Life with CO or CO_2 and H_2 as a source of carbon and energy

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ABSTRACT **An account is presented of the recent discovery of a pathway of growth by bacteria in which CO or CO_2 and H_2 are sources of carbon and energy. The Calvin cycle and subsequently other cycles were discovered in the 1950s, and in each the initial reaction of CO_2 involved adding CO_2 to an organic compound formed during the cyclic pathway (for example, CO_2 and ribulose diphosphate). Studies were initiated in the 1950s with the thermophylic anaerobic organism *Clostridium thermoaceticum*, which Barker and Kamen had found fixed CO_2 in both carbons of acetate during fermentation of glucose. The pathway of acetyl-CoA biosynthesis differs from all others in that two CO_2 are combined with coenzyme A (CoASH) forming acetyl CoA, which then serves as the source of carbon for growth. This mechanism is designated the acetyl CoA pathway and some have called it the Wood pathway. A unique feature is the role of the enzyme carbon monoxide dehydrogenase (CODH), which catalyzes the conversion of CoASH, CO, and a methyl group to acetyl CoA, the final step of the pathway. The pathway involves the reduction of CO_2 to formate, which then combines with tetrahydrofolate (THF) to form formyl THF. It in turn is reduced to CH_3-THF. The methyl is then transferred to the cobalt on a corrinoid-containing enzyme. From there the methyl is transferred to CODH, and CO and CoASH bind with the enzyme at separate sites. Acetyl CoA is then synthesized. CODH would more properly be called carbon monoxide dehydrogenase-acetyl CoA synthase as it catalyzes oxidation of CO to CO_2 and the synthesis of acetyl CoA. The solution of the mechanism of this pathway required more than 30 years, in part because the intermediate compounds are bound to enzymes, the enzymes are extremely sensitive to O_2 and must be isolated under strictly anerobic conditions, and the role of a corrinoid and CODH was unprecedented. It is now apparent that this pathway occurs (perhaps with some modification) in many bacteria including the methane and sulfur bacteria. In some humans this pathway is catalyzed by the bacteria of the gut and acetate is produced rather than methane; it is calculated that 2.3×10^6 metric tons of acetate are formed daily from CO_2. A similar synthesis occurs in the hind gut of termites. It is becoming apparent that the acetyl CoA pathway plays a significant role in the carbon cycle. The direct combination of two CO_2 to form acetate may have been used by the earliest forms of life rather than the more complicated cyclic mechanisms of autotrophism.— Wood, H. G. Life with CO or CO_2 and H_2 as a source of carbon and energy. *FASEB J.* 5: 156-163; 1991.**

Key Words: autotrophism • acetate synthesis from CO or CO_2 and H_2 • acetyl CoA pathway • corrinoid enzyme • CO dehydrogenase • Clostridium thermoaceticum

METABOLIC ESSENTIALS

It is a great honor to receive the William C. Rose Award. Rose carried out his outstanding investigations in the 1930s and 1940s. I began graduate studies in 1931 and remember Rose and his work very well. In the 1930s biochemical research provided exciting findings that explained nutritional observations, and in particular it identified the roles of vitamins in metabolism, such as that thiamine pyrophosphate is a cofactor of pyruvate decarboxylase (1). Nicotinamide was identified as a cofactor of glucose-6-phosphate dehydrogenase (2). Some enzymatic roles of vitamins remained unexplained for another 2 decades. In 1961 Lynen and co-workers showed that biotin is a cofactor of enzymes that fix carbon dioxide (3), and in 1963 I and others demonstrated directly the role of biotin in enzymes (4–6).

Side by side the biochemical rationale for the requirement for certain amino acids in the diet was worked out by Rose, first with rats and then with humans. Healthy male graduate students served as experimental subjects on a diet of cornstarch, sucrose, melted butterfat, corn oil, inorganic salts, and vitamins, that were baked by Rose's wife into wafers. The list of amino acids essential for the diets of rats and graduate students was not quite the same. For optimum growth rats require histidine and arginine, which graduate students did not (7).

TOTAL SYNTHESIS OF ACETATE

Now I want to describe an investigation we started in 1952 and has occupied my attention to this very day.

As background, let me point out that the great Russian microbiologist, Winogradsky, had shown in 1891 (8) that certain bacteria can grow with CO_2 as the sole source of carbon; long before that photosynthetic plants were known to use CO_2 as the sole source of carbon. However, no one had the slightest idea how these organisms were able to totally synthesize a multicarbon compound from CO_2. The only known mechanisms for utilization of CO_2 in the 1940s were those occurring in heterotrophs which require organic compounds for growth (9). The typical heterotroph such as you and I uses CO_2 in certain synthetic reactions that play an essential role in our metabolism, but these reactions involve addition of CO_2 to a pre-existing organic compound, such as CO_2 combining with pyruvate to form oxalacetate. They do not involve the total synthesis of an organic compound from CO_2.

However, in the late 1940s, it began to appear that there might be exceptions to the general rule that heterotrophs cannot totally synthesize an organic compound from CO_2.

An organism named *Clostridium thermoaceticum* was isolated at the University of Wisconsin in 1942 (10), which in an anaerobic fermentation of glucose formed 3 mol of acetate

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per mol of glucose. It seemed possible the glucose was converted to 2 mol of acetate and two CO_2 and then the two CO_2 were reduced to the third mol of acetate as shown in **Fig. 1.** If so, this would represent a total synthesis of an organic compound from CO_2.

Martin Kamen at Berkeley had discovered how to prepare long-lived ^{14}C-radioactive carbon in the cyclotron. Along with H. A. Barker (11), he examined this question in one of the first experiments done with ^{14}C. They fermented glucose in the presence of $^{14}CO_2$, isolated the acetate, and degraded it. They found that ^{14}C was present in both the methyl and carboxyl positions, and presented the equations shown in Fig. 1.

I was excited by these results. I thought if this fermentation involves the total synthesis of acetate, if we determine the mechanism of this synthesis, we might provide clues to how plants and autotrophic bacteria can grow and totally synthesize all material from CO_2.

There remained some doubt, at least in my mind, that there was a total synthesis of acetate. Barker and Kamen (11) found much more ^{14}C in the carboxyl group of acetate than in the methyl group. Thus it was evident that there was some method of converting CO_2 to acetate that did not involve a total synthesis of acetate from CO_2. There remained the possibility that the mechanism involved formation of the methyl group from CO_2 by one reaction, and by a separate, independent reaction, CO_2 was converted to the carboxyl group of acetate and no molecules were totally synthesized from CO_2. I thought a conclusive experiment could be done with $^{13}CO_2$ to prove whether the fermentation of glucose by *C. thermoaceticum* truly involved a total synthesis of acetate from CO_2.

We conducted fermentations of glucose in the presence of $^{13}CO_2$ so that we could weigh the carbon 13 in the resulting acetate using our mass spectrometer (12). We found (as indicated in **Fig. 2**) that acetate was formed that had a mass of 62. Thus it was evident, as acetate containing only unlabeled ^{12}C carbons has a mass of 60, that both of its carbons had arisen from $^{13}CO_2$. From the mass ratios, it was calculated that about 33% of the acetate was totally synthesized from CO_2 as had been proposed by Barker and Kamen. However, molecules of mass 61 were also present which therefore contained only one ^{13}C. It was calculated from the mass ratios that about 33% of the molecules were of this type. The remaining one-third of the acetate was made up of molecules that had a mass of 60 and contained no ^{13}CO. The latter were clearly made from the carbon of the unlabeled [^{12}C]glucose.

At first the results were puzzling. However, it was found when the bacteria were incubated in the presence of acetate and $^{14}CO_2$ in the absence of glucose, that the acetate became radioactive (12). It thus became evident the bacteria could catalyze a secondary exchange reaction with the carboxyl of acetate, and this was how the singly labeled acetate was formed. You will see that the use of this exchange reaction came in handy much later on.

Figure 1. Barker-Kamen equations of the fermentation of glucose to acetate by *Clostridium thermoaceticum.*

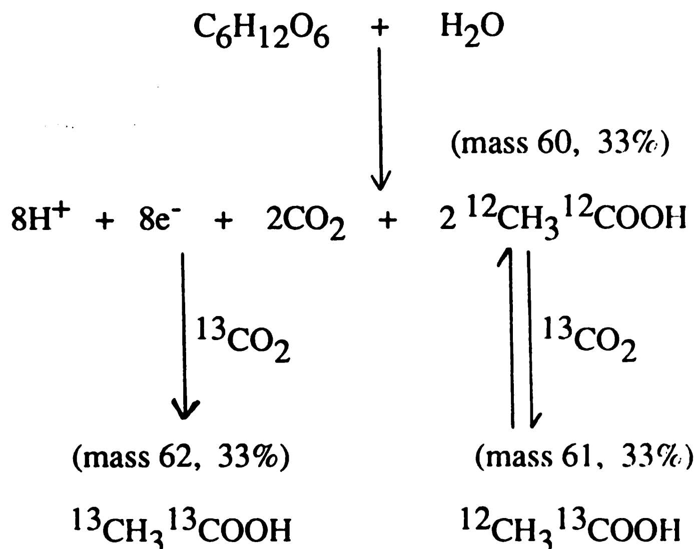


Figure 2. Evidence by mass analysis that there is total synthesis of acetate from $^{13}CO_2$ by *C. thermoaceticum.*

The ^{13}C experiments were crucial because it was possible to prove acetate was totally synthesized from CO_2.

In these days of sophisticated biochemistry these results may not seem very exciting, but they were to me. It was evident that if an autotrophic bacterium could generate protons and electrons from hydrogen by the enzyme hydrogenase, it could replace the glucose as an electron donor and totally synthesize acetate from CO_2 and grow on the resulting acetate. It also was evident that a plant could photolytically cleave water to generate the electrons for the reduction, and that it might synthesize acetate and from it synthesize its cellular material. With these thoughts in mind, we undertook the task of determining how bacteria totally synthesize acetate from CO_2.

Melvin Calvin and his colleagues and others at about the same time began investigations with $^{14}CO_2$ to determine how algae grow photosynthetically using light. By pulse labeling and the clever use of paper chromatography, they identified ^{14}C-labeled intermediates of the reductive pentose cycle and showed that CO_2 is utilized initially by combination with ribulose-diphosphate yielding 2 mol of phosphoglycerate, one of which is used for growth and the other is recycled (13).

We, on the other hand, did not solve our problem for a long time. The intermediate compounds of the acetate pathway are enzyme-bound. Thus pulse labeling with $^{14}CO_2$ gave us little information concerning the intermediate compounds. Formate was highly labeled and [^{14}C]formate was converted to the methyl group of acetate more rapidly than was $^{14}CO_2$ (14). Thus, there was a hint that formate might be a precursor of the methyl group of acetate.

The first real break concerning this pathway did not come from us, but from Earl Stadtman's laboratory (15). They knew that *C. thermoaceticum* contains many corrinoids (vitamin B_{12} derivatives) and reasoned the corrinoids might be used by the bacteria in their metabolism. They tested the effect of intrinsic factor on the metabolism. This is the compound that combines with B_{12} in the gut and aids in the transport of B_{12}. Its deficiency in humans is the cause of pernicious anemia. They found intrinsic factor inhibited the formation of acetate from CO_2. They then synthesized and used [^{14}C]methyl B_{12} as a substrate and found the methyl

group was converted to the methyl group of acetate (Reaction 1).

$$^{14}CH_3\text{-}B_{12} + CH_3COCOOH + H_2O \rightarrow {}^{14}CH_3COOH + CH_3COOH + B_{12} \qquad (1)$$

When this information became available, it was clear that the CH_3-B_{12} intermediate might be enzyme-bound and that we had not detected it because we had looked for compounds in the deproteinated solutions. Therefore, Lars Ljungdahl and Eckart Irion, in our laboratory, using large quantities of *C. thermoaceticum,* again conducted pulse labeling for 15 s with $^{14}CO_2$. They isolated the corrinoids from the bacteria and found they were present in abundance (16). In **Fig. 3**, the rectangle represents the tetrapyrrol of the corrinoid ring in which the cobalt is coordinated to the tetrapyrrol much as is Fe in heme or Mg in chlorophyll. The ^{14}C was entirely in the CH_3 linked to the cobalt, and was very radioactive.

With these results in hand, in 1966 we proposed the scheme shown in **Fig. 4** as a working hypothesis for the formation of acetate from CO_2 (17). We proposed that two pyruvates are converted to two acetates and two CO_2, then one CO_2, is reduced to formate, which in turn is converted to formyltetrahydrofolate and by a series of reactions is reduced to methyltetrahydrofolate (CH_3-THF). The methyl is then transferred to the cobalt on the corrinoid enzyme represented by the cobalt in brackets. We proposed that the second CO_2 combines with this methyl group to form acetate, and the unmethylated corrinoid enzyme is thus regenerated and the cycle is repeated.

There was precedence for this proposal based on what was known about methionine synthesis. Warwick Sakami and Robert Greenberg in our department, as well as John Buchanan at MIT, were investigating the metabolism of C_1 (one-carbon) compounds. They had shown that C_1 compounds are converted to CH_3-THF. Furthermore, Weissach and Taylor (18) had shown in methionine synthesis that the methyl of CH_3-THF is transferred to a corrinoid enzyme and the methyl is then transferred to homocysteine to form methionine. What was new about our scheme was the proposal that CO_2 combines with a methyl linked to the cobalt to form acetate. In those days, we synthesized our own carboxyl-labeled acetic acid by the Grignard Reaction, using CO_2 and a methyl Mg salt, so it was quite natural to think the CO_2 might react with a methyl attached to cobalt.

THE ACETYL CoA PATHWAY

In our more recent studies, Harold Drake and Shou-Ih Hu (19), using procedures to strictly avoid any exposure of the

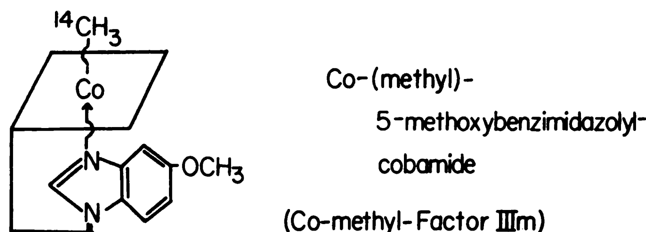


Figure 3. The CH_3-cobalt corrinoid isolated from *C. thermoaceticum* after pulse labeling with $^{14}CO_2$. The rectangle represents the tetrapyrole of the corrinoid.

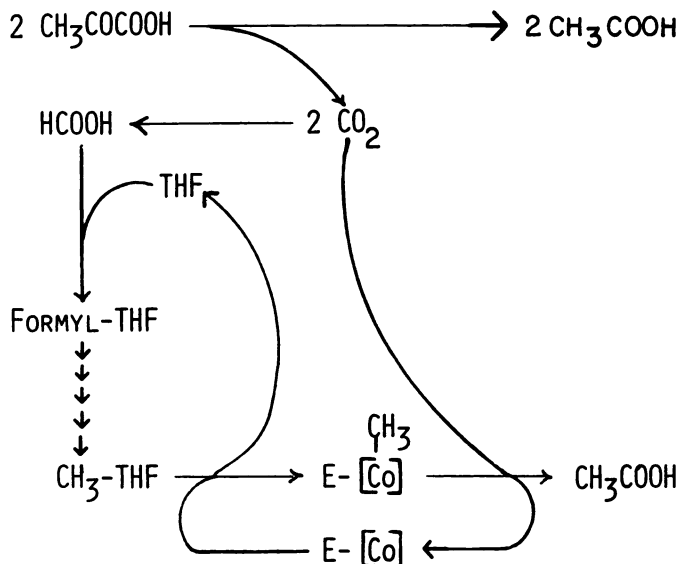
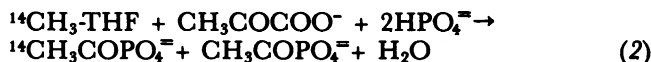


Figure 4. Scheme proposed in 1966 for the total synthesis of acetate from CO_2 during fermentation of pyruvate by *C. thermoaceticum.* THF is tetrahydrofolate and E-[Co] is the corrinoid enzyme.

enzymes to oxygen, isolated from *C. thermoaceticum* five fractions that catalyzed the conversion of CH_3-THF and pyruvate to acetylphosphate, four of which were pure enzymes. For years, no one had been able to purify the enzymes of this pathway because they are extremely sensitive to oxygen.

Drake had worked with sulfur bacteria that contain oxygen-sensitive enzymes. It is a great advantage to have young people in your laboratory with fresh ideas. This development eventually opened the way for purification of all the enzymes of the system that catalyze the conversion of CO_2 and H_2 to acetyl-CoA. **Figure 5** illustrates the type of procedure that is required. The hood is filled with N_2, and a trace of H_2 is added and a catalyst is present to convert any traces of O_2 with the H_2 to H_2O. All the purifications of the enzyme are done in this hood. It is not Drake and Hu (see Fig. 5), but Steve Ragsdale and Ewa Pezacka whose work we will consider later.

The enzymes purified were pyruvate ferredoxin oxidoreducatase, ferredoxin, methyltransferase, phosphotransacetylase, and a fifth fraction that was impure and contained three or four proteins (19). These combined fractions catalyzed the conversion of $^{14}CH_3$-THF and pyruvate to two acetylphosphates, one of which contained ^{14}C in the methyl group (Reaction 2). If CoA was substituted for the phosphate, acetyl-CoA was the product and the phosphotransacetylase could be omitted.



$$^{14}CH_3\text{-}THF + CH_3COCOO^- + 2HPO_4^{=} \rightarrow {}^{14}CH_3COPO_4^{=} + CH_3COPO_4^{=} + H_2O \qquad (2)$$

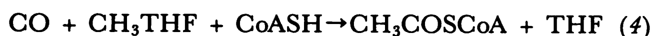
Luck is important in scientific discovery. I remember Fritz Lipmann asking about a postdoctoral fellow I was recommending. He asked, is he lucky? Curiosity is an important part of luck. We were lucky at this point.

About that time, Rolf Thauer and co-workers in Marburg, Germany, found that *C. thermoaceticum* contains the enzyme carbon monoxide dehydrogenase (20). This enzyme converts CO to CO_2 (Reaction 3).

$$CO + H_2O \rightleftharpoons C_1 \rightleftharpoons CO_2 + 2H^+ + 2e^- \qquad (3)$$

It is easily assayed using methyl viologen as an electron acceptor. We will see that this is a key enzyme in catalysis of the conversion of CO, CH_3THF, and CoA to acetyl-CoA. I had no idea that this enzyme had anything to do with the synthesis of acetate, but out of curiosity I asked Drake and Hu to determine if the impure fraction that contained three or four proteins contained carbon monoxide dehydrogenase (CODH).[1]

Drake and Hu (21) found the impure fraction was loaded with CODH and showed it is a Ni metalloenzyme (22). The luck continued. No one had been able, in the absence of pyruvate, to obtain synthesis of acetyl-CoA with CH_3-THF, CO_2, and CoA. Hu and Drake reasoned that the pyruvate might be required as an electron donor in the synthesis of $[^{14}C]$acetyl-CoA from $^{14}CH_3THF$, CO_2, and CoA. Therefore, they set up an experiment in which they left out pyruvate and replaced the pyruvate with CO. They found with CO present, pyruvate was no longer required. Furthermore, they found that the presence of CO_2 was not required, that CO per se was the source of the carbonyl group of acetyl-CoA as shown in Reaction 4 (22).

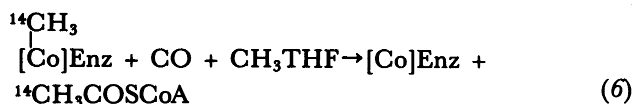


$$CO + CH_3THF + CoASH \rightarrow CH_3COSCoA + THF \quad (4)$$

These results were indeed exciting: it was evident that *C. thermoaceticum* could catalyze an autotrophic type synthesis.

Hu, as part of his Ph.D. research, was able to isolate the corrinoid enzyme (23). With the corrinoid enzyme available and using the methyltransferase, he methylated the corrinoid enzyme as shown in Reaction 5 in which [Co]Enz is the corrinoid enzyme. From its properties, it was evident that the methyl is linked to the cobalt of the corrinoid. With the methylated corrinoid enzyme, he was able to synthesize methyl-labeled acetyl-CoA from CO and CoA (Reaction 6).

$$^{14}CH_3THF + [Co]Enz \rightarrow THF + [\overset{^{14}CH_3}{Co}]Enz \quad (5)$$



$$[\overset{^{14}CH_3}{Co}]Enz + CO + CH_3THF \rightarrow [Co]Enz + {}^{14}CH_3COSCoA \quad (6)$$

The methyltransferase enzyme and CH_3THF were no longer required for the synthesis as the methyl corrinoid enzyme served as a direct source of the methyl of acetyl-CoA. These results provided clear evidence that the methyl of CH_3-THF is converted to the methyl of acetyl-CoA via the methylated corrinoid enzyme. More recently, Ragsdale et al. (24), purified the corrinoid enzyme to homogeneity and showed that it contains an $[4Fe\text{-}4S]^{1+2}$ cluster (see also ref 25).

Figure 5. Steve Ragsdale and Ewa Pezacka isolating oxygen-sensitive enzymes from *C. thermoaceticum* by use of an anaerobic hood.

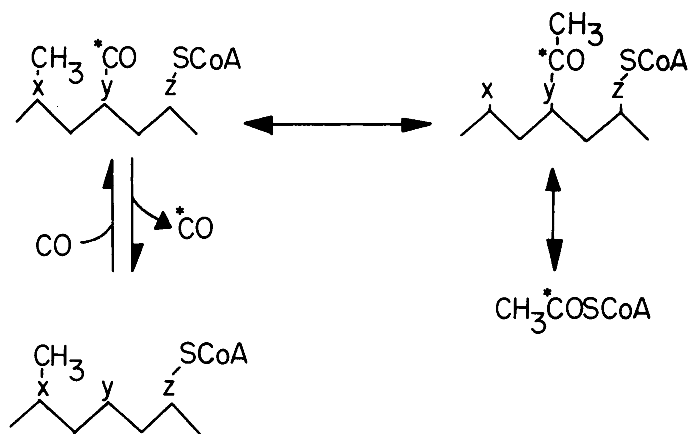


Figure 6. Illustration of the mechanism of the exchange of CO with the carbonyl group of $[1\text{-}^{14}C]$acetyl-CoA as catalyzed by CO dehydrogenase (CODH). The zigzag line represents the CODH and X, Y, Z, three binding sites, respectively, for methyl, carbonyl, and CoA groups of the CODH.

Ragsdale, in Ljungdahl's laboratory at the University of Georgia, had isolated CO dehydrogenase from *C. thermoaceticum.* He came to our laboratory as a postdoctoral fellow. During the mass spectrometer studies in 1952, I had found that *C. thermoaceticum* catalyzes an exchange of $^{14}CO_2$ with the carboxyl of acetate. I wondered if the isolated enzyme system could catalyze such an exchange. When Ragsdale investigated this exchange, he found that only CO dehydrogenase was required and adding the corrinoid enzyme had no effect on the exchange. The results were revolutionary (26). We concluded CO dehydrogenase is the central enzyme of the pathway of acetyl-CoA synthesis instead of the corrinoid enzyme as we had considered previously. Our interpretation of the results of the experiments is presented in **Fig. 6.** In this scheme, the zigzag line represents CODH and X, Y, Z represent three sites on the protein of CODH. The experiment consists of incubating in a closed system $[1\text{-}^{14}C]$acetyl-CoA and unlabeled CO. The observed results are that the CO becomes radioactive and the acetyl-CoA loses ^{14}C but there is no decrease in its concentration. For unlabeled CO to acquire ^{14}C from the $[1\text{-}^{14}C]$acetyl-CoA, the carbon-carbon bond of the acetyl-CoA and also the carbon sulfur bond must be cleaved. It is proposed that the CoA binds to the Z site of the CODH and the acetyl group to the Y site, and then the methyl is transferred to the X site. Then the ^{14}CO dissociates from the Y site and mixes with the unlabeled CO. As no acceptors are added to bind the resulting methyl or CoA group, there apparently are sites on the CO dehydrogenase per se for binding the methyl and CoA in addition to that for the CO. Therefore, the three sites were proposed for CODH (26). As the unlabeled CO acquired ^{14}C and the acetyl-CoA lost ^{14}C and there was no decrease in the amount of acetyl-CoA, it was clear that unlabeled carbon from the pool of unlabeled CO had entered acetyl-CoA.

[1]Abbreviations: CODH, carbon monoxide dehydrogenase; THF, tetrahydrofolate; CoA, coenzyme A.

Thus, it was evident the reaction is reversible. These results showed that CO dehydrogenase catalyzes two reactions: *1*) the reversible oxidation of CO to CO_2 (Reaction 3,) and *2*) the synthesis of acetyl-CoA from methyl, CO, and CoASH groups (Reaction 4). A more proper name for the enzyme would be the CO dehydrogenase-acetyl-CoA synthetase.

Let us now consider the overall pathway of acetyl-CoA synthesis. An outline of the proposed pathway of autotrophic growth via synthesis of acetyl-CoA is shown in **Fig. 7.** This is a greatly oversimplified scheme. The object is to present the general concept of the pathway. The CO_2 enters via two routes. In one, the CO_2 is reduced via formate to CH_3THF. H_2 via hydrogenase or CO by oxidation to CO_2 via CODH serves as the source of electrons in these reductions. Ljungdahl and his colleagues have isolated from *C. thermoaceticum* all enzymes required for the conversion of CO_2 to CH_3THF (27, 28). The methyl of CH_3THF is transferred to the cobalt on the corrinoid enzyme by the methyltransferase. The methyl is then transferred to the X site on the CODH.

The second CO_2 is reduced to CO via CODH, with H_2 serving as the source of electrons via hydrogenase. The CO is bound to the Y site of CODH. If CO is the source of carbon, it combines directly with the Y site of CODH. CoA combines with the Z site and then acetyl-CoA is formed by the CODH. This conversion is facilitated by carbon monoxide dehydrogenase disulfide reductase, which was isolated by Pezacka in our laboratory (29). The acetyl-CoA is used in the anabolic reactions or is converted via acetylphosphate to acetate, after which the cycle is repeated. For anabolism, the acetyl-CoA is converted to pyruvate by CO_2 fixation and from pyruvate, oxalacetate is made by CO_2 fixation; by an additional series of reactions the bacteria are able to synthesize their cellular components. The energy requirements of this pathway have been investigated by Lars Ljungdahl and colleagues. Using membranes of *C. thermoaceticum*, they (28, 30, 31) obtained evidence of a proton pump that promotes the synthesis of ATP. The protons generated from H_2 by hydrogenase or from CO by CODH drive this reaction.

A more complete representation of the pathway has been presented (28), but for our purpose it is not essential to consider each of the individual steps.

It is gratifying after all these years to have been able to isolate the enzymes that catalyze the synthesis of acetyl-CoA from CH_3THF, CoASH, CO or CO_2, and H_2 and thereby to work out the broad details of this pathway. I am pleased

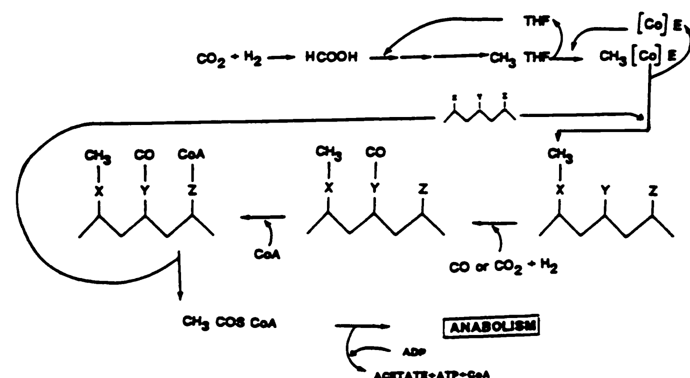


Figure 7. A simplified scheme of the mechanism of growth with CO or CO_2 and H_2 as the source of carbon and energy. THF is tetrahydrofolate, [Co]E is corrinoid enzyme, X Y Z is CO dehydrogenase with its three binding sites.

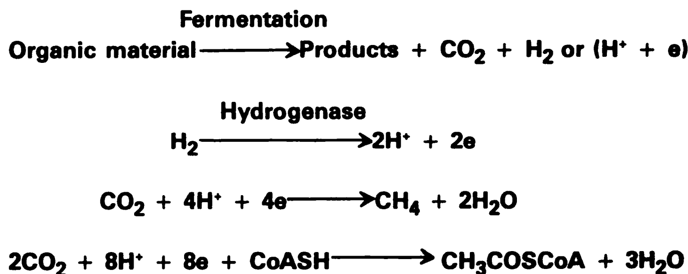


Figure 8. Examples of CO_2 serving as the electron acceptor (electron sink) for anaerobic fermentations.

some investigators have chosen to call this the Wood pathway (25, 27, 32, 33); the acetogenic pathway (or pathways) joins the Calvin cycle as a major means of autotrophic growth.

Although the details of this pathway have been derived largely from studies with *C. thermoaceticum*, more and more bacteria that produce acetate from CO_2 are being described, particularly in recent years. It is becoming apparent now that this pathway may play a significant role in the overall carbon cycle. Let us consider the findings in general terms.

Anaerobes, which grow in the absence of O_2, must use some electron acceptor in place of the O_2 for oxidation to obtain energy for growth. The acceptor is sometimes called the electron sink. The lactic acid bacteria use pyruvate as the electron sink and form lactate; yeast uses acetaldehyde and forms ethanol. Some anaerobes use CO_2 for this purpose. In **Fig. 8** two examples are shown in which CO_2 itself serves as the electron sink. In one, CO_2 is reduced to methane. The methane bacteria thrive in the sludges of swamps and lake bottoms, and if you are a duck hunter you have seen bubbles of methane rising to the surface of the swamp. For that reason, methane has sometimes been called swamp gas.

In the second type, the CO_2 is reduced to acetyl-CoA. The mechanism of this reduction is considered to be by the pathway we have been discussing.

A recent exciting development is that it is becoming apparent that CO_2 is reduced to acetate in humans. Meyer J. Wolin and co-workers at the New York Department of Health have analyzed methane in the breath of humans. Some do not produce methane. Wolin and co-workers found that in people who do not produce methane, the microflora of the gut catalyze the conversion of CO_2 to acetate, whereas the microflora of the gut of humans that form methane convert CO_2 and H_2 to methane (34), Wolin (private communication) estimates 13,000 metric tons of acetate are produced per day by bacteria in the gut by the world's total human population.

John Breznak and M. D. Kane (35) have shown that in the gut of termites there are bacteria that reduce CO_2 to acetate rather than to methane. Termites use acetate that is produced by fermentation as the principal source of carbon for growth. The reduction of CO_2 to acetate provides these insects with about one-third of their total acetate. It is very efficient metabolism. Breznak and Kane estimate 2.3×10^6 metric tons of acetate are produced in the hind gut of termites per day. Acetogens are found in the guts of many animals; ref 34 and 35 summarize the literature.

Wouldn't it be marvelous if the microflora of the cow's stomach could be altered from methanogens to acetogens? Carbon would not be wasted in the form of methane and the resulting acetate could be used as a source of energy by the cows. Such cows would be a boon to farms.

CARBON MONOXIDE DEHYDROGENASE

CODH is the central enzyme of the acetyl-CoA pathway and the enzyme from *C. thermoaceticum* has separate binding sites for CO, the CH_3 group, and CoASH.

CODH is made up of an α subunit of molecular weight 78,000 and a β subunit of 71,000. Each dimer of the hexamer contains 1 zinc, 2 nickels, 11 irons, and 15 inorganic sulfurs. CO binds at a Ni, Fe site of CODH. The evidence is based on electron spin resonance. This is a special subject in itself, which we cannot consider in detail. The investigations have been done largely by Ragsdale and Ljungdahl and co-workers. They first showed that when the enzyme is treated with CO, a novel ESR signal is observed, and when the cells are grown on ^{61}Ni there is a hyperfine broadening of the signal, thus indicating that a Ni-carbon complex is formed (36).

The effect of ^{57}Fe and ^{13}CO on the electron spin has been determined and there is hyperfine broadening of the ESR spectrum with either ^{57}Fe or ^{13}CO. These results are taken to indicate CO reacts at the Ni, Fe center of CODH (37). Recently, Ragsdale and co-workers (38, 39) have reported detailed studies of this center using electron spin resonance and Mossbauer spectroscopy.

Shanmugasundaram and Kumar (40), in our laboratory, have presented evidence that a tryptophan is involved at the CoA site. They used the fluorescent probe, 2,4-dinitrophenylsulfenyl chloride, to label the tryptophans of CODH in the presence and absence of CoA (41). In the presence of CoA, one of the tryptophan peptides is not labeled and is apparently at the CoA site. The amino acid sequence of the peptide from this site is: Ile His Asp Phe Ile Asn Tyr Gly Glu Gly Leu Trp His Thr Gly Gln Arg.

There also is evidence that an arginine residue is involved in the binding of CoA to CODH (26, 42). Phenylglyoxal, an arginine-specific reagent, inactivates CODH and CoA gives 80–85% protection against this inactivation. It was observed (42) that of a series of CoA analogs, only 3'-dephospho-CoA and adenine caused quenching of the tryptophan fluorescence of the CODH (not pantothenic acid, cysteamine, ribose, or inorganic pyrophosphate). This is taken as evidence that it is the adenine of the CoA that interacts with the tryptophan of CODH. In addition, of the analogs of CoA, only 3'-phospho-CoA and inorganic pyrophosphate protected CODH against the inactivation by phenylglyoxal. Therefore it was proposed that the pyrophosphate bridge of the CoA binds to arginine and the inorganic pyrophosphate protects against inactivation by phenylglyoxal by binding to the functional arginines.

Pezacka (43), of our laboratory, recently investigated the binding of the methyl group to CODH. She prepared $^{14}CH_3$-corrinoid enzyme and transferred the methyl to CODH and isolated the methylated CODH. She then demonstrated that the $^{14}CH_3$-CODH, when incubated with CO and CoA, yields $^{14}CH_3$-acetyl-CoA. Thus, there is no doubt CODH per se can catalyze the synthesis of acetyl-CoA.

By hydrolysis of the $^{14}CH_3$-CODH to amino acids with HCl, she showed that ^{14}C is obtained as an S-$^{14}CH_3$ cysteine. Furthermore, after formation of [^{14}C]acetyl-CoA with CO and CoA, and the CH_3-CODH, she showed that S-$^{14}CH_3$ cysteine was no longer formed when the remaining CODH was hydrolyzed to amino acids with HCl.

These results appeared to present clear evidence that the X site involves formation of S-CH_3 cysteine. However, by enzymatic hydrolysis with proteases, we have been unable to isolate S-$^{14}CH_3$-cysteine or $^{14}CH_3$ labeled peptides. Ragsdale who is now at the University of Wisconsin, Milwaukee, along with Lu and Harder (44) have shown that the methylation of CODH is greatly facilitated by a low potential (−400 mV). They consider this evidence that the metal site of CODH is involved in the binding of methyl to CODH, and they suggest that formation of S-$^{14}CH_3$-cysteine may be a dead-end side reaction. Time will tell.

The gene cluster containing CODH, the corrinoid enzyme, and methyltransferase has been cloned but only the methyltransferase was active (32). More recently, the α and β subunits of CODH have been cloned by Ragsdale, Ljungdahl and colleagues, and their sequences have been determined (45). So far, expression of monomers, which are active in reconstituting enzymatically active CODH, has not been accomplished. We hope to identify amino acid sequences at the active sites of CODH and then in collaboration with Ragsdale and Ljungdahl to make deletions and point mutations and explore the amino acid sequences that are required for catalysis by this intriguing enzyme, CODH.

Many investigations of other acetogenic bacteria have been done by Rolf Thauer and Georg Fuchs of Germany and Terry Stadtman of the U.S., especially with the methane and sulfur bacteria (see refs 28, 46–49) for reviews and references). All these organisms contain requisite enzymes such as the corrinoid enzyme and CODH, but in none has the acetyl-CoA pathway been demonstrated with purified enzymes. The biggest problem is that no one has demonstrated that CODH from these organisms catalyze the reversible exchange of CO with [1-^{14}C]acetyl-CoA (49). It may be that a second protein is required in addition to the CODH of these organisms for the exchange reaction to occur and for synthesis of acetyl-CoA. But the scheme presented here forms the basis for consideration of possible pathways. Perhaps the pathways occur with some modification.

Lipmann once said in a review (50), "A search of bacteria might uncover fossils of the earliest forms of life." The conversion of two CO_2 to acetyl-CoA is the most direct pathway possible for autotroph growth. It avoids all the complex reactions of the Calvin cycle. On the earth's crust, CO, Ni, Fe, tungsten, and selenium were all present. These are present in the metalloenzymes that catalyze the acetyl-CoA pathway.

In studies with the propionic acid bacteria, we found that inorganic pyrophosphate and inorganic polyphosphate are used by certain enzymes in place of ATP (51). Perhaps we are uncovering some reactions used by primitive forms of life before the use of ATP was developed and before CO_2 was used by the Calvin cycle.

The results I have presented are those of a large group. I owe much of my success to my collaborators. There have been too many collaborators during the 40 years we have investigated this pathway to consider each individual, so I cannot give them the credit they deserve. I still try to contribute by working at the lab bench with my own hands. We expect to apply the powerful tools of molecular biology now that we have some idea about the mechanism and enzymes and where best to attack the problems. FJ

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