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.

REFERENCES

- Appleby, C. A. & Morton, R. K. (1954). *Nature, Lond.*, **173**, 749.
- Appleyard, J. (1948). *Biochem. J.* **42**, 596.
- Askonas, B. A. (1951). *Biochem. J.* **48**, 42.
- Axelrod, B. (1948). *J. biol. Chem.* **172**, 1.
- Bailie, M. & Morton, R. K. (1955). *Nature, Lond.*, **176**, 111.
- Bublitz, C. & Kennedy, L. P. (1954). *J. biol. Chem.* **211**, 951.
- Cori, G. T., Slein, M. W. & Cori, C. F. (1948). *J. biol. Chem.* **173**, 605.
- Dixon, M. (1949). *Multi-enzyme Systems*. Cambridge University Press.
- Dixon, M. (1954). *Biochem. J.* **58**, 1.
- Dixon, M. & Webb, E. C. (1953). *Brit. med. Bull.* **9**, 110.
- Glock, G. & McLean, P. (1955). *Biochem. J.* **61**, 381.
- Gomori, G. (1943). *J. biol. Chem.* **148**, 139.
- Green, D. E. (1936). *Biochem. J.* **30**, 629.
- Hanes, C. S. & Isherwood, F. A. (1949). *Nature, Lond.*, **164**, 1107.
- Keilin, D. & Hartree, E. F. (1945). *Biochem. J.* **39**, 289.
- Keilin, D. & Slater, E. C. (1953). *Brit. med. Bull.* **9**, 89.
- Kornberg, A. & Prier, W. E. (1951). *J. biol. Chem.* **193**, 481.
- Mahler, H. R., Sarkar, N. K., Vernon, L. P. & Alberty, R. A. (1952). *J. biol. Chem.* **199**, 585.
- Martin, E. M. & Morton, R. K. (1956). *Biochem. J.* **64**, 221.
- Meyerhof, O. & Green, H. (1950). *J. biol. Chem.* **183**, 377.
- Morton, R. K. (1952). Ph.D. Thesis: University of Cambridge.
- Morton, R. K. (1954). *Biochem. J.* **57**, 595.
- Morton, R. K. (1955a). In *Methods in Enzymology*, Vol. 2, p. 556. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Morton, R. K. (1955b). *Biochem. J.* **60**, 573.
- Morton, R. K. (1955c). *Biochem. J.* **61**, 232.
- Morton, R. K. (1957). *Biochem. J.* **65**, 674.
- Morton, R. K. (1958a). *Biochem. J.* **70**, 139.
- Morton, R. K. (1958b). *Biochem. J.* **70**, 150.
- Racker, E. (1947). *J. biol. Chem.* **167**, 843.
- Racker, E. (1950). *J. biol. Chem.* **184**, 313.
- Slater, E. C. (1953). *Biochem. J.* **53**, 157.
- Theorell, H. (1936). *Biochem. Z.* **285**, 207.
- Vernon, L. P., Mahler, H. R. & Sarkar, N. K. (1952). *J. biol. Chem.* **199**, 599.

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, 1948a) 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 (1948b) 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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the nature of the enzyme catalysing this 'phosphotransferase' (Dixon, 1949) reaction.

As an essential step in this investigation, alkaline phosphatases were purified from cow's milk (Morton, 1953*a*) and from calf intestinal mucosa (Morton, 1954).

The properties of the purified enzymes were described (Morton, 1955*a*), including the substrate specificity (Morton, 1955*b*) and the kinetics of hydrolysis of a typical substrate (Morton, 1957). Sensitive spectrophotometric methods were then developed to enable the accurate estimation of selected products of transferase activity without separation from the reaction mixture (Morton, 1952*a*, 1958*a*). The results of the investigation described here show that the phosphotransferase activity is a property of both purified alkaline phosphatases, and provide some information on the mechanism of the reaction.

In contrast with the findings with the purified alkaline phosphatases, no phosphotransferase activity was detected in partially purified preparations of hexose diphosphatase of ox kidney, 5'-nucleotidase of bull testes or myosin-adenosine triphosphatase of rabbit skeletal muscle.

Certain aspects of this work have been published (Morton, 1952*b*, *c*; 1953*b*, 1955*c*).

MATERIALS AND METHODS

Organic phosphates. The sources of these are given in previous papers (Morton, 1955*a*, *b*).

Other chemicals. Redistilled glycerol was kindly supplied by Dr G. Weber. DL-Glyceraldehyde was a gift from Dr D. M. Needham, F.R.S.

Alkaline phosphatases. These were purified from cow's milk (Morton, 1953*a*) and from calf intestinal mucosa (Morton, 1954). Solutions of enzymes were prepared from freeze-dried powder which had been stored over CaCl_2 at 0°. The specific activities had declined somewhat from those obtained immediately after purification. The activities with β -glycerophosphate were not regularly tested, but may be calculated approximately from the relationship that 1 μg . of intestinal phosphatase is equivalent to 7 units, and 1 μg . of milk phosphatase to 1.5 units, of activity as previously defined (Morton, 1953*c*).

Alkaline hexose diphosphatase. Minced ox kidney (100 g.) was extracted with sodium lactate buffer (pH 4.2 at 18°) as described by Gomori (1943). The insoluble protein was removed by centrifuging at about 1800 g for 30 min. The supernatant was adjusted to pH 7.5 with 0.1*N*-NaOH and then filtered through a thin layer of Hyflo Super-Cel over no. 1 Whatman paper on a Büchner funnel.

The clear filtrate was fractionated with $(\text{NH}_4)_2\text{SO}_4$. The fraction obtained between 55 and 68% saturation was dissolved in 5 ml. of water, and dialysed in seamless cellulose tubing against several changes of glass-distilled water at 0° for 48 hr. and then centrifuged at 14 000 g for 30 min. at 0–2°. The supernatant containing the enzyme was stored at 0°. Before use, the enzyme was activated by pre-

incubation in 0.01*M*-veronal (sodium diethylbarbiturate)-HCl buffer, pH 9.2, containing 0.01*M*-cysteine hydrochloride and 0.01*M*- MgCl_2 . At 38° approx. 40 μg . of inorganic phosphate was liberated/min./mg. dry wt. of enzyme from 5 mm-fructose 1:6-diphosphate in 0.05*M*-veronal- Na_2CO_3 , pH 9.5, containing mm-cysteine hydrochloride and mm- MgCl_2 . There was no hydrolysis of 0.01*M*- β -glycerophosphate under similar conditions.

5'-Nucleotidase. The mince from two decapsulated bull's testes was placed in a muslin bag and washed in running tap water until the effluent was colourless. The mince was then extracted with 0.15*M*-NaCl (pH 7.5 at 20°) for 20 min. The extract was centrifuged at 2500 g for 30 min. and the resulting supernatant was centrifuged at 14 000 g for 1 hr. The active protein was precipitated from the supernatant by addition of solid $(\text{NH}_4)_2\text{SO}_4$ to 50% saturation, and collected by centrifuging, then dissolved in water and dialysed in seamless cellulose tubing against 0.15*M*-NaCl. The material was then centrifuged at 14 000 g for 20 min. The active supernatant was colourless but opalescent. It was stored at 0°. Whereas 0.1 ml. of the enzyme solution liberated about 3 μg . of inorganic phosphate/min. from 5 mm adenosine-5'-phosphate (AMP) in 0.02*M*-veronal-HCl buffer, pH 9.0, containing 0.01*M*- MgCl_2 , there was no inorganic phosphate liberated from 0.01*M*- β -glycerophosphate under similar conditions.

Myosin-adenosine triphosphatase. Myosin, prepared from rabbit muscle as described by Tsao (1953), was kindly supplied by Dr T. C. Tsao.

Other enzymes. The preparation of the other enzymes used is described in the preceding paper (Morton, 1958*a*).

Inorganic phosphate. This was estimated according to Weil-Malherbe & Green (1951).

Phenol. This was estimated by the procedure of King (1951) modified as previously described (Morton, 1955*b*).

Creatine. This was estimated by the method of Eggleton, Elsdon & Gough (1943).

pH values. These were estimated in replicate tubes of those used for the enzyme reactions. Unless otherwise specified, all values given refer to pH at 38°.

Estimation of phosphate transfer. The procedure is described in the preceding paper (Morton, 1958*a*).

RESULTS

Effect of phosphocreatine on the synthesis of glucose phosphate from glucose and inorganic phosphate

The progressive synthesis of glucose phosphates from sodium phosphate (0.16*M*) and glucose (0.80*M*) with intestinal and milk phosphatases was compared with that obtained with a similar reaction mixture but with the addition of phosphocreatine (14 mm). The results in Table 1 show that there was an increased synthesis with both enzymes in the presence of added phosphocreatine, but the additional glucose phosphate formed with phosphocreatine diminished with time. After prolonged incubation, the amount of hexose phosphate obtained with and without phosphocreatine was essentially the same.

In a further experiment, the amount of purified intestinal phosphatase was reduced to about one-

Table 1. *Synthesis of glucose phosphates by alkaline phosphatases from glucose and sodium phosphate, with and without phosphocreatine*

The reaction mixture (0.4 ml.) contained 0.80 M-glucose, 0.16 M-sodium phosphate, pH 8.5, approx. 90 μ g. of intestinal phosphatase or about 200 μ g. of milk phosphatase and, where added, 14 mM-phosphocreatine. The reaction was carried out at 38° and at pH 8.5.

Phosphatase	Time (hr.)	Glucose phosphate formed/tube (μ mole)		Increased synthesis with creatine phosphate	
		Without creatine phosphate	With creatine phosphate	(μ mole)	(%)
Calf intestine	0	0	0	0	—
	0.1	0.007	0.027	0.02	290
	1	0.16	0.27	0.11	69
	2.5	0.32	0.41	0.09	28
	6	0.42	0.49	0.07	17
	25	0.96	—	—	—
Cow's milk	0	0	0	—	—
	0.1	0.003	0.023	0.02	660
	1	0.07	0.13	0.06	86
	2.5	0.27	—	—	—
	6	0.31	0.37	0.06	19
	25	0.61	0.63	0.02	3

Table 2. *Synthesis of glucose phosphates by alkaline phosphatase from glucose and phosphocreatine only*

The reaction mixture (0.12 ml.) contained 1.7 M-glucose, 0.03 M-veronal (sodium diethylbarbiturate)-HCl buffer, intestinal phosphatase (approx. 9 μ g.) and either 0.1 M-sodium phosphate or 64 mM-phosphocreatine as indicated. The pH was adjusted to that shown. The reaction period was 5 min. at 38°.

pH	Glucose phosphate formed/tube (μ mole)	
	With inorganic phosphate	With creatine phosphate
7.5	0	0.072
8.5	0	0.074
9.0	0	0.084
9.3	0	0.087

tenth of that used in the former experiment, and the amount of glucose phosphate formed from glucose (1.7 M) and phosphocreatine (64 mM) was compared with that obtained from glucose (1.7 M) and sodium phosphate (0.1 M) at several pH values. Table 2 shows that, under the conditions used, there was no appreciable synthesis of glucose phosphate from glucose and inorganic phosphate, whereas there was considerable synthesis from glucose and phosphocreatine. There was no synthesis of glucose phosphate when the sodium phosphate concentration was 0.05 or 0.01 M. The reduced amount of intestinal phosphatase explains the failure to detect glucose phosphates formed from inorganic phosphate and glucose as shown in Table 2, whereas synthesis was found with a greater amount of enzyme (Table 1).

Fig. 1. Progress curve for synthesis of glucose phosphate by intestinal phosphatase at 38°. The reaction mixture (0.4 ml.) contained 1.5 M-glucose, 13 mM-phosphocreatine, 0.05 M-sodium bicarbonate-sodium carbonate buffer, pH 9.65, and about 18 μ g. of intestinal phosphatase. A, Glucose phosphate; B, creatine; C, percentage transfer.

Kinetic studies of the transferase reaction

Estimation of initial velocity of phosphate transfer. Fig. 1 (curve A) shows that the rate of formation of glucose phosphate from phosphocreatine (13 mM) and glucose (1.5 M) at pH 9.65 is linear for about 4 min., after which it rapidly declines. After 20 min., when some 75 % of the phosphocreatine has been used, the glucose phosphate reaches a maximum value, and the amount present diminishes with further incubation, probably due to hydrolysis by the phosphatase. From Fig. 1 it appears that values close to the true initial rates of

glucose phosphate formation will be obtained if the amount of enzyme is adjusted so that the concentration of newly formed ester does not exceed 0.4 mM, namely, is less than 0.2 μ mole/0.5 ml. of reaction mixture. These conditions were used in subsequent studies.

The percentage transfer is a measure of the efficiency of the phosphotransferase reaction. It was defined by Axelrod (1948*a*) as the quotient:

$$\frac{\text{moles of ester phosphate formed} \times 100}{\text{moles of donor phosphate utilized}}$$

Fig. 1 (curve *C*) shows that the percentage transfer to glucose (1.5M) is initially about 12%, but the value steadily diminishes during the course of the reaction. This clearly indicates that the accumulation of glucose phosphate is opposed by the hydrolysis catalysed by the phosphatase itself. The rate of hydrolysis of the glucose phosphate formed will increase with increasing concentration of this compound, as has been shown for other ester phosphates (Morton, 1952*a*, 1957) and is apparent from Fig. 1. To be valid therefore estimates of percentage transfers should be based on measurements of initial rates.

Effect of enzyme concentration. The rates of hydrolysis of phosphocreatine and of synthesis of glucose phosphate were found to be proportional to the amount of enzyme, provided that the conditions were such that the initial rates were reliably estimated. The percentage transfer was then independent of the amount of enzyme.

Influence of pH on the transferase reaction. The reaction was studied over the range pH 7.9–10.1. The results for intestinal phosphatase (Fig. 2, curve *A*) show that, in the absence of glucose, the inorganic phosphate and the creatine liberated closely coincide. Addition of glucose (1.5M) depresses the rate of hydrolysis of phosphocreatine, as indicated by the decreased amount of inorganic phosphate liberated (curve *C*). The excess of creatine above the inorganic phosphate liberated (cf. curves *B* and *C*) is due to the transferase reaction. The amount of glucose phosphate formed (curve *D*) so varies with the pH of the reaction that the percentage transfer (curve *E*) remains fairly constant over the pH range investigated.

The results obtained with milk phosphatase (Table 3) substantiate the findings obtained with intestinal phosphatase.

Effect of the concentration of phosphate donor. In different experiments, the concentration of phosphocreatine has been varied from 5 to 60 mM at similar glucose concentrations (1.5M). The initial velocity of glucose phosphate formation increased at the higher donor concentrations, but the percentage transfer was not changed significantly over this range. It appears therefore that the hydrolytic

Fig. 2. Influence of pH on synthesis of glucose phosphate. The reaction mixture (0.4 ml.) contained 13 mM-phosphocreatine, 0.01 M-magnesium acetate, 0.1 M-veronal-sodium acetate-HCl or 0.05 M-sodium bicarbonate-sodium carbonate buffer, about 9 μ g. of intestinal phosphatase and, where added, 1.5 M-glucose. The reaction period was 5 min. at 38°. *A*, Creatine (○) and inorganic phosphate (●) in the absence of glucose; other curves with 1.5 M-glucose; *B*, creatine; *C*, inorganic phosphate; *D*, glucose phosphate; *E*, percentage transfer.

Table 3. Influence of pH on the synthesis of glucose phosphate by phosphate transfer catalysed by milk alkaline phosphatase

The reaction mixture (0.4 ml.) contained 0.03 M-sodium bicarbonate-sodium carbonate buffer at the pH shown; 0.01 M-magnesium acetate; 13 mM-phosphocreatine; 1.5 M-glucose; 20 μ g. of milk phosphatase. The reaction was for 5 min. at 38°. Figures in parentheses show the amounts of creatine and inorganic phosphate liberated in identical reaction mixtures without glucose.

pH	Product/tube (μ mole)			Phosphate transfer (%)
	Creatine	Inorganic phosphate	Glucose phosphate	
8.75	1.27 (1.75)	1.04 (1.75)	0.10	7.8
9.55	1.38 (1.71)	1.18 (1.72)	0.11	8.0
10.05	1.13 (1.35)	1.00 (1.35)	0.08	7.1

and transferase reactions are similarly influenced by the concentration of the phosphate donor.

Effect of acceptor concentration. The influence of increasing glucose concentrations on the phosphate-transfer reaction with phosphocreatine as donor is shown in Fig. 3. The rate of hydrolysis, as indicated by curve *B*, declines continuously as the concentration of the acceptor increases up to about

1.5M-glucose. Curve *A* shows that the rate of creatine liberation (due to both hydrolysis and transfer reactions) declines more slowly up to 1.5–2.0M-glucose. At higher glucose concentrations, however, both the liberation of creatine and of inorganic phosphate fall rapidly and in a similar fashion. The rate of glucose phosphate formation (curve *C*) increases with glucose concentration, reaches a maximum value near 1.5–2.0M-glucose, and then declines at higher glucose concentrations. The similar decline in the rates of creatine, inorganic phosphate and glucose phosphate formation above 2M-glucose indicates that the phosphatase is inhibited. The percentage transfer (curve *D*) increases with increasing glucose concentration and reaches a maximum value between 1.5 and 2.0M-glucose, which is unchanged by further increase of glucose concentration.

Acceptor specificity of intestinal alkaline phosphatase

A number of potential phosphate acceptors were examined.

Glucose. The glucose phosphate formed as shown in Fig. 3 is the sum of glucose 1- and glucose 6-phosphate, since Slater's (1953) procedure B(a) estimates both. Inorganic phosphate was removed from the reaction products formed with 2M-glucose (see Fig. 3) by addition of ammoniacal-magnesia, and the esters were then precipitated as the barium salts. After washing and conversion into the ammonium salts, the products were

Fig. 3. Effect of glucose concentration on the phosphotransferase reaction. The reaction mixture (0.5 ml.) contained 26 mM-phosphocreatine; 0.01M-magnesium acetate; 0.05M-veronal-sodium carbonate buffer, pH 9.8; about 9 μ g. of intestinal alkaline phosphatase; glucose at the concentrations shown. The reaction period was 5 min. at 38°. *A*, Creatine; *B*, inorganic phosphate; *C*, glucose phosphate; *D*, percentage transfer.

applied to washed Whatman no. 1 paper and run in the propan-1-ol-aq. NH_3 soln. solvent of Hanes & Isherwood (1949). Glucose 6-phosphate was the only new ester detected with the procedure of Hanes & Isherwood (1949).

Fructose. Fig. 4 shows that fructose is also phosphorylated. Here, Slater's (1953) procedure B(a) estimates the sum of fructose 1:6-diphosphate and of fructose 6-phosphate. In one experiment a large amount of products was formed under the conditions of Fig. 4 but with a tenfold increase of enzyme. Fructose diphosphate was estimated separately from fructose 6-phosphate (see Morton, 1958*a*) and found to be between 2 and 5% of the total phosphates formed at 2 and 4M-fructose. With very large amounts of muscle fractions A and B, and with Slater's (1953) procedure B(a), the estimate of fructose phosphate differed very little from that obtained with the usual amount of muscle fractions, indicating that there is very little fructose 1-phosphate formed (see Morton, 1958*a*).

Glycerol. Fig. 5 shows that glycerol is readily phosphorylated. As both D- and L-isomers are formed, the glycerophosphate formed was estimated as twice the L- α -glycerophosphate determined enzymically as already described (Morton, 1958*a*). As shown in Fig. 5, there was substantial agreement between the amount of glycerophosphate estimated enzymically with that estimated from the difference between the liberated creatine and inorganic phosphate, indicating that very little β -glycerophosphate was formed in the reaction.

Fig. 5 shows the effect of increasing glycerol concentrations on the phosphotransferase reaction. The percentage transfer (curve *B*) reaches a maximum near 4M-glycerol and is unchanged by further increase of glycerol concentration.

Fig. 4. Effect of fructose concentration on the phosphotransferase reaction. Conditions were as for Fig. 3 except that fructose replaced glucose. *A*, Creatine; *B*, fructose phosphate; *C*, percentage transfer.

Fig. 6 (curve A) shows the coupled reaction between intestinal alkaline phosphatase and cytochrome *c*-linked L- α -glycerophosphate dehydrogenase. Reduction of cytochrome *c* due to the formation of L- α -glycerophosphate was initiated by addition of intestinal phosphatase.

Propane-1:2-diol. Table 4 shows that propane-1:2-diol is a suitable acceptor. The product was estimated as the difference between creatine and inorganic phosphate liberated enzymically.

Glyceraldehyde and dihydroxyacetone. The phosphorylated derivatives of both trioses were estimated enzymically by Slater's (1953) procedure (Morton, 1958*a*). DL-Glyceraldehyde and dihydroxyacetone (both from L. Light and Co. Ltd.) were phosphorylated (Table 5). The product with glyceraldehyde was estimated as twice the triose phosphate determined enzymically, since L-glyceraldehyde phosphate would not be determined and both optical isomers would be expected.

Fig. 6 (curve B) shows the coupled reaction between alkaline phosphatase and diphosphopyridine nucleotide (DPN)-linked L- α -glycerophosphate dehydrogenase. Oxidation of reduced DPN was initiated by addition of phosphatase.

Sucrose. No detectable amount of new ester was formed with sucrose whereas hexose phosphates were synthesized from a mixture of glucose and fructose (Table 6). With sucrose, esters were estimated by the difference method and also enzymically after hydrolysis of the reaction products with 0.1N-HCl at 60° for 5 min. or after treatment with yeast invertase at pH 5.5 for 1 hr. at 20°. Non-enzymic synthesis of hexose phosphates (Morton, 1958*b*) was observed during the acid hydrolysis.

Fig. 5. Effect of glycerol concentration on the phosphotransferase reaction. Conditions were as for Fig. 3 except that the phosphocreatine concentration was 35 mM and glycerol replaced glucose. A, DL-Glycerophosphate calculated from enzymic estimation of L- α -glycerophosphate (□), or by difference between creatine and inorganic phosphate liberated (■); B, percentage transfer; C, creatine; D, inorganic phosphate.

Serine, threonine and ethanolamine. The reaction mixtures (0.4 ml.) contained 64 mM-NaHCO₃-Na₂CO₃ buffer, pH 9.2, mM-magnesium acetate, 2 μg. of intestinal alkaline phosphatase and either DL-serine (approx. M), DL-threonine (approx. 0.5 M) or ethanolamine (M). The reaction period was 30 min. at 38°. Spots of about 0.01 ml. were applied to Whatman paper no. 1 and ascending chromatograms were run in water-saturated

Fig. 6. Coupled reactions of glycerophosphate dehydrogenases and intestinal alkaline phosphatase. Curve A shows the reaction with cytochrome *c*-linked L- α -glycerophosphate dehydrogenase. Each cuvette (1 cm.) contained, in 2.6 ml., 2M-glycerol, 0.01M-MgCl₂, approx. 0.05 mM-ferri-cytochrome *c*; 0.05M-veronal-sodium acetate-HCl buffer, pH 8.0, and 'particle-bound' L- α -glycerophosphate dehydrogenase of rabbit skeletal muscle. Cytochrome *c* was omitted from the blank cuvette. Readings were made at 550 mμ with a Beckman spectrophotometer model DU. After 4 min., 0.1 ml. of purified intestinal phosphatase was introduced. The reaction was carried out at room temperature (approx. 16°). Curve B shows the reaction with DPN-linked L- α -glycerophosphate dehydrogenase. Each cuvette (1 cm.) contained in 2.7 ml. 0.05M-glycylglycine buffer, pH 8.2; 0.1 mM-reduced DPN; approx. M-DL-glyceraldehyde; 0.01M-phenyl phosphate; an enzyme preparation from rabbit skeletal muscle containing both 'soluble' L- α -glycerophosphate dehydrogenase and triose phosphate isomerase. Readings were made at 340 mμ. After 10 min., 0.1 ml. of purified intestinal phosphatase was introduced. The reaction was carried out at room temperature (approx. 19°).

Table 4. *Phosphorylation of propane-1:2-diol catalysed by intestinal alkaline phosphatase*

The reaction mixture (0.5 ml.) contained 0.03M-sodium bicarbonate-sodium carbonate buffer, pH 9.2; 35 mM-phosphocreatine; mM-magnesium acetate; 6 μ g. of intestinal alkaline phosphatase; propane-1:2-diol at the concentrations indicated. The reaction period was 10 min. at 38°.

Concn. of propanediol (M)	Product/tube (μ moles)			Phosphate transfer (%)
	Inorganic phosphate	Creatine	Propanediol phosphate*	
0	2.40	2.38	—	—
1	1.96	2.38	0.42	18
3	1.31	2.04	0.73	36
5	0.93	1.60	0.67	42

* Difference between creatine and inorganic phosphate liberated.

Table 5. *Phosphorylation of glyceraldehyde and of dihydroxyacetone*

The reaction mixture (0.5 ml.) contained 0.05M-glycine-NaOH buffer, pH 9.8; 0.08M-phenyl phosphate; mM-magnesium acetate; approx. 1 μ g. of intestinal alkaline phosphatase, and either DL-glyceraldehyde or dihydroxyacetone at the concentrations indicated. The reaction period was 10 min. at 38°.

Concn. of triose (M)		Products/tube (μ moles)		Phosphate transfer (%)
Glycer- aldehyde	Dihydroxy- acetone	Inorganic phosphate	Triose phosphate	
0	0	2.26	0	—
1	—	1.47	0.10	7
2	—	0.57	0.07	11
—	1	1.22	0.14	10
—	2	0.63	0.12	16
—	3	0.39	0.11	22

Table 6. *Results obtained with sucrose as an acceptor for phosphate in the transferase reaction with intestinal alkaline phosphatase*

The reaction mixture (0.5 ml.) contained 0.05M-veronal-sodium carbonate buffer (King & Delory, 1940), pH 9.5; mM-magnesium acetate; 0.02M-phosphocreatine; 9 μ g. of intestinal phosphatase; sugars at the concentrations indicated. The reaction period was 5 min. at 38°.

Sugar	Concn. of sugar (M)	Products/tube (μ mole)			
		Creatine	Inorganic phosphate	Sucrose phosphate† or hexose phosphate‡	Hexose phosphate‡
Sucrose	0	0.97	0.95	+0.02	—
	0.5	0.93	1.00	-0.07	—
	1	0.51	0.63	-0.12	0
	2	0.50	0.51	-0.01	0
	3	0.31	0.24	+0.07	—
Glucose-fructose mixture*	2	0.69	0.55	+0.14	0.05
	4	0.27	0.10	+0.17	0.05

* A mixture of equal weights of glucose and fructose was used, and treated identically as sucrose.

† Difference between creatine and inorganic phosphate formed.

‡ Determined enzymically, directly or after hydrolysis of the reaction products obtained with sucrose in 0.1N-HCl at 60° for 5 min.

phenol (Dent, 1948). In each, the appearance of a new ninhydrin-positive spot in the reaction products of the tube containing the phosphatase, as compared with the control containing enzyme destroyed by boiling, was taken as presumptive evidence for the formation of some phosphorylated compound. However, no authentic marker com-

pounds were available for comparison with the suspected phosphorylated component.

Nucleosides. Approx. 0.2 ml. of 0.05M-ethanolamine-HCl buffer, pH 9.8, containing 0.02M- β -glycerophosphate or 0.01M-*p*-nitrophenyl phosphate was saturated with adenosine, guanosine, cytidine or uridine, and approx. 1 μ g. of intestinal

alkaline phosphatase added. After 30 min. at 22°, 0.02 ml. was applied to Whatman no. 1 paper and dried immediately. Similar reaction mixtures containing authentic nucleotides were used as markers.

The esters were also precipitated as the barium salts, washed and converted into the ammonium salts. They were then applied to Whatman no. 1 paper as before. Descending chromatograms were run in the propan-1-ol-aq. NH_3 soln. solvent of Hanes & Isherwood (1949), and also in the saturated $(\text{NH}_4)_2\text{SO}_4$ -sodium acetate-propan-2-ol solvent of Markham & Smith (1951). The chromatograms were examined by ultraviolet light (Markham & Smith, 1949).

A very small amount of adenosine monophosphate was detected, but, apart from adenosine, no other nucleoside was phosphorylated.

Donor specificity in the transferase reaction

Table 7 shows that a number of the typical substrates of both intestinal and milk phosphatases act as donors in the transfer reaction.

With phenyl phosphate (0.01M) and glucose (2M) at pH 10.2, similar percentage transfers were obtained with a preparation of microsomes from calf intestinal mucosa (Morton, 1954), and with partially purified and purified preparations of intestinal alkaline phosphatase.

Comparative studies with substrate-specific phosphatases and alkaline phosphomonoesterases

Kidney hexose diphosphatase and milk alkaline phosphatase. Glycerol was used as acceptor, and hexose diphosphate as phosphate donor. Synthesis of L- α -glycerophosphate was measured enzymically with cytochrome c-linked α -glycerophosphate dehydrogenase (Morton, 1958a). Whereas there was no detectable synthesis of L- α -glycerophosphate with kidney hexose diphosphatase there was considerable synthesis by milk alkaline phosphatase under similar conditions (Table 8). Under the assay conditions used, 0.02 μ mole of DL- α -glycerophosphate/tube would be estimated.

5'-Nucleotidase and intestinal alkaline phosphatase. The reaction mixture (0.5 ml.) contained

Table 7. *Phosphate transfers from various donor compounds*

Acceptor	Donor	Free energy of hydrolysis ($-\Delta F'$) of donor† (kcal./mole)	Reaction conditions		Phosphate transfer (%) at acceptor concn.*				
			Concn. of donor (M)	pH	(M)				
					1	2	3	4	5
Glucose	Creatine phosphate	10.5	0.026	9.8	12	14	14	16	—
	Phenolphthalein phosphate	—	0.01	9.8	—	14	—	—	—
	Phenyl phosphate	—	0.01	10.0	10	16	—	14	—
	Adenosine 5-phosphate	—	0.005	9.0	—	10	—	—	—
	β -Glycerol phosphate	2.1‡	0.02	9.6	—	16	—	14	—
Glycerol	Creatine phosphate	10.5	0.035	9.8	14	18	33	45	45
	Phospho-enol pyruvate	12.8	0.002	9.6	—	—	—	43	—
	Hexose diphosphate	—	0.005	9.5	—	14	—	43	—
	β -Glycerol phosphate	2.1‡	0.02	9.8	—	18	—	40	—
	<i>p</i> -Nitrophenyl phosphate	—	0.01	9.8	—	20	—	—	—

* Based on enzymic estimations of glucose phosphate and α -glycerophosphate.

† Values are given for pH 7 and 25°, as estimated by Atkinson & Morton (1958); see also Oesper (1951).

‡ Value for α -glycerophosphate.

Table 8. *Comparison of reactions catalysed by milk alkaline phosphatase and kidney hexose diphosphatase*

The reaction mixture (0.5 ml.) contained 0.05M-veronal-sodium carbonate buffer, pH 9.5; 0.01M-magnesium acetate; 5 mM-fructose 1:6-diphosphate, glycerol at the concentrations indicated; either hexose diphosphatase (approx. 0.3 mg.) or milk alkaline phosphatase (approx. 10 μ g.). The reaction period was 10 min. at 38°.

Concn. of glycerol (M)	Products/tube (μ mole)				Phosphate* transfer (%) with alkaline phosphatase
	Hexose diphosphatase		Alkaline phosphatase		
	Inorganic phosphate	α -Glycerol phosphate	Inorganic phosphate	α -Glycerol phosphate	
0	1.32	—	1.29	—	—
1	1.39	0	—	—	—
2	1.26	0	0.68	0.11	14
3	1.16	0	—	—	—
4	1.23	0	0.38	0.19	33
5	1.18	0	—	—	—

* Assuming only DL- α -glycerophosphate is formed.

5 mM-AMP, 2M-glucose, 0.01M-MgCl₂, 0.02M-veronal-HCl buffer, pH 9.0, and 0.1 ml. of either 5'-nucleotidase or intestinal phosphatase. The incubation time was 10 min. at 38°, the amount of enzyme being adjusted to liberate about 1.1 μ moles of inorganic phosphate (about 44 % hydrolysis of the substrate) in each case.

No glucose phosphate was detected in a 1 ml. portion of the reaction products of 5'-nucleotidase so that less than 0.02 μ mole was formed, whereas 0.12 μ mole was synthesized by the intestinal phosphatase (11 % transfer).

Myosin-adenosine triphosphatase and intestinal alkaline phosphatase. The reaction mixtures (0.5 ml.) contained 0.02M-veronal-HCl buffer, pH 8.5, 0.01M-CaCl₂, 25 mM-adenosine triphosphate (ATP), glucose at 0, 2 and 4M concentrations and sufficient myosin preparation (0.1 ml.) to hydrolyse about 55 % of the substrate in the incubation time (30 min.) at 20°. No glucose phosphate was detected in any tube, so that the amount formed was less than 0.02 μ mole. The amount of inorganic phosphate liberated at 4M-glucose (0.61 μ mole) was little different from that in the absence of glucose (0.69 μ mole). With intestinal phosphatase (approx. 20 μ g.) under similar conditions (except that MgCl₂ replaced CaCl₂) and also at pH 9 with 5 mM-ATP, mM-magnesium acetate and 1, 2 and 4M-glucose, there was no significant hydrolysis of ATP and no synthesis of glucose phosphate.

DISCUSSION

The two alkaline phosphatases used in this investigation were from different sources and were highly purified (Morton, 1953*a*, 1954). Although they have similar substrate specificity, they are quite different enzymes (Morton, 1955*a*). Under similar experimental conditions, the efficiency of phosphorylation of glucose by microsomes from intestinal mucosa was the same as that obtained with partially purified and purified enzyme preparations. With the purified enzymes from intestinal mucosa and from milk, the initial rates of phosphate transfer and of hydrolysis were similarly influenced by the pH, and by the concentration of phosphate donor or inorganic phosphate. These findings establish that the phosphotransferase activity is undoubtedly a property of the mucosal and milk alkaline phosphatases themselves.

Table 1 confirms the synthesis of esters from inorganic phosphate and sugars (Kay, 1928; Meyerhof & Green, 1949). Large amounts of inorganic phosphate inhibit both the hydrolytic and the transferase activities of alkaline phosphatase (cf. Tables 1 and 2). Experimental conditions may be chosen so that ester phosphate is synthesized from phosphocreatine and glucose, but not from

comparable (or smaller) concentrations of inorganic phosphate and glucose (Table 2). It is clear therefore that the transferase reaction of alkaline phosphatase involves direct transfer of the phosphate group from the donor without the intermediary formation of inorganic phosphate, a finding previously established by the use of radioactive phosphorus (Meyerhof & Green, 1950).

Meyerhof & Green (1950) suggested that both the rate of synthesis of new ester and the efficiency of phosphorylation (percentage transfer) are determined by the free energy of hydrolysis of the donor. These workers used as phosphate donors phosphocreatine and glucose 1-phosphate, and in later work (Green & Meyerhof, 1952) acetyl phosphate, phosphocreatine, phosphopyruvate and *p*-nitrophenyl phosphate. As already pointed out (Morton, 1952*a, b*; 1953*b*), the rates of synthesis of glucose and glycerol phosphates are not related to the free energy of hydrolysis of the donor, but rather are determined primarily by the affinity of the enzyme for the particular donor used (see Table 7). Under comparable conditions of pH, enzyme and acceptor concentrations, the donors which are the more rapidly hydrolysed also give the higher rates of formation of new ester. Compounds (such as ATP) which are not hydrolysed (see above) do not act as donors for the transferase reaction. With the one acceptor at a given concentration, substantially the same percentage transfer was achieved with all substrates investigated, which included β -glycerophosphate and phosphocreatine (Morton, 1953*b*, and this paper, Table 7). The same is true of the prostate acid phosphatase also (Morton, 1953*b*, 1958*b*). The percentage transfer is chiefly a function of the affinity of the enzyme for the acceptor, and the acceptor concentration. The results indicate the importance of the enzyme complexes involved in the reaction and suggest that the activation energies for the enzymic reaction, rather than the free energy of hydrolysis of the donor, determine the rate of synthesis of new ester and probably also the percentage transfer.

Although both the intestinal and milk alkaline phosphatases, and prostate acid phosphatase (Appleyard, 1948; Morton, 1952*a*, 1953*b*, 1958*b*), catalyse simultaneous phosphate-transfer and hydrolysis reactions, it cannot be concluded that this is a general property of phosphatases (Meyerhof & Green, 1950). The results with hexose diphosphatase, 5'-nucleotidase and myosin-adenosine triphosphatase suggest that these enzymes catalyse hydrolysis only. Although a far wider range of acceptors should be examined in order to substantiate this finding, the maintenance of the rate of hydrolysis in the presence of high concentrations of acceptors, and hence lowered concentrations of free water, supports this view (Table 5, and Morton,

1955c). The failure of myosin-adenosine triphosphatase to catalyse phosphate-exchange reactions (Koshland, Budenstein & Kowalsky, 1954; Koshland, 1955) further substantiates the absence of phosphotransferase activity (Morton, 1953b). Heppel & Hilmoe (1951) reported that the specific yeast inorganic pyrophosphatase fails to catalyse synthesis of organic pyrophosphates. Thus phosphatases may be classified into two groups, namely (a) those enzymes which are specific hydrolases, in that water is the only phosphate acceptor, and (b) those which are transferases as well as hydrolases, in that other hydroxy compounds in addition to water may act as phosphate acceptors (Morton, 1952a, c; 1953b).

Acceptor specificity of alkaline phosphatases

The results show that the polyols and trioses used in this study are more effective than hexoses or sucroses at similar acceptor concentrations. These compounds do not contain ionizable groups, and where these are present the percentage transfer may vary with pH, whereas it is independent of pH with glucose and similar acceptors (Fig. 2). The influence of groups adjacent to the hydroxyl, and of the configuration and size of the acceptor, probably accounts for the absence of appreciable transfer to sucrose, to the nucleosides or to diphosphopyridine nucleotide (Morton, 1955d). The preliminary findings of phosphorylation of serine, threonine and ethanolamine require confirmation.

These results indicate that the various acceptors, including water, are absorbed at specific sites of the enzyme surface (Morton, 1952a-c, 1953b, 1955c). Whereas the qualitative specificity of the two alkaline phosphatases towards hydroxy compounds is similar, there may be quantitative differences, as suggested by the lower phosphorylation of glucose and of glycerol by the milk enzyme (cf. Table 3 and Fig. 3, and Table 8 and Fig. 5).

The specificity of the alkaline phosphatases to the products synthesized is especially noteworthy. The results show that DL- α -glycerophosphate is the major product formed by phosphate transfer to glycerol, whether β -glycerophosphate or another compound is the donor. Whereas both α - and β -glycerophosphates are readily hydrolysed by alkaline phosphatases (Morton, 1955b), α -glycerophosphate is the major product formed from inorganic phosphate and glycerol (Meyerhof & Green, 1949). The synthesis of α -glycerophosphate from β -glycerophosphate (Table 7) ostensibly is a 'phosphoglyceromutase' reaction (see Dixon & Webb, 1953). Glucose 6-phosphate is the major product formed in the transferase reaction with glucose, as it is also in the synthesis from inorganic phosphate and glucose (Meyerhof & Green, 1949, and Table 1). Surprisingly, the predominant

initial product formed with fructose seems to be fructose 6-phosphate with a small amount of fructose 1:6-diphosphate (see Results section), although most of the sugar would be in the pyranose configuration and so would be expected to give fructose 1-phosphate. The results mentioned above (and Fig. 4) suggest that fructose in the furanose configuration is preferentially phosphorylated. Since, under the experimental conditions, fructofuranose is about 20-30% of the total fructose (Gottschalk, 1945) this may account for the much lower rate of phosphorylation found with fructose (Fig. 4) as compared with glucose (Fig. 3). Meyerhof & Green (1949), however, found that the product of inorganic phosphate and fructose incubated with phosphatase for 72 hr. at 38° was about 65% of fructose 1-phosphate and 28% of fructose 6-phosphate, as well as a small amount of fructose 1:6-diphosphate.

Mechanism of the phosphotransferase reaction

The plots of initial reaction rates at increasing concentrations of acceptors (Figs. 3-5) provide information concerning the mechanism of the transferase reactions of hydrolytic enzymes (Morton, 1952a-c, 1953b, 1955c). In each, the rate of synthesis of new ester increases with increasing concentration of acceptor up to a limiting concentration, beyond which further increase of acceptor concentration causes a fall in the rate of synthesis. The rate of hydrolysis decreases with increase of the acceptor concentration. The percentage transfer increases with acceptor concentration up to a maximum value, which occurs close to the concentration of acceptor which gives the maximum rate of ester synthesis. Further increase of acceptor concentration causes no further change in the percentage transfer.

The plot of synthesis rate versus acceptor concentration (Fig. 3, curve C, for example) is of a form resembling that obtained with many enzymes for the plot of rate of formation of product versus substrate concentration. It is consistent with the view that the acceptor is adsorbed at a specific site of the enzyme. It is assumed that this site is saturated when the acceptor reaches the limiting concentration giving the maximum rate of ester synthesis. From Figs. 3-5, and from Table 4, the concentrations of acceptors giving half the maximum rates of synthesis are approx. 0.4, 0.4, 0.9 and 0.9M for glucose, fructose, glycerol and propane-1:2-diol respectively.

The decline in the rate of hydrolysis with increasing concentration of acceptor (Figs. 3-5) may be due to the lowering of the concentration of water molecules and also to direct competition of the other hydroxy compound with the water. The fall in the rates of synthesis and of hydrolysis at very

high concentrations of acceptors (e.g., between 2 and 4M-glucose in Fig. 3) appears to be due to unspecific effects of low water content.

These observations suggest that the transferase activity is the result of the competition between the acceptor molecules and water molecules for sites at the enzyme surface. The two may not necessarily compete for the same site (Morton, 1955c), although this is not unlikely. The interaction of acceptor molecules with the enzyme-donor complex (or its reaction product) in some cases may lead to an increased rate of utilization of the donor as compared with the rate in the absence of added acceptor (see Fig. 3, curve A, for example.)

A direct non-enzymic transfer of phosphate from phosphocreatine to glucose may also occur under acid conditions (Morton, 1952a, b, 1958b). The difference between the products formed in this reaction and those formed by phosphotransferase action supports the suggestion that the acceptor is adsorbed at the enzyme surface. A mechanism for the transferase action, which is consistent with these kinetic results, has been proposed (Morton, 1952c, 1953b, 1955c). However, it is recognized that kinetic studies, by themselves, cannot establish any reaction mechanism, and the suggested sequence of reactions is by no means proven. Alternative interpretations of the phosphotransferase reaction have been offered (Koshland, 1955; Swoboda, 1955). Formulation of any reaction mechanism for the alkaline phosphatases must take into account not only the kinetic behaviour in respect of the transferase reaction, but also the kinetics of hydrolysis (Morton, 1957).

SUMMARY

1. Alkaline phosphatases from calf intestinal mucosa and from cow's milk catalyse synthesis of phosphate esters by direct transfer of the phosphate group from phosphomonoesters to suitable hydroxy compounds. There is no requirement for inorganic phosphate.

2. Kinetic studies of the transfer reaction were carried out with glucose, fructose and glycerol as phosphate acceptors. The products were estimated enzymically.

3. Over the range pH 8–10, the rate of synthesis of glucose phosphate varied with pH, but the percentage transfer was independent of pH. The rate of synthesis of new ester and of hydrolysis of the donor varied with the nature and concentration of the donor, but the percentage transfer was independent.

4. Whereas the rate of hydrolysis of the donor decreases, the rate of synthesis of new ester, and of percentage transfer, increases with acceptor con-

centration up to a limiting value which varies with each acceptor.

5. Neither the initial rate of synthesis of new ester nor the percentage transfer is related to the free energy of hydrolysis of the donor, but both vary considerably with the nature of the acceptor.

6. Kidney hexose diphosphatase, 5'-nucleotidase and myosin-adenosine triphosphatase failed to catalyse transfer to acceptors other than water.

7. Competition between water and other hydroxyl-containing compounds for sites at the surface of the enzyme-donor complex is postulated to explain the reactions of the alkaline phosphomonoesterases.

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REFERENCES

- Appleyard, J. (1948). *Biochem. J.* **42**, 596.
 Atkinson, M. R. & Morton, R. K. (1958). In *Comparative Biochemistry*, vol. 1. Ed. by Florkin, M. & Mason, H. New York: Academic Press Inc. (In the Press.)
 Axelrod, B. (1947). *Fed. Proc.* **6**, 235.
 Axelrod, B. (1948a). *J. biol. Chem.* **172**, 1.
 Axelrod, B. (1948b). *J. biol. Chem.* **176**, 296.
 Dent, C. E. (1948). *Biochem. J.* **43**, 169.
 Dixon, M. (1949). *Multi-Enzyme Systems*. Cambridge University Press.
 Dixon, M. & Webb, E. (1953). *Brit. med. Bull.* **9**, 110.
 Eggleton, P., Elsdon, S. R. & Gough, N. (1943). *Biochem. J.* **37**, 526.
 Gomori, G. (1943). *J. biol. Chem.* **148**, 139.
 Gottschalk, A. (1945). *Nature, Lond.*, **156**, 540.
 Green, H. & Meyerhof, O. (1952). *J. biol. Chem.* **197**, 347.
 Hanes, C. S. & Isherwood, F. A. (1949). *Nature, Lond.*, **164**, 1107.
 Heppel, L. A. & Hilmo, R. J. (1951). *J. biol. Chem.* **192**, 87.
 Kay, H. D. (1928). *Biochem. J.* **22**, 855.
 King, E. J. (1951). *Micro-Analysis in Medical Biochemistry*. 2nd ed. London: Churchill.
 King, E. J. & Delory, G. E. (1940). *Enzymologia*, **8**, 278.
 Koshland, D. E. (1955). *Disc. Faraday Soc.* **20**, 271.
 Koshland, D. E., Budenstein, Z. & Kowalsky, A. (1954). *J. biol. Chem.* **211**, 279.
 Markham, R. & Smith, J. D. (1949). *Nature, Lond.*, **163**, 250.
 Markham, R. & Smith, J. D. (1951). *Biochem. J.* **49**, 401.
 Meyerhof, O. & Green, H. (1949). *J. biol. Chem.* **178**, 655.
 Meyerhof, O. & Green, H. (1950). *J. biol. Chem.* **183**, 377.
 Morton, R. K. (1952a). Ph.D. Thesis: University of Cambridge.
 Morton, R. K. (1952b). In *Résumés des Communications, II^e Int. Congr. Biochim., Paris*, p. 262.
 Morton, R. K. (1952c). In *Phosphorus Metabolism*, vol. 2, p. 125. Ed. by McElroy, W. D. & Glass, B. Baltimore: The Johns Hopkins Press.
 Morton, R. K. (1953a). *Biochem. J.* **55**, 795.
 Morton, R. K. (1953b). *Nature, Lond.*, **172**, 65.

- Morton, R. K. (1953c). *Biochem. J.* **55**, 786.
Morton, R. K. (1954). *Biochem. J.* **57**, 595.
Morton, R. K. (1955a). *Biochem. J.* **60**, 573.
Morton, R. K. (1955b). *Biochem. J.* **61**, 232.
Morton, R. K. (1955c). *Disc. Faraday Soc.* **20**, 149.
Morton, R. K. (1955d). *Biochem. J.* **61**, 240.
Morton, R. K. (1957). *Biochem. J.* **64**, 674.
Morton, R. K. (1958a). *Biochem. J.* **70**, 134.
Morton, R. K. (1958b). *Biochem. J.* **70**, 150.

- Oesper, P. (1951). In *Phosphorus Metabolism*, vol. 1, p. 523. Ed. by McElroy, W. D. & Glass, B. Baltimore: The Johns Hopkins Press.
Slater, E. C. (1953). *Biochem. J.* **53**, 157.
Swoboda, P. A. T. (1955). *Disc. Faraday Soc.* **20**, 271.
Tsao, T. C. (1953). *Biochem. biophys. Acta*, **11**, 368.
Weil-Malherbe, H. & Green, R. H. (1951). *Biochem. J.* **49**, 286.

The Phosphotransferase Activity of Phosphatases

3. COMPARISON OF ENZYMIC CATALYSIS BY ACID PHOSPHATASE WITH NON-ENZYMIC CATALYSIS AT ACID pH VALUES

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In a preceding paper (Morton, 1958a) it was shown that highly purified alkaline phosphomonoesterases from cow's milk and from calf intestinal mucosa have associated 'phosphotransferase' (Dixon, 1949; Dixon & Webb, 1953) activity. The kinetics of the phosphorylation of glucose, fructose, glyceraldehyde, dihydroxyacetone and glycerol were investigated with sensitive enzymic methods developed for estimation of the phosphorylated products (Morton, 1958b).

This paper describes similar studies with a partially purified acid phosphatase from prostate gland, the phosphotransferase activity of which was first observed by Appleyard (1948). During this investigation it was found that glucose phosphates were formed by phosphate transfer from phosphocreatine in a non-enzymic reaction occurring at the same pH values as used for the studies with the acid phosphatase. A comparison was made of the enzymic and non-enzymic reactions (Morton, 1952a).

This work has been briefly reported elsewhere (Morton, 1952b, 1953).

MATERIALS AND METHODS

Except for the acid-phosphatase preparation, the materials and methods have been described in a preceding paper (Morton, 1958a).

Acid phosphatase. A human prostate gland was finely minced and then ground to a smooth paste in a mortar with fine acid-washed sand and 0.15M-NaCl, pH 7.0 (at 22°). The paste was diluted with 5 vol. of 0.15M-NaCl and the suspension stirred gently for 30 min. and then centrifuged

at 2000 g for 30 min. The supernatant was then re-centrifuged at 14 000 g for 30 min. The clear supernatant was fractionated by addition of $(\text{NH}_4)_2\text{SO}_4$, the pH being maintained at about 6.8 at 20°. The precipitate obtained between 55 and 65% saturation with $(\text{NH}_4)_2\text{SO}_4$ was dissolved in water and dialysed in seamless cellophane tubing against water for 24 hr. at 0–4°. A precipitate was removed by centrifuging at 14 000 g for 30 min. in a cold-room at 2°, and the clear supernatant was stored at 0°.

RESULTS

Reactions catalysed by acid phosphatase

Phosphorylation of glucose. The reaction mixtures contained acid phosphatase (except in the control tube), 8 mM-phosphocreatine, 0.1M-sodium acetate buffer, pH 5.5, and increasing concentrations of glucose up to 4M. All components except the phosphocreatine were added to the tubes, the volumes were adjusted to 0.4 ml. and the tubes brought to 38°. The reaction was commenced by addition of phosphocreatine, and stopped after 5 min. at 38° by addition of trichloroacetic acid as already described (Morton, 1958b). The analyses were carried out as described before (Morton, 1958a, b), the glucose phosphate being estimated enzymically. Fig. 1 shows that the rate of synthesis of glucose phosphate (curve C) increased continuously up to 3.5M-concentration of glucose. The rate of hydrolysis, as indicated by the liberation of inorganic phosphate (curve B), declined continuously with increasing concentrations of glucose, whereas the rate of utilization of phosphocreatine (due to both the hydrolysis and transfer reactions) remained fairly constant (curve A). Curve D shows that the percentage transfer (see Morton, 1958a) increased to about 13% with 4M-glucose.

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