RESEARCH PAPERS

THE CHEMISTRY OF ANTI-PERNICIOUS ANÆMIA FACTORS

PART VII. SOME TRANSFORMATIONS OF VITAMIN B_{12b}

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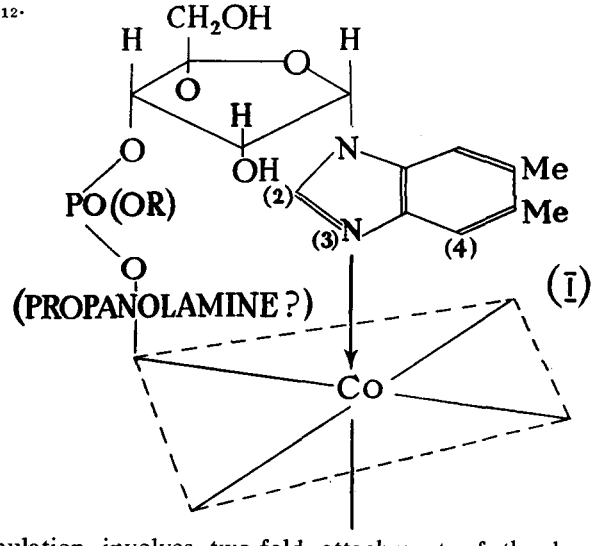
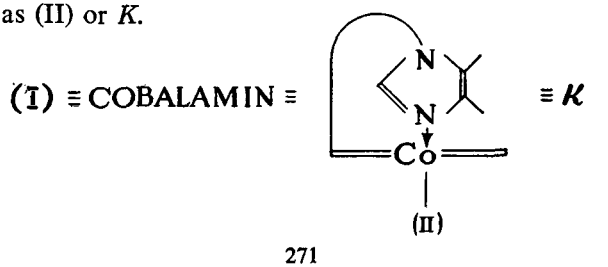
Received March 20, 1951

SPECTROSCOPIC studies outlined in Part VI of this series led Beaven, Holiday, Johnson, Ellis and Petrow[1] to assign a partial structure (I) to vitamin B_{12}.

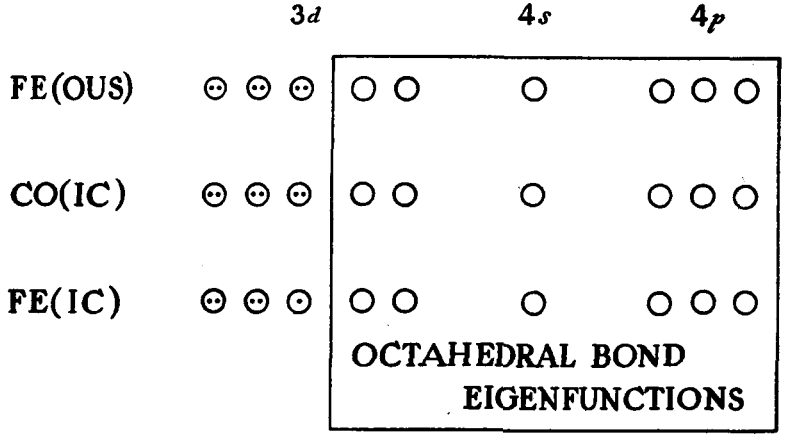


This formulation involves two-fold attachment of the benziminazole glycosidic residue to the cobalt-containing macrofragment of the B_{12} molecule, which is visualised as a planar structure spatially akin to the porphyrins. Evidence regarding the nature of the remaining group present in the co-ordination shell has now been presented by Brink, Kuehl, and Folkers[2], who have shown that vitamin B_{12} may be regarded as a cyano-cobaltic complex. The latter authors have, therefore, suggested that the name "cobalamin" be given to that part of the B_{12} molecule which is attached to the cyano-group. We propose to adopt this nomenclature and shall accordingly refer to (I) as cobalamin, which we shall represent as (II) or *K*.



The spatial and structural analogies between vitamin B_{12} (cyanocobalamin, [*K*-CN]) and the porphyrins, to which attention has already been directed in this series of publications, permits a comparison to be drawn between the transformations undergone by the B_{12} group of factors and certain pigments of the hæm type. Of these, the ferroporphyrins bear the closest analogy to complexes derived from Co^{+++}. This conclusion follows from a study of the electron arrangements in the 3*d*, 4*s*, and 4*p* orbitals of Fe^{++}, Fe^{+++}, and Co^{+++}, which are given below.

ELECTRON ARRANGEMENTS IN THE 3*d*, 4*s*, AND 4*p* ORBITALS IN COVALENT COMPLEXES OF Fe^{++}, Fe^{+++}, AND Co^{+++}

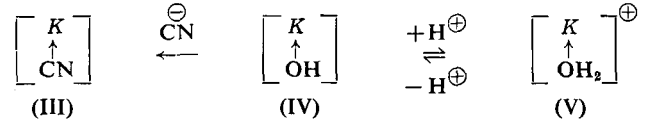


Examination of these reveals the close analogy between Fe^{++} and Co^{+++}, the atoms presenting an identical distribution of electrons in the outer orbitals which are concerned in the formation of octahedral complexes of the d^2sp^3 type. Analogies may, therefore, be drawn between the transformations of the B_{12} group and those of the ferroporphyrins.

Vitamin B_{12b} was first isolated by Lichtman, Watson, Ginsberg, Pierce, Stokstad, and Jukes[3,4] from the fermentation liquors of *Streptomyces aureofaciens* and from liver, and was later prepared by Brockman, Pierce, Stokstad, Broquist, and Jukes[5] by catalytic hydrogenation of vitamin B_{12}, when *ca.* 3 mols. of hydrogen were absorbed, followed by oxidation with air. The latter procedure had previously[6] given "vitamin B_{12a}" which is now known to be identical with B_{12b} in spectrographic and biological behaviour[7,8]. The latter designation, which appears to command wide acceptance[9], is, therefore, adopted in describing this material.

The relationship between vitamins B_{12b} and B_{12} has recently been elucidated by Kaczka, Wolf, Kuehl, and Folkers[10], who have found that an aqueous solution of vitamin B_{12b} has a *p*H of about 9, and behaves on titration as a weak base. This observation, coupled with the formulation of vitamin B_{12} as cyanocobalamin (III)[2], and the facile conversion of B_{12b} into B_{12} by the action of cyanide[2,11,12], leads to the conclusion

that vitamin B_{12b} contains an hydroxyl group coordinated to cobalt as shown in (IV) (Kaczka *et al., loc. cit.*).

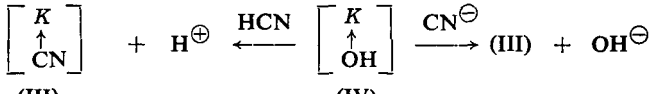


(III) (IV) (V)

The formulation of vitamin B_{12b} as hydroxocobalamin (IV), however, is not entirely satisfactory as it fails to account for its behaviour as a base[10] in aqueous solution. Again[5], the spectrum of vitamin B_{12b}, in striking contrast to that of vitamin B_{12}, undergoes a definite bathochromic shift in passing from *p*H 2 to *p*H 12. These changes may conceivably be regarded as due to an equilibrium between hydroxocobalamin (IV) and the aquocobalamin cation (V), the relative proportions of the two forms depending upon the *p*H of the solution. The observed bathochromic shift would thus be associated with the change in net charge caused by conversion of (IV) into the aquated form (V). As the spectrum of crystalline vitamin B_{12b} isolated from natural sources corresponds to that of a solution at the lower *p*H limits (hereafter referred to as the "acid" spectrum), the material probably exists in the aquated form (V). The transformations of B_{12b}, however, are more conveniently expressed on the basis of (IV), which is accordingly adopted in the present communication.

The Reaction Between Vitamin B_{12b} and Cyanide

The conversion of vitamin B_{12b} into cyanocobalamin (B_{12}) was reported independently by three groups of workers[2,11,12]. The reaction[8] occurs over the *p*H range 5·5 – 9 and may be readily accomplished employing either hydrogen cyanide or potassium cyanide. As the degree of dissociation of the prussic acid at the lower *p*H values is negligible, the reaction can evidently proceed in two different ways:



(III) (IV)

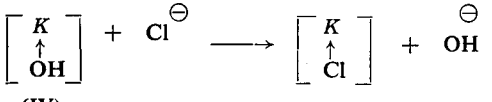
The cyanocobalamin (III) thus formed reacts further with prussic acid or cyanide to give a purple "B_{12}-cyanide" complex[13], formulated as dicyanocobalamin in an *addendum* added in proof to Part VI of this series[1]. The information that B_{12} is itself a cyano-complex[2] was not available, however, when the latter communication was sent to print. Some further remarks upon the structure of the "dicyanide" are, therefore, required.

In Part VI of this series the hypothesis was presented that the anomalous contribution of the benziminazole chromophore of (III) to the ultraviolet absorption spectrum of vitamin B_{12} was due to the co-ordination of $N^{(3)}$ with cobalt as shown in (I). Addition of cyanide to the cobalt atom of (III) was held to lead to an increase in the electronegativity of the cobalt atom, and a corresponding decrease in the electronic contribution of the

$N^{(3)}$ benziminazole nitrogen to the cobalt electron cloud. Decrease in this electronic contribution was assumed to diminish the changes produced by the $N^{(3)}$ – Co linkage in the benziminazole chromophore spectrum and lead to an absorption curve in the benziminazole region more closely resembling that of an unco-ordinated 5 : 6-dimethylbenziminazole-1-glycoside.

The relevant portions of the absorption curves of B_{12b}, B_{12}, and "B_{12}-CN" are shown in Fig. 1. Comparison of these with that of a 5 : 6-dimethylbenziminazole-1-glycoside shows that the anomalous absorption contribution in the benziminazole region is most marked in the case of B_{12b}, decreasing in the order $B_{12b} > B_{21} >$ "B_{12}-CN".

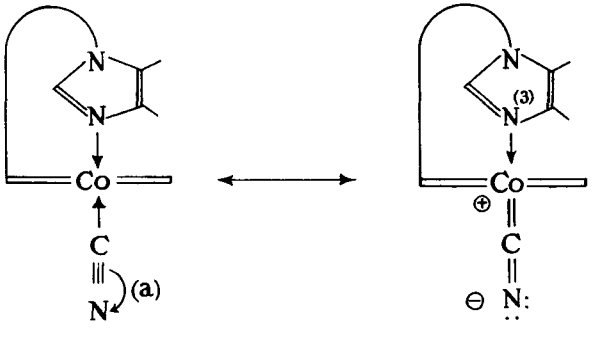
The most significant difference between B_{12b} and cyanocobalamin lies in the marked lability of the hydroxyl group in the former compound. Thus reaction of B_{12b} with chloride ions leads to the formation of chlorocobalamin (Kaczka *et al.*[10]).



(IV)

The Co-OH bond of (IV), in contrast to the Co-CN bond of (III), must, therefore, possess marked ionic character, which will presumably be compensated for by an enhanced withdrawal of electrons from the benziminazole nucleus (*cf.* I). The latter process, in turn, should be reflected by a greater degree of distortion in the benziminazole region of the spectrum, as is indeed found to be the case.

Replacement of (OH) in (IV) by cyano- leads to a notable increase in the stability of the resulting structure, which is accompanied by a marked decrease in the anomaly observed in the benziminazole region of the

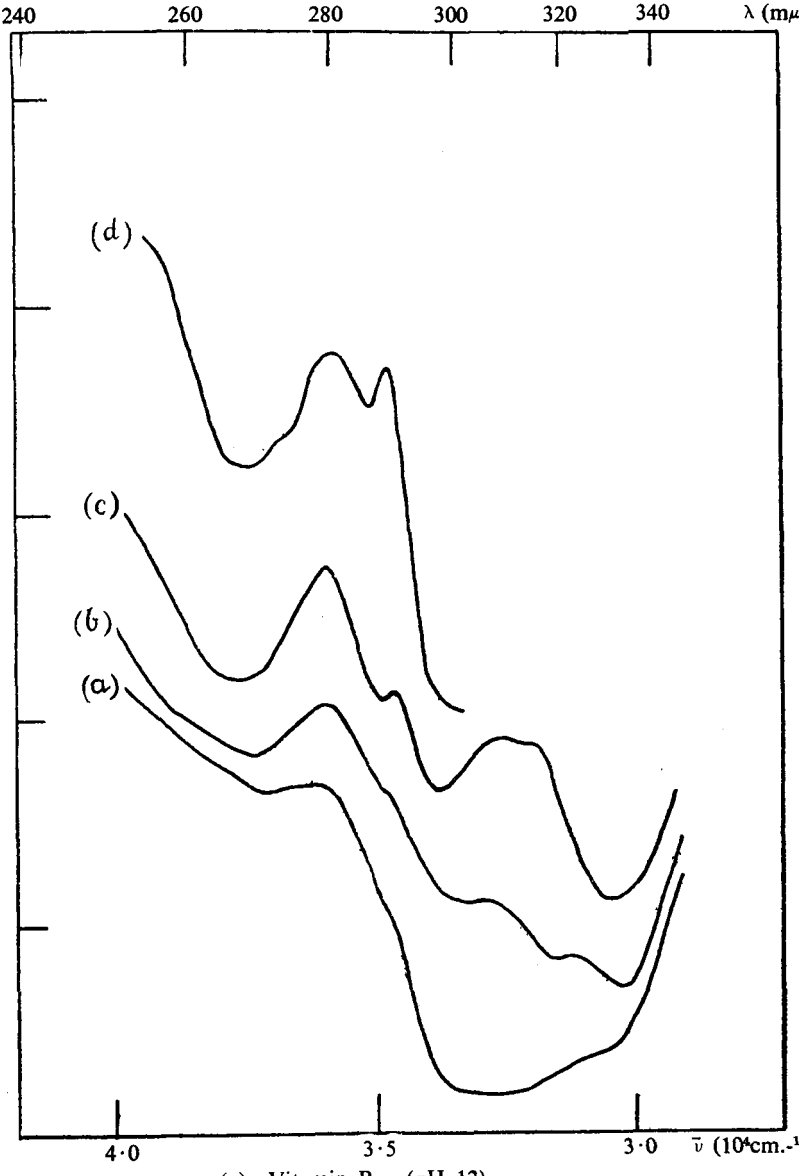


(III) (IIIa)

spectrum (*cf.* Fig. 1). Thus reconversion of (III) into (IV) has only been effected by irradiation[13,11] or by the hydrogenation technique previously referred to[5,6]. The enhanced stability of the cyanocobalamin (III) derives, in our view, from increased resonance energy produced by the contribution of structures such as (IIIa) (*cf.* Pauling[14]) to the normal state of the molecule. The formation of the Co-C double bond initiated

by the electromeric shift (a) transfers a positive charge to the cobalt atom, thereby strengthening the Co-N[3] bond as indicated.

Further action of cyanide on cyanocobalamin leads, as indicated above, to the formation of dicyanocobalamin[1] in which two cyano-groups are



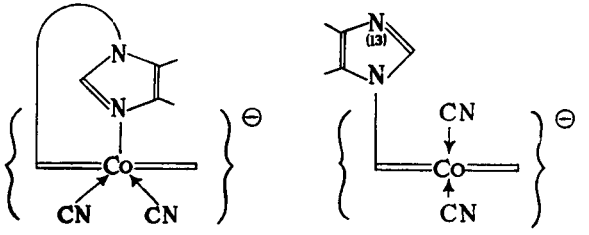
(a) Vitamin B_{12b} (*p*H 12)
(b) Vitamin B_{12} (*p*H 2 to 10)
(c) Dicyanocobalamin (*p*H 12)
(d) 5:6-Dimethylbenziminazole-l-glycoside (*p*H 12)

The curves have been displaced vertically to avoid overlapping.

FIG. 1.

co-ordinated to the central cobalt atom. The co-ordination of the second cyano-group is accompanied, as already pointed out in Part VI by a marked diminution in the anomaly present in the benziminazole region of the spectrum (see Fig. I). In fact, the contribution of the benziminazole chromophore to the spectrum of dicyanocobalamin may now be considered as "normal." (*Cf.* Pt. VI[1], p. 951.) The dicyanocobalamin thus formed, however, lacks stability and reverts readily to the parent compound (III) in the absence of excess cyanide.

Two formulations are possible for dicyanocobalamin. Addition of

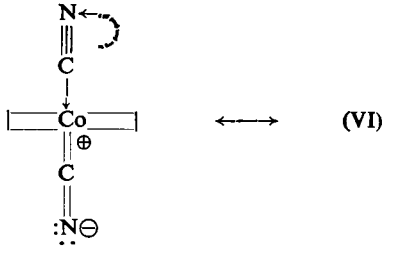


(V) (VI)

cyanide to cyanocobalamin may lead to production of the heptacoordinated structure (V). Alternatively extrusion of the benziminazole nucleus from the coordination shell may occur as shown in (VI). Decision between these alternative structures employing spectroscopic data alone is not, unfortunately, possible.

Heptacoordinated structures of cobalt have not, hitherto, been described in the literature. Co-ordination of this type, however, is encountered in columbium and tantalum compounds of the type R_2TaF_7 and R_2CbF_7. X-ray study of such compounds by Hoard[15] has revealed that the XF_7 polyhedron may be visualised as derived from an XF_6 group in the form of a trigonal prism by addition of the seventh fluorine atom through the centre of one square face, followed by the appropriate distortion. Such a situation can only obtain in the dicyanocobalamin complex by withdrawal of the cobalt atom from the plane of the cobalt chromophore. This possibility cannot be excluded in the absence of data regarding the structure of the latter complex, but must, at best, be considered unlikely.

Extrusion of the benziminazole residue from the cobalt co-ordination



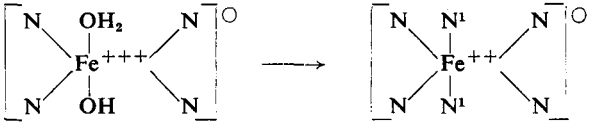
(VIa)

shell (*cf.* VI) offers no theoretical objections. The stability of the resulting dicyano-system (*cf.* VI⟷VIa), however, compares unfavourably with (III) ⟷ (IIIa) and would presumably lead to an enhanced ionic character of a Co-CN bond. The effective concentration of the benziminazole nucleus in the vicinity of the coordination shell is clearly of a high order as the fragment is still attached to the cobalt chromophore *via* the phosphate linkage. The benziminazole residue may, therefore, be expected to displace the cyanide under conditions other than those in which a high concentration of cyanide obtains.

The foregoing transformations parallel those established in the porphyrin series. Thus the stability of dinicotino-, dicyano-, and nicotino-cyanohæmochromogen falls in the order nicotino-cyano <dicyano <dinicotino- (Hill[16]). In this instance, too, structures such as (IIIa) may be used to explain the stability of the mixed cyanide-base hæmatins.

The Reaction Between Vitamin B_{12b} and Ammonia

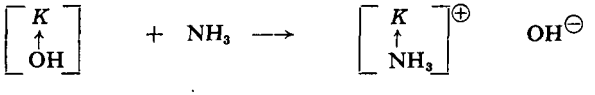
Extension of the formal analogy established in the preceding section between the mixed base:cyanide hæmatins and the B_{12} group of compounds leads to the conclusion that complexes analogous to the hæmochromes should be capable of existence. The latter types (VIII) contain two mols. of a base such as ammonia, pyridine, 4-methyliminazole, etc.,



(VII) (VIII)

in the ferrous coordination sphere. Their preparation is usually effected by direct action of the ligand on the ferroporphyrin or, more conveniently, by reduction of a hæmatin (VII) in the presence of the required base[17].

We now find that by treating vitamin B_{12b} with liquid ammonia, facile reaction occurs to give a new member of the B_{12} group which is undoubtedly the corresponding ammonia cobalichrome* (IX). Thus the



(IV) (IX)

compound has an absorption spectrum similar to that of the parent compound (IV) (see Experimental), an observation in marked accord with results established in the porphyrin field[18]. Again, exposure of its solution to ultraviolet light or to heat leads to reversion of the spectrum to that of "acid B_{12b} "(V), whilst treatment with prussic acid or cyanide effects conversion into cyanocobalamin. These facts justify its formulation as

* Note on nomenclature: The term *cobalichrome* is being employed to describe those derivatives of B_{12b} in which the hydroxyl group is replaced by basic ligands, such as ammonia, etc.

(IX) in the absence of supporting analytical data which could not, unfortunately, be obtained in view of the limited supplies of material available.

The biological activity of ammonia cobalichrome varies markedly with the method of assay as indicated in Table I.

TABLE I

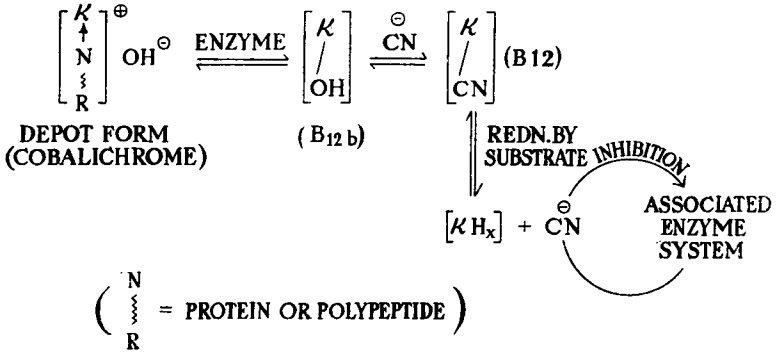
BIOLOGICAL ACTIVITY OF AMMONIA COBALICHROME

METHOD	ACTIVITY (Vitamin B_{12} = 1·0)
Lactobacillus lactis Dorner (ATCC 10697)	2·5*
Escherichia coli (NCIB 8134)	1·5*
Euglena gracilis	0·5†
Chick	1·0‡

* Kindly determined by Dr. S. W. F. Underhill (Physiological Dept. B.D.H.).
† Kindly determined by Dr. W. J. Robbins through the courtesy of Dr. T. Jukes.
‡ Kindly determined by Dr. T. Jukes (Lederle Laboratories).

The ability to utilise (IX) thus varies from species to species.

The foregoing observations assume an added interest following recent evidence (Scheid and Schweigert[19]; Wijmenga *et al.*[8]) that the B_{12} group of factors exist partly in combined form in certain natural materials. The results obtained may well be interpreted as evidence supporting the view that " cobalamin " is stored by tissues in a cobalichrome type of combination with a compound of protein or polypeptide character. This view is strengthened by our observation that vitamin B_{12b} undergoes facile conversion into histidine cobalichrome (*cf.* hæmoglobin in which a terminal histidine residue of globin is generally considered as co-ordinated with the porphyrin iron atom) and points to the following tentative scheme for the transformations undergone by the " cobalamin " residue in the living cell:



The above scheme is admittedly based on experimental evidence of a most tenuous character. The hypothesis that " cobalamin " performs its functions by reversible release of cyanide, which then becomes available for inhibiting an associated system of enzymes has, nevertheless, the merit that it provides a reason for the existence of a cobaltic complex in nature. Should this, indeed, prove to be the case, a closer under-

standing of the structural features associated with this form of biological activity may become possible.

EXPERIMENTAL

The crystalline vitamin B_{12b} employed in the present investigation was prepared by a method essentially that of Veer *et al.*[11], except that the solution of vitamin B_{12} in 0·001 N hydrochloric acid, contained in a Pyrex flask, was irradiated with a high-pressure mercury vapour arc (Mazdalux 250 w.). The progress of the reaction was followed by measuring the ratio of the optical densities at 351 and 361 mμ. This ratio is 0·605 for vitamin B_{12}, and increases during irradiation to a maximum of 1·34, which was regarded as indicating completion of the conversion into vitamin B_{12b}.

Ammonia cobalichrome. A solution of vitamin B_{12b} (18 mg.) in liquid ammonia (10 ml.) was allowed to evaporate spontaneously. Crystallisation of the red residue from aqueous acetone gave dark red needles (12 mg.) of ammonia cobalichrome. The spectrum of this compound is shown in Figure 2. (See also Table II.)

When a solution of ammonia cobalichrome (40 μg. per ml.) at *p*H 4 was irradiated with the mercury vapour arc, the spectrum rapidly reverted to that of " acid " vitamin B_{12b}.

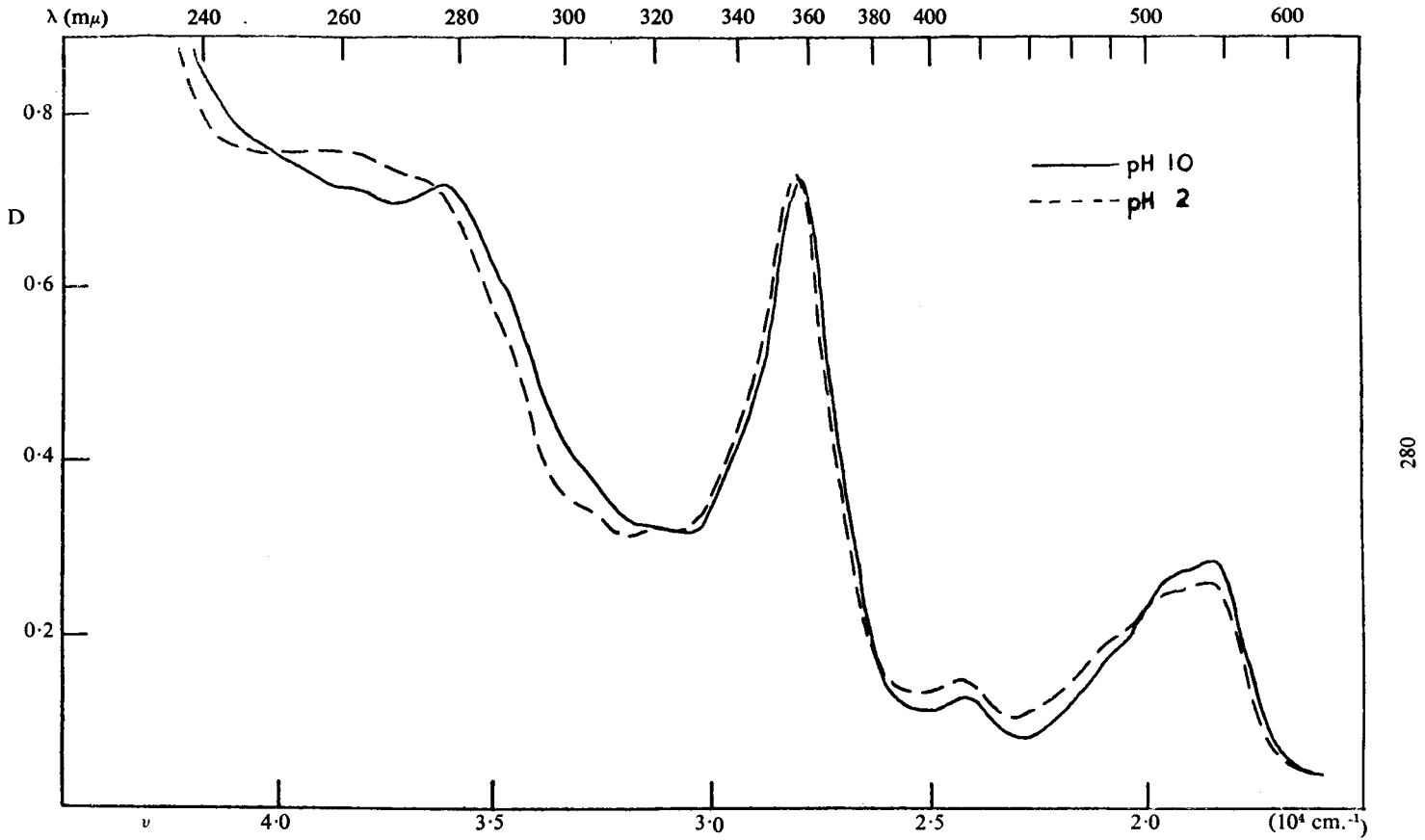
When a sealed tube containing a solution of ammonia cobalichrome in distilled water (92 μgm. per ml.) was heated for 30 minutes at 100°C., the spectrum again changed to that of " acid " vitamin B_{12b}.

The reaction between ammonia cobalichrome and cyanide appeared to proceed less readily than expected. Thus when a large excess of potassium cyanide (250 mg.) was added to an aqueous solution of the complex (200 μg. in 4 ml. of water), the change in colour from red to violet occurred very slowly, and only after some hours was the spectrum identical with that of dicyanocobalamin. Again, a change in colour was not immediately evident when a solution of ammonia cobalichrome (250 μg. in 2 ml. of water) was treated with hydrocyanic acid (1 ml. of 4 per cent.), but after storage at room temperature for 30 hours the characteristic colour of dicyanocobalamin was observed. Evaporation of the mixture to dryness gave material identified as vitamin B_{12} both spectroscopically and chromatographically[20]. The latter compound was also obtained when a mixture of ammonia cobalichrome and hydrocyanic acid was heated in a sealed tube at 100°C. for 15 minutes.

The A.P.F. (animal protein factor) activity of ammonia cobalichrome was kindly determined for us by Dr. T. Jukes, who has supplied the data in Table III.

Basal diet: 69·5 per cent. of yellow corn, 25 per cent. of soya bean protein concentrate (50 per cent. of protein), minerals and vitamins without folic acid, B_{12} or choline.

Interpretation: Ammonia cobalichrome has approximately the same potency as B_{12} for chicks either orally or by intramuscular injection. The growth produced by either 10γ of AC or 50γ of B_{12} may be con-



Absorption spectra of ammonia cobalichrome.

FIG. 2

sidered as maximal and on the plateau of the response curve. Presumably 10γ of B_{12} would have produced as rapid growth as 50γ, for 5γ of B_{12} produced almost 80 per cent. of the maximal growth response.

TABLE II

WAVELENGTHS (mμ) OF RESOLVED* MAXIMA IN ABSORPTION SPECTRA OF COBALAMIN COMPLEXES

Vitamin B_{12}	*p*H 2-10	279	—	(306)	322	361	—	(520)	550
Vitamin B_{12b}	*p*H 2	274	—	—	—	351	408	—	522
	*p*H 10	278	—	—	—	358	418	—	535
Dicyanocobalamin ...	*p*H >8	279	289	308	—	368	—	540	582
Ammonia Cobalichrome	*p*H 2	275	—	—	—	355	411	—	536
	*p*H 10	276	—	—	—	357	412	—	541
Histidine Cobalichrome	*p*H 2	275	—	—	316	357	410	(509)	535
	*p*H 10	277	—	—	—	358	412	—	539

* The values in parentheses refer to just-resolved maxima.

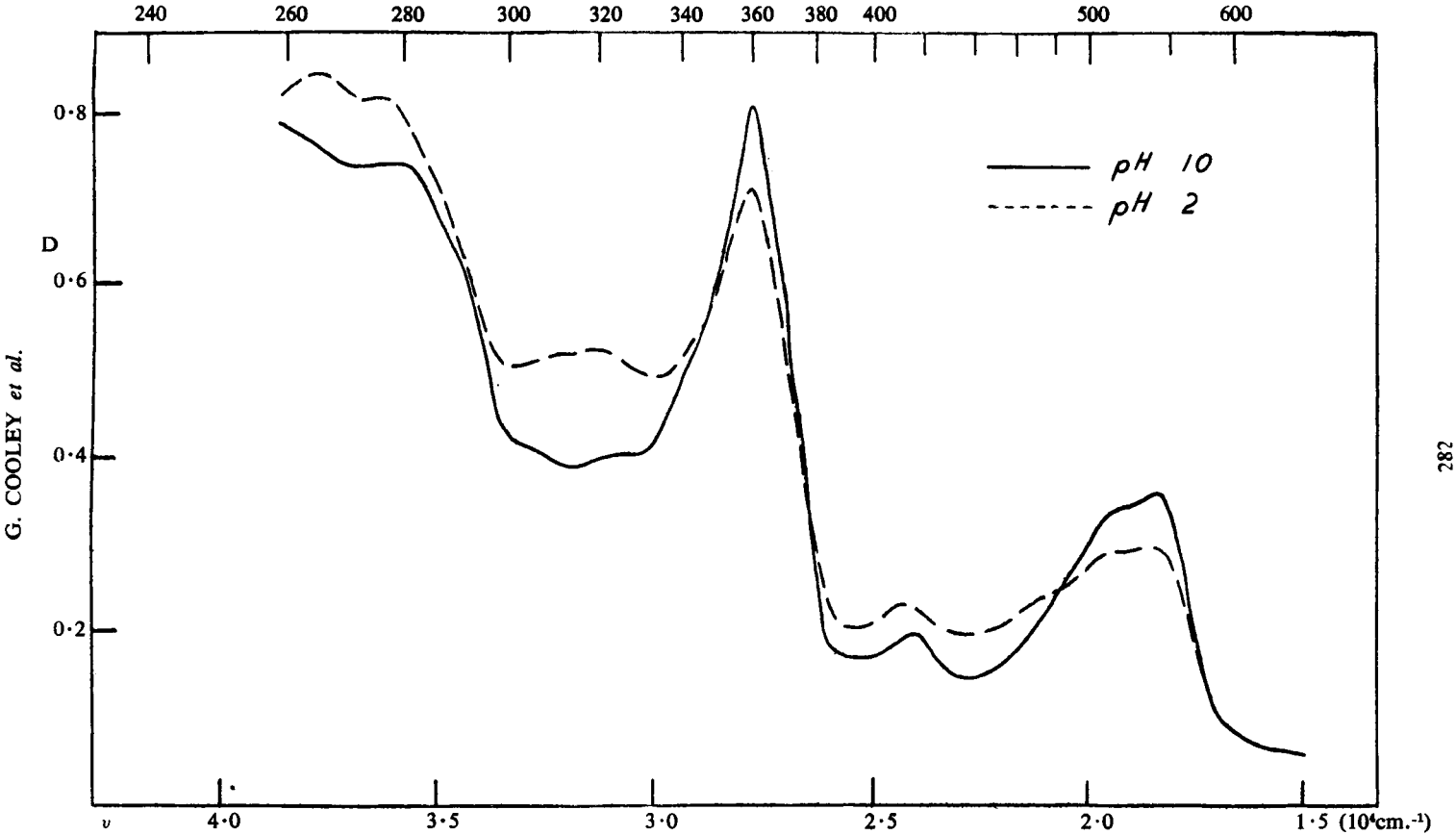
Histidine cobalichrome : dl-Histidine (1 mg.) was added to a solution of vitamin B_{12b} (1 mg.) in water (250 μl.) and aliquots of the mixture submitted to paper chromatography employing *sec*-butanol saturated with water as the irrigation solvent. Although with this solvent system both histidine cobalichrome and histidine (detected with ninhydrin) possessed low R_F values (0·05 and 0·15 respectively), the two substances were nevertheless effectively separated from each other. Aqueous eluates of the histidine cobalichrome possessed the characteristic spectra shown in Figure 3 (see also Table II).

TABLE III

CHICK ASSAY OF AMMONIA COBALICHROME (AC)

	Average weight (gm) and number of survivors ( ) Days of feeding period			
Supplement	8	22	25	26
None	68 (12)	122 (8)	142 (8)	158 (7)
None	64 (12)	127	148	156 (12)
3γ B_{12} per kilo of diet	67 (12)	185 (11)	230	244 (11)
3γ AC „ „	67 (12)	186 (10)	230	242 (10)
5γ B_{12} „ „	72 (12)	210	261	274 (12)
5γ AC „ „	73 (11)	198	244	256 (11)
10γ AC „ „	79 (12)	245	297	312 (12)
0·15γ B_{12} injected twice weekly	71 (12)	203	251	263 (12)
0·15γ AC „ „	74 (12)	202	246	258 (12)
50γ B_{12} per kilo of diet	76 (12)	236	290	304 (12)

Histidine cobalichrome, obtained as an aqueous chromatogram eluate as described above, reacted readily with potassium cyanide to give a solution possessing the spectrum of dicyanocobalamin. The ejection of



Absorption spectra of histidine cobalichrome.

FIG. 3

the histidine molecule from the cobalichrome complex during this reaction was demonstrated simply by submitting histidine cobalichrome to paper chromatography, employing as irrigation solvent *sec*-butanol saturated with $2\frac{1}{2}$ per cent. aqueous potassium cyanide solution to which a few drops of 5 per cent. aqueous ammonia solution had been added. A violet spot migrated with $R_F = 0{\cdot}16$. On air-drying the chromatogram, the colour of this spot changed to red, and spraying the paper with ninhydrin revealed a spot of histidine migrating with $R_F = 0{\cdot}06$.

The microbiological activity of histidine cobalichrome towards *E. coli* appeared to be of the same order as that of vitamin B_{12}.

Notes on Paper Chromatography: Whatman No. 1 filter paper impregnated with potassium dihydrogen phosphate[20] was employed for all chromatograms described in this section. These were irrigated at room temperature (unless otherwise stated) with *n*-butanol saturated with water. The chromatography cabinets used measured 8 × 8 × 18 in. and possessed glass sides. The materials under investigation were applied to the paper strips as visible red spots (20 to 50 μg. B_{12} factors).

(a) *Vitamin B_{12}.* In agreement with the observations of Woodruff and Foster[20] all samples of vitamin B_{12} so far examined by us have given rise to the appearance on the chromatograms of two widely separated spots. The fainter and slower moving of these has been identified with B_{12b} [20].

In view of the facility with which aqueous vitamin B_{12} at a *p*H in the region of 4 is converted into vitamin B_{12b} by exposure to daylight, it seemed possible that this agent may well be a factor influencing the formation of B_{12b} from B_{12} during chromatography of the latter compound on paper impregnated with potassium dihydrogen phosphate (*p*H 4·6). Our experiments have, indeed, indicated such a relationship. Thus chromatography of B_{12} in complete darkness was characterised by the appearance of only just visible spots of B_{12b}. Much stronger spots of this substance resulted, however, when the chromatography cabinet was situated so that the paper inside it directly faced the daylight from an unscreened window only a few feet distant. We have also observed that a considerable degree of conversion of B_{12} into B_{12b} occurs during the elution[21] of a B_{12} spot from a segment of phosphate-impregnated chromatogram when this leaching operation is performed in normal daylight. Rechromatography in the dark of such an eluate was found to give B_{12b} and B_{12} as two spots of almost equal intensity. It is perhaps relevant to add that if light is, indeed, a factor influencing the conversion of B_{12} into B_{12b} during chromatography of the former compound, it is somewhat curious that the latter substance invariably appears as a single well-defined spot and not as a streak, such as might be expected to arise under the experimental conditions.

(b) *B_{12b} Ammonia and Histidine Cobalichromes.* The substances investigated were (i) a specimen of authentic B_{12b} (Ref. NP 119.17.6; ex. Lederle Laboratories), (ii) a second specimen of authentic B_{12b} from an alternative source (ex. Pfizer), (iii) B_{12b} prepared by irradiation of B_{12},

(iv) ammonia cobalichrome prepared from (iii), and (v) histidine cobalichrome produced from (iii).

The chromatographic behaviours of (i), (ii) and (iii) were compared. In a typical experiment each of these materials was chromatographed side by side on one and the same strip of impregnated paper. In addition, a fourth spot of B_{12} applied at the starting line served as a marker. After 24 hours' irrigation in daylight, the B_{12} spot moved to a position 11·5 cm. from the point of application, and the fainter spot of B_{12b} formed from it, to a position 5·0 cm. from the origin. Specimens of B_{12b} (i), (ii) and (iii) likewise moved 5·0 cm. The behaviour of (iii) thus exactly paralleled that of authentic B_{12b} isolated from natural sources, i.e., specimens (i) and (ii). This result confirms the observations of Wijmenga *et al.*[8].

Vitamins B_{12} and B_{12b} were also chromatographed side by side on phosphate-impregnated paper at a temperature of 35°C. Vitamin B_{12} behaved normally at this elevated temperature, giving rise to an intense rapidly moving spot (B_{12}) and a faint slow-moving spot (B_{12b}). On the other hand, each sample of crystalline B_{12b} examined had clearly split into two spots, the faster moving of which corresponded in position with "B_{12b}" formed from B_{12} during the chromatography. In a particular experiment extending over 24 hours, the distances travelled by the spots were: B_{12}, 13·7 cm., B_{12b} (from B_{12}), 6·0 cm., the two spots arising from crystalline B_{12b}, 3·9 and 6·0 cm. respectively. We have consistently observed this phenomenon, the elucidation of which must necessarily await the results of further investigation.

Chromatography at room temperature of ammonia and histidine cobalichromes, (iv) and (v), revealed that these substances each migrate at the same rate as vitamin B_{12b}. The latter compound, however, appears as an orange-red spot on the paper, and is readily distinguishable from the spots of the two cobalichromes, both of which are blue-red in colour.

Summary and Conclusions

1. The bathochromic shift observed in the spectrum of vitamin B_{12b} in passing from *p*H 12 to *p*H 2 is interpreted as due to aquation of hydroxocobalamin with formation of an aquocobalamin cation.

2. The reaction mechanism underlying the stepwise conversion of vitamin B_{12b} into cyanocobalamin and dicyanocobalamin is discussed.

3. Ammonia cobalichrome, structurally analogous to ammonia hæmochrome, has been prepared from vitamin B_{12b} and its conversion into cyanocobalamin effected.

4. Evidence is presented for the existence of histidine cobalichrome, likewise converted into cyanocobalamin by cyanide.

5. A hypothetical scheme for a possible role of vitamin B_{12} in metabolic processes is presented.

The authors thank Dr. T. Jukes (Lederle Laboratories Division, American Cyanamid Company) and Dr. W. A. Lazier (Chas. Pfizer & Co.,

Inc.) for samples of B_{12b} isolated from microbiological sources. Their best thanks are also due to Dr. T. Jukes for the chick assay of ammonia cobalichrome, and to the Directors of The British Drug Houses, Ltd., for encouraging this work.

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