STUDIES ON THE MEASUREMENT OF SERUM INSULIN ACTIVITY

by

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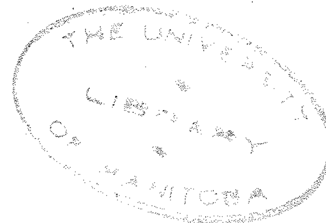

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The evidence in the current literature suggests that insulin acts by increasing the rate of transfer of glucose into the cell.

A reliable method for the estimation of serum insulin activity would be of value in helping to elucidate the physiological role of insulin, and the etiology and pathogenesis of diabetes mellitus and other disorders of carbohydrate metabolism.

The in vivo assay method measures the serum insulin activity by the drop in blood sugar produced in alloxan-diabetic hypophysectomized adrenalectomized mice. The reliability of this method has not been satisfactorily proven and, in addition, the animals are unstable and difficult to maintain.

The in vitro assay method measures the serum insulin activity by the rate of glucose uptake of the isolated rat diaphragm. This method and the in vivo method cited above, measure the net serum insulin activity. One modification of the in vitro method was investigated and found to give extremely inconsistent results in our hands.

A method for the estimation of absolute serum insulin activity was devised. This method compares the glucose uptake of a rat hemi-diaphragm immersed in serum with that of the corresponding hemi-diaphragm immersed in serum whose insulin has been inactivated by the addition of cysteine. The serum insulin activities of twelve normal fasting adults were measured. It was found that these values were enhanced by the ingestion of glucose or by the injection of insulin. A diabetic deprived of insulin was found to have no serum insulin activity while an infant with idiopathic spontaneous hypoglycemia was found to have an elevated serum insulin activity. The in vitro addition of insulin to serum was found to enhance the insulin activity of that serum. The threshold for the production of this effect was 75 milli-units per ml.

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CHAPTER I. THE MECHANISM OF INSULIN ACTION

GENERAL ACTIONS:

Insulin, the hormone produced by the beta cells of the pancreatic islets, has been observed to affect the metabolism not only of carbohydrate but also of fat and protein. The changes it leads to include lowering of blood sugar, stimulation of muscle glucose utilization and glycogen synthesis, stimulation of liver glycogen synthesis, stimulation of fat synthesis from acetate, inhibition of ketone body production and the inhibition of gluconeogenesis from protein. In investigating the mechanism of action of insulin, workers have sought to find a single site of action in the metabolic scheme which would explain all the observed effects of this hormone.

CARBOHYDRATE METABOLISM:

In order to understand the proposed theories of insulin action, it is necessary first to examine the principal reactions involved in glucose metabolism and their relation to the metabolism of fat and protein. These reactions are illustrated diagramatically in Fig. 1. From this diagram it is seen that the initial step in the metabolism of glucose is its reaction with adenosine triphosphate to form glucose-6-phosphate. This reaction is catalyzed by the enzyme gluco-hexokinase and is not reversible. The glucose-6-phosphate so formed can be used to synthesize glycogen, or can enter the glycolytic cycle in which series of reactions one mol of the six carbon compound is converted to two mols of a three carbon compound. One anerobic oxidation step takes place and the result of anerobic glycolysis of one mol of

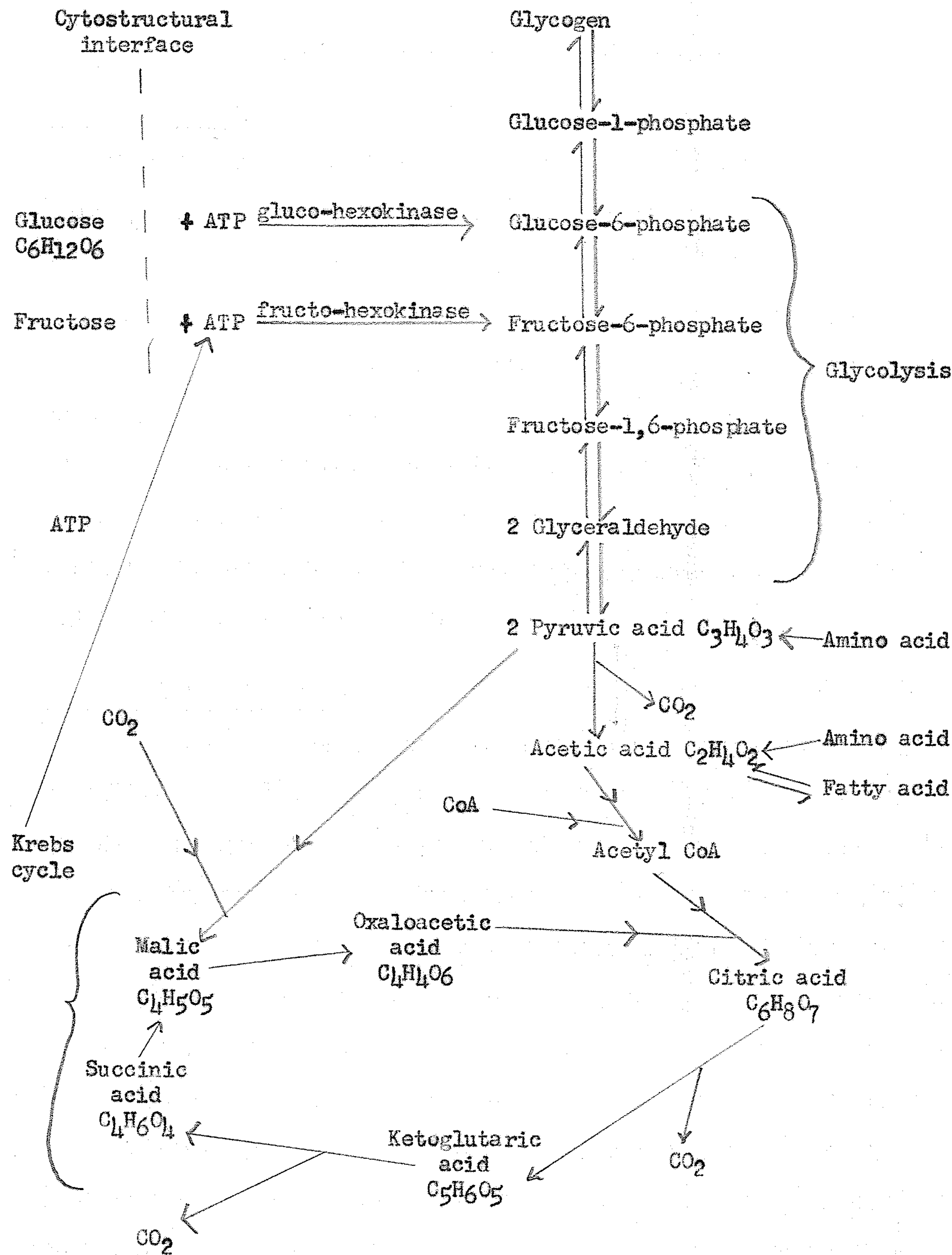

Figure 1. An Outline of Intermediary Metabolism.

glucose-6-phosphate is two mols of pyruvic acid. The glycolytic reactions are reversible and thus pyruvic acid can be converted to glucose-6-phosphate and glycogen. This end product of glycolysis, pyruvic acid, then undergoes oxidative decarboxylation to yield acetate which, after being activated by coenzyme A, condenses with oxaloacetic acid to enter the oxidative system known as the Krebs or citric acid cycle. In one complete revolution of this cycle there are three separate steps of oxidative decarboxylation which result in the oxidation of pyruvic acid to carbon dioxide and water, the regeneration of oxaloacetic acid and the liberation of energy which is stored in the high energy phosphate bonds of adenosine triphosphate or creatine phosphate to be used in the many energy-requiring reactions of the cell, including synthesis of protein and fat. The citric acid cycle is also the final common pathway for the oxidation of fats and proteins as these too can degrade to acetate. Alanine and some other amino acids can also be converted to pyruvic acid by the action of the enzyme amino acid oxidase. In this way, gluconeogenesis can occur, i.e. the synthesis of carbohydrate from non-carbohydrate precursors. The sugar fructose enters the glycolytic cycle by phosphorylation to form fructose-6-phosphate. This reaction is catalyzed by the enzyme fructo-hexokinase.

SITE OF ACTION:

From the metabolic relations which have been outlined, it can be seen that it is not inconceivable that a hormone such as insulin could exert a primary action upon carbohydrate metabolism which could have far-reaching secondary effects upon fat and protein metabolism.

In attempting to localize the site of the metabolic defect in diabetes, Chernick and Chaikoff (1) showed that the liver slices of alloxan diabetic rats oxidized glucose at a rate less than that of normal rats, while the oxidation of fructose in alloxan diabetic liver slices proceeded normally. Teng, Sinex, Deane and Hastings (2) found that, while the liver of alloxan diabetic rats was unable to synthesize glycogen from glucose at a normal rate, the synthesis of glycogen was unimpaired when pyruvate was used as a substrate. These findings suggested that the site of insulin action was somewhere before the pathways of fructose and glucose met, and did not involve the reactions leading from pyruvate to glycogen. Such a site is the formation of glucose-6-phosphate. Insulin could act at this point by speeding up the gluco-hexokinase reaction, but it could also act simply by promoting the entry of glucose into the cell so that it could be acted upon by the enzyme.

The Transfer Mechanism

Although it is not impossible that insulin has more than one site of action, the evidence to be summarized favors the concept that insulin acts by promoting the transfer of glucose into the cell and that such an action can explain all the other changes which have been observed to occur. Levine (3) has shown that in the eviscerated nephrectomized dog, insulin caused an increase in the volume of distribution of galactose. Since this preparation cannot metabolize galactose, it was concluded that the increased volume of distribution was brought about by the movement of galactose into the cells. It was inferred that insulin had a similar

action on glucose. Using the isolated rat diaphragm, Park (4) set out to demonstrate the accumulation of free glucose in the cell as a consequence of insulin action. In order to do this it was necessary to prevent the removal of this free glucose by hexokinase, and this he did by chilling the tissue, thereby slowing the enzyme activity. He was thus able to show that, whereas without insulin the amount of free glucose in the tissue corresponded to the amount in the extracellular space, when insulin was added the amount of free glucose was four to five times greater. He concluded that insulin had increased the rate of glucose transfer beyond the capacity of the chilled hexokinase system and therefore free glucose had accumulated within the cell. In the ciliary body of the rabbit's eye, insulin was clearly shown to enhance the permeability to glucose (5). By studying the rate of entry of glucose into the rat diaphragm at various temperatures, Levine and Goldstein (6) concluded that the changes in rate were such as to suggest that the transfer mechanism involved little or no energy exchange and was not under the influence of an enzyme. If so, this may be taken as evidence that any effect of insulin upon the transfer mechanism is a primary one and not secondary to changes in the availability of ATP.

According to this transfer mechanism concept of insulin action, the observed effects are explained as derivatives of the primary action in the following manner. The transfer of glucose into the cell is seen as resulting in more substrate being made available for phosphorylation by hexokinase, and thus for the synthesis of glycogen and the process of glycolysis. As glycolysis produces more pyruvate, more

substrate is made available for the citric acid cycle which then becomes capable of greater oxidation of acetate and production of high energy phosphate bonds. At the same time the increased rate of glycolysis permits more fatty acid synthesis from acetate, a process which seems to require the energy derived from glycolysis in order to proceed properly. As more acetate is utilized for oxidation and for fatty acid synthesis, less is converted into ketone bodies, and ketosis is thereby corrected. The ability of insulin to reduce gluconeogenesis is ascribed to the increased production of pyruvate from glycolysis, so that less is produced from the glycogenic amino acids such as alanine.

The Hexokinase Reaction

The second postulated site of insulin action is at the glucohexokinase reaction. This could be either a direct action upon the enzyme, or an indirect action resulting from an increased availability of ATP from the oxidative phosphorylations of the citric acid cycle.

Price, Cori and Colowick (7), using cell-free extracts containing muscle hexokinase, found that insulin was capable of releasing the inhibition imposed upon this system by anterior pituitary extracts and adrenal cortical extracts. However, Stadie and Haugaard (8) were unable to duplicate this finding and a direct stimulant effect of insulin upon the hexokinase reaction has not been shown conclusively. Indeed, apart from Price et al (7), no one has ever demonstrated that insulin is active in tissue in which cellular structure has been destroyed.

On the other hand, evidence has been presented which suggests that insulin acts by increasing the efficiency of oxidative phosphorylation, making available more high energy phosphate bonds to the hexokinase reaction and to fat and protein synthetic processes. Krebs and Eggleston (9) demonstrated that insulin accelerated the rate of oxidation of various members of the Krebs cycle by minced pigeon breast muscle. Actually insulin did not increase the initial rate of oxidation, but it slowed the decline in the rate so that after three to four hours the total amount of oxidation had been doubled. Stadie (10) points out that in tissue homogenates, such as were used by Krebs and Eggleston, glucose and glycogen break down to lactic acid and the hexokinase reaction ceases to operate. Thus these actions of insulin cannot be considered secondary to any change in the hexokinase reaction, but are interpreted as primary effects upon the citric acid cycle. These findings were confirmed by Shorr and Barker (11) and by Stare and Bauman (12) for pigeon muscle but not for mammalian muscle. Insulin effects on phosphorous metabolism have been shown many times. Kaplan and Greenberg (13) have demonstrated increased P^{32} turnover and increased ATP in rats treated with insulin. Haugaard, Marsh and Stadie (14) found a net increase in the number of mols of ATP produced in rat diaphragms incubated with insulin without any corresponding increase in oxygen consumption. They interpreted this as signifying an increase in the efficiency of oxidative phosphorylation but they have not eliminated the possibility that the extra phosphate bonds were derived from the process of glycolysis which