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THE EFFECT OF AN EXPERIMENTAL NEOPLASTIC DISEASE ON THE FLUX OF SODIUM AND POTASSIUM IONS ACROSS RED BLOOD CELLS AND ON THE LIPID COMPOSITION OF THEIR MEMBRANES

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The erythrocytes from Morris Hepatoma 5123 bearing rats took up Na^+ and K^+ ions from the incubation medium and released Na^+ into the extracellular space at lower rates than did erythrocytes from intact control rats. The lipid composition of erythrocytes membranes from the tumor-bearing rats differed from that of membranes from unaffected rats, showing increased contents of phospholipid phosphorus and a decreased content of cholesterol, resulting in decreased cholesterol:phospholipid molar ratios.

The neoplastic disease is a process that involves the whole organism of the tumor-bearing individual. Its impact may be reflected in events occuring in various tissues and cells, among others in red blood cells.

It has been shown [1] that, in erythrocytes of the Morris Hepatoma 5123 bearing rat, considerable changes occur in the carbohydrate components of red blood cells membranes as well as in the activity of enzymes catalyzing their main metabolic pathways. In the course of a neoplastic disease, the

erythrocytes may show shape deformations and decreased resistance towards hemolytic agents [1, 2, 3]. The observed quantitative as well as qulitative changes in the composition of erythrocyte membranes affect their functioning [4, 5], among others, their permeability to cations. The transport of ions across the cell membrane is accomplished by membrane protein components [6, 7], though membrane lipids may also be involved [8, 9].

Ionic composition of erythrocytes may be also disturbed in various other diseases such as arterial hypertonia, hepatic cirrhosis, muscle diseases or psoriasis [10 - 14]. The authors of these reports try to correlate the observed changes in the red blood cells permeability to ions with changes in composition of their membranes, particularly with alterations of their lipid composition. It is the molar ratio of cholesterol (C) to phospholipids (PL) which is assumed to be of importance in this respect [15, 16]. In the presented work we studied the fluxes of sodium and potassium ions across the cell membrane as well as its cholesterol and phospholipid content in erythrocytes from Morris Hepatoma 5123 bearing rats.

MATERIAL AND METHODS

Inbred male white Buffalo rats, aged 10 weeks, body weight 200 g were used. Homogenates of the Morris Hepatoma 5123 tissue were injected bilaterally into the femoral muscles of the recipient rats. Details of the transplantation procedure were described in our previous papers [1, 2].

The ionic fluxes as well as the membrane lipid composition were determined in erythrocytes from the tumor-bearing rats 20 and 30 days after implantation of tumor homogenates, and compared with the same parameters determined in red blood cells from tumor free, healthy rats of the same age kept under identical conditions (food, temperature and illumination).

Blood samples were withdrawn by puncturing the left heart chamber and collected in a heparinized medium. After centrifugation at $4^{o}C$ and $1000 \times g$ for 10 min, the plasma together with the upper cellular layer containing blood platelets, leukocytes and reticulocytes was sucked off and the sediment washed 4 times with ice-cold 110 mM $MgCl_2$ plus 10 mM Tris/HCl buffer (pH 7.4). The assays of ion fluxes through the erythrocyte membrane were performed according to Glynn *et al.* [17, 18] on suspensions of the washed erythrocytes equivalent to 10% of hematocryte, with

the aid of radioactive $^{22}Na^+$ and $^{43}K^+$ (USSR product; spec. radioactivity was 11.6×10^{10} Bq/mol, 3.18×10^{10} Bq/mol, respectively). The radioactivity of the erythrocytes preparations was measured in a scintillation counter (type USB-II, Poland).

Assay of Na^+ efflux from red blood cells. The washed erythrocytes were incubated for 4 h with constant gentle shaking at 37°C in a medium containing 146 mM NaCl, 10 mM glucose and 10 mM Tris/HCl buffer, pH 7.4, supplemented with 2.69×10^5 Bq ^{22}NaCl per ml. Thereafter, the erythrocytes were sedimented by cenrifugation at $3000 \times g$ for 3 min at 4°C and then washed 3 times with an ice cold medium made up of 110 mM $MgCl_2$ and 10 mM Tris/HCl buffer, pH 7.4, to remove the external radioactivity. The resulting sediment of washed erythrocytes was suspended in the above described incubation medium free of radioactive sodium, and was incubated at 37°C for 60 min. Samples of this suspension were taken at 15 min intervals and the erythrocytes were sedimented by centrifugation for 1 min at $5000 \times g$ at 4°C. After a washing procedure, the same to that described above, the cells were spun down and their radioactivity was counted. The efflux of sodium ions was expressed in µmoles per 1 ml of erythrocytes retained by these cells after the consecutive time intervals of incubation in the medium free of radioactive sodium ions.

The Na^+ content in the centrifuged cell population determined immediately after the 4 h preincubation in the medium containing the radioactive sodium isotope was assumed as that representing the "zero time" sample.

Assay of Na^+ influx into red blood cells. Erythrocytes destined for determination of the sodium influx were treated exactly as those prepared for the study of the sodium ion efflux. The washed cells were added to the incubation medium described above.

Assay of K^+ influx into red blood cells. Prewashed erythrocytes were incubated at 37°C in a medium of 146 mM NaCl, 8 mM KCl, 10 mM Tris/HCl buffer, pH 7.4, and 10 mM glucose. $^{43}K^+$ was added into the incubation medium at the amount of 1.11×10^5 Bq/ml.

Samples of this suspension were taken at 15 min intervals, after the start of incubation and the cells were sedimented by spinning at $5000 \times g$ for 1 min at 4°C. The sedimented cells were washed 3 times with a medium of 110 mM $MgCl_2$ and 10 mM Tris/HCl buffer, pH 7.4 at 4°C.

The radioactivity of the obtained cells and medium was measured in the scintillation counter.

The influx of potassium ions was expressed in μmole of K^+ per 1 ml of erythrocytes.

The efflux of potassium from the erythrocytes was measured exactly as that of sodium ions, except that $^{22}Na^+$ was replaced by $^{43}K^+$ in the incubation medium.

Determination of the lipid composition of erythrocyte membranes. Erythrocytes membranes were obtained according to Dodge *et al.* [19] by hemolysis of the erythrocytes in 10 mM Tris/HCl buffer, pH 7.4, at 4°C. The membrane lipids were extracted using the method of Kates [20], with methanol : chloroform mixture 1 : 2 (v/v).

The total cholesterol content was determined in the chloroform phase of the membrane extract by the colorimetric method of Zlatkis & Zak [21] with ferric chloride and conc. sulfuric acid in ethyl acetate as the colour developing medium. Quantitative determination of phospholipids was based on determination of their phosphate content by means of the Fiske-Subbarow colorimetric assay in the modification of Bartlett [22] designed for determination of organic phosphates.

The results were evaluated statistically by means of the Student's *t*-test; all mean values are presented together with standard deviations.

RESULTS AND DISCUSSION

In the present study we show that the flow rates of sodium and potassium ions through the erythrocyte membrane of the Morris Hepatoma-bearing rats differs from that of normal rats and so does their cholesterol and phospholipid content.

Figure 1 shows that, after 30 min of incubation, the efflux of sodium ions from eryhrocytes of the tumor-bearing rats differed substantially from that of control rats. These differences further increased after 45 and 60 min of incubation of the cells in the $^{22}Na^+$ free medium.

Figure 2 shows that the influx of sodium ions into erythrocytes of normal rats was greater than into cells from the tumor-bearing rats over the whole time of exposure to the incubation medium.

The results pertaining to the influx of potassium ions into erythrocytes of control and tumor-bearing rats are presented in Figure 3. It is evident that cells from the tumor-bearing rats took up less of potassium from the medium than cells from control rats. Decreased rates of uptake were noted

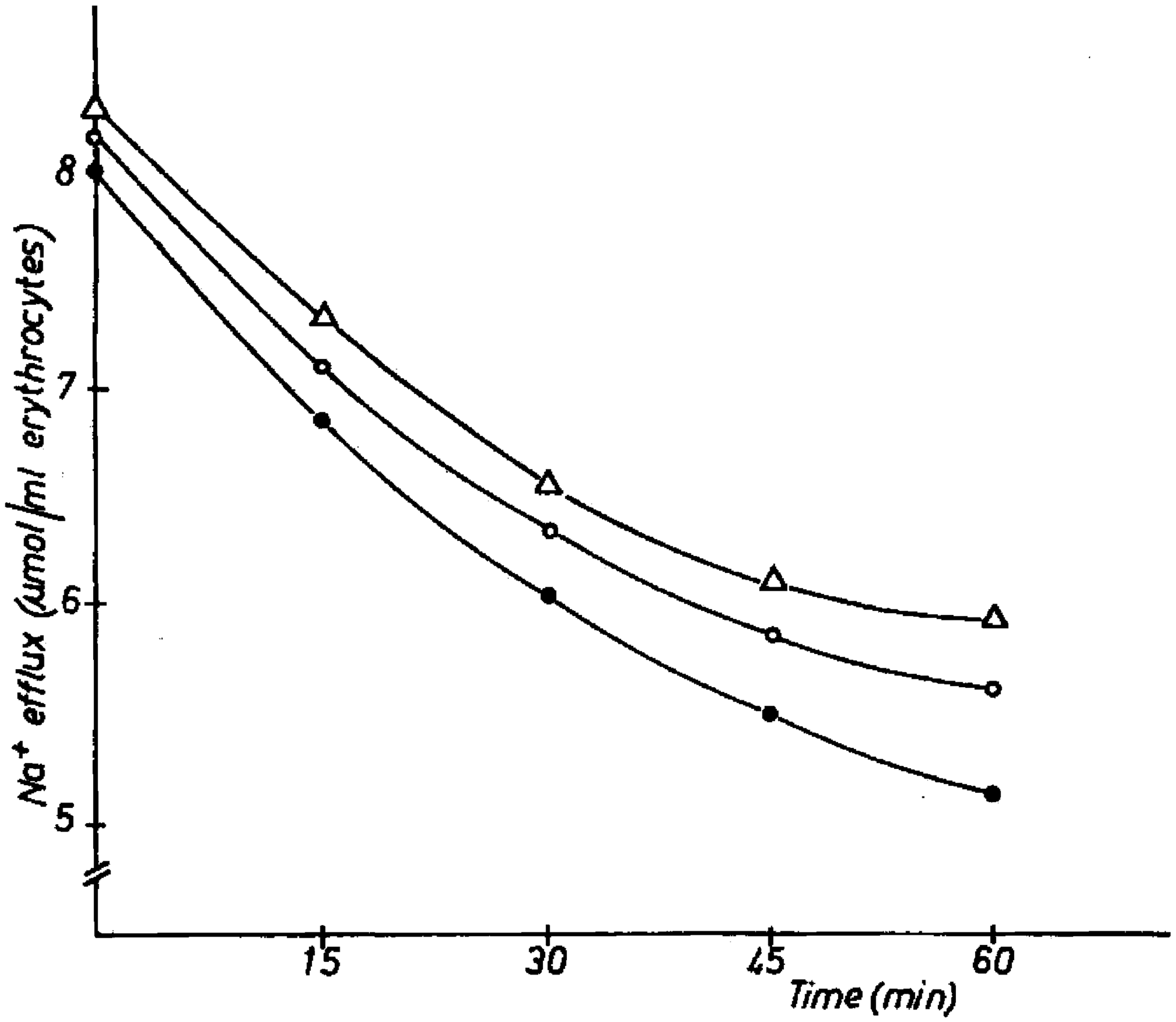

Fig. 1. Efflux of sodium ions from rat erythrocytes. ●, Control. ○, Erythrocytes from Morris Hepatoma-bearing rats, 20 days following implantation of the tumor and Δ, erythrocytes from tumor bearing rats, 30 days following tumor implantation

in samples analysed after 30, 45 and 60 min of exposure to the medium containing the radioactive potassium ions.

The efflux of potassium ions from erythrocytes of Morris Hepatoma--bearing rats did not differ significantly from that of control animals (not shown).

The cholesterol and phospholipid content of the erythrocytes membranes prepared from Morris Hepatoma-bearing rats and control animals is presented in Table 1.

The results are expressed in μmoles of the respective lipid per 1 ml of packed erythrocytes and as percentage with respect to membranes prepared from erythrocytes of control rats.

The figures clearly show that erythrocyte membranes from the tumor--bearing rats have a lower content of cholesterol than those from intact rats,

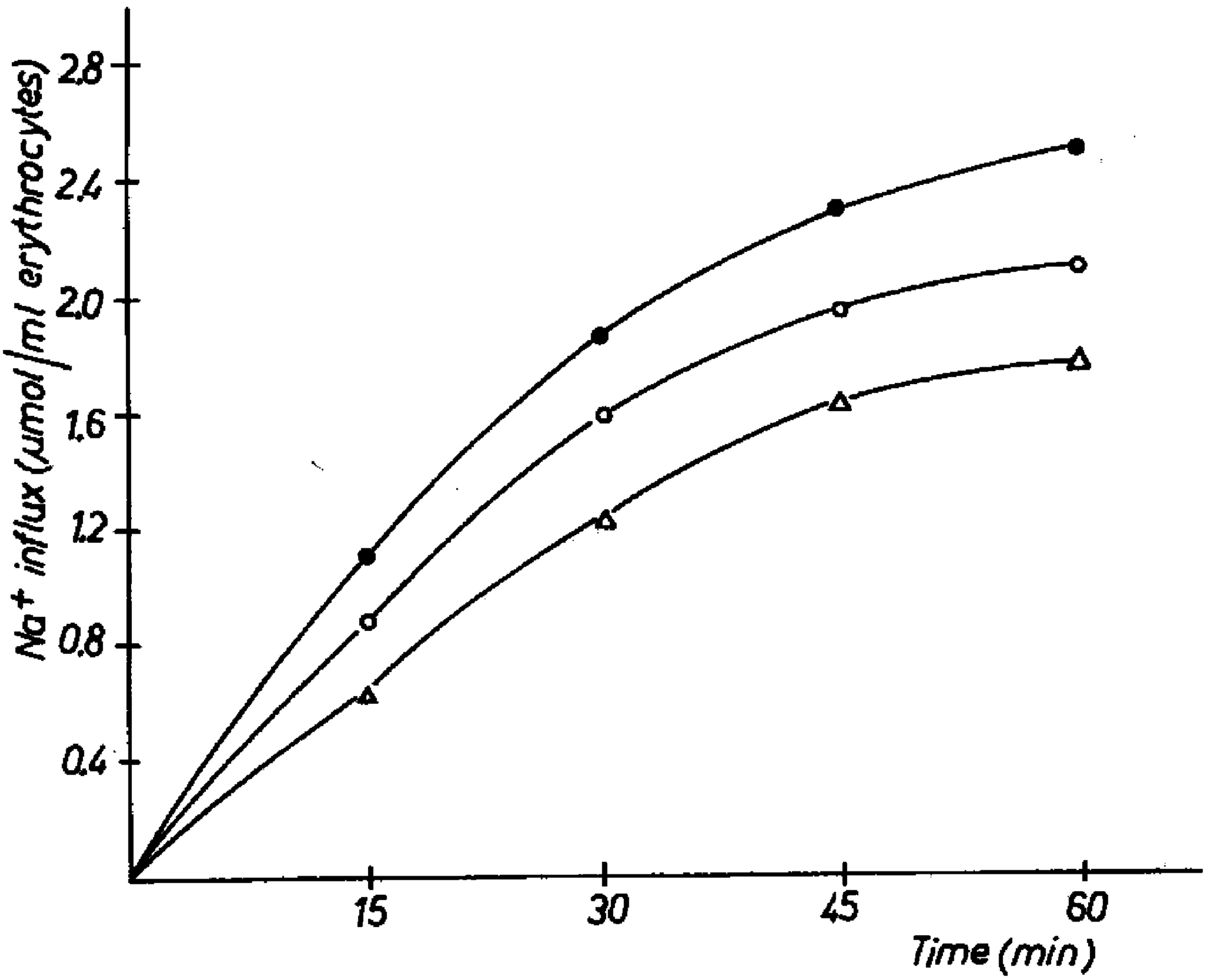

Fig. 2. Influx of sodium ions into rat erythrocytes. ●, Control. ○, Erythrocytes from Morris Hepatoma-bearing rats, 20 days following implantation of the tumor and Δ, erythrocytes from tumor bearing rats, 30 days following tumor implantation

the reduction amounting to 22% and 34%, respectively, in membranes prepared from rats 20 and 30 days following implantation of the tumor.

The phospholipid content of erythrocytes membranes from tumor--bearing rats prepared 20 days after transplantation of the tumor was increased in comparison with that of control rats (Table 1). In rats in which the tumor developed for 30 days, the phospholipid content of these membranes was comparable to that of the control group.

The molar ratios of cholesterol to phospholipid phosphorus were significantly decreased (from 1 down to 0.7) both 20 and 30 days after transplantation.

Thus the results of our experiments, show that erythrocytes from the tumor bearing rats are characterized by a decreased permeability to sodium ions as reflected by the reduced outward and inward flow of these ions, as

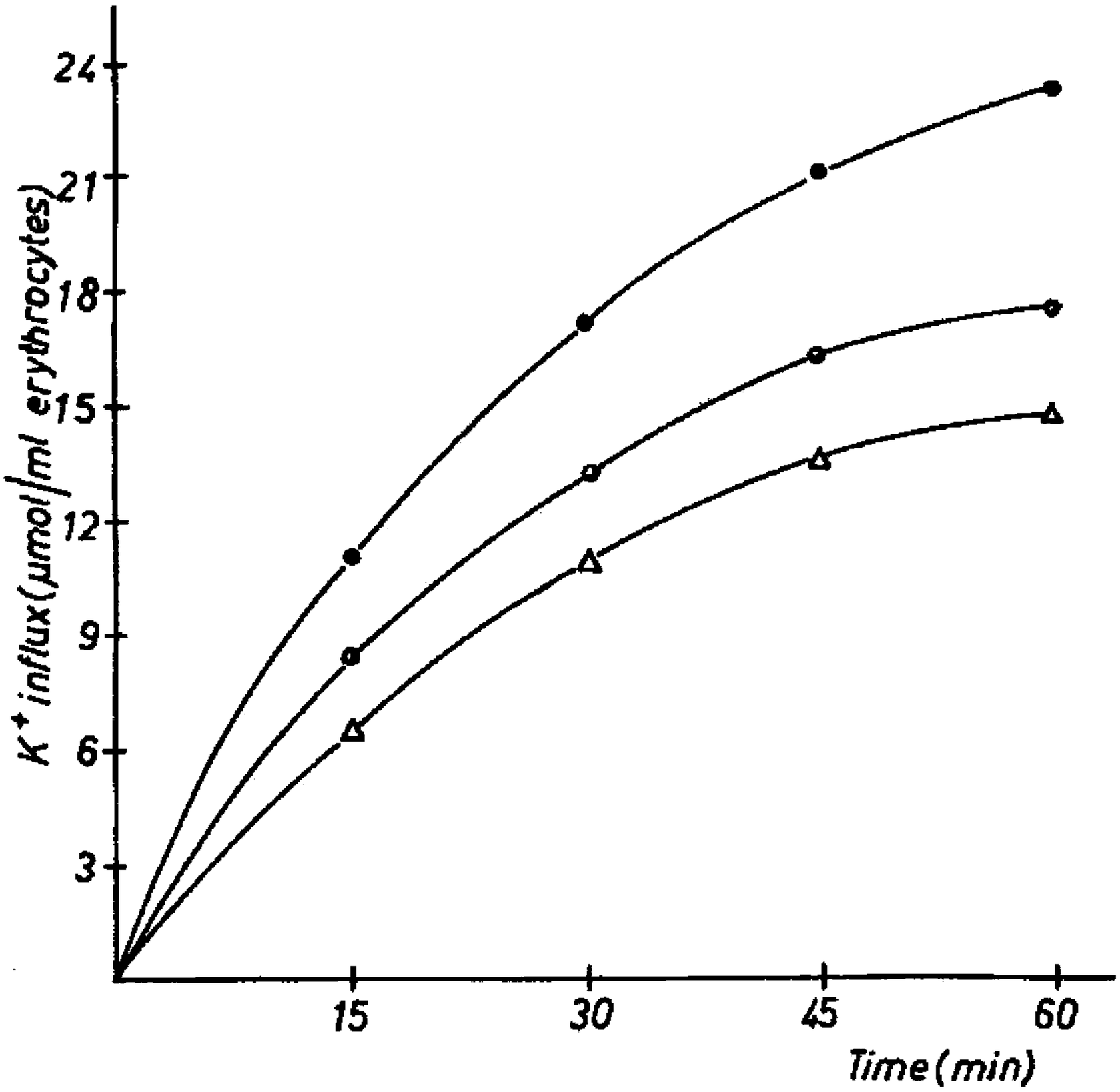

Fig. 3. Influx of potassium ions into rat erythrocytes. ●, Control. ○, Erythrocytes from Morris Hepatoma-bearing rats, 20 days following implantation of the tumor and Δ, erythrocytes from tumor bearing rats, 30 days following tumor implantation

well as by a decreased influx of potassium ions from the external environment into the cell. The cholesterol and phospholipid contents, in their cell membranes are changed resulting in a decreased molar ratio of cholesterol to phospholipid.

The studied fluxes of sodium and potassium ions are a resultant of two different transport processes, i.e. of the passive transport of these ions through the so called ion channels and the active transport against a concentration gradient, mediated by the enzymic system, the sodium : potassium pump, in which the $(Na^+ - K^+)$ATPase constitutes an important element. The chemical composition of cell membranes may be of importance for both transport systems. Kroes & Ostwald [23] have shown that erythrocyte membranes with an increased cholesterol content show a

Table 1

Cholesterol and phospholipid content and their molar ratios in membranes of erythrocytes from Morris Hepatoma-bearing rats

	Control rats (μmol/ml cells)	Tumor bearing rats (μmol/ml cells)	
		20 days after tumor implantation (I)	30 days after tumor implantation (II)
Cholesterol	3.38 ± 0.08 (100%)	2.97 ± 0.09 (88%)[a]	2.23 ± 0.09 (66%)[a,c]
Phospholipid	3.28 ± 0.25 (100%)	4.02 ± 0.28 (123%)[b]	3.30 ± 0.26 (101%)[c]
Cholesterol : phospholipid ratio	1.03	0.73	0.70

Number of animals = 6

[a]significantly less ($p < 0.001$) then corresponding value for control rats

[b] significantly greater ($p < 0.001$) then the corresponding value for control rats

[c]significantly less ($p < 0.001$) then the corresponding value for group I

decreased flux of sodium ions *via* both the passive transport through ion channels and the active one, involving the (Na^+- K^+)ATPase system.

According to these authors, the physicochemical properties of cell membranes are dependent on their chemical composition. Cell membranes with an increased cholesterol content are more rigid and have a lesser fluidity, which may alter the mobility of other components within these membranes, and this in turn may influence the ion flux across these membranes.

Giraud *et al.* [5] suggest that cholesterol influences the intracellular affinity of sodium and potassium for the enzymatic system of the (Na^+, K^+)-pump, by rearranging the structure of lipid memoranes.

According to others, the membrane binding sites for cations, in spite of being protein in nature, are dependent on, and modulated by, membrane phospholipids [24]. However, it is commonly acknowledged that an increase in the membrane cholesterol content reduces the rate of ionic transport across the membrane. The results of our experiments are not in keeping with this common view.

The erythrocytes of the Morris Hepatoma-bearing rats, in spite of the demonstrated decreased cholesterol and increased phospholipid contents, which resulted in a significantly reduced, in comparison with control

membranes, cholesterol : phospholipid molar ratio, showed a reduced transport of sodium and potassium across their membranes. These results may suggest that the reduced flux of sodium and potassium across erythrocytes membranes of the tumor bearing rats is caused by factors other than their lipid composition, which rendered the red blood cells less permeable to these ions in spite of a lipid composition favouring the transport of these ions.

The reduced export of sodium ions from the erythrocytes of the tumor bearing rats accompanied by a decreased import of potassium ions may be a result of some kind of modulation of the $(Na^+- K^+)$ATPase activity.

The activity of this enzyme is controlled by phosphatidylserine [24, 25] as well as by the amount and kind of fatty acids constituting the membrane lipids. Unsaturated fatty acids are known to increase the fluidity of membranes by decreasing their viscosity and by altering the membrane structure. Unsaturated fatty acids may thus cause some kind of disarrangement in the well organized hydrophobic zone of the membrane. This in turn may induce changes in the activity of $(Na^+- K^+)$ATPase, resulting in alterations of the ion transport capacity of the membrane.

A more detailed study on compositional changes occurring in the erythrocytes membrane as well as in the activity of membrane-bound enzymes should render possible the elucidation of the multiple questions raised in this paper.

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