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Anomalous Effects of Zinc-Insulin Upon *Trypanosoma hippicum*.*

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During the course of investigations into the nature of carbohydrate metabolism in *T. hippicum* some very interesting observations were made on the effect of zinc-insulin upon the respiration of the parasites.

Insulin has been reported to cause augmentation of the oxygen assimilation of pigeon breast muscle under certain conditions,[1-3] and a similar augmentation has been reported for mammalian tissues.[4] The increase in oxygen utilization is thought to be concerned with pyruvate oxidation.[5] *T. hippicum* responds to zinc-insulin with sizeable increases in oxygen uptake and pyruvate production, but glucose utilization is spared. Zinc-insulin inhibits the anaerobic production of pyruvate from glucose.

Experimental. Whole trypanosomes were suspended in a medium of Krebs Ringer-phosphate[6] with 0.025 M bicarbonate added. Glucose was used as a substrate. Measurements of gas exchange were made manometrically at 38°C with a Barcroft-Warburg constant volume respirometer using the methods of Dixon.[7] Glucose was analysed by the method of Nelson,[8] and pyruvate was determined according to Friedemann and Haugen.[9]

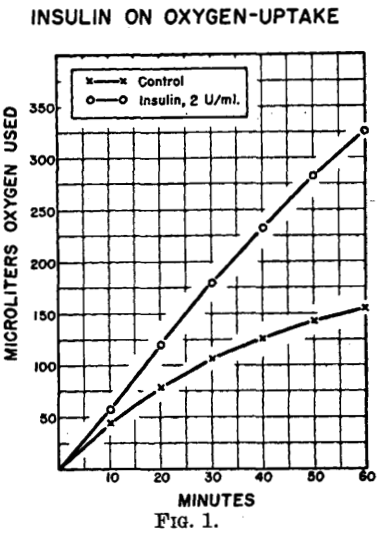

FIG. 1.

The augmenting action of zinc-insulin on the time variation of oxygen uptake. Glucose concentration, 0.01 M.

The effect of zinc-insulin (Lilly) upon time variation of oxygen-uptake of *T. hippicum* is shown in Fig. 1. The response shown may be elicited when the insulin concentration is as low as 0.1 unit per ml of medium, but below that level the effect becomes insignificant. Under conditions of optimal glucose concentration (0.01 M) the trypanosomes utilize about 100% more oxygen during the first hour when insulin is present than when it is withheld. The augmentation of the initial rate, however, only amounts to about 20%. Insulin also has a marked effect upon the oxygen uptake-glucose concentration relationship. The slope of the ascending limb of the curve is greatly increased, and the plateau level of the curve is elevated 60-100%, as seen from the example given in Fig. 2.

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TABLE I.
Specific Action of Zinc-Insulin on Oxygen-Uptake.

Added substances	Microliters oxygen used
Glucose, 0.028 M	187
Insulin, 2U/ml + glucose, 0.028 M	390
" 2U/ml	—3
Zn++, 0.005 mg/ml + glucose, 0.028 M	142
Cystine, 10^{-4} M + glucose, 0.028 M	180
*GS-SG, 10^{-4} M + glucose, 0.028 M	192
Tyrosine, 0.01 mg/ml + glucose, 0.028 M	173
Albumin, 1 mg/ml + glucose, 0.028 M	167
Insulin, 2U/ml + glucose, 0.028 M + malonate. 0.01 M	368

*Oxidized glutathione.

TABLE II.
Effect of Zinc-Insulin on Carbon Balance.*

	Insulin, 2U/ml		Control	
	Glucose used (micromoles)	Pyruvate formed (micromoles)	Glucose used (micromoles)	Pyruvate formed (micromoles)
Aerobic	1.86	7.50	2.85	5.67
Anaerobic	1.26	0.33	2.67	2.58

* Initial glucose concentration, 0.0011 M.

The specificity of the insulin action is shown in Table I. That the insulin itself was not being metabolized was indicated by the failure of the parasites to respire when glucose was withheld. Zinc in concentrations comparable to that provided by the zinc-insulin preparation did not increase the oxygen consumption and may have caused a slight inhibition. Disulfide groups were provided by cystine and oxidized glutathione in amounts approximating that found in the insulin. The data demonstrate that the action of the insulin cannot be attributed to its disulfide linkages *per se*, nor can it be attributed to a dynamic action of the tyrosine units in the molecule. That the effect was not a non-specific protein effect was tested by the addition of bovine serum albumin (fraction V, Armour) in a concentration roughly 10 times that of the insulin which it replaced. Malonate did not abolish the action of insulin, a finding which is compatible with the data of Table II in which it is shown that pyruvate oxidation was not promoted by the insulin.

Examination of Fig. 2 reveals that at a glucose concentration of 0.0012 M, for example, the oxygen utilized is about 4 times that which can be accounted for by the total amount of glucose available in the system (3 ml volume), if one assumes that pyruvate is the final product.[10] At lower concentrations the oxygen uptake cannot be accounted for by any possible metabolic scheme based upon glucose as the sole substrate. In order to determine what influence the insulin was exerting on the glucose-pyruvate relationship, these two substances were analysed from the experimental system. Table II presents data collected from aerobic and anaerobic experiments. Aerobically the glucose utilization was spared by insulin to the extent of 35%, but the pyruvate production was increased 32% over the controls. Anaerobically glucose utilization was diminished 53% in the presence of insulin, while there was a simultaneous diminution of 87% in pyruvate production.

Discussion. It is not obvious what general implications these actions of insulin may have regarding the mechanism of action of the hormone. In whole cell preparations of animal tissues the drug is generally considered to promote the disappearance of glucose. Insulin, however, was found to have no effect upon the hexokinase of the trypanosomes. Only in those instances cited has insulin been reported to affect the rate of oxygen uptake, which

[10] Harvey, S. C., to be published.

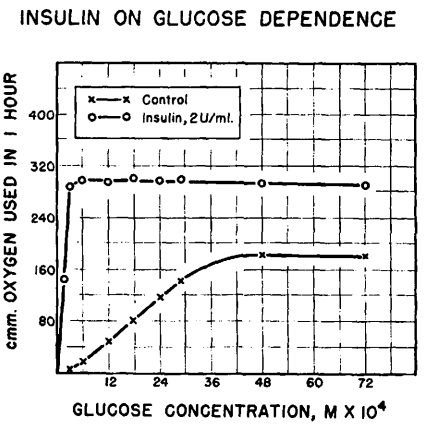

FIG. 2.

Zinc-insulin promoted alterations in the shape of the oxygen uptake-glucose concentration dependence curve.

Rice and Evans[5] purport is a result of increased pyruvate dissimilation. *T. hippicum,* however, is incapable of oxidizing pyruvate, and the accumulation of pyruvate and the failure of malonate to moderate the response to insulin show that pyruvate oxidation was not involved. It is impossible to explain an increase in aerobic pyruvate production in the face of a decrease in glucose consumption, considering the stoichiometrical relationship between glucose and pyruvate in the parasite, without assuming that endogenous substrate is brought into the sequence. Again, this is the only means by which the enormous oxygen uptake and low concentrations may be reconciled. *T. hippicum* cannot respire upon endogenous substrate alone, nor have polysaccharides been found in the organism,[10] so that it would seem that insulin is making lipoid or proteinaceous materials available for dissimilation. It should be interjected, however, that these perturbations in the carbon balance were performed at glucose concentrations low enough that the endogenous contribution to the scheme would be proportionately significant and thereby detectable. Nevertheless, at glucose concentrations even as high as 0.044 M, where the endogenous contribution is proportionately small, 60-80% augmentation of oxygen uptake was yet evident. The slope of the insulin curve in Fig. 1 does not fall off as rapidly as that of the control, therefore, it seems that a stabilizing factor is at work as well as an augmenting one.

The discovery that insulin, contrary to the aerobic case, inhibited the anaerobic pyruvate production to an extent way out of proportion to the glucose sparing effect is no less startling than it is inexplicable. It may lead to the disturbing hypothesis that the aerobic and anaerobic pathways of pyruvate production are dissimilar.

Summary. Aerobically, insulin was found to greatly increase the oxygen assimilation and pyruvate production in *T. hippicum,* while at the same time the utilization of glucose was diminished.

Anaerobically, it was found to inhibit the production of pyruvate while the diminution in glucose consumption was of the same order as that under aerobic conditions.