56. Dissimilation of Phosphoglyceric Acid by *Escherichia coli*

By M. F. Utter and C. H. Werkman, *Industrial Science Research Institute,
Iowa State College, Ames, Iowa, U.S.A.*

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Still [1940] compared the triosephosphate dehydrogenase of *Escherichia coli* with the corresponding enzyme in yeast, and Utter & Werkman [1941] similarly compared the enzymes aldolase and isomerase of *E. coli* with those in muscle and yeast. This communication extends this line of investigation to the reactions involving phosphoglyceric acid.

Embden, Deuticke & Kraft [1933] and Meyerhof & Kiessling [1933] showed that (-)3-phosphoglyceric acid was converted into pyruvic acid and phosphoric acid in muscle and Lohmann & Meyerhof [1934] established phosphopyruvic acid as an intermediate. Further investigations by Meyerhof & Kiessling [1934; 1935*a, b*] disclosed still another intermediate, (+)2-phosphoglyceric acid. The series of reactions may be represented:

The final stage of the reaction, the conversion of phosphopyruvic acid into pyruvic acid and H_3PO_4 was shown to be the only irreversible reaction of the series [Meyerhof, Ohlmeyer & Möhle, 1938].

Antoniani [1933] reported that bacteria also converted phosphoglyceric acid into pyruvic acid and H_3PO_4 . Stone & Werkman [1936; 1937] established phosphoglyceric acid as an intermediate in bacterial dissimilation by isolating that compound from fermentations by numerous genera of bacteria. Additional evidence of the presence of a phosphorylating mechanism in bacterial fermentation has been given by Tikka and Endo. Tikka [1935] investigated the fermentation of several intermediate phosphate esters and showed that the end-products were normal. Endo [1938] obtained an acetone powder from *E. coli* which showed many of the general characteristics of muscle and yeast preparations. Wiggert & Werkman [1938], working with a living culture of *Aerobacter aerogenes*, demonstrated an initial esterification of phosphate in the fermentation of glucose.

A cell-free preparation of *E. coli* is shown by the present work to contain the enzymes which convert phosphoglyceric acid into phosphopyruvic acid, and those which transfer phosphate from the latter acid to adenylic acid and to glucose.

METHODS

The preparation of the bacterial extract used in these experiments followed the general directions given in an earlier communication [Utter & Werkman, 1941]. The wet bacterial mass was mixed with powdered glass and ground as described by Wiggert, Silverman, Utter & Werkman [1940], with the following modifications. The proportion of glass to wet bacteria was 2:1 and the grinding was accomplished by passing the bacteria-glass mixture between concentric ground glass cones. The inner one was rotated by a motor. The resulting paste was extracted with 1.5 ml. of water for each gram of wet bacteria

used in preparing the mixture. After the glass had been thrown down on an angle centrifuge at 4000 r.p.m., centrifuging of the extract was continued on a small air-driven Beams' centrifuge until the cells and cell fragments were largely removed and the extract was clear although it retained a slight brownish opalescence.

The preparation used in these experiments was very similar to that employed in the study of the aldolase and isomerase equilibria [Utter & Werkman, 1941]. Although the method of preparation has been modified slightly, the extract contains essentially the same enzymes but in greater concentration. Both extracts contain the enzymes necessary for the conversion of hexosediphosphate into phosphopyruvate, and for the transfer of PO_4 from phosphopyruvate to adenylic acid and to glucose. The preparations do not attack pyruvic acid or its products and have negligible aerobic activity.

In the previous work on the aldolase-isomerase equilibria, the dismutation of the triosephosphate immediately following those equilibria, was inhibited by removing coenzyme I from the extract by dialysis. The action on hexosediphosphate was thus effectively restricted to aldolase and isomerase. Dialysis was not necessary in the present experiments since the reactions did not ordinarily proceed beyond the stage of phosphopyruvate unless a phosphate acceptor was added.

In some experiments, the extract was dialysed in a collodion tube at $4-5^\circ$ against circulating distilled water. In a few experiments, the extract was dried in a vacuum desiccator over CaCl_2 and the resulting powder was dissolved in water to form the enzyme solution. In addition to greater stability, the dried powder gave greater activity when dissolved in a smaller proportion of water than was present in the original extract. Precipitation of the enzymes by acetone or ammonium sulphate, followed by drying and redissolving, greatly diminished the activity.

Since the preparation had negligible aerobic activity, the experiments were performed in air. In the majority of experiments, 0.2–0.3 ml. of extract was added to a similar volume of substrate, together with buffer and other additions to make a total volume of 1.0 ml. The reaction was stopped after incubation by addition of 5 ml. of 4% CCl_3COOH which had been brought previously to the same temperature as the sample. Duplicate tubes in which enzyme activity was prevented by addition of the deproteinizing agent at the start of the experiment served as controls. After addition of the CCl_3COOH , the solution was diluted to 10 ml., filtered, and the various phosphate fractions determined on aliquots.

Inorganic phosphate was determined on a Klett-Summerson photoelectric colorimeter according to Fiske & Subbarow [1925]. Phosphopyruvate (PP) was determined as the fraction of phosphate liberated by treatment with alkaline iodine at room temperature [Lohmann & Meyerhof, 1934]. Since phosphopyruvate is also completely hydrolysed in 60 min. at 100° in N HCl, the alkaline-iodine fraction was compared with the 60 min. fraction in a number of experiments. Phosphoglyceric acid was determined by subtracting the inorganic + 180 min.-hydrolysable phosphate from the total phosphate as determined by ashing, since phosphoglyceric acid is not hydrolysed in 180 min. Adenosinetriphosphate (ATP) was estimated as the 7 min.-hydrolysable fraction except in the presence of phosphopyruvic acid, when adenosinetriphosphate was obtained by subtracting the alkaline- I_2 fraction (phosphopyruvate) from the 60 min.-hydrolysable fraction (phosphopyruvate + ATP).

Commercial preparations of barium phosphoglycerate and adenylic acid were used in the preparation of the substrates. It should be pointed out that the Ba salt used was a mixture of 3- and 2-phosphoglycerate and in the experiments where pure 3-phosphoglycerate was required, the salt was recrystallized several times by dissolving in dilute HCl and reprecipitating at a reaction just acid to Congo red by adding alcohol dropwise. A sample of synthetic barium phosphopyruvate was made available through the kindness of Dr Gerhard Schmidt.

EXPERIMENTAL

Equilibria between phosphoglycerate and phosphopyruvate

Table 1 (exps. 1-4) illustrates the effect of temperature on the amount of phosphopyruvate formed from phosphoglycerate. The phosphopyruvate was determined from the phosphate present in the appropriate fraction. The balance of the organic phosphate is present as phosphoglycerate. The amounts of phosphopyruvate phosphate formed at the various temperatures compare favourably with those reported by Lohmann & Meyerhof [1934] with muscle. It will be noted that in addition to the phosphopyruvate formed, small amounts of inorganic phosphate also appeared. The extent of the phosphatase action depends on the success achieved in removing the enzyme responsible, adenosinepolyphosphatase, during the centrifuging employed in the preparation of the extract. The inorganic phosphate was subtracted from the organic phosphate and did not enter into the calculation of the percentage of phosphopyruvate formed.

Table 1. *Effect of temperature and phosphoglycerate concentration on phosphopyruvate (PP) formation*

Per experiment: 0.3 ml. extract, 0.2 ml. 0.3 *M* NaHCO₃. * Total vol. 1.0 ml. Final pH, 7.4.

Exp. no.	1	2	3	4	5	6	7	8	9
Time of incubation, min.	60	30	20	10	30	30	30	30	30
Temp. °	2	24	40	59	24	24	24	24	24
PGA-P, 1×10^{-2} mg. P/ml.	27.8	27.8	27.8	27.8	6.95	13.90	27.8	41.7	55.6
After incubation, 1×10^{-2} mg./ml.									
PP	5.59	7.75	9.35	11.78	2.26	3.91	7.75	10.93	12.73
Inorg. P	0.47	0.51	1.91	1.37	0	0.71	0.61	0.40	0.34
Total organic P	27.33	27.29	25.89	26.43	6.95	13.19	27.19	41.30	55.26
% organic P as PP	20.46	28.39	36.10	44.55	32.52	29.63	28.50	26.45	23.03

* Freshly saturated with CO₂.

In exps. 5-9, Table 1, the approximate upper limit of the substrate concentration is defined. With 0.3 ml. of extract in a total volume of 1.0 ml., approximately 40×10^{-2} mg. phosphoglycerate P (2.4 mg. PGA) is the maximum concentration which can be brought to equilibrium in 30 min. The equilibrium point is fairly constant although the value rises slightly as the concentration decreases.

The upper limit of the substrate concentration depends on the incubation period and on the concentration of the enzyme. Fig. 1 shows that equilibrium was not attained in 15 min. but that the percentage of phosphopyruvate P was maximum in *ca.* 30 min. Fig. 2 illustrates the effect of dilution on the enzyme. The upper and lower curves represent two series of dilutions, incubated for 60 and 30 min. Both curves approach the theoretical value of 31-32% phosphopyruvate P.

The preceding studies have been based on the calculation of the percentage of phosphopyruvate formed. As stated previously, Meyerhof & Kiessling [1935*a*] demonstrated that another intermediate, (+)2-phosphoglycerate is involved, and the production of phosphopyruvate from 3-phosphoglycerate involves two interlocking equilibria as shown in the equations above.

Meyerhof & Schulz [1938] described a polarimetric method for the determination of both isomers of phosphoglyceric acid, in which the rotatory power of the esters is greatly increased by the addition of ammonium molybdate. In this way the $[\alpha]_D^{20}$ of (-)3-phosphoglyceric acid is increased from -13.8 to -74.0° and that of (+)2-phosphoglyceric acid from +24.5 to -68°. The large increase in rotation markedly enhances the accuracy of the determinations. Table 2 gives the results of experiments in which both the phosphopyruvate and 2-phosphoglycerate were determined, the latter by the polarimetric method. From these values, the positions of both equilibria can be determined.

The amount of 2-phosphoglycerate formed (exp. 1), calculated as percentage of total phosphoglycerate, is 11.23 % in the absence of NaF and 7.97 and 9.92 % in its presence (exps. 2 and 4). The presence of NaF should not influence the equilibrium between 3- and 2-phosphoglycerates, but possibly slowed the rate of reaction without changing the end-point. Such a retardation would explain the low values in exps. 2 and 4.

Fig. 1.

Fig. 2.

Fig. 1. Effect of time on production of phosphopyruvate from phosphoglycerate. 0.3 ml. juice, 27.8×10^{-2} mg. PGA-P per experiment. Temperature, 25°.

Fig. 2. Effect of enzyme dilution on production of phosphopyruvate from phosphoglycerate. ○—○ 30 min. incubation. □—□ 60 min. incubation. 27.8×10^{-2} mg. PGA-P per experiment. Total volume, 1.0 ml. Temperature, 27°.

Meyerhof & Schulz [1938], working at various temperatures, found values of 8.5 to 11 % of 2-phosphoglycerate in the muscle equilibrium. Approximately 30 % of the organic phosphate is present as phosphopyruvate in exp. 1, in which no NaF was added, while the amount of phosphopyruvate formed in the presence of NaF is negligible.

Table 2. *Equilibria between 3-phosphoglycerate, 2-phosphoglycerate, and phosphopyruvate**

Exps. 1, 2 and 3; 4 ml. juice (conc. preparation from dried extract), 2 ml. 0.3 M NaHCO₃; total vol. 10 ml.; stopped by addition of 1.5 ml. 40 % CCl₃COOH.

Exps. 4 and 5; 4 ml. juice, 2 ml. 0.3 M NaHCO₃; total vol. 10 ml. Polarimetric readings in 1 dm. tube. Temp. 25°.

Exp. no.	1	2	3	4	5
Time of incubation, min.	120	120	0	45	0
NaF molarity	0	0.03	0	0.03	0
Orig. conc. PGA/ml.					
mg. PGA	4.027	4.027	4.027	0.703	0.703
mg. PGA-P	0.673	0.673	0.673	0.117	0.117
After incubation, mg./ml.					
PP	0.196	0.005	0	0	0
Inorg. P	0.025	0.010	0	0.003	0
Organic P	0.648	0.662	0.673	0.114	0.117
PGA	2.706	3.933	4.027	0.683	0.703
Optical rotation	-1.81°	-2.70°	-2.97°	-0.46°	-0.52°
[α] _D ²⁵	668.2°	686.4°	737.5°	673.3°	739.6°
% PGA as 2-PGA	11.23	7.97	0	9.92	0
% organic P as PP	30.22	0.82	0	0	0

* Appreciation is expressed to Dr I. B. Johns of the Department of Chemistry for assistance in polarimetric investigations.

In the polarimetric experiments it was necessary to use longer incubation periods and more active enzyme preparations since comparatively large concentrations of phosphoglyceric acid were involved. (–)3-Phosphoglycerate obtained by recrystallization from the mixture of the 3- and 2-salts was used in these experiments. The increase in enzyme activity was accomplished by dissolving a dried powder of the extract in a small amount of water. The resulting solution contained approximately four times as much dissolved material as the original extract.

It should be mentioned that Katagiri & Murakami [1939] found that the lactic acid bacteria exhibit a different specificity in the fermentation of the various optical isomerides of phosphoglyceric acid from that which has been found for *E. coli* extract used in this work or has been reported previously by Meyerhof & Kiessling for muscle and yeast. With *E. coli*, muscle and yeast (–)3-phosphoglyceric acid and (+)2-phosphoglyceric acid are the isomerides involved. Katagiri & Murakami reported that a *d*-lactic acid producer attacked only the (–)2- and (–)3-isomerides while a *l*-lactic acid producer attacked only the dextrorotatory isomerides of the two esters. A species which formed racemic lactic acid was able to attack both optical isomers of both esters.

Table 3. *Reversible equilibria between phosphoglycerate and phosphopyruvate as demonstrated by serial temperature experiment*

Per sample removed: 0.3 ml. extract, 0.2 ml. 0.3 *M* NaHCO₃. Total volume 1.0 ml.
27.8 × 10^{–2} mg. PGA-P per sample.

Exp. no.	1	2	3	4
Temp. °	25	2	40	2
Time elapsed, min.	30	90	110	200
Values given as 1 × 10 ^{–2} mg./ml.				
PP	7.73	6.60	9.00	6.73
Inorg. P	0.20	0.40	2.27	2.93
Total organic P	27.60	27.40	25.53	24.87
% organic P as PP	28.02	24.09	35.25	27.07

Since an indication had been obtained that the equilibrium between 3- and 2-phosphoglyceric acids is similar to the equilibrium in muscle and in yeast, the remaining experiments on the nature of phosphoglyceromutase and enolase were carried out by determining phosphopyruvate, a much simpler procedure.

If the equilibria are truly reversible, the amount of phosphopyruvate P will vary with temperature. The fluctuation is apparent (Table 3) but the figure for 2° (exp. 4) is somewhat low. The failure to reach the correct value may be due to the destruction of the enzymes during the long incubation period.

Another method of establishing the reversibility of a reaction employs both the product and the reactant as substrates. If truly reversible, the equilibrium will be approached from either direction at approximately the same rate. In Fig. 3, the percentage of phosphopyruvate P present as plotted against time approaches the same equilibrium point at approximately the same rate whether the substrate is phosphoglycerate or phosphopyruvate. Use of a dialysed extract precluded reaching the equilibrium point too rapidly.

Fig. 3. Approach to phosphoglyceromutase-enolase equilibrium from either direction. □—□ Initial conc. 12.333 × 10^{–2} mg. pyro-P per experiment. ○—○ Initial conc. 13.866 × 10^{–2} mg. PGA-P per experiment. Total vol. 1.0 ml. 0.3 ml. of 1.25 hr.-dialysed extract per experiment. Temperature, 25°.

The pH range of activity of the enzymes (Table 4) is fairly wide. The sharp decrease in the acid range can be partially attributed to the precipitation of the enzymes. In the alkaline range the decrease is more gradual.

Table 4. *pH range of equilibria between phosphoglycerate and phosphopyruvate*

Per experiment: 0.2 ml. extract, 27.8×10^{-2} mg. PGA-P. Total volume 1.0 ml. 30 min. incubation. Temp. 22°.

Exp. no.	1	2	3	4	5	6	7
pH	5.1	5.8	7.4	8.2	9.2	9.7	10.1
Buffer used	Acetate	Acetate	Bicarb.	Glycine	Glycine	Glycine	Glycine
After incubation, mg. $\times 10^{-2}$ /ml.							
PP	0.33	4.47	7.73	7.56	5.80	3.60	2.93
Inorg. P	0	0.53	0	0.20	0	0	0
Total organic P	27.80	27.27	27.80	27.60	27.80	27.80	27.80
% organic P as PP	1.20	16.38	27.82	27.39	20.86	12.95	10.55

Table 5. *NaF and Na oxalate inhibitions of equilibria between phosphoglycerate and phosphopyruvate*

Per experiment: 0.3 ml. extract, 0.2 ml. 0.3 *M* NaHCO₃. 1.0 ml. total vol. 27.80×10^{-2} mg. PGA-P. Temp. 24°. Incubation, 30 min.

Exp. no.	1	2	3	4	5	6	7	8	9
Molar conc. NaF	0.04	0.02	0.01	—	—	—	—	—	—
Molar conc. Na oxalate	—	—	—	—	0.04	0.02	0.01	0.005	—
After incubation, mg. $\times 10^{-2}$ /ml.									
PP	0.07	0.67	2.07	7.60	2.87	6.27	6.60	7.00	7.60
Inorg. P	0.93	0	0	0.53	0.47	0.27	0.47	0.33	0.33
Total organic P	26.87	27.80	27.80	27.27	27.33	27.53	27.33	27.47	27.47
% organic P as PP	0.25	2.40	7.44	27.87	10.47	22.76	24.15	25.49	27.77

NaF inhibits the formation of phosphopyruvate from 2-phosphoglycerate (Table 5), but the inhibition is relatively complete only at the highest concentration of NaF, i.e. 0.04 *M*. In spite of the inhibition of the enolase reaction, some inorganic phosphate appears in the reaction. It was noted that higher temperatures increased the phosphatase effect while addition of inorganic phosphate depressed it.

Lohmann & Meyerhof [1934] reported that sodium oxalate inhibited enolase less than dephosphorylation, but it can be seen in Table 5 (exps. 5–9) that oxalate at least reduces the rate of the reaction in the bacterial system.

In the course of this work, experiments with dialysed extracts have indicated that Mg or Mn is required for the transformation of phosphoglycerate into phosphopyruvate. It has not been determined whether the ions stimulate both phosphoglyceromutase and enolase.

Table 6. *Effects of Mg and Mn on phosphoglyceromutase and enolase*

Per experiment: 0.1 ml. of 3 hr. dialysed extract, 0.2 ml. 0.3 *M* NaHCO₃, 41.7×10^{-2} mg. PGA-P. Total vol. 1.0 ml. Temp. 21°. Incubation time, 20 min.

Exp. no.	1	2	3	4
Additions, molar conc.				
MgCl ₂	—	0.005	—	—
MnSO ₄	—	—	0.005	—
NaCl	—	—	—	0.01
After incubation, 1×10^{-2} mg./ml.				
PP	2.87	5.73	6.20	2.80
Organic P	41.70	41.70	41.70	41.70
% organic P as PP	6.88	13.75	14.87	6.71

The effects of Mg and Mn are shown in Table 6. It will be noted that less of a dialysed enzyme extract, as well as a shorter incubation time, were used in these experiments because it was necessary to compare rates of reaction. This can be accomplished only if the reaction is stopped before the equilibrium is reached. The conditions in these

experiments permitted a comparison of rates of reaction. The stimulation of the rate by Mg and Mn is shown in exps. 2 and 3, while Na in exp. 4 has no effect.

This is the first report of a Mg or Mn stimulation of the phosphoglyceromutase-enolase activity of muscle, yeast, or bacteria. This problem is being further investigated and extended to other types of preparation.

Transfer of phosphate from phosphopyruvate

The previous experiments have dealt with the transformation of phosphoglycerate into phosphopyruvate. Only small amounts of phosphopyruvate were broken down to pyruvate and H_3PO_4 . Presumably, this reaction takes place in two steps:

The first step was reported by Lutwak-Mann & Mann [1935]; the second is very slow in a bacterial preparation because of the removal of adenosinepolyphosphatase by centrifuging in the preparation of the extract. The transfer of phosphate to adenylic acid is limited by the amount of adenylic acid in the extract since adenylic acid cannot be appreciably regenerated. Only catalytic concentrations of adenylic acid are present in the extract. On addition of adenylic acid to the system, phosphate was transferred from the phosphopyruvate to the added coenzyme (Table 7). In exps. 1-6 the phosphopyruvate involved in the transfer was formed before the addition of adenylic acid, by incubating the extract with phosphoglycerate. After the equilibrium of approximately 30% phosphopyruvate had been reached, this phase of the reaction was stopped by addition of NaF, and the phosphopyruvate which had been formed transferred PO_4 to added adenylic acid. The PO_4 disappearing as phosphopyruvate P reappears as ATP-P. The transfer is relatively complete in the absence of oxalate and approximately all of the original phosphopyruvate P can be accounted for as residual phosphopyruvate P, ATP-P, or inorganic P. Exp. 6 shows that ATP is not formed in the absence of phosphopyruvate, i.e. through the phosphorylation of adenylic acid by inorganic phosphate. Sodium oxalate greatly inhibits the transfer of phosphate (exps. 3 and 4). In exp. 5, an amount of adenylic acid insufficient to accept all the PO_4 from the phosphopyruvate was added and the transfer was only partially complete.

Table 7. *Transfer of PO_4 from phosphopyruvate to adenylic acid*

Exps. 1-6: 0.3 ml. extract incubated for 30 min. with 0.40 mg. PGA-P, 0.2 ml. 0.3 M NaHCO_3 , and additions to 1.0 ml. Further additions made to vol. of 1.7 ml. Incubation for 30 additional minutes.

Exps. 7-10: Same as 1-6 except synthetic phosphopyruvate used and no preliminary incubation period employed. Temp. 23°.

Exp. no.	1	2	3	4	5	6	7	8	9	10
Additions, molar conc.										
NaF	—	0.035	0.035	0.035	0.035	0.035	—	0.035	0.035	0.035
Adenylic acid	—	0.004	0.004	0.004	0.004	0.004	—	—	0.004	0.004
Na oxalate	—	—	0.018	0.009	—	—	—	—	—	—
MnSO_4	—	—	—	—	—	—	—	—	—	0.006
Values as 1×10^{-3} mg./ml.										
PP before incubation	10.53	10.53	10.53	10.53	10.53	0.00	11.40	11.40	11.40	11.40
PP after incubation	8.93	0.33	6.53	6.40	5.13	0.00	6.33	9.93	2.27	0.40
Net change in inorg. P	0.73	0.00	0.13	0.33	1.13	0.00	0.33	1.07	0.73	0.20
ATP-P	0.00	11.13	3.27	3.40	2.07	0.21	0.00	0.00	8.03	10.20
PP disappeared	1.60	10.20	4.00	4.13	5.40	0.00	5.07	1.47	9.13	11.00
Total PP accounted for (ATP, inorg. P and residual PP)	9.67	11.47	9.93	9.47	8.33	0.21	6.67	11.00	11.03	10.80

The second part of Table 7 demonstrates the same transfer from synthetic phosphopyruvate. In the absence of added NaF (exp. 7) part of the phosphopyruvate disappears and cannot be accounted for as ATP-P. This disappearance is due to the formation of phosphoglycerate through the establishment of the usual equilibrium between phosphopyruvate and phosphoglycerate. Added NaF prevents this reaction and transfer of PO_4

to adenylic acid occurs (exp. 9). Mn ions in exp. 10 have a slight stimulating effect upon the transfer.

Following the formation of ATP, and in the absence of a strong phosphatase, PO_4 is transferred from ATP to glucose by the enzyme hexokinase [Meyerhof, 1926]. Table 8

Table 8. PO_4 transfer to glucose and hexosemonophosphate

Per experiment: 0.3 ml. extract incubated with 0.4 mg. PGA-P for 20 min. (total vol. 0.8 ml.), then additions to a total vol. of 1.6 ml. made and incubation for an additional 60 min. Temp. 23°.

Exp. no.	1	2	3	4	5
Additions, molar conc.					
NaF	0.04	0.04	0.04	0.04	0.04
Adenylic acid	0.00	0.005	0.005	0.005	0.005
Glucose	—	—	0.014	0.014	—
Hexosemonophosphate	—	—	—	—	0.004
MgCl_2	—	—	—	0.007	—
Values given in mg. $\times 10^{-2}$ /ml.					
PP before incubation	7.26	7.26	7.26	7.26	7.26
PP after incubation	6.69	0.00	0.00	0.00	0.00
Inorg. P change	0.50	0.97	-0.83	-0.97	-0.37
ATP-P	0.00	6.33	3.07	4.13	6.67
PP disappeared	0.00	7.26	7.26	7.26	7.26
PP accounted for (ATP, inorg. P, remaining PP)	7.193	7.30	2.23	3.17	6.30

shows that in the presence of added glucose more than one-half of the phosphate which should reappear as ATP cannot be accounted for in that compound. The glucose has acted as a PO_4 -acceptor for ATP with the formation of an ester which was not identified but which is probably hexose-mono- or -di-phosphate. Hexosemonophosphate will not replace glucose as a PO_4 -acceptor since very little ATP is missing in Exp. 5 in which it was added.

All the individual reactions involved in the production of phosphopyruvate from hexosediphosphate and the transfer of PO_4 from phosphopyruvate to adenylic acid and glucose have now been demonstrated by the authors with the exception of the oxidation-reduction of the triosephosphate and that step has been previously reported by Still [1940] in a cell-free extract of *E. coli*. It has now been shown that the individual steps occurring in muscle and yeast metabolism as revealed by the work of Embden and Meyerhof, also occur in the metabolism of bacteria as judged from cell-free extracts.

SUMMARY

1. Cell-free extracts obtained by grinding *E. coli* with powdered glass contain the enzymes which convert phosphoglyceric acid into phosphopyruvic acid. The effect of temperature on the reaction is in agreement with the results of Meyerhof & Kiessling for muscle and yeast.
2. (-)3-Phosphoglyceric acid and (+)2-phosphoglyceric acid establish the same equilibrium in bacteria as in muscle and in yeast but differ with regard to the optical specificity reported for lactic acid bacteria by Katagiri & Murakami.
3. The equilibria were shown to be truly reversible.
4. An indication was obtained that Mg or Mn stimulates the phosphoglyceromutase-enolase equilibria.
5. Phosphopyruvate transfers PO_4 to added adenylic acid.
6. The phosphate from phosphopyruvate was partially transferred to glucose via adenylic acid when glucose was added as a PO_4 -acceptor; hexosemonophosphate did not successfully replace glucose.

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