ROBERT ROBINSON LECTURE*

Vitamin B_{12}. Retrospect and Prospects

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B_{12} is not a vitamin
It is a fraternity
Fanny Rosenblum, 1958

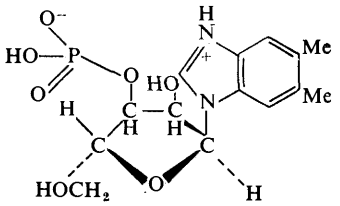
As a junior research chemist, I first met Sir Robert Robinson at the Dyestuffs Division (now Organics Division) of I.C.I. in 1944, when I had the duty of taking minutes at the monthly meetings of Consultants, who at that time were Sir Robert together with Professors Heilbron and Todd. This task was considered to be no small privilege (for me) although errors of reporting were not dismissed lightly, especially by Sir Robert. It was at these meetings that I learnt much basic dyestuffs chemistry and I recall particularly Sir Robert's discourse on one of his student's (Michael Dewar) novel aromatic, non-benzenoid, tropolone structure for the mould metabolite stipitatic acid. This compound, together with its hydroxy derivative, puberulic acid, became good friends and in due course we synthesized stipitatic acid. After a few years, I left I.C.I. and joined Professor Todd at Cambridge to broaden my horizons of natural product chemistry.

Soon after the important recognition of the effectiveness of liver therapy for the treatment of pernicious anaemia, several research groups carried out programmes on the fractionation of liver extracts aimed at the isolation of the active principle. Combined chemical and clinical, and later microbiological, studies led to the isolation of a red crystalline compound named vitamin B_{12} which possessed high biological activity. The isolation was reported by two groups almost simultaneously: by Karl Folkers and his team at Merck[1,2] and also by E. Lester Smith and his colleagues at Glaxo.[3] A collaboration of groups at Cambridge and Glaxo was established and I was very pleased to be asked to head the small team of Cambridge research students to work on the degradation of the vitamin

*The present text is based upon the lecture delivered on the 11th April, 1980 at the Annual Chemical Congress of the Chemical Society in Durham.

[1] E. L. Rickes, N. G. Brink, F. R. Koniuszy, T. R. Wood, and K. Folkers, *Science*, 1948, **107**, 396.
[2] K. Folkers, 'Vitamin B_{12}. Proceedings of the 3rd European Symposium', W. de Gruyter, Berlin, New York, 1979, p. 7.
[3] E. Lester Smith, *Nature*, 1948, **161**, 638.

with a view to the determination of its structure. Meanwhile Dr. Karl Folkers and his collaborators were carrying out a similar exercise and for the first two or three years there was intense rivalry between the two schools which led to a succession of exciting results as the nature of various hydrolytic fragments was established. These included D-(*R*)-1-aminopropan-2-ol, cyanocobalt(III), and α-ribazole-3′-phosphate [(1), along with the isomeric 2′-phosphate], a phosphori-



(1)

boside of 5,6-dimethylbenziminazole. Compounds related to vitamin B_{12} were isolated later which were shown to contain a range of alternative bases[4] such as 5-hydroxybenziminazole, adenine, and other purine bases. The major part of the B_{12} molecule was obtained as an intractable acidic red gum.[5] Use of column electrophoresis served to emphasize the complexity of this gum, the major hydrolytic product,[6] but persistence prevailed, and eventually Dr. Cannon managed to produce a crystalline hexacarboxylic acid.[7] Through a most fruitful collaboration with Dr. Dorothy Hodgkin, a suitable crystal of the acid was subjected to *X*-ray analysis and very soon, in the structure (2),[8,9] the novel corrin chromophore was seen for the first time.

The crystallographic work was quickly extended to the vitamin itself and in a short time its structure too was determined (3),[8,9] a triumph for Dorothy and a result which received special mention in her Nobel Prize citation. The extensive *X*-ray studies in the B_{12} series were described later in full detail in a series of

[4] W. Friedrich, 'Vitamin B_{12} und verwandte Corrinoide', Band III/2 of 'Fermente. Hormone. Vitamine.' Georg Thieme. Stuttgart, 1975, p. 24.

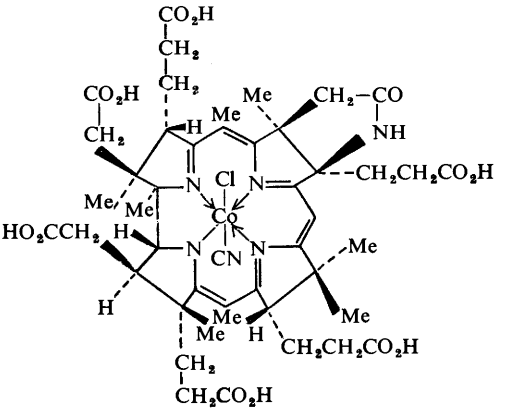
[5] A. W. Johnson and Sir Alexander Todd, 'Vitamins and Hormones', Academic Press, New York, 1957, Vol. 15, p. 1.

[6] J. B. Armitage, J. R. Cannon, A. W. Johnson, L. F. J. Parker, E. Lester Smith, W. H. Stafford, and A. R. Todd, *J. Chem. Soc.*, 1953, 3849.

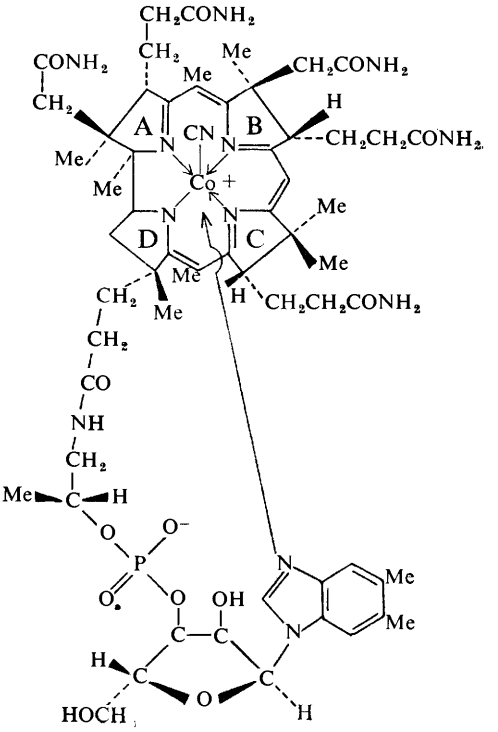
[7] J. R. Cannon, A. W. Johnson, and A. R. Todd, *Nature*, 1954, **174**, 1166.

[8] D. C. Hodgkin, J. Pickworth, J. H. Robertson, K. N. Trueblood, R. J. Prosen, and J. G. White, *Nature*, 1955, **176**, 325.

[9] D. C. Hodgkin, A. W. Johnson, and A. R. Todd, in 'Recent Work on Naturally Occurring Nitrogen Heterocyclic Compounds', ed. K. Schofield, Special Publication No. 3, The Chemical Society, London, 1955, p. 109.

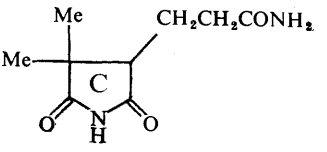


(2)

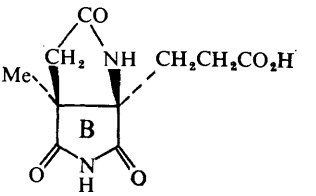


(3)

papers.[10–16] Meanwhile the chemists had managed to produce a little more supporting structural information by the isolation of (4) from ring C by oxidation of B_{12},[17] and (5) from ring B by oxidation of the hexacarboxylic acid.[18] A larger

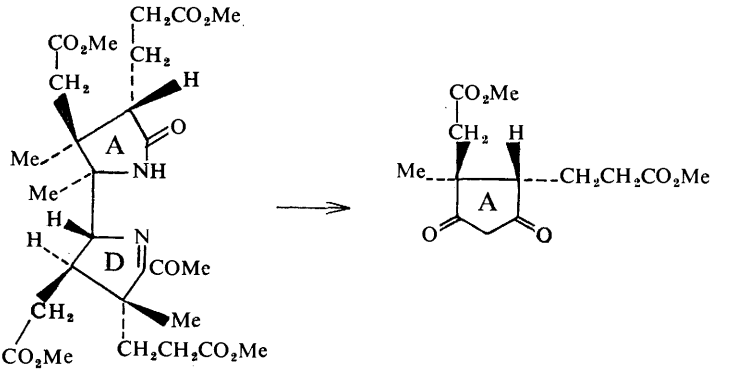


(4)



(5)

fragment (6) derived from rings A and D of methyl cobyrinate (cobyrinic acid, when it was first isolated[6] was referred to as the heptacarboxylic acid) by ozon-

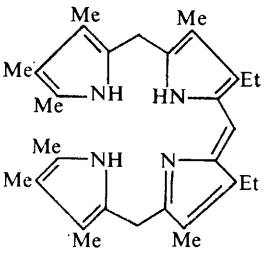


(6)

[10] D. C. Hodgkin, J. Pickworth, J. H. Robertson, R. J. Prosen, R. A. Sparks, and K. N. Trueblood, *Proc. Roy. Soc. A*, 1959, **251**, 306.
[11] D. C. Hodgkin, M. J. Kamper, J. Lindsey, M. MacKay, J. Pickworth, J. H. Robertson, C. B. Shoemaker, J. G. White, R. J. Prosen, and K. N. Trueblood, *Proc. Royal Soc. A*, 1957, **242**, 228.
[12] D. C. Hodgkin, *Fortsch. Chem.org. Naturstoffe*, 1958, **15**, 167.
[13] J. G. White, *Proc. Roy. Soc. A*, 1962, **266**, 440.
[14] D. C. Hodgkin, J. Lindsey, M. MacKay, and K. N. Trueblood, *Proc. Roy. Soc. A*, 1962, **266**, 475.
[15] D. C. Hodgkin, J. Lindsey, R. A. Sparks, K. N. Trueblood, and J. G. White, *Proc. Roy. Soc. A*, 1962, **266**, 494.
[16] D. C. Hodgkin, ref. 2, p. 19.
[17] A. Kuehl, C. H. Shunk, M. Moore, and K. Folkers, *J. Amer. Chem. Soc.*, 1952, **74**, 4521.
[18] V. M. Clark, A. W. Johnson, I. O. Sutherland, and Sir Alexander Todd, *J. Chem. Soc.*, 1958, 3283.

olysis has been described more recently.[19] Syntheses of α-ribazole,[20–22] were also reported.

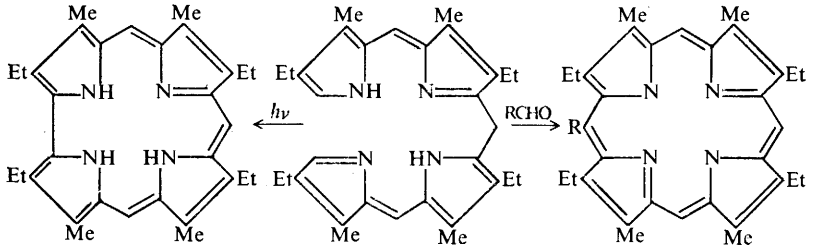
With the structure of vitamin B_{12} established securely, several synthetic programmes were initiated which were aimed firstly at the corrin chromophore and then at B_{12} itself. At Cambridge, our first objectives centred round an assessment of pyrroline chemistry and the possibility of linking two such rings directly and through a carbon atom. Although these problems were solved eventually,[23–25] the difficulty of adding further rings proved formidable and the approach, in due course, was abandoned. During the early phases of this work, I moved from Cambridge to Nottingham and there initiated a new approach in which we prepared tetradehydrocorrins containing one and two angular-methyl groups, as well as the aromatic corrole [e.g., (8)].[26] It has been of special interest that the linear tetrapyrrolic compounds, bilenes-*b* [*e.g.*, (7)] and biladienes-*ac*



(7)

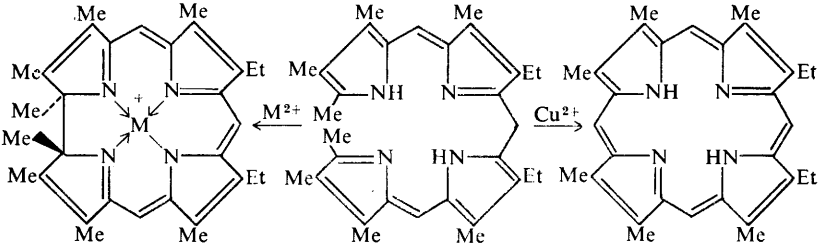
[*e.g.*, (9)—(11)], selected as starting products for these syntheses, could also be converted into porphyrins by slightly modified methods (Schemes 1—3), which have since been used widely for the synthesis of porphyrins of all types.[33]

[19] M. Imfeld, D. Arigoni, R. Deeg, and G. Müller, ref. 2, p. 315.
[20] F. W. Holly, C. H. Shunk, E. W. Peel, J. J. Cahill, J. B. Lavigne, and K. Folkers, *J. Amer. Chem. Soc.*, 1952, **74**, 4521.
[21] A. W. Johnson, G. W. Miller, J. A. Mills, and A. R. Todd, *J. Chem. Soc.*, 1953, 3061.
[22] R. B. Woodward, ref. 2, p. 37.
[23] R. F. C. Brown, V. M. Clark, I. O. Sutherland, and Sir Alexander Todd, *J. Chem. Soc.*, 1959, 2109.
[24] R. F. C. Brown, V. M. Clark, M. Lamchen, and Sir Alexander Todd, *J. Chem. Soc.*, 1959 2116.
[25] V. M. Clark, B. Sklarz, and Sir Alexander Todd, *J. Chem. Soc.*, 1959, 2123.
[26] A. W. Johnson in 'Porphyrins and Metalloporphyrins', ed. K. W. Smith, Elsevier Scientific Publishing Co., Amsterdam, 1975, p. 729.
[27] A. W. Johnson and I. T. Kay, *J. Chem. Soc.*, 1965, 1620.
[28] D. Harris and A. W. Johnson, *J.C.S. Chem. Comm.*, 1977, 771.
[29] D. Dolphin, R. L. N. Harris, J. L. Huppatz, A. W. Johnson, and I. T. Kay, *J. Chem. Soc.* (*C*), 1966, 30.
[30] A. W. Johnson and I. T Kay, *J. Chem. Soc.*, 1961, 2418.
[31] A. W. Johnson in 'The Porphyrins', ed. D. Dolphin, Academic Press, New York, 1978, Vol. IA, p. 235.
[32] D. A. Clarke, R. Grigg, R. L. N. Harris, A. W. Johnson, I. T. Kay, and K. W. Shelton, *J. Chem. Soc.* (*C*), 1967, 1648.
[33] R. L. N. Harris, A. W. Johnson, and I. T. Kay, *J. Chem. Soc.* (*C*), 1966, 22.



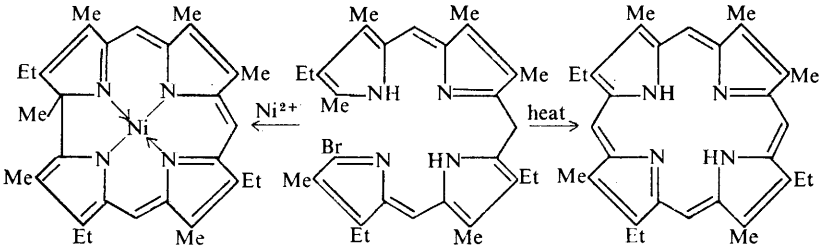
(8) (ref. 26) (9) (ref. 27)

Scheme 1 *Synthesis of corroles*



(ref. 28) (10) (refs. 29, 30)

Scheme 2 *Synthesis of metal* 1,19-*dimethyltetradehydrocorrins*



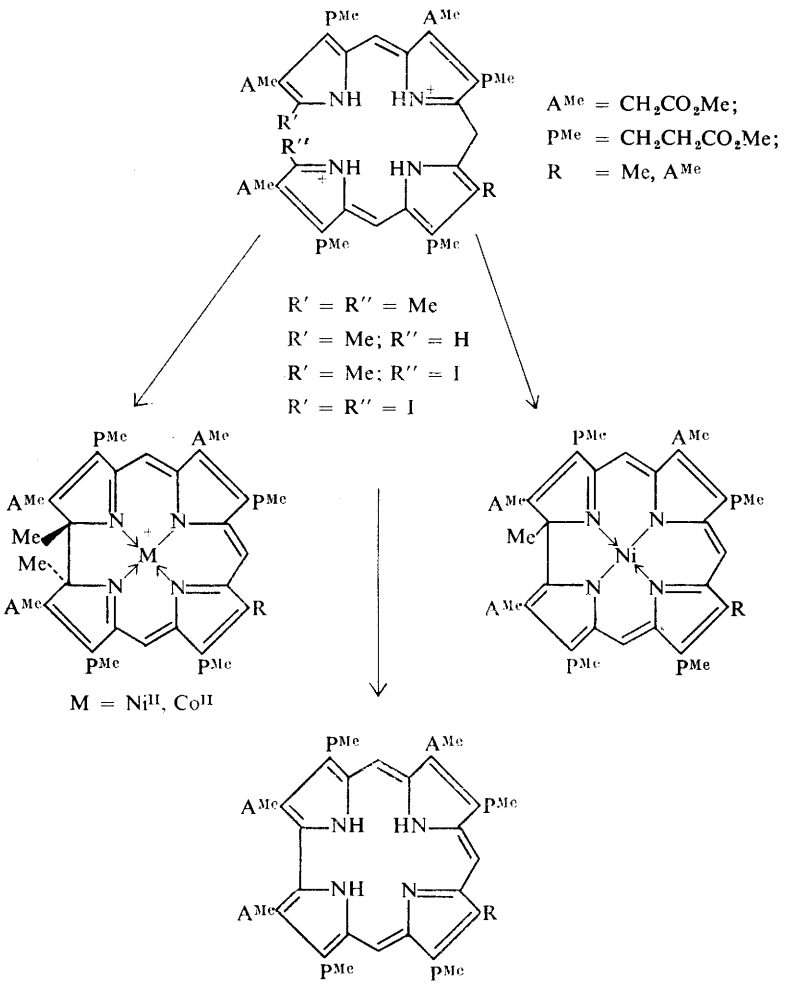
(ref. 31) (11) (ref. 32)

Scheme 3 *Synthesis of nickel* 1-*methyltetradehydrocorrins*

Thus the skeleton of the B_{12} chromophore can be constructed in a relatively simple manner, and moreover, in view of the biosynthetic connection between uroporphyrinogen III (12) and B_{12}[34,35] (see below) the syntheses were extended (in collaboration with Professor Gossauer) to tetradehydrocorrins carrying the uroporphyrin III and 12-decarboxyuroporphyrin III substitution patterns

[34] A. R. Battersby, ref. 2, p. 217.
[35] A. I. Scott, ref. 2, p. 247.

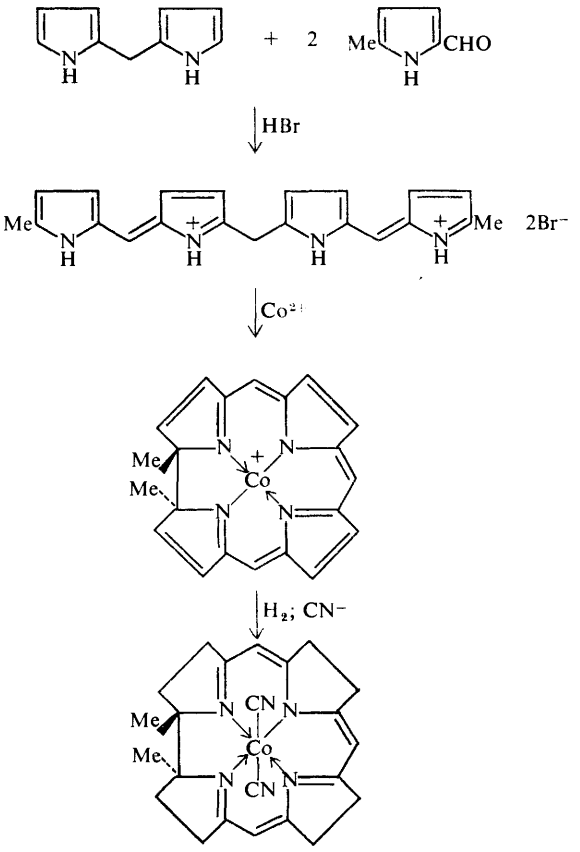
(Scheme 4).[36] The conversions of the tetrahydrocorrins to corrins involve additions to the $\beta\beta$-bonds of the pyrrolic rings. So far this has been accomplished by



Scheme 4 *Synthesis of tetradehydrocorrins with uroporphyrin* III *and* 12-*decarboxyuroporphyrin* III *substitution patterns*

hydrogenation (Scheme 5 illustrates a three-stage cobalt corrin synthesis)[37] and by hydroxylation[38,39] in the 1,19-disubstituted series, but a good method for the

[36] J. Engel, A. Gossauer, and A. W. Johnson, *J.C.S. Perkin I*, 1978, 871.
[37] A. W. Johnson and W. R. Overend, *J.C.S. Perkin I*, 1972, 2681.
[38] H. H. Inhoffen, J. Ulrich, H. A. Hoffmann, G. Klinzman, and R. Scheu, *Annalen*, 1970, **738**, 1.
[39] H. H. Inhoffen, F. Fattinger, and N. Schwarz, *Annalen*, 1974, 412.



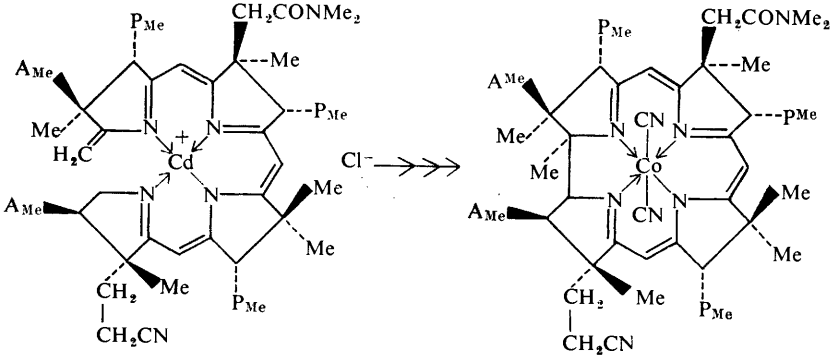
Scheme 5 *Three-stage corrin synthesis*

addition of the 'extra' β-methyl groups in each of the five-membered rings is still being sought. Introduction of angular-(1-, above) and meso-(5- and 15-) methyl groups[29] can be accomplished by adaptation of the macrocylic ring design. It should be noted that angular ester groups can be removed readily by hydrolysis and decarboxylation, or by reduction and retro-aldol elimination,[40] *i.e.* the stabilized 1-methyl-19-ethoxycarbonyl compounds act as 'protected' 1-methyl derivatives.

During the sixties and seventies, R. B. Woodward[22,41] and A. Eschenmoser,[42,43] ably supported by large research teams at Harvard and Zurich, evolved

[40] D. P. Arnold and A. W. Johnson, *J.C.S. Chem. Comm.*, 1977, 787.
[41] R. B. Woodward, *Pure Appl. Chem.*, 1968, **17**, 519; 1971, **25**, 283; 1973, **33**, 145.
[42] A. Eschenmoser, *Naturwiss.*, 1974, **61**, 513; *Chem. Soc. Rev.*, 1976, **5**, 377; *Quart. Rev.* 1970, **24**, 366.
[43] A. Eschenmoser, ref. 2, p. 89.

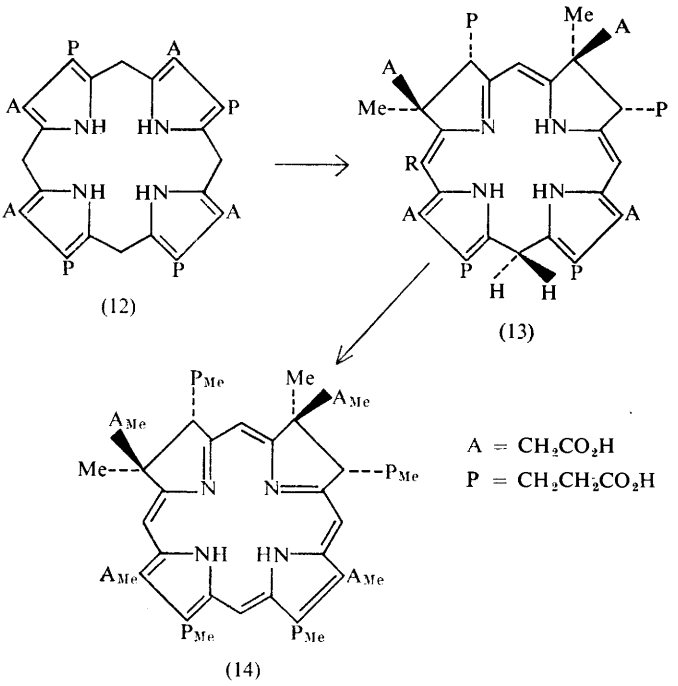
synthetic schemes of extreme elegance which culminated in a total synthesis of the vitamin. This, the largest synthetic project ever undertaken in the history of organic chemistry, was marked by the adoption of the B_{12} skeleton as the symbol of the 1968 I.U.P.A.C. London Conference on the Chemistry of Natural Products. More recently, marked progress of another synthetic scheme has been reported.[44] All of these syntheses rightly paid full attention to stereochemical detail from the outset, using suitable terpenes or other optically active compounds as precursors, and as a substantial bonus, detailed consideration of one step of the Harvard synthesis led to the conception of the Woodward-Hoffmann rules. We at Nottingham were of course gratified that the original schemes proposed at Harvard and Zurich, whereby the macrocycle was constructed in the final stage by linking rings A and B, were later extended to effect cyclization by photochemical cyclization involving rings A and D (*e.g.*, Scheme 6).



Scheme 6

Meanwhile research on the biosynthesis of haemin, and in particular the mode of biological construction of the porphyrin ring with a III-type arrangement of substituents, was extended to considerations of the biosynthesis of B_{12}. Several laboratories, *e.g.* those of Battersby,[34] Scott,[35] and Müller,[45] established that uroporphyrinogen III was a biosynthetic intermediate for B_{12} as well as for haem, and the conversion of uroporphyrinogen III to cobyrinic acid (and thence to B_{12}), which requires the introduction of seven methyl groups and a ring contraction, is currently an area of intensive study. So far it has been established (Scheme 7) that uroporphyrinogen III (12) is first methylated in ring A [46] and then in ring B to give, presumably, (13; R = H), isolated as the so-called sirohydrochlorin ester (14; R = H). Sirohydrochlorin is also the metal-free derivative of the prosthetic group of sulphite reductase.[47] The third methylation in the B_{12}

[44] R. V. Stevens, ref. 2, p. 119.
[45] G. Müller, R. Deeg, K. D. Gneuss, G. Gunzer, and H. P. Kriemler, ref. 2, p. 279.
[46] M. Imfeld, D. Arigoni, R. Deeg, and G. Müller, ref. 2, p. 315.
[47] L. M. Siegel, M. J. Murphy, and H. Kamin, *J. Biol. Chem.*, 1973, **248**, 251; M. J. Murphy and L. M. Siegel, *ibid.*, p. 6911; M. J. Murphy, L. M. Siegel, H. Kamin, and D. Rosenthal, *ibid.*, p. 2801.



Scheme 7 *Early stages in the biosynthetic conversion of uroporphyrinogen* III *to cobyrinic ester*

biosynthesis occurs at C-20, probably still in the oxidation state (13; R = Me) although again isolated as the ester (14; R = Me). This unexpected point of methylation (both the C-20 carbon and the attached methyl group need to be expelled at a later stage) is still of uncertain significance (dehydrogenation to C-20 exocyclic methylene?) and the precise stage at which ring contraction occurs is as yet unknown.

Other problems involved in the late stages of the biosynthesis, *e.g.* the origins of the D-1-aminopropan-2-ol, 5,6,-dimethylbenziminazole[48] and its nucleotide loop,[49] the introduction of cobalt, and the order of amidation of the side-chains have all been examined to an extent, but much more work is required before the full biosynthetic pathway is established.

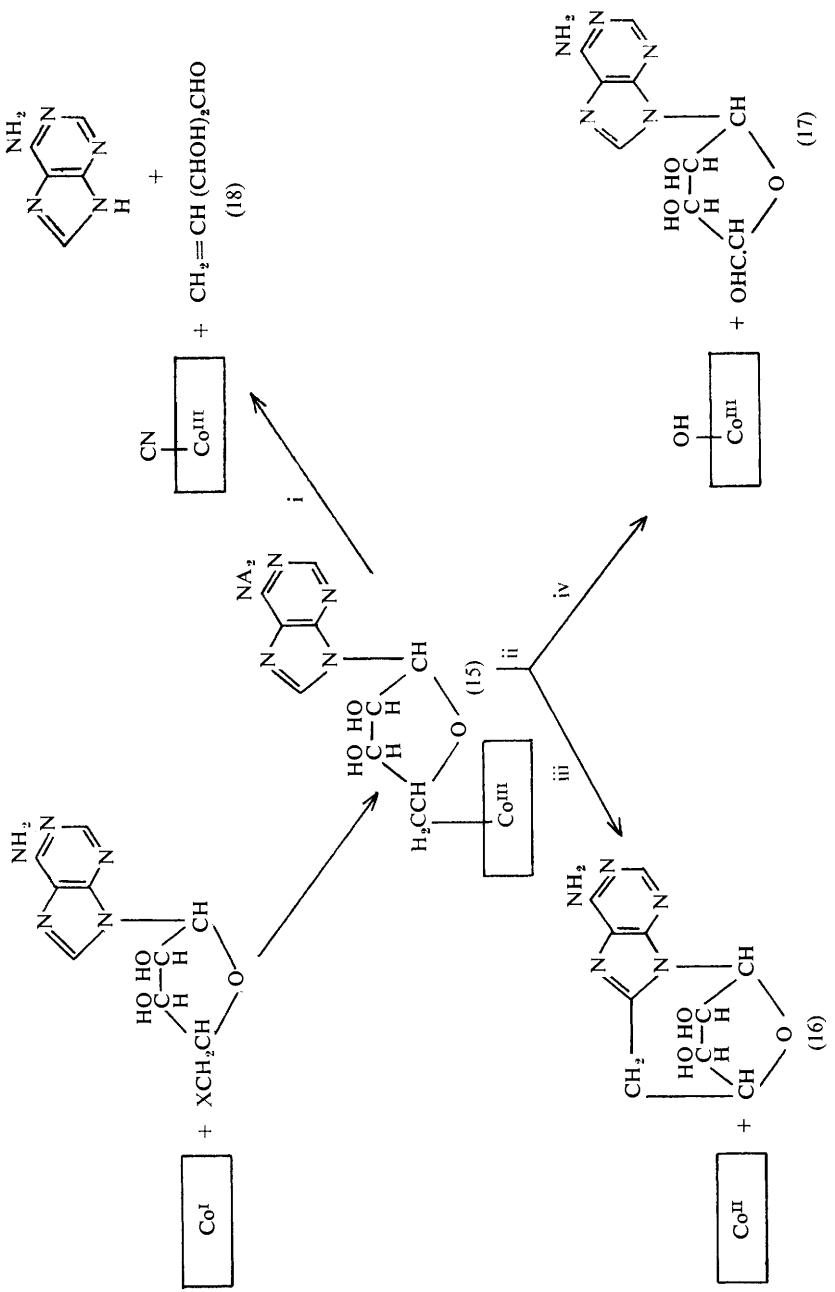
In 1960 the remarkable observation was made by Barker[50] that vitamin B_{12}, cyanocobalamin, is an artefact and that the naturally occurring B_{12} derivative is the light-sensitive co-enzyme form, adenosylcobalamin. This structure was

[48] P. Renz, J. Horig, and R. Wurm, ref. 2, p. 317.
[49] H. C. Friedmann, ref. 2, p. 331.
[50] H. A. Barker, 'Vitamin B_{12} and Intrinsic Factor,' ed H. C. Heinrich, F. Enke Verlag, Stuttgart, 1962, p. 82.

determined by Lenhert and Hodgkin[51] and a synthesis of the cobalt–alkyl structure [partial formula, (15)] of the co-enzyme and a range of other alkylcobalamins was discovered whereby B_{12s}, the cobalt(I) complex, was treated with[52,53] 5′-*p*-toluenesulphonate or a 5′-halogeno derivative of adenosine (Scheme 8). Early experiments[54] on the properties of the co-enzyme showed that the action of light on the co-enzyme caused the homolytic fission of the cobalt—carbon bond; under anaerobic conditions there were formed the cobalt(II) complex B_{12r}, together with the adenosyl radical which cyclized rapidly to (16). We have since[55] demonstrated unequivocally the existence of the adenosyl radical from aerobic photolyses of the co-enzyme by spin-trapping experiments using nitroso compounds. In the presence of air, hydroxocobalamin B_{12b}, and 5′-aldehyde (17) (or acid) of adenosine result from photolytic decomposition of the co-enzyme. Cyanide ions, as in the early B_{12} preparations, gave cyanocobalamin together with the unsaturated sugar (18) (Scheme 8).

The co-enzyme synthesis was used for the preparation of a range of alkylcobalamins including the important naturally occurring methylcobalamin. Detailed studies of the chemistry of these light-sensitive derivatives soon followed which in turn led to studies of a large number of other octahedral cobalt(III)–alkyl complexes, all so-called 'B_{12} models' of which the cobaloximes of Schrauzer[56] are the best known. However, it cannot be overstressed that the mechanism of the reactions of these compounds, albeit of great interest as organometallics, cannot be assumed to be identical with the mechanism of the reactions of the B_{12} enzyme-controlled reactions, and many unnecessary polemics could have been avoided with an appreciation of this obvious point. The B_{12}-containing enzymes use either methylcobalamin or adenosylcobalamin as the co-enzymes. The former is involved in a range of biological methylation reactions including certain routes to methionine and biomethylation of a range of toxic elements, such as mercury, arsenic, thallium, tin, and lead, which lead to widespread environmental problems.[57] Enzymes containing the co-enzyme adenosylcobalamin are involved in about ten rearrangement reactions and the studies of the detailed mechanism of these are very interesting but unfinished stories. For our part we have selected one of these, ethanolamine ammonia-lyase, originally because of ease of preparation and for simplicity of structure. The enzyme was described originally by Kaplan and Stadtman[58] and it has been subjected to detailed study by Babior[59] whom it is a pleasure to thank for cordial exchange of

[51] P. G. Lenhert and D. C. Hodgkin, ref. 50, p. 105; *Nature*, 1961, **192**, 937.
[52] E. Lester Smith, L. Mervyn, A. W. Johnson, and N. Shaw, *Nature*, 1962, **194**, 1175; *J. Chem. Soc.*, 1963, 4146.
[53] K. Bernhauer, O. Müller, and G. Müller, *Biochem. Z.*, 1962, **336**, 102.
[54] D. Dolphin, A. W. Johnson, and R. Rodrigo, *Annals New York Acad. Sci.*, 1964, **112**, 590; A. W. Johnson and N. Shaw, *J. Chem. Soc.*, 1962, 4608.
[55] K. N. Joblin, A. W. Johnson, M. F. Lappert, and B. K. Nicholson, *J.C.S. Chem. Comm.*, 1975, 441.
[56] G. N. Schrauzer, *Angew. Chem., Internat. Edn.*, 1976, **15**, 417.
[57] J. M. Wood and Y. T. Fanchiang, ref. 2, p. 539.
[58] B. H. Kaplan and E. R. Stadtman, *J. Biol. Chem.*, 1968, **243**, 1787, 1794.
[59] B. M. Babior, *Acc. Chem. Res.*, 1975, **8**, 376; ref 2. pp. 461, 485.

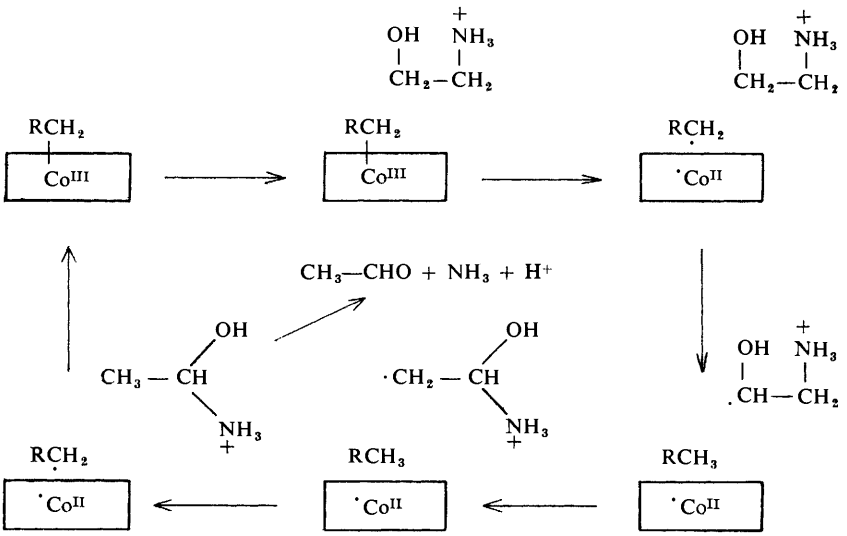


Reagents: i, CN^-; ii, *hν*; iii, anaerobic; iv aerobic; $\boxed{Co^{III}}$ ≡ cobalamin

Scheme 8 *Reactions of B_{12} co-enzyme*

information. Ethanolamine ammonia-lyase catalyses the formation of acetaldehyde and ammonia from the natural substrate 2-aminoethanol, and propionaldehyde and ammonia from 2-aminopropanol (2*S* or 2*R*), an unnatural substrate, in reactions which involve migration of an amino group between adjacent substrate carbon atoms. The views of most research groups, including ours, on the mechanism of the ethanolamine rearrangement are summarized in Scheme 9,[60] and similar overall mechanisms can be written for all of the other rearrangements. However, it is possible that the migrating group could be anionic or cationic in nature and that the mechanism of the migration step need not necessarily be the same in all cases. The ribonucleotide-reductase system additionally involves a redox system.

It is implicit in Scheme 9 that the approach of the substrate causes homolytic



Scheme 9 *Mechanistic scheme for the rearrangement of 2-aminoethanol catalysed by ethanolamine ammonia-lyase*

fission. Thus the adenosyl radical is intermediate both in the natural enzyme-induced reactions as well as in the photolyses of the B_{12} enzymes and co-enzyme. Several workers have therefore examined the possibility of effecting rearrangement of the B_{12}-enzyme substrates by mixing them with co-enzyme or other cobalt–carbon containing compounds followed by irradiation. We found[61] that adenosylcobalamin is essentially inert under conditions of anaerobic irradiation

[60] R. H. Abeles, ref 2, p. 373. R. H. Abeles and D. Dolphin, *Acc. Chem. Res.*, 1976, **9**, 114.
[61] A. J. Hartshorn, A. W. Johnson, S. M. Kennedy, M. F. Lappert, and J. J. MacQuitty, *J.C.S. Chem. Comm.*, 1978, 643.

at 30 °C towards either 2-aminoethanol or ethylene glycol because of the more facile intramolecular cyclization to 8,5′-cyclo-5′-deoxyadenosine (Scheme 8).

When, however, the irradiation experiment with adenosylcobalamin and ethylene glycol was carried out in the presence of a thiol, dihydrolipoic acid amide, 30 % of acetaldehyde was obtained[62] and indeed there is evidence to suggest that sulphur-containing intermediates may be involved in B_{12}-mediated rearrangement reactions. Photolyses of 8-methoxy-5′-deoxyadenosylcobalamin, methylcobalamin, or methyl(aquo)cobaloxime in the presence of ethylene glycol or 2-aminoethanol caused the formation of acetaldehyde,[61] and the pH dependence of the yield paralleled that quoted for hydroxy-radical-induced reactions.

It should be stressed that as yet there is no experimental evidence in favour of the direct attachment of either substrate or product to cobalt in the B_{12}-mediated rearrangement reactions. Indeed a theoretical analysis of e.s.r. spectra obtained in the course of several such rearrangements suggests that the substrate and product radicals are separated from the cobalt atom by at least 10 Å in all cases,[63] although this does not preclude the existence of other intermediate electron-carriers, a possible role for sulphur-containing metabolites, as in the case of ribonucleotide reductase. Several authors have shown that rearrangements of the same types as those catalysed by the B_{12} co-enzymes are possible by irradiation of compounds where the 'substrate' is attached directly to cobalt in cobaloxime or cobalamin complexes. Examples are illustrated in Scheme 10.

The features of the B_{12}-controlled ethanolamine rearrangement which involve changes in the immediate environment of the cobalt atom as shown in Scheme 9, make the metal an effective probe for mechanistic studies, *e.g.* e.s.r. experiments, which indicated that the paramagnetic cobalt(II) species is formed at a kinetically competent rate, after mixing enzyme with co-enzyme and (2*S*)-2-aminopropanol.[69] Variation of the cobalt valency state also gives rise to marked changes in the electronic spectra and we, in a collaboration with Dr. M. R. Hollaway, have used stopped-flow rapid wavelength scanning spectrophotometry[70] in an investigation of the mechanism of the B_{12}-controlled rearrangement of 2-aminoethanol. The method involves mixing enzyme and substrate solutions in about 3 ms and then recording spectra over the 345—570 nm range at the rate of 800 spectra per second, each spectrum taking 1 ms with a 0.25 ms gap between spectra. The data-capture system used enabled the storage of up to 32 spectra at preselected times during the reaction or, alternatively, 64 spectra could be

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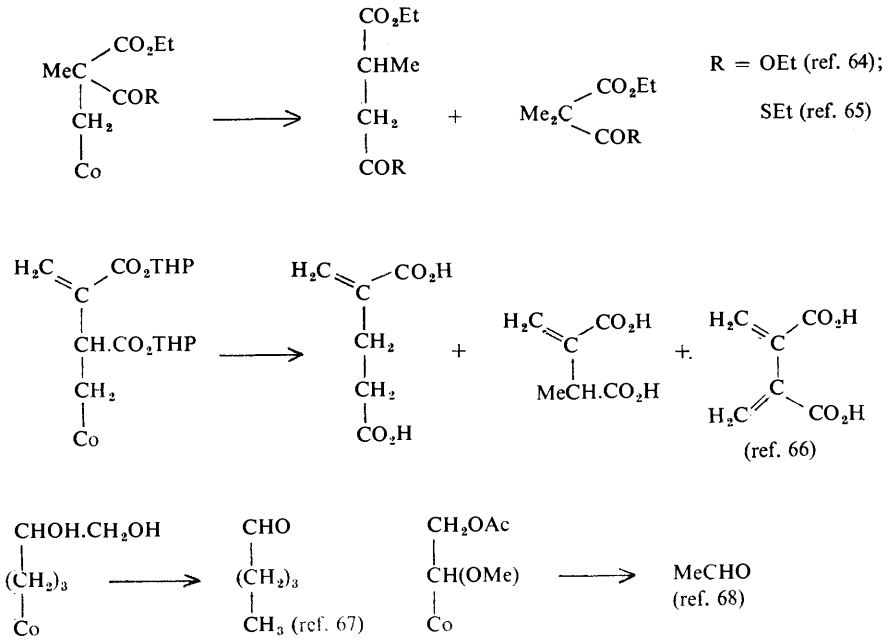


Scheme 10 *Rearrangements of cobalt-ligands after irradiation*

summed in a single register. The simplest expression of the rearrangement reaction is in terms of a three-step mechanism (Scheme 11) involving binding of the substrate (k_{+1} step), cob(II)alamin formation (k_{+2} step), and cob(II)alamin breakdown (k_{+3} step). The representation E*.Co^{II}.X, however, probably corresponds to at least three sequential intermediates which are not resolved by the present method.

$$E + Co^{III} \rightleftharpoons E^*.Co^{III} + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} E^*.Co^{III}.S \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}}$$

$$E^*.Co^{II}.X \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} E^*.Co^{III} + P$$

Reactants: Co^{III}, adenosylcobalamin; Co^{II}, cob(II)alamin; S, substrate; P, product; E, enzyme; E*.Co^{II}.X, the sum of all intermediates containing Co^{II}

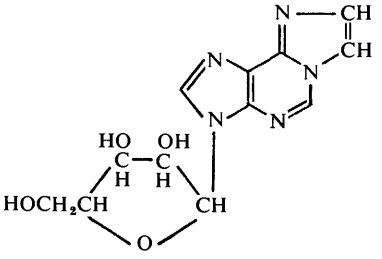
Scheme 11 *Kinetic model*

It was found that the binding of adenosylcobalamin to the enzyme protein was followed by a slow (on the time scale of subsequent catalytic steps) change in the conformation of the enzyme molecule (no spectral change) to give a catalytically active species. Once this had been formed, the first order of rate constants for the formation of the cob(II)alamin were 90 s^{-1} for (2*S*)-2-aminopropanol, and > 300 s^{-1} for 2-aminoethanol. In a typical experiment with (2*S*)-2-aminopropanol, the duration of the steady-state phase of the reaction was about 60 s (depending on the relative amounts of substrate and enzyme) corres-

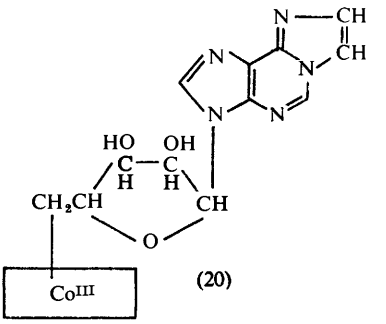
ponding to a catalytic-centre activity (k_{cat}) of 1–2 s^{-1}. For 2-aminoethanol the lifetime of the intermediate was about 12.5 s and the k_{cat} value about 170 s^{-1}. Cob(II)alamin thus fulfils the requirements of a true intermediate on the reaction pathway: it is formed in a process with a rate constant (> 300 s^{-1}) greater than the overall k_{cat} value (170 s^{-1}) and it has a lifetime consistent with that calculated from the k_{cat} value. The difference between the substrates 2-aminoethanol and (2*S*)-2-aminopropanol is expressed mainly in the k_{+3} step (240 s^{-1} and about 1.5 s^{-1} respectively) the corresponding k_{+2} values being 336 s^{-1} and 90 s^{-1}. The difference between the respective k_2 and k_3 values thus suggest that the enzyme discrimination between the substrates is at a relatively late stage in the catalytic process.

Work on the protein of ethanolamine ammonia-lyase showed[58] that it had M_r approximately 520 000. We have shown[71] that it contains two sub-units in equimolecular proportions, six of each type, *i.e.* I_6II_6, per molecule of enzyme. These sub-units, M_r 51 000 and 36 000 respectively, can be separated using sodium dodecyl sulphate-acrylamide gel electrophoresis.[71] A novel kinetic study of the ethanolamine ammonia-lyase catalysed rearrangement of (2*S*)-2-aminopropanol, under conditions of high adenosylcobalamin concentration so that full saturation of the enzyme with co-enzyme was achieved, has led to the conclusion that there are six functional active-sites per molecule of enzyme, thus correlating with the sub-unit structure, and that all the active-sites function at the same rate.[72]

Finally, work has been reported from several schools on the incorporation of modified adenosines and adenosyl linkages in the co-enzyme with a view to establishing correlations between structure and biological activity in the various enzyme systems, for example diol dehydrase[73] and ribonucleotide reductase.[74] For our part, we have examined the effect of substituting the cycloadenosine (19)[75] for adenosine in the co-enzyme, *i.e.* use of the analogue (20), and we hope thereby to utilise fluorescent spectra as an additional diagnostic tool.



(19)



(20)

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I must express my deep debt of gratitude to my many collaborators over a period of nearly a third of a century. I have been able to present only a fraction of their results but I hope my story will have sufficed to emphasize their skills and their enthusiasm for this most remarkable molecule, vitamin B_{12}.