1949

Role of hexokinase in carbohydrate metabolism

Isaac Jacob Levine
University of Nebraska Medical Center

THE ROLE OF HEXOKINASE IN CARBOHYDRATE METABOLISM

by

Isaac J. Levine

Senior Thesis
Presented to the College of Medicine
University of Nebraska
Omaha, 1949

FOREWORD: The early studies being almost without exception in German journals, and abstracts generally unavailable or inadequate, the resumé of preliminary hexokinase studies is quoted almost entirely from an excellent review of this work by Colowick and Kalckar (5)

INTRODUCTION: Revived interest in hexokinase, the enzyme first described by Meyerhof (23) in 1927, has yielded, particularly to the Cori group at St. Louis, results which promise to be of far-reaching significance in the studies of carbohydrate metabolism, most directly as they relate to the problem of diabetes mellitus in man. In addition, the epoch-making discovery by the Cori group of an enzymal-hormonal control of the hexokinase reaction opens up vast new avenues to workers in related fields. It is with these exciting prospects in mind that the present author undertakes a review and analysis of research on the hexokinase reaction and corollary studies.

The enzyme which catalyzed the phosphorylation of glucose and fructose was described in 1927 by Meyerhof(5). He found that muscle extracts which split polysaccharides and hexose phosphates to lactic acid but were unable to ferment hexoses could be enabled to do so by the addition of a protein fraction from baker's yeast.

The same protein fraction activated the aerobic oxidation of hexoses in the hemolysate from red blood cells (24). Meyerhof named the yeast protein hexokinase to indicate that it initiates the metabolism of hexoses.

The nature of the hexokinase reaction was revealed by studies of von Euler and Adler (9) and of Meyerhof (25) in 1935. Von Euler and Adler noted that the crude hexose monophosphate dehydrogenase (Zwischenferment) obtained from yeast by Warburg and Christian in 1943 (40), was able not only to oxidize hexosemonophosphate, but also unphosphorylated glucose and fructose provided that adenosine triphosphate was added to the system.

Von Euler and Adler (9) showed that the action of the adenosine triphosphate was due to an enzymatic transfer of the labile phosphate group of this nucleotide thus forming hexose monophosphate, the substance to be oxidized. They named the yeast enzyme catalyzing this transfer, heterophosphatese. Meyerhof (25) then established that his "activator" of 1927, which he named hexokinase, had the same enzymatic properties as Euler's "heterophosphatese".

This explains the action of hexokinase on the fermentation of monohexoses in muscle extracts. Since

small amounts of ATP are formed continuously in muscle as soon as the fermentation is started, the limiting factor therefore being the phosphate transferring enzyme, hexokinase (heterophosphatese), the reaction was tentatively formulated as follows:

Adenosine triphosphate + 2 Hexose → Adenylic Acid + 2 hexose monophosphate

Colowick and Kalckar (5) confirmed the earlier work but showed that actually only one half of the labile phosphate was transferred. They deduced that the reaction was more likely to be

ATP + hexose → Adenosine diphosphate + hexose monophosphate.

The reaction products were identified and their deductions confirmed.

Hexokinase has been prepared in crystalline form and its physical properties studied (3, 18). Kunitz and McDonald's method and studies are set down in the book "Crystalline Enzymes" edited by Northrop et al (27) from which the following material is taken.

Crystalline hexokinase is a protein of the albumin type. It is crystallized at 5°C in the presence of ammonium sulfate and dilute phosphate buffer pH 7.0 The crystals become relatively pure after two or three crystallizations as tested by solubility, electrophoresis,

and ultracentrifuge measurements. The hexokinase activity of the crystals is associated with the protein nature of the material.

Solutions of crystalline hexokinase in dilute buffers of pH 4.5-7.5 are stable for several days when kept at a temperature of 5°C. or lower. At higher temperatures the hexokinase activity is rapidly lost. The loss in activity is accompanied by denaturation of the protein. The point of maximum stability is around pH 5.0 which is near the iso-electric point of the material. The elementary composition of crystalline hexokinase is that of a typical protein. It contains 0.11 percent phosphorous wich would indicate a minimum molecular weight of about 30,000. Sedimentation and diffusion measurements in acetate buffer pH 5.5 at 1°C. gave a molecular weight of 96,000.

The basis of the present method of preparation is the technique of purification and crystallization of proteins by means of ammonium sulfate from concentrated protein solutions as developed by Northrop, Kunitz, and others for the isolation of crystalline enzymes. Details of preparation and other physiochemical minutia are included in the Northrop (27) book.

As stated earlier, only one half the labile

phosphate from adenosine triphosphate (ATP) was found to be transferred in the hexokinase reaction. Colowick and Kalckar at St. Louis subsequently noted that Adenosine diphosphate (ADP) can be made available for the phosphorylation of hexose if there is added to the hexokinase system an enzyme prepared from skeletal muscle (5). This enzyme has been named myokinase because skeletal muscle has been found to be by far the best source.

The ~~augmented~~ Hexokinase enzyme system has been used by these workers for the preparation of ADP from ATP. Glucose and Fructose are phosphorylated equally well. The ester formed in both cases consists of 2/3 Glucose 6 Phosphate (G6P) and 1/3 Fructose 6, Phosphate (F6P) owing to the presence of isomerase.

Myokinase (5) is a protein which possesses a high degree of stability to boiling with mixed acids as well as to precipitation with trichloroacetic acid. The enzyme is inactivated by oxidation with H_2O_2. Subsequent reduction with cysteine or glutathione restores the activity completely. Myokinase is found in large amounts in the skeletal muscles of rabbit and frog. It is present in traces in the heart and brain, but absent in liver, kidney and endocrine organs.

In 1943 Kalckar (13) noted that the addition of ADP with myokinase alone did not give rise to any

change in the amount of acid labile phosphate. Dr. M. Johnson in a personal communication to Kalckar as reported (13) suggested that myokinase might catalyze a reversible transfer of phosphate from one molecule of ADP yielding ATP and Adenylic acid (AA). Such a reaction, it is seen, would not give rise to any change in the total amount of acid labile phosphate. Through an analysis of the reaction products, such proved to be the case. The hexokinase reaction as it was now understood was as follows:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{G6P} + \text{ADP}$$

$$\text{Glucose} + \text{ADP} \xrightarrow{\text{Myokinase}} \text{G6P} + \text{AA}$$

The same applies for fructose.

Additional components of the hexokinase reaction were brought out by Price and Colowick in 1935 (28). Working with fluoride poisoned extracts of minced rat muscle they studied the above reactions. They noted that the enzymes catalyzing these reactions are stable at 25°C. in the pH range 7-8 but are rapidly inactivated at pH 6. They can be rapidly reactivated by the addidtion of small amounts of dihydrocozymase. They demonstrated this in an iodoacetate poisoned system which renders the oxidized form inactive.

In aqueous extracts prepared from acetone-dried

rat muscle, the enzymes were stable through pH 6-8 but could be inactivated at pH 6 by the addition of fresh muscle extract. Extracts of fresh acetone-dried rat muscle could be inactivated by dialyzing against saline and reactivated by the addition of Phosphate and Magnesium ions. The dihydrocozymase had no effect. When fresh muscle was inactivated at pH 6, dihydrocozymase and inorganic ortho phosphate were needed for the reaction. The same authors (29) note that the dihydrocozymase action is not related to its well known action in hydrogen transport since with a flouride, and iodo-acetate inhibited system, oxidation could not possibly occur.

Further elucidation of this problem came in 1945 when these workers (30) noted that the phosphate is necessary for the formation of Guanine from a precursor present in dialized extracts. They based their work on a theory brought forward by Kalckar (14) in 1945.

That investigator presented evidence that the purine nucleosidases hitherto thought to split nucleosides into purine and free ribose (or desoxyribose) liberated instead Ribose 1 Phosphate. Working with the reaction

Inosine (hypoxanthine riboside) phsosphate

hypoxanthine ribose 1 phosphate.

he noted that phosphate was required mole for mole

with the ribose liberated.

The dialyzing procedure mentioned earlier does not remove the dihydrocozymase but still inactivates the preparation. It can be reactivated with phosphate but there is a lag of 5-30 minutes and the reactivation is completely prevented by iodoacetate. A substrate has been obtained from the dialyzed extract which when incubated with phosphate causes reactivation of the dialyzed preparation in the absence of phosphate. This substance was found to be Guanine. The reactivation occurs without a lag and is not abolished by iodoacetate. Phosphate is necessary for, and iodoacetate prevents the appearance of the guanine. It was concluded by the authors (30) that Mg^{++} Dihydrocozymase and Guanine are necessary for the hexokinase reaction. Incidentally, a possible mechanism for the Pasteur phenomenon was here suggested. The sudden influx of oxygen might oxidize sufficient quantities of dihydrocozymase to render the hexokinase enzyme system temporarily inactive thus rendering unavailable considerable quantities of body glucose. The above material refers only to animal hexokinase. Yeast hexokinase does not require dihydrocozymase nor guanine. (30)

With the basic scheme of the hexokinase reaction firmly established, various investigators began to re-examine this particular phase of carbohydrate metabolism. A required primary step for the oxidative utilization of ingested carbohydrate is the phosphorylization of glucose (7) so that any factors or conditions which inhibit or enhance the hexokinase reaction could well be of prime importance in understanding the regulation of carbohydrate metabolism.

Pursuing their earlier work along this line, Price, Cori, and Colowick (31) announced in 1945 that they had shown anterior pituitary extract (APE) to inhibit the hexokinase reaction and insulin to release this inhibition. Further details of this discovery will be set down.

They noted that APE could be added to the preparation or fed to the rats whose muscle was to be used for the experiment with equal effectiveness. The APE produced a lag of utilitzation lasting about 15 minutes which was gradually released presumably due to an inactivation of the factor, if phosphosaline extracts of various rat tissue (muscle, liver, kidney, heart or brain) were used. ~~If purified preparations of~~ If purified preparations of muscle hexokinase are used, the inhibition is not released with time. In contrast to animal hexokinase, yeast hexokinase was not inhibited by APE.

These authors (31) showed further that rats made diabetic by injection of alloxan yield tissue extracts which show the same activity as those injected with APE. In other words, the inhibition seen in the alloxanized animals was equivalent to the inhibition seen in normal rats which had been injected with APE. Brain tissue did not show inhibition in the alloxanized or injected rats, but this tissue showed inhibition of the hexokinase reaction when APE was a added in vitro.

The conversion of glycogen to lactic acid in muscle extract, which does not involve the hexokinase reaction was not affected by APE. Oxidation of glucose was inhibited by APE while the oxidation of Fructose 6 Phosphate was not. This evidence is taken by the authers to show that the chief, if not sole point of action of the hexokinase system is in the phosphorylization of glucose and that neither the hexokinase nor the inhibitory factors (APE) have any effect beyond that point. Evidence to be cited later appears to cast considerable doubt upon the latter part of this assertion.

Further shown in this experiment, insulin releases hexokinase from APE inhibition but does not by itself enhance hexokinase activity; an important

point later to be discussed. Within a certain range, the release of inhibition is proportional to the amount of insulin added in vitro. That the in vivo action of insulin is similar is indicated by the fact that muscle extracts prepared from diabetic rats showed normal hexokinase activity after injection of insulin. When insulin was reduced by cysteine, it no longer exerted its antagonistic effect against APE.

Broh-kahn and Mirsky in 1947 (22) confirmed the hexokinase reaction as set down by the St. Louis group but their results and conclusions differed in the matter of APE and insulin effect. They noted that the inhibitory effect of APE was not constant, some preparations almost inactive. They were careful to point out, however, that their preparation of APE was different from that of the St. Louis group.

On the basis of their experiments they postulated a hexokinase inhibiting factor quite distinct from the diabetogenic factor of APE. They also raised the question of non-specificity by demonstrating inhibition of the hexokinase reaction with splenic extract. This inhibition was, however, weak and inconstant.

Inexplicably their alloxanized rats failed to show any decrease of hexokinase activity. In their experements the addition of insulin failed to increase the

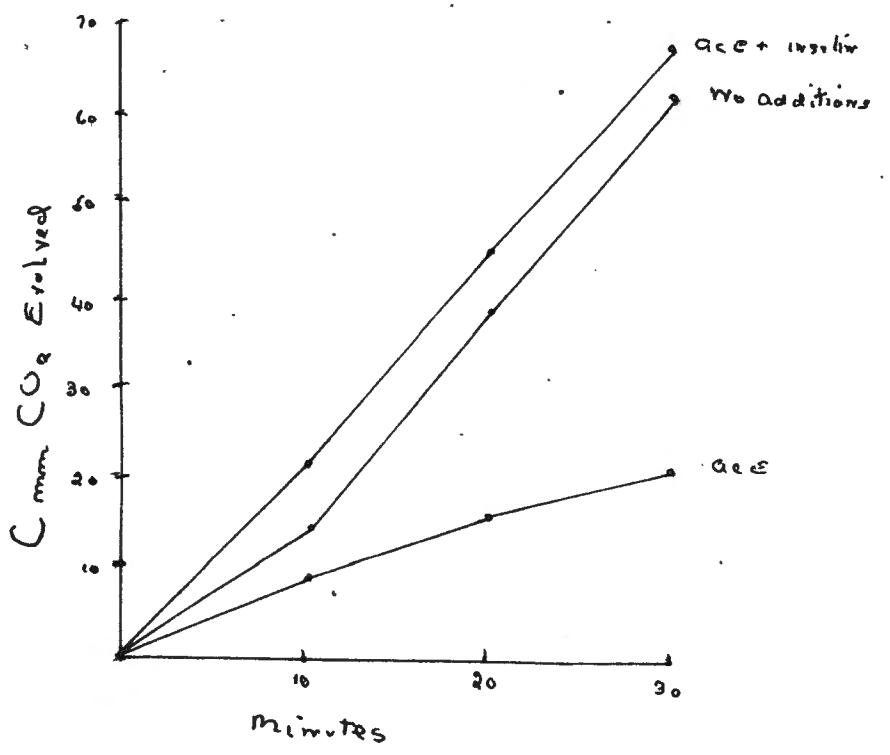
the hexokinase activity of either normal or diabetic extracts, in contrast to the previously noted results of the St. Louis group. Broh-kahn and Mirsky conclude that the disorders of glucose mechanism encountered in diabetes do not necessarily result from any inhibition of hexokinase activity. For the present, this disturbing bit of contradictory evidence must be set aside to be viewed in the light of subsequent reports from other laboratories.

In that same year (1947) Colowick, Cori, and Slein (6) published the results of experiments which apparently add yet another link to the intriguing hormonal-enzymal relationships surrounding the hexokinase reaction.

They noted that the hexokinase activity of extracts of diabetic rats can be inhibited by the addition of adrenal cortical extract (ACE). Out of 30 different rats made diabetic by injection of alloxan, 15 showed inhibition ranging from 21-76% while 15 showed inhibition of 14% or less. These figures are cited to show the considerable variability of the results. In contrast to this, the hexokinase activity of normal rat muscle or beef brain is not inhibited by ACE but can be inhibited 30-75% by the addition of 1-2 mg of a protein fraction from the Anterior Pituitary. APE inhibition is greatly enhanced by ACE in normal muscle.

About 50 gamma of insulin was required to counteract .1 cc of ACE on diabetic muscle extracts. It would appear from this experiment that the inhibitory factor of AP is mediated through ACE. Further light will be shed on this problem when the inhibitory effect of ACE is tested on the muscle extracts of alloxanized hypophysectomized rats.

Effect of Adrenal Cortical Extract (ACE) and Insulin on Hexokinase activity of Diabetic Rat Muscle. (6, 42)



- A brief resume on methods and materials may be included here. Colowick and Kalckar in 1943 (5) developed a manometric method for the determination of hexokinase activity which is based on the fact that one acid equivalent is formed for each phosphate group transferred from ATP to glucose. They also showed that enzyme activity can be followed chemically by determining the disappearance of glucose or of ATP. In most of the experiments discussed, the reaction was followed manometrically, and a terminal analysis was made on the contents of the Warburg vessels.

The animal hexokinase has so far been purified only partially (31). Hexokinase activity has been demonstrated in the Cori's laboratory in extracts of liver, kidney brain, skin, intestine, anterior pituitary, heart and skeletal muscle. In the case of muscle and other tissues the hexokinase activity was found to be of sufficient magnitude to account for the rate of metabolism of glucose observed in these tissues in the intact animal. (42).

The instability of the pituitary factor has so far prevented progress in its purification (31, 42). Cori (42) reports that at the present stage of purification it did not seem profitable to try to identify the pituitary inhibitor of hexokinase with other

factors known to be present in anterior pituitary extracts. Highly purified preparations of adrenotrophic, lactogenic, and growth hormones, however, were tested for inhibitory effect with negative results (31).

The inhibitory effedt of Upjohn cortical extract could be reproduced with an amorphous fraction of the adrenal cortex, but now with crystalline compounds A, B, and E of Kendall.

The chief point of contention faced by those who would postulate the hexokinase reaction to be the sole point of insulin action is the remarkable sensitivity of the hypophysectomized animal to insulin (2, 10, 11, 32). If the release from APE inhibition were the only function of insulin then no effect should be expected in the absence of that gland. Soskin (39) refuses even to consider the Cori theory until this point is solved since he believes that it negates all that has gone before. Similar ideas in the minds of other investigators have cast this problem to the fore as one of the most pressing and crucial problems of endocrine regulation of carbohydrate metabolism.

The hypersensitivity of the hypophysectomized animal to insulin was first pointed out by Houssay (10). It has recently been re-investigated by Ben-

nett and Roberts in 1946 (2). Working with eviscerated rats they found that by the criteria of 1) Actual fall in blood glucose level, 2) degree of decrease in blood sugar level, and 3) the final blood glucose level expressed as a percentage of the original level there was no significant effect in the normal rats when amounts of insulin less that .04 Units/Kg were used. But by all three criteria they found a highly significant effect in the hypophysictomized animals with insulin dosages of .005 Units/kg or .02 Units/kg. The hypersensitivity of the hypophysectomized, eviscerated preparations was on the horder of four-fold. They concluded that the increased sensitivity to insulin in this case is due to an increased glycogen uptake by the non-hepatic tissues.

They also demonstrated that the administration of APE 1½ hours prior to evisceration protected partly the effect of insulin administration.

Russell in 1944 (32) showed that the glucose requirement for the mainenance of physiological blood sugar levels in normal eviscerated rats is reduced about 40% by previous treatment with APE. In eviscerated hypophysectomized rats, the glucose requirement found to be greater than normal was reduced by anterior pituitary and adrenal cortical hormones.

That the insulin sensitivity is not based primar-

ily upon the lack of epinephrine response in hypophysectomized animals was indicated by Safford, Wells, and Gellhorn in 1946 (33). They demonstrated that the hyperglycemic response to conditions of anoxia or adrenalin administration persists for several days after hypophysectomy. The insulin sensitivity is present almost immediately (32).

Together, these data suggest that there is a greater glucose requirement in the hypophysectomized animal rendering it acutely sensitive to any change in the blood sugar level influenced by insulin through some mechanism other than the hexokinase reaction.

It may be, in the opinion of the present writer, that in the absence of APE a secondary inhibiting mechanism at the tissue or cellular level is called into play <u>in vivo</u> which though strong enough to maintain a semblance of homeostasis, is grossly more sensitive to insulin inhibition. In view of the multitudinous other compensatory adjustments of which the body is capable this seems reasonable to assume.

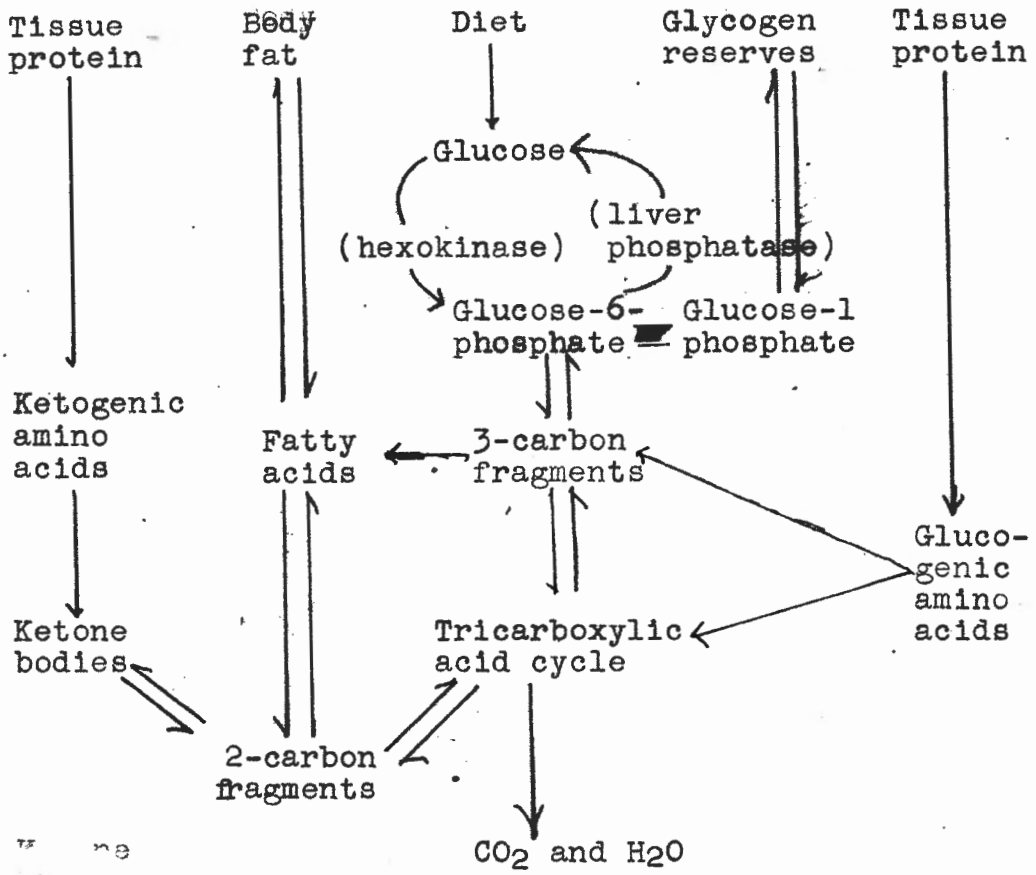
Another possibility is that a secondary insulin action, masked in the presence of APE comes to the fore in its absence due to an prolongation and intensification of its effect consequent to a drop in liver reduced glutathione (GSH). It has been shown (8)

t' ~+

that this occurs in rats following hypophysectomy. It seems likely that inactivation of insulin in the body is accomplished by sulfhydryl compounds (1) and the inactivation would be enhanced by the fall of GSH (1, 19).

As will be noted, the amelioration of diabetes mellitus in the adrenalectomized animal (21) fits into the Cori pattern with much great facility. If the hexokinase inhibition were mediated through ACE, its lack would greatly decrease the inhibition so that the metabolic unbalance would be less severe.

The place of the hexokinase reaction within the general scheme of carbohydrate metabolism will now be considered. It is not within the scope of the present paper to enlist separately the vast number of studies, which went into the making of this scheme. Rather, the reader is referred to three excellent reviews (7, 17, 26,) the total sum of which encompasses most of the current concepts. Stetten (39) has embodied grossly these concepts in a brilliantly conveived diagram upon which the following discussion is based.



--Fate of Glucose in the body--

It will be seen from the diagram that there are three chief sources of body glucose. The first and major of these is the intestinal absorbtion of the p products of carbohydrate digestion. The second, a process called glycogenolysis has been shown to proceed from glycogen by an initial phosphorolysis which yields glucose 1 phosphate, and this product, in the body rapidly comes into equilibrium with its isomer, glucose 6 phosphate. The next step, the hydrolysis of glucose 6 phosphate to give glucose, is governed by a specific phosphatase which is abundantly present in

the liver but appears to be lacking in muscle. Because of this, liver glycogen is not a source of body glucose. The third source of glucose is termed gluconeogenesis and under this heading is included glucose production from any non-carbohydrate sources. Among these are the glucogenic amino acids, glycerol, possibly fatty acids which have arisen from fats, and also products which have themselves arisen in the course of carbohydrate catabolism. Essentially, each step in the degradation of glucose 6 phosphate has been shown to be reversible hence any of these degradation products or any material which m y give rise to them is essentially gluconeogenic. As in the process of glycogenolysis, the liver has the enzyme to effect the last step in gluconeogenesis, namely the hydrolysis of glucose 6 phosphate to yield glucose.

The magnitudes of each of these various contributions to the body glucose is unknown in man, but Stetten has shown (35) that in rats 2/3 was of dietary origin, about 1/3 from gluconeogenesis and only about 3% from glycogenolysis.

In considering the fate of glucose it must first be pointed out that glucose must undergo phosphorylation through the hexokinase reaction before it can enter into the dynamic mechanism of glucose disposal.

This reaction must be clearly separated from the previously mentioned hydrolysis of glucose 6 phosphate. Both reactions as indicated in the diagram are energy yielding and therefore irreversible.

Once glucose has been phosphorylated, many pathways are open to it (See diagram). In the liver, glucose 6 phosphate may be split to regenerate glucose. In addition, glucose 6 phosphate may be converted into glycogen by a reversal of the same process by which glycogen is broken down. Glucose 6 phosphate may enter the so-called Embden-Meyerhof cycle to be broken down to pyruvate, lactate and other three-carbon fragments. A portion of these is utilized each day in the synthesis of fatty acids (lipogenesis), while another portion is burned over the Krebs tricarboxylic acid cycle to give carbon dioxide and water. Once again it is pointed out that this is only the general scheme.

Again, the magnitudes of these several fates of glucose are unknown in man. Stetten and Böxen have shown (35) that while only 3% of glucose ingested by well nourished rats si converted to glycogen, 30% is consumed in the manufacture of fatty acids. In the rat at least, much more glucose is used each day to replenish fat depots than in the formation of glycogen.

The relative proportions of ingested glucose employed in these various pathways have been shown to be influenced by the insulin level in the animal. Thus in the diabetic animal, it has been shown that the conversion of glucose to pyruvate is impaired.(16). Although the diabetic animal continues to make glycogen, it makes it from small fragments rather than from glucose (36). In addition it has been found that the rate of lipogenesis drops appreciably in the absence of a normal supply of insulin (36, 38) In alloxan diabetes in rats, 3/4 of the urinary glucose was found to have originated in ingested carbohydrate. (36).

Conversely, if insulin is injected into a normal animal, both glycogen and fatty acids are synthesized from glucose at a much higher rate (38)

These and other findings which have as a common step, the hexokinase reaction, tie in closely with the experimental observations on this reaction. For with an inhibition of the hexokinase reaction due either to an excess of APE or a dearth of insulin the major source of body glucose (intestinal absorption) would be chiefly unavailable. Glucose would continue to arise from this and other normal sources so that the blood sugar would rise and the renal threshold be exceeded. For the continuance of life, however, the main sequence of energy-

yielding reactions must be kept in operation. In the functional absence of ingested glucose the body must draw on other stores; glycogen, proteins, and fats, to contribute to this sequence. As a result there would be a fall in liver glycogen, a tendency toward ketosis and a negative nitrogen balance. All these sequelae which would be expected from the action of insulin as described by Cori are in agreement with the facts observed.

Conversely an excess of insulin would overcome the physiological inhibition of the hexokinase reaction by APE and the phosphorylation of glucose would proceed with undue rapidity. The blood glucose would fall and all the reactions of glucose 6 phosphate would be enhanced; that is, glycogenesis, lipogenesis and combustion would all be enhanced. This too tallies with the observed facts.

How the work of the St. Louis group will affect ideas concerning the conflicting theories of diabetes mellitus, it is impossible to tell at the present time. Actually, both the proponents of the Non-Utilization theory and the Overproduction theory (34) may find evidences in the work suited to the respective theories. Soskin for example is gratified to note that by this evidence insulin takes no actual part in oxidation but

is concerned only with pre-oxidative mechanisms.

From the equation

$$\text{Glucose} + \text{ATP} \longrightarrow \text{G6P} + \text{ADP}$$

it can be seen that an increased levelof blood sugar would tend to drive the reaction to the right regardless of the enzymal control, thus reasserting the importance of the blood sugar level itself upon body physiology.

On the other hand, the proponents of the non-Utilization theory may point out that in the diabetic state the dietary glucose is not properly phosphorylated, and since it is generally agreed that dietary glucose is to all intents and purposes intert until phosphorylation, this is tantamount to non-utilization. They would have to yield, however, concerning the mechanism of ketosis in hypoinsulinism.

In general, it may be said that there are yet many points concerning insulin and diabetes which are not yet understood and which do not yield to the pattern offered by the St. Louis group. The massive dosages of insulin required in some cases of ketosis; the mechanism of insulin resistance in toxemic states (39) the apparent lack of significant pancreatic pathology in many cases of diabetes; the hormonal relationship between diabetes and obesity; and finally, the cause of

insulin sensitivity in the hypophysectomized animal--these and many other questions must await further expe imentation.

But despite these drawbacks, the Cori's have presented so provocative and far-reaching a piece of evidence that few workers in carbohydrate metabolism may safely ignore it. In fact a goodly percentage of the work on insulin and carbohydrate metabolism of the past 25 years is subject to re-appraisal in the light of it.

It may yet be, that when the full mechanism of the hexoki ase reaction is understood the final solution of the problem of diabetes mellitus will be at hand, including, it is hoped, its cure.

BIBLIOGRAPHY

1. Barron, E.S.G., and Singer, T.
Enzyme systems containing active sulfhydryl groups. The role of glutathione.
Science, 97: 356-8, 1943

2. Bennett, L., and Roberts, L.
Hypersensitivity to insulin in eviscerated hypophysectomized rats.
Am. J. Physiol., 146: 502-6, 1946

3. Berger, L., Slein, M., Colowick, S., and Cori, C.
Isolation of pure hexokinase from yeast.
J. Gen.Physiol., 29: 143, 1946

4. Colowick, S., and Kalckar, H.
An activator of the hexokinase system.
J. Biol. Chem., 137: 789-90, 1941

5. Colowick, S., and Kalckar, H.
The role of myokinase in transphosphorylations. I.The enzymatic phosphorylation of hexoses by adenyl pyrophosphate.
J. Biol. Chem., 148: 117-26, 1943

6.- Colowick, S., Cori, G.T., and Slein, M.
Effect of anterior pituitary and adrenal cortical extract and insulin on the hexokinase reaction.
J. Biol. Chem., 168: 583-96, 1947

7. Cori, C.F., Cori, G.T., and Luck, M.
Carbohydrate Metabolism.
Annual Rev. of Biochem., 10: 151-75, 1941

8. Ennor, A.
Influence of anterior pituitary extract on the content and rate of oxidation of glutathione in tissues.
Austral. J. of Exp. Biol. and Med. Sci., 17: 173-80, 1939

9. Euler, H. von, and Adler, E.
Uber die Komponenten der Dehydrasesysteme. VI. Dehydrierung von Hexosen unter Mitwirkung von Adenosintriphosphorsaure.
Z. Physiol. Chem., 235: 122-53, 1935 Cited by (5) and (19)

10. Houssay, B.
The hypophysis and carbohydrate metabolism.
New Eng. J. Med., 214: 971 , 1936

11. Houssay, B., and Biasotti, A.
Hypophysis, carbohydrate metabolism and diabetes.
Endocrinology, 15: 511, 1931

12. Jacobs, H.R.
Effect of cysteine on the action of insulin.
Proc. Soc. Exp. Biol. and Med., 38: 305-8, 1938

13. Kalckar, H.
The role of myokinase in transphosphorylations. II. The enzymatic action of myokinase on adenine nucleotides.
J. Biol. Chem., 148: 127-37, 1943

14. Kalckar, H.
Enzymatic synthesis of a nucleotide.
J. Biol. Chem., 158: 723-4, 1945

15. Kaplan, N., and Greenberg, D.
Radioactive phosphate as an indicator of the relationship between the phosphate changes of blood muscle and liver following the administration of insulin.
Am. J. Physiol., 140: 598-602, 1944

16. Klein, D.
Effects of administration of glucose and insulin on pyruvate and lactate in diabetes mellitus.
J. Biol. Chem., 145: 35-43, 1942

Krebs, H.A.
The intermediary stages in the biological oxidation of carbohydrate.
Adv. in Enzymol., 3: 191-252, 1943

18. Kunitz, M., and McDonald, M.
Isolation of crystalline hexokinase and other proteins from yeast.
J. Gen. Physiol., 29: 143-44, 1946

19. Levine, R., Hechter, O., Grossman, A., and Soskin, S.
Reduced glutathione of tissue and increased insulin sensitivity.
Proc. Soc. Exp. Biol. and Med., 40: 525-8, 1939

20. Lohman, K.
Konstitution der Adenylpyrophosphorsaure und adenosinediphosphorsaure.
Biochem. Z., 282: 120-3, 1935 Cited by (5)

21. Long, C., Katzin, B., and Fry, E.
Adrenal cortex and carbohydrate metabolism.
Endocrinology, 26: 309, 1940

22. Mirsky, I., and Broh-kahn, R.
Hexokinase activity and diabetes mellitus.
Science, 106: 148-9, 1947

23. Meyerhof, O.
Die Mischsaurebildung aus den garfahrigen Hexosen.
Biochem. Z., 183: 176, 1927 Cited by (5)

24. Meyerhof, O.
Uber die Abtrennung des Milchsaurebildenden ferments aus Erythrocyten.
Biochem. Z., 246: 249-84, 1932 Cited by (5)

25. Meyerhof, O.
The Action of Hexokinase.
Naturwisse., 23: 850, 1935 Cited by (5)

26. Meyerhof, O.
The chemistry of anaerobic recovery of muscle.
New Eng. J. of Med., 220: 49-56, 1939

27. Northrop, J., Kunitz, M., and Herriot, R.
Crystalline Enzymes. Columbia University Press 2nd Ed. revised 1948
Chapter IX and appendix p.282

28. Price, W., and Colowick, S.
The role of dihydrocozymase and inorganic orthophosphate in enzymatic transphosphorylation.
Federation Proc., 4: 100, 1945

29. Price, W., and Colowick, S.
The role of dihydrocozymase in enzymatic transphosphorylation.
J. Biol. Chem., 157; 417-8, 1945

30. Price, W., and Colowick, S.
Guanine as a coenzyme in enzymatic transphosphorylations.
J. Biol. Chem., 159: 563-4, 1945

31. Price, W, Cori, C., Colowick, S.,
Effect of anterior pituitary extract and insulin on the hexokinase reaction.
J. Biol. Chem., 160: 633-4, 1945

32. Russell, J.
The adrenals and the hypophysis in the carbohydrate metabolism of the eviscerated rat.
Am. J. Physiol., 140: 98-105, 1944

33. Safford, H., Wells, L., and Gellhorn, E.
Sympathetico-adrenal discharges in hypophysectomized rats.
Am. J. Physiol., 146: 386-8, 1946

34. Soskin, S., and Levine, R.
Carbohydrate Metabolism. University of Chicago press. 1946

35. Stetten, D.,Jr., and Boxer, G.
Studies in carbohydrate metabolism. I. The rate of turnover of liver and carcass glycogen studied with the aid of deuterium.
J. Biol. Chem., 155: 231-4, 1944

36. Stetten, D.,Jr., and Boxer, G.
Studies in carbohydrate metabolism. III. Metabolic defects in alloxan diabetes.
J. Biol Chem., 156: 271-7, 1944

37. Stetten, D.,Jr., and Klein, B.
Studies in carbohydrate metabolism. V. Effects of adrenalin and insulin upon glycogenesis in rats.
J. Biol Chem., 159: 593-601, 1945

38. Stetten, D.,Jr., and Klein, B.
Studies in carbohydrate metabolism. VI. The effect of hypo and phyperinsulinism in rabbits.
J. Biol. Chem., 162: 377-382, 1946

39. Stetten, D., Jr.,
The endocrine regulation of carbohydrate. metabolism.
J.A.M.A., 132:373-5, 1946

40. Taubenhaus, M., and Soskin, S.
Mechanism of insulin resistance in toxemic states.
J. Clin. Endocr., 2: 171-5, 1942.

41. Warburg, O., and Christian, W.
Uber das gelbe Oxydationsferment.
Biochem. Z., 263: 228-9, 1935 Cited by (5)

42. Cori, Carl F.
Enzymatic reactions in carbohydrate metabolism.
The Harvey Lectures, 1945-6, Lancaster, Pa.,
Science Press, 1946 pp253-272