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

$$\text{Co en}_3^{+++} + e- \rightleftarrows \text{Co en}_3^{++} \qquad (3)$$

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

$$\text{Co en}_3^{++} \overset{K_1}{\rightleftarrows} \text{Co en}_2^{++} + \text{en} \overset{K_2}{\rightleftarrows}$$
$$\text{Co en}^{++} + \text{en} \overset{K_3}{\rightleftarrows} \text{Co}^{++} + \text{en} \qquad (4)$$

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

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

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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TABLE I

THE INFRARED AND RAMAN SPECTRA AND THEIR INTERPRETATION FOR TETRACHLORODIBORINE

Raman (cm.$^{-1}$)	Infrared (cm.$^{-1}$)	Intensity and polarization	Interpretation
107	..	m	ν_9(e; β-BCl$_2$)
177	..	m	ν_8(e; β-BCl$_2$)
291	..	w,p	ν_3(a$_1$; δ BCl$_2$)
347	..	vw	2 × 177 (e) = 354(A$_1$ + B$_1$ + B$_2$)
401	..	vs,p	ν_2(a$_1$; ν-BCl)
550	..	vvw	107 (e) + 445 (b$_2$) = 552 (E); 2 × 291(a$_1$) = 582 (A$_1$)
..	622	w	177(e) + 445 (b$_2$) = 622 (E); 730(b$_2$) − 107(e) = 623 (E)
..	689	w	107(e) + 2 × 291(a$_1$) = 689 (E)
..	720	s	
..	729	vs	ν_5(b$_2$; ν-BCl)
..	739	vs	
..	746	s	291(a$_1$) + 445(b$_2$) = 736(B$_2$)*
750	..	vvw	291(a$_1$) + 445(b$_2$) = 736(B$_2$)*
..	760	vw	177(e) + 2 × 291(a$_1$) = 759 (E)
..	821	ms	107(e) + 729(b$_2$) = 836(E)
900	..	w	2 × 445(b$_2$) = 890 (A$_1$); 177 (e) + 729(b$_2$) = 906 (E)
..	920	vvs	ν_7(e; ν-BCl)
927	..	w	ν_7(e; ν-BCl)
948	..	w	107(e) + 401(a$_1$) + 445(b$_2$) = 953 (E); 2 × 107(e) + 729 (b$_2$) = 943 (A$_1$ + A$_2$ + B$_1$ + B$_2$)
..	1029	w	291(a$_1$) + 729(b$_2$) = 1020(B$_2$); 107(e) + 920(e) = 1027(A$_1$ + A$_2$ + B$_1$ + B$_2$)
..	1057	w	177(e) + 2 × 445(b$_2$) = 1067 (E)
..	1090	w	177(e) + 920(e) = 1097 (A$_1$ + A$_2$ + B$_1$ + B$_2$)
1123	..	w,p	ν_1(a$_1$; ν-B^{11}B^{11})
1150	..	w,p	ν_1(a$_1$; ν-B^{11}B^{10})
..	1232	w	107(e) + 1123(a$_1$) = 1230 (E)
1259	1259	vw	107(e) + 1150(a$_1$) = 1257 (E)
..	1324	w	401(a$_1$) + 920(e) = 1321 (E)
..	1362	vw	445(b$_2$) + 920(e) = 1365 (E)
..	2058	w	920(e) + 1123(a$_1$) = 2043 (E); 920(e) + 1150(a$_1$) = 2070 (E)

* Shifted by Fermi resonance with ν_5 fundamental.

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RECEIVED JUNE 5, 1953

BUTYRYL CoA-DEHYDROGENASE, A CUPRO-FLAVOPROTEIN

Sir:

Butyryl CoA dehydrogenase, previously shown to be a flavoprotein of vivid green color,[1] contains copper as part of its prosthetic group. This identification rests on the following observations: (1) a solution of the enzyme of highest purity isolated from a preparative electrophoresis run, and shown to be homogeneous in the analytical ultracentrifuge, was dialyzed exhaustively against 10^{-4} *M* tris-hydroxymethyl-aminomethane buffer of *p*H 8.0. Part of the preparation was then denatured by boiling for three minutes and the supernatant flavin solution, the denatured protein and the original preparation were examined by arc and spark spectroscopy.[2] The results summarized in Table I clearly indicate the presence of copper tightly bound to the enzyme protein. (2) Aliquots of two preparations shown to be approximately 22% and 98% pure by analytical electrophoresis (in 0.1 *M* tris-hydroxymethyl-aminomethane buffer of *p*H 8.0), and containing 4.7×10^{-5} and 2.6×10^{-5} *M* flavin,[3] respectively, were washed in platinum crucibles for 1 hour at 1000° F. Manometric copper determination with internal standards[4] showed the presence of 6.9 γ and 3.2 γ of cupric ion per ml. in the two preparations. Thus the mole ratios Cu^{++}/flavin were 2.23/1 and 1.90/1 or approximately equal to 2, at two purity levels of the dehydrogenase.

TABLE I

SPECTROSCOPIC METAL DETERMINATION

	Cu^a	Mg^a
Buffer before dialysis	−	−
Buffer after dialysis	−	−
Enzyme after dialysis	++	+
Supernatant of boiled enzyme	−	+
Residue of boiled enzyme	++	−

^{a} Only metals found present, all others, specifically Fe, Zn, Mn, Mo, Co, Ca, absent in all samples.

(3) The four-banded spectrum of the enzyme (λ_{max} at 265, 355, 432.5 and 685 mμ) undergoes the following transformations: (a) on treatment with butyryl CoA the peaks at 355, 432.5 and 685 mμ all disappear and the extinctions at these wave lengths are reduced to 0.86, 0.47 and 0.45 times their original value; (b) after dialysis against 5×10^{-3} *M* KCN,[5] buffered at *p*H 7.5, for 36 hours, the flavin peaks at 265, 355 and 432.5 are unchanged in both position and extinction while the peak at 685 mμ disappears.

(4) The cyanide-treated enzyme can still be reduced by butyryl CoA as shown by changes in the flavin region (390–480 mμ) of the spectrum, and is capable of catalyzing the interaction between butyryl CoA and dyes such as 2,6-dichlorophenolindophenol, a two-electron change. However, when the enzyme is tested with oxidizing agents mediating one-electron changes such as ferricytochrome c

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(2) We are indebted to Mr. Rubin Shapiro, Department of Chemistry, University of Wisconsin for performing the spectroscopic analyses.

(3) Flavin was determined by assuming a molecular extinction coefficient $\epsilon = 10.6 \times 10^3$ for flavoproteins at their visible maximum (O. Warburg and W. Christian, *Biochem. Z.*, **298**, 150 (1938)). The value so found, was in good agreement with the amount of flavin liberated by boiling the enzyme preparations and calculated assuming an extinction coefficient at 450 mμ $\epsilon = 11.3 \times 10^3$ (E. Dimant, D. R. Sanadi and F. M. Huennekens, THIS JOURNAL, **74**, 5440 (1952)).

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