# EFFECT OF TEMPERATURE ON POTASSIUM – DEPENDENT STIMULATION OF TRANSCELLULAR MIGRATION IN NORMAL AND NEOPLASTIC CELLS

R.STROM, P.CAIAFA, B.MODOVÌ and A.ROSSI FANELLI
*Institute of Biological Chemistry, University of Rome, Rome, Italy*

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## 1. Introduction

Neoplastic cells have been shown [1–5] to be more sensitive than normal cells to the effect of relatively high temperatures. Oxygen consumption, as well as incorporation of radioactive precursors into nucleic acids and proteins, are partially or fully inhibited in neoplastic cells by exposure to 42°–43° [3–5]. Therapeutic applications of these studies have been successfully carried out [1,2]. Since the effects observed were strictly dependent on the integrity of the cellular structure, and were considerably affected by agents acting on cell membranes, the biochemical mechanism of the peculiar heat sensitivity of tumor cells was tentatively ascribed to an alteration at the cell membrane level.

In the present study, we investigated the effect of "elevated" temperature on transcellular migration of glutamate through a complex membrane of normal, regenerating or neoplastic hepatocytes on a Millipore filter.

## 2. Materials and methods

Male Sprague-Dawley rats were used as the source of normal liver, regenerating liver and Novikoff hepatoma cells, prepared as previously described [2,4]. The suspension fluid was essentially a potassium-free Krebs-Ringer bicarbonate buffer [6], diluted with 1/15 of 0.154 M (isotonic )Na-glutamate, and containing 100 I.U./ml of benzylpenicillin and 0.1 mg/ml of streptomycin sulfate. The cell-coated Millipore filter was prepared as described by Harris and Friedman [7]. The complex membrane was formed by two cell-coated filters, sandwiched in an all-glass apparatus similar to that described by the same authors [7]; it separated a large compartment (200 ml) from a smaller one (20 ml). Both compartments were filled with the potassium-free Krebs-Ringer bicarbonate-glutamate buffer, and the whole apparatus immersed in a water bath at the desired temperature.

Experiments were started by addition of 50 $\mu$l of a L-U-$^{14}$C-glutamate solution (New England Nuclear Corp., Boston, Mass.; 0.1 millicurie/ml, 1.5 mc/mg) to the larger compartment. Migration was followed by repetitive sampling of 100 $\mu$l from the smaller compartment, and scintillation counting in a Nuclear Chicago mod. 725 liquid scintillation system, after mixing 20 ml of a dioxane-naphtalene solution [8].

## 3. Results

Normal liver cells, as well as cells from regenerating liver and from Novikoff hepatoma, followed, at 38°, the same pattern reported for Ehrlich ascites cells [7,9]: when potassium-free Krebs-Ringer bicarbonate medium containing 10 mM glutamate was present on both sides of the membrane, the rate of migration of $^{14}$C-glutamate into the smaller compartment was constant over a period of at least 3 hr. However, a lag-time of 15–30 min was almost constantly present.

Addition of isotonic KCl-glutamate to the smaller compartment (final concentration in this compartment: KCl = 12 mM, glutamate = 10 mM) resulted, with all kinds of cells at 38°, in a stimulation of the migration rate, as described by Harris and Friedman

Table 1

Influence of $K^+$ ionic gradient upon the rate of transcellular migration. The migration rate is expressed as increase per minute of the concentration of radioactive glutamate in the smaller compartment, i.e. as increase of c.p.m./100 μl of the fluid of that compartment.

| | Normal liver cells | | | Regenerating liver cells | | | Novikoff hepatoma cells | | |
|---|---|---|---|---|---|---|---|---|---|
| | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 1 | Exp. 2 | Exp. 3 |
| 38°, no KCl | 6.90 | 4.80 | 4.20 | 3.81 | 3.20 | 3.70 | 6.48 | 6.00 | 4.83 |
| 38° + KCl in the smaller compartment | 9.60 | 6.90 | 5.54 | 4.62 | 4.40 | 4.78 | 10.20 | 7.90 | 7.60 |
| $R^{+KCl}_{no\ KCl}$ | 1.39 | 1.44 | 1.32 | 1.21 | 1.38 | 1.29 | 1.57 | 1.32 | 1.57 |

Table 2

Influence of temperature upon the rate of transcellular migration. The migration rate is expressed as indicated in table 1.

| | Normal liver cells | | Regenerating liver cells | | | Novikoff hepatoma cells | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Exp. 1 | Exp. 2 | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 4 |
| 38° | 4.72 | 4.20 | 2.58 | 3.68 | 3.20 | 6.00 | 8.89 | 6.06 | 4.83 |
| 40° | – | – | 3.34 | 4.78 | – | 8.39 | 11.90 | – | – |
| 40° + KCl | – | – | 3.98 | 5.74 | – | 8.36 | 11.90 | – | – |
| $R^{40°}_{38°}$, no KCl | – | – | 1.29 | 1.30 | – | 1.40 | 1.34 | – | – |
| 42° | 5.00 | 4.33 | – | 5.78 | 4.50 | – | – | 9.00 | 7.70 |
| 42° + KCl | 6.23 | 5.54 | – | 5.88 | 4.60 | – | – | 9.00 | 7.73 |
| $R^{42°}_{38°}$, no KCl | 1.09 | 1.03 | – | 1.57 | 1.40 | – | – | 1.48 | 1.59 |

[7] for Ehrlich ascites cells. The extent of this stimulation, although somewhat variable with different batches of cells, ranged around 30–45% for normal hepatocytes, 20–40% for regenerating liver cells, and 30–60% for Novikoff hepatoma cells (table 1). Addition of KCN, NaCN or 2,4-dinitrophenol to any of the two compartments did not, in our hands, produce clearcut results.

A similar, or even identical, pattern was followed also at 42° by normal liver cells (fig. 1). The rate of migration through Novikoff hepatoma cells was instead significantly higher at 42° than at 38°, and no stimulation by $K^+$ ions could be evidenced any more at 42°, nor, as shown by fig. 2, even at 40°. The enhancement by temperature was relatively fast, being complete within the equilibration time (about 10 min) and was not reversed by changing the temperature back to 38° (fig. 3b).

The behavior of regenerating liver cells is intermediate between that of normal and that of neoplastic hepatocytes; the rate of migration at 40° in the absence of $K^+$-ions, although higher than at 38°, can still be enhanced by addition of KCl (fig. 4). At 42°, instead, no further stimulation by KCl can be observed.

The rate of migration through cells, whose membranes have been disrupted by repetitive freezing and thawing, is constantly high, and no enhancement either by higher temperatures or by KCl addition is possible (fig. 3c).

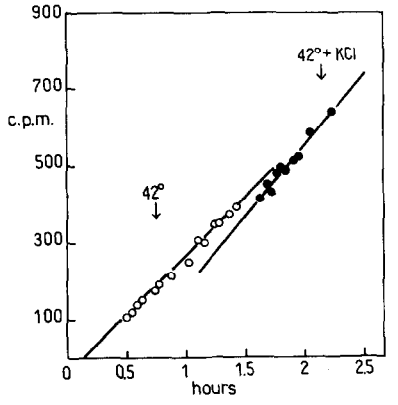


Fig. 1. Transcellular migration of glutamate at 42° through normal liver cells. After 90 min, KCl was added to the smaller compartment to a final concentration of 12 mM. On the ordinate axis are indicated the counts per minute in 0.1 ml of the content of the smaller compartment.

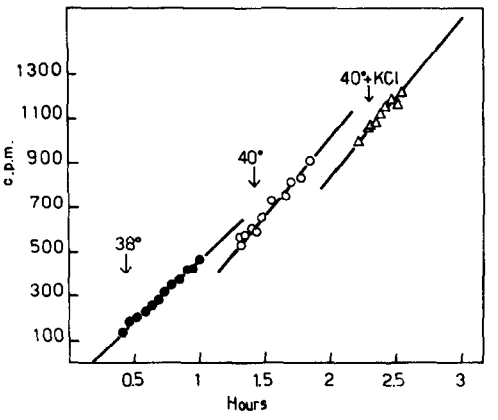


Fig. 2. Effect of temperature increase on transcellular migration of glutamate through Novikoff hepatoma cells. After 70 min at 38°, the apparatus was transferred to 40°. Addition of KCl to the smaller compartment was subsequently performed as indicated previously.

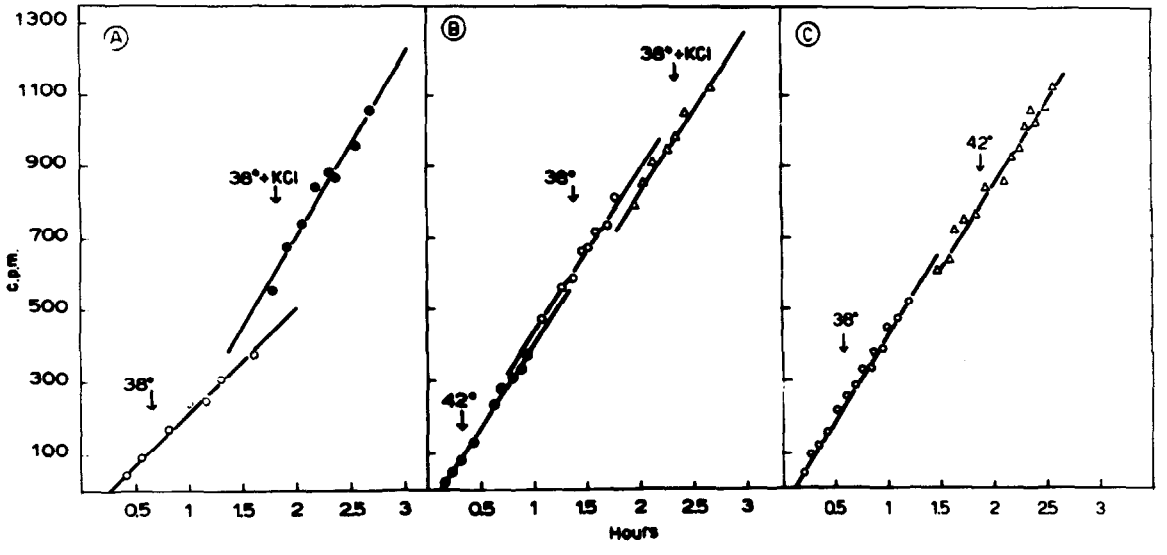


Fig. 3a. Effect of KCl on Novikoff hepatoma cells at 38°. b. Irreversibility of the temperature effect in Novikoff hepatoma cells. c. Ineffectiveness of temperature increase in disrupted Novikoff hepatoma cells. In all cases, the same preparation of Novikoff hepatoma cells was used. In experiment c, cells were disrupted, prior to the preparation of the membrane, by freezing and thawing 3 times.

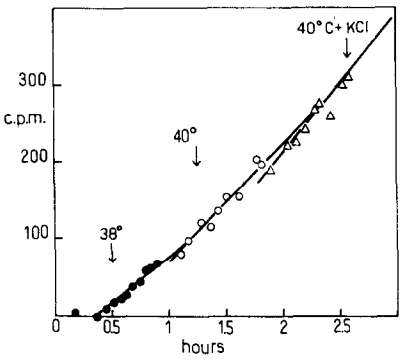


Fig. 4. Effects of temperature increase and of KCl addition on transcellular migration of glutamate through regenerating liver cells. Experimental conditions as for fig. 2.

## 4. Discussion

Transcellular migration of glutamate across an ionic gradient is a complex phenomenon, where the effects of simple diffusion are superimposed on those due to facilitated and active transport.

The extent of stimulation by temperature of diffusion phenomena may hardly be evaluated from the literature [10], as there is a large variability according to the diffusing molecule, the type of membrane, the temperature range considered, and even the method used. Four sets of data, however, rule out simple diffusion as being sole responsible for the temperature effect:

(1) Disrupted Novikoff cells do not show a temperature dependence;

(2) Temperature effects on normal regenerating and neoplastic hepatocytes differ considerably;

(3) At 40° or 42°, $K^+$ ions do not produce any further stimulation of migration through Novikoff hepatoma cells. This requires that facilitated transport be inactivated by the rise in temperature, i.e. that more complex phenomena occur within the cell membrane;

(4) Simple temperature dependence of diffusion rates would not be irreversible, as is stimulation by heat in Novikoff hepatoma cells.

On the other hand, investigations by several authors [11–15] on the effect of heat on either cellular or model membranes have shown that in many cases profound modifications occur at temperatures which are critical for the membrane considered, and which may be around 40°–42° [11,13,16]. Thermal transitions, in the 38°–42° range, have also been observed with pure lipid compounds, such as for instance lauroyl-1,2-diglyceride [17], 2,3-dipalmitoyl-1-phosphatidylcholine [18,19], 2-oleoyl-3-stearoyl-1-phosphatidylcholine [16], cholesterol [16] and even more markedly with binary or multiple systems of lipid compounds [17].

Although these thermal transitions, in the pure compounds or in well-defined mixture of them, should be reversible, irreversible phenomena can easily occur in a complex membrane structure. This may result, in membranes of appropriate composition and/or structure, either in a modification of the diffusion properties of the membrane, or in a loss of specific transport capabilities, or in both. This hypothesis would imply that membranes from normal, regenerating and neoplastic hepatocytes have different composition and/or structure, so that thermal transition occurs, for each kind of cells, at a different temperature. These transitions would either lead, as a secondary effect, to irreversible phenomena, or might even be themselves practically irreversible within a finite time, as indicated by some experiments on synthetic phospholipids [20].

It appears, as if for every kind of cells there was a limiting rate of transcellular migration, which may be reached either by addition of $K^+$ ions to one side of the membrane or through an appropriate raise in temperature. In the latter case, addition of $K^+$, or even gross membrane damage, cannot increase the migration rate above the maximum level. The temperature required for maximum migration rate in the absence of a $K^+$ ions gradient would be $38° < t_1 \leqslant 40°$ for Novikoff hepatoma cells, $40° < t_2 \leqslant 42°$ for regenerating liver cells, and $t_3 > 42°$ for normal hepatocytes. Although regenerating liver cells also show a certain heat lability, which may be a property of rapidly growing cells in general, Novikoff hepatoma cells are characterized by a peculiar heat sensitivity, especially if compared to normal hepatocytes. It may well be that these alterations at the membrane level would be reflected in the respiratory and biosynthetic activities of the cell itself, and produce the behaviour previously described [1–5].

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