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OFFICE OF NAVAL RESEARCH

(15) Contract N00014-76-C-1166 new

Task No. NR 207-039

TECHNICAL REPORT NO. 1

(6) Ion Exchange in Normal and Leukemic Human Lymphocytes: Description by the Association-Induction Hypothesis and Experimental Testing of its Predictions.

by

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(9) Technical rept. no. 1 (Annual) Oct 76 – Oct 77,

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(11) 4 January 1978

(12) 16 p.

406 784

Annual Report Number 1 - Office of Naval Research Contract
Ion-exchange in Normal and Leukemic Human Lymphocytes: Description by the Association-induction Hypothesis and Experimental Testing of its Predictions
Contract # N00014-76-C-1166

PII Redacted

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[redacted]

Period of Report: 10/76-10/77

The long-range goal of this project is to define the physiological factors that control cell division in both normal and malignant cells. To do this, an integrated concept of cell function is required. The cell surface membrane plays a major role in this regard; however, the correlation of cell surface, cytoplasmic, and nuclear events in the control of cell division remains poorly defined. Hence, we asked whether the association-induction hypothesis of Ling, which provides a detailed physical chemical concept of cell function applicable to a wide variety of phenomena, might not provide a useful base from which to begin to comprehend the complexity of both the normally dividing cell, and the malignant cell.

The human lymphocyte was chosen, first, because it is readily stimulated to undergo cell division in culture, and second, because in the form of the lymphatic leukemias it provides the only readily available human cancer cell that can be easily separated from other, normal, cells.

Since the association-induction hypothesis has focused upon potassium and sodium as "probes", as it were, of the physical state of the cell, since potassium and sodium flux or content are altered during mitogenic transformation of lymphocytes, and since altered content and physical state of potassium, sodium and water have been described in cancer cells, including the leukemic lymphocyte, we have focused our attention first upon potassium, sodium and water metabolism in human lymphocytes.

There are three sorts of studies that might be done in cells, in order to define and specify the nature of ion distribution and exchange. These three sorts of studies contain the fewest variables, and hence are most amenable to use in testing theoretical predictions. They are: 1) the equilibrium (or steady-state) contents of ions; 2) the exchange of one ion for another under the most extreme non-equilibrium conditions; and

(cont'd p 2)

(3) the exchange of a labeled ion for its unlabeled counterpart under conditions in which the total ion contents are in equilibrium (or steady-state) with regard to the (stable) external ion content. Experiments strictly fulfilling these criteria are quite rare in the scientific literature. The first two sets of experiments have been completed by us, and were carefully reported in the six month report for this contract. Two manuscripts were included with that report, and had been submitted to the Journal of Cell Biology for review.

This report will cover three major activities: (1) Discussion of some additional data and of the exchange with the Journal of Cell Biology over the two papers submitted; (2) Results of experiments done over the past six months having to do with equilibrium ion transport; and (3) Experiments recently done to clarify the question of ion content changes in mitogenically-transformed lymphocytes.

1. Equilibrium ion contents and potassium exchange from extreme non-equilibrium state.

A major conclusion from these studies, reported in the six month report, was that there are two components of potassium, a saturable one, and a non-saturable one. The non-saturable component has a distribution ratio between cell water and external water of 0.65. Because the standard deviations of these data were overlapping, we carried the experiments further, up to an external potassium level of 64 mM, in order to be absolutely certain that the non-saturable component of cell potassium was reproducible. In fact, these experiments gave a distribution ratio of 0.5, and showed unequivocably the reality of this component of cell potassium (Figure A).

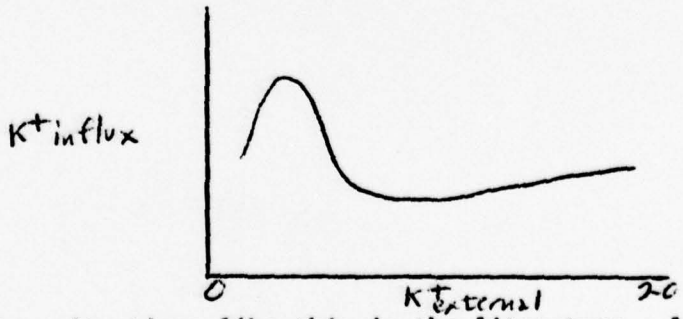
The two manuscripts that were included in the six month report were reviewed by the Journal of Cell Biology for six months and then were emphatically rejected on the basis of the reviewers' comments. The reviews were quite inadequate; therefore, I prepared a detailed rebuttal and re-submitted the papers to the Journal of Cell Biology. A copy of that rebuttal is attached to this report, as it contains a good deal of information immediately relevant to the major issue at hand, whether

or not the association-induction hypothesis is a viable model for cell function. As a result of my rebuttal, the Journal of Cell Biology has agreed to review the papers again and reconsider them for publication.

2. Exchange of potassium under equilibrium conditions.

This sort of ion flux study is in fact the "standard" sort of short-term ion flux studies that have been done and are reported throughout the literature, in red cells, squid axon, and a wide variety of other cells. In those studies, initial ion flux was determined as a function of change in the external or internal ion concentrations, and the results usually show a Michaelis-Menten or a sigmoid relation. The claim that these are in "steady-state", appears to be based on the absence of a macroscopic change in cell ion levels during the course of the experiment, usually 10-30 minutes. However, most of those studies have not insured a genuine steady-state (or equilibrium) at the microscopic level, as this requires not only an unequivocal stability of ion contents, and not only that influx equal efflux, but also that the rate of change of influx and efflux itself is stable in time.

Using conditions, described in the first manuscript by Negendank and Shaller, that was attached to the six month report, and that are unequivocably equilibrium conditions, we have studied potassium influx in human lymphocytes as a function of external potassium. Preliminary experiments done a couple of years ago showed rather peculiar results as diagrammed below:



There are two situations like this in the literature. 1. Hempling (1), in the Ehrlich ascites tumor cell, in 1962, showed the decrease and then increase in influx with increasing external potassium. 2. Jones and Karreman (2), using the

dog carotid artery muscle, found that the rate constant for potassium influx varied with external potassium by the empirical relation A + Bexp ($-K^+$) and that the flux was influenced by the cooperative relation between internal and external potassium. These studies, in contrast to the "standard" ones, have two things in common. First they are close to a true equilibrium over the entire range of external potassium studied, and second, as external potassium was increased, external sodium was proportionately decreased.

Another piece of information derived from our preliminary studies was that potassium exchange appeared to be surface limited, and not limited by cytoplasmic bulk phase diffusion. Both the single exponential function of flux (see below), as well as analysis by using a technique derived by Crank (3), indicated this.

Over the past six months we have pursued these phenomena, have in essence repeated the experiments and shown again the peculiar nature of potassium influx with a peak followed by a trough and then progressive rise in the rate of exchange of potassium through the saturable component of cell potassium.

The technique, briefly, is to pre-equilibrate cells at a given external potassium level for 24 to 48 hours, under conditions described previously and carefully studied in our first manuscript attached to the six month report. We then transferred the cells to media containing radioactive potassium, but with identical total potassium and sodium contents. The exchange of radioactive for labeled potassium was then studied, using a cell separation technique in which cells were not washed and were not exposed to any other media whatsoever. Cells were spun down at 6,000 g's into microtubes, and the tip of the plastic microtube was then cut off and analyzed for radioactivity, and for total cell potassium and sodium contents. Previous studies had defined the water contents under identical conditions, and had shown that the trapped space is 4%.

The results were then plotted as the influx, expressed as 1 minus the ratio of specific activity of potassium in the cell to potassium in the external medium at three different times. The results are shown in the attached figures (B,C,D). The stability of external potassium levels was assured by monitoring the medium, and the

stability of internal potassium levels is shown in the attached figure (E). As can be seen from these figures, the previously determined two components of cell potassium are also reflected here, with a rapid component of potassium influx followed by a single slower exponential element of influx. The components may be separated simply by extrapolating the slower curves back to the ordinate, since the entire system is in equilibrium, and the amount of potassium participating in the slow and the fast exchange components determined. These show that the amount of potassium in the rapid component is very similar to the amount previously found to be in the non-saturable component of cell potassium.

We then plotted the rate of influx as a function of external potassium for the saturable component of cell potassium, figure F. These results show the reproducibility of our preliminary data, and the rather peculiar nature of the relationship of the influx to the external potassium.

How may these results be interpreted? Clearly, there must be at least three quasi-independent variables operating. The association-induction hypothesis views potassium permeation as being limited by adsorption to and desorption from the fixed negative charges at the cell surface itself. Ling, in his book in 1962 (4), predicted that the equilibrium exchange of a strongly adsorbed ion, such as potassium in the resting cell, would be surface limited if the surface sites that adsorb potassium also have the same high affinity for potassium that the internal sites have; and that sodium would potentiate potassium permeation by a triplet adsorption-desorption mechanism. A diagram of this process was given on page 336, and the triplet adsorption-desorption described by equation 11-13 on page 300:

$$[\rho_i^{ex}\cdot f\cdot \rho_j^{ex}]^{I}_{surface} = \frac{\exp(\gamma F/RT)}{1+\exp(\gamma F/RT)}\,[\rho_i^{ex}\cdot f]_{surface} \times$$

$$\frac{[\rho_j]_{ex}\,\dfrac{(\rho f)^{triplet}_{ij}}{(\rho f)^{free}_{j}}\,\exp\left[\dfrac{-(\Delta E_{ij}+\Delta E_i)}{RT}\right]\exp\dfrac{(\delta_t[\Delta E_{ij}-\Sigma E_j]-E_t^{\cdot}}{RT}}{1+\left\{\sum_j[\rho_j]_{ex}\,\dfrac{(\rho f)^{triplet}_{ij}}{(\rho f_i)^{free}_{j}}+\sum_j[\rho_j]_{ins}\,\dfrac{(\rho_i f_i)^{triplet}_{ij}}{(\rho f_i)^{free}_{j}}\right\}\exp\left[\dfrac{-(\Delta E_{ij}+\Delta E_i)}{RT}\right.}$$

where $i=K^+$, $j=Na^+$, $f=$ surface sites, $ins=$ interstitial (in cell water).

However, Ling did not at that time take into account the cooperative relation between internal and external potassium. If the surface sites bear the same cooperative relation to external potassium that the inner sites do, then the apparent dependence of potassium influx on internal potassium may actually be due to the fact that, at equilibrium, the concentration and affinity of the surface sites are directly related to those of the total internal potassium sites. One may qualitatively describe the data of figure F in the following manner.

A. At external potassium below 1 mM, much of the cell, including the surface fixed negative charges, is in the sodium state and is near the cooperative transition (which occurs at 0.6 mM potassium as determined in our prior equilibrium distribution experiment). The labeled potassium influx rises steeply, and is very high because 1. labeled potassium competes readily with sodium for surface sites containg sodium and 2. there remains a lot of sodium near the surface to facilitate labeled potassium entry via the triplet adsorption-desorption mechanism which in turn is relatively efficient because of an average potassium/sodium affinity that is relatively low.

B. At external potassium between 1 and 10 mM, the cell, and the surface sites, are almost entirely in the potassium state. Labeled potassium influx reaches a minimum because 1. labeled potassium is now competing with unlabeled potassium for surface sites containing potassium, and having a higher tendency to hold onto potassium than sodium, and 2. facilitation of potassium entry via the triplet mechanism is less efficient because the potassium/sodium affinity ratio of surface sites is now much higher.

C. At external potassium above 10 mM, the cell remains in the potassium state, but the rate of labeled potassium influx rises because there is greater facilitation of potassium entry by potassium itself, in essence a mass action effect.

The next question to be answered is whether these ideas may be tested quantitatively. Dr. George Karreman has developed an appropriate mathematical treatment of these ideas, and we will attempt to apply this to our data. Unfortunately, we cannot directly test

theoretical predictions without some degree of curve fitting, because there are too many variable parameters. Therefore we will fit the mathematical description to the data described in Figure F, and will then determine the parameters in that way. We will next use these parameters and the theoretical equations to attempt to predict the results of another type of flux study, specifically the influx of labeled potassium under equilibrium conditions, but in which a) the external sodium is maintained constant at 150 mM, b) external sodium is replaced by sucrose, and c) external potassium is replaced by rubidium.

3. Ion contents of mitogenically-transformed lymphocytes.

There have been a very large number of physiological and biochemical changes described in lymphocytes being treated with mitogenic lectins, such as PHA or Con-A. Among these are an increased influx and eflux of potassium. There have been variable reports of ion contents, in which sometimes they seem to go up, sometimes down, and sometimes are not changed at all. In our own previous work, we described a decrease in cell potassium which was not accompanied by an increase in cell sodium but in which there was no change in the total water content. These results were reported (5), and were then repeated by an entirely different cell preparation technique, and reported at the Eleventh Leucocyte Culture Conference (6). In the first set of studies, the cells were separated through 12% sucrose into microtubes. In the second set of studies they were separated through an oil-dibutylphthalate mixture. Because of the possibility of artifact associated with these separations, we repeated our study with lymphocytes treated with PHA or Con-A, and determined cell ion contents in the manner described above and reported in our previous manuscripts associated with this report. With this technique, no washing of cells was done at all, and they were exposed to no other media. The results are shown in Table I. It is evident that potassium content does drop, but it is also evident that it is replaced by sodium. It seems likely therefore that in our previous experiments sodium was washed out of the cells in the separation process. Nevertheless, the fact that the sodium could be washed out, and the total sodium + potassium decreased without a change in the total water content, remains a mystery.

References

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2. Jones, A. and G. Karreman. 1969. Biophysical Journal 9:910.

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4. Ling, G.N. 1962. A Physical Theory of the Living State, Blaisdell, New York

5. Negendank, W.G. and C.R. Collier. 1976. Exp. Cell Res. 101:31.

6. Negendank, W.G. and C. Shaller. 1977. In Regulatory Mechanisms in Lymphocyte Activation, Academic Press, N.Y., p. 429.

Table I

Equilibrium ion contents at 24 hours (mmol/kg):

	Control	Con A 20µg/ml	PHA 20µg/ml
K^+	126 ± 8.7	85.7 ± 8.4	108 ± 7.1
Na^+	37 ± 5.5	75 ± 6.0	53 ± 6.0
$K^+ + Na^+$	163 ± 9.5	161 ± 3.0	161 ± 0

Figure A

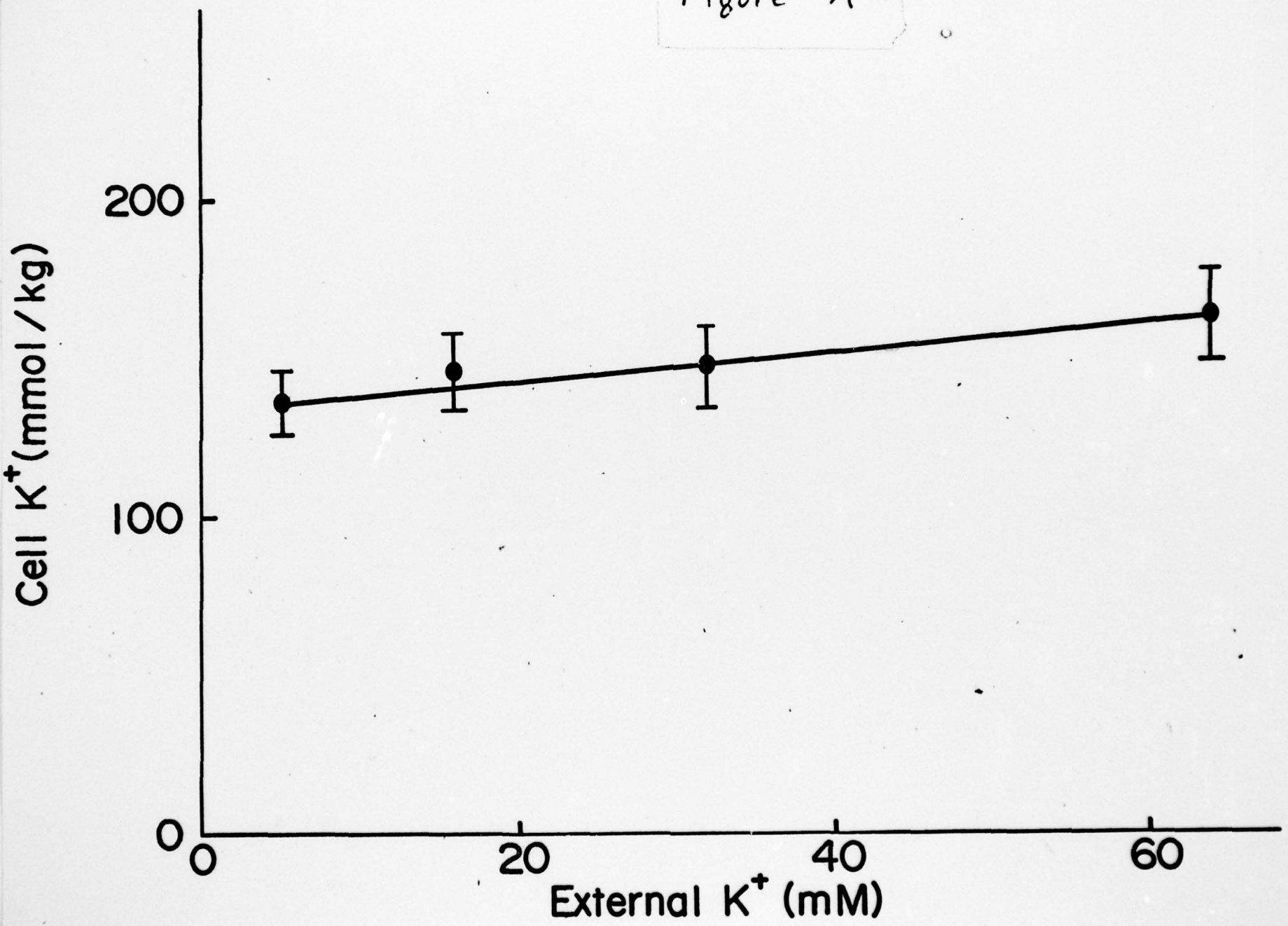


Figure B

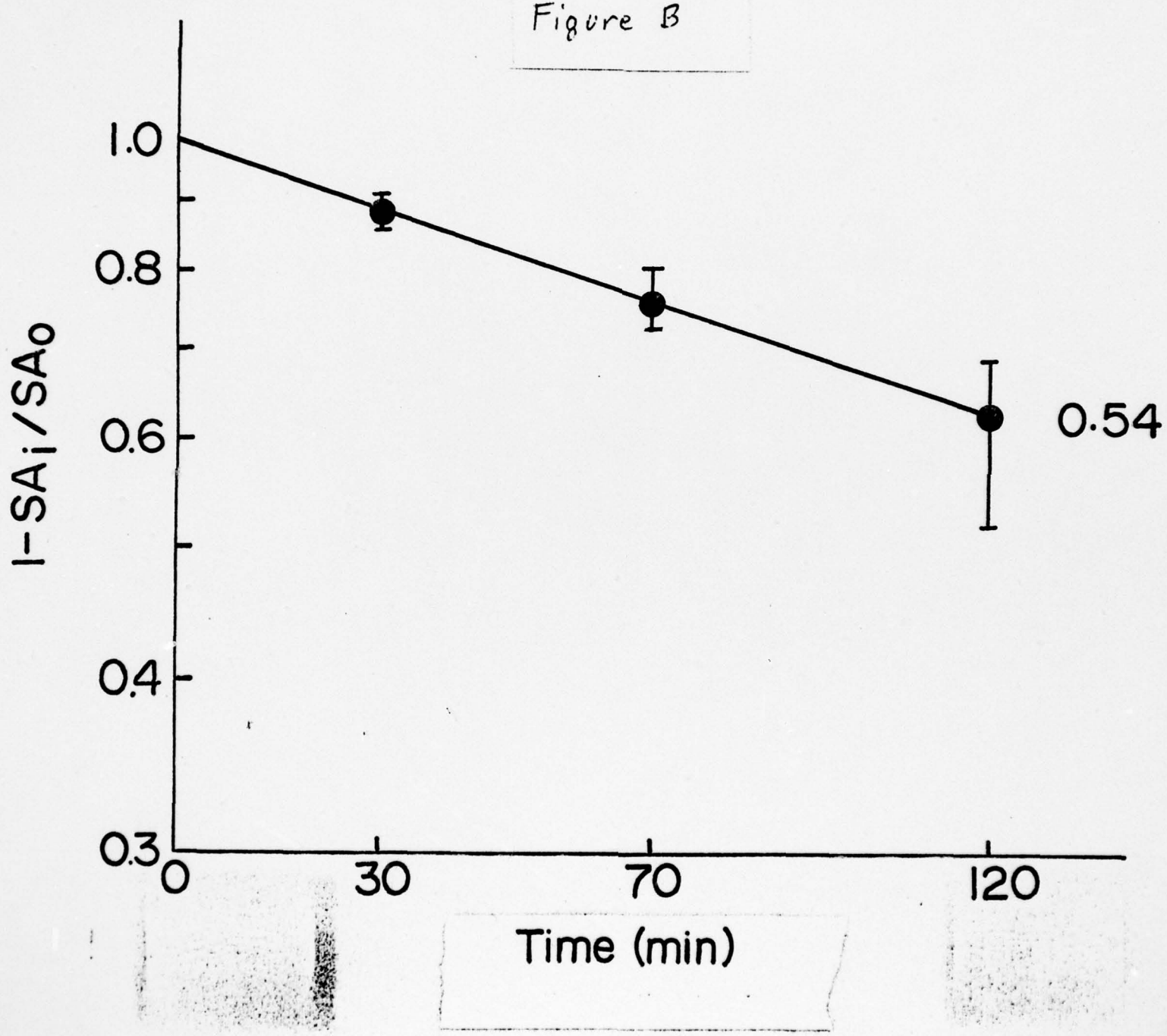


Figure C

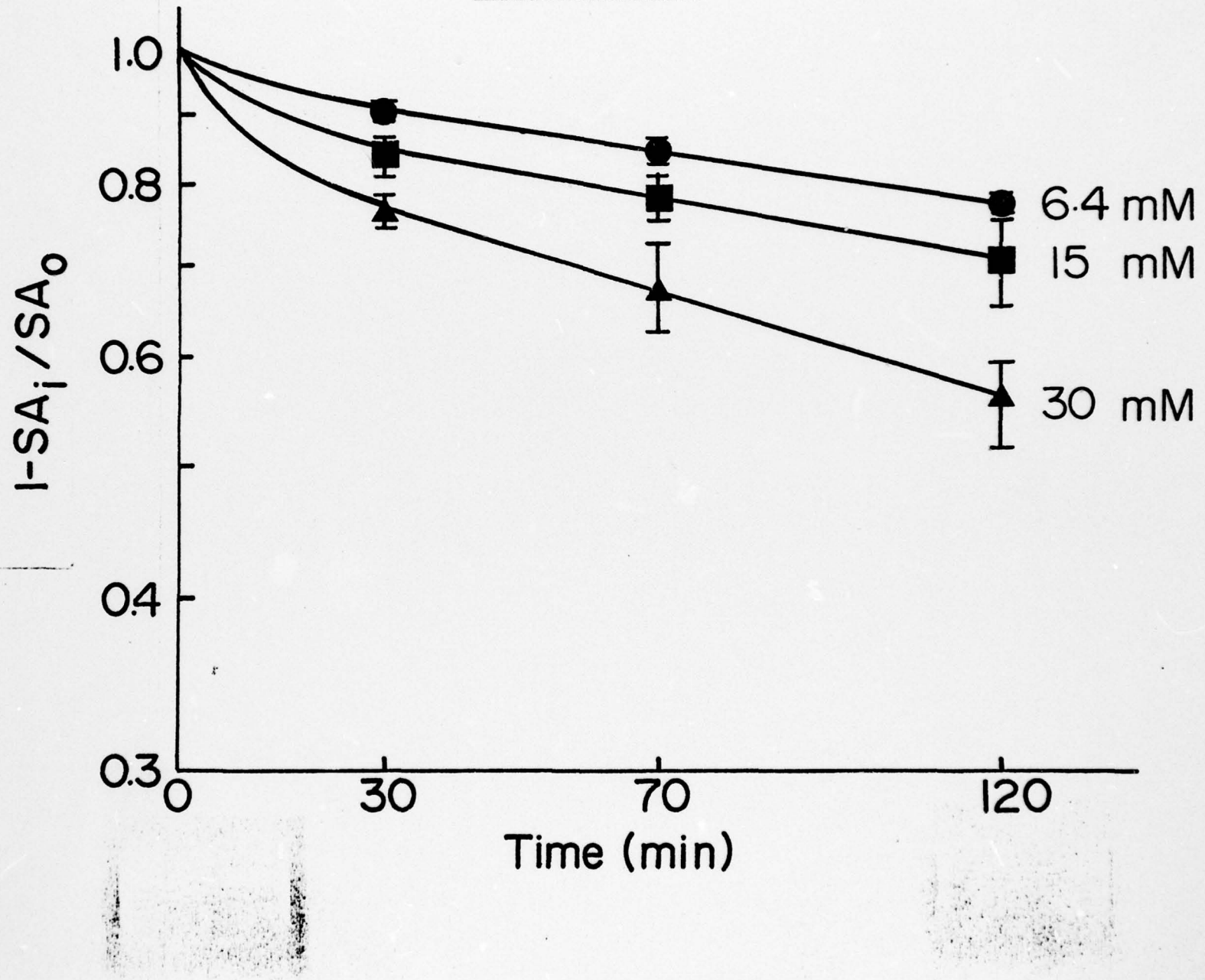


Figure D

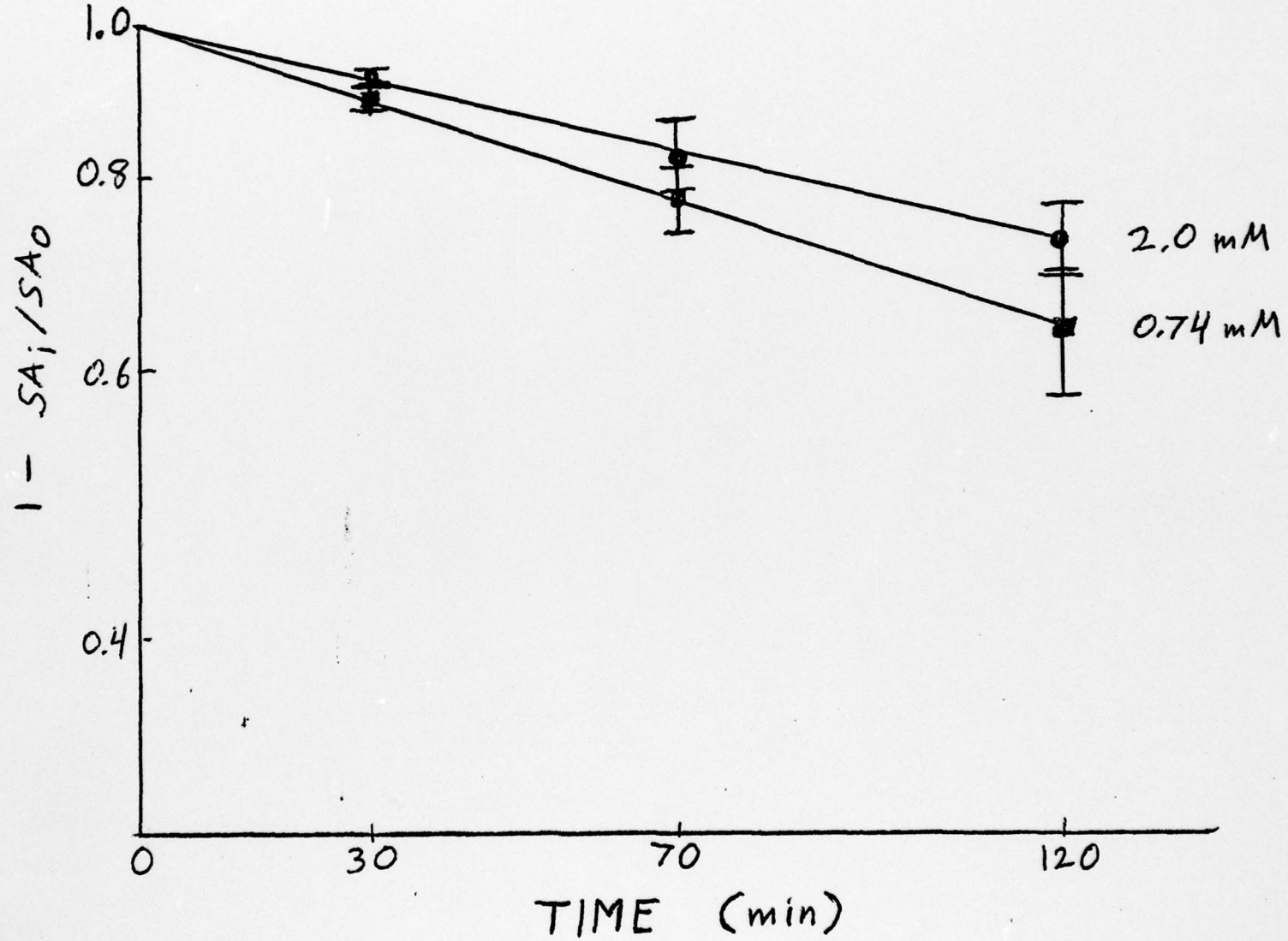


Figure E

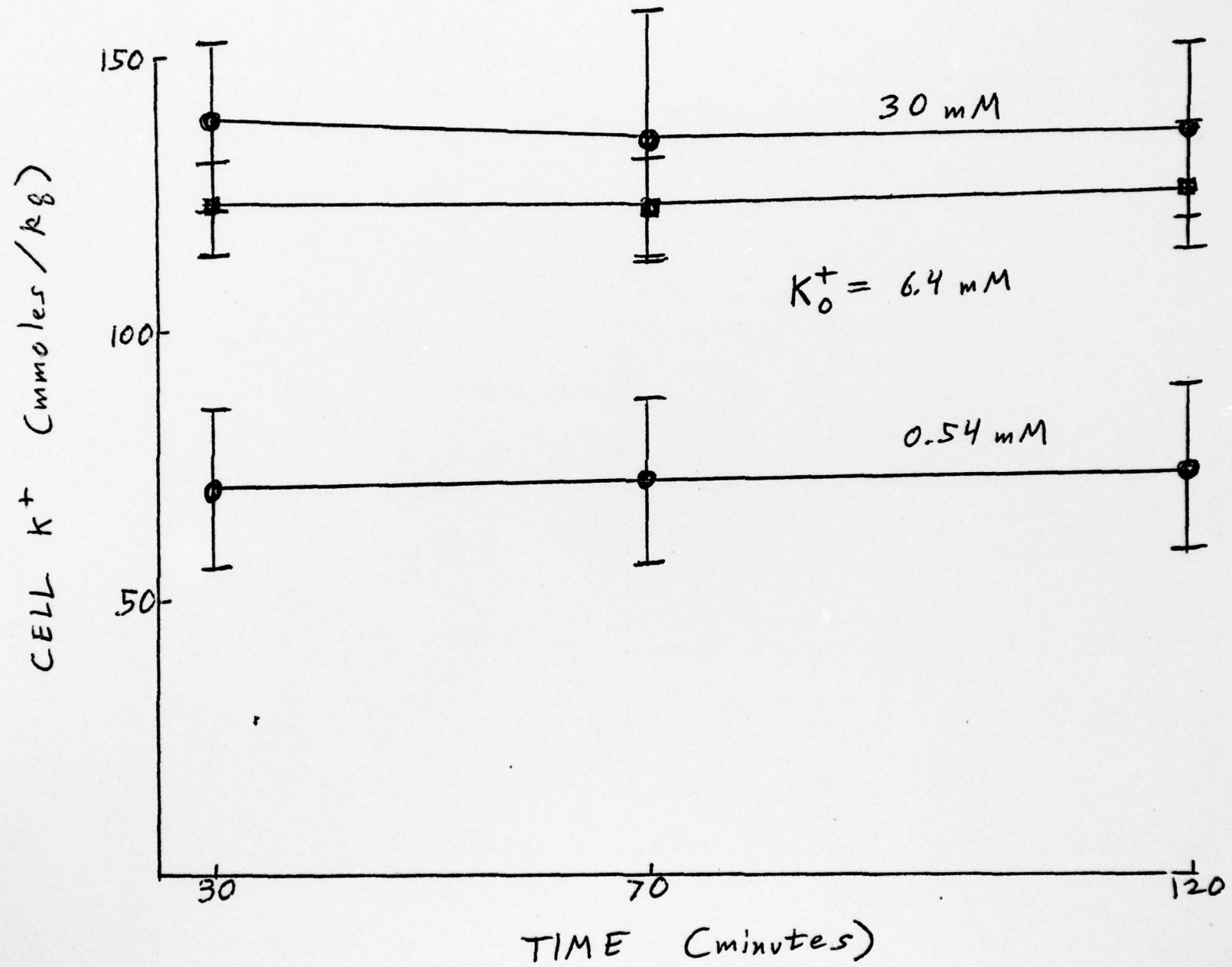
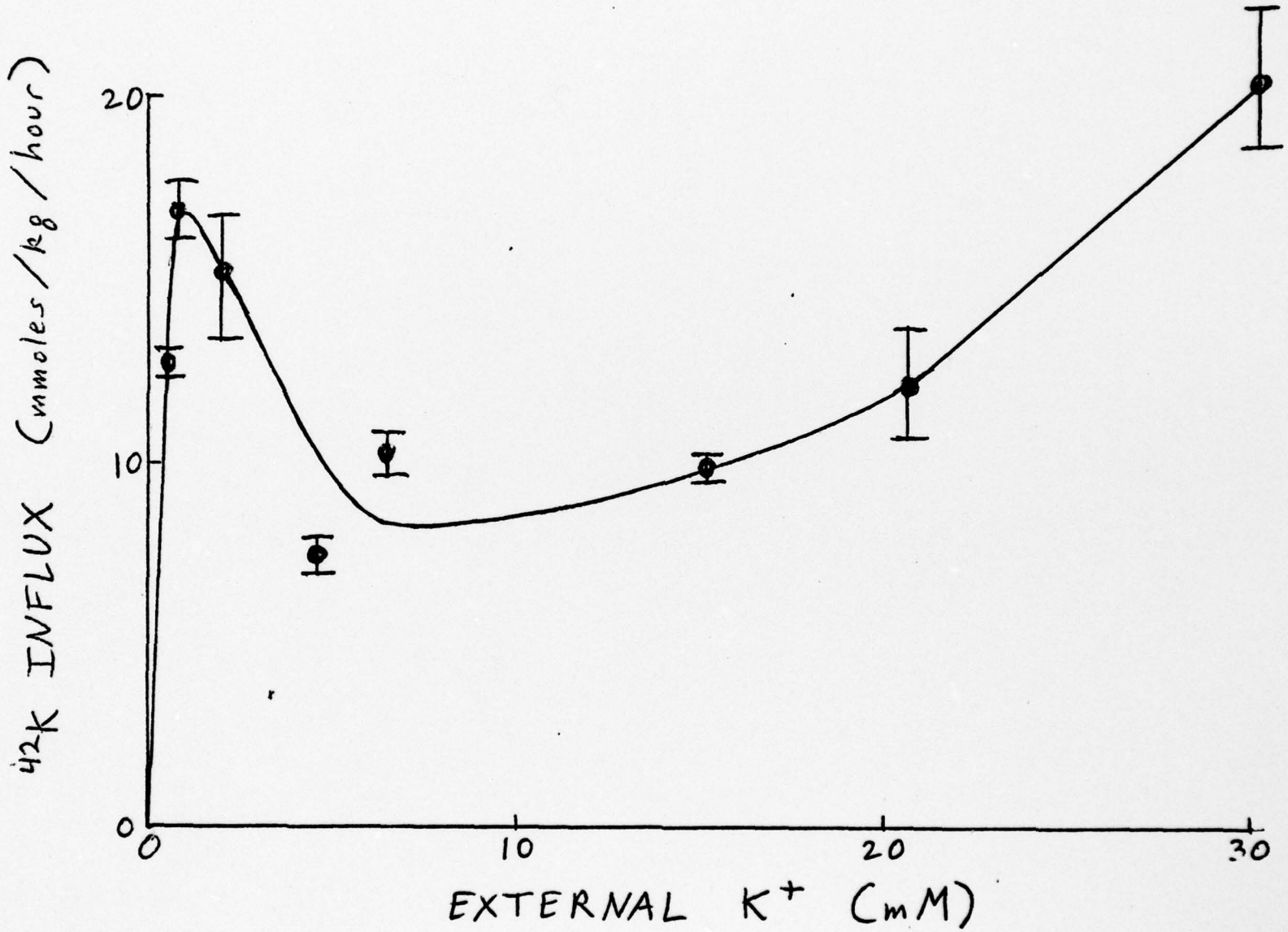

150
100
50
CELL K+ (mmoles / kg)
30 mM
K_o^+ = 6.4 mM
0.54 mM
30
70
120
TIME (minutes)

Figure F.



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