2. The enzyme was isolated in an electrophoretically homogeneous form by adsorption on to cadmium sulphate, followed by fractional precipitation with ammonium sulphate.

3. On electrophoresis the purified enzyme had an isoelectric point of about pH 5·3. Ultracentrifuging a slightly impure preparation showed a minimum mean molecular weight of approximately 44 000.

4. The material was extremely toxic when injected intravenously into rabbits, and a concentration of 75 $\mu\mu$g./ml. clotted human plasma in 24 hr.

Our best thanks are due to Dr H. J. Rogers for many helpful discussions and for laboratory facilities, to Dr P. A. Charlwood for examination of material in the ultracentrifuge at the National Institute for Medical Research, Mill Hill, London, and to Glaxo Laboratories, Greenford, Middlesex for laboratory facilities. We also wish to thank Mr J. W. Ottewill and Mr W. Phillippe for their valuable help in constructing some of the equipment used. The work was made possible by a research grant from the South West Metropolitan Regional Hospital Board.

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The Phosphotransferase Activity of Phosphatases

1. SPECTROPHOTOMETRIC METHODS FOR THE ESTIMATION OF SOME PHOSPHATE ESTERS AND OTHER COMPOUNDS

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For the kinetic study of the 'phosphotransferase' (Dixon, 1949; Dixon & Webb, 1953) reaction catalysed by phosphatases (Morton, 1952, 1955*a*, 1958*a*, *b*) it became necessary to estimate phosphate esters without separation from the reaction mixture. It was required that the methods used would be accurate, sufficiently sensitive to measure between 0·05 and 0·2 μmole of a compound, and convenient enough to enable a large number of determinations to be handled.

Methods based on reactions catalysed by highly specific dehydrogenases appeared to meet all the above criteria; dehydrogenation of a substrate is coupled with the reduction of cytochrome *c* or diphosphopyridine nucleotide, which is estimated by changes in light-absorption at appropriate wavelengths. This paper describes the estimation of D-hexose phosphates, L-α-glycerophosphate, D-glyceraldehyde phosphate, dihydroxyacetone phosphate and phosphocreatine. Extension of these methods enables succinate, L-lactate and a variety of other compounds to be estimated.

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MATERIALS

Enzyme preparations

Yeast alcohol dehydrogenase. The crystalline enzyme was prepared from dried Fleischman's yeast by the method of Racker (1950).

Rabbit muscle L-α-glycerophosphate dehydrogenase. The preparation was based on the observations of Green (1936). Rabbit skeletal muscle (about 300 g.) was chilled in crushed ice immediately after removal from the animal, and finely minced and extracted twice (30 and 15 min.) with two

portions of about 300 ml. of 3 mM-KOH. The suspension was filtered through muslin and the filtrate used as source of the rabbit-muscle fractions A and B (see below).

The residual muscle was washed three times by suspending in about 1 l. of water at about 2°, and filtering through muslin and then squeezing the retained material.

The residue was ground with about 250 g. of acid-washed sand in a prechilled earthenware mortar with addition of about 100 ml. of 0·2N-Na_2HPO_4. The paste was dispersed in 2 vol. of water at 0° and then centrifuged at 2500 g for 30 min. at 0–4°. The supernatant was cooled to 0°, then centrifuged at 14 000 g for 1 hr. at 0–4°. The precipitate was dispersed with a Potter–Elvehjem-type homogenizer in 10 ml. of 0·15M-NaCl and the suspension was adjusted to pH 7·5 with 0·1N-NaOH. The dispersion was again centrifuged at 14 000 g for 1 hr. at 0–4° and the precipitate redispersed in about 3 ml. of 0·15M-NaCl at 0°. The suspension was stored at 0°. Activity rapidly declined after about 3 days.

Rabbit-muscle fractions A and B. The alkaline extracts from the rabbit skeletal muscle (see above) were combined. Fraction A was prepared from this extract as described by Racker (1947) for the 'glycerophosphate dehydrogenase and aldolase' fraction. Fraction B was also prepared from the same extract by Slater's (1953) modifications of Racker's (1947) 'phosphohexokinase' preparation. Fractions A and B were stored and used as described by Slater (1953).

Rabbit-muscle glyceraldehyde-3-phosphate dehydrogenase. Crystalline enzyme was prepared from rabbit muscle essentially as described by Cori, Slein & Cori (1948). The crystals were collected by centrifuging at 10 000 g for 10 min. at 0–4° and dissolved in 0·03M-sodium pyrophosphate–HCl buffer, pH 8·0, just before use.

Creatine phosphokinase. This was kindly supplied by Dr B. A. Askonas.

Reduced diphosphopyridine nucleotide–cytochrome c *reductase.* Initially, a suspension of microsomes from calf intestinal mucosa (Morton, 1954; Bailie & Morton, 1955) was used. The sedimented microsomes were suspended in 0·05M-sodium phosphate buffer, pH 7·4, with a Potter–Elvehjem-type homogenizer, and centrifuged at 3000 g for 30 min. at 0°. The cloudy supernatant was stored at 0°.

In later work the enzyme was prepared from pig-heart muscle according to Mahler, Sarkar, Vernon & Alberty (1952), up to the first ammonium sulphate precipitation. The precipitate was dissolved in 0·05M-glycylglycine buffer, pH 7·4, containing 0·5% of serum albumin, and stored at −14°.

Yeast lactic dehydrogenase. The crystalline preparation of Appleby & Morton (1954, and in preparation) was used. The crystals were washed with 0·05M-sodium lactate, and dissolved in 0·5M-NaCl and then dialysed anaerobically against a solution containing 0·5M-NaCl, 0·05M-sodium pyrophosphate–HCl buffer, pH 6·6, and 10 μM-sodium ethylenediaminetetra-acetate.

Muscle lactic dehydrogenase. This was prepared from rabbit skeletal muscle according to Askonas (1951).

Fructose diphosphatase. This was prepared from ox kidney as described by Morton (1958*a*).

Chemicals

Diphosphopyridine nucleotide (DPN), about 33% pure, and adenosine triphosphate (ATP) prepared by the late Mr J. Morgan were used in the initial studies. The ATP present in the DPN seriously interfered with some of the determinations (see later), and was converted into other compounds by hydrolysis in HCl (pH 1·5) for 4 min. at 100°. The solution was then cooled and adjusted to pH 6·8 with N-NaOH.

In later work DPN (92% pure) from the Sigma Chemical Co. was used.

Reduced diphosphopyridine nucleotide (DPNH) was prepared by enzymic reduction of DPN as described by Racker (1950).

Cytochrome c. This was prepared from horse heart or ox heart according to Keilin & Hartree (1945).

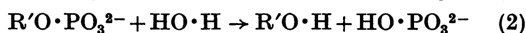
Phosphate esters. Previous papers (Morton, 1955*b, c*) give the sources of these compounds.

METHODS AND RESULTS

Measurement of phosphate transfer

Preliminary studies. Investigation of the kinetics of hydrolysis of phenyl phosphate (Morton, 1952, 1957) had shown that substrates of alkaline phosphatases are hydrolysed very slowly, or not at all, at about pH 9·5 (at 37°) and if the substrate is low in concentration (about 0·1 mM to 10 μM). It therefore appeared possible that conditions could be chosen such that any new ester synthesized by a phosphotransferase reaction (1) would be only slowly hydrolysed by the phosphatase (2), whereas hydrolysis of the initial substrate by the phosphatase (3) would proceed quite rapidly.

$$RO{\cdot}PO_3^{2-} + R'O{\cdot}H \rightarrow RO{\cdot}H + R'O{\cdot}PO_3^{2-} \quad (1)$$



$$R'O{\cdot}PO_3^{2-} + HO{\cdot}H \rightarrow R'O{\cdot}H + HO{\cdot}PO_3^{2-} \quad (2)$$

$$RO{\cdot}PO_3^{2-} + HO{\cdot}H \rightarrow RO{\cdot}H + HO{\cdot}PO_3^{2-} \quad (3)$$

The methods described by Axelrod (1948), Appleyard (1948), Meyerhof & Green (1950) and by Hanes & Isherwood (1949) were found unsuitable for estimation of small quantities of ester phosphate (e.g. glucose 6-phosphate) in the presence of large amounts of donor phosphate (e.g. phosphocreatine). However, it was found that with glucose, fructose or glycerol as the acceptors for the transferred phosphate group the product formed is capable of estimation by specific and sensitive enzymic methods.

General procedure. Glass tubes (approx. 6 mm. × 60 mm.) were marked at 0·35, 0·4 and 0·45 ml. and at 2 ml. volumes. Rubber stoppers for the tubes were extracted with warm 5% (w/v) trichloroacetic acid and then with boiling water. The stoppers were immersed in boiling water and dried immediately before use.

The acceptor was weighed into a tube and buffer, phosphate donor and magnesium salt were added. The acceptor was dissolved and the volume was adjusted to 0·35, 0·4 or 0·45 ml. (as appropriate) with water. The tube was brought to 38° in a water bath and enzyme (0·05–0·1 ml.) was added. The stopper was immediately inserted, the contents of the tube were thoroughly mixed by inversion and the tube was replaced in the water bath. This method was

used since some of the solutious, such as 4 M-glucose and 5 M-glycerol, were very viscous. After the reaction period (usually 5 min. at 38°), 0·1 ml. of 20% (w/v) trichloroacetic acid was added, the stopper was re-inserted and the contents of the tube were mixed for 15 sec. and then adjusted to pH 8·0 with a predetermined volume of 0·5 N-NaOH. The volume was then adjusted to 2 ml. with water, and the tube stoppered and the contents were mixed. The tube was then held in an ice bath. Suitable portions (0·1–0·5 ml.) were used for estimation of inorganic phosphate, phenol or creatine (according to the donor used) and acceptor phosphate formed. Enzyme was added to the control tube immediately after the trichloroacetic acid.

Unless otherwise specified, all estimations of newly formed phosphate esters were carried out with a Beckman Model DU spectrophotometer at room temperature with quartz cuvettes (1 cm. light path).

Estimation of phosphorylated compounds

Fructose 1:6-*diphosphate*, D-*glyceraldehyde* 3-*phosphate and dihydroxyacetone phosphate*. Procedure A of Slater (1953) was followed. There was no reaction with added glucose 1-phosphate, fructose 6-phosphate or fructose 1-phosphate. Some preparations reacted with glucose 6-phosphate owing to contaminant phosphohexokinase. This was removed by dissolving the enzyme paste in a minimum of water at 0°, and adjusting the pH to about 6·0 and holding for 30 min. After readjusting to pH 7·5, the mixture was filtered through a thin layer of Hyflo Super-Cel and the filtrate was stored at 0° until used.

Hexose monophosphates. Procedure B (a) of Slater (1953) was used. This procedure estimates glucose 6-phosphate, glucose 1-phosphate and fructose 6-phosphate, as well as hexose diphosphate which, however, may be estimated separately as above. Synthetic fructose 1-phosphate reacted rather slowly in this system. However, an estimate of fructose 1-phosphate was obtained by using about three times the normal amount of rabbit-fractions A and B. Because of the relatively high blank value, however, this estimate is less accurate than that for the other compounds mentioned.

With very high concentrations of glucose, there was a continuous slow oxidation of reduced DPN. However, extrapolation to obtain the true change in optical density (see Slater, 1953, fig. 1) enabled an accurate determination of the hexose monophosphate present to be made.

Differentiation of fructose diphosphate, glyceraldehyde phosphate and dihydroxyacetone phosphate. Slater's (1953) procedure A estimates the sum of fructose diphosphate and triose phosphates. It was found that the amount of fructose diphosphate in a mixture containing triose phosphates could be estimated from the loss in reactive component after hydrolysis catalysed by the specific fructose diphosphatase of ox kidney (Gomori, 1943). Slater's procedure A was used for the determinations since the products of hydrolysis of fructose diphosphate by the kidney enzyme are fructose 6-phosphate and inorganic phosphate.

The D-glyceraldehyde phosphate in the same mixture was then specifically estimated by measuring the reduction of DPN at 340 mμ in the presence of sodium arsenate and glyceraldehyde phosphate dehydrogenase essentially as described by Cori *et al.* (1948).

Phosphocreatine. This was estimated with creatine phosphokinase and procedure B (b) as described by Slater (1953).

L-α-*Glycerophosphate*. The method depends on the reduction of heart-muscle ferricytochrome *c* by the particle-bound L-α-glycerophosphate dehydrogenase of skeletal muscle according to reaction 4:

L-α-Glycerophosphate + ferricytochrome c $\rightarrow$
dihydroxyacetone phosphate + ferrocytochrome c (4)

This reaction is catalysed by an enzyme which is firmly attached to lipoprotein particles and which, at the time of these studies, had not been obtained in true solution.

Four cuvettes are used for the estimation. The first contains 1·5 μmoles of KCN (at pH 8·0), 0·1 μmole of ferricytochrome *c*, 30 μmoles of $MgCl_2$, 150 μmoles of glycylglycine buffer, pH 8·0, about 0·2 ml. of enzyme and 0·2–0·5 ml. of the test solution to bring the volume to 3 ml. The test solution is omitted from the second cuvette, cytochrome from the third and enzyme from the fourth. Water replaces the missing component. Optical densities were measured before and after addition of the final components. The contents of the cuvettes were mixed with plastic stirrers (see Dixon, 1954) before all readings, which were continued until there was no change between successive 10 min. readings.

Fig. 1 shows a typical determination. E_2 and E_3 are the initial optical densities (corrected for the dilution on adjustment to 3 ml. volume) and E_2' and E_3' the extrapolated

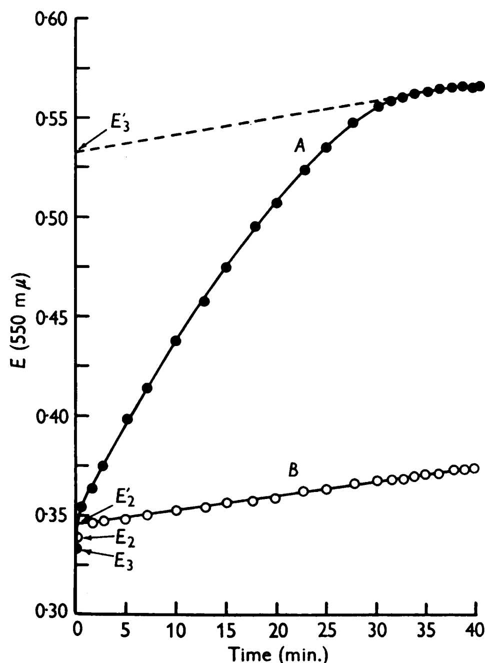


Fig. 1. Estimation of α-glycerophosphate with rabbit-muscle cytochrome *c*-linked L-α-glycerophosphate dehydrogenase and cytochrome *c*. E_2 and E_3 are the initial optical densities (corrected for the dilution on adjustment to 3 ml. volume) and E_2' and E_3' the extrapolated densities for the control (curve *B*) and test (curve *A*) respectively. Other details are given in the text.

optical densities for the control and test cuvettes respectively at the completion of the reaction (cf. fig. 1, Slater, 1953). The change in optical density (ΔE_{550}) due to the compound being estimated thus equals $(E_3' - E_3)-(E_2' - E_2)$ When the final volume of reactants is 3 ml., the amount (in μmoles) of L-α-glycerophosphate is

$$0{\cdot}5 \times (E_{550} \times 0{\cdot}154),$$

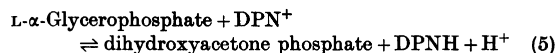
since 2 moles of cytochrome *c* are reduced for each mole of L-α-glycerophosphate. The factor 0·154 was calculated from the molar-extinction coefficients of reduced and oxidized cytochrome *c* (see Theorell, 1936).

The recovery of added DL-α-glycerophosphate from 0·05 to 0·2 μmole/cuvette was between 90 and 94%.

Although the enzyme preparation is substantially free of ferrocytochrome *c* oxidase, normally KCN (0·5 mM) was included in the reaction mixture as a precaution against oxidation of the cytochrome. Potassium ferricyanide and 2:6-dichlorophenol indophenol as hydrogen acceptors were found to be unsatisfactory for the microdetermination of α-glycerophosphate. Neither glycerol (up to 4M) nor β-glycerophosphate caused inhibition of the enzyme and did not interfere with the estimations of α-glycerophosphate.

However, inorganic phosphate at concentrations above 0·02M caused substantial inhibition of the reduction of cytochrome *c* (Morton, 1952).

L-α-Glycerophosphate was later estimated by the coupling of the DPN-linked muscle L-α-glycerophosphate dehydrogenase (reaction 5) with the specific reduced DPN–cytochrome *c* reductase (reaction 6) thus:



L-α-Glycerophosphate + DPN^+
⇌ dihydroxyacetone phosphate + DPNH + H^+ (5)

DPNH + H^+ + 2 ferricytochrome *c*
→ DPN^+ + 2 ferrocytochrome *c* (6)

The overall reaction will proceed to complete reduction of L-α-glycerophosphate since the equilibrium of the reductase reaction is displaced very much in favour of oxidation of reduced DPN.

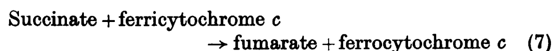
Four cuvettes (0·5 cm. light path) were used for the estimation. The first contained (in 1·2 ml.) 60 μmoles of glycylglycine buffer, pH 8·5; 0·08 μmole of ferricytochrome *c*; 0·2 μmole of DPN; 0·05 ml. of rabbit-muscle fraction A (0·5 ml. of paste diluted to 3 ml. with water); 0·025 ml. (approx. 100 μg. of enzyme protein) of reduced DPN–cytochrome *c* reductase from pig-heart muscle and the test solution. The second cuvette omitted the test solution, the third the cytochrome, and from the fourth the enzymes were omitted. Water replaced the missing components. The reaction was started by the addition of the two enzymes. The optical density (at 550 mμ) was determined before and after additions of the enzymes, and measurements were continued (usually for 25 min.) until there was no change between successive 10 min. readings, or the rate of change in the cuvette containing the test solution was the same as that in the control. The amount of L-α-glycerophosphate was calculated from the change in optical density due specifically to the test solution. Fig. 2 shows a typical determination. As previously, ΔE_{550} equals $(E_3' - E_3)-(E_2' - E_2)$. Recoveries of added DL-α-glycerophosphate (0·08–0·25 μmole/cuvette) of 86–92% were obtained.

In some studies, 0·1 ml. of a suspension of intestinal microsomes (about 4 mg. dry wt.) was used instead of purified heart-muscle cytochrome *c* reductase, 60 μmoles of sodium phosphate buffer, pH 7·8, replaced the glycylglycine and the reaction mixture contained 1 μmole of KCN. This system was less satisfactory.

Estimation of other compounds

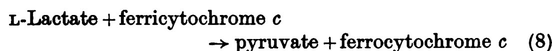
Although not used in the investigation of the phosphotransferase reaction, some procedures are described here which have been found useful for the spectrophotometric estimation of succinate and lactate in pure solution.

Succinate. The preparation of cytochrome *c*-linked α-glycerophosphate dehydrogenase from skeletal muscle also contains an active succinic dehydrogenase system which catalyses reaction 7. Succinate was estimated by a similar method to that used for estimation of α-glycerophosphate. The recovery of 0·03 μmole of sodium succinate/cuvette was 94%.



Succinate + ferricytochrome *c*
→ fumarate + ferrocytochrome *c* (7)

Lactate. L-Lactate was estimated by measurement of the reduction of ferricytochrome *c* (reaction 8) catalysed by yeast lactic dehydrogenase (cytochrome b_2). The method was similar to that used for estimation of α-glycerophosphate except that the yeast lactic dehydrogenase replaced the muscle glycerophosphate dehydrogenase, and sodium pyrophosphate–HCl buffer, pH 8·0, replaced the glycylglycine buffer.



L-Lactate + ferricytochrome *c*
→ pyruvate + ferrocytochrome *c* (8)

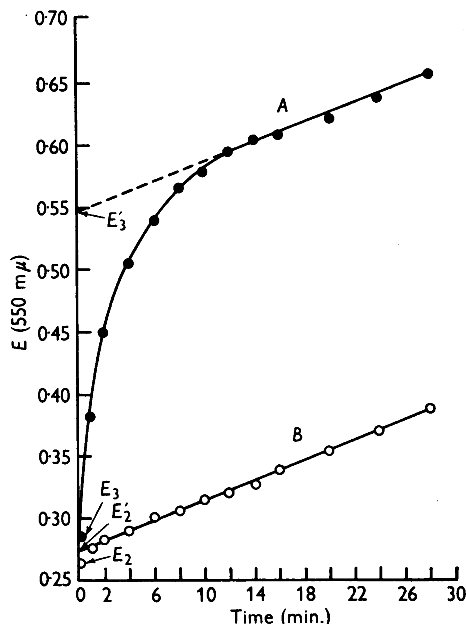


Fig. 2. Estimation of α-glycerophosphate with rabbit-muscle DPN-linked L-α-glycerophosphate dehydrogenase, reduced DPN–cytochrome *c* reductase and cytochrome *c*. E_2 and E_3, and E_2' and E_3' have the same meaning as in Fig. 1 for the control (curve *B*) and test (curve *A*) respectively. Other details are given in the text.

DISCUSSION

For estimation of products of a phosphotransferase reaction, it seemed desirable to avoid, if possible, separation of the newly formed phosphate ester from the other reactants. Specific, sensitive enzymic methods for estimation of phosphate esters were therefore used for kinetic studies of phosphate transfers catalysed by acid and alkaline phosphatases (Morton, 1958*a*, *b*).

The methods developed by Slater (1953) are particularly valuable in that a number of different compounds can be estimated with the same enzyme preparations. Some of the methods described here are extensions of Slater's procedures. The differentiation of fructose diphosphate from the triose phosphates, and the separate estimation of glyceraldehyde phosphate, for example, should add to the usefulness of Slater's procedures. These methods have not, as yet, been used in the analysis of tissue extracts. However, it would be expected that they would be quite satisfactory for this purpose. Unfortunately, the product of hydrolysis of fructose 1:6-diphosphate by fructose diphosphatase is fructose 6-phosphate, which will react in Slater's procedure B(a). Hence fructose diphosphatase cannot be used for differentiation of hexose monophosphates and fructose diphosphate. Where both of these are present in a mixture, the amount of hexose monophosphate must be calculated from the difference between separate determinations by Slater's procedures A and B(a). Glucose 6-phosphate, of course, is readily estimated with triphosphopyridine nucleotide and yeast glucose 6-phosphate dehydrogenase as described by Kornberg & Pricer (1951).

The sensitivity of the spectrophotometric methods described here is conveniently increased by almost threefold by reducing the volume of the cuvette (1 cm. light path) to 1·2 ml. with the insert described by Martin & Morton (1956).

Estimations based on enzymic reduction of cytochrome c

Estimation of enzyme substrates with cytochrome *c* as the hydrogen (or electron) acceptor are particularly sensitive because of the high molar extinction coefficient of ferrocytochrome *c* (28×10^3 at 550 mμ; Theorell, 1936; Keilin & Slater, 1953). Moreover, two molecules of cytochrome *c* are reduced for each molecule of substrate oxidized. The reactions proceed to completion since the equilibria of the cytochrome *c*-linked systems are very greatly in favour of reduction of cytochrome *c*. Hence the choice of the cytochrome *c*-linked α-glycerophosphate dehydrogenase for estimation of α-glycerophosphate (Morton, 1952, 1955*a*). Estimation of this compound by determining the amount of DPN reduced with the DPN-linked α-glycerophosphate dehydrogenase of muscle in the presence of a trapping agent for dihydroxyacetone phosphate was found to be unsatisfactory (Morton, 1952). Bublitz & Kennedy (1954), however, recently reported successful estimations with this enzyme.

In addition to α-glycerophosphate, succinate and lactate, with the appropriate cytochrome *c*-linked dehydrogenases, choline, xanthine and hypoxanthine, probably could be estimated by similar procedures to those described here. However, the range of substrates which could be estimated spectrophotometrically is greatly extended by the coupling of the reactions of the DPN-linked dehydrogenases with the reaction catalysed by reduced DPN–cytochrome *c* reductase. L-α-Glycerophosphate and L-lactate have both been successfully estimated. However, the method is not as satisfactory as was at first anticipated. With the reduced DPN–cytochrome *c* reductase of microsomes, difficulties arise due to changes in turbidity during the course of the reaction. Although the enzyme extracted from heart muscle is readily soluble, trouble has been found owing to a slow non-specific reduction of cytochrome *c* catalysed by this preparation. Moreover, the enzyme is rather unstable at room temperature, and is inhibited by a variety of metal salts (see Vernon, Mahler & Sarkar, 1952). Conditions for estimation of any particular substrate require extensive investigation.

Glock & McLean (1955) have used the coupled reaction of alcohol dehydrogenase and reduced DPN–cytochrome *c* reductase of heart muscle for estimation of DPN in tissue extracts. These workers measure the rate of reduction of cytochrome *c* under carefully standardized conditions, rather than the total amount of cytochrome *c* reduced, possibly because of difficulties due to non-specific reduction of cytochrome *c*.

SUMMARY

1. Methods are described for kinetic study of the phosphotransferase reaction. The phosphate esters synthesized by phosphate transfer are estimated spectrophotometrically with substrate-specific enzymes. The methods avoid the separation of the phosphate esters from the other components of the reaction mixtures.

2. Slater's (1953) procedures were used for estimation of triose phosphates and hexose phosphates. Extensions of these procedures for differentiation of fructose diphosphate, glyceraldehyde phosphate and dihydroxyacetone phosphate are described.

3. Methods for estimation of L-α-glycerophosphate, succinate and L-lactate are described.

Cytochrome *c* is used as the hydrogen acceptor with the appropriate cytochrome *c*-linked dehydrogenase system, or with the appropriate diphosphopyridine nucleotide-linked dehydrogenase together with reduced diphosphopyridine nucleotide-cytochrome *c* reductase.

I wish to thank Professor E. C. Slater for suggesting the use of enzymic methods for estimation of the products of phosphate transfer to hexoses, and for making available in 1951 the details of his procedures before publication. I am most grateful to Dr M. Dixon, F.R.S., for his valuable guidance and interest in this work, which was carried out in 1951 with the financial assistance of the Agricultural Research Council of Great Britain. The assistance of the Council is gratefully acknowledged.

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The Phosphotransferase Activity of Phosphatases

2. STUDIES WITH PURIFIED ALKALINE PHOSPHOMONOESTERASES AND SOME SUBSTRATE-SPECIFIC PHOSPHATASES

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The synthesis of organic phosphates by enzymic transfer of the phosphate group from organic 'donor' compounds (such as *p*-nitrophenyl phosphate) to a suitable alcohol (such as propan-1-ol) was first demonstrated by Axelrod (1947, 1948*a*) and by Appleyard (1948). These workers used crude acid phosphatase preparations from citrus fruits and from prostate gland respectively. In both cases there was net synthesis of new ester. Much earlier Kay (1928) had shown that alkaline phosphatases may catalyse synthesis of an organic ester from an alcohol and inorganic phosphate, but Axelrod (1948*b*) established that the biosynthesis catalysed by the citrus-fruit acid phosphatase preparation did not involve the intermediary formation of inorganic phosphate. Meyerhof & Green (1950) subsequently showed that partially purified alkaline phosphatase from calf intestinal mucosa catalysed an exchange reaction between the phosphate group of phosphocreatine and that of glycerophosphate.

These workers all showed that hydrolysis of the donor phosphate accompanied synthesis of the new ester. However, it was by no means certain that the same enzyme catalysed both reactions. Appleyard (1948) had proposed that a 'transphosphorylase', distinct from the phosphatase, was present in the extract of prostate gland. In 1949 therefore investigations were initiated to establish

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