3. The presence of an inositol-containing phosphoglyceride in rat liver has been confirmed.

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The Effect of Nucleotides on the Formation of Phosphopyruvate

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Utter & Kurahashi (1954*a, b*) have isolated from chicken liver an enzyme that catalyses the reactions

where ATP, ITP, ADP and IDP represent adenosine triphosphate, inosine triphosphate, adenosine diphosphate and inosine diphosphate respectively. ITP was found to accelerate the decarboxylation more effectively than ATP. In studies on sheep-kidney cyclophorase Bartley (1954) showed that the phosphate of phosphopyruvate was derived from ATP. In this paper a comparison has been made between ATP, ITP and inorganic phosphate as a source of the phosphate in phosphopyruvate. It was found that when oxaloacetate or malate is oxidized phosphopyruvate can be formed without participation of either nucleotide.

EXPERIMENTAL

Tissue preparations. Twice-washed particles (cyclophorase) of pigeon-liver homogenates, prepared according to Bartley & Davies (1954), were used in most experiments. The homogenates were made in a stainless-steel homogenizer of the Potter-Elvehjem type. In a few experiments washed mitochondria were used.

Media. In most experiments 2 ml. of the suspension (about 70 mg. dry wt.) were added to 2.5 ml. of a solution containing K phosphate buffer, pH 7.4 (0.008M), MgCl₂ (0.0016M), aminotrihydroxymethylmethane (tris) buffer pH 7.4 (0.04M), substrate (0.02M) and the nucleotide under test; the amounts of the nucleotide are given in the description of the individual experiments.

Special chemicals. ITP was prepared by deamination of ATP by the method of Kleinzeller (1942); inosine monophosphate (IMP), adenosine monophosphate (AMP) and coenzyme I (DPN) were commercial products. The other chemicals were those used by Bartley & Avi-Dor (1955).

Analytical methods. The procedures described by Bartley & Avi-Dor (1955) were used.

RESULTS

Effect of ATP or ITP on malate or oxaloacetate metabolism

Table 1 gives the results of preliminary experiments, with malate or oxaloacetate as substrate, in which phosphopyruvate formation by pigeon-liver cyclophorase was measured in the presence or absence of ATP or ITP. ATP increased oxygen-uptake, malate consumption and also, in most experiments, phosphopyruvate formation. The increase in phosphopyruvate production was smaller when the amount of ATP added was large (Expts. 1 and 2). The stimulation by ATP of oxygen and malate consumption was less when the tissue concentration was high (Expts. 2 and 4). ITP stimulated respiration in the presence of malate (Expt. 4), but did not influence malate utilization or phosphopyruvate formation. When oxaloacetate was substrate, ATP but not ITP stimulated respiration (Expt. 5), but neither nucleotide in the concentration used affected phosphopyruvate formation. At higher concentrations of nucleotide (Expt. 6) the yield of phosphopyruvate was greater in the presence of ITP than of ATP. The addition of ATP and ITP

together reduced the amount of phosphopyruvate formed (Expt. 6).

Oxidation and phosphorylation occurred at a relatively high rate in the cyclophorase preparations without the addition of ATP (Expts. 1 and 5). This was taken to indicate a saturation of the particles by the endogenous ATP. If this is correct, washing of the preparation should simultaneously lower the amount of endogenous nucleotides and reduce ability to form phosphopyruvate. However, washing did not lower the

oxygen uptake or malate consumption or affect phosphopyruvate formation from malate or oxaloacetate (Table 2), although it greatly decreased the amounts of ATP and ITP in the suspension. The small amounts of nucleotides remaining in the particles at the end of the incubation were estimated in the following way. Labelled orthophosphate was added at the start of incubation and the specific activities of the orthophosphate and phosphopyruvate in a sample of the tissue suspension were measured at the end of the incubation

Table 1. *Effect of ATP or ITP on pigeon-liver cyclophorase oxidizing malate or oxaloacetate*

Pigeon-liver cyclophorase incubated in Warburg vessels at 20° in O₂ for 20 min. Each vessel contained 2 ml. of the tissue suspension in 0.9% (w/v) KCl solution and 2.5 ml. of a solution containing K phosphate buffer pH 7.4 (0.008M), MgCl₂ (0.0016M), tris buffer pH 7.4 (0.04M) and substrate (0.02M). The amounts of nucleotide and the dry wt. of the tissue added are given below.

Expt. no.	Dry wt. of tissue (mg.)	Substrate added	Nature and amount (μmoles) of nucleotide added	O ₂ uptake (μmoles)	Phosphopyruvate formed (μmoles)	Malate used (μmoles)
1	72	Malate	None ATP (4)	4.7 7.3	5.6 8.1	7.4 13.2
2	108	Malate	None ATP (10)	9.5 10.8	10.6 11.6	9.7 12.1
3	51	Malate	None ATP (6)	— —	5.3 5.5	— —
4	57	Malate	None ITP (4) ATP (10)	3.0 5.0 6.2	6.3 5.6 6.8	6.5 5.9 9.8
5	50	Oxaloacetate	None ATP (6) ITP (6)	2.3 6.1 2.1	3.5 3.6 3.6	— — —
6	70	Malate	ATP (10) ITP (10) ATP (10) + ITP (10)	— — — —	6.3 9.1 7.6	7.2 9.5 8.6
		Oxaloacetate	ATP (10) ITP (10) ATP (10) + ITP (10)	2.6 2.7 — —	4.2 7.8 6.1	— — —

Table 2. *Effect of washing with KCl solution on the production of phosphopyruvate by pigeon-liver cyclophorase*

100 mg. (dry wt.) of pigeon-liver cyclophorase incubated in Warburg vessels at 20° for 20 min. Conditions as in Table 1 except that no nucleotide was added. Washing consisted in resuspending the cyclophorase particles in 0.9% (w/v) KCl solution with the aid of a mechanical stirrer and then recentrifuging, the particles being finally suspended in KCl solution for addition to the vessel.

Expt. no.	No. of washings with KCl	Substrate	Phosphopyruvate formed (μmoles)	Δ malate (μmoles)	O ₂ uptake (μmoles)	ATP present in vessel after 20 min. incubation (μmole)
1	2	Malate	7.8	-10.3	6.6	0.27
2	4	Malate	10.7	-17.9	7.4	0.05
3	5	Malate	12.4	-14.7	8.6	0.03
4	5	Oxaloacetate	11.2	+ 9.1	4.0	0.01
5	5	Oxaloacetate	11.1	+ 9.7	4.2	0.01

after separation of the phosphate esters by paper chromatography. Carrier ATP was added to a further sample of the deproteinized incubation mixture. The phosphate esters were separated on the paper chromatogram and the radioactivity found in the ATP area was measured. The amount of ATP present was then calculated on the assumption that the β and γ phosphorus atoms of the endogenous ATP were in equilibrium with the orthophosphate at the end of the incubation. The amount of ATP found was of the same order as that found by Bartley & Davies (1954) by direct analysis of the phosphate compounds in unincubated washed sheep-kidney mitochondria, namely 0.01–0.03 μ mole/100 mg. of tissue preparation. The amount of ITP was calculated on the assumption that only the γ phosphorus atom of ITP exchanges with orthophosphate. After five washings the amounts of ITP were of the same order as those of ATP. Although the addition of nucleotide is not necessary for phosphopyruvate formation from malate or oxaloacetate, the addition of 2:4-dinitrophenol or sodium azide (0.001 M) completely inhibits phosphopyruvate formation. However, phosphopyruvate can be formed in the presence of 2:4-dinitrophenol only when α -oxoglutarate is the substrate (Mudge, Neuberg & Stanbury, 1954). Oxidative phosphorylation is therefore normally involved in phosphopyruvate production, but when this mechanism is inhibited phosphopyruvate can be formed by the following series of reactions, involving phosphorylation at the substrate level:

Table 3. *Effect of ATP concentration on the formation of phosphopyruvate from malate*

Pigeon-liver cyclophorase washed 5 times with 0.9% (w/v) KCl solution (84 mg. dry wt.) incubated in Warburg vessels at 20° in O₂ for 20 min. Each vessel contained 2 ml. of the tissue suspension in 0.9% (w/v) KCl and 2.5 ml. of a solution containing K phosphate buffer pH 7.4 (0.008 M), MgCl₂ (0.0016 M), tris buffer pH 7.4 (0.04 M), malate (0.02 M) and ATP as given below. The centre well contained 0.2 ml. of 2N-NaOH and filter paper.

Amount of ATP added at the start of incubation (μ moles)	Malate consumed (μ moles)	Phosphopyruvate formed (μ moles)
0	8.0	5.9
2	9.3	7.2
4	9.5	8.8
8	10.5	6.9
12	12.6	5.5
20	14.3	5.6

*Effect of concentration of added ATP
or ITP on phosphopyruvate formation*

Malate (Tables 3 and 4) or oxaloacetate (Tables 5 and 6) were incubated for 20 min. with pigeon-liver cyclophorase and different amounts of ATP or ITP. Table 3 shows that the consumption of malate rose with the amounts of ATP added, but the phosphopyruvate formation did not parallel the

Table 4. *Effect of ITP concentration on the formation of phosphopyruvate from malate*

Pigeon-liver cyclophorase washed 5 times with 0.9% (w/v) KCl solution (69 mg. dry wt.) incubated in Warburg vessels at 20° in O₂ for 20 min. Conditions as in Table 3 except that the nucleotide added was ITP.

Amount of ITP added at the start of incubation (μ moles)	Malate consumed (μ moles)	Phosphopyruvate formed (μ moles)
0	12.4	7.4
2	12.5	7.3
4	11.6	7.6
8	11.5	7.0
12	12.2	5.7
20	8.8	5.3

Table 5. *Effect of ATP concentration on the formation of phosphopyruvate from oxaloacetate*

Pigeon-liver cyclophorase washed 5 times with 0.9% (w/v) KCl solution (92 mg. dry wt.) incubated in Warburg vessels at 20° in O₂ for 20 min. Oxaloacetate added 0.02 M; other conditions as in Table 3.

Amount of ATP added at the start of incubation (μ moles)	Phosphopyruvate formed (μ moles)
0	10.3
2	8.0
4	7.3
8	6.2
12	5.5
20	4.1

Table 6. *Effect of ITP concentration on the formation of phosphopyruvate from oxaloacetate*

Pigeon-liver cyclophorase washed 5 times with 0.9% (w/v) KCl solution (69 mg. dry wt.) incubated in Warburg vessels at 20° in O₂ for 20 min. Oxaloacetate added 0.02 M; other conditions as in Table 4.

Amount of ITP added at the start of incubation (μ moles)	Phosphopyruvate formed (μ moles)
0	9.6
2	9.9
4	8.9
8	8.4
12	7.2
20	4.6

malate consumption; it reached a maximum when 4 μ moles of ATP were added initially and declined with greater amounts of ATP. In a similar experiment with ITP (Table 4) there was no consistent trend in malate consumption. Phosphopyruvate formation was not stimulated by small amounts of ITP and larger amounts were markedly inhibitory. When oxaloacetate was the substrate (Table 5)

Bartley, 1953; Whittam, Bartley & Weber, 1955). When the incubation medium contains labelled phosphate there is a rapid equilibration of the label with the β and γ phosphorus atoms of ATP, indicating a continual phosphorylation of ADP. In contrast with the other substrates malate does not maintain ATP in pigeon- or rat-liver preparations (Table 7). As malate is readily oxidized, the dis-

Table 7. *Comparison of ATP maintenance by different tissue preparations oxidizing malate or succinate*

The tissue preparations were incubated in Warburg vessels at 20° in O₂ for 10 min. The substrate concentration was 0.02 M. In Expt. 3 the data are from Whittam, Bartley & Weber (1955) and in Expt. 4 from Krebs *et al.* (1953).

Expt. no.	Preparation	Substrate	ATP present initially (μ moles in 4 ml.)	ATP present after incubation (μ moles in 4 ml.)
1	Pigeon-liver cyclophorase	Succinate	6.0	1.1
2	Rat-liver cyclophorase	Succinate	6.0	6.1
3	Rat-liver mitochondria	Succinate	6.0	6.1
4	Rat-liver homogenate	Succinate	6.0	5.9
5	Pigeon-liver cyclophorase	Malate	6.0	0.15
6	Pigeon-liver mitochondria	Malate	6.2	0.2
7	Rat-liver cyclophorase	Malate	6.0	1.0
8	Rat-liver homogenate	Malate	6.0	3.0

there was a progressive decline in phosphopyruvate formation, with increasing ATP concentration. ITP did not affect phosphopyruvate formation at quantities of 8 μ moles or less of ITP (Table 6). At higher concentrations inhibition occurred.

The solutions of the sodium salts of ATP and ITP used in these experiments were prepared from the barium salts by precipitation of the barium with excess of sodium sulphate solution. To test whether the inhibition of phosphopyruvate formation by large amounts of nucleotides was due to concomitant addition of sulphate the tissue preparations were incubated with and without sulphate (0.005 M), with either malate or oxaloacetate as substrate. The phosphopyruvate formation was the same in the presence as in the absence of this amount of sulphate. These results with ATP confirm the observation of Bartley & Avi-Dor (1955) that in the presence of 4–10 μ moles of ATP malate was a more effective precursor of phosphopyruvate than oxaloacetate. Utter & Kurahashi (1954*a, b*) showed that ITP was more effective than ATP in reaction (1) when it was catalysed by their purified enzyme preparation but, as shown in Tables 4 and 6, added ITP did not stimulate phosphopyruvate formation from malate or oxaloacetate in pigeon-liver cyclophorase.

Maintenance of ATP during the oxidation of malate. Many tissues (rat liver, sheep kidney, sheep heart, pigeon breast muscle) can maintain an ATP concentration of 0.0025 M during the oxidation of citrate, α -oxoglutarate, succinate, pyruvate and β -hydroxybutyrate (see Krebs, Ruffo, Johnson, Eggleston & Hems, 1953; Bartley, 1953; Hems &

appearance of ATP suggests that another reaction affecting the accumulation of ATP takes place when malate is the substrate. This is the formation of phosphopyruvate (Bartley, 1954). Phosphopyruvate can be formed from ATP or ITP and oxaloacetate (Utter & Kurahashi, 1954*a, b*), but in the experiments described here it must be formed by another route, not involving ATP or ITP, as the following results show.

Experiments with $^{32}\text{P}_4$. It was previously shown (Bartley, 1954) that the specific activity of phosphopyruvate formed by sheep-kidney cyclophorase during the oxidation of α -oxoglutarate in the presence of added ATP and $^{32}\text{P}_4$ was initially less than that of ATP. The same observation was made when pigeon-liver mitochondria were incubated with succinate or α -oxoglutarate. The findings confirm that ATP can act as a phosphate donor in the formation of phosphopyruvate. Since Utter & Kurahashi (1954*a, b*) found that ITP was more active than ATP in the formation of phosphopyruvate from oxaloacetate, experiments were carried out in which ATP and ITP were both present in the incubation mixture. In one experiment malate was the substrate and in the other oxaloacetate, and in both cases the orthophosphate was labelled with ^{32}P . Incubation varied from 1 to 15 min., and after the reaction was stopped the phosphate esters were separated on the paper chromatogram and the amounts and radioactivity of the phosphate compounds were determined. In the presence of malate (Table 8) the rate of formation of phosphopyruvate was very high and the specific activity of phosphopyruvate was below

that of orthophosphate during the short incubation times. Moreover, the specific activities of the γ phosphorus atoms of ATP or ITP were considerably below the specific activity of phosphopyruvate. The specific activities of the γ phosphorus atoms have been calculated by assigning the whole of the observed counts to the terminal phosphorus atom. Thus the true specific activities of the γ phosphorus atoms must be below the values given, if any activity is present in the β phosphorus

phosphopyruvate into pyruvate. The specific radioactivity of an immediate precursor must be initially greater and later less than that of the end product. The plot of the specific activity of the precursor will cross the plot of the specific activity of the product at its maximum (see Zilversmit, Entenman & Fishler, 1943; Reiner, 1953). If the precursor is not immediate the plot of the specific activity will cross that of the product before its maximum. It follows that since the specific activity of phospho-

Table 8. *Changes in amounts and specific activities of ATP, ITP, phosphopyruvate and orthophosphate on incubation with pigeon-liver cyclophorase and malate*

Each Warburg vessel originally contained pigeon-liver cyclophorase (67 mg. dry wt.) suspended in 4.5 ml. total volume made up as follows: 2.0 ml. of 0.9% (w/v) KCl, 1.0 ml. of 0.1M tris buffer pH 7.4, 0.2 ml. of 0.05M-MgCl₂, 0.1 ml. of 0.5M malate and the phosphate compounds given at time 0 below. The nucleotides, together with tracer amounts of ³²PO₄, were placed in the side arm of the vessel. The gas phase was oxygen, the bath temperature 20° and the centre wells contained NaOH and filter paper. The vessel contents were mixed as the manometers were put in the bath and the reaction was stopped at the times given below by removal to an ice bath and addition of 0.5 ml. of 30% (w/v) TCA.

Incubation time (min.)	ITP phosphorus		ATP phosphorus		Phosphopyruvate		Orthophosphate	
	Amount (μ moles)	Specific activity (counts/min./ μ g. P)	Amount (μ moles)	Specific activity (counts/min./ μ g. P)	Amount (μ moles)	Specific activity (counts/min./ μ g. P)	Amount (μ moles)	Specific activity (counts/min./ μ g. P)
0	17.1	0	15.5	0	0	0	15.4	3100
1	10.0	110	7.8	610	1.1	1260	19.1	2340
2	9.6	170	6.7	950	1.6	1440	19.2	2000
3	5.4	310	3.8	1040	2.2	1560	23.9	1760
5	1.9	500	2.5	1140	2.5	1760	26.0	1600
8	1.5	780	2.6	1260	2.8	1720	25.7	1580
15	0.8	1040	1.1	1660	3.6	1660	27.4	1440

Table 9. *Changes in amounts and specific activities of ATP, ITP, phosphopyruvate and orthophosphate on incubation with pigeon-liver cyclophorase and oxaloacetate*

Conditions as in Table 8, except that the substrate was oxaloacetate and 81 mg. (dry wt.) of cyclophorase were added.

Incubation time (min.)	ITP phosphorus		ATP phosphorus		Phosphopyruvate		Orthophosphate	
	Amount (μ moles)	Specific activity (counts/min./ μ g. P)	Amount (μ moles)	Specific activity (counts/min./ μ g. P)	Amount (μ moles)	Specific activity (counts/min./ μ g. P)	Amount (μ moles)	Specific activity (counts/min./ μ g. P)
0	17.2	0	15.5	0	0	0	14.9	4680
1	8.3	260	6.9	1000	0.68	2880	21.0	3130
2	4.8	560	4.7	1400	1.2	3280	24.5	2630
3	3.3	720	3.1	1560	1.5	2930	25.7	2470
5	2.0	980	1.8	1880	2.1	2720	27.4	2280
8	0.94	1240	1.2	1920	2.7	2640	28.5	2130
15	0.96	1600	0.7	3000	4.3	2580	27.6	2020

atoms, as is probable at least for ATP. The specific activities of ADP, IDP, AMP and IMP were at all times lower than that of ATP or ITP. The amounts of ATP and ITP present rapidly declined, especially during the first minute. The fact that phosphopyruvate accumulates in the presence of the large amounts of ADP or IDP shows that the phosphopyruvic kinase cannot be appreciably active in these preparations, since in these circumstances the equilibrium would favour the conversion of

pyruvate is much greater than that of either ATP or ITP there must be a route of phosphopyruvate formation where ATP and ITP are not the immediate suppliers of phosphate for phosphopyruvate. The fact that the specific activity of phosphopyruvate is lower (in the early stages) than that of orthophosphate indicates that orthophosphate is not the immediate donor of phosphate.

When oxaloacetate was the substrate (Table 9) the specific activity of the phosphopyruvate rose

much faster than with malate. Within 1 min. the specific activity was very close to that of orthophosphate and by the second minute had surpassed it. In this case very little phosphate can have come from ATP or ITP. The more rapid rise in specific activity of phosphopyruvate is expected if oxaloacetate is the immediate precursor of the carbon skeleton of phosphopyruvate. The findings can be accounted for by the assumption that the primary phosphate acceptor is a nucleotide other than ADP or IDP, say guanosine diphosphate (GDP), which can donate phosphate to ADP, IDP or oxaloacetate.

0.1 μ g. of IMP phosphorus. In other experiments where ATP but no inosine was added to the incubation mixture up to 0.5 μ g. of labelled phosphorus was found in the IMP area after an incubation time of 15 min. As mentioned above, IMP and presumably inosine are rapidly formed from ATP by dephosphorylation and deamination. The greater formation of labelled IMP when ATP is added to the incubation mixture suggests that ATP may phosphorylate the inosine present. Krebs & Hems (1955) have previously shown that liver or brain preparations incubated with γ P-labelled ITP and AMP gave rise to IMP containing radioactivity.

Table 10. *Changes in amount and radioactivity of ADP, IDP, AMP and IMP on incubation with pigeon-liver cyclophorase*

Data obtained from the experiments described in Tables 8 and 9. In Expt. 1 the substrate was malate and in Expt. 2 oxaloacetate.

Expt. no.	Incubation time (min.)	ADP phosphorus		AMP phosphorus		IDP phosphorus		IMP phosphorus	
		Amount (μ moles)	Radio-activity (counts/min.)	Amount (μ moles)	Radio-activity (counts/min.)	Amount (μ moles)	Radio-activity (counts/min.)	Amount (μ moles)	Radio-activity (counts/min.)
1	0	1.85	19	0.25	12	3.0	50	0.2	20
	5	5.42	572	1.75	36	8.7	179	1.6	210
	15	2.56	581	2.75	36	4.1	358	4.6	257
2	0	1.16	73	0.21	60	2.23	169	0.47	69
	5	2.19	614	3.15	66	6.05	528	1.7	268
	15	0.85	703	2.97	74	5.1	760	5.4	872

Changes in amounts and specific activities of other nucleotides. AMP, IMP and IDP, but not ADP, showed a low activity at the end of the incubation time. Even ADP cannot have been a phosphate donor to phosphopyruvate, because of the low activities at the earlier stages. ADP formed initially disappears on further incubation; the IDP formed initially also disappears later, but relatively slowly (Table 10). At the same time very much more IMP appears than can be accounted for by the disappearance of IDP or ITP, which shows that most of the IMP is formed by the deamination of AMP. It will also be seen from Table 10 that the counts in AMP do not increase whereas those in IMP do. This suggests that some IMP must arise by a route other than the deamination of AMP or the hydrolysis of IDP, but the possibility cannot be excluded that the chromatogram area of IMP contains another phosphate ester. It is of interest that IDP also accumulates some radioactivity.

A few experiments were made to test the possibility of the phosphorylation of inosine. When well-washed pigeon-liver cyclophorase was incubated for 15 min. with malate, [32 P]orthophosphate and 0.001 M inosine in the absence of added nucleotides, radioactivity appeared on the paper chromatogram in the IMP area. The amount of radioactivity corresponded to the formation of

Fig. 1. Postulated routes of phosphorus transfer giving phosphopyruvate. X-P may be guanosine triphosphate.

DISCUSSION

In experiments where phosphopyruvate was formed from oxaloacetate or malate in the presence of radioactive phosphate the specific activities of the ATP and ITP added were always below that of the phosphopyruvate formed. These observations may be explained if it is assumed that there is a form of phosphate intermediate between orthophosphate and ATP (or ITP) and that this primary product of oxidative phosphorylation (X-P) donates its phosphate to oxaloacetate rather than to ADP or IDP. Fig. 1 shows the reaction sequences

envisaged. Thus when α -oxoglutarate or succinate is oxidized in the circumstances described by Bartley (1954) only small amounts of oxaloacetate are formed and ADP successfully competes for the high-energy phosphate of X-P; ATP is maintained at a high level and phosphopyruvate formation is thus by route (a). In this case the specific activity of ATP will always be greater than that of phosphopyruvate. When malate or oxaloacetate is oxidized the large amount of oxaloacetate present successfully competes with ADP for the phosphate of X-P, and (b) becomes the main route of phosphopyruvate formation. When this occurs a high concentration of ATP cannot be maintained; this is in accordance with the experimental observations in Table 7, which shows that ATP was not maintained when malate was oxidized by different tissue preparations. It is feasible that the low specific activity of ATP, compared with that of phosphopyruvate, is due to part of the ATP (possibly that within the mitochondria) becoming radioactive at a higher rate than the rest, because of impermeability of the mitochondrial membrane to external ATP, and that this fraction specifically donates its phosphorus to phosphopyruvate. This explanation could not simultaneously account for the inability of malate to maintain ATP. Although the possibility of the ATP reacting in an inhomogeneous way cannot be excluded, it is thought to be unlikely; it is known that with other tissues and substrates (Krebs *et al.* 1953; Bartley, 1953; Whittam, Bartley & Weber, 1955) under comparable conditions the γ phosphorus atom of ATP rapidly equilibrates with labelled orthophosphate (in 8–10 min. at 20°). Moreover, it seems that when succinate or α -oxoglutarate is a substrate the mitochondrial membrane is permeable to external ATP, for in this case the specific activity of the ATP is greater than that of the phosphopyruvate. The direct phosphorylation of oxaloacetate by X-P would also explain the inability of ATP or ITP to stimulate phosphopyruvate production in tissue preparations even where ATP and ITP had been reduced to a very low level by washing. It is possible that X-P may be guanosine triphosphate (GTP), which is known to act as a phosphate donor to ADP in the oxidative decarboxylation of α -oxoglutarate (Sanadi, Gibson & Padmasini Ayengar, 1954). Guanosine diphosphate is also more active than IDP or ADP in the fixation of carbon dioxide by reaction (1) (Utter, 1955). This lends support to the suggestion that X-P is GTP.

SUMMARY

1. Phosphopyruvate is formed at a high rate from malate or oxaloacetate by well-washed pigeon-liver cyclophorase without the addition of

adenosine triphosphate (ATP) or inosine triphosphate (ITP).

2. When phosphopyruvate is formed from malate or oxaloacetate in the presence of ATP, ITP and radioactive phosphate, the specific activity of the phosphopyruvate increases faster than that of either nucleotide. The results suggest that phosphopyruvate in these conditions does not receive phosphate primarily from ATP or ITP, but from some other endogenous phosphate rapidly in equilibrium with the added orthophosphate. A possible mechanism involving guanosine triphosphate is suggested.

3. Rat-liver homogenate, cyclophorase or mitochondria maintain ATP at a steady concentration of approximately 0.0015M when oxidizing succinate, but fail to maintain ATP when oxidizing malate. Similar preparations from pigeon liver fail to maintain ATP when oxidizing either succinate or malate, but the breakdown of ATP is much more rapid in the presence of malate. This is explained by the assumption that ADP and oxaloacetate compete for the phosphate fixed in GTP by oxidative phosphorylation.

4. Observations on the effects of ATP and ITP on the consumption of malate and the formation of phosphopyruvate also suggest that these nucleotides are not primary phosphate donors in the formation of phosphopyruvate.

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