for tissue respiration. Since this correction (*7*) would have tended to reduce errors attributable to low leaf permeability, it was considered worthwhile to make corrected measurements. Comparison with similar measurements for the Spanner psychrometer would indicate the residual error attributable to low leaf permeability. Tests were carried out with paired samples from opposite halves of the same leaf, the samples being placed in separate chambers with one of the two types of psychrometer. The same four species were tested at approximately the same water potentials (Table 2). Each correction for respiration was made by combining the output of the dry psychrometer (with no water on the silver ring) with the normal output (with water on the silver ring), the required temperature difference of chamber dry-bulb minus chamber wet-bulb being given. The dry-bulb reading was obtained by removing the psychrometer from the chamber after a constant wet-bulb reading had been obtained, drying the silver ring, and replacing the dry psychrometer in the chamber. The pairs of corrected water potentials agree well, although not as closely as the values shown in Table 1. This is probably largely because observations were made on pairs of samples with different psychrometers, rather than on one sample with the same psychrometer. However, differences are similar in size to the experimental errors found by Waister (*9*) for the Spanner psychrometer (standard error for mean of duplicate samples less than $\pm$ 0.25 bars) and by Ehlig (*2*) for the Richards and Ogata psychrometer (less than $\pm$ 0.3 bars), and are randomly distributed. The results offer no evidence that the Richards and Ogata psychrometer underestimates water potential, provided a correction is applied for respiration, and Rawlins's conclusion that this instrument can be grossly affected by leaf permeability is not substantiated for the four species investigated.

Rawlins reported an error of at least 60 percent in the determination of the water potential of pepper leaves with the Richards and Ogata psychrometer, which he attributed to low leaf permeability. The results reported here do not support this explanation, and suggest an error of only 10 to 20 percent, which has been attributed to respiration effects. Since this leaves a possible further error of 40 percent unaccounted for, and our conclusions have been reached as a result of different types of experiment, it seemed worthwhile to try to establish whether the differing approaches adopted were responsible. Accordingly, Rawlins's pepper-plant experiment (*3*) was repeated. A pepper plant was grown for 3 months in nutrient solution, transferred to nutrient solution containing carbowax 4000 late one afternoon, and kept in the dark overnight prior to sampling. The low transpiration conditions would be expected to bring about approximate equality between water potentials in the leaves and in the culture solution. With the Spanner psychrometer, and with corrections being made for respiration to the leaf water potentials, values found were 10.2 bars for the nutrient solution and 9.6, 10.2, and 10.9 bars (average 10.2 bars) for three leaves. There was reasonable agreement between the two sets of data and no evidence of any large error in leaf water-potential measurement in addition to that of the respiration effect. Although there is no obvious explanation for the agreement reported here between leaf and solution water potentials, in contrast to Rawlins's result, it should perhaps be pointed out that the correction for leaf respiration can be much higher than the 10 to 20 percent (Table 2) when more than one layer of leaf tissue lines the equilibration chamber (*6*).

Thus for the four species examined, leaf permeability is not sufficiently low to affect determinations of leaf water potential when either the Spanner or the Richards and Ogata psychrometer is used. Good agreement exists between the two instruments provided corrections for respiration effects are applied. The results, from the culture-solution experiment, were not significantly affected by any additional residual errors, such as extraneous moisture sinks (*3*).

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Insulin: Inducer of Pyruvate Kinase

Abstract. *The hepatic pyruvate kinase activity markedly decreased when rats were made diabetic by alloxan injection. Insulin treatment induced new synthesis of pyruvate kinase which was prevented by injection of ethionine and actinomycin D. The evidence indicated that the increased pyruvate kinase activity induced by insulin entails at a certain stage a stimulation of the synthesis of certain RNA species relevant to the production of this enzyme.*

From the point of view of their strategic metabolic role and similar responsiveness to hormonal regulatory action, the four key hepatic gluconeogenic enzymes appear to be produced on the same functional genome unit (*1*). The evidence for their strategic role was presented by Krebs (*2*) and the regulatory responsiveness in terms of the action of adrenocortical hormone as inducer and insulin as suppressor of the biosynthesis of the key gluconeogenic enzymes was summarized (*1, 3*).

Another group of key enzymes, glucokinase, phosphofructokinase and pyruvate kinase, is thought to be rate-limiting for glycolysis (*1, 3*). These enzymes have low activities, govern one-way reactions and operate in the opposite direction to the function of the key gluconeogenic enzymes. The work of Weinhouse (*4*) and, subsequently, other investigators (*5*) indicated that liver glucokinase decreased in diabetic rats and was returned to normal by insulin injections. Since the rise in glucokinase activity was blocked by inhibitors of protein synthesis, it was concluded that the insulin-induced elevation of glucokinase activity was *de novo* enzyme biosynthesis (that is, synthesis of new enzyme) (*6*). Glucokinase was not affected by adrenocortical hormone treatment (*4, 6*). We suggested that the key glycolytic enzymes—glucokinase, phosphofructokinase, and pyruvate kinase—might occupy the same functional genome unit (*1, 3*), and in consequence a similar hormonal response was expected from all three enzymes. In order to subject this concept to experimental studies the behavior of pyruvate kinase was examined first under conditions similar to that reported for glucokinase. Our results show that hepatic pyruvate kinase

activity was markedly decreased in alloxan diabetes, and insulin administration returned the enzyme to normal range. The insulin-induced rise in pyruvate kinase activity was blocked by actinomycin; therefore, the pyruvate kinase induction by insulin may represent hormone-induced biosynthesis dependent on new RNA synthesis.

Male Wistar rats (90 to 110 g) were kept in separate cages, with Purina laboratory chow and water available as desired unless otherwise specified. The techniques for preparation of tissue homogenate and supernatant fluid, and counting of cell nuclei have been described (*7*). In animals starved for 30 hours, diabetes was induced by intraperitoneal injection of 12 mg or intravenous injection of 4 mg of Alloxan monohydrate (Eastman) per 100 grams of body weight. Blood sugar was determined according to Nelson's adaptation of the Somogyi method (*8*). Pyruvate kinase was assayed in the supernatant fluid on the Cary 11 recording spectrophotometer which was thermostated to permit the assay to run at 37°C. Protamine zinc insulin (Lilly) was used in treatment of diabetic rats.

Since administration of cortisone did not influence glucokinase activity in short-term experiments, the action of this hormone on pyruvate kinase was explored, and a similar lack of effect was expected. In experiments to test this, the more potent steroid, triamcinolone, was administered in three daily doses of 1 mg per 100 g of body weight. On the 4th day the pyruvate kinase activity in liver (μmole of substrate metabolized per cell per hour) was assayed. Under these conditions, steroid administration failed to have any action on hepatic pyruvate kinase activity. Thus, pyruvate kinase, as glucokinase, was not affected by short-term steroid treatment.

Next we examined the action of diabetes and insulin on pyruvate kinase (Fig. 1). Alloxan diabetes in this series was caused by intraperitoneal injection of alloxan, and the diabetic rats had blood sugars of 500 mg per 100 ml of blood or higher. In diabetes the enzyme activity of the average liver cell decreased to 60 percent at 24 hours, to 52 percent at 72 hours, and to 30 to 40 percent at 96 to 144 hours after alloxan injection. Thus, when most of the insulin disappeared in the alloxan-treated rat, pyruvate kinase activity decreased and remained at a low level. To investigate the action of insulin, hormone injections were given to groups of diabetic rats starting 24 hours after alloxan administration. As a result of insulin treatment pyruvate kinase activity returned to normal range and remained there during the experimental period of 144 hours. Similar relations exist when the data are presented on the basis of body weight.

In another experimental series alloxan diabetes was permitted to progress for 96 hours. Then the diabetic rats were divided into five groups: (i) untreated diabetics, (ii) insulin-treated diabetics, (iii) diabetics injected with triamcinolone and insulin, (iv) diabetics injected with actinomycin and insulin, and (v) diabetics injected with ethionine and insulin (Fig. 2). In diabetic animals pyruvate kinase decreased over 96 hours to approximately 30 percent of the values normal for fed rats, and it remained on this level for the duration of the experiment. Insulin treatment returned the enzyme activity to normal range in 24 hours. Triamcinolone did not influence the action of insulin. However, in animals treated with actinomycin or ethionine before insulin injections were given, enzyme activity remained in diabetic range. Thus, insulin is required for maintaining normal pyruvate kinase activity in rat liver. When most of the insulin was lost as a result of alloxan injection, pyruvate kinase was markedly decreased, and insulin was able to restore enzyme activity to normal. Since ethionine, a blocker of

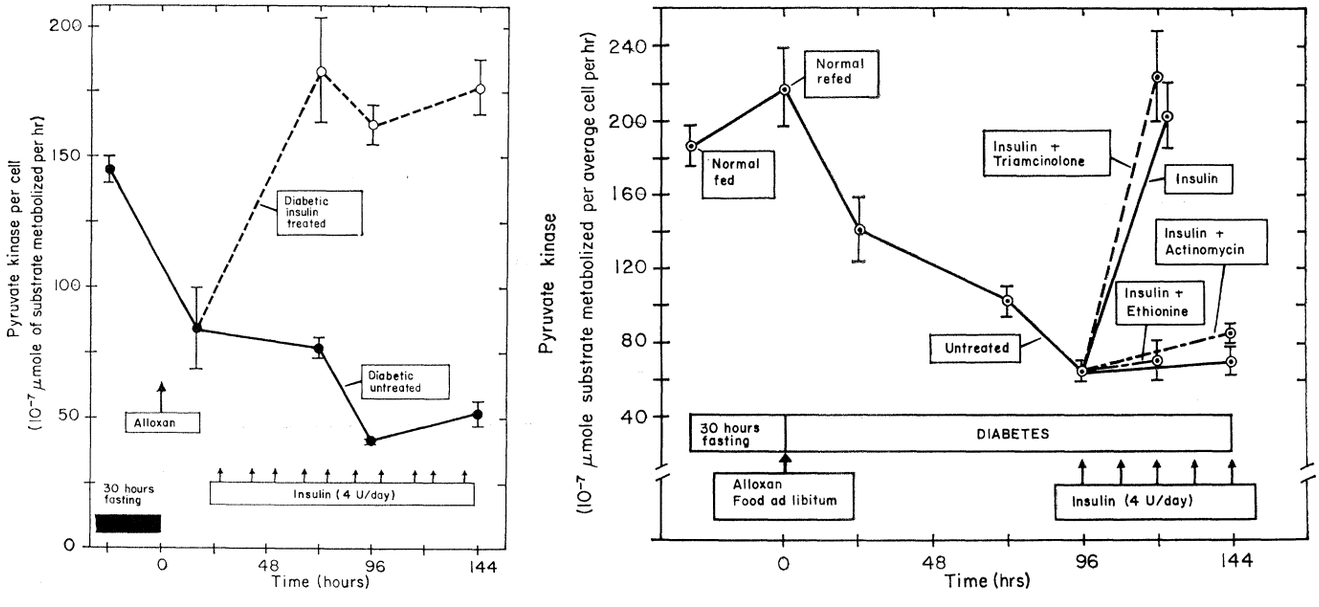

Fig. 1 (left). Action of diabetes and insulin on hepatic pyruvate kinase activity. The means (closed and open circles) and standard errors (vertical bars) represent a group of four or more rats. The reaction mixture contained in a final volume of 3 ml the following components in the order of addition: tris buffer *p*H 7.4, 125 μmole; $MgSO_4$, 125 μmole; KCl, 300 μmole; phosphoenolpyruvate, 100 μmole; ADP, 4 μmole; DPNH, 1.3 μmole; lactic dehydrogenase, 0.1 mg protein; supernatant fluid of rat liver, 0.4 mg tissue equivalent; 37°C. Fig. 2 (right). Actinomycin and ethionine inhibition of insulin-induced biosynthesis of hepatic pyruvate kinase. Results were calculated as enzyme activity per cell at 37°C. Means (circles) and standard errors (vertical bars) represent a group of four or more rats. Protamine zinc insulin (4 units per 100 g of body weight) was injected subcutaneously in two divided doses per day. Triamcinolone (5 mg per 100 g of body weight) was injected intraperitoneally in the morning, one-half hour before treatment with insulin. Actinomycin (Merck, 4 μg per 100 g of body weight) or ethionine (50 mg per 100 g of body weight) was injected intraperitoneally in two divided doses, one-half hour before insulin was administered.

protein synthesis, and actinomycin, an inhibitor of RNA synthesis (*9*), blocked the insulin-induced increase in hepatic pyruvate kinase activity, it appears that the insulin-induced rise in this enzyme activity is due to synthesis of new enzyme which is dependent on new RNA production.

As a consequence of the foregoing action of insulin on hepatic pyruvate kinase, one would expect a decrease of this enzyme in starvation (when insulin secretion is decreased or absent), and a return of enzyme activity to normal upon refeeding, especially with a high carbohydrate diet (which results in insulin release). Thus, the reported decrease of liver pyruvate kinase in starved rats and the return to normal upon refeeding high carbohydrate diet (*10*) are in accord with our experimental data and may be interpreted on the action of insulin as inducer of hepatic pyruvate kinase activity.

An economy of insulin action is apparent from our data and from other developments in the elucidation of insulin function. Insulin exerts its action in facilitating the entrance of glucose from the blood stream into the peripheral cells, by a process not involving enzyme synthesis (*11*). In contrast, other functions of insulin involve an action on biosynthetic response of functionally related enzymes. Insulin acts as a suppressor (*1*) of the four key gluconeogenic enzymes, glucose 6-phosphatase, fructose 1,6-diphosphatase, phosphoenolpyruvate carboxykinase (*12*) and pyruvate carboxylase (*13*). In contrast, insulin acts as an inducer to glycogen synthetase (*14*). Furthermore, it induces glucokinase (*4–6*) and pyruvate kinase, two of the enzymes studied out of the three strategic glycolytic enzymes. Insulin also induces the key enzymes in fatty acid synthesis (*11*). Thus, insulin acts as a hormone in coordinating metabolic reactions, functioning in lowering the blood sugar, and increasing metabolic utilization and storage. These considerations further emphasize the economy of hormone action in that insulin attacks receptor sites at the source of enzyme production and thus turns on or off the biosynthetic action of whole genetic units governing the production of key rate-limiting enzymes.

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Active Uptake of Sodium by Softshell Turtles (Trionyx spinifer)

Abstract. *Aquatic softshell turtles* (Trionyx spinifer spinifer) *show a net active uptake of sodium from solutions of this cation as dilute as 5 micromoles per liter. This probably occurs in the pharynx, a site of aquatic respiration. Inhibition of active sodium transport by low temperatures causes an extreme lowering of the sodium concentration in the plasma. Other fresh-water turtles may utilize this mechanism of ionic regulation. The ultrastructure of the pharyngeal villi is similar to that of frog skin and toad bladder.*

Aquatic softshell turtles have the ability to procure oxygen from the water, primarily through the pharynx and cartilaginous plastron (*1*). Water is brought into contact with highly vascularized pharyngeal villi by rhythmic movements of the hyoid apparatus. Although fresh-water reptiles are generally considered to be effectively impermeable to water and salts, the contact of water with respiratory surfaces may create special problems of electrolyte balance. In an initial effort to determine the extent of these problems, sodium balance in the softshell turtle (*Trionyx spinifer spinifer*) was analyzed.

If softshell turtles are to maintain sodium balance, losses of sodium into the environment will have to be made up in the food or by active uptake. To test for active uptake of sodium animals were placed in 2 liters of distilled water in covered aquariums. The concentration of sodium in the medium was measured by flame photometry in order to determine the net sodium influx or efflux. An initial net loss of sodium into the medium was followed by a net uptake until an equilibrium between net sodium influx and efflux occurred (Fig. 1). Initial net sodium loss per gram of body weight for eight turtles was inversely related to body weight. This might be expected since the efflux relative to weight from a large turtle would be less than from a small turtle. Sodium equilibrium was achieved in two turtles in water containing a 5 μM concentration of sodium. The results for one turtle are shown in Fig. 1. The external concentration was increased by adding NaCl at points *A*, *B*, and *C*. After each addition the turtle quickly reduced the concentration to the previous equilibrium value. Net chloride flux was less than net sodium flux (Fig. 1). The failure of chloride to follow sodium particle for particle indicated that other anions, possibly bicarbonate, were participating in the maintenance of the electrical equilibrium. Net potassium efflux was roughly linear for 692 hours, and was not affected by sodium and chloride fluxes.

When ouabain (Sigma) (10 μmole/lit.) was added to the medium (at *D* in Fig. 1), no change occurred in net sodium and chloride flux for 48 hours. For the next 52 hours, until death, net sodium and chloride efflux increased at about the same rate. Net potassium efflux was greatly increased immediately after the addition of ouabain (Fig. 1). A lower concentration (1 μmole/lit.) of ouabain did not significantly affect net sodium, chloride, or potassium fluxes of another turtle. The effect of the 10 μM concentration of ouabain on net sodium flux suggests either that sodium uptake involves active transport, or that renal sodium reabsorption is inhibited.

Net rates of sodium influx were calculated from the rates of sodium uptake following the addition of NaCl