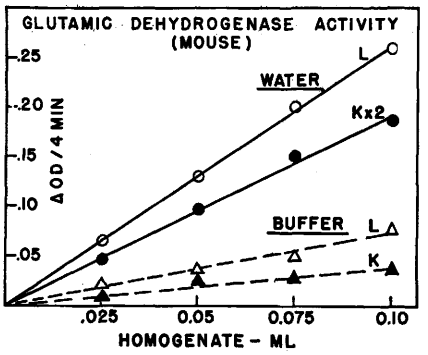

FIG. 3. Relationship of glutamic dehydrogenase activity of mouse liver and kidney to amount of homogenate used in the assay. The homogenates were: liver 1% and kidney 2%. Twice the amount of kidney homogenate prepared in water was used.

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)

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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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late kinase prompted this study of the possible presence of this enzyme in the potato preparation.

Materials and methods. ATP, as the barium salt, was isolated from rabbit muscle according to modification of method of Dounce *et al.*(7). ADP was prepared by modification of the procedure of Bailey(8). Yeast hexokinase was purified to the 3a stage as described by Berger *et al.*(9). The potato enzyme was prepared according to Lee and Eiler(1). Inorganic phosphate was determined by the method of Fiske and Subbarow (10). The labile phosphate of either ATP or ADP was measured by increase in inorganic phosphate due to hydrolysis of either substance in 1 N sulfuric acid at 100° for 10 minutes. The reaction mixture and conditions for the enzymic reactions are given in Table I. It should be noted that the potato enzyme can be activated with either calcium or magnesium serving as the divalent cation. Hexokinase was used to test activity of adenylate kinase. With ADP as substrate, the presence of adenylate kinase in the preparation would provide ATP, which, in the presence of added hexokinase and glucose, would account for loss in labile phosphate not accompanied by an increase in inorganic phosphate. Such a loss in labile phosphate is assumed to represent hexose phosphate in these experiments. In this test, hexokinase must compete with ATPase activity of the preparation for the ATP that is to be released if adenylate kinase is present. Further both enzymes will be competing for low concentrations of ATP since both enzymes are highly active in comparison to the ADP splitting activity of the preparation. It can be shown that factors influencing competition between two enzymes for a single substrate are related as follows: $\frac{v''}{v'} = \frac{V'' \cdot |Km' + (S)|}{V' \cdot |Km'' + (S)|}$ where the v's are the respective velocities at a given concentration of substrate (S), the V's are the respective velocities at high substrate concentration; and, the Km's, assumed to approximate true dissociation constants for the respective enzyme-substrate complexes, are the Michaelis constants. At concentrations of substrate less than the Km's, as expected to be obtained in these studies, the above relation reduces to the approximation: $\frac{v''}{v'} = \frac{V''}{V'} \cdot \frac{Km'}{Km''}$. To adjust the concentrations of the competing enzymes, hexokinase and the ATP splitting enzyme of the potato preparation, so that they will be competitive at low concentrations of ATP, it is desirable that the Km's relative to ATP for both enzymes be known. The Km for yeast hexokinase has been reported by Slein *et al.*(11). We determined the Km for the ATPase activity of the potato preparation, using the conditions given in Table I, over a 100-fold range in concentration of ATP.

TABLE I. Hydrolysis of ATP and ADP by Potato Apyrase and Hexokinase. Each tube contained: $MgCl_2$ (0.006 M); succinate buffer, pH 6.8 (0.02 M); glucose (0.05 M); ATP (395 μg acid-labile-P) or ADP (350 μg acid-labile-P) and enzymes* as indicated in the table. Final vol was 2.8 ml. Incubation time for ATP as substrate was 5 min. at 30°C, and for ADP as substrate was 30 min. at 30°C.

	After incubation†		
	Inorg. P	Acid-labile-P	Hexophosphate-P
ATP as substrate			
No enzyme	0	395	
Potato enzyme	180	218	
Hexokinase	0	251	144
Potato enz. & hexokinase	102	197	96
ADP as substrate			
No enzyme	0	347	
Potato enzyme	92	258	
Hexokinase	0	350	
Potato enz. & hexokinase	105	245	

* See text for enzyme concentrations.
† All quantities in μg.

Results. From the data presented in Fig. 1, calculations yielded a Km of 1.4 x 10^{-4} M for the ATPase activity of the preparation. This is very close to the value of 9.5 x 10^{-5} M reported for yeast hexokinase(11). According to the approximate equation given above, the two enzymes would be competitive for low concentrations of ATP if their concentrations were adjusted so that they would be competitive at high concentration of ATP, since their Km's are nearly equal. In obtaining the data presented in Table I with ATP

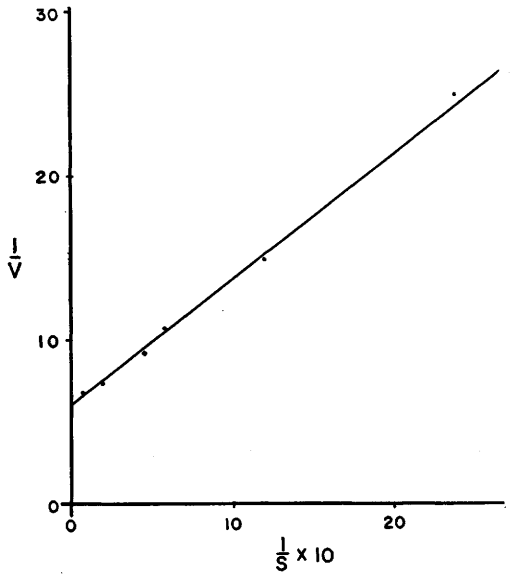

FIG. 1. Lineweaver-Burk plot on effect of ATP conc. on rate of hydrolysis by potato enzyme. Conditions as in Table I. Ordinate is reciprocal of rate of hydrolysis of ATP (in millimoles/l) at 30° during 5 min., and abscissa is reciprocal of conc. of ATP (in millimoles/l).

as substrate, the concentrations of hexokinase and the ATP splitting activity of the potato preparation were adjusted so that their respective activities were approximately equal when the reaction mixture contained 395 μg acid-labile phosphate (ATP) per 2.8 ml. When tested separately, or in competition with each other, the two enzymes showed approximately equal activity, as judged by the increase in inorganic phosphate and loss of labile phosphate not giving rise to inorganic phosphate (hexose phosphate). The same concentrations of the two enzymes were then tested separately and in competition with each other with ADP serving as the substrate. The data in Table I with ADP as substrate show, both in the presence and in the absence of hexokinase, that inorganic phosphate accounted completely for the decrease in acid-labile phosphate. Clearly, ATP is not an intermediate in the hydrolysis of ADP and adenylate kinase is not present in the preparation.

Since ADP in moderate to high concentrations does not inhibit the rate of hydrolysis of ATP at pH 6.8,‡ it seems probable that at least two sites of enzymic activity are present in the preparation. Presumably, the second site may be described as having ADPase activity. The two enzymic functions have different pH optima. In the presence of 0.033 M succinate buffer, the ATP splitting activity shows a sharp optimum at pH 6.4-6.5, while in the same buffer the ADP splitting activity shows an equally sharp optimum at pH 4.4.‡

Summary. With the use of hexokinase, it has been possible to show that the adenylpyrophosphatase from potato does not contain adenylate kinase. The lack of competitive inhibition of ATP splitting by ADP suggests two sites of enzymic activity, one acting on ATP, the other on ADP.

‡ Unpublished data.

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