salt of α-lipoic acid, prepared in a similar manner, was amorphous.

Biosynthetic studies[4] provided the initial evidence favoring the straight chain carbon skeleton. A mutant strain of *Escherichia coli*, which can synthesize α-lipoic acid but which requires a more complex form of this factor for growth, produces appreciably more α-lipoic acid in the presence of acetate or more particularly 2,4,6-octatrienoic acid. This effect was observed in resting cell suspensions and was favored by the presence of cysteine. *n*-Caprylic acid and pyruvate were inactive in these tests.

Further confirmation of this structure was obtained by comparing the infrared spectrum of α-lipoic acid with that of various fatty acids. The infrared spectrum revealed no resolved methyl absorption at high dispersion in the 3.4 λ region.

It has thus been established that the carbon skeleton of α-lipoic acid is the straight chain C_8 acid, and α-lipoic acid is therefore the intramolecular disulfide of a dimercapto-*n*-octanoic acid, unsubstituted in the α- and β-positions.

The optical activity of crystalline α-lipoic acid was found to be $[\alpha]^{25}D$ +96.7° (1.88% in benzene.

(4) L. J. Reed and B. G. DeBusk, unpublished results.

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ENZYMATIC PYROPHOSPHORYLATION OF COENZYME A BY ADENOSINE TRIPHOSPHATE[1]

Sir:

It is well known that acetate may be enzymatically activated by a reaction chain involving adenosine triphosphate (ATP) and coenzyme A (CoA). Acetyl-CoA was found to be the product of this reaction,[2] the mechanism of which became of particular interest since Lynen, *et al.*,[3,4] have shown acetyl-CoA to be an acetyl mercaptoester.

We studied the ATP-CoA-acetate reaction with an enzyme obtained from yeast extract by protamine and ammonium sulfate fractionation. The generation of acetyl-CoA was followed by the use of hydroxylamine as chemical acetyl-acceptor,[2] determining acethydroxamic acid, according to Lipmann and Tuttle.[5] An important lead toward the understanding of the mechanism was obtained when it was found that acethydroxamic acid may accumulate without an equivalent liberation of inorganic phosphate from ATP. In the presence of fluoride, ATP was found to be converted to an acid-labile phosphate, identified eventually as inorganic pyrophosphate, and to adenosine monophosphate (AMP). Fluoride preserves the pyrophosphate by inhibiting pyrophosphatase, which is a contaminant of our enzyme. A balance experiment is shown in Table I.

ATP was determined by the hexokinase-hexosemonophosphate-dehydrogenase-TPN procedure of Kornberg,[6] AMP spectrophotometrically according to Kalckar[7] using Schmidt's deaminase. Pyrophosphate was determined by manganese precipitation according to Kornberg.[6] The pyrophosphate was further identified by the use of a five times recrystallized pyrophosphatase,[8] kindly supplied to us by Dr. Kunitz.

TABLE I

Each vessel contained: 29 μM ATP, 250 μM acetate, 860 μM NH_2OH (*p*H 6.5), 80 μM glutathione, 160 μM potassium fluoride, 640 μM tris-(hydroxymethyl)-aminomethane buffer (*p*H 7.4), and 32 μM $MgCl_2$ in 3.2 ml. total volume. Each vessel contained 0.32 ml. of the yeast enzyme.

CoA, units	Incubation time, min.	ATP, μM	AMP, μM	Acethydroxamic acid, μM	Pyrophosphate, μM
0	0	29.0	0.2	0	0
	150	22.9	1.2	2.7	0
290	0	28.7	2.8	0	0
	150	0.4	19.7	32.5	24.9 23.1[a]

[a] Value determined with pyrophosphatase.

In view of these results, the ATP-CoA-acetate reaction is formulated as a two-step reaction

$$\text{ATP} + \text{CoA} \rightleftharpoons \text{AMP} + \text{CoA-pyrophosphate} \quad (1)$$

$$\text{CoA-pyrophosphate} + \text{acetate} \rightleftharpoons \text{acetyl-CoA} + \text{pyrophosphate} \quad (2)$$

The exchange of pyrophosphate for acetyl in acetyl-CoA as indicated by reverse reaction (2) was confirmed in the following experiment:

TABLE II

Each sample contained, per 5 ml.: 1.8 μM acetyl-CoA, 200 μM potassium pyrophosphate or arsenate or phosphate, 200 μM magnesium chloride, 100 μM potassium fluoride, 1 ml. yeast enzyme. Incubate 30 minutes at 37°. The *p*H was 7.1.

	μM acetyl-CoA[a]
Initial	1.8
Incubated with pyrophosphate	0.5
Substituted arsenate for pyrophosphate	1.85
Substituted phosphate for pyrophosphate	1.8

[a] Determined as acethydroxamic acid.

When, after partial conversion of acetyl-CoA to CoA-pyrophosphate, excess acetate was added and reincubated, acetyl-CoA reformed. The equilibrium between acetyl-CoA and pyrophosphate is in favor of the acetyl compound. Nevertheless, if acetyl phosphate with Stadtman's transacetylase[9] is used as acetyl "feeder," CoA, pyrophosphate,

(1) This work was supported by the National Cancer Institute of the National Institutes of Health, Public Health Service; the Atomic Energy Commission; and the National Foundation for Infantile Paralysis.

(2) T. C. Chou and F. Lipmann, *J. Biol. Chem.*, **196**, 89 (1952).

(3) F. Lynen and E. Reichert, *Angew. Chem.*, **63**, 47 (1951).

(4) F. Lynen, E. Reichert and L. Rueff, *Ann.*, **574**, 1 (1951).

(5) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **158**, 505 (1945).

(6) A. Kornberg, *ibid.*, **182**, 779 (1950).

(7) H. M. Kalckar, *ibid.*, **167**, 445 (1947).

(8) M. Kunitz, *J. Gen. Physiol.*, **35**, 423 (1952).

(9) E. R. Stadtman, G. D. Novelli and F. Lipmann, *J. Biol. Chem.*, **191**, 365 (1951).

and AMP will yield considerable quantities of ATP by a reversal of reactions (2) and (1).

Experiments with pigeon liver extract have shown that the mechanism of the ATP-CoA-acetate reaction there is the same as described here. Preliminary studies of the properties of CoA-pyrophosphate have been made. The compound is stable to acid at room temperature and may be heated at neutral reaction with only slight loss of activity. Further characterization by use of chromatography is in progress.

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ACTIVATION OF PURIFIED GLUTAMIC-ASPARTIC APOTRANSAMINASE BY CRYSTALLINE PYRIDOXAMINE PHOSPHATE

Sir:

Several years ago, Snell[1,2] suggested that reversible interconversion between pyridoxamine and pyridoxal might be involved in the mechanism of enzymatic transamination. There is now ample evidence that pyridoxal phosphate (PALPO) is a coenzyme of transaminase. However, Umbreit, *et al.*,[3] found that although impure preparations of PALPO and pyridoxamine phosphate (PAMPO) activated crude glutamic–aspartic apotransaminase of *S. faecalis*, only PALPO was effective with purified pig heart glutamic–aspartic apotransaminase. These findings were interpreted to indicate that either the proposed mechanism of transamination was incorrect, or, less likely, that the mechanisms of transamination for the heart and bacterial systems were different. Because of the significant implications of these results in terms of the mechanism of transamination, we have performed similar experiments using purified pig heart apotransaminase,[4] crystalline PAMPO,[5] and crystalline sodium PALPO.[6]

The present studies demonstrate that PAMPO[7] as well as PALPO activates purified pig heart transaminase. In general agreement with O'Kane and Gunsalus,[4] it was found that simultaneous mixing of PALPO, enzyme, and substrates resulted in low activity, and that pre-incubation of enzyme and PALPO was necessary for appreciable activation with low concentrations of PALPO. As described in Table I, PALPO, but not PAMPO, significantly activated transamination with a pre-incubation period of five minutes. When the enzyme was incubated with PALPO or PAMPO for longer periods before the addition of substrates, equivalent activity was observed with both phosphate esters at the same concentrations. Dialysis (*p*H 7.5, 5°, 48 hours) of the enzyme-coenzyme mixtures before addition of substrate did not affect the activity. Thoroughly dialyzed PALPO-reconstituted enzyme preparations contained 1 to 3% of the added PALPO[8] as determined at *p*H 5.5[9] with tyrosine apodecarboxylase.[10] Similar values for PALPO binding were obtained in experiments with PALPO labeled with P^{32}. On the other hand, P^{32}-PAMPO-reconstituted preparations retained only about 0.1% of the added radioactivity,[8] and no PALPO was detected[10] either in dialyzed PAMPO-reconstituted preparations or in the concentrated dialysates.

TABLE I

ACTIVATION OF PIG HEART APOTRANSAMINASE BY PYRIDOXAL PHOSPHATE AND PYRIDOXAMINE PHOSPHATE

Duration of incubation of enzyme with coenzyme prior to addition of substrates, min.	Transaminase activity[a] (microliters per 15 minutes) With pyridoxal phosphate	With pyridoxamine phosphate
0	32.0	12.7
5	93.0	17.6
10	118	37.2
30	119	75.2
60	112	116
90	111	123
120	118	120
120[b]	124	126
120[c]	70.0	62.0
120[d]	205	213

[a] The enzyme preparation (50γ) was incubated with 5γ of coenzyme in 1.7 cc. of 0.059 *M* potassium phosphate buffer (*p*H 7.5) in the main compartment of a Warburg vessel at 37°. At the indicated intervals, a side arm containing 100 micromoles of L-aspartate and 200 micromoles of sodium α-ketoglutarate (0.5 cc.) was tipped. After 15 minutes 0.5 cc. of aniline–50% citric acid (1:1) was added from a second side arm, and the evolved carbon dioxide was recorded. There was no activity in the absence of coenzyme. [b] Dialyzed for 48 hours at *p*H 7.5. [c] Concentration of coenzyme = 2.5γ. [d] Concentration of coenzyme = 10.0γ.

The data are consistent with the hypothesis that activation requires enzyme–coenzyme combination, and demonstrate that after suitable pre-incubation equal concentrations of PALPO and PAMPO produce equivalent activity. The observation that P^{32} binding was greater with P^{32}-PALPO than with P^{32}-PAMPO suggests that most of the PALPO is bound at non-functional sites, or, less likely, that PAMPO enzyme combination involves some dissociation of the phosphate group. Conversion of PAMPO to PALPO *prior* to addition of substrates was not demonstrated by these studies, and such a reaction appears unlikely. Interconversion of PAMPO and PALPO probably occurs in the presence of substrates after enzyme-coenzyme combination has been established. Conversion of PAMPO to PALPO by transamination with pyruvate and

(1) E. E. Snell, *J. Biol. Chem.*, **154**, 313 (1944).

(2) E. E. Snell, THIS JOURNAL, **67**, 194 (1945).

(3) W. W. Umbreit, D. J. O'Kane, and I. C. Gunsalus, *J. Biol. Chem.*, **176**, 629 (1948).

(4) D. E. O'Kane, and I. C. Gunsalus, *ibid.*, **170**, 425 (1947).

(5) E. A. Peterson, H. A. Sober and A. Meister, THIS JOURNAL, **74**, 570 (1952).

(6) E. A. Peterson, H. A. Sober, and A. Meister, *Federation Proceedings*, **11**, 268 (1952).

(7) The crystalline PAMPO preparation did not activate tyrosine apodecarboxylase indicating the absence of PALPO.

(8) These studies were carried out under conditions similar to those described in Table I, with 0.625 to 200γ of coenzyme, 220γ of enzyme preparation, and a pre-incubation period of 120 minutes.

(9) PALPO can be released from the enzyme preparation with 0.05 *M* sodium acetate buffer of *p*H 5.5.

(10) W. W. Umbreit, W. D. Bellamy, and I. C. Gunsalus, *Arch. Biochem.*, **7**, 185 (1946).