globin reduction and has a different pyridine nucleotide specificity. Furthermore, extracts prepared by their method of treatment of hemolyzates with chloroform and ethanol, or by boiling, were inactive in our system.

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1 E. M. SCOTT AND D. D. HOSKINS, *Blood*, 13 (1958) 795.
2 H. EDELHOCH, O. HAYAISHI AND L. J. TEPLY, *J. Biol. Chem.*, 197 (1952) 97.
3 P. S. GERALD AND P. GEORGE, *Science*, 129 (1959) 393.
4 H. BARCROFT, Q. H. GIBSON, D. C. HARRISON AND J. MCMURRAY, *Clin. Sci.*, 5 (1945) 145.
5 R. F. SIEVERS AND J. B. RYON, *Arch. Int. Med.*, 76 (1945) 299.
6 Q. H. GIBSON, *Biochem. J.*, 42 (1948) 13.
7 F. M. HUENNEKENS, R. W. CAFFREY, R. E. BASFORD AND B. W. GABRIO, *J. Biol. Chem.*, 227 (1957) 261.

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Pyrophosphate formation in cell-free extracts of *Escherichia coli*

In respiring cells of *Acetobacter suboxidans*, yeast[1], and *Merulius lacrymans*[2], the acid-labile phosphate in PP has a more rapid turnover than that in ATP and the other nucleoside di- and tri-phosphates. This suggests that the formation of PP is to some extent independent of ATP, and consequently arises by some separate phosphorylating pathway.

Intact cells of *Escherichia coli* behave similarly to other micro-organisms studied. PP is rapidly labelled when *E. coli* cells respire in the presence of $^{32}P_i$. ATP is more slowly labelled. In the presence of excess carrier P_i, the specific activity of PP approaches about half the specific activity of P_i, while the specific activity of ATP approaches that of PP.

Cell-free extracts of *E. coli* contain a very active PPase, which rapidly splits added PP. The extracts contain, however, a small amount of PP, which seems to be protected from the action of the hydrolyzing enzyme. This PP fraction is rapidly labelled by added $^{32}P_i$.

An extract of *E. coli* was made by treating the cell paste in aqueous suspension with an ultrasonic vibrator. The pH in the liquid after ultrasonic treatment was close to 5.5. The suspension was first freed from remaining cells, then centrifuged at 10,000 $\times$ *g* for 10 h, and then the speed was increased to 35,000 $\times$ *g* for 45 min. The precipitate was resuspended in Tris buffer, pH 8.0, to give a highly viscous liquid of pH 7.5. After centrifugation at 35,000 $\times$ *g* for 45 min, a slightly turbid, reddish supernatant, and a ropy precipitate were obtained. The supernatant was used in these experiments to study the incorporation of $^{32}P_i$ into PP and ATP. Endogenous respiration and phosphorylation was high. In some experiments alcohol dehydrogenase, DPN, and alcohol were added to test the effect of DPNH.

Abbreviations: PP, inorganic pyrophosphate; P_i, inorganic orthophosphate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; Tris, tris(hydroxymethyl)aminomethane; DPN, DPNH, oxidized and reduced diphosphopyridine nucleotide.

TABLE I

INCORPORATION OF ^{32}P INTO PP, ATP, AND ADP IN EXTRACTS OF *Escherichia coli*

Each flask contained: 30–50 ml extract, pH 7.5 (1.5–2.0% protein), 0.001 *M* ethylenediamine tetraacetate, and 0.003 *M* Mg^{++}. The flasks were shaken at 37°, and carrier P_i (30–100 μmoles), 50 μmoles ADP, and 100 μC $^{32}P_i$ were added in that order. The time was measured from the addition of $^{32}P_i$, to the interruption of the experiment by the addition of trichloroacetic acid to a final concentration of 5%. Protein was removed by centrifugation, aliquots were taken for determination of P and radioactivity, 500 μmoles P_i were added, the phosphates were isolated as barium salts, and fractionated as described earlier[1].

Expt.	*Incubation time (sec)*	*Specific activity (counts/min/μmole acid-labile P)*			
		P_i	*PP*	*ATP*	*ADP*
1*	30	11,800	1,340	1,070	684
2	6	14,500	1,540	1,413	472
	30	14,100	2,480	3,600	2,533
	180	10,350	4,540	8,440	8,700
3	4	24,700	7,180	5,550	3,710

* Extract stored for one week at — 5°.

Table I summarizes the results of some experiments designed to test whether PP is mostly labelled through ATP, or receives its isotopic P by some independent pathway.

With short incubation times PP was found to have a higher specific radioactivity than ATP, whereas after continued incubation ATP became the most radioactive. This is incompatible with any mechanism of pyrophosphate formation via ATP as an intermediate. The specific activity of PP was found to level off at about half the activity of P_i, and rise only slowly above that value.

In contrast to what happens in intact cells, the specific activity of ATP in the extract rapidly rose above the activity of PP, reaching a level of about 90% of the activity of P_i. This indicates a direct phosphorylation of ADP, either on substrate level, or perhaps linked to electron transport. The failure of ATP to reach the specific activity of P_i may indicate that part of the ATP is formed from PP, for example through a polynucleotide pyrophosphorylase.

The oxidative state of the extract was found to influence the amount and turnover rate of PP. Anaerobic conditions, and the addition of a DPNH-generating system, reduced the amount of PP, and the rate of incorporation of ^{32}P into PP, compared to an aerobically-incubated control without added DPNH. This indicates that the PP-protecting agent in the extract may be identical with the oxidized form of a respiratory enzyme. Recently WADKINS AND LEHNINGER[3] reported that the rate of incorporation of ^{32}P into ATP in digitonin extracts of mitochondria is reduced under anaerobic conditions.

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[1] L. KLUNGSÖYR, T. E. KING AND V. H. CHELDELIN, *J. Biol. Chem.*, 227 (1957) 135.
[2] J. GOKSÖYR AND L. KLUNGSÖYR, *Acta Chem. Scand.*, (1959) in the press.
[3] C. L. WADKINS AND A. L. LEHNINGER, *J. Biol. Chem.*, 234 (1959) 681.

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