nol *comparably* labeled with deuterium or tritium. Thus, in metabolic studies involving reactions where such fractionation may occur, conclusions regarding the possible pathways of a compound depend on the particular isotope of hydrogen chosen as the tracer, since the data obtained under identical conditions with a compound labeled identically with either deuterium or tritium allow different interpretations.

The second significant point, related closely to the isotope effect, is concerned with the ambiguity that can arise from replacing different numbers of equivalent hydrogen atoms in the individual molecules of a metabolite with the same labeling isotope of hydrogen. If one allows that the change in the D:C^{14} ratio observed between the methyl of methanol and the methyl of tissue choline and creatine is due primarily to a *metabolic* change in the hydrogen–carbon ratios during intermediary steps, then the results of the previous experiments[1,2] utilizing the CD_3-species of methanol indicate the possibility that "formate" is an intermediate in the neogenesis of methyl from methanol whereas the present experiment with the CH_2D- species shows that labile methyl arises *de novo* from a "formaldehyde" intermediate without passage through a "formate" step. On the other hand, it can be seen that the deuterium content of formate isolated in this experiment from the total pooled urine has been increased relative to C^{14}. The ratio of D:C^{14} in the methyl of tissue choline, that is suggestive of a "formaldehyde" precursor, could be a reflection of deuterium enrichment at all intermediary steps including a "formate" level. Thus "formate" as a required intermediate would not be ruled out. In view of the large degree of selection among the hydrogen isotopes during these metabolic reactions, it is not possible, from the type of experimentation employed in the present or the previous isotopic studies, to make an unequivocal deduction with regard to a particular intermediate.

It should be pointed out that the multiple labeling of carbon and hydrogen employed in the above mentioned experiments was intermolecular as a result of admixture of separately labeled species of methanol. The double labeling of a carbon and its bonded hydrogen within the same molecule, *i.e.*, intramolecularly, should minimize the influence of hydrogen isotope selection on the ratio of isotopic hydrogen to isotopic carbon. Any change in this last named ratio should be more an indication of the nature of intermediates and less a reflection of isotopic selection. However, if more than one hydrogen atom is linked to the carbon-labeling atom, *e.g.*, C^{14}, in a molecular grouping under study, the possibility of fractionation between the lighter and the heavier isotopes of hydrogen within the molecular grouping, and therefore between labeled carbon and labeled hydrogen, still can exist. The ideal case would be that in which the carbon-labeling atoms *only* are directly linked to only hydrogen-labeling atoms in pertinent carbon–hydrogen bonds. At the present time, this objective in labeling can be approached most closely with the available concentrations of deuterium, tritium and C^{13}, and to a lesser degree with the available C^{14}. Experiments in which metabolites are doubly labeled with isotopes of carbon and hydrogen within the same molecule have been undertaken in one of these laboratories (CUMC).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

The Polarography of Vitamins B_{12r} and B_{12a}

BY BRUNO JASELSKIS AND HARVEY DIEHL

RECEIVED MARCH 26, 1954

Unlike vitamin B_{12}, which undergoes a single two-electron, polarographic reduction, B_{12a} undergoes two one-electron reductions corresponding to changes in valence of the cobalt from three to two and from two to one. Vitamin B_{12r}, in which the cobalt is bivalent, shows one anodic and one cathodic wave agreeing in position and height with the waves of B_{12a}.

The catalytic hydrogenation of an aqueous solution of vitamin B_{12} yields a brown solution containing a reduced cobalt compound[1] which has been designated as B_{12r}.[2] Potentiometric titration of B_{12r} with potassium ferricyanide in a completely oxygen-free atmosphere has indicated that one equivalent is required to oxidize the B_{12r}; the cobalt atom is thus in the bivalent state.[2] During hydrogenation the cyanide group of B_{12} is reduced to methylamine.[3] The polarography of vitamin B_{12r} has been reported briefly[4] and there also has been described the polarographic behavior of a related but distinctly different product resulting from the action of chromous ethylenediaminetetraacetate on B_{12r}.[5]

In the present paper the polarographic behavior of B_{12r} is given in further detail. In particular the polarogram has been extended into the region positive to the saturated calomel electrode by using potassium sulfate as the supporting electrolyte and another wave has been found having a half-

(1) E. Kaczka, E. E. Wolf and K. Folkers, THIS JOURNAL, **71**, 1514 (1949).

(2) H. Diehl and R. Murie, *Iowa State Coll. J. Sci.*, **24**, 555 (1952).

(3) J. L. Ellingboe, J. I. Morrison and H. Diehl, in press.

(4) H. Diehl and J. I. Morrison, *Record of Chem. Progress*, Kresge-Hooker Library, **13**, 17 (1952).

(5) R. N. Boos, J. E. Carr and J. B. Conn, *Science*, **117**, 603 (1953).

wave potential −0.04 v. toward the S.C.E. This makes a total of three waves in the polarogram of B_{12r}, the half-wave potentials being −0.04, −0.95 and −1.55 v., respectively, toward the S.C.E. The first wave is anodic and the second and third cathodic in nature. The first two waves are one-electron steps. The height of the third wave depends on the time of hydrogenation and other conditions. The characteristics of these waves and their relation to those of B_{12} and B_{12a} were examined. In this connection new values for the diffusion constants of vitamins B_{12} and B_{12a}[6] have been used.

Experimental Work

Materials.—Vitamin B_{12}, obtained from the Squibb Institute for Medical Research, New Brunswick, New Jersey, was recrystallized from water and dried in a vacuum over anhydrous magnesium perchlorate. B_{12a} was prepared by hydrogenation of recrystallized B_{12} over platinum, oxidation by air, and precipitation by acetone. Moisture determinations were made on the preparations of both B_{12} and B_{12a}, and samples were weighed out accurately on a semi-micro balance; nevertheless, the concentrations of both B_{12} and B_{12a} given in the following work were obtained by direct colorimetric determination of the cobalt following ashing with perchloric acid.

Potassium sulfate, A.C.S. grade, was recrystallized from distilled, carbonate-free water. Solutions exactly 0.1 *N* and free of carbonate were prepared from this potassium sulfate for use as the supporting electrolyte.

Cylinder nitrogen was freed of oxygen by passage through a vanadous sulfate train, alkali and water. Glass tubing was used in connecting the scrubbing train to the apparatus.

Apparatus.—Polarograms were recorded on a Sargent Model XXI polarograph. The functional operations of the polarograph were checked frequently by calibration against a standard resistance. A saturated calomel electrode having an agar–potassium chloride salt bridge was used as anode; the internal resistance of this electrode was shown to be sufficiently low to have no effect on the polarograms.

Hydrogenation of vitamin B_{12} and vitamin B_{12a} was carried out in the hydrogenation apparatus described in a previous publication.[2]

The hydrogen used was cylinder hydrogen purified by passage through alkaline permanganate and ascarite.

Polarograms of Vitamins B_{12} and B_{12r}.—A weighed amount of vitamin B_{12} was dissolved in a definite volume of 0.1 *N* potassium sulfate to give a solution approximately 5×10^{-4} *M*. A 1.00-ml. aliquot was taken for a cobalt analysis. Another aliquot was transferred to the polarograph cell and the polarogram obtained in the usual manner. Another aliquot of the solution was transferred to the hydrogenation vessel, platinum oxide equal to about half the weight of the vitamin was added, and hydrogen was passed through the solution for seven hours after which no further change in *p*H occurred.[3] The resulting solution of B_{12r} was transferred through the fritted glass filter to the polarograph cell, the entire system being well flushed with oxygen-free nitrogen. The polarogram was then taken. An aliquot of the residual solution was then taken for a cobalt determination; this obviated any error caused by the slight changes in concentration which occurred during hydrogenation.

In another experiment this same procedure was followed but polarograph cell was used having a wide mouth rubber sheet pierced to accommodate glass and calomel electrodes, capillary, and the tip of a Machlett buret. Standard hydrochloric acid was added and the polarogram of B_{12r} obtained at various values of *p*H.

Polarograms of Vitamins B_{12a} and B_{12r}.—Starting with crystalline B_{12a} polarograms were obtained using the procedure described in the preceding section.

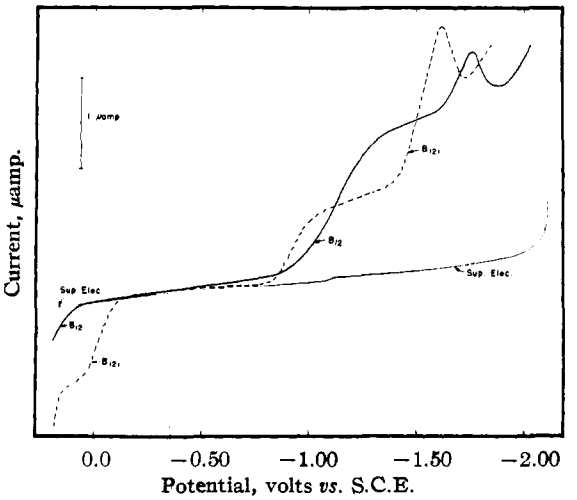


Fig. 1.—Polarograms of vitamins B_{12} and B_{12r}; potassium sulfate, 0.100 *N* as supporting electrolyte.

(6) B. Jaselskis and H. Diehl, unpublished work.

Results and Discussion

The polarogram obtained for B_{12}, identical with that reported earlier,[7,8] and that of the B_{12r} derived from the B_{12} are shown in Fig. 1. As will be seen the polarogram of B_{12r} shows three waves, the first wave being anodic and the second and third cathodic. The first and second waves are each just half the height of the B_{12} wave, and by inference each involves a one-electron change. This is borne out by an application of the Ilkovič equation, using for the diffusion constant of B_{12r}, the recently obtained value for the diffusion constant of B_{12}. The pertinent data are given in Table I.

TABLE I

POLAROGRAPHIC CHARACTERISTICS OF VITAMINS B_{12}, B_{12r} AND B_{12a}

Supporting electrolyte: 0.100 *N* potassium sulfate

Material	Concn., m*M*	$E_{1/2}$ v. *vs* S.C.E.	i_d, amp.	D, cm.²/sec. × 10^6	$605\ m^{2/3}t^{1/6}$ (coul.)/(cm.) (equiv.) (sec.)$^{1/2}$ × 10^{-3}	*n*
B_{12}	0.416	−1.11	1.44	2.95[c]	1.002	1.98
B_{12}[a]	.434	−0.04 (anodic)	0.81	2.95[d]	1.003	1.06
		−0.95	0.76	2.95	1.002	1.01
		−1.55	[f]			
B_{12}[a]	.303	−0.06	0.39	2.33[c]	1.003	0.84
		−0.55[g]	.12	2.33	1.002	.25
		−1.02	.45	2.33	1.002	.96
B_{12r}[b]	.306	−0.04	.40	2.33[e]	1.003	.85
		−0.94	.50	2.33	1.002	1.05

[a] Obtained by hydrogenation of vitamin B_{12} for 7 hours. [b] Obtained by hydrogenation of vitamin B_{12a} for 6 hours. [c] Ref. 6. [d] Assumed to be the same as that of the B_{12} from which derived. [e] Assumed to be the same as that of the B_{12a} from which derived. [f] Diffusion current depends on time of hydrogenation. [g] Probably due to impurity.

The height of the third wave of B_{12r} depends on various factors which have not been elucidated; it is possible, as happened in our very early work with B_{12r}, to have this wave the height of a one-electron reduction but this is quite accidental. This wave apparently involves the reduction of the organic portion of the molecule and probably also

(7) H. Diehl, R. R. Sealock and J. I. Morrison, *Iowa State Coll. J. Sci.*, **24**, 433 (1950).

(8) H. Diehl, J. I. Morrison and R. R. Sealock, *Experientia*, **7**, 60 (1951).

the further reduction of the cobalt as we postulated earlier.

The first two waves of B_{12r} are independent of *p*H; the data are presented in Table II. In preliminary experiments to determine the *p*H dependence of the half-wave potentials of B_{12r}, various buffer solutions were used and difficulty was encountered in filtering off completely the platinum catalyst. In the presence of platinum both the wave height and half wave potential were altered and in view of this, the same supporting electrolyte, 0.1 *N* potassium sulfate with which no such difficulty was encountered was used throughout the work. Admittedly the solutions were not well buffered but it may be definitely concluded that the half wave potential is independent of *p*H.

TABLE II

POLAROGRAPHIC CHARACTERISTICS OF VITAMIN B_{12r}

Supporting electrolyte: 0.1000 *N* potassium sulfate; *p*H adjusted by addition of oxygen-free hydrochloric acid.

*p*H	$E_{1/2}$	$E_{1/2}$
10.08	−0.06	−0.96
8.38	− .04	− .94
6.85	− .04	− .95
5.32	− .04	− .93

The polarogram of B_{12a} shows two one-electron cathodic waves, Fig. 2. The half-wave potential of the first wave is the same as that of the first wave of B_{12r}. Moreover the polarogram of B_{12r} prepared from B_{12a} is identical with that of B_{12r} prepared from B_{12}; Table I. The addition of methylamine to the solutions did not affect the polarograms. The anodic, first wave of B_{12r} and the cathodic, first wave of B_{12a} are therefore for the same couple

$$RCo(III)^{+} + e^{-} = RCo(II)$$

The small wave falling between the first and second waves in the polarogram of B_{12a} is quite likely due to an impurity, produced during the air oxidation of B_{12r}. This small wave decreases in height and shifts in the negative direction with decreasing *p*H. The height of the wave varies from one preparation to another.

The second wave of B_{12a}, $E_{1/2} = -1.02$ v. *vs.* S.C.E., is a one-electron step. Like the first wave of B_{12a} it is independent of *p*H. It has about the same half-wave potential as the second wave of B_{12r}. Both waves correspond to the reduction of cobalt from two to one

$$RCo(II) + e^{-} = RCo(I)^{-}$$

the variation in half wave potential resulting from the difference in the composition of the main body of the electrolyte.

The most negative wave of B_{12a} is a multi-electron step the nature of which, as with the corresponding waves of B_{12} and B_{12r}, is not understood. Quite possibly it is a catalytic hydrogen wave similar to those found with certain other cobalt complexes.

In another paper[3] we have shown that the hydrogenation of B_{12} takes place according to the reaction

$$\underset{B_{12}}{RCoCN} + {}^{5}/_{2}H_2 = \underset{B_{12r}}{RCo} + CH_3NH_2$$

We have confirmed also our earlier[2] finding that one equivalent is required to oxidize B_{12r} to B_{12a}, both by repetition of the ferricyanide titration and by

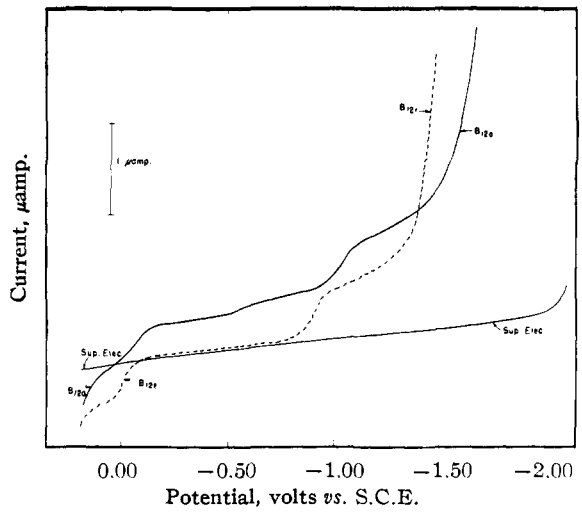


Fig. 2.—Polarograms of vitamin B_{12a} and B_{12r}; potassium sulfate, 0.100 *N* as supporting electrolyte.

titration in acid solution with iodine.[9] Inasmuch as the hydrogen absorbed is all accounted for and again that only one equivalent of oxidizing agent is necessary under different conditions, it is likely that the B_{12r} solutions contain only a single material. The poor yield of B_{12a} obtained by the hydrogenation of B_{12} and air oxidation of B_{12r} results from side reactions in the oxidation step.[3]

In a cyanide solution the two-electron wave of B_{12} shifts in half-wave potential (but not in height) from −1.12 to −1.35 v. *vs.* S.C.E.,[8] a change which is consonant only with the explanation that this wave involves cobalt and results from the reduction of cobalt from three to one. The same shift, of course, happens with B_{12} and B_{12r} which are converted to the B_{12}-cyanide purple on treatment with cyanide, the conversion of B_{12r} happening with great speed and without the benefit of oxygen. That the waves of B_{12r} and B_{12a} having half-wave potentials at −1.0 v. involve the reduction of cobalt to the univalent state is perfectly reasonable in view of the firmness with which cobalt is attached to the organic portion of the molecule. In this connection it is of interest to note that univalent and zero-valent cobalt compounds have now been actually isolated.[10]

The reduced B_{12} compound reported by Boos and others, prepared by the reduction of B_{12} by bivalent chromium, is a distinctly different material than the B_{12r} studied in this paper; the numbers of equivalents of reducing agent required in the preparations differ and the absorption spectra are markedly different. Probably cyanide is still present in the Boos compound and the material would be expected to show a reduction wave more negative than the −0.95 v. wave of B_{12r}. It is unfortunate that Boos and co-workers did not report the complete polarogram of this material.

The great difference in the reactivities of B_{12a} and B_{12} is nowhere more evident than in the ease with which they are reduced as evidenced by their po-

(9) J. M. Brierly, J. L. Ellingboe and H. Diehl, *Iowa State Coll. J. Sci.*, **27**, 428 (1953).

(10) W. Hieber and C. Bartenstein, *Naturwissenschaften*, **39**, 300 (1952).

larography. Whereas the cobalt of B_{12a} is easily reduced to the bivalent state, $E_{1/2} = -0.04$ v., and at a more negative potential reduced to the univalent state, $E_{1/2} = -1.02$ v., B_{12} undergoes only a single two-electron reduction, $E_{1/2} = -1.12$ v. *vs.* S.C.E. We have observed also that B_{12a} can be hydrogenated to B_{12r} without a catalyst.

There appear, then, to be four classes of cobaltic ammines with respect to their polarography:

Class	Material	Half-wave potential E'	Half-wave potential E''	n	Valence change
I	Most ammines,[11] *e.g.*, $Co(NH_3)_6$-	−0.00 to	−1.3 to	1	3 to 2
	Cl_3	−0.04	−1.4	2	2 to 0
II	B_{12a}	−0.04		1	3 to 2
	B_{12a}, B_{12r}		−1.02	1	2 to 1
III	B_{12}[7]	−1.12		2	3 to 1
	$B_{12}(CN)^{-8}$	−1.35		2	3 to 1
	$K_4Co(CN)_5H_2O$ [12]	−1.3		1	2 to 1
IV	$K_3Co(CN)_5H_2O$ [12]	−1.45		2	3 to 1

(11) J. B. Willis, J. A. Friend and D. P. Mellor, THIS JOURNAL, **67**, 1680 (1945).

Acknowledgment.—The authors wish to express their appreciation of the aid they have received from The Squibb Institute for Medical Research.

(12) D. N. Hume and I. M. Kolthoff, *ibid.*, **71**, 867 (1949).

AMES, IOWA

[CONTRIBUTION FROM THE RADIATION LABORATORY AND DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

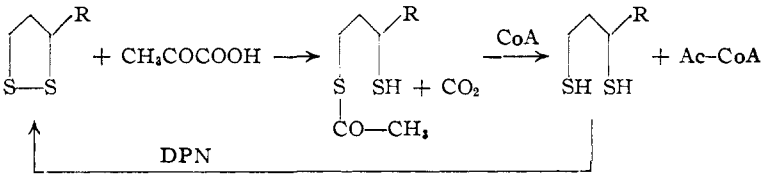
The Chemistry of 1,2-Dithiolane (Trimethylene Disulfide) as a Model for the Primary Quantum Conversion Act in Photosynthesis[1a]

BY J. A. BARLTROP,[1b] P. M. HAYES AND M. CALVIN

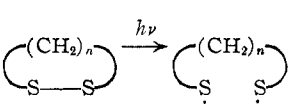
RECEIVED MARCH 24, 1954

Some chemical and photochemical observations on 1,2-dithiolane and its derivatives with particular reference to the possible mode of function of the naturally occurring system, 6-thioctic acid, are discussed. Experimental evidence is presented to demonstrate that the strain energy in this 5-membered ring is not less than 6.5 kcal. and probably larger. Reagents which both oxidize and reduce this ring are described together with the conditions required for its reformation from the corresponding dithiol. Evidence is adduced to indicate that the primary product of photolysis of this ring in acidic media is very likely a thiol and sulfenic acid or derivative thereof.

Although plants which are allowed to photosynthesize in $C^{14}O_2$ rapidly assimilate labeled carbon into a series of compounds, very little of the C^{14} finds its way into the intermediates of the Krebs tricarboxylic acid cycle during illumination. If, after a period of photosynthesis, the light is turned off, the compounds of the Krebs cycle rapidly become labeled.[2] Thus there is a reaction path linking the photosynthesis and Krebs cycles which becomes blocked during illumination. With the discovery[3-6] that 6-thioctic acid is a coenzyme for the oxidative decarboxylation of pyruvate to active acetyl groups, which through CoA feed carbon into the Krebs cycle.[7,8] Calvin and Massini[9] suggested that the process could be formulated as



Moreover, since the coenzyme must be present in its oxidized form in order that the oxidation of pyruvic acid may proceed, these authors suggested that the reducing power formed in the presence of light shifted the steady-state conditions of the coenzyme toward its reduced (dithiol) form and thus reduced the rate at which intermediates of the photosynthetic cycle entered the TCA system. At this point, specimens of the isomeric 4-, 5- and 6-thioctic acids became available to us through the courtesy of Dr. T. H. Jukes of Lederle Laboratories. The ultraviolet absorption spectra of these compounds (Fig. 1) showed a displacement of the absorption peak to progressively longer wave lengths as the size of the disulfide ring diminished, a phenomenon which might well be due to ring-strain. If one supposes that the absorption band is due to a transition



then assuming to a first approximation that the excited states of all the disulfides have the same energy

(1) (a) The work described in this paper was sponsored by the U. S. Atomic Energy Commission; (b) Rockefeller Fellow, 1952–1953, while on leave of absence from Brasenose College and the Dyson Perrins Laboratory, Oxford University, England.

(2) A. A. Benson and M. Calvin, *J. Exptl. Bot.*, **1**, 63 (1950).

(3) (a) L. J. Reed, I. C. Gunsalus, *et al.*, THIS JOURNAL, **73**, 5920 (1951); (b) E. L. Patterson, *et al.*, *ibid.*, **73**, 5919 (1951).

(4) I. C. Gunsalus, L. Struglia and D. I. O'Kane, *J. Biol. Chem.*, **194**, 859 (1952).

(5) L. J. Reed and B. G. DeBusk, THIS JOURNAL, **74**, 3457 (1952).

(6) M. W. Bullock, *et al.*, *ibid.*, **74**, 3455 (1952).

(7) S. Ochoa, J. R. Stern and M. C. Schneider, *J. Biol. Chem.*, **193**, 691 (1951).

(8) S. Korkes, A. DelCamilo, I. C. Gunsalus and S. Ochoa, *ibid.*, **193**, 721 (1951).

(9) M. Calvin and P. Massini, *Experientia*, **8**, 445 (1952).