carbon with upwelling water into these lakes. The activity of the upwelling water alone is probably about 20 percent below the recent activity (*3*). The activity actually found is about 8 percent below normal; it is not possible to apply a correction for fractionation, for there is probably also no equilibrium for the C^{13} content of the lakes and the atmosphere.

The difference in the C^{14} concentration of the atmosphere and the ocean is of considerable interest. The present measurements may add some information to the data discussed previously in the literature (*5*). The new data cannot be fitted into the picture, however, before an international C^{14} standard is available. Such a standard will permit the expression of all activities relative to the same standard.

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References and Notes

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11 February 1958

Some Kinetic Parameters of Liver Glucose-6-Phosphatase in Normal and Diabetic Rats

Several laboratories have recently reported an increase in the activity of liver glucose-6-phosphatase in the diabetic animal (*1–4*) and in animals subjected to increased levels of adrenal cortical hormones (*2, 3, 5*). Insulin appeared to reverse the effect in the diabetic animal within 12 to 48 hours (*1, 2*). Ashmore *et al.* (*1*) further observed that mixtures of normal and diabetic rat liver homogenates possessed additive activities. This fact suggested that the difference was not due to the presence of an inhibitor in normal liver or an activator in diabetic liver, although the possibility of limited amounts of a tightly bound activator or inhibitor is not entirely eliminated. We have undertaken an investigation of the comparative properties of normal and diabetic rat liver glucose-6-phosphatase with the view that the results might bear on the problem of the biochemical mechanism whereby the hormonal effects are mediated (*6*).

In general, there appear to be two underlying enzymic mechanisms which could account for the elevated glucose-6-phosphatase activity in the diabetic animal. Either more of the normal enzyme may be present, or the amount of enzyme present may be unchanged but its properties altered in the direction of an increase in catalytic potency (or a combination of both). The latter implies some structural change in the enzyme which would presumably be manifested in one or more measurable properties. In this connection, the very obvious possibility of an increase in the rate constant for the decomposition of the enzyme-substrate complex into products (k_3) must await extensive purification of the enzyme before it can be tested. However, several kinetic parameters, such as the Michaelis constant (K_s), the equilibrium constant for inhibitor binding (K_i), and the enthalpy of activation of the reaction (ΔH^*) can be measured even in a crude system (bearing in mind the possibility that other substances present in the enzyme preparation might affect the values of these parameters). In all of the experiments reported here, conditions were employed in which it had previously been established that the amount of product liberated was proportional to time, the velocity was proportional to enzyme concentration, and the stoichiometry was that expected.

In Fig. 1 are presented the entire series of determinations of apparent Michaelis constants (K_s) and maximum velocities at infinite substrate concentration (V_m) of the liver glucose-6-phosphatase of normal rats of the Carworth strain and their diabetic litter mates (*7*). Several groups of animals, and both males and females, are included in the series. The diabetic animals had been injected subcutaneously with 20 mg of alloxan monohydrate per 100 g at least 3 weeks previous to sacrifice; they had blood sugar concentrations of at least 400 mg/100 ml. From Fig. 1 it can be seen that the average K_s of the diabetic animals was almost twice as great as that of the normal animals, and that there was very little overlap in the values. In addition, a marked linear correlation between the K_s and V_m values for individual animals is apparent ($r = 0.87$). An alteration in k_3 produces a relationship of this kind (*8*).

The ΔH^* values of the reactions catalyzed by the normal and diabetic enzymes over the range 25° to 40°C were 12,480 cal and 16,700 cal, respectively, at a substrate concentration of $0.01M$. The thermal instability of glucose-6-phosphatase at 37°C and *p*H 5.0, which has been reported for the normal enzyme (*9*), was also found in the case of the diabetic enzyme.

Studies of the kinetics of glucose inhibition revealed that the inhibition was of a noncompetitive nature (Fig. 2). The possibility that this effect was the result of an exchange between the substrate and the inhibitor was tested and excluded. The K_i of glucose binding was 0.11 to $0.15M$ (av. $0.12M$) for the enzyme from four normal animals, and 0.11 to $0.18M$ (av. $0.13M$) for the enzyme from seven diabetic animals (*10*).

The results reported here make it clear that while there is an increased *capacity* of the diabetic liver to hydrolyze glucose-6-phosphate, as expressed by the activity per milligram of tissue at high substrate concentration (V_m), the

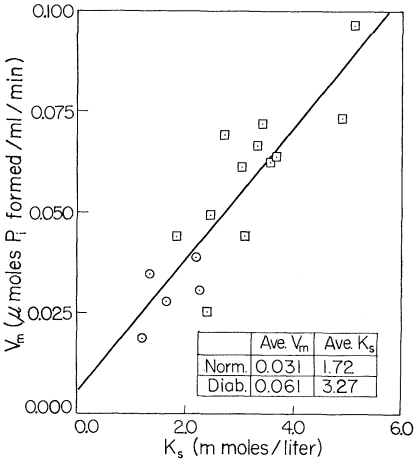

Fig. 1. Kinetic parameters of normal and diabetic liver glucose-6-phosphatase. The normal values are shown by circles, the diabetic by squares. Each value was calculated by the method of least squares from the initial velocities at four to six initial substrate concentrations over the range 1 to 30 μmole/ml of K glucose-6-phosphate (see Fig. 2). In addition, each milliliter of reaction mixture contained 0.3 ml of $0.25M$ sodium cacodylate (*p*H 6.4) and 2.5 mg of liver homogenate. Incubation was for 10 min at 30°C with shaking. Inorganic phosphate (P_i) was determined by the Fiske-Subbarow method. The slope of the line of best fit through the points is 0.017 ± 0.002 min^{-1}.

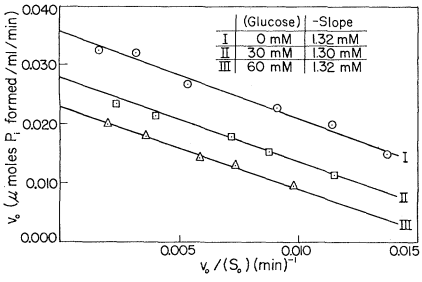

Fig. 2. Glucose inhibition. Incubation conditions were the same as those shown for Fig. 1, except that glucose was added to initial concentration indicated. Initial velocity is plotted against initial velocity divided by initial substrate concentration. Slopes were calculated by the method of least squares.

actual difference between the rates of hydrolysis of glucose-6-phosphate in the diabetic and normal liver decreases progressively as the substrate concentration diminishes. For example, from the average K_s and V_m values given in Fig. 1, it can be calculated that at a glucose-6-phosphate concentration of 10 m*M* the rate of hydrolysis per milligram of tissue is about 75 percent more rapid in the diabetic liver than in the normal liver, whereas, at a substrate concentration of 1 m*M* the difference is about 25 percent. It is obvious that any extension of differences in glucose-6-phosphatase activities observed in vitro to an explanation of the altered carbohydrate metabolism of the diabetic liver must be based upon an actual difference in the rate of hydrolysis of glucose-6-phosphate in vivo rather than upon a difference in potential capacity.

It will be of interest to discover whether the increased K_s values observed with the enzyme from diabetic animals represents an intrinsic difference in property which is retained in the purified enzyme or is a result of an altered milieu in the diabetic liver.

Note added in proof. In recent experiments it has been found that the high K_s values of the enzyme from diabetic animals was restored to the normal level after 48 hours of insulin treatment, and the V_m values were also markedly decreased (*11*).

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6. This investigation was supported by a research grant (A-875, C-2) from the National Institutes of Health, U.S. Public Health Service.
7. Values of 2 m*M* for the K_s of the glucose-6-phosphatase of the microsome fraction of rat liver [R. K. Crane, *Biochim. et Biophys. Acta* 17, 443 (1955)], and of 2.5 m*M* for the K_s of partially purified glucose-6-phosphatase from dog liver (*4*) have been reported. We have obtained an average value of 3.2 m*M* for the enzyme in homogenates of eight normal dog livers.
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10. Langdon and Weakley (*4*) have reported a *competitive* inhibition by glucose for the partially purified enzyme from dog liver, with a K_i of 0.029*M*.
11. A paper describing the details of these experiments is in preparation.

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Method for Adapting the Virus of Rinderpest to Rabbits

The difficulty of adapting the virus of rinderpest to rabbits by serial passage is well known. Baker (*1*) succeeded in adapting the rinderpest virus to rabbits by alternating the inoculations between calves and rabbits. The calf served to maintain sufficient virus in the inocula to overcome the threshold of infection in the rabbits. After a number of alternations sufficient adapted virus was produced to assure the maintenance of serial passage infections.

Shope *et al.* (*2*) have shown that the minimal infective dose of rinderpest virus for the chorioallantoic membrane is roughly in agreement with the subcutaneous minimal infective dose of virus for the calf. It was, therefore, thought that the chorioallantoic membrane might be substituted for the calf in the alternate passing referred to above. With this in mind an experiment was conducted in which a virulent strain of rinderpest virus was successfully adapted to the rabbit. Although this method has no particular advantage other than economic over that evolved by Baker, it is possible that it might be of value in the study of other viruses.

The Kabete strain of rinderpest virus was propagated on the chorioallantoic membrane through 45 serial transfers after the manner described by Shope *et al.* Calf inoculations of earlier passage membranes usually resulted in death on the 8th, 9th, or 10th day. Chorioallantoic membranes from the 42nd passage eggs were inoculated into a calf, and there was no evidence of attenuation of the virus.

Rabbits of various breeds were employed. Only healthy, mature subjects whose normal temperature fell in the range of 102 to 103.5°F were selected for the experiment. The fertile eggs employed were from a healthy flock of mixed breeds.

The inoculum of chorioallantoic membranes for each rabbit consisted of 2.0 ml of a suspension of three chorioallantoic membranes comminuted in 3.0 ml of allantoic fluid. The rabbit spleen suspensions were prepared by grinding each rabbit spleen in 9.0 ml of normal saline. The amount of this suspension deposited on each chorioallantoic membrane was 0.3 ml. None of the tissue suspensions were centrifuged, and the rabbit inoculations (all intravenous) were made slowly in order to avert shock. The 10-day embryonated eggs were incubated for 4 days, and all rabbits whose spleens were employed for inocula were electrocuted on the fourth day after injection.

1) The series of inoculations was begun by inoculating 43rd passage chorioallantoic membranes into rabbit No. 1. The highest temperature recorded was 104.8°F. The spleen from rabbit No. 1 was inoculated on to the first group of chorioallantoic membranes.

2) The first group of membranes was inoculated into rabbits No. 2 and 3. The peak temperature of the former was 103.9°, and that of the latter 104.1°. The spleen from rabbit No. 3 was inoculated on to the second group of chorioallantoic membranes.

3) The second group of membranes was inoculated into rabbits No. 4 and 5. Rabbit No. 4 died as a result of shock. Rabbit No. 5 had a peak temperature of 104.6°. The spleen was inoculated on to the third group of chorioallantoic membranes.

4) The third group of membranes was inoculated into rabbit No. 6. The highest temperature was 104.0°. The spleen was inoculated on to the fourth group of chorioallantoic membranes.

5) The fourth group of membranes was inoculated into rabbit No. 7.

The inoculations were then continued serially in rabbits. The spleen from rabbit No. 7 was inoculated into rabbits No. 8 and 9. The highest temperatures were 103.4° and 104.1° respectively.

The spleen from rabbit No. 9 was inoculated into rabbit No. 10. The highest temperature recorded was 106.2°. The spleen from this rabbit was inoculated into rabbits No. 11 and 12. The post-injection temperatures are listed in Table 1.

The spleen of rabbit No. 12 was removed and each of two calves was given 1.0 ml of a 1:10 dilution in saline.

Calf No. 1, which had been previously immunized against rinderpest, showed no evidence of the disease.

Calf No. 2, which had not been immunized, succumbed to characteristic rinderpest in 12 days. The first high temperature was recorded on the third day following injection, indicating that there was considerable amount of virus present in the spleen of rabbit No. 12.

The temperature curves of rabbits 10, 11, and 12 for the 96-hour postinjection period conformed in general with a typical curve drawn by Baker. These rabbits also exhibited substantially the same signs of illness and gross pathology as Baker observed in his rabbits of comparable passages, except that rabbits 11 and 12, but not rabbit 10, displayed a considerable number of small white foci in the swollen Peyer's patches, sacculus rotundus, tonsilla cecalis major, and appendix. The number of white foci was not as great in either rabbit as is usually observed in rabbits injected with the Nakamura lapinized strain.

Although 43rd-passage membrane virus was employed to initiate the alter-