powder; cold-acid hydrolysed; hot-acid hydrolysed. In addition there was a significant quantity of metabolites remaining water-soluble after all forms of hydrolysis.

4. When 1 or 2 hr. fractions of bile were analysed the proportion of radioactivity excreted as glucuronide always decreased in successive samples, whereas water-soluble metabolites generally increased. In some animals the proportion of cold-acid-hydrolysed metabolites increased and in others decreased. No consistent change was detectable in hot-acid-hydrolysed radioactivity. A similar trend was seen in successive urine samples.

5. The relationship of these results to similar studies of progesterone in man and other animals is discussed.

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Purification and Properties of Muscle Phosphoglycerate Kinase

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The enzyme phosphoglycerate kinase catalyses the reversible transphosphorylation:

This reaction is an integral part of the Embden-Meyerhof glycolytic scheme. The existence of the reaction was originally mentioned by Warburg & Christian (1939), and the first detailed investigations of the enzyme involved were made by Bücher (1947). The kinase has been partially purified from pea seeds by Bandurski & Axelrod (1953) and has been detected in blood by Blancher, Williams & Weiss (1955) and in liver by Ichihara & Greenberg (1957). Some statements about the behaviour of this enzyme upon fractionation of an

aqueous extract of muscle have been made by Beisenherz *et al.* (1953), but, despite some erroneous statements in the literature, he has not described the crystallization of phosphoglycerate kinase. The present paper deals with the purification and properties of the enzyme from rabbit skeletal muscle. Some differences in properties of the enzymes from yeast and from muscle are also described. A preliminary report was presented earlier by Rao & Oesper (1959).

MATERIALS AND METHODS

Abbreviation. P_i, Inorganic phosphate.

Unless otherwise stated, all reagents were obtained from Sigma Chemical Co., St Louis, Mo., or Schwarz Bio-Research Inc., Mount Vernon, New York. All optical

measurements were made with a Beckman model DU spectrophotometer. The barium salts of the various nucleotides and of phosphoglyceric acid were dissolved with HCl and converted into the sodium salts by treatment with a stoichiometric amount of Na_2SO_4 . The BaSO_4 was removed by centrifuging at 0–5° and the solution was neutralized to pH 7 with NaOH. However, for a study of the metal requirement and of nucleotide specificity, the solutions were neutralized with tris. All solutions were stored at 0–5°. P_i was determined by the method of Lohmann & Jendrassik (1926). Glyceraldehyde 3-phosphate was made enzymically by the method of Meyerhof & Junowicz-Kocholaty (1943), and dihydroxyacetone phosphate according to Oesper & Meyerhof (1950). Phosphoglycerol phosphate was prepared by the method of Negelein & Brömel (1939) with the modification of Oesper (1954). A neutralized solution was kept frozen at –20° and used as required. It was convenient to distribute the stock solution into several small tubes, rather than freeze and thaw the bulk of the solution repeatedly. The concentration was determined by the method of Negelein & Brömel (1939). An extinction coefficient of $6.27 \times 10^6 \text{ cm}^2/\text{mole}$ for DPNH has been used in all calculations.

Buffer systems. Imidazole was a product of Eastman Kodak Co., Rochester, N.Y., U.S.A. Tris was a product of Sigma Chemical Co. Veronal was a product of Abbot Laboratories, Chicago, Ill.

Inhibitors. *p*-Chloromercuribenzoic acid, *p*-chloromercuriphenylsulphonic acid and iodosobenzoic acid were products of Sigma Chemical Co. Iodobenzoic acid was a product of Eastman Kodak Co. Phenylloxarsine was a product of Delta Chemical Works and a gift of Dr J. S. Roth. Oxophenarsine hydrochloride was a product of Parke Davis Company, Detroit, Mich.; dichlorophenarsine hydrochloride, of Abbot Laboratories; *N*-ethylmaleimide, of Schwarz BioResearch Co.; and iodoacetamide, of Eastern Chemical Corp., Newark 2, N.J.

Estimation of protein. Protein was determined by the method of Robinson & Hogden (1940) after precipitation with trichloroacetic acid. Human serum albumin was used as a standard. In some cases, protein was also determined by the method of Warburg & Christian (1941), with corrections for nucleic acids as suggested by Kalckar (1947).

Determination of enzyme activity. The enzyme reactions were run at room temperature (23–27°). The enzymes were diluted in 0.2% human serum albumin just before use, because the highly purified yeast enzyme (Bücher, 1947) lost 70% of its activity in dilute buffer solutions over a period of 3–4 hr. at 0°. The purified enzyme from muscle was more stable. Enzymes from both sources were definitely more stable in 0.2% albumin solution.

Back-reaction: assay method I. The back-reaction was studied by the method of Bücher (1947) in an optical assay system that utilized the two reactions:

Sum:

Reaction (1) is catalysed by phosphoglycerate kinase and reaction (2) by glyceraldehyde 3-phosphate dehydrogenase.

The reaction mixture consisted of 50 μmoles of imidazole or tris buffer, pH 7.0, 10 μmoles of glutathione, 16 μmoles of Mg^{2+} ions, 13–25 μmoles of phosphoglycerate, 4–65 μmoles of ATP, 0.5 μmole of DPNH and an excess of glyceraldehyde 3-phosphate dehydrogenase (Krebs & Najjar, 1948) in a total volume of 2.8 ml. The kinase (0.2 ml.; diluted to give an extinction change of 0.12–0.15/min.) was added to start the reaction, which was followed by determining the extinction change at 340 $\text{m}\mu$ every 30 sec. for a period of 4 min. All reactions were run at two different concentrations of enzyme.

One unit of enzyme activity is defined as an extinction change of 0.10/min. under the above assay conditions.

Specific activity is defined as the number of units/mg. of protein. The change in extinction used to calculate the specific activity is either from $\frac{1}{2}$ to $1\frac{1}{2}$ min. or from 1 to 2 min. During this interval the rate is linear and proportional to enzyme concentration.

Assay method II. The method described is essentially that devised by Bandurski & Axelrod (1953).

A mixture was prepared, containing 50 μmoles of imidazole buffer, pH 7.1, 12.5 μmoles of phosphoglycerate, 4.5 μmoles of ATP, 10 μmoles of MgCl_2 and 750 μmoles of hydroxylamine in 2 ml. of solution. The final pH was adjusted to 7.1, if necessary, by cautious addition of *N*-HCl, and the mixture equilibrated in centrifuge tubes in a water bath at 37° for 5 min. At appropriate time intervals, suitably diluted enzyme preparations were added so that the final volume was 2.2 ml. and the tubes were incubated for 30 min. at 37°. The blanks contained all components except the enzyme. At the end of this period, 3 ml. of FeCl_3 -HCl-trichloroacetic acid (8.3 g. of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 20 g. of trichloroacetic acid and 42 ml. of conc. HCl in 500 ml. of water) was added, followed after 5 min. by 2.5 ml. of 95% ethanol. The mixture was stirred and centrifuged to remove denatured protein, and the absorption of the supernatant at 490 $\text{m}\mu$, which was due to the Fe^{3+} -phosphoglycerohydroxamic acid complex, was determined in a Beckman spectrophotometer. A large-scale incubation was also carried out, essentially by the same procedure, and the phosphoglycerohydroxamic acid identified by paper chromatography.

The hydroxylamine stock solution was made by the method of Beinert *et al.* (1953); it was not neutralized, but frozen in solid CO_2 and stored at –20°. The concentration of hydroxylamine was determined by titration with standard HCl.

Specific activity is defined as the extinction change/mg. of protein under the above assay conditions. The method is relatively insensitive, requires long incubation periods and is wasteful of material, but was used to determine the distribution of enzyme in various tissue extracts, and also to study the effect of inhibitors and metal ions on the enzyme. Assay method I is not suitable for crude extracts because the triose phosphate isomerase present will convert the glyceraldehyde phosphate into dihydroxyacetone phosphate, which reacts with DPNH in the presence of α -glycerophosphate dehydrogenase, which may also be present in the crude preparation. The change in extinction is, accordingly, quite misleading.

Forward reaction. The reaction in the forward direction can be studied by assay method I but is liable to inter-

ference by contaminating enzymes, especially triose phosphate isomerase and α -glycerophosphate dehydrogenase (Bücher, 1947) (see above). Consequently a separate assay method was devised.

Assay method III. The reaction mixture contained 50 μ moles of imidazole buffer, pH 7.0, 4.68 μ moles of ADP, 8 μ moles of Mg^{2+} ions, 0.1 or 0.2 ml. of a suitably diluted enzyme preparation in a volume of 1.8 ml. The reaction was started by adding 2 μ moles of phosphoglyceryl phosphate. The final volume was 2 ml. At the end of 5 min. at room temperature (23–27°) 0.3 ml. of 30% (w/v) trichloroacetic acid was added, the tubes were well shaken and centrifuged, and the inorganic phosphate was determined. The acyl phosphate of phosphoglyceryl phosphate is hydrolysed by the acid molybdate in the phosphate reagent, whereas the ATP into which it is converted is not, so that the extent of reaction is immediately given by this assay.

Determination of Michaelis constants lying below 10⁻⁴ moles/l. The Michaelis constant for phosphoglyceryl phosphate is so small that it is impossible to measure it directly; when the concentration of substrate is small enough to affect the reaction rate, the amount of reaction is too small to measure by ordinary means (spectrophotometry or phosphate determinations).

We have devised an isotopic method for measuring the reaction rate at very low substrate concentrations. In this procedure, the enzyme is incubated with labelled substrate, the reaction stopped, a known amount of unlabelled product added, and the specific activity of the purified product is determined; the activity of the product is obviously proportional to the extent of the reaction.

In our particular case, phosphoglyceryl phosphate containing ³²P in the 1-position was incubated with enzyme and excess of ADP for 30 sec. at 25° in 5 ml. of 0.05 M-tris buffer, pH 7. The reaction was stopped with 1 ml. of 30% trichloroacetic acid, ATP and P_i were added as carriers, the mixture was heated at 60° for 30 min. to decompose the remaining phosphoglyceryl phosphate, and centrifuged to remove the coagulated protein. The pH was adjusted to 1.7 and the ATP precipitated at 0° with 10 vol. of acetone. The ATP was redissolved in water and reprecipitated twice more, additional carrier P_i being added before each reprecipitation. The carrier P_i dilutes out the [³²P]P_i derived from the active phosphoglyceryl phosphate, so that the ATP finally isolated is essentially free of the activity of the phosphoglyceryl phosphate remaining at the end of the reaction. Details of the separation of ATP from P_i are described by Oesper (1954).

RESULTS

Activity of the enzyme in different tissues

Table 1 shows the activity of phosphoglycerate kinase in various tissues of the rabbit, studied by assay method II.

Preparation of phosphoglycerate kinase

Rabbit skeletal muscle was used as the source of phosphoglycerate kinase because of its high initial specific activity and easy availability. Initial attempts to purify the enzyme by ammonium sulphate fractionation were unsuccessful, as

Beisenherz *et al.* (1953) had also found. Therefore, attempts were made to develop a method for the isolation of the enzyme by fractionation with organic solvents.

All procedures were carried out at the temperatures noted below. Contact of the solutions and various fractions with metal was avoided throughout the fractionation. Measurements of pH were made at or near 20°, with a Beckman pH meter. Whenever a protein-precipitating agent was used, the mixture was stirred vigorously but without foaming, and kept for at least 5 min. before centrifuging. The concentration of acetone is expressed in terms of volume per cent, assuming that the volumes are additive. The temperature during the acetone fractionations was held at –5°. A typical protocol is as follows

Extraction of tissue. A rabbit was anaesthetized by intravenous injection of a 5% solution of sodium pentobarbital. The carotid artery was cut and the animal bled. The leg and back muscles were dissected in a cold room at 2–4° and passed twice through a chilled meat grinder; 430 g. of muscle was obtained. It was stirred with twice its volume (860 ml.) of water and kept at 2–5° for 3 hr. with occasional stirring. The suspension was centrifuged at 3500g for 25 min. and the clear supernatant decanted through glass wool.

Acetone fractionation; '40% acetone' fraction. To 800 ml. of muscle extract at approximately 0°, 534 ml. of chilled acetone (below –20°) was added over a period of 10–15 min. at –5°. The precipitate had essentially no activity and over 40% of the total protein was removed in this step.

'45% acetone' fraction. Chilled acetone (120 ml.; about –10°) was added to the supernatant from the previous step over about 5 min. and the mixture centrifuged at 1020g for 25 min. at –5°. The precipitate had about 25% of the original activity but the specific activity was low. The clear supernatant was used for the next step.

'50% acetone' fraction. Chilled acetone (146 ml.; about –10°) was added to the supernatant from

Table 1. *Activity of phosphoglycerate kinase in various tissues*

Rabbit liver, kidney, brain and muscle were dissected out immediately after killing the animal with sodium pentobarbital. The tissues were weighed and homogenized with 2½ vol. of water at 0–5° in a glass homogenizer. The homogenate was centrifuged at 3500g for 15 min. and the clear supernatant used for the determination of the activity by assay method II.

Tissue	Activity/g. of tissue	Activity/g. of protein
Muscle	27.3	624
Brain	14.0	320
Kidney	12.9	114
Liver	12.8	81

the previous step over about 5 min. and the mixture centrifuged at 1020g for 30 min. at -5° . The supernatant was rejected and the precipitate was dissolved in 30 ml. of ice-cold water, frozen and freeze-dried to remove acetone. The freeze-dried material could be stored at -20° for a day or two. It was dissolved in ice-cold water and centrifuged at 3500g to remove any insoluble material. The clear solution was used for the next step.

Heating in the presence of ammonium sulphate. The volume of the solution was adjusted to give a protein concentration of 10 mg./ml. Solid ammonium sulphate was added to give a final concentration of 10%. The pH was 6.0–7.0. The solution was heated to 50 – 52° for 5 min. in a water bath, cooled to 10° , centrifuged to remove denatured protein, and decanted through glass wool. This fraction could be kept frozen at -20° for a month without loss of activity.

Adsorption on alumina C γ . The above fraction was dialysed to remove ammonium sulphate and the pH adjusted to 8.0 with 0.5M-tris. Alumina C γ gel (Willstätter & Kraut, 1923) was added (protein: gel ratio, 5–6:1). The mixture was stirred in the cold room at 2 – 5° for 20 min. with a magnetic stirrer and centrifuged at 3000g. The packed gel was eluted successively with 0.5, 1, 2, 4, 5, 7.5 and 10% ammonium sulphate solutions and each of the fractions was assayed separately. In a typical experiment 66 ml. of a solution containing 225 mg. of protein was stirred with 1300 mg. of alumina C γ gel as described above. Twenty per cent of the activity was recovered in the supernatant and the purification was about twofold. Ammonium sulphate solution (25 ml.) was used for each elution. The first fraction contained 6% of the activity and the purification was threefold. Succeeding fractions had lower specific activity.

The conditions for the gel-adsorption step are rather critical and the results variable. The gel-adsorption procedure eliminated the trace of α -glycerophosphate-dehydrogenase activity present in the earlier fraction.

A summary of results of a typical fractionation procedure is given in Table 2.

Properties of the purified phosphoglycerate kinase

Most of the experiments as well as the properties recorded here apply to the preparations that were

not subjected to purification by alumina C γ -gel procedures but were carried through the heating step.

Tests for the presence of other enzymes in the phosphoglycerate-kinase preparation. The enzyme fractions after heat treatment were free of aldolase, triose phosphate dehydrogenase, DPNH oxidase, adenosine triphosphatase, myokinase and acyl phosphatase (Harary, 1957) when tested at concentrations 100 times greater than that used in the standard-activity determination (method I). Triose phosphate isomerase and a trace of α -glycerophosphate dehydrogenase were detected in all enzyme preparations. The latter could be removed by the treatment with alumina C γ .

Stability. The enzyme could be repeatedly frozen and thawed over short intervals without loss of activity. About 50% of the activity was lost in a period of 3 months with repeated freezing and thawing. The enzyme was stable in the frozen state in the presence of ammonium sulphate.

Activation by metal ions. The effect of cations on the activity of phosphoglycerate kinase was tested by assay methods I and III. Both muscle and yeast kinases required Mg^{2+} or Mn^{2+} ions (approximately 2 mM) for full activity. Higher concentrations of the metal ions were slightly inhibitory. Even after extensive dialysis, both kinases showed a small residual activity, which could be completely eliminated by adding EDTA. The inhibitory effect of EDTA could be abolished by adding Mg^{2+} or Mn^{2+} ions. There was no activation of either muscle or yeast kinase by K^{+} , Li^{+} , Zn^{2+} , Co^{2+} , Ni^{2+} or Al^{3+} ions.

Nucleotide specificity. The nucleotide specificity of the muscle phosphoglycerate kinase was investigated by assay methods I and III. The enzyme is specific for ATP and ADP in the back and forward reactions respectively. None of the other adenine nucleotides (adenosine 2', 3' or 5'-monophosphate) were effective in replacing ATP or ADP. Uridine triphosphate, inosinic acid and creatine phosphate were also ineffective. Inosine triphosphate, however, did have some activity (up to 40% of that found with ATP). ADP was not active in the back-reaction.

Effect of inhibitors on phosphoglycerate-kinase activity. The effect of inhibitors on the muscle and yeast kinases was studied in the back and forward

Table 2. *Purification of phosphoglycerate kinase from muscle*

Assay method I was used to follow the degree of purification.

Fraction	Total protein (g.)	Specific activity	Purification	Total units	Recovery (%)
Muscle extract	13.28	24	0	319 900	100
'50% acetone' fraction	0.640	135	5.6	86 400	27
Heat treatment at 50 – 52°	0.484	164	7.0	79 380	24

reactions, by assay methods II and III. The muscle kinase is highly sensitive to *p*-chloromercuribenzoate and *p*-chloromercuriphenylsulphonic acid; 100 % inhibition was obtained at concentrations of 0.26 and 0.43 mM respectively. The yeast enzyme, however, was not inhibited even by 10 mM-*p*-chloromercuribenzoate. The activities of both the muscle and yeast enzymes were unaffected by iodoacetamide, *o*-iodobenzoate, iodosobenzoate or *N*-ethylmaleimide at concentrations of 4.36 mM. Oxophenarsine hydrochloride, dichlorophenarsine hydrochloride and phenyloxarsine had no effect at concentrations of 1 mM.

The inhibition due to mercurial sulphhydryl reagents could be reversed by addition of GSH or cysteine. A slight activating effect of EDTA was also noticed in some instances in the presence of excess of Mg^{2+} ions. This is presumably due to the

removal of heavy-metal contaminants in the assay system or enzyme.

Determination of Michaelis constants for phosphoglyceric acids, adenosine tri- and di-phosphate, and Mg^{2+} and Mn^{2+} ions. The Michaelis constants for phosphoglycerate, ATP, Mg^{2+} and Mn^{2+} ions were determined in the back-reaction, by assay method I. The Michaelis constant for ADP was determined in the forward-reaction, by the assay method described by Bücher (1947) for the yeast enzyme, and method III for the muscle enzyme.

As we have already observed, the Michaelis constant for phosphoglyceryl phosphate is so small that it is impossible to measure it in the usual way; Bücher (1947) overcame this difficulty by measuring the competition between phosphoglyceryl phosphate and phosphoglycerate or ATP, and computing what the enzyme-substrate dissociation

Table 3. *Isotopic determination of the Michaelis constant of yeast phosphoglycerate kinase for phosphoglyceryl phosphate*

Phosphoglyceryl phosphate (activity, 4 820 000 counts/min./ μ mole) was incubated at the concentrations given below, in the presence of 1.5 mM-ADP and 0.033 μ g. of enzyme. Total volume equalled 5 ml.; 44.5 μ moles of ATP and 49 μ moles of P_i were added as carriers, with an additional 147 μ moles of P_i being added before each of the two reprecipitations of ATP.

Phosphoglyceryl phosphate concn. (μ mole/ml.)	Total counts/min. recovered*	ATP recovered (μ moles)	Counts/min./ μ mole of ATP	ATP formed/ml. of reaction mixture (μ mole)†
0.00516	4643	21.7	214	0.000395
0.00258	3202	20.4	157	0.000291
0.00134	2517	21.7	116	0.000215

* Corrected for background and blank.

† Counts/min./ μ mole $\times$ (44.5/4 820 000) $\times$ ($\frac{1}{4}$).

Table 4. *Isotopic determination of the Michaelis constant of muscle phosphoglycerate kinase for phosphoglyceryl phosphate*

Experiment similar to that described in Table 3. Activity of phosphoglyceryl phosphate was 1 570 000 counts/min./ μ mole; volume, 4.0 ml.; ADP, 2 mM; 0.02 μ g. of enzyme. Carrier ATP (51.4 μ moles) was added.

Phosphoglyceryl phosphate concn. (μ mole/ml.)	Total counts/min. recovered*	ATP recovered (μ moles)	Counts/min./ μ mole of ATP	ATP formed/ml. of reaction mixture (μ mole)†
0.00495	1040	24.6	42.3	0.000346
0.00248	570	27.3	20.9	0.000171
0.00124	390	20.4	19.1	0.000156

* Corrected for background and blank.

† Counts/min./ μ mole $\times$ (51.4/1 570 000) $\times$ ($\frac{1}{4}$).

Table 5. *Michaelis constants for substrates and activators*

K_m values are given in moles/l.

Enzyme source	pH	Phosphoglycerate	ATP	Phosphoglyceryl phosphate	ADP	Mg^{2+}	Mn^{2+}
Yeast	6.0	8.5×10^{-4}	—	—	—	—	—
Yeast	7.0	13.0×10^{-4}	3.2×10^{-4}	2.0×10^{-6}	4.0×10^{-4}	2.8×10^{-4}	5.9×10^{-4}
Yeast	6.9*	2.0×10^{-4}	1.1×10^{-4}	1.8×10^{-6}	2.0×10^{-4}	2.5×10^{-4}	—
Yeast	8.0	10.7×10^{-4}	3.3×10^{-4}	—	—	—	—
Muscle	6.0	13.6×10^{-4}	—	—	—	—	—
Muscle	7.0	12.2×10^{-4}	4.8×10^{-4}	2.2×10^{-6}	3.5×10^{-4}	2.7×10^{-4}	3.0×10^{-4}
Muscle	8.0	10.7×10^{-4}	4.8×10^{-4}	—	—	—	—
Pea seeds	7.3†	7.6×10^{-3}	4.1×10^{-3}	—	—	—	—

* Bücher (1947).

† Bandurski & Axelrod (1953).

constant for phosphoglyceryl phosphate must have been. We have used the isotopic method described above. Typical protocols are given in Tables 3 and 4.

The K_m values for the various substrates and activators are given in Table 5, the results having been obtained from graphs drawn according to Dixon (1953).

DISCUSSION

The present work has shown that a protein possessing phosphoglycerate-kinase activity can be precipitated from an aqueous extract of rabbit skeletal muscle by fractionation with acetone. About 30 % of the total activity was recovered in the 50 % acetone fraction, an approximately six-fold purification being obtained in this single step. In general, ammonium sulphate fractionation has proved unsatisfactory for purifying transphosphorylating enzymes. Similar observations were made by Askonas (1951), who adopted organic solvents such as acetone, propanol etc. for the purification. Kuby, Noda & Lardy (1954) and Noda & Kuby (1957) have used acetone fractionation in the presence of bivalent metal ions such as Mg^{2+} , Mn^{2+} or Zn^{2+} (for which the enzyme protein has a high affinity) and have succeeded in obtaining crystalline preparations of creatine phosphokinase and myokinase.

The entire fractionation procedure was highly reproducible up to the alumina-gel stage, and an overall purification of 12- to 15-fold was repeatedly obtained after the gel treatment. Further attempts to purify the enzyme with organic solvents in the presence of Mg^{2+} , Mn^{2+} or Zn^{2+} ions, by isoelectric precipitation, selective denaturation of inactive protein with chloroform or by subsequent ammonium sulphate fractionations, were not encouraging. Failure to achieve further purification by these methods raised the question whether the phosphoglycerate kinase might be a particulate enzyme like brain hexokinase (Crane & Sols, 1953). However, repeated centrifugings for various lengths of time at 100 000g did not change the specific activity of the enzyme. The purified kinase from rabbit skeletal muscle was contaminated with triose phosphate-isomerase activity and was not electrophoretically homogeneous.

There is an absolute requirement for a bivalent metal ion such as Mg^{2+} or Mn^{2+} . For the muscle enzyme Mg^{2+} ions are more effective than Mn^{2+} ions at high concentrations, whereas at low concentrations Mn^{2+} ions are more effective. Lardy & Ziegler (1945) observed the effect of K^+ ions on pyruvic phosphokinase in dialysed muscle extracts but thought that phosphoglycerate kinase was probably not activated by K^+ . These findings have now been confirmed with purified phosphoglycerate kinase from muscle.

The inhibition of muscle phosphoglycerate kinase by -SH group-binding reagents like *p*-chloromercuribenzoate, *p*-chloromercuriphenylsulfonic acid, and heavy-metal ions like Cu^{2+} ions indicates the need for -SH groups for the activity of the enzyme. The lack of inhibition by organic arsenicals or iodoacetamide is not surprising, since there are instances of -SH group enzymes that are highly sensitive to *p*-chloromercuribenzoate but not to organic arsenicals or iodoacetamide (Rose, Grunberg-Manago, Korey & Ochoa, 1954). Since both the yeast and muscle enzymes catalyse the same reaction, but only the latter appears to need -SH groups for activity, interesting possibilities regarding the mechanism of action of the enzymes arise. Dixon (1949) has proposed a mechanism for transphosphorylation, in which -SH groups combine to form -SH-magnesium-phosphate complexes. The more recent proposals of Koshland (1954) or Boyer & Harrison (1954) do not appear to assign any role to the -SH group in transphosphorylation reactions.

Some aspects of the substrate and nucleotide specificity of the phosphoglycerate-kinase reaction have been investigated, and, on the basis of the present work and the findings of Bandurski & Axelrod (1953), one may state that the enzymes from three different sources (plants, yeast and muscle) are absolutely specific for phosphoglycerate, ATP, ADP and phosphoglyceryl phosphate. The slight activity noticed with inosine triphosphate may be due to the presence of ADP or ATP in the sample. If it is due to ADP this would imply the presence of a nucleoside diphosphokinase in the phosphoglycerate kinase which would catalyse the reaction:

The Michaelis constants determined in the present study for all the substrates and activators of both yeast and muscle enzymes are comparable with the values reported by Bücher (1947). The Michaelis constant for phosphoglyceryl phosphate determined here by an isotopic method agrees closely with the value found by Bücher from studies of competitive inhibition. It is also consistent with direct measurements of the binding of the enzyme to the substrate (Oesper, 1961).

SUMMARY

1. A partial purification of the enzyme, phosphoglycerate kinase, from an aqueous extract of rabbit skeletal muscle has been achieved. The purified enzyme was not homogeneous electrophoretically and contained two components. It was free of most of the enzymes of the anaerobic

glycolytic pathway but contained triose phosphate isomerase.

2. A new assay method for studying the phosphoglycerate-kinase reaction in the forward direction was developed. The method has the advantage that it can be used with partially purified enzyme preparations containing triose phosphate isomerase and α -glycerophosphate dehydrogenase. The assay method also facilitated an independent study of inhibitors and metal ions in the enzyme reactions.

3. Phosphoglycerate kinase is activated by Mg^{2+} and Mn^{2+} ions, and by no other metal ions tested. It reacts specifically with adenosine di- and tri-phosphate, but with no other nucleotides.

4. The study of the effect of various thiol-binding agents on the muscle and yeast phosphoglycerate kinases revealed that the muscle phosphoglycerate kinase is a sulphhydryl enzyme whereas the yeast enzyme is not.

5. The Michaelis constants for the various substrates and activators obtained with the muscle enzyme are similar to the values obtained with the yeast enzyme. These values are also in good agreement with the data originally reported by Bücher (1947). The Michaelis constants of the muscle and yeast enzymes for phosphoglyceryl phosphate have been determined independently by an isotopic method.

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Digestion and Absorption of Disaccharides in Man

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Disaccharides and starch are well known to constitute the major part of the carbohydrates present in our diet. The general conception of the digestion of disaccharides, as concluded from current text-books of biochemistry, is that disaccharide hydrolases (glycosidases) are secreted with the 'succus entericus' into the intestinal lumen,

where the hydrolysis of disaccharides is believed to occur.

During a recent study on intestinal digestion and absorption in man (Borgström, Dahlqvist, Lundh & Sjövall, 1957), it was observed, however, that the glycosidase activity of the intestinal contents during digestion was too low to account for any