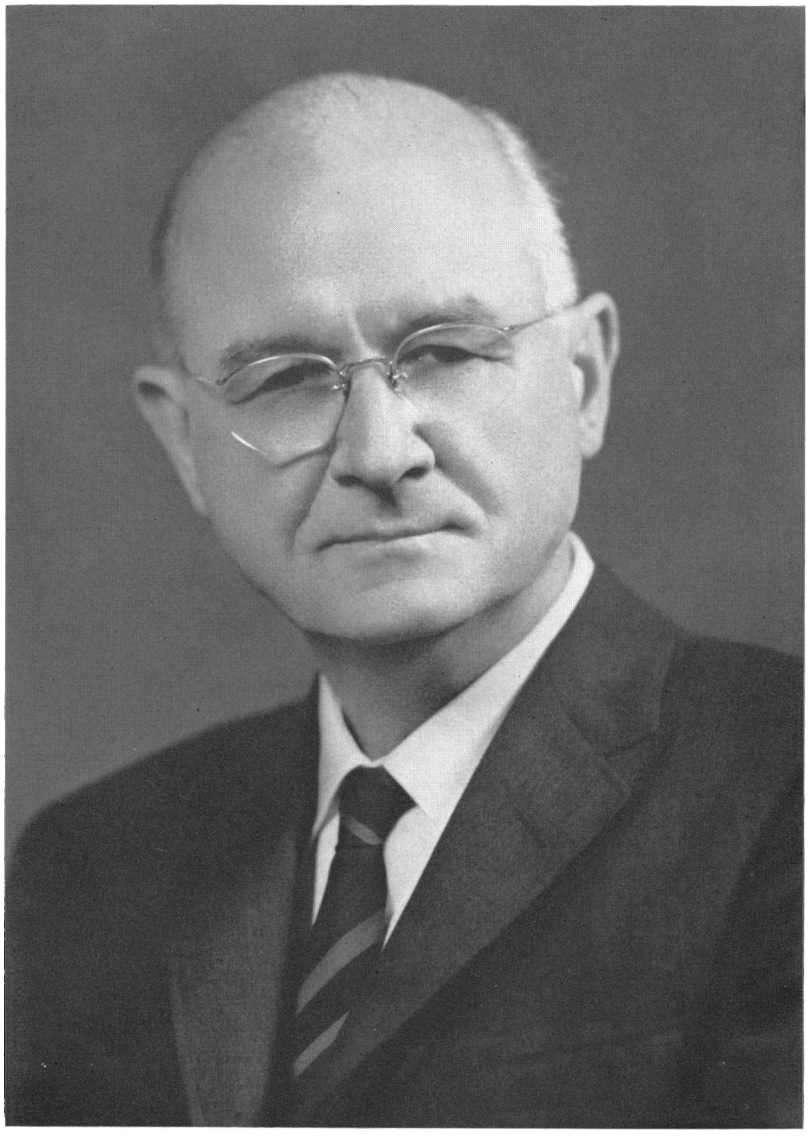

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Biochemical Functions of Corrinoid Compounds

THE SIXTH HOPKINS MEMORIAL LECTURE

BY H. A. BARKER*

Delivered on 13 *April* 1967 *in University College London, Gower Street, London, W.C.* 1

The invitation to give the Hopkins Memorial Lecture is an honour that I greatly appreciate. I expect that I am the first Lecturer in this series who has not had the privilege of knowing Professor Hopkins personally or working in the Cambridge Biochemical Laboratory. But I can claim a sort of second-generation connexion with the Laboratory through Professor J. Murray Luck of Stanford University. My first contact with biochemistry came from Professor Luck in 1929, shortly after he had obtained his Doctorate at Cambridge. He brought to the Far West some of the attitudes and flavour of the Cambridge Laboratory.

My interest in corrinoid compounds developed from studies on the fermentation of glutamic acid by *Clostridium tetanomorphum* (Barker, 1961*a*). Experiments with ^{14}C-labelled glutamate had shown that this organism does not use the classical tricarboxylic acid-cycle reactions in the fermentation (Wachsman & Barker, 1955). Studies with cell-free extracts led to the identification of mesaconic acid, a branched-chain C_5 dicarboxylic acid, as an early intermediate in glutamate breakdown (Wachsman, 1956). Mesaconic acid was found to be formed by deamination of β-methylaspartic acid, which has the same carbon 'skeleton' (Barker, Smyth, Wawszkiewicz, Lee & Wilson, 1958; Barker, Smyth, Wilson & Weissbach, 1959). Attention was then focused on the reversible conversion of glutamate into β-methylaspartate. This rearrangement of the carbon chain of glutamate was found to be dependent on a heat-stable cofactor. This cofactor, which was isolated after much effort and considerable frustration, turned out to be a bright-orange compound that could be readily inactivated and converted into pseudovitamin B_{12} by treatment with cyanide or by exposure to light (Barker, Weissbach & Smyth, 1958; Barker *et al.* 1960*a*). A little later the analogous coenzyme form of vitamin B_{12} was isolated in crystalline form from *C. tetanomorphum* and propionic acid bacteria (Weissbach, Toohey & Barker, 1959; Barker, 1960*b*). In retrospect, the initial obstacle to isolation of the coenzyme form of pseudovitamin B_{12} was its unsuspected sensitivity to light. Once this was recognized, the purification of the coenzyme presented no unusual difficulties.

Examination of the products formed by the conversion of the coenzymes into the corresponding vitamins by the action of light showed the presence of an 'extra' mole of adenine in two nucleosides closely related to but not identical with adenosine (Weissbach, Ladd, Volcani, Smyth & Barker, 1960). The identity of the precursor of these products and its mode of attachment to the vitamin were soon determined by Lenhert & Hodgkin (1961) by X-ray diffraction analysis of the crystalline coenzyme. They showed that vitamin B_{12} coenzyme contains a 5′-deoxyadenosyl moiety attached to the vitamin by a bond between the cobalt and the 5′-methylene carbon atom. The deoxyadenosyl group occupies the same co-ordinate position on the cobalt as the cyanide group in cyanocobalamin, the commercial form of vitamin B_{12}.

The discovery of the novel Co–C bond led to a search for a method of synthesizing the coenzymes and analogous cobalt alkyl corrinoids from the vitamins. A simple synthetic method was developed by Lester Smith and his associates (Smith, Mervyn, Johnson & Shaw, 1962; Johnson, Mervyn, Shaw & Smith, 1963) and independently by Bernhauer, Müller & Müller (1962). This synthesis involves the reduction of the tervalent cobalt of vitamin B_{12} through the bivalent state (vitamin B_{12r}) to the univalent state (vitamin B_{12s}), which shows nucleophilic properties. Vitamin B_{12s} is a strong reducing agent that reacts with water at an appreciable rate to form hydrogen gas and vitamin B_{12r}. It also reacts rapidly with alkylating agents to form cobalt alkyl compounds. Cobalt methylcobalamin is formed by reaction with methyl bromide, and other alkyl or acyl corrinoids can be readily synthesized in the same way. Deoxyadenosylcobalamin is prepared by first allowing vitamin B_{12s} to react with the 5′-toluene-*p*-sulphonyl derivative of isopropylideneadenosine, and then removing the protecting isopropylidene group with acid. The purified synthetic coenzyme is identical in every respect with the natural product.

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It is important to emphasize that the chemical synthesis of the coenzyme and other cobalt alkyl corrinoid compounds appears to require the univalent cobalt of vitamin B_{12s}; no similar reaction occurs with the bivalent cobalt of vitamin B_{12r}. We shall see that several enzymic reactions of corrinoid compounds also required reduction of the cobalt, probably to the univalent state, although the evidence for this is not always conclusive.

Enzymic synthesis of deoxyadenosyl corrinoids

Vitamin B_{12} and its analogues, in either the cyano or the hydroxo form, are readily converted into their deoxyadenosyl derivatives by some bacteria and animal tissues (Fenrych, Pawelkiewicz & Magas, 1962). An enzyme catalysing this conversion has been found in *Propionibacterium shermanii* (Brady, Castanera & Barker, 1962) and *C. tetanomorphum* (Peterkofsky & Weissbach, 1964) and has been partially purified. The reaction requires ATP, a thiol or dithiol (Vitols, Walker & Huennekens, 1964; Walker, Murphy, Schmidt & Huennekens, 1967) and a reduced flavine or reduced ferredoxin (Weissbach, Brot & Lovenberg, 1966). ATP serves as the biological alkylating agent; the deoxyadenosyl moiety of ATP is transferred intact to the vitamin to form the coenzyme (Peterkofsky & Weissbach, 1964). The three phosphate groups are released by the clostridial enzyme as inorganic tripolyphosphate. The *P. shermanii* enzyme differs from the clostridial enzyme in this respect; it releases the phosphate groups as inorganic pyrophosphate and orthophosphate, possibly as the result of a secondary cleavage of enzyme-bound tripolyphosphate.

The reducing agents appear to be required, as in the chemical synthesis of deoxyadenosyl corrinoids, to form vitamin B_{12s}. This interpretation is supported by the observation that chemically prepared vitamin B_{12s} readily reacts with ATP in the enzymic system in the absence of other reducing agents, whereas chemically prepared vitamin B_{12r} is inert under the same conditions (Vitols, Walker & Huennekens, 1964, 1966). However, the spectrum of the corrinoid compound in the enzymic system, containing a thiol and a reduced flavine as reductants, shows that most of the cobalt is in the bivalent state. To reconcile these observations it has been postulated that in the presence of excess of reducing agents an equilibrium exists between the reactive vitamin B_{12s} and the unreactive vitamin B_{12_r} that greatly favours the latter (Smith, 1965). As vitamin B_{12s} is adenosylated, it is regenerated from vitamin B_{12r}. Vitols *et al.* (1964) have shown that the reduction of vitamin B_{12} is usually rate-limiting in the enzymic synthesis of deoxyadenosylcobalamin. Walker *et al.* (1967) recently reported that the physiological reducing agent in *C. tetanomorphum* is a small protein, similar to thioredoxin, containing a dithiol grouping. This protein is kept reduced by an NADH-dependent flavoprotein functioning as a reductase.

Observations on the occurrence of corrinoid vitamins and coenzymes in biological materials have established that the deoxyadenosyl derivatives are generally the most abundant corrinoids in bacteria (Volcani, Toohey & Barker, 1961) and mammalian liver (Toohey & Barker, 1961). In most organisms 50–90% of the ethanol-soluble corrinoids are deoxyadenosyl derivatives of complete B_{12} vitamins, although similar derivatives of incomplete vitamins, lacking part or all of the nucleotide side chain, are also commonly present in smaller amounts, as are the hydroxo forms of the vitamins. Cyano corrinoids do not occur in Nature in significant amounts. Cobalt methyl corrinoids have been detected in only very small amounts in biological materials. Lindstrand (1964) found methylcobalamin in *Escherichia coli*, calf liver and human blood plasma (Lindstrand & Stahlberg, 1965), and Irion & Ljungdahl (1965) isolated a methyl corrinoid from a *Clostridium*. A small amount of what appeared to be a carboxymethyl corrinoid was isolated from the latter source. Higher plants apparently do not contain corrinoid compounds, except in root nodules and other tissues containing symbiotic micro-organisms (Evans, Russell & Johnson, 1965).

Corrinoid-dependent enzymic reactions

Corrinoid-dependent enzymic reactions may be conveniently divided into two groups on the basis of the type of corrinoid compound required. The first group contains enzymic reactions requiring vitamin B_{12} or one of its analogues; a cobalt methyl corrinoid is probably formed during the reactions. This group includes several transmethylations and other reactions of methyl or carboxymethyl groups. The known examples are the methylation of homocysteine, the formation of methane and the synthesis of acetate from carbon dioxide. The second group contains enzymic reactions requiring a deoxyadenosyl corrinoid. These reactions involve a transfer of hydrogen and, in most cases, its replacement by another adjacent group. They include two mutase reactions, the diol dehydratase, glycerol dehydratase and ethanolamine deaminase reactions, the reduction of ribonucleotides to deoxyribonucleotides and a step in the fermentation of lysine. I shall try to summarize what is known about the role of corrinoid compounds in reactions of these two types.

Reactions requiring vitamin B_{12}

Methylation of homocysteine. First I shall review some recent studies on the role of vitamin B_{12} and other cofactors in the enzymic methylation of homocysteine. This methyl transfer reaction has been studied more intensively than any other vitamin B_{12}-dependent reaction and the conclusions reached on the role of the vitamin appear to be applicable in some degree to other related systems.

It is well known that the final enzymic reaction in methionine biosynthesis is a methyl group transfer from N^5-methyltetrahydrofolate to homocysteine:

N^5-Methyltetrahydrofolate + homocysteine
$\xrightarrow{\text{B12-dependent methyltransferase + adenosylmethionine + FADH2}}$
tetrahydrofolate + methionine

The identification of N^5-tetrahydrofolate as the methyl donor was made by Buchanan and his associates (Larrabee, Rosenthal, Cathou & Buchanan, 1961, 1963). The catalytic function of vitamin B_{12} in homocysteine methylation in extracts of an *E. coli* mutant was first demonstrated by Woods and his colleagues at Oxford (Helleiner & Woods, 1956; Guest, Helleiner, Cross & Woods, 1960; Kisliuk & Woods, 1960), and the binding of vitamin B_{12} to an essential protein was discovered as soon as attempts were made to purify the catalytically active proteins from cells grown in the presence of the vitamin (Hatch, Takeyama & Buchanan, 1959; Hatch, Larrabee, Cathou & Buchanan, 1961; Kisliuk, 1961; Takeyama, Hatch & Buchanan, 1961). Mangum & Scrimgeour (1962) discovered that catalytic amounts of *S*-adenosylmethionine activate the coenzyme B_{12}-dependent methyltransferase from pig liver, and replace the previously reported requirement for ATP. Subsequently *S*-adenosylmethionine was found also to be required for homocysteine methylation by the *E. coli* system (Rosenthal & Buchanan, 1963; Weissbach, Peterkofsky, Redfield & Dickerman, 1963). The need for a reduced flavine in catalytic amounts was recognized in both Woods's and Buchanan's Laboratories (Hatch *et al.* 1961; Guest *et al.* 1960).

A possible role for a cobalt methyl corrinoid in homocysteine methylation was suggested by the discovery by Guest and his associates (Guest, Friedman, Woods & Smith, 1962) that synthetic methylcobalamin can serve as a methyl donor in the *E. coli* system.

Methylcobalamin + homocysteine →
cobalamin + methionine

The participation of free methylcobalamin as an intermediate appeared to be excluded by the relatively low rate of methionine synthesis with methylcobalamin, as compared with N^5-methyltetrahydrofolate. Consequently it was postulated (Guest, Friedman, Dilworth & Woods, 1964; Foster, Dilworth & Woods, 1964; Woods, Foster & Guest, 1965) that enzyme-bound methylcobalamin is the true intermediate; the bound vitamin is presumably methylated by reaction with N^5-methyltetrahydrofolate, and then the methyl group is transferred to homocysteine. Since chemical alkylation of vitamin B_{12} requires reduction of the cobalt to the univalent state, it was further postulated that a similar reduction is essential for the formation of enzyme-bound methylcobalamin (Guest *et al.* 1964; Weissbach *et al.* 1963). Later studies of the methionine-forming enzyme have sought more specific information about the roles of protein-bound cobalamin, methylcobalamin and *S*-adenosylmethionine in methyl transfer.

For such studies, a highly purified enzyme is desirable and, for some purposes, essential. Corrinoid-dependent methyltransferases have been purified from liver (Buchanan, Elford, Loughlin, McDougall & Rosenthal, 1964; Loughlin, Elford & Buchanan, 1964; Dickerman, Redfield, Bieri & Weissbach, 1964) and from several species of bacteria. The bacterial enzymes are generally easier to purify. Jeanicke (1966) obtained a highly purified, apparently homogeneous, methyltransferase from *Streptococcus faecalis*. This enzyme contained about 1 mole of an incomplete corrinoid compound (possibly cobyric acid)/mole of protein. Unfortunately, no detailed description of this enzyme has yet been published. *E. coli* is the most common source of purified methyltransferase (Hatch *et al.* 1961; Foster, Jones & Woods, 1961; Kisliuk, 1961). The best preparation so far reported from this source, recently obtained by Taylor & Weissbach (1967*b*), contained 0·35 mole of corrinoid compound (undoubtedly a cobalamin)/mole of protein of molecular weight about 140000. Since no apoenzyme was present, about two-thirds of this preparation must have been contaminating protein.

The absorption spectrum of this salmon-coloured *E. coli* enzyme in the visible region is similar to that of vitamin B_{12r}, which contains bivalent cobalt (see also Takeyama & Buchanan, 1961). However, unlike free vitamin B_{12r}, the prosthetic group lacks the prominent extinction peak at 312 mμ and, moreover, is stable to oxygen. Taylor & Weissbach (1967*b*) have suggested that the cobalt may be coordinated to a thiol group in the protein, since such adducts (Dolphin & Johnson, 1963) are known to have a spectrum similar to that of vitamin B_{12r}. Weissbach and his associates (Brot & Weissbach, 1965; Weissbach & Taylor, 1966) have shown that

the corrinoid prosthetic group can be readily propylated, and the enzyme inactivated, by reaction with propyl iodide in the presence of a reduced flavine and a thiol. The requirement for the reducing agent suggests again that the cobalt is reduced to the univalent state, although direct spectral evidence for this is lacking. There is, however, substantial evidence that the cobalt is propylated. The absorption spectrum of the enzyme is altered by treatment with the alkylating agent, although not quite in the way expected for formation of a cobalt alkyl compound. Nevertheless, it was shown that the propyl group can be removed from the holoenzyme by exposure to light in a reaction typical of an alkyl corrinoid compound. The removal of the propyl group is accompanied by more or less complete reactivation of the enzyme. The correlation of propylation and depropylation with loss and recovery of methyltransferase activity supports the view that the cobalt of the prosthetic group is directly involved in methyl transfer. This conclusion is further strengthened by the demonstraton that exposure of the holoenzyme to N^5-methyltetrahydrofolate and a catalytic amount of *S*-adenosylmethionine protects it against chemical propylation and inactivation. This is interpreted to mean that enzymic methylation of the cobalt by N^5-methyltetrahydrofolate prevents chemical propylation, just as chemical propylation prevents enzymic methylation and therefore methyl transfer.

The conversion of inactive apomethyltransferase into active holoenzyme by addition of various corrinoid compounds has been used to provide evidence as to the probable oxidation state of the active prosthetic group (Weissbach, Redfield, Dickerman & Brot, 1965; Brot & Weissbach, 1966). Active holoenzyme can be formed by addition of either hydroxocobalamin, containing tervalent cobalt, or vitamin B_{12r}, containing bivalent cobalt, only when a thiol and a reduced flavine are also present. This indicates that these compounds must be further reduced, presumably to the univalent state, before they can bind to the apoenzyme. Addition of methylcobalamin, on the other hand, converts apoenzyme into the active holoenzyme in the absence of a reduced flavine, presumably because methylcobalamin, after methyl transfer, provides fully reduced cobalt (Woods *et al.* 1965).

Recently Taylor & Weissbach (1967*a*,*e*) showed that when N^5[^{14}C]-methyltetrahydrofolate is incubated with highly purified methyltransferase in the presence of *S*-adenosylmethionine and reduced flavine, but in the absence of homocysteine, ^{14}C-labelled protein is obtained, i.e. the methyl group is transferred from N^5-methyltetrahydrofolate to the vitamin B_{12} protein. About 0·3 g.atom of methyl carbon was bound/mole of vitamin B_{12} chromophore. On subsequent incubation of the ^{14}C-labelled protein with homocysteine, ^{14}C-labelled methionine was formed in nearly stoicheiometric yield, indicating that most of the methyl group was attached to an active site on the enzyme from which it could be transferred to homocysteine. Chemical propylation of the vitamin B_{12} chromophore on the methyltransferase inhibited methionine synthesis and diminished the amount of protein-bound methyl carbon to about the same extent.

An unexpected property of the methylated enzyme is its insensitivity to light; the methyl group is not affected by an exposure to light that would completely remove the propyl group from chemically propylated enzyme. This observation raised the possibility that the methyl group on the enzyme was attached to bound tetrahydrofolate rather than to cobalamin. However, by using variously labelled methylfolates as substrates, R. T. Taylor & H. Weissbach (personal communication) have shown that only the methyl group, and not the pteridine moiety of N^5-methyltetrahydrofolate, is bound to the enzyme. Further, they found that, although the methylated native enzyme is stable to light, the denatured enzyme readily loses the radioactive methyl group when exposed to light. Consequently the stability to light is dependent on the normal structure of the protein. Finally, [^{14}C]-methylcobalamin was isolated from the methylated enzyme after treating the protein with 80% (v/v) ethanol. Although details of these experiments are not yet available, the results appear to provide convincing evidence for the hypothesis that enzyme-bound methylcobalamin participates in methyl transfer in this system.

The role of *S*-adenosylmethionine in methyl transfer has been extensively studied, but still is not well understood. It is clearly established that *S*-adenosylmethionine is essential for methyl transfer from N^5-methyltetrahydrofolate, but is not required for methyl transfer from methylcobalamin and has no stimulating effect on this process (Foster *et al.* 1964; Taylor & Weissbach, 1967*b*). *S*-Adenosylmethionine can serve as an enzymic methyl donor to homocysteine, but the rate of this transfer is very low compared with that from N^5-methyltetrahydrofolate (Rosenthal, Smith & Buchanan, 1965). Further, *S*-adenosylhomocysteine cannot replace *S*-adenosylmethionine as a catalyst for methyl transfer from N^5-methyltetrahydrofolate (Elford, Katzen, Rosenthal, Smith & Buchanan, 1965). Consequently free *S*-adenosylmethionine cannot be an intermediate in methyl transfer from folate and there is no direct evidence that enzyme-bound *S*-adenosylmethionine functions in this way. It seems likely that *S*-adenosylmethionine is required in catalytic amounts to methylate an unidentified site on the holoenzyme that is essential for activity. This interpretation is

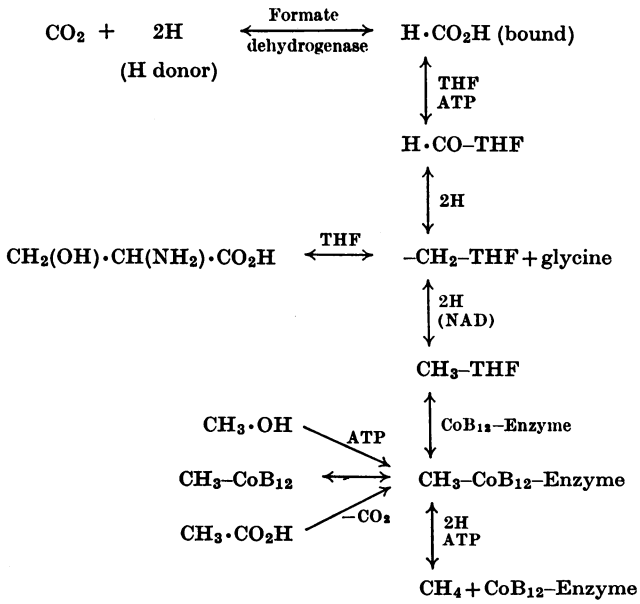
strengthened by the recent demonstration that methyl iodide, ethyl iodide and some other alkylating agents can partially replace *S*-adenosylmethionine as a cofactor, and also by kinetic evidence that the methyl groups from N^5-methyltetrahydrofolate and from *S*-adenosylmethionine do not compete and therefore presumably attach to different sites on the enzyme (Taylor & Weissbach, 1967*c*,*d*; Rosenthal *et al.* 1965). Elford *et al.* (1965) have suggested that the methyl group from N^5-methyltetrahydrofolate may be transferred to the upper axial position on the cobalt atom of the prosthetic group, whereas the methyl group from *S*-adenosylmethionine is transferred to the lower axial position. The presence of the second methyl group, derived from *S*-adenosylmethionine, presumably would facilitate methyl transfer at the other position. Unfortunately, direct confirmation of this or other hypotheses (Kerwar, Mangum, Scrimgeour, Brodie & Huennekens, 1966) for the role of *S*-adenosylmethionine is still lacking.

Methane formation. Let us now turn to the problem of biological methane formation. It is well established by the investigations of Blaylock and Stadtman and of Wolfe and his associates that the formation of methane by bacteria involves a participation of corrinoid compounds (Stadtman & Blaylock, 1966; Stadtman, 1967). The most abundant corrinoids that can be extracted with ethanol from whole cells of the most intensively studied species, *Methanobacillus omelianskii* and *Methanosarcina barkeri*, have been identified as the hydroxo and 5′-deoxyadenosyl derivatives of factor III, a cobalamin analogue containing 5′-hydroxybenzimidazole in place of 5,6-dimethylbenzimidazole (Lezius & Barker, 1965; A. G. Lezius, personal communication). Cobalt methyl corrinoids have not yet been detected in methane-producing bacteria.

The carbon of methane, formed by living bacteria, is derived from only a few sources, mainly carbon dioxide, formate, methanol, the methyl carbon of acetate and the β-carbon of serine (Barker, 1956) (Scheme 1). All of the many compounds utilized by methane bacteria serve either as sources of the above compounds or as reducing agents or both. Cell-free extracts of methane bacteria, under suitable conditions, can form methane from carbon dioxide, formate, formaldehyde, methanol and serine, but have not as yet been shown to decompose acetate (Stadtman, 1967). Unlike living bacteria, extracts can also use methyl corrinoids, N^5-methyltetrahydrofolate and $N^{5,10}$-methylenetetrahydrofolate as substrates for methane formation.

The conversion of methylcobalamin into methane by extracts of *Methanosarcina* was first observed by Blaylock & Stadtman (1963, 1964*a*), after the discovery that this compound can serve as a methyl donor in methionine synthesis:

$$\text{Methylcobalamin} \xrightarrow[+\text{ATP}]{\text{Reducing system}} CH_4 + \text{vitamin } B_{12r}$$



Scheme 1. Pathways of methane formation. THF, Tetrahydrofolate; CoB_{12}, B_{12} coenzyme.

They found that either pyruvate or hydrogen gas can serve as a reductant in the enzymic system. Wolin, Wolin & Wolfe (1963) subsequently observed the same reaction with extracts of *Methanobacillus* and made the further important observation that ATP, or another nucleoside triphosphate, is an essential component of the system. In their initial study, the stoicheiometry of the ATP requirement could not be determined because of the presence of an active adenosine triphosphatase. However, later Wood & Wolfe (1966*a*) (see also Wolfe, 1966) reported that the phosphatase could be controlled by the addition of a suitable concentration of ouabain. In the presence of this inhibitor approx. 1 mole of ATP is decomposed/mole of methane formed. Unfortunately, the products of ATP decomposition have not been identified. In the conversion of methylcobalamin into methane the main corrinoid product that accumulates is vitamin B_{12r} (Wolin, Wolin & Wolfe, 1964; Wood, Wolin & Wolfe, 1966). However, in view of the easy interconversion of the various oxidation states of corrinoid compounds under the conditions of the experiment, no firm conclusion can be reached about the valence of cobalt in the immediate reaction product. Consequently the valence of the transferred methyl carbon is also uncertain, although a carbonium ion seems probable. A similar non-enzymic reduction of methylcobalamin to methane occurs in the presence of hydrogen and a platinum catalyst (Dolphin, Johnson & Rodrigo, 1964). The other more or less immediate precursors of methane in the enzymic systems are N^5-methyltetrahydrofolate (Wood & Wolfe, 1965; Wood, Allam, Brill & Wolfe, 1965) and methanol (Blaylock & Stadtman, 1964*b*, 1966). The conversion of the methyl groups of these compounds into methane also requires a reducing system and ATP. The utilization of N^5-methyltetrahydrofolate for methane formation obviously indicates a relation between this system and the N^5-methyltetrahydrofolate–homocysteine methyltransferase of other organisms.

In the *Methanosarcina* system, methanol can serve, not only as a substrate for methane formation, but also as a methyl donor to vitamin B_{12s} with the formation of methylcobalamin (Blaylock & Stadtman, 1966). As in other similar reactions, vitamin B_{12s} cannot be replaced by the more oxidized forms of the vitamin. In this reaction, the methyl group of the alcohol is transferred intact, without loss or exchange of hydrogen. Another noteworthy feature of this methyl transfer is a requirement for ATP. Unfortunately, neither the stoicheiometry of the ATP requirement nor the role of ATP has been established. It is tempting to speculate that ATP activates the alcohol by formation of methyl-ADP in which the methyl group is linked to the terminal phosphate by a C–O–P bond. Such a compound could probably serve as an effective methyl donor just as ATP serves as a deoxyadenosyl donor in the synthesis of *S*-adenosylmethionine and adenosylcobalamin.

The fact that methylcobalamin can be formed from vitamin B_{12s} and methanol, and can be converted into methane, suggested that methylcobalamin or one of its analogues may be a normal intermediate in the conversion of methanol into methane. However, further evidence did not support this conclusion. As mentioned above, a cobalt methyl derivative could not be detected among the corrinoids extractable from methane bacteria. Further, free corrinoids appear not to be essential for the conversion of N^5-methyltetrahydrofolate or methanol into methane by partially purified enzyme preparations. However, the formation of methane from free methylcobalamin or other substrates for the *Methanosarcina* system is strongly inhibited by intrinsic factor (Blaylock & Stadtman, 1966), which binds corrinoid compounds. Methane formation is also strongly inhibited by treatment of enzyme preparations with propyl iodide and can be restored by exposure of the propylated preparation to light (Wood & Wolfe, 1966*b*). Consequently it may be concluded that protein-bound corrinoids are required for methane formation as they are for methionine synthesis.

Because of the relative instability of the methane-forming system, most of the early studies were done with unfractionated extracts. However, within the past year substantial progress has been reported in the purification of corrinoid-containing proteins involved in the formation of methane and methylcobalamin. Wood & Wolfe (1966*c*) partially purified a corrinoid-containing protein from *Methanobacillus* that, in association with unfractionated crude extract, catalyses methane formation from methylcobalamin or N^5-methyltetrahydrofolate. This protein, which is easily inactivated during the usual isolation procedures, was stabilized and labelled by treating the proteins of an unfractionated extract with ^{14}C-labelled propyl iodide. The most abundant radioactive propylated protein was purified about 100-fold and found to be an inactive red protein that could be reactivated for methane formation by exposure to light. The absorption spectrum of the propylated protein did not permit specific identification of the bound corrinoid, but the chromophore was separated from the partially purified protein and shown to contain three corrinoid compounds, one of which was radioactive. This compound was identified as cobalt propyl-factor III. The two non-radioactive corrinoids were identified as hydroxo- and deoxyadenosyl-factor III. Stadtman & Blaylock (1966) have reported the separation of a red protein from *Methanosarcina* extracts that also apparently contains factor III as a prosthetic

group, and is active, in association with other proteins, in the formation of methylcobalamin and methane from methanol.

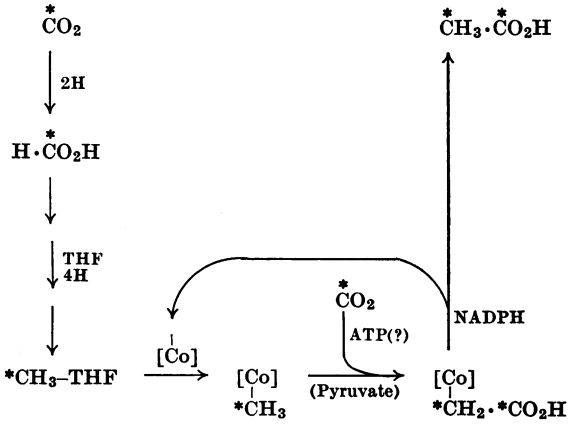
The above evidence indicates that some form of factor III is the prosthetic group of an enzyme catalysing a step in methane formation from N^5-methyltetrahydrofolate or methanol. The inactivation by propylation and reactivation by light again suggests that the upper axial co-ordination position of the cobalt atom is the site of methyl activation for methane formation.

Acetate synthesis from carbon dioxide. Another metabolic sequence involving methyl corrinoids was found by Poston, Kuratomi & Stadtman (1964) in *Clostridium thermoaceticum*. This organism ferments glucose to form 3 moles of acetate/mole of sugar (Fontaine, Peterson, McCoy, Johnson & Ritter, 1942). By fermenting glucose in the presence of $^{14}CO_2$, Kamen and I showed (Barker & Kamen, 1945) many years ago that about 2 moles of acetate and 2 moles of carbon dioxide are formed/mole of glucose, and the carbon dioxide is reutilized to form the third mole of acetate. More direct proof of the total synthesis of acetate from carbon dioxide was provided by Wood (1952) by mass analysis of acetate formed from $^{13}CO_2$. This type of acetate synthesis has been found to be a quantitatively important process in several anaerobic bacteria. Early attempts to determine the path of acetate synthesis by tracer methods were unsuccessful (Wood & Stjernholm, 1962).

The first indication of the involvement of a corrinoid in acetate synthesis was obtained by Poston *et al.* (1964), who tested the effect of intrinsic factor on acetate formation from $^{14}CO_2$ in extracts of *C. thermoaceticum*. Intrinsic factor caused a partial inhibition of carbon dioxide fixation; and, more significantly, the incorporation of carbon dioxide into the methyl carbon of acetate was markedly decreased relative to that into the carboxyl carbon. This observation, coupled with the knowledge that methylcobalamin can serve as a methyl donor in methionine synthesis, suggested that methylcobalamin may also be involved in acetate synthesis. This was soon confirmed. Methylcobalamin was found to be an effective precursor of the methyl carbon of acetate in this system. The methyl group of methylcobalamin was later shown to be formed from $^{14}CO_2$ and vitamin B_{12} in the presence of pyruvate and a suitable low-potential reducing system (Poston & Kuratomi, 1965; Kuratomi, Poston & Stadtman, 1966).

The identities of the corrinoid compounds in *C. thermoaceticum* were established by Irion & Ljungdahl (1965), who found the major components to be the 5′-deoxyadenosyl derivatives of cobyric acid and 5-methoxybenzimidazolylcobamide or factor IIIm. Small amounts of the cobalt methyl derivatives of cobyric acid and factor IIIm and several related compounds were also isolated from 400 g. of cells; about 0·6% of the total corrinoids were cobalt methyl derivatives. This small quantity suggested that the methyl corrinoids are bound to proteins. The relation of these compounds to carbon dioxide fixation was established by showing that, when actively fermenting cells of the *Clostridium* were exposed to $^{14}CO_2$ for 15 sec., ^{14}C-labelled methylcobyric acid and methyl-factor IIIm were formed. These corrinoids were highly radioactive, and the radioactivity was confined entirely to the cobalt methyl group (Ljungdahl, Irion & Wood, 1965). In addition, a very small amount of a third ^{14}C-labelled corrinoid was isolated and tentatively identified as a carboxymethyl corrinoid. This compound appeared to be labelled in both the carboxyl and methylene groups.

The role of these compounds in the synthesis of acetate from carbon dioxide is shown schematically in Scheme 2. The postulated steps from carbon dioxide to cobalt methyl corrinoid will not be considered in detail; suffice it to say that some evidence, as yet inconclusive, indicates that formate and N^5-methyltetrahydrofolate are probable intermediates in this conversion (Lentz & Wood, 1955; Ljungdahl, Irion & Wood, 1966). The cobalt methyl corrinoid formed from carbon dioxide has now been shown by Ljungdahl, Glatzle, Goodyear & Wood (1967) to be protein-bound. They observed that, after cell-free extracts are exposed to $^{14}CO_2$, a protein fraction can be separated that contains ^{14}C-labelled cobalt methyl corrinoid. Since most of the radioactivity is lost from the protein on exposure to light, it appears that only the methyl group is labelled. The methyl group of this protein-bound

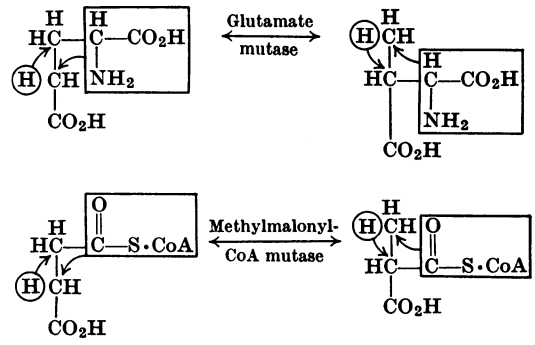


Scheme 2. Pathway of acetate synthesis in *C. thermoaceticum* (Ljungdahl *et al.* 1966). THF, Tetrahydrofolate.

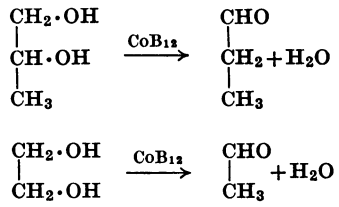
[^{14}C]methyl corrinoid can be converted, like free [^{14}C]methylcobalamin, into methyl-labelled acetate by incubation with a cell-free extract under suitable conditions. At least two other protein fractions, plus pyruvate, CoA and ferredoxin or a functionally related compound, appear to be required for this reaction (Poston & Kuratomi, 1965; Poston, Kuratomi & Stadtman, 1966).

The conversion of protein-bound cobalt methyl corrinoid into cobalt carboxymethyl corrinoid has not been demonstrated directly in an enzymic system. This step is postulated because of the isolation, mentioned above, of what appeared to be a labelled carboxymethyl corrinoid from whole cells exposed to $^{14}CO_2$, and because Ljungdahl *et al.* (1965) have shown that synthetic carboxymethylcobalamin is readily converted into acetate by extracts of *C. thermoaceticum*. NADPH serves as a reductant for this step. A protein fraction that catalyses the reductive formation of acetate from carboxymethylcobalamin has recently been separated from crude extracts (Ljungdahl *et al.* 1966; Poston *et al.* 1966). This protein fraction was shown to contain hydroxo-factor IIIm and a derivative of this corrinoid containing a radioactive ligand derived from $^{14}CO_2$ (Ljungdahl *et al.* 1967). All this evidence indicates that protein-bound corrinoids are essential catalysts or group carriers in the formation of acetate from carbon dioxide.

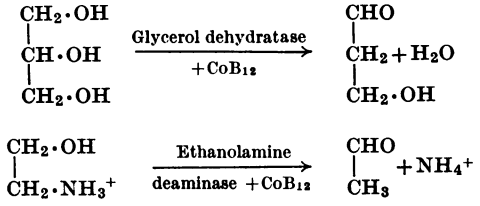
In comparing this system with those previously discussed, it is apparent that homocysteine methylation, methane formation and acetate synthesis involve increasingly complex systems utilizing cobalt methyl corrinoids. Homocysteine methylation consists of a relatively simple methyl group transfer, methane formation involves methyl transfer and methyl group reduction, and acetate synthesis apparently involves methyl transfer, methyl group carboxylation and carboxymethyl group reduction to acetate.



Scheme 3. B_{12} coenzyme-dependent mutase reactions.



Scheme 4. Diol dehydratase reactions. CoB_{12}, B_{12} coenzyme.



Scheme 5. Glycerol dehydratase and ethanolamine deaminase reactions. CoB_{12}, B_{12} coenzyme.

Reactions requiring 5′-deoxyadenosyl corrinoids

Mutase, diol dehydratase and related reactions. I shall now turn to the reactions requiring deoxyadenosyl corrinoids, which are quite different from those reactions requiring corrinoid vitamins. As mentioned above, the former reactions always involve a hydrogen transfer, generally between two adjacent carbon atoms and, usually, the transfer of another group in the opposite direction between the same carbon atoms.

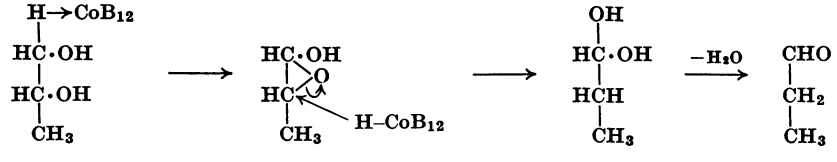
The glutamate mutase reaction (Scheme 3) was the first enzymic conversion of this type to be discovered (Barker, 1961*a*). In this reaction, a hydrogen atom moves from C-4 of L-glutamate to C-3 to form a methyl group, and a glycine residue simultaneously moves from C-3 to C-4 to form *threo*-β-methyl-L-aspartic acid (Barker, Suzuki, Iodice & Rooze, 1964*a*; Barker, Rooze, Suzuki & Iodice, 1964*b*). The reaction is fully reversible, the equilibrium strongly favouring the formation of L-glutamate. By carrying out the reaction in D_2O it was shown that the hydrogen transfer does not involve a free proton; no deuterium is incorporated into the products (Iodice & Barker, 1963). Also, free glycine is not formed as an intermediate (Barker *et al.* 1964*a*).

In the methylmalonyl-CoA mutase reaction, the pattern is essentially the same (Wood, Kellermeyer, Stjernholm & Allen, 1964). A hydrogen atom moves reversibly from C-3 of succinyl-CoA to C-2, and the carbonyl thio ester group moves in the opposite

direction (Eggerer, Stadtman, Overath & Lynen, 1960). Here again the transferred hydrogen does not exchange with the solvent, indicating that it probably moves as an H^-ion (Overath, Kellerman, Lynen, Fritz & Keller, 1962).

The diol dehydratase reaction (Abeles & Lee, 1961), and the closely related glycerol dehydratase (Smiley & Sobolov, 1962, 1964; Schneider & Pawelkiewicz, 1966) and ethanolamine deaminase (Bradbeer, 1965; Kaplan, 1966; Babior, 1967) reactions, appear at first inspection to be quite different from the mutase reactions because they do not involve a rearrangement of a carbon chain (Schemes 4 and 5). Purified diol dehydratase (Lee & Abeles, 1963) catalyses an irreversible decomposition of two diols, propane-1,2-diol and ethylene glycol. Both the D- and L-isomers of propane-1,2-diol are used by the enzyme at similar rates (Abeles & Lee, 1962). Propane-1,2-diol is converted into propionaldehyde and water, and ethylene glycol is converted into acetaldehyde and water. Although the overall reaction is a dehydration, the conversion must be regarded as an intramolecular oxido-reduction, since the primary alcohol group is oxidized to an aldehyde and the adjacent primary or secondary alcohol group is reduced to a methyl or methylene group. Brownstein & Abeles (1961) have shown that in this reaction, as in the mutase reactions, a hydrogen is moved from C-1 to C-2 without exchange with the solvent. This transfer occurs, as in the mutase reaction (Sprecher, Switzer & Sprinson, 1966; Sprecher, Clark & Sprinson, 1966), in a stereospecific manner; only one of the two hydrogen atoms on C-1 of D- or L-propane-1,2-diol is transferred and this occurs with inversion of the configuration at C-2 (Frey, Karabatsos & Abeles, 1965; Zagalak, Frey, Karabatsos & Abeles, 1966; Retey, Umani-Ronchi & Arigoni, 1966*a*). Retey, Umani-Ronchi, Seibl & Arigoni (1966*b*) have shown, by the use of propane-1,2-diol appropriately labelled with ^{18}O, that the oxygen atom of the hydroxyl group on C-2 moves in a stereospecific manner to C-1 with the probable formation of a hydrated aldehyde (Scheme 6). The hemiacetal form of lactaldehyde has been postulated to be an intermediate in this transfer. The hydrated aldehyde finally loses a molecule of water in an enzyme-controlled stereospecific reaction to form propionaldehyde. This transfer of the oxygen atom from C-2 to C-1 is completely analogous to the transfer of a carbon-containing group in the mutase reactions. Consequently it may be concluded that all of these deoxyadenosyl corrinoid-dependent reactions are basically similar. This generalization probably applies also to the less completely studied glycerol dehydratase and ethanolamine deaminase (Babior, 1967) reactions.

The transfer of a non-exchangeable hydrogen atom was recognized rather early as the common feature of deoxyadenosyl corrinoid-dependent reactions, and it was postulated that the corrinoid participates directly in this process (Barker, 1961*b*), but evidence for such participation has been obtained only recently. Abeles & Zagalak (1966) first obtained convincing evidence that the hydrogen transfer is an intermolecular rather than an intramolecular process by allowing diol dehydratase to act simultaneously on [1-^{3}H]propane-1,2-diol and on unlabelled ethylene glycol. Examination of the products showed that ^3H was present on C-2 of both propionaldehyde and acetaldehyde. This formation of ^3H-labelled acetaldehyde from unlabelled ethylene glycol could be most readily explained by postulating that ^3H from labelled propane-1,2-diol was transferred to either the enzyme or the coenzyme, and then was returned to a molecule of reacting ethylene glycol to form labelled acetaldehyde. If only a single position in the catalyst were available to accept and donate a hydrogen atom, such a transfer could not occur. Consequently it was concluded that the enzyme or coenzyme must contain at least two equivalent hydrogen atoms at the catalytic site. This would permit one hydrogen atom to be received and another to be returned to a reacting molecule. Abeles & Frey (1966) then demonstrated that the hydrogen is transferred from the substrate to the corrinoid coenzyme rather than to the enzyme. This was done by carrying out a reaction with [1-^{3}H]propane-1,2-diol and then isolating the coenzyme from the reaction mixture and showing that it contained ^3H derived from the substrate. The specific radioactivity of the isolated coenzyme indicated the presence of about 2 atoms of substrate hydrogen/molecule. In a second experiment, the labelled coenzyme was incubated with enzyme and unlabelled substrate. The result-



Scheme 6. Postulated mechanism of the diol dehydratase reaction (Retey *et al.* 1966*b*). CoB_{12}, B_{12} coenzyme.

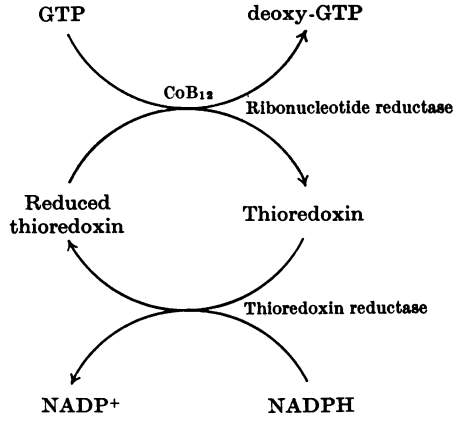
ing propionaldehyde was oxidized to propionic acid, which was shown to contain about 50% of the ^3H added as coenzyme. These experiments proved that the coenzyme can both accept and transfer hydrogen atoms from the substrate.

The next problem was to establish the position of the transferable hydrogen atoms in the coenzyme. Ingraham (1964) had postulated on purely theoretical grounds that the cobalt-linked 5′-methylene group of 5′-deoxyadenosylcobalamin may serve as a hydrogen acceptor in the mutase reactions with the transient formation of 5′-deoxyadenosine as an intermediate. Wagner, Lee, Frey & Abeles (1966) later tentatively identified 5′-deoxyadenosine as a product of a non-catalytic reaction between glycolaldehyde and the diol dehydratase–coenzyme complex. This result, and the knowledge that at least two equivalent hydrogen atoms are involved in coenzyme-catalysed hydrogen transfer, focused attention on the cobalt-linked methylene group as the site of the transferable hydrogen atoms. Frey & Abeles (1966) therefore synthesized deoxyadenosylcobalamin labelled with ^3H in the 5′-methylene group. When this labelled coenzyme was used in the enzymic conversion of unlabelled propane-1,2-diol, much of the ^3H was found in the resulting propionaldehyde. Under some conditions virtually all of the ^3H in the labelled coenzyme was transferred to the product, indicating that both of the methylene hydrogen atoms participated in the reaction. In the coenzyme these hydrogen atoms are not sterically equivalent. This suggests that during the enzymic reaction the Co–C bond is modified so that the hydrogen atoms become equivalent. This may occur by cleavage of the bond and formation of a methyl group as a transient intermediate.

The hydrogen transfer function of deoxyadenosylcobalamin has also been demonstrated for the two mutase reactions. Retey & Arigoni (1966) prepared ^3H-labelled coenzyme by means of the diol dehydratase reaction with (*RS*)-[1-^3H]propane-1,2-diol as a substrate. In a separate experiment they showed that methylmalonyl-CoA mutase of *P. shermanii*, acting on unlabelled succinyl-CoA in the presence of the labelled coenzyme, transferred up to 32% of the coenzyme-bound ^3H to the products of the reaction. This demonstrated that the same hydrogen atoms are transferred in both the diol dehydratase and the methylmalonyl-CoA mutase reactions. In my Laboratory, we have used synthetic ^3H-labelled coenzyme, generously supplied by Dr Abeles, to test for hydrogen transfer in the glutamate mutase reaction and have found that up to 90% of the ^3H can be transferred from coenzyme to glutamate and β-methylaspartate (R. L. Switzer, B. G. Baltimore & H. A. Barker, unpublished work). In this system the fraction of the total coenzyme-bound ^3H transferred to products depends on the amount of purified enzyme (Suzuki & Barker, 1966; Switzer & Barker, 1967) added. The results show that only enzyme-bound coenzyme is active in hydrogen transfer, and little exchange occurs between free and bound coenzyme, even during a rather long incubation. The same situation exists in the diol dehydratase system.

The reactivity of the hydrogen atoms on C-5′ indicates that these hydrogen atoms must be readily accessible to the substrates of the various reactions. A model of the coenzyme shows that the two reactive hydrogen atoms lie in a rather shallow depression on the surface of the coenzyme, into which all of the substrates can be fitted so that the transferable hydrogen of the substrate is in contact with the reactive methylene group.

Ribonucleoside triphosphate reductase. Let us now turn to the corrinoid coenzyme-dependent ribonucleotide reduction. A connexion between vitamin B_{12} and deoxyribose synthesis in *Lactobacillus leichmannii* was first established by nutritional studies. Kitay, MacNutt & Snell (1949) observed that any one of several deoxyribonucleosides can replace the vitamin B_{12} requirement of this organism, whereas ribonucleosides are ineffective. Later tracer experiments with intact cells carried out in several Laboratories established that ribose and ribonucleosides are converted into deoxyribonucleotides of DNA only when vitamin B_{12} is available (Wacker, Kirschfeld & Trager, 1959; Dinning, 1959; Manson, 1962). Finally Blakley, working first in my Laboratory and later in Canberra, obtained a cell-free system from *L. leichmannii* with which the chemistry and cofactor requirements of deoxyribose synthesis could be



Scheme 7. Ribonucleotide triphosphate reductase system of *L. leichmannii* (Blakley, 1966*a*). CoB_{12}, B_{12} coenzyme.

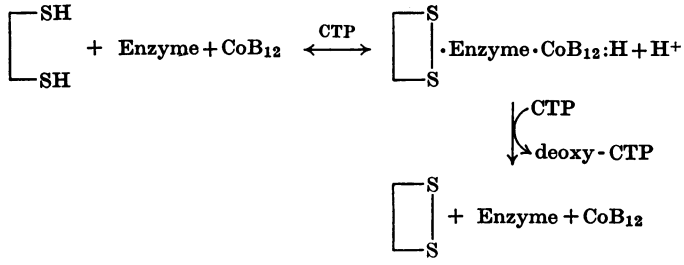
studied more effectively (Blakley & Barker, 1964; Blakley, 1965, 1966*a*). He demonstrated the conversion of CMP into deoxy-CMP when ATP, a reducing system and deoxyadenosylcobalamin were supplied as cofactors. The ATP was thought to be required for the phosphorylation of CMP, but initially it was uncertain whether CDP or CTP is the actual substrate for reduction. Later, after purification of the reductase, it was established by Abrams (1965), and independently by Blakley, Ghambeer, Nixon & Vitols (1965), that only the triphosphate is used; when CTP is provided, ATP is no longer required (Scheme 7). The purified enzyme (Goulian & Beck, 1966) also reduces other ribonucleoside triphosphates, including GTP, ATP, ITP and UTP (Beck, Goulian, Larsson & Reichard, 1966*b*; Blakley, 1966*b*; Vitols, Brownson, Gardiner & Blakley, 1967). The requirement for a nucleoside triphosphate distinguishes this enzyme from the ribonucleotide reductase of *E. coli*, which uses only nucleoside diphosphates (Reichard, 1962).

The reducing system first used by Blakley with crude extracts consisted of mercaptoethanol and an NADPH-generating system. Subsequent studies (Orr & Vitols, 1966) have indicated that the immediate physiological reducing agent in *L. leichmannii* is a low-molecular-weight dithiol protein similar to the reduced thioredoxin of *E. coli* (Moore & Reichard, 1963; Laurent, Moore & Reichard, 1964). The disulphide form of this protein is reduced by reaction with NADPH in the presence of a specific reductase. The *L. leichmannii* and *E. coli* systems are sufficiently similar so that *E. coli* thioredoxin and thioredoxin reductase can substitute for the analogous *L. leichmannii* proteins (Vitols & Blakley, 1965; Beck *et al.* 1966*b*; Goulian & Beck, 1966). In both systems, reduced lipoic acid and related dithiols can substitute for reduced thioredoxin, and are commonly used in experimental work.

The reductase of *L. leichmannii* shows an absolute requirement for 5′-deoxyadenosylcobalamin or one of its analogues containing another benzimidazole or purine base in the nucleotide side chain (Blakley, 1966a). Other alkyl corrinoids or corrinoid vitamins are entirely inactive.

The chemistry of the enzymic reduction of the ribosyl moiety has been investigated by carrying out the reaction in the presence of ^2H- or ^3H-labelled water. Since the thiol groups of the reductant are in isotopic equilibrium with the solvent, the deoxyribosyl moiety of the product should contain one or more labelled hydrogen atoms, depending on the course of the reaction. Because of the obvious chemical similarity of the nucleotide reductase reaction to the diol dehydratase reaction, Fukui, Tamao, Kato & Shimizu (1965) and others have suggested that a 2′-deoxy-3′-oxoribosyl moiety may be formed as an intermediate; the 3′-oxo group would then be reduced to form the deoxyribonucleotide. This would put a labelled hydrogen atom from the solvent on C-3′. This mechanism has been proved to be incorrect. Gottesman & Beck (1966) and Blakley, Ghambeer, Batterham & Brownson (1966) found that solvent hydrogen is added exclusively to C-2′, as it is also in the *E. coli* ribonucleotide reductase system (Larsson, 1965). Further, Batterham, Ghambeer, Blakley & Brownson (1967) concluded by examining the proton-magnetic-resonance spectrum of ^2H-labelled deoxyadenosine derived from deoxy-ATP, which had been prepared by enzymic reduction of ATP in ^2H-labelled water solution, that the ^2H is added to C-2′ with retention of configuration. It has been suggested that the reaction involves a displacement of an OH^- ion by an H^- ion.

The role of the corrinoid coenzyme in the ribonucleotide reductase reaction was first examined by Beck, Abeles & Robinson (1966*a*). Since the coenzyme acts as a hydrogen-transferring agent in the diol dehydratase and mutase reactions, they looked for a similar hydrogen transfer from ^3H-labelled coenzyme to product in the reduction of CTP. The result was completely negative; no ^3H was detected in the deoxy-CTP formed in the reaction. However, ^3H was transferred rapidly and quantitatively from the 5′-position of the coenzyme to water. This transfer was found to depend on the same components as the reduction of ribonucleotides, namely enzyme, substrate and reduced lipoate; oxidized lipoate could not replace the reduced form. Later Hogenkamp, Ghambeer, Brownson & Blakley (1967) discovered that the ribonucleotide substrate could be replaced as an activator of the exchange reaction by any one of several deoxyribonucleotide triphosphates, of which deoxy-GTP was the most effective. These requirements, plus the fact that the experiments were done with highly purified enzyme, indicate that the reductase catalyses the exchange with water. Beck *et al.* (1966*a*) have postulated that the loss of coenzyme hydrogen to water is caused by a rapid reversible oxidoreduction and hydrogen transfer reaction between reduced lipoate and the enzyme–coenzyme complex (Scheme 8). This reaction, in the reverse direction, transfers coenzyme hydrogen to the thiol groups, which rapidly exchange their hydrogen atoms with water. If this reaction is sufficiently fast compared with the transfer of coenzyme hydrogen to substrate, little or no coenzyme hydrogen will be found in the reduced nucleotide. As yet no precise information is available on the relative rates of the exchange and reduction reactions, although it has been reported that the exchange is at least fast enough to account for the observed rate of ATP reduction (Hogenkamp *et al.* 1967). Although the proposed



Scheme 8. Postulated role of B_{12} coenzyme in ribonucleotide triphosphate reductase reaction (Beck *et al.* 1966*a*). CoB_{12}, B_{12} coenzyme.

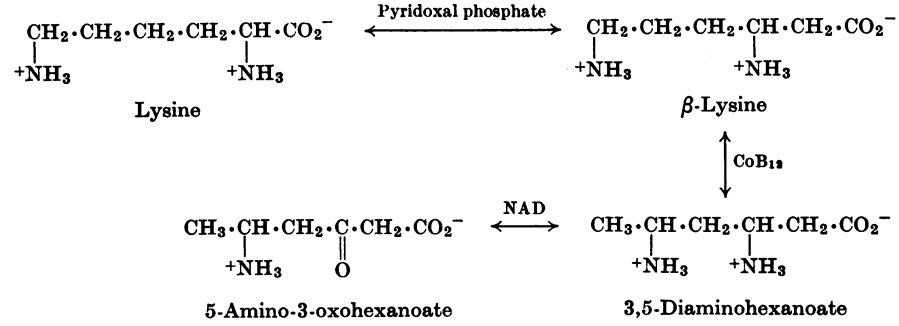
scheme for the reductase reaction is plausible, the occurrence of the rapid hydrogen exchange with water has so far made it impossible to obtain direct evidence that the coenzyme functions as a hydrogen carrier between reductant and substrate in this system.

Conversion of β-lysine into 3,5-diaminohexanoate. Finally I shall present some evidence for the participation of a corrinoid coenzyme in a new reaction involved in the fermentation of lysine by clostridia. Several years ago Stadtman (1955, 1963, 1964) reported that cell-free extracts of *Clostridium sticklandii* and a related organism convert lysine under appropriate conditions into acetate, butyrate and ammonia. Several cofactors, including deoxyadenosylcobalamin, were shown to be essential or stimulatory for this fermentation. The participation of a corrinoid coenzyme was indicated by the observation that the enzyme system could be inactivated by charcoal treatment, by exposure to light or by addition of purified intrinsic factor, and could be reactivated, at least partially, by addition of deoxyadenosylcobalamin or one of its analogues.

Attempts to analyse the system further by determining the path of lysine degradation were unsuccessful until Costilow and Rochovansky (Costilow, Rochovansky & Barker, 1966), working in my Laboratory with extracts of still another lysine-fermenting *Clostridium*, succeeded in detecting, isolating and identifying 3,6-diaminohexanoate or β-lysine as the first product of lysine metabolism (Scheme 9). β-Lysine is formed from lysine by a readily reversible transfer of the amino group from C-2 to C-3. This appears to be a pyridoxal phosphate-dependent reaction. The next step in lysine fermentation is a transfer of the amino group on C-6 of β-lysine to C-5 to form 3,5-diaminohexanoate, which in a following step (E. Rimerman & H. A. Barker, unpublished work) is oxidized at C-3 to form the corresponding 5-amino-3-oxo acid. 3,5-Diaminohexanoate was isolated and characterized by Stadtman & Renz (1967) at the National Institutes of Health, and independently by E. E. Dekker (unpublished work) in my Laboratory. Both investigators obtained evidence that the conversion of β-lysine into 3,5-diaminohexanoate is dependent on a deoxyadenosyl corrinoid. The formation of the new diamino acid from lysine or β-lysine was shown to be specifically inhibited by intrinsic factor, and the inhibition could be largely prevented by addition of deoxyadenosylcobalamin. T. C. Stadtman (unpublished work) also fractionated crude bacterial extracts and found that at least two protein fractions were required for the utilization of β-lysine. One of these fractions was colourless, whereas the other was orange and contained a chromophore tentatively identified as deoxyadenosyl-pseudovitamin B_{12}. Neither of these fractions alone was active, but they were active when combined. Some stimulation of activity was also observed on the addition of deoxyadenosylcobalamin to these partially purified fractions. These observations provide strong evidence for participation of a corrinoid coenzyme in this reaction. Further information will undoubtedly be available before long.

The conversion of β-lysine into 3,5-diaminohexanoate appears to be rather similar to the diol dehydratase reaction in its basic features, since it involves a shift of an amino group from C-6 to C-5 and a simultaneous transfer of a hydrogen in the opposite direction. The differences between the two reactions consist only in the kind of group transferred and the fact that the acceptor carbon atom already has an hydroxyl group in one reaction and not in the other. So the participation of a corrinoid coenzyme in this amino transfer is not surprising.

This completes the list of well-defined enzymic reactions known to require a corrinoid prosthetic group or coenzyme. Undoubtedly more corrinoid-dependent reactions will be found before long, since there are a number of organisms from bacteria to man that either require or synthesize corrinoid compounds for as yet unknown functions. The information already available clearly establishes the kinds of reactions in which corrinoids participate



Scheme 9. Reactions in the fermentation of lysine by clostridia. CoB_{12}, B_{12} coenzyme.

and the general nature of the catalytic processes, and will facilitate future explorations in this area.

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