# THE RÔLE OF INTRACELLULAR CATIONS ON LIVER GLYCOGEN FORMATION IN VITRO

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The importance of specific inorganic ion concentrations in the extracellular fluids of the body for the maintenance of the integrity and normal function of cells has long been recognized. The development of "balanced" salt solutions, such as Ringer's solution, as a substitute for natural extracellular fluid is an illustration of this. In recent years, attention has turned, however, to the importance of the intracellular ion concentrations for the maintenance of normal intracellular enzyme activity.[1,2] This becomes of special urgency in *in vitro* studies in which metabolic functions of tissues are under investigation. For example, the intracellular fluid of mammalian liver is high in potassium and magnesium and low in (or perhaps free from) sodium and calcium; whereas the extracellular fluid of the tissue is similar to blood plasma, high in sodium and low in potassium, magnesium and calcium. When liver tissue is incubated *in vitro* at body temperature in an artificial extracellular medium such as Ringer's solution, there is a progressive change in permeability of the cells with a resultant exchange of intracellular and extracellular ions until equilibrium is established. Such an exchange of ions results in an intracellular ionic environment which is quite different from that which normally obtains in the body.

However, by placing tissues in a fluid similar to the intracellular fluid with respect to its cations, it should be possible to maintain the normal intracellular cation environment in spite of exchange of ions across the cell boundary.

The present communication reports the effects the differences in ionic environment produced upon one metabolic function of rat liver—namely, the production of glycogen from glucose *in vitro*. Our experiments were stimulated by the work of Ostern, Herbert and Holmes[3] who reported in 1939 the *in vitro* production of glycogen by rabbit liver when incubated with glucose in Ringer's solution enriched with calcium. These experiments with rabbit liver were repeated and confirmed in our laboratory, but rat liver, under the same conditions, failed to produce significant amounts of glycogen. We, therefore, turned to a more critical examination of the effect of intracellular versus extracellular cations on glycogen formation.

The specific problem undertaken was the comparison of the glycogen content of liver slices before and after they had been incubated for one hour at 38°, in isotonic glucose-containing solutions: (*a*) similar to intracel-

lular fluids with respect to cations—i.e., high in potassium and magnesium, and (*b*) similar to extracellular fluids—i.e., high in sodium with small amounts of potassium, calcium and magnesium. Comparable experiments were also performed in the absence of glucose. These provided information on the rôle of cations on net glycogen formation and breakdown. In view of the complexity of the intracellular organic phosphate anions, it is impractical at present to provide a normal intracellular anionic environment in the extracellular medium.

It may be stated at once that incubation of the liver in the high potassium, high magnesium medium favored glycogen synthesis, whereas incubation in the high sodium medium favored glycogen breakdown.

*Experimental.*

| SOLUTION | Na, mM/L | K, mM/L | Ca mM/L | Mg, mM/L | Cl, mM/L | $HCO_3$, mM/L | GLUCOSE % |
|---|---|---|---|---|---|---|---|
| I | 0 | 130 | 0 | 20 | 130 | 40 | 1.0 |
| II | 152 | 5 | 1 | 1 | 121 | 40 | 1.0 |
| III | 0 | 130 | 0 | 20 | 130 | 40 | 0 |
| IV | 152 | 5 | 1 | 1 | 121 | 40 | 0 |

The above solutions were equilibrated with 5% $CO_2$:95% $O_2$. When 2 cc. of such solutions were incubated in 25 cc. vessels with 300–500 mg. of rat liver slices for one hour, the resultant pH was found to be between 7.35 and 7.45. The glycogen content of liver plus the solution of each vessel was determined by the method of Good, Kramer and Somogyi[4] as modified by Sjörgen.[5] The total phosphorus content of each vessel was also determined by the method of Fiske and Subbarow.[6] The initial glycogen and phosphorus contents of the liver were determined before incubation in order to provide a basis for comparison. It was found to be more convenient to estimate the amount of liver in each vessel from the phosphorus determinations than to depend upon the weights of the slices of liver which was introduced. Results were calculated in terms of grams of glycogen per 100 grams of wet liver. The rats were starved for 18 hours prior to experimentation.

*Results.*—In 37 out of 42 experiments in which rat liver, whose initial glycogen was less than 0.3 per cent, was incubated with Solution I (i.e., high K and Mg), there was an absolute increase (average 0.05–0.15% ) in the amount of glycogen present at the end of one hour. In experiments in which the initial glycogen was greater than 0.3 per cent, a decrease in glycogen was usually observed. It was concluded from these experiments that, contrary to the previous experience in which little or no increase in glycogen occurred when Ringer's solution was used, rat liver would quite consistently produce glycogen *in vitro* when incubated in a potassium-mag-

nesium medium providing the initial glycogen concentration did not exceed 0.3 per cent.

The results obtained in seven experiments in which the effects of using Solutions I, II, III, IV were compared are shown graphically in figures 1

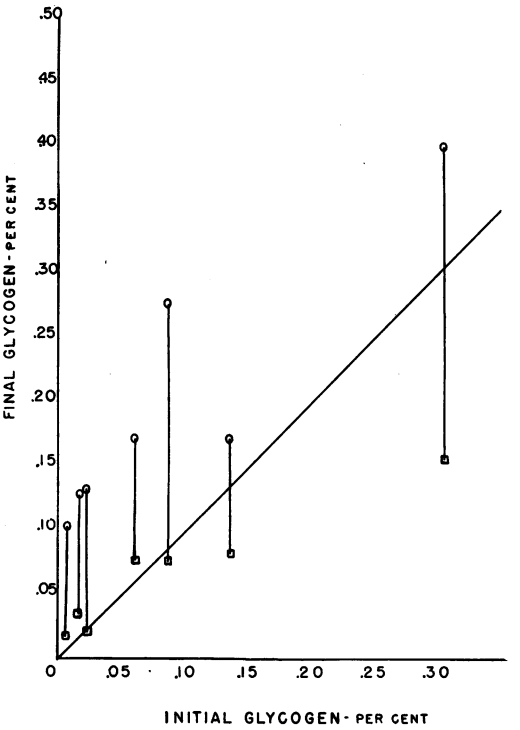


FIGURE 1

Final liver glycogen concentration is plotted against initial glycogen concentration. Circles designate experiments with Solution I (high potassium and magnesium, containing glucose); squares designate experiments with Solution II (high sodium containing glucose). Points lying below the diagonal line indicate net glycogen breakdown, points above the line indicate net glycogen formation.

and 2. In figure 1, it will be seen that, in the presence of glucose, the final glycogen concentration was absolutely greater than the initial concentration when the high potassium-magnesium medium (I) was used; whereas, when the high sodium medium (II) was used, the final glycogen concentration was less than or essentially the same as the initial concentration. Figure 2 shows that, in the absence of glucose, there was no loss of glycogen

in the high potassium-magnesium medium (III), whereas, the glycogen practically disappeared in the high sodium medium (IV). These results may be interpreted as indicating that high sodium and low potassium, cal-

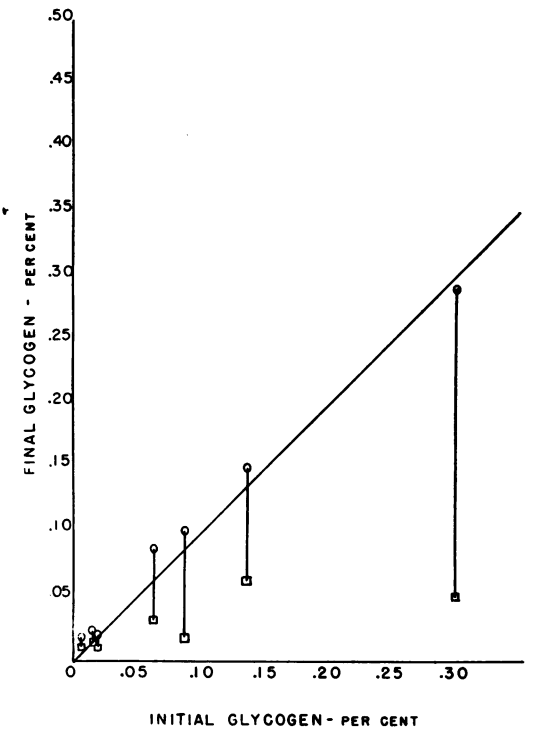


FIGURE 2

Final liver glycogen concentration is plotted against initial glycogen concentration. Circles designate experiments with Solution III (high potassium and magnesium, without glucose); squares designate experiments with Solution IV (high sodium, without glucose). Points lying below the diagonal line indicate net glycogen breakdown, points above the line indicate net glycogen formation.

cium and magnesium favored glycogen breakdown in excess of glycogen synthesis, whereas, the high potassium and high magnesium solution favored glycogen synthesis in excess of glycogen breakdown.

The above observations are to be regarded as only preliminary to a more detailed and complete analysis of the role of ions in influencing intracellular reactions. For example, it has already been observed in this laboratory by one of us (J. M. B.) that calcium is more effective than magne-

sium in promoting glycogen synthesis in rat liver *in vitro*. It is not the intention of the present communication to consider the question of what solution would provide for optimum rate of glycogen formation, but rather to call attention to the importance of considering the maintenance of the normal intracellular ionic environment in the study of intracellular enzymic reactions.

*Summary.*—1. The presence of concentrations of potassium and magnesium comparable to those occurring in intracellular fluid has been found to favor glycogen formation by rat liver *in vitro*, whereas incubation of liver in media comparable to extracellular fluid favors glycogen breakdown.

2. The importance of maintaining a normal intracellular ionic environment in the study of intracellular reactions is pointed out.

[1] Fenn, W. O., *Physiol. Rev.*, **20**, 377 (1940).
[2] Hastings, A. B., *Harvey Lectures*, **36**, 91 (1940–1941).
[3] Ostern, P., Herbert, D., and Holmes, E., *Biochem. Jour.*, **33**, 1858 (1939).
[4] Good, C. A., Kramer, H., and Somogyi, M., *Jour. Biol. Chem.*, **100**, 485 (1933).
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[6] Fiske, C. H., and Subbarow, Y., *Jour. Biol. Chem.*, **66**, 375 (1925).

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## *ON THE MATERIAL EJECTED FROM NOVAE*

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The extremely complex spectroscopic problems connected with the outbursts of novae present many angles of approach. The one that has been most thoroughly exploited is concerned with the identification of the several absorption and emission spectra, moving with different radial velocities through the ejected envelope. The problem cannot, however, be regarded as solved until not only the motion and distribution, but also the quantity and composition of the ejected matter has been analyzed. The present paper is concerned with an approach to the second of these questions.

In studying the changes in the spectra of novae during and after the outbursts it has been usual to estimate and describe the intensities of the dark and bright lines as seen against the continuous background of the spectrum. Such estimates, however, are deceptive, since the intensity of the background is itself changing rapidly (and not necessarily uniformly), so that the true changes, especially in the intensities of bright lines, may be falsified.