THE INCORPORATION OF CARBOXYL AND BICARBONATE CARBON INTO GLUTAMIC ACID BY THE RAT*

BY ROGER E. KOEPPE AND ROBERT J. HILL

(From the Department of Biochemistry, University of Tennessee, Memphis, Tennessee)

(Received for publication, January 28, 1955)

The isotope pattern found in rat liver glycogen following administration of a variety of carbon-labeled compounds has been used by Wood and his coworkers (1-6) in studies concerning carbon metabolism. Experiments of a similar nature involving glutamic acid as the target molecule will be described in this paper.

Its relative abundance, ease of isolation, and interconversion in vivo with α-ketoglutarate, a key intermediate in the Krebs tricarboxylic acid cycle, make glutamate a useful compound for studying metabolic pathways. Such studies with microorganisms (7-11) and rats (12-14) have been described. However, no data are available concerning the complete isotope pattern found in glutamate derived from mammalian tissue following the administration in vivo of carbon-labeled substrates.

With the exception of purine synthesis, those in vivo mammalian CO₂ fixation reactions which result in synthesis of carbon to carbon bonds involve compounds of the Krebs tricarboxylic acid cycle. The principal reaction is the formation of oxalacetic (15) or malic acid (16). Fixation of C¹⁴O₂ in this manner gives rise to α-ketoglutarate, and hence glutamate, labeled only in carbon 1 (17-21). It would be predicted, therefore, that carboxyl-labeled 4-carbon dicarboxylic acids (oxalacetic), 3-carbon glycolytic acids (pyruvic), or their biological precursors (aspartic acid, alanine) would also give rise to glutamic acid labeled only in carbon 1. However, administration of mβ-glyceric acid-1-C¹⁴, bicarbonate-C¹⁴ (12), and C¹⁴O₂ to rats (13), and mβ-aspartic acid-4-C¹⁴ to rabbits, resulted in tissue glutamic acid having as low as 70 to 83 per cent of its total radioactivity located in carbon 1. Since in none of these investigations was a complete degradation of glutamate accomplished, the location of activity other than in carbon 1 is not known.

* Aided by a grant from the National Science Foundation. Presented in part at the Thirty-eighth annual meeting of the Federation of American Societies for Experimental Biology at Atlantic City, April, 1954.

1 The International unit carbon-numbering system for glutaric acid derivatives will be used throughout the presentation. Thus the α-carboxyl of glutamate is designated carbon 1 and γ-carboxyl, carbon 5.

2 Sallach, H. J., and Rose, W. C., personal communication.

3 Koeppe, R. E., and Rose, W. C., unpublished data.
To elucidate further the pathway of incorporation of carbon dioxide and carboxyl carbon into glutamate, bicarbonate-C\textsuperscript{14}, DL-alanine-1-C\textsuperscript{14}, and glycine-1-C\textsuperscript{14} have been injected into rats, tissue glutamate has been isolated and degraded, and each carbon assayed for radioactivity. Although most of the radioactivity of the isolated glutamic acid was located in carbon 1, significant activity was also present in carbon 5, while trace amounts were found in positions 2, 3, and 4.

In contrast, administration of acetate-1-C\textsuperscript{14} gave rise to glutamic acid labeled predominantly in carbon 5 and having the remaining one-third of its activity in carbon 1.

**EXPERIMENTAL**

Male albino rats (of Wistar origin) from our own colony were used in these investigations. The various isotopic compounds were dissolved in saline and administered by intraperitoneal injection. During the period between injection and sacrifice the animals were placed in an all-glass metabolism chamber which was swept with a slow stream of air. The expired CO\textsubscript{2} was trapped in alkali.

Rats 1 through 9 were fed Purina checkers ad libitum. Rats 11 and 13 were fed, for 2 weeks prior to injection, a mixture of ground Purina checkers and 10 per cent L-leucine, also ad libitum. Their respective litter mates, Rats 10 and 12, were fed ground checkers for the same period. The leucine-fed animals gained weight at a rate very nearly comparable to that of the controls. Rat 9 was partially hepatectomized 3 days before injection.

The isotopic compounds used were DL-alanine-1-C\textsuperscript{14} (Tracerlab, Inc., 1.06 mc. per mmole), sodium acetate-1-C\textsuperscript{14} (Isotopes Specialties Company, Inc., 2.0 mc. per mmole), glycine-1-C\textsuperscript{14} (Isotopes Specialties, 0.82 mc. per mmole), and sodium bicarbonate-C\textsuperscript{14} (Nuclear Instrument and Chemical Corporation, 1.54 mc. per mmole).

Data concerning the quantities injected and the duration of experiments are given in Tables I and II.

**Isolation Procedures**—Immediately after sacrifice of the rat by a blow on the head, the liver was removed and a protein powder prepared from the liver and “carcass” by conventional means involving extraction of the ground tissue with 10 per cent trichloroacetic acid, acetone, and ether. “Carcass” refers to the entire animal, except liver, including the washed gastrointestinal tract.

The carcass protein was hydrolyzed by boiling under a reflux for 24 hours with 10 volumes of 6 N HCl. After filtration with Celite and removal of excess HCl by vacuum distillation, the hydrolysate was brought to pH 2.2 by addition of the free base form of a weak anion resin (Amberlite IR-45,
Rohm and Haas) and treated with charcoal (Nuchar) (22). Glutamic and aspartic acids were then quantitatively removed from the charcoal filtrate by passage through an Amberlite IR-45 column in the acetate cycle.

A partial separation of aspartic and glutamic acids was achieved when the amino acids were eluted from the column with 1 N acetic acid. After evaporation to dryness of the eluates containing glutamic acid, the residue was dissolved in a small volume of water and glutamic acid hydrochloride crystallized by saturation with HCl; crystalline glutamic acid was obtained by neutralization with pyridine and precipitation with ethanol.

Sufficient L-glutamic acid was added to the liver protein powders of Rats 1 to 9 to dilute 10-fold the 12.2 per cent glutamate reported present in rat liver protein (23). This mixture was then hydrolyzed by boiling under a reflux with 6 N HCl. The hydrolysate was concentrated in vacuo to a small volume, decolorized with charcoal, and glutamic acid hydrochloride crystallized by saturation with HCl. Free glutamic acid was then obtained as described above.

Glutamic acid was isolated, without addition of carrier, from the liver protein of Rats 10 to 13 by a method identical to that described above for carcass glutamate. Carrier L-glutamic acid was then added prior to recrystallization and degradation.

Each glutamic acid sample was characterized by paper chromatography, C analysis, and ninhydrin-released CO2. With the exception of the liver glutamates of Rats 1, 3, and 4, all samples were recrystallized to constant specific activity.

_Degradation Procedures_—Carbon 1 of glutamate was liberated as CO2 with ninhydrin and measured manometrically as described by Van Slyke et al. (24).

Glutamic acid was completely degraded by the method of Mosbach, Phares, and Carson (25, 26). This procedure selectively converts each carbon of glutamate to CO2. The authors have experienced only minor difficulties in repeating this excellent method of degrading glutamate. Yields of butyric acid have been found to be more consistent if a pH of 6.5 to 7.0 is reached prior to treatment with chloramine-T. Also, it has been helpful, following oxidation of amines, to remove excess permanganate by the addition of FeSO4. As a further check on radioactivity in carbon 5, we have run Schmidt reactions (25) on glutamic acid by using a 3-fold excess of azide. Approximately 50 to 70 per cent yields of CO2 from carbon 5 were obtained by this procedure. Recently Miller and Bale (27) have described a similar reaction.

_Combustion Methods_—The glutamate samples isolated from Rat 1 were converted to CO2 by the persulfate wet oxidation technique of Anthony and Long (28). All others were oxidized by the macrocombustion method of
Van Slyke et al. (29, 30). The amount of CO₂ liberated during degradation and combustion of tissue glutamates isolated from Rat 1 was determined as BaCO₃ (28), whereas that obtained from all subsequent degradations and combustions was measured manometrically according to the method of Van Slyke and Folch (29).

Assay of Radioactivity—All measurements of radioactivity were made with a vibrating reed electrometer (Applied Physics Corporation, Pasadena, California). The BaCO₃ samples obtained from Rat 1 were treated with H₃PO₄ and the liberated CO₂ introduced into an ion chamber as described by Anthony and Long (28). The CO₂ measured manometrically was transferred directly from the Van Slyke apparatus to the ion chamber via a Drierite trap and mercury valve (28, 31). Reproducible data have been obtained readily in this laboratory by use of the wet oxidation manometric technique for total C and the vibrating reed electrometer for radioactivity assay. Liquid nitrogen and dry methane, two essentials of the Van Slyke, Steele, and Plazin method (32), are not required in this procedure, yet the times of analysis and assay are essentially the same. It should be emphasized, however, that samples analyzed in our laboratory have always contained more than 2 mg. of C. Whether equally precise results would be obtained with smaller samples has not been determined.

RESULTS AND DISCUSSION

Results of the glutamate degradations are presented in Tables I and II. The data from Rat 10 differ strikingly from those of the other bicarbonate-treated animals and will be discussed separately. No significant differences were found in the labeling patterns of corresponding liver and carcass glutamates. The data for carcass glutamic acids, which are more complete and more consistent from one animal to another, have been used as the basis for most of the following discussion.

With the exception of those rats which received acetate-1-C¹⁴, most of the C¹⁴ in the rat glutamates was located, as expected, in carbon 1. The high percentage of labeling in carbon 1 is in general agreement with results cited earlier²,⁵ (12, 13). However, all these samples also had appreciable radioactivity in carbon 5. Although in most instances definite activity was detected in positions 2, 3, and 4, the amounts were often small in comparison to the background and hence subject to considerable error. Indeed, the activity found in these carbons is reminiscent of that found in carbons 1, 2, 5, and 6 of liver glycogen by Shreeve et al. (33) after administration of bicarbonate-C¹⁴. How carboxyl or bicarbonate carbon is incorporated into these positions of glutamate and glycogen is not clear. It should be emphasized, however, that the small amount of activity found in position 2, as compared to 5, precludes any considerable conversion in vivo of succinate to α-ketoglutarate.
The finding of significant amounts of radioactivity in carbon 5 following administration of bicarbonate-C\textsuperscript{14} is, to our knowledge, the first demonstration of a CO\textsubscript{2} fixation \textit{in vivo} into this carbon of glutamate. Any mechanism whereby CO\textsubscript{2} is fixed into the carboxyl of acetate would explain labeling in carbon 5 (2, 34). Bicarbonate might be incorporated into the carboxyl of acetate via the conversion of succinate to “acetyl” (35, 36) or the fixation of CO\textsubscript{2} into the carboxyl of acetate during the metabolism of leucine and isovalerate (37-39). Rats 11 and 13 were fed a high leucine diet prior to injection of bicarbonate in an effort to increase CO\textsubscript{2} fixation into carbon 5. Although the expected increase was not observed, the results should not be construed as evidence against the Coon mechanism of leucine metabolism. Much of the ingested leucine may have been excreted as a conjugate or may have been so rapidly oxidized that at the time of injection leucine metabolism had returned to normal. Recently, Hendler and Anfinsen (40)

| Rat weight, gm. | 130 | 155 | 152 | 185 | 170 |
| Dose injected, μg | 100 | 150 | 150 | 150 | 150 |
| Duration, hrs. | 6.5 | 4.0 | 4.0 | 4.0 | 4.0 |
| Per cent injected, exhaled as CO\textsubscript{2} | 85 | 76 | 87 | 85 | 92 |

**Table I**

\textbf{C\textsuperscript{14} Distribution in Rat Glutamic Acid after Administration of NaHCO\textsubscript{3}O\textsubscript{2}}

<table>
<thead>
<tr>
<th></th>
<th>Rat 9\textsuperscript{*}</th>
<th>Rat 10</th>
<th>Rat 11\textsuperscript{†}</th>
<th>Rat 12</th>
<th>Rat 13\textsuperscript{†}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carbon 1</strong>&lt;br&gt;COOH&lt;br&gt;“ 2, CHNH\textsubscript{2}&lt;br&gt;“ 3, CH\textsubscript{2}&lt;br&gt;“ 4, CH\textsubscript{2}&lt;br&gt;“ 5, COOH</td>
<td>11.4</td>
<td>19.8</td>
<td>15.1</td>
<td>11.3</td>
<td>15.1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>13.0</td>
<td>37.5</td>
<td>17.8</td>
<td>14.0</td>
<td>18.2</td>
</tr>
<tr>
<td><strong>Liver</strong>&lt;br&gt;Sum,\textsuperscript{‡} carbons 1-5&lt;br&gt;<strong>Total</strong>&lt;br&gt;Carbon 1, COOH&lt;br&gt;“ 5 ”</td>
<td>69</td>
<td>138</td>
<td>78</td>
<td>54</td>
<td>56</td>
</tr>
<tr>
<td><strong>“ 5 ”</strong>&lt;br&gt;Carbon 1, COOH&lt;br&gt;“ 5 ”</td>
<td>60</td>
<td>91</td>
<td>65</td>
<td>90</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>5.1</td>
<td>22.4</td>
<td>16.2</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.9</td>
</tr>
</tbody>
</table>

\* Partially hepatectomized 3 days before the experiment.

\† For 2 weeks before the experiment these rats were fed a diet supplemented with L-leucine to the extent of 10 per cent.

\‡ The values in the per cent total columns were obtained by dividing the sum of the millimicrocuries per millimole found in each carbon by the total millimicrocuries per millimole and multiplying by 100.

\§ The values from Rats 9, 12, and 13 are corrected for a 10-fold dilution with carrier; those from Rats 10 and 11 for a 20-fold dilution.

of acetate via the conversion of succinate to “acetyl” (35, 36) or the fixation of CO\textsubscript{2} into the carboxyl of acetate during the metabolism of leucine and isovalerate (37-39). Rats 11 and 13 were fed a high leucine diet prior to injection of bicarbonate in an effort to increase CO\textsubscript{2} fixation into carbon 5. Although the expected increase was not observed, the results should not be construed as evidence against the Coon mechanism of leucine metabolism. Much of the ingested leucine may have been excreted as a conjugate or may have been so rapidly oxidized that at the time of injection leucine metabolism had returned to normal. Recently, Hendler and Anfinsen (40)
<table>
<thead>
<tr>
<th></th>
<th>Rat 1</th>
<th>Rat 3</th>
<th>Rat 4</th>
<th>Rat 6</th>
<th>Rat 8</th>
<th>Rat 5</th>
<th>Rat 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat weight, gm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose injected, µg</td>
<td>101</td>
<td>129*</td>
<td>103</td>
<td>105</td>
<td>115</td>
<td>133</td>
<td>130</td>
</tr>
<tr>
<td>Duration, hrs.</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per cent injected, exhaled as CO₂</td>
<td>66</td>
<td>79</td>
<td>72</td>
<td>29</td>
<td>36</td>
<td>64</td>
<td>78</td>
</tr>
</tbody>
</table>

### C₁₄ in glutamic acid

<table>
<thead>
<tr>
<th></th>
<th>C₁₄</th>
<th>Glycine-₁⁻₀⁴</th>
<th>Acetate-₁⁻₀⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat 7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Administered in three equal portions, 3 hours apart.
† These values are corrected for a 10-fold dilution with carrier.
‡ See foot-note to Table I.
have reported the incorporation of $\text{C}^{14}\text{O}_2$ into carbon 5 of glutamate by minces of hen oviduct.

Partial hepatectomy prior to bicarbonate administration (Rat 9) caused no striking change in labeling of either carcass or liver glutamic acid.

It is of interest to compare the labeling patterns produced by the carboxyls of alanine and glycine with those of bicarbonate. The carboxyl carbons may be converted to glutamate via the bicarbonate pool or by a more direct pathway. Prior conversion to bicarbonate would result in a specific activity in glutamate less than that observed with bicarbonate-$\text{C}^{14}$.

**Table III**

Corrected Specific Activity of Carcass Glutamic Acid Carbons after Administration of $\text{NaHC}^{14}\text{O}_3$, Alanine-$1-\text{C}^{14}$, and Glycine-$1-\text{C}^{14}$

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Compound administered</th>
<th>Correction factor*</th>
<th>Corrected specific activity, mcg. per mmol</th>
<th>Total CO$_2$</th>
<th>Carbon 1 COOH</th>
<th>Carbon 2 CH$_3$</th>
<th>Carbon 3 CH$_2$</th>
<th>Carbon 4 CH$_2$</th>
<th>Carbon 5 COOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>9†</td>
<td>$\text{NaHC}^{14}\text{O}_3$</td>
<td>1.53</td>
<td>19.9  17.5</td>
<td>1.87</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>&quot;</td>
<td>1.36</td>
<td>51.0  27.0</td>
<td>10.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11‡</td>
<td>&quot;</td>
<td>1.17</td>
<td>20.8  17.7</td>
<td>2.26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>&quot;</td>
<td>1.44</td>
<td>20.2  16.3</td>
<td>2.19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13‡</td>
<td>&quot;</td>
<td>1.23</td>
<td>22.4  18.6</td>
<td>2.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Alanine-$1-\text{C}^{14}$</td>
<td>3.33</td>
<td>31.3  29.3</td>
<td>1.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>1.76</td>
<td>28.0  25.0</td>
<td>0.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>1.34</td>
<td>27.7  25.0</td>
<td>2.17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Glycine-$1-\text{C}^{14}$</td>
<td>3.98</td>
<td>33.1  27.5</td>
<td>3.94</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>3.14</td>
<td>32.3  28.1</td>
<td>4.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Obtained by dividing the rat weight by the microcuries of $\text{C}^{14}\text{O}_2$ exhaled.
† This rat was partially hepatectomized 3 days before the experiment.
‡ For 2 weeks before the experiment these rats were fed a diet supplemented with L-leucine to the extent of 10 per cent.

However, incorporation into glutamate without previous formation of bicarbonate might lead to a higher specific activity in glutamic acid. Since the animal weights, amounts of activity administered, and rates of $\text{C}^{14}$ excretion are not the same, the data for alanine, glycine, and bicarbonate are more readily analyzed if first corrected for the total bicarbonate-$\text{C}^{14}$ available to each gm. of tissue during the course of the experiment. The microcuries of $\text{C}^{14}$ excreted as CO$_2$ per gm. of body weight of animal are roughly proportional to the correction mentioned above. In Table III, the specific activities of the carcass glutamates have been multiplied by the microcuries excreted per gm. of body weight. The "corrected" data are remarkably constant for each group of animals, Rat 10 again being the exception.
If alanine-1-C¹⁴ and glycine-1-C¹⁴ are converted to glutamate exclusively through bicarbonate, the “corrected” data should be nearly identical for all animals. However, the data indicate that the carboxyls of alanine and glycine yield higher activity in carbon 1 of glutamate than does bicarbonate. This may be explained by the fact that these amino acids can be converted to carboxyl-labeled pyruvate. The latter can give rise to glutamate labeled in carbon 1 (via oxalacetate and fumarate) without prior conversion to bicarbonate. The carboxyl of glycine appears to be a better precursor of carbon 5 than bicarbonate or alanine. This observation is compatible with a rather direct conversion of small amounts of glycine to acetate and offers a plausible explanation for the conversion of glycine-1-C¹⁴ to acetate as reported by Sprinson and Coulon (41).

The results with acetate-1-C¹⁴ are in precise agreement with current concepts of acetate metabolism. A carbon 5 to carbon 1 ratio of 2:1 would be observed after one tour of the Krebs tricarboxylic acid cycle. Wang et al. (10) have obtained comparable results with yeast. It is apparent that glutamate, in contrast to glycogen, can be used to distinguish between metabolic pathways proceeding through the carboxyl of the 2-carbon fragment and those proceeding through the carboxyl of the “trioses.”

The data from Rat 10 are difficult to explain. Careful analysis of this experiment has led the authors to conclude that these apparently anomalous data did not result from experimental error. Although the specific activity of glutamate from Rat 10 is about double that of other bicarbonate-treated animals, the activity in carbon 1 is only slightly greater. The major difference, a 10-fold one, is in carbons 2, 3, and 4. CO₂ fixation leading to a 2,3-labeled triose would explain the results. Recent reports by Horecker et al. (42, 43) provide at least plausible explanations for incorporation of CO₂ into the 2, 3 positions of trioses. However, the magnitude of this incorporation by Rat 10 is surprising and represents the first demonstration of a CO₂ fixation in vivo into all positions of glutamate.

SUMMARY

The pattern of labeling in rat glutamate has been used to study in vivo pathways of carbon metabolism.

Bicarbonate-C¹⁴ has been found to yield glutamic acid labeled predominantly in carbon 1. Significant amounts of C¹⁴ were also observed in carbon 5, probably indicating CO₂ fixation into the carboxyl of acetate. Feeding a high leucine diet did not enhance the incorporation of bicarbonate into carbon 5. With the exception of one rat, which incorporated significant amounts of bicarbonate into all carbons of glutamate, only trace amounts of radioactivity were found in positions 2, 3, and 4 of the isolated glutamates.
The labeling patterns observed in tissue glutamic acid following administration of DL-alanine-1-C\textsuperscript{14} and glycine-1-C\textsuperscript{14} were similar but not identical to those observed with bicarbonate.

Acetate-1-C\textsuperscript{14} yielded glutamate with 65 to 70 per cent of its activity in carbon 5, most of the remaining activity being in carbon 1.

BIBLIOGRAPHY

LABELING PATTERNS IN GLUTAMATE

THE INCORPORATION OF CARBOXYL AND BICARBONATE CARBON INTO GLUTAMIC ACID BY THE RAT
Roger E. Koeppe and Robert J. Hill


Access the most updated version of this article at [http://www.jbc.org/content/216/2/813.citation](http://www.jbc.org/content/216/2/813.citation)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/216/2/813.citation.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/216/2/813.citation.full.html#ref-list-1](http://www.jbc.org/content/216/2/813.citation.full.html#ref-list-1)