Carbon dioxide fixation in the brain / Soll Berl, Genkichiro Takagaki, Donald Dudley Clarke, and Heinrich Waelsch From the New York Psychiatric Institute and the College of Physicians and Surgeons, Columbia University, New York, New York

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Carbon Dioxide Fixation in the Brain*

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Upon intracarotid administration of ammonia to cats, there is a significant increase of cerebral glutamine without a corresponding decrease in the level of cerebral glutamic acid (3). Similar observations in dogs have been reported by others (4). Since the supply of glutamic acid from the circulating blood is insufficient for the formation of the additional amount of glutamine, the dicarboxylic acid has to be synthesized in the brain.1 The continuous utilization of α-ketoglutarate for this purpose would rapidly deplete the citric acid cycle of its intermediates unless they can be replenished by CO₂ fixation. Therefore, the occurrence of significant CO₂ fixation in brain in vivo was assumed (1, 2). Such an assumption seemed particularly justified, since carbon dioxide fixation had been demonstrated in the isolated retina 10 years ago (5), and the presence of the malic enzyme has also been shown in brain tissue (6).

We wish to present experiments in which NaHCO₃ was administered by intracarotid infusion to cats with and without ammonia; the results show a significant fixation of CO₂ into amino acids by the cerebral cortex in vivo.

EXPERIMENTAL PROCEDURE

General Procedures and Materials—The cats were prepared as described previously (7), and glutamine, glutamic and aspartic acids, ABA,² and GSH were isolated from cerebral cortex, liver, and blood by the methods described in the preceding paper (3).

Sodium bicarbonate-C₁⁴ solutions were made up in 0.9% sodium chloride solution with or without Na₂N₁⁵-ammonium acetate (see legends to tables). Sodium bicarbonate-C₁⁴, DL-glutamic acid-1-C₁⁴, DL-aspartic acid-4-C₁⁴, and standard benzoic acid-C₁⁴ were obtained from New England Nuclear Corporation, Waltham, Massachusetts.

The isolated samples of ABA were of low specific activity and contained large amounts of salt; therefore, these samples were desalted (8), and the ABA content was redetermined by the hydroxamate procedure (9) before counting.

The radioactivity of the samples was measured with a Nuclear-Chicago model D-47 thin-window gas flow counter. Aliquots of the various fractions were pipetted on to weighed stainless steel planchettes (1.25 inches in diameter) and dried under an infrared lamp and reweighed. The weight of the sample was used to apply self-absorption corrections. Samples weighing less than 0.1 mg did not require any correction.

Decarboxylation of L-Glutamic Acid—Glutamic acid fractions obtained from Dowex 1 (AG-1) columns were evaporated to dryness in a rotary flash evaporator and taken up in a measured volume of water (usually 1.2 ml). An aliquot (0.5 ml) was placed in a double side-armed Warburg flask and decarboxylated by the procedure of Gale (10) with Escherichia coli ATCC 11246 (Worthington Biochemical Corporation). The side arm containing the enzyme suspension was stopped, and the empty side arm was closed with a gassing tube, which was sealed with a rubber vial cap. When CO₂ release had ceased and the pressure was recorded, the CO₂ was absorbed into 0.2 ml of Hyamine (Packard Instrument Company), which was injected by syringe through the vial cap into the empty side arm. Shaking was continued until the manometer had come to equilibrium (approximately 90 minutes). The flasks were opened, and the Hyamine was transferred to a 20-ml Crystallite vial; the side arm was washed with four 0.5-ml portions of toluene, which were added to the counting vial. Toluene (10 ml) containing 0.4% diphenyloxazole and 0.01% p-bis-2-(5-phenyloxazolyl)benzene were added.

The samples were counted in a Packard Tri-Carb liquid scintillation counter. The total radioactivity of the glutamic acid was determined by counting 0.05-ml aliquots of the initial solution in 10 ml of toluene containing diphenyloxazole and p-bis-2-(5-phenyloxazolyl)benzene, and in addition 2 ml of ethanol. These counts were corrected for quenching by use of benzoic acid-C₁⁴ as an internal standard. Since the glutamic and aspartic acids (see below) were counted by both methods, the radioactivity of the CO₂ could be related to that of the compounds counted by the gas flow counter.

ABA resulting from the decarboxylation of the glutamic acid was isolated as follows. To the main compartment of the Warburg flask, which contained 2 ml of solution, was added 0.2 ml of 50% trichloroacetic acid; 2 ml were removed and centrifuged at approximately 30,000 x g. The supernatant fluid was adjusted to pH 7.0 and was used for the isolation (3) and counting of the ABA.

When DL-glutamic acid-1-C₁⁴ was carried through the above decarboxylation procedure, 40% of the radioactivity was found in the CO₂, and the specific activity of the resultant ABA accounted for less than 0.1% of the radioactivity of the L-glutamic acid in the sample.

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† Fellow of The Rockefeller Foundation.

¹ In this paper, “brain” is used synonymously with cerebral cortex.

² The abbreviation used is: ABA, γ-aminobutyric acid.
Decarboxylation of \( \text{L-Aspartic Acid} \)—Tissue aspartic acid was decarboxylated by methods similar to those used for glutamic acid, except for the enzyme preparation and the incubation conditions, which are those of Krebs and Bellamy (11). The organism used, \textit{Nocardiopsis globulosa} 13130, (American Type Culture Collection), was transferred to new medium at intervals of not longer than 5 days. This organism decarboxylates aspartic acid to produce CO\(_2\) and \( \alpha \)-alanine (12). Only 10\% of the \( Q_{\text{CO}_2} \) values reported (11) was obtained with this culture, but our values were close to those obtained by Crawford (12).

The solution from the main compartment of the Warburg flask was treated similarly to the decarboxylated glutamic acid (see above) and placed on a column of Dowex 1. The fraction containing the neutral amino acids was counted to determine the C\(_4\) activity remaining in the alanine. The validity of this method was checked with aspartic acid-4-C\(_4\).

**RESULTS AND DISCUSSION**

In both experiments in which sodium bicarbonate-C\(_{14}\) was administered to cats by intracarotid infusion, the specific activities of the compounds isolated from brain cortex are equal to or higher than those obtained from blood (Table I). In previous papers (3, 13), it has been shown that blood glutamic acid and glutamine exchange with the respective cerebral metabolites despite an insignificant or small net uptake by the brain; however, the specific activities of the cerebral amino acids stay far below the blood amino acids within the experimental period. Hence, the fixation of carbon dioxide into glutamic and aspartic acids as well as glutamine must have occurred in the cerebral tissue at an appreciable rate. The highest specific activity among the brain metabolites was found in aspartic acid. It is, therefore, probable that carbon dioxide fixation occurs by condensation with pyruvate or phosphoenolpyruvate. The data suggest that the mechanism for carbon dioxide fixation in brain is analogous to that in liver.

Free ABA isolated from brain had 5\% or less of the specific activity of cerebral glutamic acid (Tables I and II). In addition, upon decarboxylation of the cerebral glutamic acid and glutamine, 95\% or more of the C\(_4\) activity was found in the \( \alpha \)-carboxyl group of the glutamic acid moiety. Since C-1 of \( \alpha \)-ketoglutarate labeled by carbon dioxide fixation would be lost by decarboxylation, these findings are also in accord with current concepts of carbon dioxide fixation and the operation of the citric acid cycle (14).

When, simultaneously with radioactive bicarbonate, ammonium acetate was administered by intracarotid infusion, there was an increase of specific activity of glutamine relative to that of aspartic acid (cf. Tables I and II).

The two experiments given in Table II differ by the length of the period of infusion and by the amount of labeled bicarbonate administered. Since the glutamine level in brain increases under these conditions of ammonia infusion, the data indicate that oxaloacetate is being utilized for the synthesis of \( \alpha \)-ketoglutarate, glutamate, and glutamine at a faster rate than it is converted to aspartic acid. The shift of the C\(_4\) labeling pattern from aspartic acid toward glutamine in brain during ammonia infusion also can be seen when the balance sheet of the distribution of the fixed C\(_4\) is considered (Table III). Thus, in Experiments 9 and 10, in which no ammonia was given, aspartic acid contained 28 and 30\% of the radioactivity in brain, whereas glutamine contained 9 and 19\% of the C\(_4\), respectively. In Experiments 6 and 7 (Table III), in which ammonia was infused, aspartic acid contained 6 and 11\% of the label, respectively, and glutamine had 35 and 38\% of the C\(_4\) activity found in brain.

The fact that the specific activity of cerebral glutamine is equal to or higher than that of glutamic acid in all four experi-
ments (Tables I and II) gives further support to the concept of compartmentation of glutamic acid and glutamine synthesis in brain developed in previous papers (3, 13, 15). The  

It is interesting to note that the specific activity of liver glutamine is close to that of liver glutamic acid in animals that did not receive ammonia (Table I). However, in animals receiving ammonia, the specific activity of liver glutamine was only 20 to 50% of that found in liver glutamic acid. This suggests that in liver the compartmentation of glutamine synthesis also exists  

It should be noted that the specific activities of glutamic acid, glutamine, and aspartic acid of liver are much lower in Experiment 10 than in Experiment 9 (Table I). In addition, the total C14 activities in blood (36,200 c.p.m. per g of tissue) and liver (67,700 c.p.m. per g) are much higher in Experiment 9 than in Experiment 10 (blood, 12,500 c.p.m. per g of tissue; liver, 38,800 c.p.m. per g of tissue), whereas brain shows comparable values of C14 activity in these experiments (Table III). This is probably due to the facts that the respiratory pump was adjusted to a respiratory rate decreased by 25% in Experiment 9, and that the cat used in Experiment 9 weighed 25% less than the one used in Experiment 10. There is little difference between the radioactivity contained in brain in both of these experiments. This is not surprising, since this organ was exposed first to the NaHCO3-containing blood, and any small differences in brain size could not influence the results significantly.

### Table III

*Relative distribution of C14 in brain amino acids*

<table>
<thead>
<tr>
<th></th>
<th>During infusion of NaHCO3</th>
<th>N14-ammonium acetate and NaHCO3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment No.</td>
<td>c.p.m. per g of tissue</td>
<td>Per cent of total counts</td>
</tr>
<tr>
<td>Trichloacetic acid extract</td>
<td>9</td>
<td>19,700</td>
<td>100</td>
</tr>
<tr>
<td>Glutamine</td>
<td>3,650</td>
<td>19.6</td>
<td>480</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>3,816</td>
<td>19.5</td>
<td>170</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>5,580</td>
<td>28.4</td>
<td>146</td>
</tr>
<tr>
<td>ABA</td>
<td>(0)*</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td>GSH (glutamic acid)</td>
<td>82</td>
<td>0.4</td>
<td>(6)</td>
</tr>
<tr>
<td>Total</td>
<td>66.9</td>
<td>58.6</td>
<td></td>
</tr>
</tbody>
</table>

|                | 10 | 21,200 | 100 | 7 | 18,000 | 100 |  |
| Glutamine       | 1,995 | 9.4 | 6,800 | 37.8 |  |
| Glutamic acid   | 4,100 | 19.4 | 2,400 | 13.3 |  |
| Aspartic acid   | 6,380 | 30.2 | 1,000 | 5.6 |  |
| ABA             | 65 | 0.3 | 14 | 0.1 |  |
| GSH (glutamic acid) | 34 | 0.2 | 32 | 0.2 |  |
| Total           | 50.5 | 57.0 |  |

* Parentheses indicate measurement at the limit of sensitivity of the counter.

### Table IV

*Decarboxylation of aspartic acid (brain)*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Aspartic acid</th>
<th>CO2</th>
<th>Per cent of activity in CO2</th>
<th>Alanine</th>
<th>Per cent of activity in alanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>1800</td>
<td>1140</td>
<td>63</td>
<td>580</td>
<td>32</td>
</tr>
<tr>
<td>Liver</td>
<td>5270</td>
<td>2630</td>
<td>50</td>
<td>2450</td>
<td>46</td>
</tr>
</tbody>
</table>

Data of Experiment 9 are presented here.

but reverses the pattern of that in brain; viz., the liver pool of glutamic acid used for glutamine synthesis appears to come mainly from a large tissue pool of this amino acid, which does not mix very rapidly with newly synthesized glutamic acid, the amino group of which is channeled into aspartic acid formation; these conclusions are in accord with the results of the experiments in the preceding paper (3).

The radioactivity in blood and liver amino acids accounts for only a small portion of the total radioactivity in these tissues. In brain, also, only approximately half of the radioactivity was recovered in the amino acids analyzed, whereas in the N14 experiments (3), the isotope content of these compounds accounted for close to 90% of the isotope content of the trichloroacetic acid extract. It is probable that a large portion of the radioactivity not accounted for in these tissues is present in glucose and members of the citric acid cycle, since, as known from studies on liver, this organ incorporates C14O2 rapidly into carbon atoms 3 and 4 of glucose (16, 17).

The aspartic acid of liver and brain in Experiment 9 was decarboxylated to give the beta-carboxyl group (as carbon dioxide) and alpha-alanine (Table IV). In brain, 63% of the radioactivity was found in the CO2, and 32% remained in the alanine fraction, whereas in liver, the counts were almost equally distributed between the CO2 and alanine. This distribution of counts in aspartic acid supports the operation of the "dicarboxylic acid shuttle" (18). The demonstration that brain can fix carbon dioxide to a significant extent raises the question whether ammonia toxicity can be explained by depletion of the citric acid cycle intermediates of brain (19). Whether or not the rate of carbon dioxide fixation in brain is sufficient to counteract the drain on citric acid cycle intermediates cannot be evaluated without data on the rate of CO2 fixation into these intermediates. Work along these lines is now in progress. In addition, for a better understanding of the effects of the increased ammonia levels on brain metabolism, the capacity of the glutamine-forming system as well as the fate of the glutamine synthesized in response to the elevated ammonia levels will have to be known.

Investigations of cerebral tissue have shown that it contains all of the enzymatic activities of the major metabolic pathways found in other organs; the demonstration of a potent carbon dioxide-fixing mechanism underlines the similarity of brain to other metabolically highly active tissues, such as liver, and adds another essential feature to the autonomy of cerebral metabolism. On the other hand, the extent and rate of carbon dioxide fixation is not restricted to the mammalian central nervous system. Recent experiments in this laboratory have demonstrated incorporation of CO2 into the dicarboxylic amino acids and into the intermediates of the citric acid cycle in the isolated lobster nerve (20).
fixation may introduce another controlling factor in the operation of the citric acid cycle, a fact of particular significance for cerebral tissue, in which glucose is the main, if not the sole, source of energy of metabolism. Our findings also raise the question whether some effects on nervous tissue of CO₂ ascribed to its influence on the physicochemical environment (e.g. pH) may not be the result of the direct participation of CO₂ in the metabolism of the tissue mediated by CO₂ fixation.

SUMMARY

Carbon dioxide fixation in brain was studied in cats to which NaHCO₃ was administered by intracarotid infusion. Glutamic and aspartic acids, glutamine, glutathione, and γ-aminobutyric acid were isolated from blood, brain, and liver, and their specific activities were determined. The data indicate a significant incorporation of CO₂ into the amino acids of the cerebral cortex, presumably by way of the citric acid cycle. Without simultaneous ammonia infusion, the specific activity of aspartic acid is 3 times that of glutamine, whereas in the presence of ammonia the ratios of specific activity of both compounds are closer to unity or reversed. The data suggest that in the presence of ammonia the oxaloacetic acid is channeled into glutamine formation. γ-Aminobutyric acid isolated from the tissue, as well as that obtained after decarboxylation of glutamic acid or glutamine, has less than 5% of the counts of the precursor. These findings give additional support to the assumption that the operation of a CO₂ fixation mechanism in brain is similar to that in liver. Additional data on the compartmentation of glutamic acid and glutamine synthesis are presented.

The significance of the findings for an interpretation of ammonia metabolism in brain is pointed out.

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REFERENCES