Ionic Strength of Homogenizing Medium on Glutamic Dehydrogenase Activity of Tissues.*† (22895)

GERALD L. ENDAHL AND CHARLES D. KOCHAKIAN
Oklahoma Medical Research Foundation, Oklahoma City.

Glutamic dehydrogenase activity in mammalian tissues has been studied by a number of investigators(1). No study, however, has been made as to relationship of activity with the medium used for homogenization of the tissues. In most instances, the buffer medium has been used but occasionally water has been employed. The low activity obtained while using a reported method(2) which prepared the tissue homogenate in phosphate buffer prompted this study.

Methods. Adult male mice of Swiss strain, (National Laboratories, Creve Coeur, Mo.) and rats (Sprague-Dawley) were used. Tissues were homogenized in all-glass apparatus and diluted to 1% for liver and 2% for kidney. The glutamic dehydrogenase activity was determined at 37° by DPNH oxidation method of Olson and Anfinsen(2) with a Cary Spectrophotometer.‡ The DPNH preparation was 91% as compared to 60% used by Olson and Anfinsen(2). Therefore, 50% more DPNH was used. This amount of DPNH gave a linear oxidation with time while higher and lower amounts were not completely satisfactory. The DPNH was prepared just before use from pure Pabst DPN by the method of Green and Dewan(3). The average of conversions was 91 ± .3% as determined by extinction coefficients at 260 and 340 mμ. Solutions were prepared in distilled water which had been passed through a deionizer column (Deiminizer, A. S. Aloe Co.).

Results. The relative glutamic dehydrogenase activity of mouse liver and kidney decreased rather slowly until an ionic strength of about 3 x 10^{-3} was used in the homogenizing medium and then decreased very rapidly (Fig. 1). The results were not due to any specific ion because identical results were obtained with the 3 different salts. The same phenomenon was observed in the preparation of rat liver and kidney homogenates with phosphate buffer (Fig. 2).

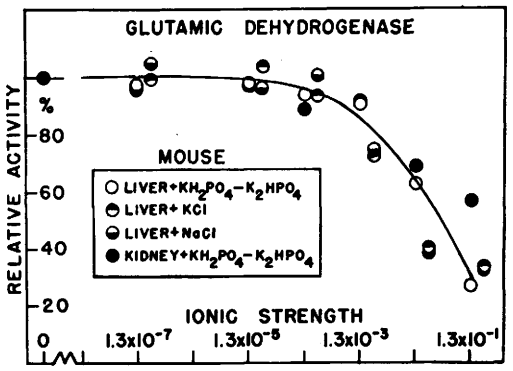

FIG. 1. Relationship of glutamic dehydrogenase activity of mouse liver and kidney to ionic strength of homogenization medium.

The decrease in activity was not due to presence of ions in the assay medium, for addition of KCl at an ionic strength of 3 x 10^{-1} after homogenization in water did not decrease the activity. Addition of much higher concentrations of several ions to the assay medium has been reported(2) to decrease en-

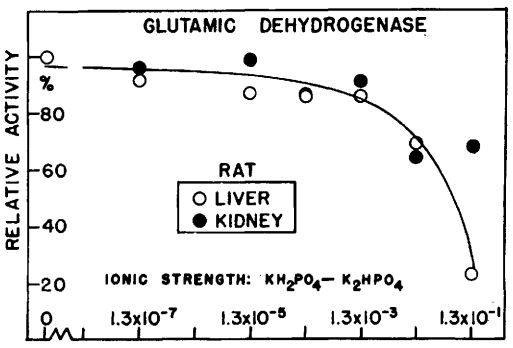

FIG. 2. Relationship of glutamic dehydrogenase activity of rat liver and kidney to ionic strength of phosphate buffer, pH 7.4, used for homogenization.

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‡ The Cary Spectrophotometer was provided by the Amer. Cancer Soc.

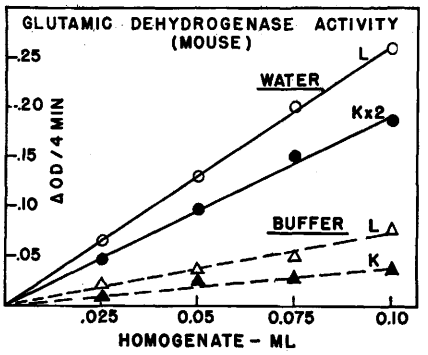

FIG. 3. Relationship of glutamic dehydrogenase activity of mouse liver and kidney to amount of homogenate used in the assay. The homogenates were: liver 1% and kidney 2%. Twice the amount of kidney homogenate prepared in water was used.

zyme activity.

Activity of glutamic dehydrogenase of liver and kidney homogenized in water was proportional to the amount of homogenate used in the assay (Fig. 3).

Discussion. The increase in glutamic dehydrogenase activity when the homogenates are prepared in water is probably due to a greater release of the enzyme from the mitochondria. It has been reported that the mitochondria swell in ionic solutions and burst in water(4).

Summary. Glutamic-dehydrogenase activity of liver and kidney of the mouse and rat was greater when the tissues were homogenized in water than in .05 M phosphate buffer, or NaCl and KCl of equivalent ionic strength. The decrease in activity could be varied by variations in ionic strength of the medium.

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Mode of Action of Potato Apyrase.* (22896)

KWAN-HUA LEE, J. Z. KREZANOSKI AND JOHN J. EILER†
University of California School of Pharmacy, San Francisco.

In a previous paper(1), we reported on an enzyme preparation from potato which selectively catalyzes hydrolysis of acid-labile phosphates of adenosinetriphosphate (ATP) and shows only negligible activity toward adenosine-5-phosphate and inorganic pyrophosphate. Several laboratories(2,3,4) have since verified our results bearing on the selectivity of action and, recently, Whittam, Bartley and Weber(4) confirmed our observation that at low temperatures (0°-7°), the enzyme preparation catalyzes hydrolysis of ATP only to adenosinediphosphate (ADP) and inorganic phosphate, while at higher temperatures both labile phosphates of ATP are removed.

Despite frequent practical uses to which the preparation has been put(2,4,5), its mode of action remains obscure. Conceivably, the preparation may consist of a single active protein showing greatly different activities toward ATP and ADP. However, we have not been able to demonstrate any evidence of competition between the two substrates. It is also possible that the preparation may consist of a mixture of enzymes whose ratio remains constant during various fractionation procedures we have employed. Either a mixture of an ATPase and an adenylate kinase, or of an ATPase and an ADPase would explain the enzyme activities observed. The observations(6) that plant materials contain adeny-

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