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ACTIONS OF LITHIUM IONS AND INSULIN ON GLUCOSE UTILIZATION, GLYCOGEN SYNTHESIS AND GLYCOGEN SYNTHASE IN THE ISOLATED RAT DIAPHRAGM*

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Abstract—The effects of lithium ions and insulin on carbohydrate metabolism of the isolated rat diaphragm were studied and compared. Like insulin, lithium ions caused a conversion of glycogen synthase D to the more active I form of the enzyme. Maximal activation of the enzyme was produced by about 5 mM LiCl. Lithium ions markedly increased glucose utilization and glycogen synthesis by the diaphragm, but the action of this ion appears to be exerted by a mechanism different from that of insulin, since the effects of maximal concentrations of the two agents are additive. In their action on glucose metabolism, lithium ions had a unique ability to direct the glucose taken up by the cell toward glycogen. Insulin and lithium ions had opposite effects on the tissue content of glucose 6-phosphate; insulin increased the tissue level of this metabolite, whereas lithium ions decreased it. ATP and creatine phosphate concentrations were not affected by insulin or lithium ions. The effects of lithium ions on carbohydrate metabolism are exerted at relatively low concentrations of Li, and our results indicate that significant alterations of carbohydrate metabolism may occur when therapeutic or toxic amounts of lithium salts are ingested by man.

IT HAS BEEN demonstrated by many investigators that monovalent cations markedly influence glucose metabolism and the action of insulin in muscle preparations incubated *in vitro*. In particular, it has been observed that the lithium ion has an "insulin-like" action in that it stimulates both glucose utilization and glycogen synthesis in muscle.[1–3] In an earlier paper,[3] we reported the results of experiments on carbohydrate metabolism and the action of insulin in the isolated rat diaphragm as influenced by high concentrations of lithium, sodium and potassium ions. The present study, which is an extension of the previous investigation, is concerned with the action of lithium ions on glucose metabolism in the diaphragm and with the similarities and differences between the metabolic effects of lithium and insulin in this tissue.

METHODS

Animals. Male Wistar strain rats weighing between 125 and 150 g were used. The animals were fasted overnight and killed by decapitation. Hemidiaphragms were carefully dissected out and collected in ice-cold 0·15 M NaCl. They were blotted lightly on filter paper and weighed on a torsion balance. The fact that all animals used were fasted for the same period of time assured a uniform initial tissue glycogen level (*ca.* 17 μmoles glucose equivalents/g).

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Incubation. The tissues were placed in Erlenmeyer flasks containing 2 ml medium of the following composition: 0·040 M HEPES (*N*-2-hydroxyethyl piperazine-*N*-2-ethane sulfonic acid, neutralized with NaOH or KOH), 0·005 M $MgCl_2$, and NaCl, KCl or LiCl in varying proportions to give a total concentration of 0·110 M. Glucose or U-^{14}C-glucose (*ca.* 20,000 cpm/μmole) was present in a concentration of 0·006 M. Insulin (Lilly, amorphous lot No. A2023SB-188) when present was added to give a concentration of 0·06 unit/ml. The flasks were gassed with 100% oxygen and incubated for various times at 37° in a Dubnoff shaker.

Analytical measurements. The initial and final glucose in the incubation medium was determined by the glucose oxidase method after deproteinization with $ZnSO_4$ and $Ba(OH)_2$. Lactate was determined by an enzymatic method according to Hohorst.[4]

In experiments in which the synthesis of glycogen from U-^{14}C-glucose was determined, the tissue was digested with hot KOH and the glycogen precipitated by alcohol in the cold. The precipitate was dissolved in warm water and the glycogen purified essentially according to the method of Thomas *et al.*[5] Aliquots of the aqueous solution of glycogen were placed on pieces of Whatman No. 4 or 41 filter paper. The papers were washed four times in 66% alcohol and one time in acetone. After drying in air, they were placed in counting vials containing 10 ml LSC-complete (from Yorktown Research) and radioactivity was measured with a Packard liquid scintillation counter. Control experiments showed that all ^{14}C-glucose and other contaminating radioactive metabolites were removed by the washing procedure.

Glycogen synthase determinations. Glycogen synthase activity was measured in extracts of diaphragm by the method of Thomas *et al.*[5] After preincubation under different experimental conditions, the tissues were blotted lightly on filter paper and ground in ice-cold mortars with 6 vol. of extraction medium of the following composition: 0·02 M NaF, 0·01 M sodium EDTA and 0·05 M Tris buffer (pH 7·8). After centrifugation in the cold at 3000 *g* for 10 min, the supernatant fluid was collected and used for the enzyme assay. In each assay, 0·05-ml extract was incubated for 10 min at 37° with 0·1-ml medium of the following composition: 0·05 M Tris buffer (pH 7·8), 0·005 M sodium EDTA, 0·0067 M UDP-^{14}C-glucose (*ca.* 40,000 cpm/μmole) and 10 mg/ml of glycogen. The assay was carried out in the presence and absence of 0·0067 M glucose 6-phosphate in the medium in order to determine both total enzyme activity and the activity of the I form of the enzyme. The reaction was stopped by addition of 0·25 ml of 30% KOH and the mixture heated to 100° for 5 min. The radioactivity of the glycogen was determined after purification on filter paper as described above, except that the precipitation of glycogen was omitted and the number of alcohol washings of the filter papers was increased to six.

Determinations of metabolites. In some experiments, the diaphragms were ground in the cold with perchloric acid and ATP, creatine phosphate and glucose 6-phosphate were determined in the perchloric acid extracts after centrifugation and neutralization with KOH.[6]

RESULTS

Effects of monovalent cations and insulin on glycogen synthase activity. In an earlier publication, we described the results of studies of carbohydrate metabolism of diaphragm muscle *in vitro* as influenced by the monovalent cations present in the incu-

bation medium.[3] With potassium as the predominant cation, glycogen synthesis was depressed and the formation of lactate increased. In contrast, lithium ions promoted glycogen synthesis and decreased glycolysis. The effects of insulin on glucose utilization and glycogen synthesis were large in media in which the predominant monovalent cations were sodium or lithium, but in a medium in which all the monovalent cations were potassium the action of insulin was markedly inhibited.

Our first experiments in this study were concerned with the state of the glycogen synthase enzyme system under these different experimental conditions. The results are presented in Table 1.

TABLE 1. EFFECT OF MONOVALENT CATIONS AND INSULIN ON THE ACTIVITY OF GLYCOGEN SYNTHASE IN RAT HEMIDIAPHRAGMS INCUBATED *in vitro**

N	Main cation in preincubation medium	Insulin	Independent synthase activity (μmoles/g/min)	Total synthase activity (μmoles/g/min)	% Independent enzyme
9	Na	0	0·21 ± 0·013	1·79 ± 0·063	12·3 ± 0·53
10	Na	+	0·51 ± 0·037	2·31 ± 0·128	22·4 ± 1·42
	Sig. of insulin effect		$P < 0·001$	$P < 0·01$	$P < 0·001$
8	K	0	0·21 ± 0·019	1·78 ± 0·073	12·1 ± 1·15
10	K	+	0·36 ± 0·029	2·05 ± 0·068	16·9 ± 1·24†
	Sig. of insulin effect		$P < 0·01$	$P < 0·05$	$P < 0·05$
6	Li	0	0·52 ± 0·069	2·14 ± 0·094	24·7 ± 1·98
6	Li	+	0·55 ± 0·034	2·41 ± 0·034	23·1 ± 0·66
	Sig. of insulin effect		NS‡	NS	NS

* The diaphragms were incubated at 37° for 30 min (gas phase, oxygen) in media of the composition described in Methods. The media contained 0·110 M NaCl, 0·110 M KCl or 0·110 M LiCl. Insulin when present, 0·06 unit/ml.

† This value is significantly smaller ($P < 0·01$) than the value obtained in the all-sodium medium plus insulin.

‡ NS = not significant.

After 30 min of incubation in an all-sodium medium, the enzyme was mostly in the glucose 6-phosphate dependent form (D form). As expected, the presence of insulin promoted the conversion of the enzyme to the form independent of glucose 6-phosphate for activity (I form). Insulin also caused a small but significant increase in total enzyme activity. Substitution of sodium by potassium ions had no effect on enzyme activity in the absence of insulin but significantly decreased the effect of insulin on the conversion of the D to the I form of glycogen synthase. In a medium in which 85 per cent of the monovalent ions consisted of lithium and 15 per cent of sodium (those involved in the neutralization of the HEPES buffer), the glycogen synthase was activated and insulin had no further effect. The level of activity of glycogen synthase I was actually the same as that seen in an all-sodium medium in the presence of insulin. Addition of 50 mM NaCl, KCl or LiCl to the assay system for glycogen synthase had no effect on the enzyme activity, showing that large variations in the concentrations of these ions did not affect the determination of glycogen synthase.

Effects of low concentrations of lithium on glucose utilization and glycogen synthesis. In view of the marked "insulin-like" effects of lithium on glycogen synthesis and glycogen synthase in rat diaphragm, it was of interest to determine the effects of dif-

ferent concentrations of this ion on glucose metabolism in this tissue. Diaphragms were incubated for different times in media containing radioactive glucose, and the utilization of glucose and the incorporation of labeled glucose into glycogen were determined. The medium was the usual all-sodium or one in which increasing amounts of LiCl were substituted for NaCl. The results of the studies are reported in Fig. 1.

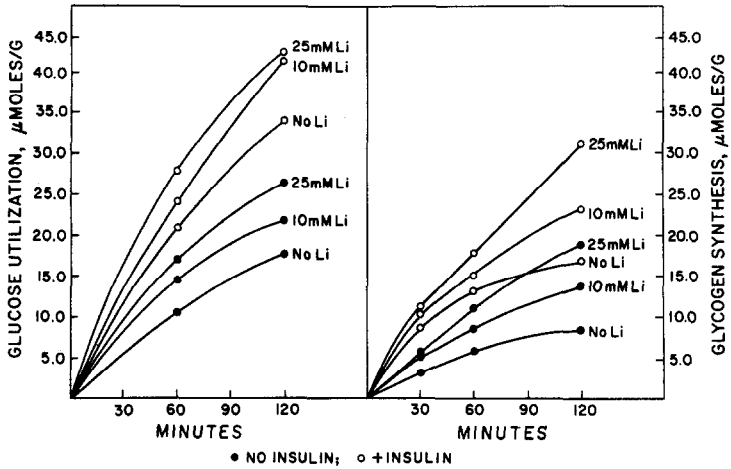

FIG. 1. Effects of lithium ions and insulin on glucose utilization and glycogen synthesis by rat diaphragm incubated *in vitro*. Experimental conditions are as described in Methods. The values are means of seven to eleven experiments.

In the left panel, the utilization of glucose in the presence and absence of insulin is recorded as a function of time, at 10 and 25 mM lithium and in the all-sodium medium. The right panel depicts the incorporation of glucose into glycogen under the same conditions. The results demonstrate the stimulation by relatively low concentrations of lithium ions of both glucose utilization and glycogen synthesis. Proportionately, the effect of lithium on glycogen synthesis is actually larger than the effect of the ion on glucose uptake, and the action of lithium on glycogen formation increases with time tending to make the curve of glycogen synthesis linear with time. Insulin has the usual action in the sodium medium of stimulating glucose utilization and the incorporation of glucose into glycogen. In the presence of 10 or 25 mM Li, insulin causes a further increase in glucose uptake and glycogen synthesis in a manner that is additive to that of lithium.

The accumulation of lactate in the medium was also determined. Lithium ions up to 25 mM had no effect on lactate production but decreased it at higher concentrations. In contrast, insulin stimulated lactate output by the tissue in the presence or absence of lithium.

Studies of the actions of lithium and insulin on glycogen synthase and on glycogen formation. In order to study the role of glycogen synthase in the actions of lithium and insulin on glycogen synthesis, we carried out a series of experiments in which diaphragms were incubated for 30 min in media containing increasing concentrations of lithium ions, and the state of the glycogen synthase enzyme system at the end of the incubation was compared with the incorporation of glucose into

glycogen during the 30-min experiment. The results of these short-term experiments are recorded in Fig. 2.

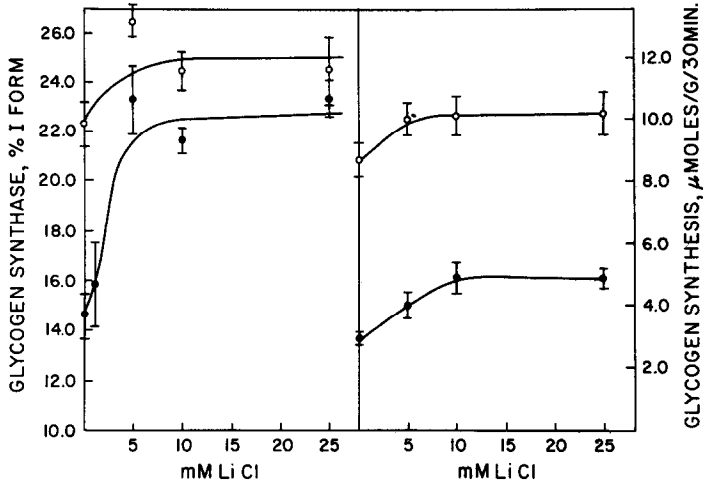

FIG. 2. Effects of lithium ions and insulin on glycogen synthase and glycogen synthesis. Incorporation of U-^{14}C-glucose into glycogen was determined after a 30-min incubation. Glycogen synthase activity was measured at the end of a 30-min incubation. N = 8–10. Filled circles, no insulin; open circles, + insulin.

In the panel on the left it is seen that both insulin and lithium ions activate the enzyme but that the effects are not additive in that insulin in the presence of lithium at 5 mM and above causes very little if any further stimulation of the enzyme already activated by lithium. The results (right panel) in which glycogen synthesis was measured are quite different. It is seen that lithium increases glycogen formation from glucose up to a concentration of 10 mM. The effect of insulin is much larger, however, and is independent of the concentration of lithium in the incubation medium. Lithium increases glycogen synthase activity at a relatively low concentration (half maximal activation at less than 3 mM) but despite the fact that this enzyme can be activated by lithium to an extent almost equal to the activation produced by insulin, the hormone causes a much larger and more immediate effect than lithium on glycogen synthesis. It appears that a process different from the glycogen synthase reaction is involved in the main action of insulin on the over-all synthesis of glycogen. The data in Fig. 2 are the results of experiments in which diaphragms were incubated for relatively short periods of time. However, as seen from Fig. 1, the effects of lithium ions on glycogen synthesis are greater during longer term incubations, and at the highest concentration of LiCl used (25 mM) glycogen synthesis was larger than when LiCl was present at a concentration of only 10 mM. Additional experiments, not presented here, showed that increases in the concentration of LiCl above 25 mM had little or no further effect. Due to the differences in the action of lithium ions during short- and long-term experiments, we studied the state of the glycogen synthase enzyme system after 90 min of incubation of the diaphragm. The results are presented in Table 2.

It is seen that the action of insulin on glycogen synthase activity is not present after 90 min of incubation in a sodium medium. However, in the presence of 25 mM

TABLE 2. EFFECTS OF LITHIUM IONS AND INSULIN ON GLYCOGEN SYNTHASE OF RAT DIAPHRAGM AFTER PROLONGED INCUBATION*

LiCl (mM)	Insulin	N	% Synthase I ±S.E.M.	Significance of insulin effect
0	0	10	16·4 ± 0·60	
0	+	10	17·6 ± 0·78	NS
25	0	10	23·7 ± 0·85†	
25	+	10	19·4 ± 0·76	$P < 0·001$

* Diaphragms were incubated for 90 min at 37° in media of composition as described in Methods, and containing 110 mM NaCl (sodium medium) or 85 mM NaCl–25 mM LiCl (gas phase, oxygen).
† $P < 0·001$ compared to activity without lithium.
NS = not significant.

LiCl the percentage of the I form of the enzyme is higher than in the control experiments, showing that lithium stimulation of glycogen synthase is maintained with time. Curiously, in the presence of lithium ions, insulin significantly inhibited glycogen synthase in the long-term experiments; in effect it overcame the activation produced by lithium. Danforth[7] reported that high levels of glycogen in skeletal muscle of rats and mice were associated with a lowering of the percentage of synthase 1. The highest concentration of glycogen in our experiments was observed upon prolonged incubation of diaphragms in the presence of lithium ions and insulin, and it is possible that the low level of synthase I under these conditions could be related to the extremely high tissue content of glycogen after 90 min of incubation. In general, however, there does not appear to be any definite relationship between glycogen content and synthase I activity in our experiments. The finding that the stimulation of glycogen synthase activity by insulin is not present after 90 min of incubation does not appear to be due to a lack of hormonal action in the tissue, since at that time glucose utilization was still increased in the presence of insulin.

Effects of lithium and insulin on ATP, creatine phosphate and glucose 6-phosphate concentrations in the diaphragm. One possible way in which insulin or lithium ions could alter carbohydrate metabolism in muscle would be to cause changes in the intracellular concentrations of high-energy phosphate constituents or glycolytic inter-

TABLE 3. TISSUE CONTENT OF GLUCOSE 6-PHOSPHATE, ATP AND CREATINE PHOSPHATE AFTER INCUBATION OF RAT DIAPHRAGM *in vitro**

Compound	Incubation (min)	N	Sodium medium No insulin (μmoles/g wet wt ± S.E.M.)	Sodium medium Plus insulin (μmoles/g wet wt ± S.E.M.)	Sodium medium with 25 mM LiCl No insulin (μmoles/g wet wt ± S.E.M.)	Sodium medium with 25 mM LiCl Plus insulin (μmoles/g wet wt ± S.E.M.)
Glucose 6-phosphate	30	15	0·167 ± 0·006	0·192 ± 0·014	0·092 ± 0·006	0·137 ± 0·011
	90	6	0·190 ± 0·012	0·253 ± 0·011	0·106 ± 0·005	0·135 ± 0·003
ATP	30	15	4·11 ± 0·08	3·96 ± 0·11	4·00 ± 0·10	4·10 ± 0·07
	90	6	3·26 ± 0·21	3·17 ± 0·16	3·27 ± 0·13	3·49 ± 0·15
Creatine phosphate	30	15	9·97 ± 0·27	10·09 ± 0·32	9·82 ± 0·27	10·18 ± 0·23
	90	6	8·74 ± 0·19	9·28 ± 0·24	9·29 ± 0·25	9·26 ± 0·36

* The diaphragms were incubated at 37° in media of compositions described in Methods and containing 110 mM NaCl (sodium medium) or 85 mM NaCl–25 mM LiCl (gas phase, oxygen).

TABLE 4. SIGNIFICANCE OF THE EFFECTS OF INSULIN AND LITHIUM ON TISSUE GLUCOSE 6-PHOSPHATE

	30-Min incubation Δ ± S.E.M.	P	90-Min incubation Δ ± S.E.M.	P
Na, no insulin vs Na + insulin	+0·025 ± 0·015	NS*	+0·063 ± 0·017	<0·005
Li, no insulin vs Li + insulin	+0·045 ± 0·012	<0·005	+0·029 ± 0·006	<0·001
Na, no insulin vs Li, no insulin	−0·075 ± 0·008	<0·001	−0·084 ± 0·013	<0·001
Na + insulin vs Li + insulin	−0·055 ± 0·018	<0·005	−0·118 ± 0·012	<0·001

* In paired experiments, the difference was highly significant ($P < 0·001$). Effects of insulin or lithium on ATP and creatine phosphate were not statistically significant.

mediates. Such changes could affect the rate of glycolysis, the phosphorylation of glucose or the glycogen synthase reaction itself. Accordingly, we determined ATP, creatine phosphate and glucose 6-phosphate in hemidiaphragms incubated in the presence or absence of insulin for 30 and 90 min at 37° in an all-sodium medium or in a medium containing 25 mM LiCl. The results are reported in Tables 3 and 4.

The results show clearly that lithium ions caused a marked depression of the glucose 6-phosphate content of the tissue while insulin produced a small but significant increase in the concentration of this metabolite. The values for ATP and creatine phosphate decreased somewhat with time of incubation, possibly due to a decrease in efficiency of oxidative phosphorylation, but neither insulin nor lithium affected the cellular concentrations of these tissue constituents.

Lithium activation of glycogen synthesis. In Fig. 3 we have illustrated the effect of lithium ions on the conversion of medium glucose to tissue glycogen. The values are obtained from the second hr of incubation in the experiments presented in Fig. 1. The percentage of the radioactive glucose entering the cell that is incorporated into

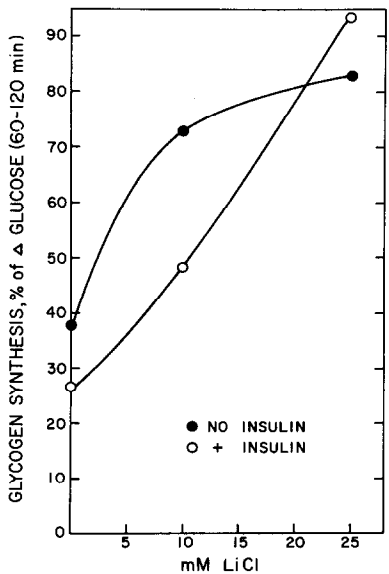

FIG. 3. Effect of lithium ions on the proportion of glucose incorporated into glycogen. The values were calculated from the results obtained in the second hr of incubation of diaphragm in the experiments reported in Fig. 1.

glycogen is expressed as a function of the concentration of lithium in the incubation medium. It is seen that as the lithium concentration in the medium is increased, a greater proportion of the glucose taken up by the tissue is incorporated into glycogen. In the absence of lithium, or with lithium present in a concentration of 10 mM, the incorporation of glucose into glycogen is somewhat greater in the absence than in the presence of insulin. This is probably a reflection of the greater stimulatory action of insulin on glucose transport than on glycogen synthesis during prolonged incubation of muscle *in vitro*. With 25 mM Li in the incubation medium, almost all glucose entering the cell is incorporated into glycogen, whether or not insulin is present.

DISCUSSION

The experiments reported here have demonstrated that relatively low concentrations of lithium ions increase glucose utilization, as well as glycogen synthesis of muscle incubated *in vitro*. These are metabolic effects shared by insulin. However, our results indicate strongly that the lithium ion exerts its action on carbohydrate metabolism by a mechanism that is different from that of insulin. At concentrations of insulin or lithium that have maximal effects on metabolism, the actions of the two agents, both on glucose uptake and incorporation of glucose into glycogen, are additive. Furthermore, lithium ions exert metabolic actions that are not produced by insulin. Lithium ions at low concentrations have no effect on lactate production by the diaphragm and at high concentrations decrease glycolysis while insulin increases lactate formation.[3] Lithium markedly decreases the tissue content of glucose 6-phosphate, while insulin increases the intracellular concentration of this metabolite. Finally, on prolonged incubation of diaphragm, lithium ions are more effective than insulin in promoting glycogen synthesis, while the action of insulin on glucose utilization is more sustained than that of lithium.

In a previous study,[3] we emphasized that the extra glucose entering the tissue in response to insulin was directed mainly toward glycogen. This is also true in the experiments reported here, particularly during the first hr of incubation. However, there is not necessarily a parallelism between an increase in glucose uptake by muscle and glycogen synthesis. In the present experiments, the action of insulin on glucose utilization is sustained during the 2-hr incubation, while the insulin effect on glycogen synthesis diminishes with time. An example of an agent that markedly increases glucose utilization but causes a decrease in glycogen content of the tissue is the dithiol, dithiothreitol.[8] Anoxia also brings about an increase in glucose uptake by muscle but leads to depletion of cellular glycogen.[9] In contrast, lithium ions have a pronounced ability to stimulate both glucose uptake and glycogen formation in muscle. With 25 mM LiCl in the medium, fully 90 per cent of the glucose entering the cell can be accounted for in the muscle glycogen. In effect, lithium causes a coupling between glucose uptake and glycogen synthesis. In our experiments, glucose was measured directly while glycogen synthesis was determined by the incorporation of radioactive glucose into tissue glycogen. However, there is little glycogenolysis in diaphragms incubated aerobically in the presence or absence of glucose,[10] and figures obtained for glycogen formation by a direct method are close to those observed here.[3] The

present method has the advantage of greater accuracy and measures the medium glucose that has actually become part of the glycogen molecule at any given moment, rather than the glycogen content which has to be corrected for the initial tissue glycogen level.

The activation of glycogen synthase produced by both insulin and lithium undoubtedly plays an important role in the stimulation of glycogen synthesis caused by these agents. However, other factors must be involved since the effects of lithium and insulin on glycogen synthesis are additive, while the effects on glycogen synthase are not.

The effect of insulin to increase the activity of the I form of glycogen synthase was first demonstrated by Villar-Palasi and Larner.[11] The conversion of the D to the glucose 6-phosphate independent I form of the enzyme was found later to be catalyzed by a protein phosphatase, while the reverse reaction involves the action of a protein kinase.[12,13] The kinase itself was found to exist in two forms, a D form, dependent on cyclic AMP for activity, and an I form independent of the presence of cyclic AMP.[14] In skeletal muscle, insulin was found to promote the conversion of the I to the less active D form of this enzyme leading subsequently to a shift in the equilibrium of the glycogen synthase enzymes toward the more active I form.[14] Recently, Horn *et al.*[15] have demonstrated that lithium ions increase glycogen synthase activity in rat diaphragm incubated *in vitro* and that addition of LiCl to partially purified preparations of glycogen synthase I kinase caused an inhibition of enzyme activity. Increasing concentrations of LiCl up to 50 mM caused a progressive inhibition of enzyme activity both in the presence and absence of cyclic AMP. However, the inhibition of kinase activity by lithium ions, even at the highest concentration used, was only about 20 per cent, and it appears likely that additional factors are involved in the activation of glycogen synthase by lithium.

The effects of insulin and lithium on the glucose 6-phosphate content of the tissue are strikingly different, with insulin causing a significant increase in the concentration of this metabolite in the diaphragm and lithium producing a decrease in tissue glucose 6-phosphate. Neither insulin nor lithium ions affected the tissue contents of high-energy phosphate compounds, so that availability of ATP for glucose phosphorylation does not appear to play a role in producing the differences in glucose utilization and glycogen synthesis observed in our experiments.

The finding that insulin increases the glucose 6-phosphate content of diaphragm muscle is in accord with earlier studies of Newsholme and Randle[16] who observed this phenomenon in intact rat diaphragm preparations. Khac *et al.*[17] showed that insulin caused an increase up to 6-fold in the concentration of glucosamine 6-phosphate in the isolated rat diaphragm incubated in the presence of glucosamine. A possible interpretation of our findings is that insulin stimulates the phosphorylation of glucose as well as glycogen synthesis whereas lithium ions act only on the glycogen synthetic process.

The metabolic consequences of changes in the intra-cellular concentrations of glucose 6-phosphate are difficult to predict, since this compound has many effects on the activity of glycolytic enzymes. One well-known action of glucose 6-phosphate in muscle is an inhibition of hexokinase activity,[18] so that phosphorylation of glucose would tend to become inhibited as glucose 6-phosphate accumulates in the cell. However, it is difficult to see how this action of glucose 6-phosphate can play a major

role in the experiments reported here. Lithium and insulin both stimulate glucose uptake and glycogen synthesis by the muscle but have opposite effects on the tissue level of glucose 6-phosphate.

Another action of glucose 6-phosphate is that of inhibiting the activity of phosphorylase.[19] As glucose 6-phosphate accumulates, this would tend to contribute to the conservation of glycogen formed from glucose. Again, lithium and insulin would exert opposite effects by this mechanism and lithium, which is more effective than insulin in producing glycogen synthesis during prolonged incubations, causes a decrease rather than an increase in glucose 6-phosphate. Finally, glucose 6-phosphate influences the glycogen synthase reaction itself in that it increases the activity of the D form of the enzyme[12] and also, as desctibed by Kato and Bishop,[20] in some unknown manner increases the rate of conversion of the D to the I form of the enzyme. Both of these effects of glucose 6-phosphate would tend to reinforce the stimulating action of insulin on glycogen synthesis but to oppose the action of lithium on this same process. Again, as with the other actions of glucose 6-phosphate discussed, there is no obvious relation between the changes in cellular content of glucose 6-phosphate and the metabolic alterations produced by lithium or insulin in these experiments. It should be noted that the K_m for activation of glycogen synthase D by glucose 6-phosphate[7] is about 9×10^{-4} M, well above the tissue concentrations observed here and the I form of the enzyme is the more active form in the diaphragm.

An important question in interpreting our results is the role, if any, that cyclic AMP plays in the effects of insulin and lithium on carbohydrate metabolism in muscle. Craig *et al.*[21] made a study of the possible involvement of cyclic AMP in the effects of insulin on glycogen synthase activity of the rat diaphragm incubated *in vitro* and came to the conclusion that alterations in the cellular content of cyclic AMP could not be responsible for the effects of insulin on carbohydrate metabolism in muscle. Even when diaphragms were exposed to both insulin and epinephrine, the increase in cyclic AMP concentration was the same as with epinephrine alone. However, it is known that cyclic AMP activates glycogen synthase kinase which would lead in the cell to an increase in the less active D form of glycogen synthase and inhibition of glycogen synthesis. It was also shown by Craig *et al.*[21] that when diaphragms were pre-exposed to insulin, the subsequent rise in cyclic AMP during the first few minutes after the addition of epinephrine was delayed. In the case of lithium, it had been demonstrated by Dousa and Hechter[22] that lithium ions inhibit adenyl cyclase activity of brain tissue and also oppose the activation of this enzyme by vasopressin in preparations obtained from rabbit renal medullary tissue.[23] Frazer *et al.*[24] showed that lithium ions decreased the effect of norepinephrine on phosphorylase activity of the isolated guinea pig heart, an indication of an interference with cyclic AMP formation. Lithium ions have also been shown to decrease the activation of adenyl cyclase by prostaglandin E_1 in preparations from human platelets.[25] Further experiments are needed to determine the role that cyclic AMP may play in the action of insulin and lithium ions on muscle metabolism.

Preparation of lithium salts are now being used widely in the therapy of patients with mental illness.[26–28] In such treatment, systemic and metabolic effects limit the doses of lithium salts that can safely be given to man. In therapy, plasma levels of about 1 mM Li are routinely attained.[29] Although higher concentrations of lithium are used in most of the experiments presented here, it is clear that our experiments

provide evidence that lithium at therapeutic or toxic levels in man may exert significant effects on carbohydrate metabolism.

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REFERENCES

1. G. Bhattacharya, *Biochem. J.* **79,** 369 (1961).
2. T. Clausen, *Biochim. biophys. Acta* **150,** 66 (1968).
3. E. S. Haugaard, E. Serlick and N. Haugaard, *Biochem. Pharmac.* **22,** 1023 (1973).
4. H-J. Hohorst, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), p. 267. Academic Press, New York (1963).
5. J. A. Thomas, K. K. Schlender and J. Larner, *Analyt. Biochem.* **25,** 486 (1968).
6. H. U. Bergmeyer (Ed.), *Methods in Enzymatic Analysis*, pp. 543 and 610. Academic Press, New York (1963).
7. W. H. Danforth, *J. biol Chem.* **240,** 588 (1965).
8. E. S. Haugaard, M. J. Smith and N. Haugaard, *Biochem. Pharmac.* **21,** 517 (1972).
9. P. J. Randle and G. H. Smith, *Biochem. J.* **70,** 490 (1958).
10. N. Haugaard, J. B. Marsh and W. C. Stadie, *J. biol. Chem.* **189,** 59 (1951).
11. C. Villar-Palasi and J. Larner, *Archs Biochem. Biophys.* **94,** 436 (1961).
12. M. Rosell-Perez, C. Villar-Palasi and J. Larner, *Biochemistry, N.Y.* **1,** 763 (1962).
13. D. L. Friedman and J. Larner, *Biochemistry, N.Y.* **2,** 669 (1962).
14. L. C. Shen, C. Villar-Palasi and J. Larner, *Physiol. Chem. Physics* **2,** 536 (1970).
15. R. S. Horn, O. Walaas and E. Walaas, *Biochem. biophys. Acta* **313,** 296 (1973).
16. E. A. Newsholme and P. J. Randle, *Biochem. J.* **80,** 655 (1961).
17. L. D. Khac, D. Eboué-Bonis, A-M. Chambout and H. Clauser, *Eur. J. Biochem.* **31,** 86 (1972).
18. R. K. Crane and A. Sols, *J. biol. Chem.* **203,** 273 (1953).
19. H. E. Morgan and A. Parmeggiani, *J. biol. Chem.* **239,** 2440 (1964).
20. K. Kato and J. S. Bishop, *J. biol. Chem.* **247,** 7420 (1972).
21. J. W. Craig, T. W. Rall and J. Larner, *Biochem. biophys. Acta* **172,** 213 (1969).
22. T. Dousa and O. Hechter, *Lancet* **1,** 834 (1970).
23. T. Dousa and O. Hechter, *Life Sci.* **9,** 765 (1970).
24. A. Frazer, A. Hancock, J. Mendels and R. McIntire, *Biol. Psychiat.* **5,** 79 (1972).
25. Y-C. Wang, G. N. Pandey, J. Mendels and A. Frazer, *Biochem. Pharmac.* **23,** 845 (1974).
26. J. F. J. Cade, *Med. J. Aust.* **36,** 349 (1949).
27. M. Schou, *J. psychiat. Res.* **6,** 67 (1968).
28. J. Mendels, S. K. Secunda and W. L. Dyson, *Archs gen. Psychiat.* **26,** 154 (1972).
29. J. Mendels and A. Frazer, *J. psychiat. Res.* **10,** 9 (1973).