_4 at 80°C for 1 h. *N*-Acetylneuraminic acid (Sigma) was used as a standard.

Carbohydrates were determined by gas-liquid chromatography, according to Bhatti *et al.* [14], in the samples previously hydrolysed with 1 M HCl in anhydrous methanol. *Myo*-inositol (Sigma) was used as an internal standard. The analysis was performed in a type GCHF 18.3 apparatus (of Czech production) provided with a column (2 m × 4 mm) filled with Chromosorb W DMCS (10/100 mesh) with 3% SE-30. A flame ionization detector was used for the analyses with nitrogen as a carrier gas.

Groups of six animals were used for each experiment. Standard deviations were calculated for all the results. Statistical significance of differences between groups of animals was evaluated by Student's *t* test.

RESULTS AND DISCUSSION

Qualitative analysis of carbohydrates in erythrocyte membranes by gas-liquid chromatography confirmed the presence of *N*-acetylneuraminic acid, galactose, mannose, glucose, fucose, *N*-acetylgalactosamine and *N*-acetylglucosamine in glycoproteins. Cholesterol and phospholipids were not detected in the glycoprotein fraction obtained. Large differences in the content of carbohydrate components in erythrocyte membrane glycoproteins were found between the group of control animals and Morris hepatoma bearing rats.

In animals with transplanted tumour tissue, the content of *N*-acetylneuraminic acid, *N*-acetylgalactosamine and galactose in erythrocyte membranes was lowered on the 30th and 40th day after transplantation (Table 1). Lowering of the content of mannose was observed on the 40th day after transplantation of neoplastic cells. On the other hand, no significant differences were found in the content of glucose and *N*-acetylglucosamine in erythrocyte membrane glycoproteins between the Morris hepatoma 5123 bearing rats and control animals.

A different tendency was observed in the case of fucose. Its content was raised as early as on the 10th day after transplantation of neoplastic cells, and then remained at the same level throughout the course of experiment.

Parallelly, we determined haemoglobin concentration and the number of erythrocytes in blood. From those determinations, as well as from earlier experiments [2] it follows that symptoms of anaemia appear in the rat not earlier that after 50 days of the tumour growth.

The lowered *N*-acetylneuraminic acid content in erythrocyte membranes of rats bearing Morris hepatoma 5123, as compared with normal rats, could be due to enhanced activity of plasma neuraminidase which hydrolyticaly cleaves *N*-acetylneuraminic acid from carbohydrate chains of erythrocyte membranes [15], or to lowered activity of the transferase responsible for incorporation of residues of this acid into saccharide chains of the membranes [16].

Probably a similar mechanism is involved in lowering of aminosugar, galactose and man-

Table 1

The content of N-acetylneuraminic acid, aminosugars, hexoses and fucose in glycoproteins of rat erythrocyte membranes at various stages of development of Morris hepatoma 5123

For details see Material and Methods. The results are expressed as μmol/100 mg protein ± S.D.

Monosaccharide	Control rats	Tumour-bearing rats			
		Day after transplantation of tumour cells			
		10	20	30	40
N-Acetylneuraminic acid	74.20 ± 4.30	81.00 ± 5.20	71.28 ± 4.60	52.96 ± 3.80[a]	43.79 ± 3.10[a]
N-Acetylgalactosamine	98.18 ± 7.32	101.48 ± 8.40	88.43 ± 6.35	67.74 ± 4.80[a]	66.78 ± 5.60[a]
N-Acetylglucosamine	33.56 ± 2.40	34.50 ± 1.70	31.75 ± 1.90	30.44 ± 2.10	29.68 ± 1.80
Galactose	101.60 ± 8.20	107.60 ± 7.90	93.20 ± 6.30	71.60 ± 5.40[a]	72.40 ± 5.30[a]
Mannose	10.60 ± 0.60	10.32 ± 0.72	9.80 ± 0.76	9.00 ± 0.80	7.80 ± 0.52[a]
Glucose	5.30 ± 0.40	5.26 ± 0.36	4.90 ± 0.32	4.96 ± 0.34	5.02 ± 0.46
Fucose	7.81 ± 0.64	10.64 ± 0.80[b]	10.86 ± 0.72[b]	10.40 ± 0.68[b]	10.20 ± 0.70[b]

[a]Values significantly lower ($P \leq 0.001$) than those for control rats.
[b]Values siginificantly higher ($P \leq 0.001$) than those for control rats.

nose content in erythrocyte membrane glycoproteins of Morris hepatoma bearing rats.

Diminished synthesis of carbohydrate fragments in transformed BHK cells reported by Den *et al.* [17] was probably caused by low activity of some transferases. It also seems probable that the changes in the content of aminosugars, galactose and mannose, observed in our experiments, were due to enhanced activity of plasma glycosidase resulting from disintegration of transformed cells.

So far, there is no explanation for the amount of fucose in the erythrocyte glycoproteins of the Morris hepatoma 5123 bearing rats, being increased over that found in control animals. This increase could be due to some changes in the processes of L-fucose biosynthesis in the endoplasmic reticulum. GDP-L-fucose, incorporated by fucosyl transferase into glycoproteins and glycolipids of cellular membranes, is formed from GDP mannose in the presence of NADPH and H^+. It has been found that the content of the terminal saccharides of carbohydrate chain of glycoproteins, i.e. fucose and sialic acid, is dependent on the presence of nucleotide coenzymes, as well as the activity of appropriate enzymes within the cell [18]. Previous work from our Laboratory indicated that NADPH + H^+ concentration in erythrocytes of tumour bearing rats was altered [1]. This might also explain the presence on the erythrocyte surface of some components contained an extra fucose residue, derived from disintegrated tumour cells. This suggestion is in agreement with the results of Hakamori [19] who also found a significant increase in L-fucose content on the surface of tumour cells.

On the basis of the results obtained, assuming that the erythrocyte can serve as a sort of a model cell, it can be concluded that the neoplastic disease in general is a process affecting the whole organism. It affects not only the cellular metabolism but also, to a significant extent, the structure of membranes, leading to changes in some of their properties.

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