

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<sup>1,2</sup> 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.*,<sup>3</sup> 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,<sup>4</sup> crystalline PAMPO,<sup>5</sup> and crystalline sodium PALPO.<sup>6</sup>

The present studies demonstrate that PAMPO<sup>7</sup> as well as PALPO activates purified pig heart transaminase. In general agreement with O'Kane and Gunsalus,<sup>4</sup> 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

(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.

before the addition of substrates, equivalent activity was observed with both phosphate esters at the same concentrations. Dialysis (pH 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<sup>8</sup> as determined at pH 5.5<sup>9</sup> with tyrosine apodecarboxylase.<sup>10</sup> Similar values for PALPO binding were obtained in experiments with PALPO labeled with P<sup>32</sup>. On the other hand, P<sup>32</sup>-PAMPO-reconstituted preparations retained only about 0.1% of the added radioactivity,<sup>8</sup> and no PALPO was detected<sup>10</sup> 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 <sup>a</sup> (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 <sup>b</sup>	124	126
120 <sup>c</sup>	70.0	62.0
120 <sup>d</sup>	205	213

<sup>a</sup> The enzyme preparation (50 $\gamma$ ) was incubated with 5 $\gamma$  of coenzyme in 1.7 cc. of 0.059 M potassium phosphate buffer (pH 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  $\alpha$ -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. <sup>b</sup> Dialyzed for 48 hours at pH 7.5. <sup>c</sup> Concentration of coenzyme = 2.5 $\gamma$ . <sup>d</sup> Concentration of coenzyme = 10.0 $\gamma$ .

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<sup>32</sup> binding was greater with P<sup>32</sup>-PALPO than with P<sup>32</sup>-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

(8) These studies were carried out under conditions similar to those described in Table I, with 0.625 to 200 $\gamma$  of coenzyme, 220 $\gamma$  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 pH 5.5.

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

other  $\alpha$ -keto acids in *Clostridium welchii* has been described.<sup>11</sup> Although the mechanism of coenzyme binding and function in the heart system requires further study, the evidence supports the hypothesis that both PAMPO and PALPO

(11) A. Meister, H. A. Sober and S. V. Tice, *J. Biol. Chem.*, **189**, 577 (1951).

play significant roles in enzymatic transamination.

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## BOOK REVIEWS

**Principles of Chemical Thermodynamics.** First Edition.

By MARTIN A. PAUL, Ph.D., Professor of Chemistry, Harpur College, State University of New York. McGraw-Hill Book Company, 330 West 42nd Street, New York 18, N. Y. 1951. viii + 740 pp. 16 × 23.5 cm. Price, \$7.50.

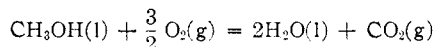
In the preface the author states: "This book is intended to serve a course in chemical thermodynamics which may accompany or immediately follow the introductory course in physical chemistry, at either the senior undergraduate or the first-year graduate level. The emphasis throughout is on general principles and their origins, with specific applications to a limited number of fields which are primarily the concern of the chemist." Actually a one-year course in physical chemistry would seem to be a prerequisite for the study of this text. This book, in common with other recently published texts in chemical thermodynamics, is fairly satisfactory in the presentation of those applications considered by Lewis and Randall, but is entirely unsatisfactory in the discussion of the fundamentals of thermodynamics. The author gives for the most part correct equations and presents some of the mathematical methods employed in thermodynamics, although the derivation of thermodynamic relations, particularly the problem of change in independent variables, should be discussed more fully. The main trouble with the text is a dearth of precise definitions, lack of logic, and vagueness in interpretation. There is a noticeable tendency toward the long and sometimes ambiguous discussion that is apt to confuse the student.

One general criticism is that the author does not distinguish between the process and the change in state. He does not seem to have defined the concept of the change in state of a system. He writes far too few changes in state and those that he does give are not sufficiently defined.

After a long chapter on temperature and its measurement, the author introduces the first law by a discussion of the mechanics of conservation and non-conservative systems. This is an unfortunate starting point since the term work is used in thermodynamics in a different sense from that in which it is used in mechanics. Quantity of heat is correctly defined (p. 55). However, the author employs the ambiguous terms work done on or by the system and heat received by the system, which may lead to confusion. Apparently the author does not define the concept of the boundary, the location of which is frequently of paramount importance in discussing work and heat. The first law is announced as the conservation of energy, and then the equation  $\Delta U = Q - W$  appears during a discussion of the internal energy of a system. This would seem to be the wrong order of presentation. If it is the author's intention to define work and heat first, then he should state the first law in terms of these quantities and proceed to prove the existence of the energy function.

The next two chapters deal with the thermal behavior of simple systems and with thermochemistry. The author discusses open systems (pp. 75-78) apparently without the realization of all of the implications of Eq. (3-12). Equation (3-15) is incorrect since the substance  $i$  has energy which it carries across the boundary of the system. The same error does not occur in the treatment of partial molal enthalpy. The writing of a change in state would have

made the error obvious. Equations (3-16) and (4-36) would hardly be called Gibbs-Duhem equations. Early in the chapter on the application of the first law, the author introduces a second law equation. This is a dangerous pitfall for the student and one into which the author falls. In the chapter on thermochemistry a different convention regarding the sign of quantity of heat is used. Under the paragraph heading "Heat of a Chemical Reaction" (p. 132) we find the typically vague statements: "The heat of a chemical reaction is defined in general as the quantity of heat evolved when a given quantity of the reaction takes place, as represented by its chemical equation. For example, the combustion of methanol is represented by the thermochemical equation



$$Q_{25^\circ\text{C}} = 173.64 \text{ kcal.}''$$

The change of  $\Delta H$  (of a chemical change in state at constant pressure and temperature) with temperature is not discussed for the case that a change in aggregation-state of a reactant or product occurs.

The author does not use a Carnot cycle to derive the second law equation but a cycle consisting of two isothermals and two isometrics, with an ideal gas as the working fluid. In the derivation he makes use of the relation proved in an earlier chapter from a second law equation that the constant volume heat capacity of an ideal gas is independent of the pressure! From the discussion under "Maximum Work and Thermodynamic Reversibility" (pp. 192-199) the student is apt to draw the incorrect conclusion that a maximum work function exists, although the author does not actually state this. The derivations of the Clausius inequalities are not satisfactory. In the discussion of the conditions of equilibrium in an isolated system the author follows the German treatment rather than the more general methods of Gibbs. Thus his Eq. (5-39),  $(\Delta S)_{U,V} \leq 0$ , contains the unnecessary subscript  $V$ .

A chapter on the thermodynamic behavior of simple systems is followed by a long one on solutions (including gaseous solutions) and heterogeneous mixtures. The treatment of gas mixtures is not rigorous and the student may well draw the incorrect conclusion that the equation (p. 325) for the fugacity coefficient of a gas in a mixture can be derived from Dalton's law (p. 310) or Amagat's law (p. 311). The discussion of liquid solutions would be improved if the relation of the activity to the choice of standard state and of activity coefficient to the choice of reference state were treated in greater detail. This portion of the text contains some excellent tables on the computation of activity coefficients and the effect of the choice of the reference state on the numerical values of activity coefficients.

In the chapter on chemical equilibrium the author introduces the ideal gas assumption too early in the argument in some of the examples discussed and he does not consider the effect of pressure on the activity of a condensed phase. This leads to some peculiar misconceptions. Thus he states that his Eq. (8-1-8) for the effect of temperature on the dissociation pressure of  $\text{CaCO}_3$  "bears a close relationship to the Clausius-Clapeyron equation for the vapor pressure of a pure liquid or solid." If care had been taken