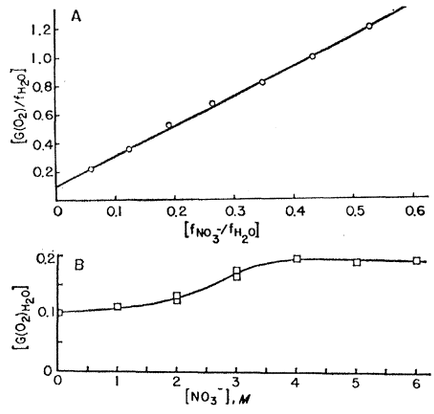

Fig. 1. *A*, G of total oxygen evolution as a function of energy fractionation between water and nitrate. *B*, G of O^{18}-labeled oxygen from the radiolysis of solutions enriched in H_2O^{18}, as a function of nitrate concentration.

cies or reactions. Detailed considerations (*4*) of the results enable us to determine the following values for primary product yields:

$$ge^- + gH = 3.41 \pm 0.23;\ gOH = 2.53 \pm 0.17;\ gH_2O_2 = 0.75$$

which, together with $g(H_2) = 0.44$ (*5*), give 4.3 for the sum of reducing species and 4.0 for the oxidizing species. Apparently a material-balance deficiency of ~0.3 exists.

However, another set of experimental data is available for this system. Mahlman (*5*) has reported G values for oxygen formation over the concentration range 1.0 to 7.0*M* NO_3^-. Although one may expect that most of this oxygen results from energy deposition in the nitrate ion, it is by no means obvious that all the oxygen originates in this way. Accordingly we have treated these data by the following relation:

$$G(O_2) = G(O_2)_{H_2O} f_{H_2O} + G(O_2)_{NO_3^-} f_{NO_3^-}$$

where f_{H_2O} and $f_{NO_3^-}$ are the fractions of energy deposited in the water and nitrate ion, respectively, and $G(O_2)_{H_2O}$ and $G(O_2)_{NO_3^-}$ represent the oxygen yields from water and nitrate. Figure 1*A* shows that the data fit this linear funtion well, and we obtain a value $G(O_2)_{H_2O} = 0.1$ from the intercept at infinitely dilute solution, which, within the limits of experimental error, is the amount needed to remove the material-balance deficit.

The unusual nature of this conclusion —that oxygen is produced from water in the radiolysis of dilute nitrate solutions—requires that other interpretations of these data be considered. Thus there may be a systematic error in the O_2 analysis that is not found in the (simultaneous) H_2 analysis; this error must also be linearly related to $f_{NO_3^-}$: f_{H_2O}, which we consider to be somewhat unlikely. Also, extrapolation of the linear relation to ~0.1*M* may not be valid; the relations may possibly change near the origin.

However, further evidence that the oxygen does in fact originate from the water is explicitly obtained by isotope methods. Mahlman, using 1.6 percent H_2O^{18}, has presented results (*6*) from which $G(O_2)_{H_2O}$ may be obtained as a function of NO_3^- concentration [Fig. 1*B*; the value of $G(O_2)_{H_2O}$ at $(NO_3^-) = 0$ is taken from the intercept of Fig. 1*A*]. Even in quite concentrated nitrate solutions, oxygen is produced from water, and the variation of production with nitrate concentration indicates that it is formed with a G of ~0.1 in dilute solution. We believe this to be clear evidence of oxygen being a product of water radiolysis.

The state of the oxygen on formation is not apparent, but we point out that the variation of $G(O_2)_{H_2O}$ with NO_3^- concentration can be nicely accounted for as a transition from the reaction $2O \rightarrow O_2$ in dilute solution to $O + NO_3^- \rightarrow O_2 + NO_2^-$ in concentrated solution; this suggestion implies, of course, that the oxygen originates in atomic form. Comparison with other (*7*) recently determined stoichiometries is interesting: Hochanadel, using the CO + O_2 system, finds $g(OH) = 2.59$ and $gH_2O_2 = 0.72$, in essential agreement with our results; he also finds stoichiometry with

$$\overset{\text{ox.}}{\Sigma} = 4.03 = \overset{\text{red.}}{\Sigma}$$

Dainton *et al.* (*8*), working with the identical system, also find stoichiometry, but their individual G values differ considerably from Hochanadel's. Seddon and Sutton (*9*) find stoichiometry in the NO system, but their evaluation is gOH = 2.9, as is Fielden's (*10*) in the MnO_4^-:HCO_2^- system; this value of gOH coincides with the sum of our gOH + g O_2 (in equivalents).

Thus the situation concerning the primary species in neutral solutions must still be regarded as unsettled. It may well be true that O atoms may be measured in most systems as the stoichiometric equivalent in OH radicals; or they may appear as O_2—which may not be expected. The scavenger systems used to determine primary species must be carefully evaluated for specificity.

MALCOLM DANIELS
ERIC WIGG

Radiation Center and
Department of Chemistry,
Oregon State University,
Corvallis 97331

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5 May 1966

Phosphorylase Kinase of the Liver: Deficiency in a Girl with Increased Hepatic Glycogen

Abstract. *Studies of a child with glycogenosis revealed an increased concentration of glycogen and low phosphorylase activity in her liver. Using mixtures of homogenates of the patient's liver and of normal liver, we found the low phosphorylase activity to be caused by a deficiency of phosphorylase kinase and not of hepatic phosphorylase. The fact that phosphorylase activity was restored to normal values by the addition of phosphorylase b kinase from rabbit muscle substantiates this conclusion.*

Since Hers (*1*) reported studies of two patients with low activities of hepatic phosphorylase and elevated concentrations of glycogen in the liver, a decrease in the activity of phosphorylase has come to be equated with a deficiency in hepatic phosphorylase (Type VI glycogenosis) (*2*). Such an interpretation does not take into account the complexity of the phosphorylase system (Fig. 1) which comprises at least three other enzymes (*3*).

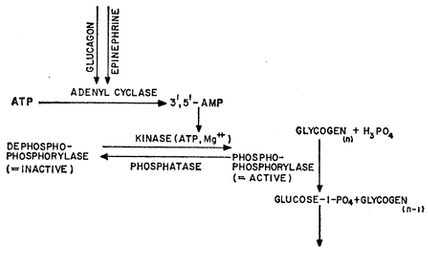

Fig. 1. Hepatic phosphorylase system.

Deficiencies other than that of phosphorylase can be postulated which, nevertheless, would result in a low phosphorylase activity.

A 4-year-old girl with marked hepatomegaly since birth was studied. The edge of her liver was 13 cm below the costal margin in the midclavicular line. Physical examination revealed no other abnormality. During the period of 1 year, the patient's liver was biopsied six times with a Menghini needle (*4*). Between 7.7 and 12.1 percent of the six biopsy specimens was glycogen (in normal liver up to 6.5 percent of wet tissue weight is glycogen), and the activity of phosphorylase varied between 0 and 6.0 μmole of phosphate per gram per minute. In liver tissue of 48 normal individuals, phosphorylase activity ranged from 19.5 to 46.9 μmole of phosphate per gram per minute with an average of 27.4 (*5*). Histologically, the patient's muscle tissue was normal, as were the glycogen content of the muscle and its phosphorylase activity as judged by biochemical analysis. Similarly, the activities of dextrin-1,6-glucosidase (amylo-1,6-glucosidase) and lysosomal acid α-1,4-glucan glucohydrolase (α-1,4-glucosidase) in muscle and in liver, and the activity of glucose-6-phosphatase in liver were normal (*6*).

The liver tissue was homogenized with a glass hand-homogenizer. Immediately after biopsy and before the beginning of the first period of incubation, the initial phosphorylase activity was determined in a portion of the homogenate which had been prepared in ice-cold tris(hydroxymethyl)aminomethane (Tris) and sodium β-glycerophosphate buffer. After the first period of incubation of 15 to 20 minutes, the following were added to all tubes: adenosine triphosphate (ATP), adenosine-3′,5′-phosphate (3′5′-AMP), magnesium chloride, and sodium fluoride, the latter to inhibit phosphorylase phosphatase. The details of concentration are listed in Fig. 2, in which the time of these additions is marked 0. In this report the additions will be referred to as zero-time additions.

A homogenate of normal human liver was incubated at 37°C. The phosphorylase activity at the beginning of the incubation and immediately after the biopsy was 25.5 μmole of phosphate per gram per minute or 13.6 μmole of phosphate per millimole of nitrogen in liver tissue per minute. During the first incubation period of 15 to 20 minutes, the phosphorylase was readily deactivated. The deactivation could be reversed by the zero-time additions. The phosphorylase activity increased within 10 to 20 minutes to a value of 22.4 μmole of phosphate per millimole of nitrogen per minute.

This normal pattern of response differed from the results obtained in two separate attempts at activation of the patient's liver homogenate in vitro under the same conditions. The two experiments provided comparable sets of data, one of which is illustrated in Fig. 2. Phosphorylase activity in the patient's liver immediately after biopsy was 5.2 μmole of phosphate per gram per minute or 3.9 μmole of phosphate per millimole of nitrogen per minute. This activity was lost during the 15 minutes of the first period of incubation. After the zero-time additions, activation of phosphorylase was obtained at 10 percent of the rate which occurred in the control homogenate.

In the same experiment, at time 0, deactivated homogenate from the patient was combined with deactivated homogenate from the control. After the zero-time additions, the combined homogenate was similarly incubated. Phosphorylase activity increased rapidly to a value of 21 μmole of phosphate per millimole of nitrogen per minute. This amount of activation is similar to that of the control homogenate.

Phosphorylase b kinase from rabbit muscle was used in a fourth reaction mixture included in the same experimental run. The phosphorylase kinase was prepared according to a standard procedure (*7*) and was used in a dilution such that the phosphorylase impurities of the preparation did not contribute any activity to the final phosphorylase assay of the experiment. When muscle phosphorylase kinase was added to a reaction mixture the same as that contained in the deactivated homogenate of the patient's liver with the usual zero-time additions, rapid activation of the patient's liver phosphorylase occurred resulting in a final activity of 25 μmole of phosphate per millimole of nitrogen per minute. This amount of activation is similar to that of the control homogenate and of the combined homogenate.

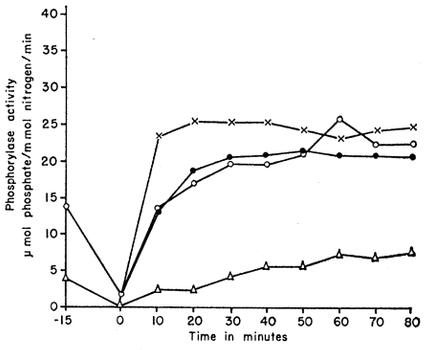

Fig. 2. Reactivation of liver phosphorylase in vitro. The homogenates contained 80 mg of tissue per milliliter in 4 × $10^{-2}M$ Tris—sodium β-glycerophosphate buffer, *p*H 7.8, for the first incubation period of 15 minutes at 37°C. At time 0, additions were made to give these final concentrations: $10^{-3}M$ ATP; $10^{-6}M$ 3′5′-AMP; 5 × $10^{-2}M$ NaF; 5 × $10^{-3}M$ $MgCl_2$; 2 × $10^{-2}M$ Tris—sodium β-glycerophosphate buffer, *p*H 7.8; 20 mg of liver tissue per milliliter except for the tube "patient plus control" which contained 40 mg of liver tissue per milliliter. Control, ○; patient's liver, △; patient's liver plus control, ●; patient's liver plus phosphorylase b kinase from rabbit muscle, X.

A reduction of phosphorylase kinase activity in the patient's liver by 90 percent or more would account for these results.

GEORGE HUG
WILLIAM K. SCHUBERT
GAIL CHUCK

Children's Hospital Clinical Research Center and *Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio*

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