The Relationship of Cobalt Requirement to Propionate Metabolism in *Rhizobium*

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(Received for publication, February 3, 1964)

Cobalt is known to be essential for symbiotic nitrogen fixation by legumes (1–5), but a requirement of the element for growth of leguminous plants supplied with adequate fixed nitrogen has not been shown. Ahmed and Evans (3) provided evidence that cobalt was required for the bacteria within the nodules of inoculated legumes but was not required for the leguminous plants per se. Lowe, Evans, and Ahmed (6) and Lowe and Evans (7) subsequently showed that cobalt is necessary for normal growth of five species of *Rhizobium*. More recently, Kliewer and Evans (8–10) analyzed nodules of a variety of symbionts and cells from several species of *Rhizobium* and observed the presence of cobamide coenzymes in each of the organisms. In addition, they (9) isolated and identified the dimethylbenzimidazolylcobamide coenzyme in extracts of cells isolated from soybean nodules and in cells of a pure culture of *Rhizobium* and observed the presence of cobamide coenzymes in each of the organisms. The *B*23 coenzyme has been shown to be involved in propionate metabolism in animals (11–13) and in *Propionibacterium shermanii* (14, 15). In addition, Arnstein and White (16) have shown that *B*23 deficiency in *Ochromonas malhamensis* resulted in a reduced capacity of the organism to oxidize propionate. Ayers (17) has described similar effects of *B*23 deficiency in a *Flavobacterium* species. On the basis of these reports, experiments were conducted to determine whether *Rhizobium* would metabolize propionate and the possible effect of cobalt deficiency on propionate utilization in these bacteria. In a preliminary communica-

**EXPERIMENTAL PROCEDURE**

**Biological Materials**—The biological materials utilized in this investigation were: soybeans (*Glycine max* (L.) Merr. var. Chippewa), peas (*Pisum sativum* L. var. American Wonder), lupine (*Lupinus angustifolius* L. var. Dorre Blue), cowpeas (*Vigna sinensis* Savi, var. Iron Clay), *Rhizobium meliloti* F-29, *Rhizobium japonicum* 61A76, *Rhizobium phaseoli* K-11, *Rhizobium leguminosarum* C-56, and *Rhizobium trifolii* P-28. The seeds of the various legumes were purchased from commercial sources and the *Rhizobium* species were kindly supplied by Dr. J. C. Burton of the Nitragin Company, Milwaukee, Wisconsin.

Reagents—ATP (dipotassium salt) and GSH were obtained from Nutritional Biochemical Corporation, coenzyme A from Sigma Chemical Company, and propionate-1-*14C*, propionate-2-*14C*, and propionate-3-*14C* from New England Nuclear Corporation. The adenylcobamide coenzyme was a gift from Dr. David Perlman, Squibb Institute for Medical Research, New Brunswick, New Jersey, and the dimethylbenzimidazolylcobamide coenzyme was kindly supplied by Dr. Karl Folkers, Merck Institute for Therapeutic Research, Rahway, New Jersey. All other chemicals were reagent grade and were purchased from commercial sources.

**Cultural Procedures**—Seeds of the legumes, inoculated with the appropriate *Rhizobium* species, were grown in a greenhouse according to the cultural method described by Ahmed and Evans (2, 3). Nodules were harvested from roots of plants at ages listed in the legends of figures and tables that present the results of the various experiments.

Medium A, used for the culture of *R. japonicum*, *R. phaseoli*, *R. leguminosarum*, and *R. trifolii*, contained the following components in a final volume of 1 liter: *K*2*HPO*4, 1.00 g; *KH*2*PO*4, 1.00 g; *MgSO*4·7*H*2*O*, 0.36 g; *CaSO*4·2*H*2*O*, 0.13 g; *KNO*3, 0.70 g; *FeCl*2·6*H*2*O*, 4 mg; yeast extract (Difco), 1.00 g; *L*-arabinose, 1 g; glycerol, 4 ml. The components of the medium were dissolved in distilled water and the pH was adjusted to 6.3 with 1 N *HCl* or 1 N *NaOH*.

Medium B, which was used in certain experiments for the culture of *R. meliloti*, was the same as Medium A except that *L*-arabinose, glycerol, and *KNO*3 were omitted and 3.00 g of *n*-mannitol and 0.06 mg of *CoCl*2·6*H*2*O* were added to each liter. The pH was adjusted to 6.8.

Media C and D were identical with Media A and B, respec-

* This is technical paper 1776 of the Oregon Agricultural Experiment Station. This investigation was supported in part by Research Grant G18556 to Harold J. Evans from the National Science Foundation.

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The method employed for purifying components of the culture medium and the cultural procedure used for the growth of *R.* *meliloti* with varying concentrations of added cobalt have been described in previous communications (2, 3, 7, 18).

The various *Rhizobium* species were transferred from slants to 50-ml culture flasks containing 20 ml of the appropriate medium. When the organisms reached the log phase of growth, a series of 1-liter flasks, each containing 400 ml of the appropriate medium, were inoculated with 4 ml of the appropriate culture. For the study of cobalt deficiency, it was necessary to transfer at least one-half the quantity of all components listed. The flasks were shaken continuously in a controlled temperature bath at 30°. The air flow was maintained at 00 ml per minute and the 14C1 was trapped in 10 ml of a mixture composed of 2 parts absolute ethanol and 1 part 2-aminoethanol. Samples were collected at 20-minute intervals. To determine the amount of 14C1 recovered, the trapping solution was collected and diluted to 15 ml with absolute ethanol. A 5-ml aliquot was placed in a 20-ml counting vial containing 10 ml of phosphor solution (0.3% 2, 5-diphenyloxazole and 0.003% 1, 4-bis-(4-methyl-5-phenyloxazolyl) benzene in toluene). This was counted in a Packard Tri-Carb model 314 DC liquid scintillation counter. Counting was usually carried out to a standard deviation of no greater than 2%. Counting efficiency was determined by use of 14C-toluene as an internal standard.

**Enzyme Assays**—The procedure used for the determination of propionyl and acetate activation is a modification of that described by Berg (25) for the activation of acetate. The complete reaction mixture in a final volume of 1.0 ml consisted of the following components in micromoles: Tris buffer, pH 8.0, 10; Tris-acetate or Tris-propionate, pH 8.0, 10; MgCl2, 20; ATP, 10; GSH, 10; NH4OH-HCl, 20; KCl, 50; coenzyme A, 0.25; and enzyme extract containing 1 to 3 mg of protein. In those experiments in which acetate was used as a substrate, the absorbance at 540 μm was converted to micromoles of acetylated ascorbate with use of the conversion factor reported by Berg (25). Where Tris-propionate was used as the substrate, absorbance measurements at 540 μm were converted to concentrations of propionyl-hydroxamate by use of a standard curve prepared with propionic anhydride (26).

Propionyl-CoA carboxylase was assayed by the procedure described by Lane and Halen (22). The complete reaction mixture in a final volume of 1.0 ml consisted of the following components in micromoles: Tris buffer, pH 8.5, 100; KH2CO3, 15; ATP, 4.0; MgCl2, 4.0; GSH, 5.0; propionyl-CoA, 1.0; and enzyme extract containing 1 to 3 mg of protein. For some experiments, other acyl-CoA compounds were substituted for propionyl-CoA (26).

Methylmalonyl-CoA mutase activity was determined by the procedure of Hegre, Miller, and Lane (28), which involves coupling of a purified propionyl-CoA carboxylase from beef liver and the methylmalonyl-CoA mutase of the enzyme extract. The complete reaction mixture in a final volume of 1.0 ml consisted of the following components in micromoles: Tris buffer, pH 8.5, 100; KH2CO3, 15; ATP, 4.0; MgCl2, 4.0; GSH, 5.0; propionyl-CoA, 1.0; propionyl-CoA carboxylase (20 units per mg of protein), 1 to 3 mg; and enzyme extract containing 0.1 to 1.0 mg of protein. The enzyme extract was added following a 10-minute preincubation at 37° of all other reaction components. An amount of coenzyme listed in the legend of the tables was added to the reaction mixture immediately after the enzyme extract was added. The complete system was then incubated for an additional 20 minutes. The permanganate oxidation and plating procedures were identical with those described by Hegre et al.
RESULTS

Radiorespirometric Experiments—The radiorespirometric patterns for the oxidation of specifically labeled propionate by bacteroids from soybean nodules are shown in Fig. 1. These data show that propionate is oxidized rapidly after an initial slower rate of oxidation. The curves in Figs. 2 and 3 indicate that cell suspensions from pure cultures of *R. japonicum* and *R. meliloti* also oxidize propionate. Fig. 3 shows that propionate oxidation by *R. meliloti* was enhanced by the addition of propionate, succinate, and methylmalonate to the culture medium.

This effect was not observed with cell suspensions of *R. japonicum*. Regardless of the organism tested or the medium used for culture, the patterns of 14CO2 recovery from propionate labeled in different positions were similar. The rate of recovery of 14CO2 from the oxidation of propionate-1-14C was always greater than rates of recovery of 14CO2 from propionate-2- and -3-14C. The rates of recovery of 14CO2 from the latter two compounds were similar.

Since methylmalonyl-CoA mutase from animal tissues (11-13) or from *P. shermanii* (14, 15) has been reported to require cobamide coenzymes, the effect of varying concentrations of cobalt on the capacity of *R. meliloti* to oxidize propionate-1-, -2-, and -3-14C was studied. The results presented in Fig. 4 show trends in propionate-1-14C oxidation that are very similar to those previously obtained with manometric experiments (18). Cobalt-deficient cells exhibited a greatly reduced capacity to oxidize propionate as indicated by the rate of evolution of 14CO2 from propionate-1-14C. The increased rate of formation of 14CO2 from deficient cells that occurred after a lag period may have been caused by contamination of cell suspensions with cobalt, resulting...
in the synthesis of cobamide coenzyme. The effect of the intermediate concentration of cobalt on the rate of oxidation of propionate was not as striking as that previously observed with the manometric experiments (18). The results of radiorespirometric experiments in which propionate-2-14C and -3-14C were utilized as substrates showed trends similar to those obtained with propionate-1-14C and are not presented.

**Enzymes of Propionate Oxidative Pathway**—Propionate utilization by organisms in all known cases is initiated by a reaction with ATP and coenzyme A yielding propionyl-CoA, adipic acid, and pyrophosphate. The enzyme catalyzing this reaction will function with either propionate or acetate (30). In the experiments in which the enzyme activity of cell-free extracts of soybean bacteroids and pure cultures of *R. japonicum* and *R. meliloti* were studied, both acetate and propionate were utilized as substrates. Data are presented in Table I which show that these extracts contained an enzyme system capable of activating both substrates.

The second reaction in the conversion of propionate to succinate is the carboxylation of propionyl-CoA to form methylmalonyl-CoA. With the use of extracts prepared from soybean bacteroids and pure cultures of *R. japonicum* and *R. meliloti*, experiments were conducted to determine if a carboxylase was present which would utilize propionyl-CoA as a substrate.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Soybean bacteroids</th>
<th><em>R. japonicum</em></th>
<th><em>R. meliloti</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl-CoA</td>
<td>0.02</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Butyryl-CoA</td>
<td>0.05</td>
<td>0.23</td>
<td>0.09</td>
</tr>
<tr>
<td>Propionyl-CoA</td>
<td>1.06</td>
<td>1.61</td>
<td>0.21</td>
</tr>
</tbody>
</table>

**TABLE II**

*Requirements of propionyl-CoA carboxylase in cell-free extracts of soybean bacteroids, *R. japonicum*, and *R. meliloti***

Bacteroids were isolated from nodules of 26-day-old soybean plants. *R. japonicum* and *R. meliloti* were grown in Media C and D, respectively. Enzyme activity was measured as described in "Experimental Procedure." The complete reaction mixture minus acetate or propionate served as a negative control. Essentially no activity was obtained when either CoA, enzyme, acetate, or propionate was omitted from reactions.

**TABLE III**

*Substrate specificity of propionyl-CoA carboxylase in cell-free extracts of soybean nodule bacteroids, *R. japonicum*, and *R. meliloti***

Bacteroids were isolated from nodules of 31-day-old soybean plants. *R. japonicum* and *R. meliloti* were grown in Media C and D, respectively. Enzyme activity was measured as described in "Experimental Procedure." The complete reaction mixture minus acyl-CoA served as a negative control.

**TABLE IV**

*Distribution of propionyl-CoA carboxylase and methylmalonyl-CoA mutase in cell-free extracts of bacteroids from nodules of legumes and of cells from pure cultures of *Rhizobium***

Propionyl-CoA carboxylase and methylmalonyl-CoA mutase activity was measured as described in "Experimental Procedure." For the carboxylase assays, the complete reaction mixture minus propionyl-CoA served as negative control. In the methylmalonyl-CoA mutase assay, a complete reaction was grown in Medium B. *R. japonicum*, *R. phaseoli*, *R. leguminosarum*, and *R. trifolii* were grown in Medium A. Bacteroids were collected from nodules of the various legumes grown for the number of days indicated in the table.

From the data presented in Table II, it is apparent that propionyl-CoA was carboxylated and that the enzyme from these organisms required an ATP-Mg++ mixture for activity. The addition or deletion of GSH had no significant effect on the overall carboxylation reaction. Since Halen and Lane (31) have described a carboxylase from beef liver that is specific for propionyl-CoA, experiments were conducted to determine the substrate specificity of the carboxylase in extracts of soybean bacteroids and of pure cultures of *R. japonicum* and *R. meliloti*. The results (Table III) provide evidence that propionyl-CoA is carboxylated at a greater rate than either butyryl-CoA and...
The cell-free extract was prepared from R. meliloti grown in Medium D. Enzyme activity was measured by the method described in “Experimental Procedure.” A complete reaction mixture, see “Experimental Procedure” without enzyme extract served as a negative control for Reactions 1 and 5. In Reactions 2, 3, and 4 and Reactions 6, 7, and 8, a complete reaction mixture without enzyme extract but with AC, BC, or DBC coenzyme as indicated served as the negative controls. The quantity of AC and BC coenzyme added to reaction mixtures (final volume, 1.1 ml) as indicated was 2.6 × 10⁻³ μmole, and the quantity of DBC coenzyme was 2.3 × 10⁻³ μmole. Certain reaction mixtures indicated in the table contained extract that had been illuminated in direct sunlight for 30 minutes at 0°.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Enzyme activity</th>
<th>μmole H⁺CO₂ fixed into permanganate compounds/mg protein/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Complete</td>
<td></td>
<td>1.17</td>
</tr>
<tr>
<td>2. Complete + AC</td>
<td></td>
<td>1.06</td>
</tr>
<tr>
<td>3. Complete + BC</td>
<td></td>
<td>1.26</td>
</tr>
<tr>
<td>4. Complete + DBC</td>
<td></td>
<td>1.23</td>
</tr>
<tr>
<td>5. Complete, extract illuminated</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>6. Complete, extract illuminated, + AC</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>7. Complete, extract illuminated, + BC</td>
<td></td>
<td>0.48</td>
</tr>
<tr>
<td>8. Complete, extract illuminated, + DBC</td>
<td></td>
<td>0.78</td>
</tr>
</tbody>
</table>

The effects of varying concentrations of cobalt in the growth medium on the activities of propionyl-CoA carboxylase and methylmalonyl-CoA mutase in cell-free extracts of R. meliloti

Propionyl-CoA carboxylase and methylmalonyl-CoA mutase activity was measured as described in “Experimental Procedure.” For the propionyl-CoA carboxylase assays, the complete reaction mixture without propionyl-CoA served as negative control. For the methylmalonyl-CoA mutase assays in which the coenzyme was omitted, the complete reaction mixture without enzyme extract served as negative control. For the reaction mixture to which DBC coenzyme was added, the complete reaction mixture without enzyme extract plus coenzyme served as the negative control. The quantity of DBC coenzyme added to the reaction mixture (final volume, 1.1 ml) was 1.65 × 10⁻³ μmole. The abbreviations p.p.b. refers to micrograms per liter.

<table>
<thead>
<tr>
<th>Concentration of cobalt added to culture medium</th>
<th>Propionyl-CoA carboxylase activity</th>
<th>Methylmalonyl-CoA mutase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.p.b.</td>
<td>μmole H⁺CO₂ fixed/mg protein/hr</td>
<td>μmole H⁺CO₂ fixed into permanganate stable compounds/mg protein/hr</td>
</tr>
<tr>
<td>None</td>
<td>1.83</td>
<td>0.00</td>
</tr>
<tr>
<td>0.01</td>
<td>2.70</td>
<td>0.09</td>
</tr>
<tr>
<td>1.00</td>
<td>2.41</td>
<td>1.07</td>
</tr>
</tbody>
</table>

The abbreviations used for the cobamide coenzymes are: DBC, dimethylbenzimidazolylcobamide; BC, benzimidazolylcobamide; AC, adenyloleibamide.

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These results suggest that formation of the apoenzyme is independent of the cobalt supply, but that the catalytic activity of the enzyme may be limited by coenzyme concentration and that synthesis of the coenzyme is directly dependent on cobalt. The data obtained from the radiorespirometric (Fig. 4) and manometric experiments (18) support this conclusion.

Identification of the products of the methylmalonyl-CoA mutase reaction (complete reaction effected with cells grown with 1 p.p.b. of cobalt, Table VI) showed that after the permanganate treatment only succinate was radioactive; however, if the reaction mixture was extracted and chromatographed prior to the permanganate treatment methylmalonate also was found to be radioactive.

An experiment was conducted with normal R. meliloti cells to determine the fate of 14C from sodium propionate-1-14C when this compound was supplied to intact cells. As shown in Fig. 5, the major compounds that were radioactive after a 10-minute incubation period with sodium propionate-1-14C corresponded with malate, succinate, methylmalonate, and fumarate in cochromatography experiments. These data are consistent with the other evidence indicating that propionate is converted to succinate via the propionyl-CoA carboxylase and methylmalonyl-CoA mutase route. The pathway that has been reported to be operative in Clostridium kluyveri (36) and which involves the same intermediates as the higher plant system would be expected to result in the most rapid recovery of the methyl carbon of propionate followed in turn by the carboxyl carbon and then the methylene carbon. In contrast, in the pathway whereby propionate is converted to succinate via methylmalonate (20), the expected rate of recovery is greatest for carbon 1. The recovery of carbon atoms 2 and 3 is expected to be equal since they become the methylene carbon atoms of succinate and should become randomized in the citric acid cycle.

To obtain information on the capacity of rhizobia to oxidize propionate and on the possible pathway whereby it is utilized, a series of radiorespirometric experiments were performed with propionate labeled in specific positions. It was found (Figs. 1, 2, and 3) that the rate of recovery of 14C as 14CO2 from propionate labeled in different positions was greatest when the cells were incubated with propionate-1-14C. In addition, the radiorespirometric patterns for 14CO2 formation from propionate-2-14C and propionate-3-14C were essentially the same regardless of the conditions or the Rhizobium species used. These patterns of recovery would be expected only if propionate were utilized by a sequence of reactions in which it was converted to succinate and then oxidized via the citric acid cycle.

When R. meliloti was grown with varying concentrations of cobalt in the culture medium, the capacity to oxidize propionate was significantly reduced in cobalt-deficient cells (Fig. 4). The rate of oxidation of propionate by the cells was strikingly increased by an increasing cobalt supply in the culture medium. Arnstein and White (16), using O. malhamensis, and Ayers (17), using Flavobacterium sp., have conducted similar experiments in which they showed an increase in the rate of propagation oxidation of vitamin B12-deficient cells by the addition of vitamin B12 and certain B12 analogues to cell suspensions. Experiments were conducted, therefore, to determine whether B12 compounds would restore the capacity of cobalt-deficient R. meliloti cells to oxidize propionate. In each instance, experiments of this type proved
to be negative. In contrast, the addition of B_{12} coenzyme to extracts of cobalt-deficient *R. meliloti* restored activity of methylmalonyl-CoA mutase to a normal level (Table VI). It is apparent, therefore, that cobalt-deficient cells contained a normal level of the apoenzyme of methylmalonyl-CoA mutase and that the content of B_{12} coenzyme was the limiting factor. These findings are consistent with the report of Kliewer and Evans (10) showing that cobalt deficiency decreased the cobamide coenzyme content of *Rhizobium*.

The results presented (Tables II and IV) show that cells from pure cultures of *R. meliloti*, *R. japonicum*, and bacteroids from soybean nodules have the necessary enzymes to convert propionate to succinate via a pathway in which methylmalonate is an intermediate. Detailed studies of the properties of the propionyl-CoA carboxylase from these sources provided evidence (Table III) that the enzyme preferentially carboxylated propionyl-CoA. The enzyme from these organisms, therefore, exhibits properties similar to those of the propionyl-CoA carboxylase purified from beef liver (31) and pig heart (37). Propionyl-CoA carboxylase is widely distributed in bacteroids from nodules of leguminous species and in pure cultures of *Rhizobium* (Table IV), and it is suggested that the enzyme may play an important role in the metabolism of these organisms.

Methylmalonyl-CoA mutase activity was observed in a variety of nodules of legumes and in pure cultures of *Rhizobium* (Table IV). An examination of various fractions from soybean nodules and soybean roots indicated activity only in the nodule bacteroids. It is of interest to compare the properties of the methylmalonyl-CoA mutase in a crude extract from *R. meliloti* with those reported for the methylmalonyl-CoA from other sources. Lengyel, Manzumer, and Ochoa (13) made a comparative study of the properties of methylmalonyl-CoA mutase isolated from both sheep kidney and *P. shermanii*. They observed that activity was restored to the coenzyme-free enzyme from sheep kidney by the addition of the DBC and BC coenzymes. The apoenzyme from sheep kidney showed the strongest affinities for the DBC and BC coenzymes but was inactive with the AC coenzyme. In contrast, the activity of the coenzyme-free enzyme from *P. shermanii* was restored by the addition of each of the three of the coenzymes. From our experiments with the coenzyme-free methylmalonyl-CoA mutase from *R. meliloti*, it is apparent that the greatest activation was obtained by the DBC coenzyme and less activation by the BC coenzyme (Table V).

The *R. meliloti* enzyme, therefore, behaved like the enzyme from sheep kidney in that the AC coenzyme was inactive. The methylmalonyl-CoA mutase isolated from *R. meliloti* was similar to the enzyme from *P. shermanii* in that the endogenous B_{12} coenzyme of both was easily destroyed by light. In contrast, the enzyme from the sheep kidney could be resolved into a coenzyme-free apoenzyme only by acid precipitation of the enzyme from ammonium sulfate solution (13). Further characterization of the methylmalonyl-CoA mutase from *Rhizobium* must be delayed until a highly purified preparation is obtained from this source.

**SUMMARY**

An investigation was conducted to determine biochemical sites at which cobalt, in the form of B_{12} coenzyme, functions in the metabolism of legumes and their associated *Rhizobium* species. The results of these experiments may be summarized as follows.

1. Data are presented which indicate that propionate is oxidized by cell suspensions of bacteroids from soybean nodules and of *Rhizobium japonicum* and *Rhizobium meliloti*. The oxidation of propionate by cell suspensions of *R. meliloti* was enhanced by the addition of sodium propionate, succinate, and methylmalonate to the culture medium, whereas, the addition of these organic acids to the culture media of *R. japonicum* failed to produce this effect.

2. The rate of propionate oxidation by cell suspensions of *R. meliloti* grown with adequate cobalt was markedly greater than that of cells grown in a cobalt-deficient medium.

3. The radiorespirometric patterns obtained by incubation of propionate labeled in specific positions with cell suspensions of *R. meliloti*, *R. japonicum*, and soybean bacteroids are consistent with expected patterns if propionate was converted to succinate via methylmalonate and was oxidized by the citric acid cycle.

4. Cell-free extracts of *R. meliloti*, *R. japonicum*, and soybean bacteroids exhibit the capacity to catalyze (a) the activation of propionate and acetate, (b) the carboxylation of propionyl coenzyme A, and (c) the conversion of methylmalonate to succinate.

5. The methylmalonyl coenzyme A mutase in extracts of *R. meliloti* was easily inactivated by exposure of the extracts to direct light. Enzyme activity was restored by the addition of dimethylbenzimidazolylcobamide or benzimidazolylcobamide coenzyme to the extracts but the adenylcobamide coenzyme was ineffective in restoring activity.

6. Propionyl coenzyme A carboxylase activity in extracts of *R. meliloti* cells was not greatly affected by the concentration of cobalt in the medium in which cells were grown. In contrast, the activity of the methylmalonyl coenzyme A mutase in the extracts was strikingly influenced by the cobalt content of the culture medium. Methylmalonyl coenzyme A mutase activity, comparable to that obtained with extracts from cells grown with adequate cobalt, was obtained by the addition of dimethylbenzimidazolylcobamide coenzyme to enzyme extracts of cobald-deficient cells or to extracts of cells grown with 0.01 p.p.b. of cobalt.

It is concluded, therefore, that cobalt deficiency in *R. meliloti* prevents the synthesis of quantities of B_{12} coenzyme adequate for the normal function of methylmalonyl coenzyme A mutase and that the inactive mutase results in the failure of the organism to oxidize propionate.

**REFERENCES**

The Relationship of Cobalt Requirement to Propionate Metabolism in Rhizobium
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