heating to red heat radioactive K_2HPO_4; purified yeast enzyme was used for the exchange experiments. As shown in Table I, pyrophosphate exchanged with the pyrophosphoryl group in ATP very rapidly and, significantly, in the absence of CoA. This observation excluded immediately a CoA pyrophosphate as an intermediary. Instead, it indicated an initial reaction between ATP and enzyme, resulting in an AMP~enzyme link with liberation of inorganic pyrophosphate.

TABLE I

PYROPHOSPHATE EXCHANGE BETWEEN RADIOACTIVE INORGANIC PYROPHOSPHATE AND ATP WITH YEAST ENZYME

Enzyme, units	CoA, μM	Pyrophosphate cts./min./ml.	Pyrophosphate cts./min./μM	ATP cts./min./ml.	ATP cts./min./μM	Exchange, %
...	..	97,100	29,400	140	60	0.3
10	..	68,600	21,100	28,270	13,400	74.3
10	1	79,700	23,100	16,350	7,680	44.7
10	2	80,700	24,700	13,920	6,360	36.8

The vessels were incubated at 37° for 20 minutes. Each vessel contained in 1 ml.: 3.4 μM P^{32}-potassium pyrophosphate buffered at *p*H 7.5; 2.2 μM ATP; 50 μM KF; 10 μM $MgCl_2$; 20 μM H_2S; 200 μM tris-(hydroxymethyl)-aminomethane buffer, *p*H 7.5 in addition to the enzyme and CoA as noted above. The enzyme fraction used was purified 11-fold over the original extracts from quick-frozen baker's yeast. The separation of ATP and pyrophosphate was carried out by charcoal adsorption of ATP in the manner described by Crane and Lipmann.[6]

As shown in the last two lines of Table I, CoA inhibits the ATP ⇄ PP exchange proportional to concentration. This indicates that AMP~enzyme subsequently exchanges AMP for CoA. Therefore, the presence of CoA decreases the concentration of AMP~enzyme and thereby slows down the rate of exchange with pyrophosphate. The correctness of this mechanism is further suggested by the results of the exchange of isotopic acetate with acetyl CoA. The data of Table II demonstrate the last step of the sequence to be an exchange of enzyme~S·CoA with acetate to form acetyl ~ S·COA and free enzyme. The combined results prompt us to propose the following sequence of reactions:

$$\text{Ex} + \text{Ad-P}\sim\text{PP} \rightleftharpoons \text{Ex}\sim\text{P-Ad} + \text{PP} \quad (2)$$

$$\text{Ex}\sim\text{P-Ad} + \text{HSCoA} \rightleftharpoons \text{Ex}\sim\text{SCoA} + \text{Ad—P} \quad (3)$$

$$\text{Ex}\sim\text{SCoA} + CH_3COOH \rightleftharpoons \text{Ex} + CH_3CO\sim\text{ScOA} \quad (4)$$

TABLE II

EXCHANGE OF ACETATE BETWEEN $CH_3C^{14}OOH$ AND ACETYL CoA WITH YEAST ENZYME

Enzyme, units	AMP μM	Acetate cts./min./ml.	Acetate cts./min./μM	Acetyl CoA cts./min./ml.	Acetyl CoA cts./min./μM	Exchange, %
...	..	77,900	39,000	108	0.6	0.003
20	..	58,100	26,800	16,830	10,650	53.3
20	5	56,400	26,000	16,900	10,700	54.6

The vessels were incubated at 37° for 40 minutes. Each vessel contained in 1 ml.: 1.58 μM acetyl CoA; 2.17 μM C^{14}-acetate; 10 μM $MgCl_2$; 200 μM tris-(hydroxymethyl)-aminomethane buffer, *p*H 7.5 in addition to the enzyme and AMP as noted above. The enzyme fraction used was the same as in Table I. *Note that, compared with the experiment of Table I, twice as much enzyme and double the incubation time was used here.* Reaction 4, therefore, is considerably slower than reaction 2. The separation of acetyl CoA and acetate was carried out by charcoal adsorption of acetyl CoA in a manner similar to that used for separation of ATP and pyrophosphate.[6] Acetate activity was determined in the supernatant of the charcoal adsorbate. Acetyl CoA was decomposed on the charcoal with hydroxylamine and the hydroxamic acid activity measured in the supernatant.

It is noteworthy that, as shown in the last two lines of Table II, addition of AMP does not appreciably influence the rate of acetyl~CoA ⇄ acetate exchange. It therefore seems that the bond between enzyme and CoA is less energy-rich than the AMP~enzyme bond. This is confirmed further through the inhibition by CoA of the ATP ⇄ PP exchange which indicates that CoA favorably competes with AMP for the enzyme. In other words, a fall in free energy occurs in the direction from AMP~enzyme to CoA·S~enzyme. The enzyme used in these experiments was practically free of Mg. Through exchange experiments with and without magnesium we were able to show that magnesium is involved, in the ATP-enzyme reaction, but is not involved in the enzyme~CoA-acetate reaction.

Although no definite suggestions can be made at the present time with regard to the grouping on the enzyme which binds AMP and CoA·SH, it is attractive to presume the group is enzyme-bound phosphate. In such a case, the primary reaction between enzyme and ATP would closely resemble the type of reaction described by Kornberg,[7] namely, a pyrophosphate split of ATP with simultaneous formation of a pyrophosphate bridge from the residual AMP to another molecule. Furthermore, the interchange of AMP and sulfhydryl-CoA then would lead to enzyme-phosphoryl~S·CoA. It seems rather attractive, on the other hand, to speculate that the here-observed formation of an enzyme-mononucleotide may well foreshadow this as a rather general biosynthetic mechanism involved, for instance, possibly in nucleic acid synthesis.

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[6] R. K. Crane and F. Lipmann, *J. Biol. Chem.*, **201**, 235 (1953).

(7) A. Kornberg, in McElroy and Glass, "Phosphorus Metabolism," Vol. I, Johns Hopkins Press, Baltimore, Md., 1951, p. 392.

(8) Postdoctoral fellow of the Atomic Energy Commission.

A THEORY OF A CERTAIN TYPE OF IRREVERSIBLE POLAROGRAPHIC WAVE

Sir:

The present study was undertaken in an attempt to elucidate polarographic processes in which, regardless of the fact that the electron transfer reaction is faster than the diffusion rates, "irreversible" slopes of the plot of log $(i_d - i)/i$ against the potential[1] are obtained. This type of polarographic process belongs to a group in which the product of

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(1) J. Tomes, *Coll. Czechoslov. Chem. Communs.*, **9**, 12, 81, 150 (1937).

the electron transfer reaction is involved in a chemical reaction.

The theoretical part of this investigation is based upon the use of steady state[2,3] and thermodynamic treatments. Consider the simplest possible case represented by the reaction scheme:

$$\begin{tikzcd} X \arrow[r, shift left, "k_x"] & X_0 + ne- \arrow[l, shift left] \arrow[r, shift left, "k_1"] & Y_0 \arrow[l, shift left, "k_2"] \arrow[r, shift left, "k_3"] \arrow[d, "k_y"] & Z_0 \arrow[l, shift left, "k_4"] \arrow[r, "k_z"] & {} \\ & & {} & & \end{tikzcd} \qquad (1)$$

in which the concentrations of the species involved are denoted by X, Y and Z. The subscript $_0$ refers to surface concentrations. The various processes occurring at the electrode are considered as unimolecular rate processes, the rate constants are defined as heterogeneous constants. Application of the steady state treatment to the above mentioned scheme, on the assumptions that $i = nAF\,(X_0k_1 - Y_0k_2)$ and that $i_d = nAFXk_x$, leads to the following result

$$\frac{i_d - i}{i} = \frac{k_x}{k_1}\left[\frac{k_2k_4 + k_2k_z + k_3k_z + k_4k_y + k_yk_z}{k_4k_y + k_3k_z + k_yk_z}\right] \qquad (2)$$

This expression is valid whether or not the process is reversible. Depending upon the relative magnitude of the various rate constants (*e.g.*, for the reversible case $k_1, k_2 \gg k_3, k_4, k_x, k_y, k_z$) and invoking the potential dependence of the electron transfer rate constants,[4] equations of polarographic waves can be derived, which show log plots with varying degree of deviation from the reversible behavior. A suitable reaction for testing some of the implications of the theory was found in the system

$$\mathrm{Co\,en_3}^{+++} + e- \rightleftarrows \mathrm{Co\,en_3}^{++} \qquad (3)$$

The trisethylenediaminecobalt(III, II) couple in excess of ethylenediamine has been shown to be reversible potentiometrically by Bjerrum[5] and polarographically by Grieb.[6] The couple was found to show irreversible log plot slopes in the absence of the complexing agent in spite of the fact that the process was diffusion controlled, and that the electron transfer reaction was reversible, which was shown using alternating current polarography.[7] Using the successive complex constants for the divalent form

$$\mathrm{Co\,en_3}^{++} \overset{K_1}{\rightleftarrows} \mathrm{Co\,en_2}^{++} + \mathrm{en} \overset{K_2}{\rightleftarrows}$$
$$\mathrm{Co\,en}^{++} + \mathrm{en} \overset{K_3}{\rightleftarrows} \mathrm{Co}^{++} + \mathrm{en} \qquad (4)$$

as determined by Bjerrum[5] and assuming that establishment of the various equilibria is instantaneous an expression for the current-voltage curve was derived

$$E_{\mathrm{d.e.}} = E_0' - \frac{RT}{F}\ln \alpha\cdot\frac{[\mathrm{Co\,en}^{++}]_0}{[\mathrm{Co\,en}^{+++}]_0} \qquad (5)$$

where α is a function of the concentration of divalent complex. The predicted shift of the half-wave potential and change of the log plot slope were confirmed using a 50-fold concentration variation. It is believed that this type of polarographic wave is found in inorganic systems although it might be more numerous in organic polarography as pointed out by Kolthoff and Lingane.[8]

At a later date the details of this investigation, which is in progress, will be published.

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RECEIVED APRIL 20, 1953

THE VIBRATIONAL SPECTRUM OF TETRACHLORODIBORINE

Sir:

While structural investigations in the field of boron chemistry have not been so numerous as might be desired there is now abundant evidence that the presence of two or more boron atoms in a molecule may result in its having an uncommon or even unique structure.[1a,b,c,d] An interesting example is afforded by tetrachlorodiborine (B_2Cl_4) in which boron exhibits a normal tricovalence while being singly bonded to another boron atom. We have determined its infrared and Raman spectra and achieved a satisfactory assignment. The spectral data suggest, in agreement with the results of a recent electron diffraction study,[2] that B_2Cl_4 has the symmetry (V_d) of a non-planar ethylene model.

Approximately 4 ml. of liquid B_2Cl_4 was prepared by the method of Wartik, Moore and Schlesinger.[3] Raman exposures were made with the liquid sample held at $-35°$. The infrared spectra were obtained with a Perkin–Elmer spectrometer (equipped with NaCl and KBr prisms) on about 5 mm. pressure of gaseous B_2Cl_4 at room temperature. The small amount of BCl_3 formed as a result of the instability of the compound at room temperature was corrected for by running blanks on pure BCl_3.

The observed frequencies and their present interpretation are given in Table I. The frequency to be associated with the b_1 torsional mode cannot yet be estimated. The b_2 deformation is assigned the value 445 cm.$^{-1}$ as deduced from combination bands. The a_1 B-B stretch appears as a polarized doublet in the Raman spectrum as a result of the B^{10}-B^{11}, B^{11}-B^{11} isotopic shift. The observed intensity ratio accords with the natural isotopic distribution of boron. A fuller discussion of the assignment, together with the results of a normal-coördinate, force-constant treatment, will be given elsewhere.

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