*Special Article*

# Glucose Transport and Phosphorylation in Muscle of Diabetic Animals

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It is generally thought that the impaired peripheral utilization of glucose in the diabetic animal is due in part to a depression of glucose uptake by muscle tissue. In the present paper, we shall attempt to analyze the disturbances in cell function causing this depression.

In a series of papers by Cori and his associates,[1-3] the proposal was developed that the low uptake was due to inhibition of the hexokinase system. The hexokinase reaction, in which glucose is phosphorylated to glucose-6-phosphate, was regarded as the first step in glucose metabolism and rate-limiting for glucose uptake by muscle. From studies with extracts from diabetic muscle,[2] it was proposed that the reaction was inhibited by pituitary and adrenal factors and that this inhibition was relieved by insulin. It was not possible, however, to obtain entirely satisfactory support for this theory. The inhibition was noted to be very unstable and could not be obtained with regularity. An effort by Stadie and associates[4,5] to duplicate the original observations did not succeed. Furthermore, it was not established experimentally that the hexokinase reaction was in fact the rate-limiting step for uptake by intact muscle, and subsequent studies showing the marked inhibitory effect of glucose-6-phosphate on hexokinase[6,7] have made it doubtful whether the enzyme was the site of inhibition in the original extract studies. Nevertheless, the "hexokinase theory" continued to receive serious attention, since many studies with intact tissue preparations indicated the presence of a metabolic block in the early phases of glucose metabolism at or near the level of the hexokinase reaction.[8]

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Following the observations of Levine and associates,[9,10] a large body of evidence has been assembled to show that insulin accelerates the transport of glucose through the cell membrane of muscle.[8,11,12] Membrane transport has been shown to involve a specific interaction between glucose and a membrane component and to be a step in glucose metabolism that antecedes glucose phosphorylation.[12] In the diabetic muscle, it has been shown that transport is depressed[13,14] and is the major limiting step for glucose uptake.

While these studies established an action of insulin on transport, they did not exclude an additional effect of the hormone on phosphorylation as envisioned in the hexokinase concept. In this connection, recently developed experimental technics make it possible to distinguish effects on phosphorylation from effects on transport in intact tissues.[16-18] By use of these methods, it has been shown that phosphorylation is in fact depressed as well as transport.[19-21] Furthermore, the inhibition of phosphorylation appears to be due in part to pituitary and adrenal activity and can be relieved by insulin under certain conditions as will be seen below. In light of these observations, it would now appear that the disturbances in the diabetic muscle are best described by a concept which includes elements of both the transport and hexokinase theories.

Representative data that support this concept are shown in the table. These data are taken from studies of glucose uptake by the perfused, isolated rat heart. The methods employed and some of the results have been described in earlier publications,[12,21] and a full description will appear shortly.

In the heart from normal, fasted rats the glucose uptake is 4.7 mg./gm./hr. under the present experimental conditions. This value (experiment 1) is taken as a reference against which other rates are compared. As can be seen, no free glucose accumulates inside the cell during uptake. This is an important observation on which the following argument is based. Since the product

TABLE 1

The effect of insulin and pituitary and adrenal factors on glucose uptake by the isolated, perfused rat heart

| Experiment number | Treatment of rats | Insulin *in vitro* 3 μg. per ml. | Glucose uptake mg. per gm. per hr. | Glucose uptake per cent of normal | Intracellular free glucose mg. per 100 ml. | Glucose phosphorylation mg. per gm. per hr. | Glucose phosphorylation per cent of normal capacity* |
|---|---|---|---|---|---|---|---|
| | | | Normal rats | | | | |
| 1 | None | 0 | *4.7±0.2* | 100 | Ca.0 | 4.7±0.2 | — |
| 2 | None | + | 10.7±0.6 | 230 | 15±4 | *10.4±0.6* | 100 |
| | | | Alloxan diabetic rats | | | | |
| 3 | None | 0 | *2.0±0.3* | 40 | Ca.0 | 2.0±0.3 | |
| 4 | None | + | 3.5±0.3 | 70 | 58±3 | *3.3±0.3* | 30 |
| 5 | Adrenalectomy | 0 | *2.0±0.3* | 40 | Ca.0 | 2.0±0.3 | |
| 6 | Adrenalectomy | + | 5.6±0.7 | 120 | 15±15 | *5.5±0.7* | 60 |
| 7 | Hypophysectomy | 0 | *1.8±0.4* | 40 | Ca.0 | 1.8±0.4 | |
| 8 | Hypophysectomy | + | 7.6±0.5 | 160 | 26±7 | *7.3±0.5* | 70 |
| 9 | Hypophysectomy and *growth hormone* | + | 5.9±0.4 | 130 | 35±10 | *5.7±0.4* | 50 |
| 10 | Hypophysectomy and hydrocortisone | + | 4.6±0.5 | 100 | 39±10 | *4.3±0.5* | 40 |
| 11 | Hypophysectomy, growth hormone and hydrocortisone | + | 2.3±0.2 | 50 | 59±8 | *1.8±0.2* | 10 |
| 12 | Insulin (24 hours) | + | *11.8±0.3* | 250 | Ca.0 | 11.8±0.3 | >110 |
| 13 | Insulin (24 hours) and growth hormone and hydrocortisone | + | *11.2±0.2* | 240 | Ca.0 | 11.2±0.2 | >110 |
| 14 | None | + anoxia | *14.1±0.6* | 300 | Ca.0 | 14.1±0.6 | >140 |

The glucose uptake values in italics provide measurements of the rate of unidirectional transport into the cell, since transport is the rate-limiting step for uptake in these experiments (see text). Uptake values not italicized indicate net transport rates only since free glucose is present inside the cell. The values in italics for phosphorylation provide estimates of the phosphorylation capacity. Values not in italics do not estimate the capacity, since the availability of substrate is restricted in these instances by the relatively slow rates of transport (see text).

All rats except those receiving insulin in vivo were fasted eighteen hours before removal of the heart. Diabetic rats were prepared by the intravenous injection of 6 mg. of alloxan per 100 gm. of body weight forty-eight hours or longer before use. Adrenalectomy was carried out four days and hypophysectomy three to six weeks before death. Growth hormone (a gift of the Endocrinology Study Section of the National Institutes of Health) was injected twenty-four hours and again twelve hours prior to sacrifice in a dosage of 100 μg./100 gm. Hydrocortisone was administered twenty-four, twelve and three hours before sacrifice in dosages of 2 mg./100 gm.

The perfusions were carried out for thirty minutes at 37° with 100 mg./100 ml. of glucose in oxygenated, bicarbonate buffer. Other procedures will be described in detail elsewhere.[23]

*These percentages take into account the variations in intracellular glucose concentration since they are referred to a curve obtained for phosphorylation in the normal heart as a function of intracellular glucose concentration.[23]

of transport into the cell is the free sugar, as shown in earlier studies,[12,13] the absence of free sugar indicates that glucose is phosphorylated as rapidly as it penetrates. As a consequence, the rate of phosphorylation is limited by the rate of transport. Transport is, in fact, the rate-limiting step for the over-all uptake of glucose, since it has been established that extracellular diffusion of sugars from the blood to the cell surface is relatively very fast.[21]

When insulin is added in vitro (experiment 2), glucose uptake is markedly stimulated and free glucose now is found inside the cell. This is interpreted to mean that transport is accelerated by the hormone to such an extent that the phosphorylation capacity is exceeded. Under these conditions, phosphorylation becomes the major limiting step for glucose uptake. It may be noted that the rate of phosphorylation is slightly less than the rate of uptake, the difference being due to the amount of free sugar accumulating inside the cell.

In hearts from severely alloxan-diabetic rats (experiment 3), uptake is reduced to 40 per cent of normal.

No free glucose is present in the cell, and transport remains the limiting step for uptake. Thus a low rate of transport must be the cause of the depressed uptake. When insulin is added (experiment 4), uptake rises, but the hormone effect is much smaller than in the normal tissue. There is, however, a very marked rise in intracellular free glucose indicating that transport into the cell has been accelerated. The failure of glucose uptake to rise to the normal extent under these conditions, where phosphorylation becomes the limiting step, provides evidence that phosphorylation is also inhibited. It is also clear that this inhibition is not relieved by insulin in vitro in the thirty-minute period of these experiments.

Further experiments show that adrenal and pituitary factors inhibit phosphorylation but not transport. In the absence of insulin, where uptake reflects the rate of transport, neither adrenalectomy nor hypophysectomy has any effect (experiments 5 and 7). On the other hand, in the presence of insulin, where uptake reflects the rate of phosphorylation, either adrenalectomy or hypophysectomy causes a substantial increase in uptake indicating removal of factors inhibiting the phosphorylation process (experiments 6 and 8). Further evidence for this inhibition is supplied in experiments 9 and 10. Here it is seen that pretreatment of the hypophysectomized-diabetic animal with growth hormone or cortisone partially restores the inhibition of phosphorylation. When both substances are administered together (experiment 11), phosphorylation is depressed below the level found in the simple diabetic (experiment 4). It can also be seen, in experiment 11, that the intracellular free glucose rises to a very high level, suggesting that the transport step is not grossly impaired.

In the above experiments the failure of insulin in vitro to relieve the depressed phosphorylation by diabetic muscle is not consistent with the fact that insulin in vivo completely restores to normal the peripheral utilization of glucose in the diabetic animal. In order to study this point further, experiments were undertaken to determine the effect of pretreatment with insulin in vivo on the subsequent phosphorylation by the isolated heart. It was found that treatment of the animal for one hour improves phosphorylation rates in some instances but not in others; treatment for four hours consistently restores the rate to normal and treatment for twenty-four hours leads to above normal rates (experiment 12). It is not clear whether this insulin effect is direct or indirect. If direct, it is slow, possibly because of slow penetration of the hormone through the membrane. If indirect, it does not appear to be due to suppression of pituitary and adrenal activity since the concomitant administration of growth hormone and cortisone does not modify the insulin response (experiment 13).

The depression of phosphorylation with diabetes and the restoration to normal with insulin in vivo are not due to adaptive changes in the enzyme content of the muscle. This is shown by the fact that exposure of the heart to anoxia immediately elevates the phosphorylation rate to a high level (experiment 14), and a subsequent re-exposure to aerobic conditions immediately returns phosphorylation to the usual diabetic rate. These rapid changes in activity demonstrate that we deal with inhibitory and not adaptive phenomena. It may be noted in passing that the sensitivity of phosphorylation to anoxia may be related to the lability of the inhibition of phosphorylation in muscle extracts mentioned earlier.

In the present discussion, it appears desirable to refer to effects on phosphorylation rather than to effects on the hexokinase reaction specifically. Studies of glycogen and glucose-6-phosphate levels in the heart, to be reported later, suggest that the primary site of pituitary and adrenal activity may be on a step beyond the hexokinase reaction. Inhibition of hexokinase may be secondary to the accumulation of a metabolic intermediate, possibly the known inhibitor glucose-6-phosphate.

As already mentioned, pituitary and adrenal secretions do not appear to inhibit the transport step. It should be emphasized, however, that these observations were made in tissues virtually free of insulin. Evidence has been presented earlier that pituitary and adrenal factors may affect transport rates in hearts from hypophysectomized animals where some insulin remains in the muscle.[22] Futhermore, it has been shown that the acceleration of transport with insulin is slow in diabetic muscle where pituitary and adrenal activity is probably greater than normal.[22] It seems therefore possible that pituitary and adrenal factors, while having no direct effect on transport, may nevertheless reduce the sensitivity of this step to insulin acceleration.

Kipnis[20] has recently carried out studies of transport and phosphorylation of the glucose analog, 2-deoxyglucose, in diabetic muscle using the isolated rat diaphragm as the test system. The conclusions he has reached are very similar to those presented here and make it seem likely that the general concept outlined below can be properly applied to muscle tissue in general.

## SUMMARY

The role of glucose transport and phosphorylation in the uptake of glucose by diabetic muscle is discussed and illustrative data are presented. Transport of glucose

through the cell membrane is reduced due to a deficiency of insulin. This is the primary cause for the depressed uptake in the diabetic tissue since uptake is limited by the transport step. Insulin causes a marked increase in the rate of glucose transport. Glucose uptake does not increase to the normal extent, however, since phosphorylation is also depressed and becomes the rate-limiting step for uptake under these conditions. The depression of phosphorylation is due in part to adrenal and anterior pituitary activity, probably involving the growth hormone and hydrocortisone. Insulin relieves this depression but the hormone effect is delayed and may be indirect.


## SUMMARIO IN INTERLINGUA

*Transporto e Phosphorylation de Glucosa in le Musculo de Animales Diabetic*

Le rolo del transporto e del phosphorylation de glucosa in le fixation de glucosa per musculo diabetic es discutite, e datos illustrative es presentate. Le transporto de glucosa a transverso le membrana cellular es reducite a causa de un deficientia de insulina. Isto es le causa primari del deprimite fixation de glucosa in le tissu diabetic, proque le fixation es limitate per le limitation del transporto. Le administration de insulina causa un marcate intensification del transporto de glucosa. Tamen, le fixation de glucosa non es augmentate usque al nivello normal, proque le phosphorylation es etiam deprimite e deveni sub iste conditiones le factor que restringe le fixation de glucosa. Le depression del phosphorylation es causate in parte per le activitate del adrenales e del pituitario anterior, in le qual le hormon de crescentia e hydrocortisona es probabilemente interessate. Insulina allevia iste depression, sed le effecto hormonal es retardate e es forsan indirecte.



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