A Spectrophotometric Method for the Determination of Creatine Phosphokinase and Myokinase

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In the past, methods for the assay of creatine phosphokinase have been based on the chemical determination of one or other of the products of the reaction:

creatine + ATP ⇌ creatine phosphate + ADP,

where ATP = adenosine triphosphate and ADP = adenosine diphosphate. The substances most commonly determined have been creatine or creatine phosphate; e.g. Banga (1943), Askonas (1951), Narayanaswami (1952), Ennor & Rosenberg (1954), Kuby, Noda & Lardy (1954) and Chappell & Perry (1954). These methods may not always be convenient for the determination of creatine phosphokinase activity in tissue homogenates, since the amount of tissue used in reaction mixtures makes the introduction of significant amounts of endogenous substances a distinct possibility. The method described here, although not more sensitive than the methods based on the colorimetric estimation of creatine, makes it possible to measure enzymic activities in tissue homogenates and extracts diluted 2000 to 20 000 times, and to follow continuously the time course of the reaction in a total volume of about 4 ml. Under these conditions effects due to endogenous substances are negligible.

The method is based on Kornberg's assay procedure for ATP (Kornberg, 1950), whereby the formation of ATP from ADP and creatine phosphate is linked to the reduction of triphosphopyridine nucleotide (TPN). When the rate-limiting step in the reaction sequence is that catalysed by creatine phosphokinase, spectrophotometric measurement of the rate of reduction of TPN gives the rate of ATP formation, and thus the activity of the enzyme.

The method has also been applied to the determination of myokinase activity by measuring the rate of formation of ATP from ADP. The methods for the assay of myokinase due to Kalckar (1943, 1947) are laborious. The most sensitive method is probably that based on the firefly luminescence system for the estimation of ATP (Strehler & Totter, 1952), but is not convenient for general use. The coupled activities of myokinase and creatine phosphokinase have been used by Chappell & Perry (1954) to assay myokinase. The present method has the advantage of convenience over most of the older methods. The reactions are followed continuously in the spectrophotometer, and the rates catalysed by small amounts of tissue can be measured with ease.

An outline of the method has been given elsewhere (Oliver, 1954). Complete experimental details are included here.

EXPERIMENTAL

Materials and methods

Adenosine triphosphate. ATP was prepared by the method of LePage (1949); the chromatographic procedure described by Krebs & Hems (1953) showed that more than 90% of the phosphate was present in the form of ATP.

Adenosine diphosphate. ADP was obtained from Schwartz Laboratories Inc. and contained less than 1% of ATP when assayed by the chromatographic procedures above, or enzymic procedures based on those of Kornberg (1950).

Adenylic acid (AMP). This was obtained from Roche Products Ltd. Solutions of AMP were brought to pH 7·0 with NaOH before use.

Sodium creatine phosphate hexahydrate. This was synthesized from creatine by the method of Ennor & Stocken (1948).

Glucose 6-phosphate. This was a gift from Dr T. H. Wilson, Walter Reed Army Medical Center, Washington 12, D.C.

6-Phosphogluconic acid. This was prepared by bromine oxidation of glucose 6-phosphate according to Seigmiller & Horecker (1951). Chromatographic procedures (Oliver, unpublished experiments) showed that the preparation was free of glucose 6-phosphate.

Triphosphopyridine nucleotide (TPN). This was prepared by Mr D. H. Williamson after Horecker (private communication). Preparations containing 65–90% TPN were prepared by gradient elution from Dowex-1 resin and contained no diphosphopyridine nucleotide (DPN) or adenylic acid (AMP), since these substances were eluted from the column at an earlier stage. Crude preparations, in which the major impurity was AMP, contained about 10% TPN and 25% DPN.

Hexokinase and glucose 6-phosphate dehydrogenase. These were obtained together in a crude preparation of yeast hexokinase similar to that described by Slater (1953). The activity of glucose 6-phosphate dehydrogenase in the preparation was increased by taking the protein fraction precipitated from aqueous extracts of baker's yeast by 50–75% saturation with ammonium sulphate. After dialysis

to remove ammonium sulphate, it has been found convenient to freeze-dry the enzyme preparation, and to store it in the refrigerator.

Glucose 6-phosphate dehydrogenase activity. This was assayed by following the rate of reduction of TPN in the spectrophotometer at 340 mμ. when glucose 6-phosphate was added to a reaction mixture consisting of 0·05 M barbitone buffer pH 8·6, 0·01 M-$MgCl_2$, 0·1 M-KCl, 10^{-4} M TPN, and the yeast preparation which had previously been incubated until the optical density became steady.

Hexokinase activity. This could not be determined quantitatively in such a system, since hexokinase is more active than glucose 6-phosphate dehydrogenase in the yeast preparation. Activities less than maximum were measured by following the rate of reduction of TPN in the spectrophotometer at 340 mμ. when ATP (final concentration 0·001 M) was added to a reaction mixture containing 0·05 M barbitone buffer pH 8·6, 0·01 M-$MgCl_2$, 0·1 M-KCl, 0·01 M glucose, 10^{-4} M TPN, and the yeast preparation which had previously been incubated until the optical density became steady.

Spectrophotometer. A Unicam spectrophotometer model SP 500 was used for the determination of optical density at 340 mμ. Cuvettes (1 cm.) were used and readings were made against a blank of barbitone buffer. The temperature of the cuvettes was controlled by a water-jacketed cell compartment made by Mr G. Fletcher according to Campbell & Simpson (1953).

Syringe. An Agla micrometer syringe (Burroughs Wellcome and Co.), fitted with an elongated glass tip bent through a right angle, was used to deliver volumes up to 50 μl. of the muscle homogenates to the reaction cuvettes.

Muscle homogenates. These were prepared from the hind-leg muscles of male albino rats immediately after killing by a blow on the head. The muscle was minced in a cold mincer, a sample weighed and then treated for 3 min. in a chilled Waring Blendor (micro cup) with 6 vol. of ice-cold 0·1 M-KCl. The homogenates were strained through gauze to remove coarse particles and ligaments and stored at 0° until required. For assay, samples were diluted tenfold with ice-cold 0·1 M-KCl immediately before use. If it was necessary to retain a preparation overnight, it was stored at −15°.

Buffer system. In using barbitone buffer at pH 8·6 we have followed the work of Banga (1943). At this pH hexokinase is stable and of high activity.

Principle of the method

Use is made of the assay procedure for ATP (Kornberg, 1950) in which ATP reacts with glucose in the presence of Mg^{2+} and hexokinase, forming glucose 6-phosphate (Eqn. 1), which is then oxidized by glucose 6-phosphate dehydrogenase with simultaneous reduction of TPN (Eqn. 2).

$$\text{Glucose} + \text{ATP} \rightarrow \text{glucose 6-phosphate} + \text{ADP}, \quad (1)$$

$$\text{glucose 6-phosphate} + \text{TPN} \rightarrow \text{6-phosphogluconic acid} + \text{TPNH}_2. \quad (2)$$

The rate of reduction of TPN is measured by observing the rate of change of optical density in the spectrophotometer at 340 mμ. (Fig. 1). Although the yeast preparation containing the hexokinase and glucose 6-phosphate dehydrogenase is very impure, it is free from several enzymes whose presence would be disadvantageous. Fig. 2 shows that two of such enzymes, glucose dehydrogenase and 6-phosphogluconic acid dehydrogenase, are completely absent. The system is completely specific for TPN, and it is therefore unnecessary, except where other considerations arise, to use purified preparations of TPN. In practice crude preparations containing about 10% of TPN and 25% of DPN have often been used in these studies.

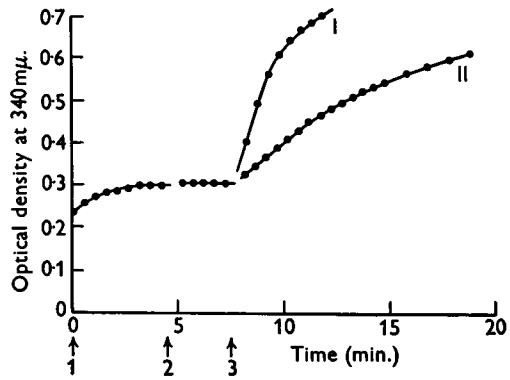

Fig. 1. Hexokinase and glucose 6-phosphate dehydrogenase in yeast enzyme preparation. At zero time both reaction mixtures consist of 0·05 M barbitone buffer pH 8·6, 0·01 M-$MgCl_2$, 0·1 M-KCl and 10^{-4} M TPN (final concentrations). At time (1), 0·1 ml. of 'coupling enzyme' containing 1 mg. dry wt. was added to both mixtures I and II with stirring. At time (2), 0·2 ml. of 0·5 M glucose added to both, with stirring, bringing final concentration to 0·02 M. At time (3), 0·1 ml. of 0·02 M glucose 6-phosphate added to I, and 0·1 ml. of 0·02 M ATP added to II with stirring. Final concentrations of these two substrates was 0·001 M.

In these coupled reactions, in order that the final rate of reduction of TPN shall be equal to the rate of formation of ATP, the activities of hexokinase and glucose 6-phosphate dehydrogenase must be in excess of the activity of the ATP-forming reaction, i.e. the latter reaction must be the rate-determining step. This situation can be arranged by trial. Reaction mixtures containing buffer, TPN, glucose, ADP, creatine phosphate and increasing amounts of the 'coupling enzyme' (yeast preparation containing hexokinase and glucose 6-phosphate dehydrogenase) were used to measure the rate of ATP formation catalysed by a constant amount of a highly diluted muscle homogenate. Under these conditions, ATP formed by creatine phosphokinase and myokinase is detected. The first level of addition to give a maximum rate was used in subsequent quantitative experiments. In our experiments 4 mg. of the dried preparation of the 'coupling enzyme' gave quantitative assay of creatine phosphokinase or myokinase activity in 0–20 μl. of a muscle homogenate of dilution one part in 60, i.e. 0–0·33 mg. wet weight of muscle. Under these conditions the activity of an enzyme which catalyses the formation of ATP is taken as shown by the initial steady rate of reduction of TPN, which can be obtained graphically from the rate curves of optical density versus time. Using the extinction coefficients for reduced TPN given by Horecker

& Kornberg (1948), i.e. $6{\cdot}22 \times 10^6$ cm.²/mole, activities can be expressed in terms of micro-moles of ATP formed per unit time, since each molecule of ATP formed gives rise to one molecule of reduced TPN. This relation holds only if the enzyme preparation is free of 6-phosphogluconic acid dehydrogenase, and glucose 6-phosphate is the only substance effective for the reduction of TPN. Our system has been tested in this respect.

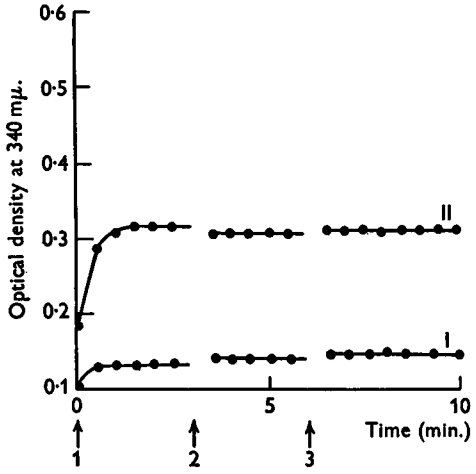

Fig. 2. Yeast enzyme preparation. At zero time reaction mixtures consist of 0·05 M barbitone buffer pH 8·6, 0·01 M-$MgCl_2$ and 0·1 M-KCl. Mixture I contains 10^{-4} M DPN and II contains 10^{-4} M TPN. At time (1), 0·1 ml. of 'coupling enzyme' added to both with stirring. At time (2), 0·2 ml. of 0·5 M glucose added to both with stirring. At time (3), 0·1 ml. of 0·02 M glucose 6-phosphate added to I, and 0·1 ml. of 0·02 M 6-phosphogluconic acid added to II with stirring. The DPN contained no TPN.

In all experiments it was found necessary to incubate the 'coupling enzyme' with the substrates for about 5 min. before starting the reaction to be studied. During this preliminary incubation some reduction of TPN takes place (see Fig. 1) which is allowed to come to completion before the required reaction is started. This effect is due to traces of ATP or glucose 6-phosphate in the reagents, and also to a substrate in the yeast preparation which has not been fully identified.

RESULTS

Measurement of creatine phosphokinase activity

The work for which this assay procedure has been developed has been concerned with enzymic activities in homogenates or tissue extracts. Accordingly, the isolation of a single phosphate transfer reaction for study has been one of the problems. The reaction catalysed by creatine phosphokinase (Eqn. 3) can be measured in terms of the production of ATP, but in tissue homogenates ATP will also arise from ADP by the activity of myokinase (Eqn. 4).

$$\text{Creatine phosphate} + \text{ADP} \rightleftharpoons \text{creatine} + \text{ATP}, \quad (3)$$

$$2\text{ADP} \rightleftharpoons \text{ATP} + \text{AMP}. \quad (4)$$

This effect has been overcome by adding excess of AMP to the reaction mixture. In Fig. 3 is seen the effect of AMP on the rate of reduction of TPN in a reaction mixture containing TPN, the 'coupling enzyme', glucose, ADP and a muscle homogenate.

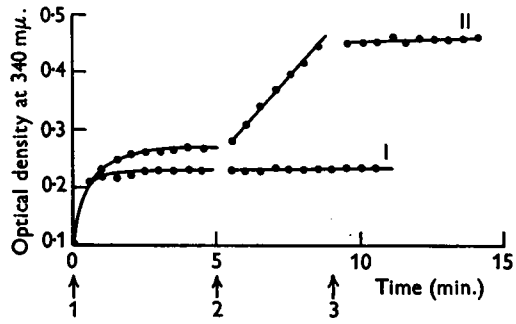

Fig. 3. Effect of AMP on myokinase. At zero time reaction mixtures consist of 0·05 M barbitone buffer pH 8·6, 0·01 M-$MgCl_2$, 0·1 M-KCl, 0·001 M ADP, 0·02 M glucose and 10^{-4} M TPN. Mixture I contains in addition, 0·01 M AMP. At time (1), 0·4 ml. of 'coupling enzyme' (containing 4 mg. dry wt.) was added to both with stirring. At time (2), 10 μl. of a muscle homogenate diluted 1 in 60 with 0·1 M-KCl were added to both with stirring. At time (3), 0·2 ml. of 0·2 M AMP was added to II, bringing final concentration to 0·01 M.

When the concentration of AMP is ten times that of ADP, the myokinase activity is completely inhibited. This makes it possible to distinguish between the ATP-forming reactions of creatine phosphokinase and myokinase. The measurement of creatine phosphokinase activity in the presence of myokinase then becomes possible. In practice, a control from which residual myokinase activity can be determined is always included in assays of creatine phosphokinase. The control contains, besides TPN, glucose and the 'coupling enzyme', only AMP and ADP in the ratio of 10 to 1 (see Fig. 4). Errors caused by myokinase activity under the conditions of assay for creatine phosphokinase can thus be evaluated, but in fact these are rarely significant.

The effect of high concentrations of AMP on the activity of creatine phosphokinase was tested. In experiments in which the ADP concentration remained constant at 0·001 M and the AMP concentration was increased from 0·0025 to 0·015 M, the activity of creatine phosphokinase did not change appreciably until the highest concentration of AMP

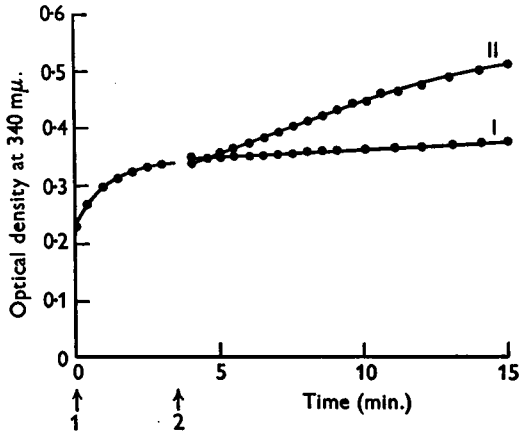

Fig. 4. Determination of creatine phosphokinase. At zero time the reaction mixtures consist of 0·05 M barbitone buffer pH 8·6, 0·01 M-$MgCl_2$, 0·1 M-KCl, 0·02 M glucose, 0·001 M ADP, 0·01 M AMP and 10^{-4} M TPN. Mixture II contains also creatine phosphate, 0·01 M. At time (1), 0·4 ml. of 'coupling enzyme' (containing 4 mg. dry wt.) is added to both mixtures. At time (2), 10 μl. of a muscle homogenate diluted 1 in 60 with 0·1 M-KCl are added to both I and II with mixing. Curve I is the control from which residual myokinase activity can be determined. The tangent to the linear portion of Curve II gives the rate of optical density change which is proportional to the ratio of reduction of TPN. This allows the evaluation of the rate of ATP formation, which is defined as the activity of the enzyme.

Table 1. *Effect of AMP on activity of creatine phosphokinase*

Reaction mixture consists of 'coupling enzyme' in 0·05 M barbitone buffer pH 8·6, 0·01 M-$MgCl_2$, 0·1 M-KCl, 0·02 M glucose, 0·001 M ADP, 0·01 M creatine phosphate, 10^{-4} M TPN, and 10 μl. of muscle homogenate containing about 20 mg. wet wt./ml.

Concn. of AMP (mM)	Activity of creatine phosphokinase (μmoles ATP/hr./mg. wet wt.) (i)	(ii)	(iii)
2·5	4·85	—	—
5·0	5·17	6·20	2·75
10	—	6·00	2·64
15	—	5·18	2·55

Table 2. *Effect of creatine phosphate on activity of creatine phosphokinase*

Reaction mixture otherwise as in Table 1. AMP 0·01 M.

Concn. of creatine phosphate (mM)	Activity of creatine phosphokinase (μmoles ATP/hr./mg. wet wt.)
10	7·55
20	7·55
30	7·61
40	7·90

Table 3. *Effect of ADP on activity of creatine phosphokinase*

Reaction mixture otherwise as in Table 1. AMP 0·01 M.

Concn. of ADP (mM)	Activity of creatine phosphokinase (μmoles ATP/hr./mg. wet wt.) (i)	(ii)	(iii)
0·5	8·25	5·38	5·80
1·0	9·90	5·44	—
2·0	8·72	4·40	5·62

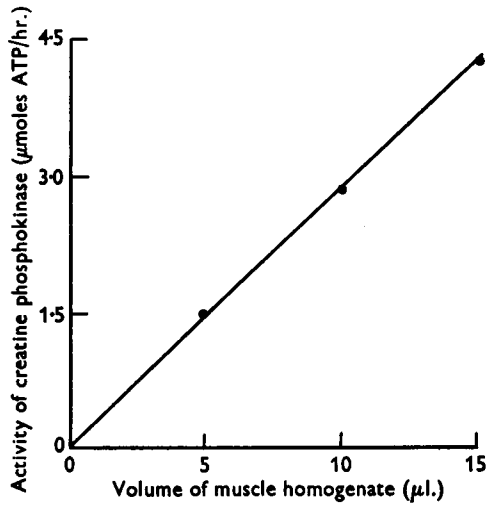

Fig. 5. Concentration of creatine phosphokinase and activity. The data were obtained from measurements of the linear rate of reduction of TPN, catalysed by increasing amounts of muscle homogenate. Reaction mixtures as in Fig. 1 with the addition of 0·01 M AMP, 0·01 M creatine phosphate, 0·001 M ADP and 0·02 M glucose. Activity expressed as μmoles ATP produced/hr.

was reached, when a slight decrease in activity was seen. Accordingly the highest concentration of AMP used was 0·01 M (see Table 1).

When the concentration of creatine phosphate was raised through the range 0·01–0·04 M in four steps, only a small increase in activity could be measured, and the concentration usually used, in order to minimize the amount of creatine phosphate required, was therefore 0·01 M (see Table 2).

When the concentration of ADP was varied from 0·0005 to 0·002 M no consistent increase in the activity of creatine phosphokinase in a muscle homogenate could be measured, and the usual concentration of ADP in the system was 0·001 M (see Table 3).

The above concentrations of ADP, AMP and creatine phosphate were used in experiments to establish the validity of the assay method. In Fig. 5 the activity of creatine phosphokinase in a

muscle homogenate is plotted against the relative concentration of the enzyme. The activities calculated from the initial steady rate of reduction of TPN (see Fig. 4) are directly proportional to relative concentration. In a further experiment the activity of creatine phosphokinase was determined in two identical samples of a muscle homogenate by the spectrophotometric method, and an independent method previously used in this laboratory. This method depends on the analysis of creatine phosphate in a reaction mixture after its separation on a paper chromatogram. An alkaline solvent described by Hanes & Isherwood (1949) consisting of a mixture of *n*-propanol, concentrated ammonia and ethylenediaminetetraacetic acid, has been used by the author for the separation of creatine phosphate from adenosine phosphates, inorganic phosphate, triose and hexose phosphates in tissue extracts. This technique is easily applicable to enzyme reaction mixtures after deproteinization with trichloroacetic acid and neutralization of the extracts, and in this way creatine phosphate was isolated for analysis from a reaction mixture similar to those used in the other method. The rate of breakdown of creatine phosphate determined by phosphate analysis on spots cut from the chromatogram according to Eggleston & Hems (1952) then gave the activity of creatine phosphokinase. This method is very much less sensitive than the spectrophotometric method and in order to adjust conditions so that the decomposition of creatine phosphate could be detected analytically, the 'coupling enzyme' was added to the reaction mixture together with glucose. This has the effect of maintaining the starting concentration of ADP, which in turn extends the time of breakdown of creatine phosphate and also the degree of decomposition. It is then possible to follow the reaction, using concentrations of ADP and creatine phosphate identical with those in the spectrophotometric system. Results of a typical experiment gave the activity of creatine phosphokinase at 25° to be 2·85 by the spectrophotometric method and 2·33 by the analytical procedure (μmoles ATP formed/hr./mg. wet weight of muscle). Since the analytical procedure is less accurate than the spectrophotometric method, these values represent fair agreement.

Measurement of myokinase activity

The reaction catalysed by myokinase

$$2ADP \rightleftharpoons ATP + AMP$$

gives rise to ATP, and the activity of this enzyme can thus be measured by a method similar to that described for creatine phosphokinase. When the formation of ATP is linked to the reduction of TPN in the presence of glucose, hexokinase and glucose 6-phosphate dehydrogenase, the reaction proceeds to the right, and since each molecule of ATP gives rise to one molecule of reduced TPN and one molecule of ADP, the activity can be calculated.

In this system purified TPN was used, since the impure samples of TPN contained adenylic acid as a major impurity. This AMP may inhibit the ATP-forming reaction and so give rise to spuriously low values for activity. Accordingly, in a system similar to that used for the assay of creatine phosphokinase, purified TPN, the 'coupling enzyme', glucose and ADP form the reaction mixture necessary to assay myokinase activity. Working in such a system, with the 'coupling enzyme' added in excess, the effect of ADP concentration on the activity of myokinase in a muscle homogenate was studied. Raising the concentration of ADP from 0·001 to 0·004 M did not increase the activity measured and in fact a slight decrease was noticed (see Table 4). Accordingly, all assays

Table 4. *Effect of ADP on activity of myokinase*

No AMP or creatine phosphate. Reaction mixture otherwise as in Table 1.

Concn. of ADP (mM)	Activity of myokinase (μmoles ATP/hr./mg. wet wt.)
1·0	9·03
2·0	8·60
3·0	8·12

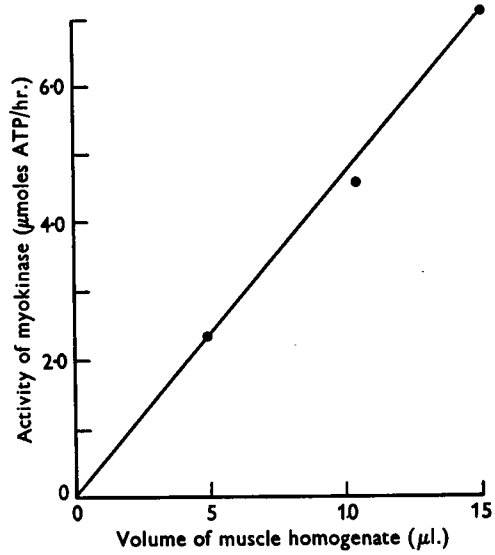

Fig. 6. Concentration of myokinase and activity. The data were obtained from measurements of the linear rate of reduction of TPN catalysed by increasing amounts of muscle homogenate. Reaction mixtures as in Fig. 1 with the addition of 0·001 M ADP and 0·02 M glucose. Activity expressed as μmoles ATP produced/hr.

were carried out at a concentration of 0·001 M ADP. At 0·001 M ADP assays of myokinase in different amounts of the same muscle homogenate gave a straight line when plotted against relative concentration of enzyme (see Fig. 6).

Application to the assay of enzyme in tissue

The method has been applied to the assay of enzymic activities in various tissues of the rat. With skeletal muscle and heart muscle the activities were determined in 10–20 μl. of homogenates diluted one part in 60. The homogenates of heart muscle in 0·1 M-KCl were prepared by grinding the heart with sand in a chilled mortar. Assays on brain, kidney, liver and spleen were obtained with extracts since the diluted homogenates were too opaque. The tissue was homogenized in 6 vol. of ice-cold 0·1 M-KCl, centrifuged, and the tissue debris re-extracted twice with 3 vol. of saline. The combined supernatants were clarified by centrifuging in the cold at 19 000 *g*, and activities determined by the usual methods. Further extraction of the tissue residue yielded insignificant amounts of enzyme (less than 5% of the total activity obtained by the above procedure).

DISCUSSION

In this work the average time taken for a single assay once the necessary reagents are to hand is about 20 min. In the course of a routine working day, assays of creatine phosphokinase and myokinase can be carried out on all the principal tissues of a single experimental animal.

The sensitivity of the spectrophotometric method enables the use of highly diluted homogenates and tissue extracts, and in this way reactions can be studied free of interfering effects resulting from the introduction of unknown amounts and kinds of endogenous substances. Very small amounts of ATP or ADP can function catalytically in promoting decomposition of creatine phosphate when AMP is added to systems containing creatine phosphokinase and myokinase (see Ennor & Rosenberg, 1954). The occurrence of small amounts of ADP in tissue preparations as used by Narayanaswami (1952) probably accounts for the results obtained by this author, who observed creatine phosphate decomposition in brain homogenates in the presence of AMP as the sole added acceptor. However, using the present method, it has been shown (Oliver, 1954) that there is no reaction between creatine phosphate and AMP in muscle homogenates or brain extracts which were highly diluted, and that reaction occurred only when ADP was added.

Table 5. *Activities of creatine phosphokinase and myokinase in rat tissues*

Results stated in μmoles ATP/hr./mg. wet wt. Temperature 30°. pH 8·6.

	Creatine phosphokinase				Myokinase	
			Rat 3			
Tissue	Rat 1	Rat 2	No cysteine	0·01 M cysteine	Rat 1	Rat 2
Skeletal muscle	14·9	18·0	12·1	26·0	18·6	23·3
Heart muscle	2·38	2·95	3·50	7·01	5·90	7·40
Brain	0·96	1·36	0·87	1·62	1·88	2·03
Kidney	0·16	0·01	—	—	1·22	1·68
Liver	0·02	—	0·06	0·07	0·38	0·93
Spleen	2·10	1·42	0·14	0·22	1·80	5·36

Table 5 presents the results of two typical assays on various tissues of the rat. Tissues were obtained from a single animal immediately after killing by a blow on the neck.

The effect of cysteine on the activity of creatine phosphokinase in tissue homogenates and extracts was also measured (see also Chappell & Perry, 1954). At a concentration of 0·01 M cysteine, the activity of creatine phosphokinase is about double that measured in its absence (Table 5). In tissue preparations aged 24–48 hr. at 0°, the activity in the presence of cysteine is about the same as that in the fresh preparations, but the activity in the absence of cysteine is lowered 10–20% by ageing.

No effect of cysteine on the activity of myokinase in tissue preparations could be demonstrated.

In the determination of creatine phosphokinase activity by the present method the reactions are such that the concentration of ADP, although small, is maintained approximately constant over the time required for the assay. In the assay of myokinase, the ADP concentration falls by less than 10% over the whole of the reaction curve. This does not appear to affect the evaluation of reaction velocities since these curves are usually linear for the first 3–5 min. after mixing, giving between six and ten points on which to calculate the velocity.

A disadvantage of the method is connected with the activity of ATPase in tissue homogenates. If the activity of this enzyme is high compared with the ATP-forming enzyme, then the ATP cannot be coupled to the reduction of TPN. The extent to which the activity of the 'coupling enzyme' can be raised is limited, and so successful competition between the 'coupling enzyme' and the ATPase cannot always be arranged. If too much 'coupling enzyme' is added, then the reduction of TPN due to an unidentified substance in the preparation makes the optical density too high to be measured with the accuracy required for the determination of reaction velocities. For this reason it has been found advantageous to reduce the ATPase activity of some tissues, e.g. brain (see also Oliver, 1954), liver, kidney and spleen. If the tissues are homogenized in 0·1 M-KCl and centrifuged at 19000 *g* for 15 min., then much of the ATPase is removed.

The rates of reaction catalysed by 0·1–0·5 mg. wet weight of muscle are usually measured in routine work, while for other tissues ten times this amount, i.e. about 5 mg., can be satisfactorily assayed. The use of such a method for those interested in studies where only small amounts of tissue are available can be appreciated.

SUMMARY

1. A rapid spectrophotometric method has been developed for the assay of creatine phosphokinase and myokinase in tissue extracts and homogenates. The formation of ATP by these enzymes is coupled to the reduction of TPN and the rate of reduction gives a measure of the activities.

2. The sensitivity of the method enables the determination of enzymic activities in tissue preparations diluted as much as one part in 20000 corresponding to a quantity of tissue of about 300 μg. wet weight. Under these conditions reactions due to endogenous substances are negligible.

3. The method has been applied to the measurement of creatine phosphokinase and myokinase in various tissues of the rat.

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Peroxidatic Activity of Catalase

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The peroxidatic function of catalase consists in its ability to catalyse the oxidation by hydrogen peroxide of different substances, such as alcohols (Keilin & Hartree, 1936, 1945*b*), nitrite (Heppel & Porterfield, 1949), and formate (Chance, 1948). This property was first revealed in experiments (Keilin & Hartree, 1936) in which the peroxide was produced as the result of a primary enzymic reaction, as for example the oxidation of hypoxanthine by xanthine oxidase. When this reaction was allowed to take place in the presence of catalase and ethanol, the latter underwent oxidation to aldehyde. Attempts to obtain peroxidatic oxidation of ethanol by preformed free H_2O_2 in presence of catalase were at first unsuccessful (Keilin & Hartree, 1936). Later on, however, very small

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