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# EFFECTS OF MONOVALENT CATIONS AND INSULIN ON GLUCOSE METABOLISM OF THE ISOLATED RAT DIAPHRAGM*

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**Abstract**—The effects of monovalent cations on glucose utilization, glycogen synthesis and lactate formation of rat diaphragm incubated *in vitro* were studied in the presence and absence of insulin. It was found that the monovalent ions markedly influenced glucose metabolism and the action of insulin in this tissue. Sodium and particularly lithium ions promoted glucose uptake and glycogen synthesis, while in the presence of a high concentration of potassium, glucose utilization and glycogen synthesis were low and lactate formation increased. The maximal effect of insulin was obtained in media high in sodium or lithium ions. The sulfhydryl reagent, 2,2′-dithiodipyridine (DTP), inhibited glucose uptake and glycogen synthesis to the same extent. Lactate production was unchanged by DTP when the tissues were incubated in a sodium medium and was increased by DTP in the presence of lithium. The monovalent cations strongly influence the metabolic action of insulin in the diaphragm preparation and appear to act by affecting an early rapid reaction catalyzed by the hormone.

IT WAS demonstrated originally by Bhattacharya[1] that the rate of glucose utilization by the rat diaphragm incubated *in vitro* was influenced markedly by the nature of the monovalent ions present in the incubation medium. The highest rate of glucose uptake was observed in a medium containing lithium ions as the predominant cation. A lower rate of glucose utilization was observed in a medium high in sodium; potassium and rubidium ions further decreased the rate of glucose utilization. Clausen[2–4] confirmed and extended these observations, and also studied the action of ouabain on glucose metabolism in diaphragm.

The experiments to be reported here are concerned with the effects of lithium, sodium, potassium and rubidium ions on glucose metabolism in the rat diaphragm. The metabolic effects of insulin *in vitro* in this tissue were studied under different ionic conditions, in order to determine the role that monovalent cations play in regulating the action of insulin.

## METHODS

*Animals.* Male Wistar strain rats weighing between 125 and 150 g and fed *ad lib.* were killed by decapitation. Hemidiaphragms were removed and collected in ice-cold 0·15 M NaCl, KCl, LiCl, RbCl or in solutions containing mixtures of these salts

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representing the proportions of monovalent cations in the preincubation or final incubation media. The pieces of hemidiaphragms were lightly blotted on filter paper and weighed on a torsion balance before the experiments.

*Incubation.* The tissues were incubated at 37° in 2 ml media containing 0·040 M HEPES (*N*-2-hydroxyethyl piperazine *N'*-2-ethane sulfonic acid) neutralized with KOH or NaOH as indicated, 0·005 M $MgCl_2$, 0·006 M glucose and 0·110 M NaCl, KCl, LiCl or RbCl to give an osmolarity of 0·300. The incubation was carried out in Erlenmeyer flasks placed in a Dubnoff shaker and the flasks were gassed with 100% oxygen. In some experiments, the hemidiaphragms were preincubated in similar media containing no glucose and 0·113 M monovalent cation chlorides. Insulin, when present, was at a concentration of 0·05 unit/ml.

*Analytical determinations.* The initial and final glucose content of an aliquot of the incubation medium was determined by the glucose oxidase method after deproteination with $ZnSO_4$ and $Ba(OH)_2$. Lactate was determined by an enzymatic method as described by Hohorst[5] and glycogen was measured by the method of Montgomery[6] after digestion of the tissue in 30% KOH and precipitation with alcohol. Glycolytic intermediates and adenine nucleotides were determined by specific enzymatic methods.[7]

*Chemicals.* The insulin preparation used was Lilly amorphous insulin (lot No. A2-235B-188). Dithiodipyridine was obtained from Aldrich Chemical Company.

## RESULTS

*Glucose utilization and lactate formation at different concentrations of sodium and potassium ions.* Diaphragms were incubated for 2 hr in media in which the monovalent ions were varied from 100% potassium ions to 100% sodium ions and the utilization of glucose and the formation of lactate were measured. The results of the experiments are recorded in Fig. 1.

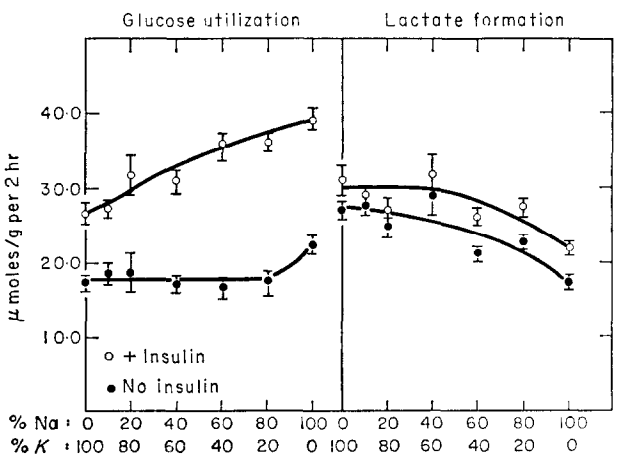


FIG. 1. Rat hemidiaphragms incubated at 37° for 2 hr as described in Methods. All-sodium medium and all-potassium medium were mixed in the proportions given in the abscissa (N = 11).

In the absence of insulin there was no change in the rate of uptake of glucose as the sodium ion concentration of the medium was raised to 80 per cent of the total monovalent cations. There was, however, a small but significant increase when the total

sodium was further increased to 100 per cent. The output of lactate was highest in the all-potassium medium and decreased as the proportion of sodium ions was elevated. In the presence of insulin, the glucose uptake increased consistently as the sodium ion concentration was raised, i.e. the insulin effect on glucose uptake became larger the greater the concentration of sodium ions. Insulin stimulated lactate formation to a small extent and this effect of insulin appeared to be relatively unaffected by the change of monovalent ions in the medium. The main effect of increasing the sodium ion concentration was to decrease glycolysis and to increase the effect of insulin on glucose utilization.

*Effects of potassium and lithium ions on glucose metabolism.* Glucose uptake, lactate output and glycogen formation were measured in hemidiaphragms incubated in media containing different proportions of potassium and lithium ions. The results of these experiments are recorded in Fig. 2.

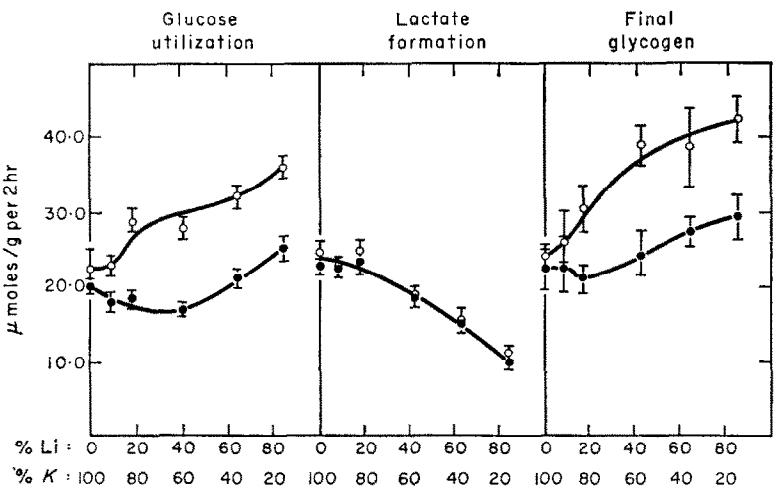


FIG. 2. Rat hemidiaphragms incubated for 2 hr as described in Methods. All-potassium medium and and 85% lithium–15% potassium medium were mixed in the proportions given in the abscissa (N = 6). Open circles, with insulin; solid circles, without insulin.

In a medium containing potassium as the only monovalent cation, a major proportion of the glucose metabolized appeared as lactate in the incubation medium and the insulin effect on glucose metabolism was small. When lithium ions were substituted for potassium, there was some increase in glucose utilization and glycogen synthesis, and a depression of lactate formation. The effect of insulin on glucose uptake and glycogen formation was restored as the lithium ion concentration was increased.

*Preincubation experiments in sodium or potassium media.* In order to magnify the ionic effects observed, hemidiaphragms were preincubated in the absence of glucose for 30 min in media containing all sodium or all potassium as the monovalent ions. The preincubation period was followed by the usual 2-hr incubation in the presence of glucose with or without insulin, and glucose utilization, lactate formation and final glycogen content of the tissues were determined. The results are reported in Table 1.

TABLE 1. EFFECTS OF SODIUM AND POTASSIUM IONS ON CARBOHYDRATE METABOLISM OF RAT DIAPHRAGM INCUBATED *in vitro**

| N | Medium in preincubation (0·5 hr) | Medium in incubation with glucose (2 hr) | Insulin | Glucose utilization (μmole/g/2 hr ± S.E.M.) | Final glycogen (μmole glucose equivalents/g ± S.E.M.)† | Lactate formation (μmole/g/2 hr ± S.E.M) |
|---|---|---|---|---|---|---|
| 16 | Na | Na | 0 | 21·1 ± 0·82 | 23·8 ± 1·65 | 10·1 ± 0·53 |
| 16 | Na | Na | + | 33·9 ± 0·75 | 29·3 ± 1·21 | 14·7 ± 0·89 |
| | | | Insulin effect | +12·8 ± 1·11‡ | +5·5 ± 2·21‡ | +4·6 ± 1·02‡ |
| 13 | Na | *K* | 0 | 13·4 ± 0·91 | 16·9 ± 1·63 | 16·2 ± 0·85 |
| 13 | Na | *K* | + | 21·4 ± 0·73 | 24·7 ± 1·74 | 18·4 ± 0·67 |
| | | | Insulin effect | +8·0 ± 1·17‡ | +7·8 ± 2·34‡ | +2·2 ± 1·08‡ |
| 13 | *K* | Na | 0 | 19·0 ± 0·64 | 16·0 ± 1·00 | 13·7 ± 0·70 |
| 13 | *K* | Na | + | 23·9 ± 1·44 | 17·9 ± 2·10 | 14·7 ± 0·90 |
| | | | Insulin effect | +4·9 ± 1·57‡ | +1·9 ± 2·32‡ | +1·0 ± 1·14‡ |
| 13 | *K* | *K* | 0 | 14·7 ± 0·45 | 15·3 ± 1·38 | 16·5 ± 0·76 |
| 13 | *K* | *K* | + | 17·9 ± 0·78 | 17·3 ± 1·70 | 18·3 ± 0·72 |
| | | | Insulin effect | +3·2 ± 0·90‡ | +2·0 ± 2·19‡ | +1·8 ± 1·05‡ |

* Rat hemidiaphragms were preincubated at 37° in media without glucose, rinsed briefly in 10 ml of 0·15 M NaCl or KCl depending on the cation of the incubation mixture) at room temperature, and incubated with glucose with or without insulin (0·05 unit/ml) at 37° as described in Methods.

† Mean initial glycogen of freshly excised diaphragms = 21·6 ± 1·16 μmole glucose equivalents/g (N = 28).

‡ S.E.M. of the insulin effect based on paired differences.

Preincubation of the hemidiaphragms in all-sodium medium had little effect on the subsequent ability of the tissues to take up glucose in an all-sodium medium, and the insulin effect was only slightly smaller than that found in the absence of preincubation (12·8 ± 1·11 μmole/g/2 hr compared to 16·6 ± 1·68 μmole/g/2 hr).

When hemidiaphragms had been preincubated in an all-sodium medium, glucose uptake in a potassium medium was decreased and, although diminished, the effect of insulin on glucose utilization was still considerable. As in the experiments illustrated in Fig. 1, potassium ions increased lactate formation and this was reflected in a lower final glycogen concentration.

When the tissues had been preincubated for 30 min in an all-potassium medium, the effect of insulin was further depressed, although the final incubation with glucose was in an all-sodium medium. The preliminary treatment with potassium had made the diaphragm quite unresponsive to the action of insulin. Lactate formation under these conditions was greater than in diaphragms pre-exposed to an all-sodium medium and the final glycogen content was lower. No significant effects of insulin on glycogen synthesis or lactate formation were observed.

When diaphragms were preincubated in an all-potassium medium and then incubated in the same medium in the presence of glucose, utilization of glucose and glycogen synthesis were depressed and the output of lactate was increased. There was a significant but very small effect of insulin on the utilization of glucose and no significant effect of insulin on glycogen synthesis or lactate formation. It is apparent that preincubation of the tissue in a medium high in potassium ions was more effective in decreasing the action of insulin than was the presence of potassium, during the actual incubation with glucose. However, the uptake of glucose and formation of lactate in the absence of insulin were determined mainly by the cation composition of the final incubation medium.

In some experiments, the final glycogen content of the hemidiaphragms was measured after the 30-min preincubation in the absence of glucose. There was only a small decrease in glycogen during this period, and no significant difference between the results from sodium- and potassium-incubated tissues.

*Effects of lithium and rubidium ions on the action of insulin.* Experiments similar to those reported in Table 1 were done with media in which the predominant monovalent ions were lithium or rubidium (Table 2).

After preincubation in an 85% lithium–15% sodium medium and incubation with glucose in a medium with the same ionic composition, glucose utilization was markedly increased compared to similar experiments in all-sodium media. Glycogen formation was elevated and lactate output was suppressed. There was a significant effect of insulin on glucose utilization, but the effects of insulin on the final content of glycogen and on lactate output were not statistically significant. When diaphragms were preincubated in the lithium medium and then incubated for 2 hr in a sodium medium, the results were identical to those obtained when the predominant monovalent ion was lithium in both the preincubation and the final incubation medium. The metabolic effects of lithium pretreatment were maintained during incubation with glucose in the all-sodium medium.

An entirely different result was obtained when diaphragms were pretreated with lithium and then incubated in an all-potassium medium. Under these conditions, the effects of lithium were abolished; there was a relatively slow rate of glucose utilization,

TABLE 2. EFFECTS OF LITHIUM AND RUBIDIUM IONS ON CARBOHYDRATE METABOLISM OF RAT DIAPHRAGM INCUBATED *in vitro**

| N | Medium in preincubation (0·5 hr) | Medium in incubation with glucose (2 hr) | Insulin | Glucose utilization (μmole/g/2 hr ± S.E.M. | Final glycogen (μmole glucose equivalents/g ± S.E.M.)† | Lactate formation (μmole/g/2 hr ± S.E.M.) |
|---|---|---|---|---|---|---|
| 10 | Li | Li | 0 | 33·5 ± 1·12 | 51·7 ± 2·30 | 5·7 ± 0·33 |
| 10 | Li | Li | + | 41·6 ± 1·74 | 60·0 ± 4·95 | 6·9 ± 0·59 |
| | | | Insulin effect | +8·1 ± 2·32‡ | +8·3 ± 4·16‡ | +1·2 ± 0·55‡ |
| 14 | Li | Na | 0 | 32·6 ± 0·76 | 55·8 ± 4·34 | 6·2 ± 0·28 |
| 14 | Li | Na | + | 38·2 ± 0·94 | 56·6 ± 3·69 | 7·1 ± 0·43 |
| | | | Insulin effect | +5·6 ± 1·25‡ | +0·8 ± 2·62‡ | +0·9 ± 0·30‡ |
| 14 | Li | *K* | 0 | 15·3 ± 1·41 | 21·6 ± 1·65 | 12·2 ± 0·73 |
| 14 | Li | *K* | + | 18·5 ± 1·44 | 25·7 ± 1·67 | 11·8 ± 0·80 |
| | | | Insulin effect | +3·2 ± 0·80‡ | +4·1 ± 1·33‡ | −0·4 ± 0·51‡ |
| 10 | Rb | Rb | 0 | 17·0 ± 1·04 | 28·2 ± 3·05 | 24·3 ± 1·65 |
| 10 | Rb | Rb | + | 21·3 ± 0·87 | 32·9 ± 3·55 | 25·8 ± 1·38 |
| | | | Insulin effect | +4·3 ± 0·92‡ | +4·7 ± 1·71‡ | +1·5 ± 1·68‡ |

* Rat hemidiaphragms were preincubated at 37° in media without glucose, rinsed briefly in 10 ml of 0·15 M LiCl, NaCl, KCl or RbCl (depending on the cation of the incubation mixture) at room temperature and incubated with glucose with or without insulin (0·05 unit/ml) at 37° as described in Methods.

† Mean initial glycogen of freshly excised diaphragms = 21·6 ± 1·16 μmole glucose equivalents/g (N = 28).

‡ S.E.M. of the insulin effect based on paired differences.

a low final glycogen content, and the formation of lactate was increased. In parallel experiments in which lithium was determined in diaphragms pretreated with this ion, it was found that the tissue lithium concentration was approx. 7 mM after 1 hr of incubation in either sodium or potassium medium.

Rubidium ions behaved to some extent like potassium ions in that glucose utilization was low and lactate formation higher than in the presence of sodium, and the effect of insulin on glucose utilization was depressed.

*Pre-exposure to insulin under different ionic conditions.* The results reported so far have shown that insulin stimulation of glucose utilization by diaphragm is depressed by potassium ions present either in the incubation medium itself or during a 30-min pretreatment of the tissue in the absence of glucose. In additional experiments, not shown here, we observed that when the pretreatment in an all-potassium medium was decreased to 10 min, the insulin effect on glucose utilization was almost normal during the subsequent 2-hr incubation with glucose in an all-sodium medium. This indicates that a relatively short-lasting depolarization of the cell with the concomitant changes in membrane properties did not cause a permanent loss of sensitivity of the tissue to insulin. We, therefore, decided to study the action of insulin in experiments in which the tissues were exposed to the hormone only for short periods of time. In such experiments, it should be possible to determine whether monovalent ions influenced the binding of insulin to receptor sites or an initial fast reaction initiated by insulin. Hemidiaphragms were exposed to insulin for 2 or 10 min in media of different compositions, thoroughly rinsed, and incubated in all-sodium medium with glucose but no insulin. Values for glucose utilization under these different experimental conditions are reported in Table 3.

It is seen that hemidiaphragms exposed to insulin for only 2 min in a sodium medium, followed by three washings in 0·15 M NaCl, exhibited a large insulin effect on glucose utilization when subsequently incubated for 2 hr in an all-sodium medium. When the tissue was pretreated with insulin for 2 min in an all-potassium medium, followed by washings in 0·15 M KCl, the insulin effect on glucose uptake was very small in the final period of incubation with glucose in the all-sodium medium. In other experiments, the hemidiaphragms were preincubated for 10 min with and without insulin, and similar results were obtained. Tissues exposed to insulin in a sodium medium responded well to the hormone; hemidiaphragms exposed to insulin in a potassium medium exhibited subsequently a much smaller hormonal response. When hemidiaphragms were exposed to insulin in a lithium medium for 10 min, the subsequent insulin effect on glucose utilization was large.

These experiments show that monovalent ions exert an influence on an initial binding of insulin with the tissue or on a rapid reaction that is stimulated by insulin. The presence of sodium or lithium ions is necessary for insulin to exert its maximum effect. It is interesting that the uptake of glucose in the absence of insulin is independent of the ionic composition of the preincubation medium, except when the hemidiaphragms are preincubated with lithium. The stimulation of glucose uptake by lithium is clearly seen after only 10 min of preincubation.

*Effects of washings of the tissue on the action of insulin.* A number of experiments were carried out in which hemidiaphragms were exposed to insulin for 10 min in an all-sodium medium (Table 4). Tissues were then "washed" in different salt solutions and subsequently incubated for 2 hr with glucose in the usual sodium medium. It is

TABLE 3. EFFECT OF MONOVALENT CATIONS ON THE INITIAL REACTION OF INSULIN WITH RAT DIAPHRAGM *in vitro**

| N | Preincubation: Cation of medium | Preincubation: Min | Preincubation: Insulin | Rinse (0·15 M) | Glucose uptake (μmole/g/2 hr ± S.E.M.) | Insulin effect (μmole/g/2 hr ± S.E.M.)† | P† |
|---|---|---|---|---|---|---|---|
| 7 | Na | 2 | 0 | NaCl | 25·2 ± 3·07 | | |
| 7 | | | + | | 34·4 ± 1·03 | 9·2 ± 1·54 | < 0·001 |
| 8 | *K* | 2 | 0 | KCl | 22·2 ± 1·02 | | |
| 8 | | | + | | 25·8 ± 0·99 | 3·6 ± 1·42 | < 0·05 |
| 10 | Na | 10 | 0 | NaCl | 22·0 ± 1·31 | | |
| 10 | | | + | | 33·0 ± 1·94 | 11·0 ± 1·25 | < 0·001 |
| 6 | *K* | 10 | 0 | KCl | 19·7 ± 1·68 | | |
| 6 | | | + | | 23·2 ± 1·62 | 3·5 ± 0·88 | < 0·01 |
| 6 | Li | 10 | 0 | LiCl | 33·6 ± 1·37 | | |
| 6 | | | + | | 43·3 ± 1·95 | 9·7 ± 1·52 | < 0·001 |

* Preincubation at 37° as indicated, in media without glucose and with or without insulin, 0·05 unit/ml. Hemidiaphragms were then rinsed three times at room temperature in 10-ml salt solutions. Incubation was at 37° for 2 hr in sodium medium containing glucose, but no insulin, as described in Methods.

† S.E.M. and statistical significance of insulin effect based on paired differences.

TABLE 4. EFFECTS OF SODIUM AND POTASSIUM IONS ON GLUCOSE UTILIZATION OF HEMIDIAPHRAGMS PRE-EXPOSED TO INSULIN*

| N | Preincubation (10 min) Insulin | Rinse | "Washing" | Glucose uptake ($\mu$mole/g/2 hr $\pm$ S.E.M.) | Insulin effect ($\mu$mole/g/2 hr $\pm$ S.E.M.)† | P† |
|---|---|---|---|---|---|---|
| 8 | 0 | NaCl | 2 min in 0·15 M NaCl; 37° | 20·3 $\pm$ 1·49 | | |
| 8 | + | | | 28·1 $\pm$ 2·54 | 7·8 $\pm$ 1·60 | < 0·005 |
| 8 | 0 | KCl | 2 min in 0·15 M KCl; 37° | 21·2 $\pm$ 1·67 | | |
| 8 | + | | | 29·5 $\pm$ 1·17 | 8·3 $\pm$ 1·53 | < 0·001 |
| 8 | 0 | NaCl | 30 min in Na medium; 22° | 22·1 $\pm$ 1·71 | | |
| 8 | + | | | 28·5 $\pm$ 1·19 | 6·4 $\pm$ 1·03 | < 0·001 |
| 8 | 0 | *K*Cl | 30 min in *K* medium; 22° | 21·4 $\pm$ 1·26 | | |
| 8 | + | | | 27·8 $\pm$ 1·14 | 6·4 $\pm$ 0·87 | < 0·001 |
| 12 | 0 | NaCl | 30 min in Na medium; 37° | 19·7 $\pm$ 0·86 | | |
| 12 | + | | | 23·1 $\pm$ 0·93 | 3·4 $\pm$ 0·67 | < 0·001 |
| 5 | 0 | *K*Cl | 30 min in *K* medium; 37° | 19·8 $\pm$ 1·01 | | |
| 5 | + | | | 25·0 $\pm$ 1·76 | 5·2 $\pm$ 1·02 | < 0·005 |

* Preincubation was for 10 min at 37° in sodium medium without glucose, with or without 0·05 unit insulin/ml. Hemidiaphragms were rinsed three times at room temperature in salt solutions as indicated. The tissues were subsequently treated with glucose-free solutions as described in the column titled "Washing". Final incubation was at 37° for 2 hr in sodium medium with glucose, but no insulin, as described in Methods.

† S.E.M. and statistical significance of insulin effects based on paired differences.

seen that brief washing in 0·15 M NaCl or in 0·15 M KCl had little effect on the subsequent action of insulin. When the washing period was extended to 30 min, there was a considerable decrease in the insulin effect, greater at 37° than at 22°, but quite independent of the ionic composition of the medium. It appears from these experiments than monovalent cations influence the binding of insulin to receptor sites or an initial effect of insulin on the cell, but these ions do not interfere with the effect of insulin once exerted.

*Role of sulfhydryl groups in glucose metabolism.* In a previous publication, we have reported that the thiol reagent, 2,2′-dithiodipyridine (DTP) inhibited the action of insulin in the rat diaphragm.[8] DTP is a disulfide and reacts with sulfhydryl groups to form mixed disulfides.[9] We studied the action of this compound at different concentrations on glucose utilization, lactate formation and glycogen synthesis on hemidiaphragms incubated in all-sodium or lithium media. The results of these experiments are reported in Figs. 3 and 4.

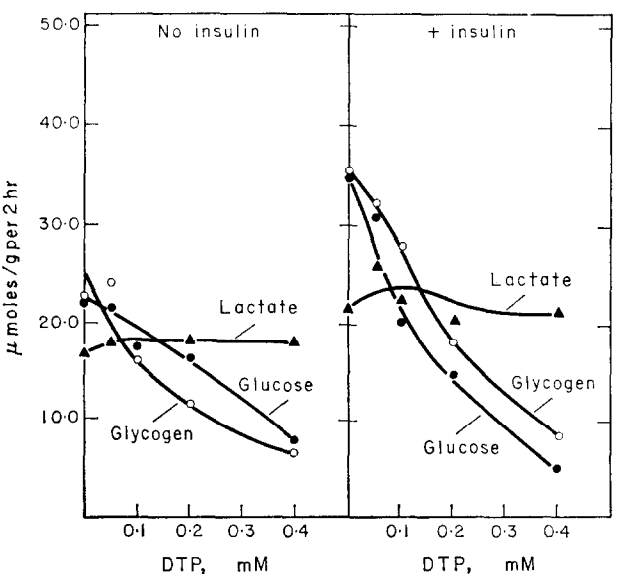


FIG. 3. Rat hemidiaphragms incubated at 37° for 2 hr in all sodium medium as described in Methods. Final concentration of DTP as indicated (N = 6).

In the sodium medium (Fig. 3), DTP at concentrations up to 0·4 mM caused a progressive decrease in glucose uptake and final glycogen content, both in the absence and presence of insulin. In contrast lactate formation was unaffected by the disulfide. It can be seen that, in agreement with our previous finding,[8] DTP at low concentrations strikingly inhibits the effect of insulin.

Similar experiments with hemidiaphragms incubated in an all-lithium medium are reported in Fig. 4. In the absence of insulin (left panel) there is the usual large utilization of glucose and high glycogen values characteristic of the lithium medium. The addition of DTP inhibited glucose uptake and glycogen synthesis, and in this case increased the formation of lactate. With insulin present in the lithium medium,

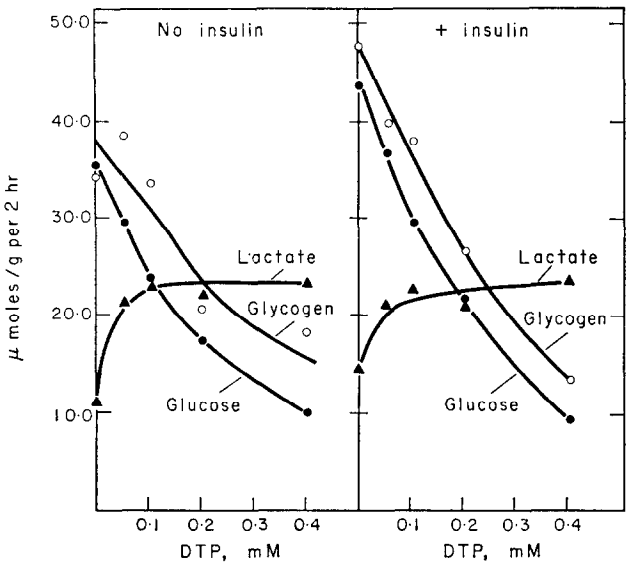


FIG. 4. Rat hemidiaphragms incubated at 37° for 2 hr in 85% lithium–15% sodium medium as described in Methods. Final concentration of DTP as indicated (N = 6).

glucose uptake and final glycogen contents reached still higher values, and there was a progressive inhibition of the insulin and lithium-stimulated glucose metabolism as the concentration of DTP was increased. As in the absence of insulin, lactate formation was increased to a small extent by the addition of DTP.

Taken together, these experiments show that glucose utilization and glycogen synthesis are inhibited to the same extent over the entire concentration range of the disulfide inhibitor. Lactate formation, on the other hand, is unaffected and not increased by DTP. The importance of these results is that the decrease in final glycogen content produced by DTP is in every case about the same as the diminution in glucose uptake. Glycolysis, as measured by lactate production, is influenced relatively little by DTP. As the concentration of DTP is increased, a reaction leading from glucose to glycogen is inhibited while the formation of lactate from glucose proceeds uninterrupted.

## DISCUSSION

The experiments reported here demonstrate clearly the profound effects that monovalent ions exert on glucose metabolism in muscle and on the action of insulin on carbohydrate metabolism in this tissue. Lithium and sodium ions, when present in excess in the incubation medium, stimulate glucose utilization and glycogen synthesis, while potassium in excess causes the tissue to take up less glucose and diminish the synthesis of glycogen. Lactate formation is stimulated by rubidium and potassium ions, intermediate in the presence of sodium, and markedly suppressed by lithium ions. These conclusions are in general agreement with earlier studies on the action of cations on carbohydrate metabolism in muscle.[1–4] In addition, our experiments show that sodium or lithium ions are necessary for the maximal effect of insulin to be exerted.

The preincubation experiments reported here reveal several new important aspects of the effects of monovalent ions on glucose metabolism in muscle and on the action of insulin. It is evident from the experiments in Table 1 that the rates of glucose utilization and lactate formation are determined mainly by the ionic composition of the final incubation medium with glucose, while the insulin effects on glucose utilization and glycogen synthesis are determined primarily by the ionic composition of the medium during the preincubation period. The results with lithium ions are somewhat different (Table 2). This ion stimulates glucose utilization, promotes glycogen synthesis, and depresses the formation of lactate. These effects of lithium are fully evident after 10 or 30 min of preincubation in a medium high in lithium, although the final incubation with glucose is carried out in an all-sodium medium (Tables 2 and 3). While sodium ions did not overcome the effects of lithium, potassium completely reversed the action of lithium on glucose metabolism.

The influence of monovalent ions on the effects of insulin is clearly exerted at an early stage of the action of the hormone. Pre-exposure of the tissue to insulin is much more effective in a sodium or lithium medium than in a medium high in potassium ions, irrespective of the ionic composition of the final incubation medium with glucose. Similarly, solutions containing high concentrations of potassium are no more effective than solutions high in sodium in reversing the insulin effect, once exerted.

There are several indications from the literature that an inhibition of the sodium pump leads to an insulin-like effect on muscle. Clausen,[2] for example, showed that ouabain caused an increase in glycogen synthesis in rat diaphragm incubated in Krebs–Ringer bicarbonate medium. Our experiments show that the highest rate of glucose utilization occurs when the sodium pump is most likely to be inhibited, i.e. in the absence of external potassium ions and in the presence of high concentrations of sodium or lithium. It is likely that the presence of lithium or an elevated sodium concentration inside the cell concomitant with a decrease in cellular potassium will act to stimulate glucose uptake and, in particular, increase the synthesis of glycogen. Bihler and Sawh[10] found that influx of 3-*O*-methyl-D-glucose into cells of the intact rat diaphragm preparation was stimulated in potassium-free media and observed that the increase in transport was correlated with intracellular changes in potassium and sodium ions. These authors concluded that their results did not support the existence of a direct link between the sodium pump and sugar transport, but than an increased sodium and decreased potassium content of the cell enhanced sugar transport in and out of the cell.

Our experiments show very strongly that potassium ions tend to direct glucose metabolism in muscle toward glycolysis, while lithium ions direct the glucose taken up by the tissue toward the formation of glycogen. Sodium is intermediate in that both glycogen synthesis and lactate formation are stimulated in the presence of an excess of this ion.

The experiments with insulin and with the sulfhydryl reagent, DTP, also indicate that the pathways of glucose to glycogen or glucose to lactate may be influenced by experimental conditions in different ways. Glycogen formation is highly responsive to insulin and quite easily inhibited by DTP, while glycolysis is less influenced by insulin and not greatly affected by DTP.

Experiments on the metabolism of [$^{14}$C]glucose in rat diaphragm by Shaw and Stadie,[11] Beloff-Chain *et al.*,[12] Antony *et al.*,[13] and Kalant and Beitner[14] have provided

evidence for different pathways of glucose metabolism in muscle. Two possibilities have been proposed: (1) that glucose can be converted into glycogen by reactions not involving glucose 6-phospate; or (2) that two anatomically distinct pools of glucose 6-phosphate exist, one on the pathway of glucose toward glycogen and one on the pathway toward lactate. The evidence for the existence of two pathways of glucose metabolism in muscle is not conclusive and further experiments are needed in this important field. In some of our experiments, we determined the tissue contents of glycolytic intermediates and adenine nucleotides. Lithium ions did not significantly alter the concentrations of these substances so that the effect of this ion on glucose metabolism does not appear to be related to alterations in glycolytic reactions. Inhibition of glucose metabolism by DTP was not accompanied by accumulation of glycolytic intermediates. However, a significant fall in ATP content of the tissue was observed (2·59–1·69 $\mu$mole/g after 60 min of incubation, N = 4). This is probably related to the known action of DTP in inhibiting oxidative phosphorylation,[15] and may account for the increase in lactate accumulation produced by this compound.

Since the early introduction of the concept of insulin binding to tissue by Stadie *et al.*,[16–18] it has become generally accepted that the first step in the action of insulin is a combination of the hormone with receptors on or close to the surface of the cell. Recently, Cuatrecasas[19] has demonstrated a specific binding of $^{125}$I-insulin to cell membrane preparations obtained from fat cells and has studied this phenomenon in detail. The binding sites have a very high affinity for insulin ($K = ca.\ 5 \times 10^{-11}$ M). Most of the studies of binding of insulin to the cell membrane preparations were carried out in Krebs–Ringer bicarbonate medium. However, when solutions with widely different ionic compositions were used, it was observed that cations had little or no effect on insulin binding. The amount of insulin bound per milligram of membrane protein was the same, for example, in 0·1 M sodium phosphate as it was in 0·1 M potassium phosphate. If insulin binding sites in muscle are similar to those in adipose tissue, it is unlikely that the effects of monovalent ions on the action of insulin in muscle involve the initial binding of the hormone to the cell. Since we have shown here that monovalent cations have marked effects on the action of insulin when present during a 2- or 10-min preincubation of the tissue with insulin, it appears reasonable to propose that these ions promote or inhibit an early and rapid reaction catalyzed by insulin. Experiments are in progress to study this problem.

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