The tricarboxylic acid cycle is usually conceptualized as a unified series of biochemical reactions that mediate the oxidation of the major nutrients. The rates of individual steps may differ from one another, but the conventional view is that the cycle virtually always "begins" with the synthesis of citrate and then proceeds until 2 mol of CO₂ are liberated and the system again is poised to resynthesize citrate from oxaloacetate and acetyl-CoA.

This conceptualization has the advantage of simplicity, but, in many instances, it may be an inadequate rendering of a more complex physiology. Some evidence suggests that the tricarboxylic acid cycle is comprised of two major components, a segment leading from 2-oxoglutarate to oxaloacetate and another that extends from oxaloacetate to 2-oxoglutarate and includes the condensation reaction to citrate. In a given tissue under particular circumstances, the system might function primarily to yield either isocitrate or oxaloacetate from a given precursor without the necessity of directing carbon through the entire cycle.

Tricarboxylic Acid Cycle in Rat Brain Synaptosomes
FLUXES AND INTERACTIONS WITH ASPARTATE AMINOTRANSFERASE AND MALATE/ASPARTATE SHUTTLE

Marc Yudkoff, David Nelson, Yevgeny Daikhin, and Maria Erecińska
From the Departments of Pharmacology, Biochemistry and Biophysics, and Pediatrics, University of Pennsylvania School of Medicine and Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104-6884

The flux through different segments of the tricarboxylic acid cycle was measured in rat brain synaptosomes with gas chromatography-mass spectrometry using either deuterated glutamine or [14C]aspartate. The flux between 2-oxoglutarate and oxaloacetate was estimated to be 3.14 and 4.97 nmol/min/mg protein with and without glucose, respectively. These values were 3-5-fold faster than the flux between oxaloacetate and 2-oxoglutarate (0.92 nmol/min/mg protein) measured in the presence of glucose. The pattern of intermediates labeling suggests that the overall rate-controlling reaction involves either citrate synthase or pyruvate dehydrogenase but not 2-oxoglutarate or isocitrate dehydrogenase. The enrichment in [3,3,4,4-2H₅]glutamine from [2,3,3,4,4-2H₅]glutamine was as rapid as in [2,3,3,4,4-2H₅]glutamate, which indicates that the aspartate aminotransferase reaction is several fold faster than the flux through the tricarboxylic acid cycle. [14C]Aspartate was rapidly converted to [13C]malate, suggesting that in intact synaptosomes aspartate entry into the mitochondrion is very slow. The finding that aspartate is taken up by mitochondria as malate, along with the observed high enrichment in [3-²H]malate (from [2,3,3,4,4-²H₅]glutamine), is consistent with the substantial synaptosomal activity of the malate/aspartate shuttle.

Our prior studies of synaptosomal metabolism of [2-¹⁵N]glutamate and [2-¹⁵N]glutamine suggested that this might be the case in nerve endings (1–3). We found that [¹⁵N]aspartate was a major product of [2-¹⁵N]glutamine handling in the nerve endings and that this process was greatly intensified by the omission of glucose from the incubation medium, a condition that favored the utilization of glutamine as a metabolic fuel. Therefore, we postulated that the consumption of glutamate derived from glutamine (through the glutaminase reaction) was mediated in large part by a sequence of reactions involving aspartate aminotransferase (which produces 2-oxoglutarate) and the segment of the tricarboxylic acid cycle between 2-oxoglutarate and oxaloacetate. Such a "mini-cycle" consumes glutamine and produces aspartate but requires only catalytic amounts of oxaloacetate (Fig. 1).

There are three steps within the tricarboxylic acid cycle proper that are considered potential regulatory sites of the overall flux through the system: the citrate synthase reaction and the isocitrate and 2-oxoglutarate dehydrogenases. In addition, pyruvate dehydrogenase, the enzyme that generates 2 carbon units for condensation with oxaloacetate, may provide an additional point of control. It is evident that the segment between 2-oxoglutarate and oxaloacetate contains only one controlling step, whereas the remaining portion has three. The present study was undertaken to compare flux through the two segments of the tricarboxylic acid cycle and to determine the overall rate-controlling step. Flux between 2-oxoglutarate and oxaloacetate was estimated by incubating synaptosomes with [2,3,3,4,4-²H₅]glutamine and monitoring the formation of [¹⁵N]aspartate, while that from oxaloacetate to 2-oxoglutarate was evaluated by following the metabolism of [3-¹⁵N]aspartate and measuring the formation of [¹⁵C]glutamate.

EXPERIMENTAL PROCEDURES

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Filmmaker correspondence should be addressed. Tel.: 215-898-3861; Fax: 215-573-2236.
oil as above. Supernatants and pellets were further treated as previously described in detail (1).

Processing of Samples for Detection of 2H or 13C Metabolites—An aliquot of the neutralized pellet extract (0.6 ml) or supernatant (1 ml) was combined with 2 ml (pellet) or 5 ml (supernatant) of 0.05 m sodium azide buffer, pH 8.6, and slowly applied onto an AG-1 (Cl-; X-8, 100-200 mesh; 0.5 x 4 cm) column. The column was washed with 9 ml of deionized water, and acidic amino acids and carboxylic acids were eluted with 4 ml of 2 N HCl. The eluates were processed for the determination of enrichment of either 2H or 13C in the amino acids (aspartate and glutamate) and carboxylic acids (malate, citrate, and succinate).

Quantification of 2H and 13C—Gas chromatography-mass spectrometry was used to measure isotopic abundance (atom percent excess) in amino acids and in malate, succinate, and citrate. All eluates were lyophilized, and the t-butyldimethylsilyl derivatives were prepared according to Mawhinney et al. (5). Acetonitrile was utilized as the solvent. Analysis of isotopic enrichment was carried out with selected ion monitoring on a Hewlett-Packard 5970 mass-selective detector. The following ion ratios were used glutamate, m/z 437/432 and 436/432 (for the 2H species) and 438/432 (for the 13C species); aspartate, m/z 419/418 (for both 2H and 13C species); citrate, m/z 592/591; malate, m/z 420/419; and succinate, m/z 280/289. Isotopic abundance was calculated from the area of each peak according to the formula of Biemann (6).

Other Procedures—The concentrations of amino acids were determined by high pressure liquid chromatography of their o-phthalaldehyde derivatives (7). Protein content was measured by the biuret reaction (8) with bovine serum albumin as the standard.

RESULTS

Formation of Deuterated Glutamate and Aspartate in the Pellet—The distribution of 2H from [2,3,3,4,4,2H5]glutamine to other metabolites is illustrated in Fig. 2. Two different species of labeled glutamate were generated: [2,3,3,4,4,2H5]glutamate (glutamate+5) and [3,3,4,4,2H4]glutamate (glutamate+4). The latter represents glutamate produced following the transamination of glutamate+5.

\[
[2,3,3,4,4,2H5]\text{Glutamate} + \text{oxaloacetate} \rightarrow 2[2,3,3,4,4,2H5]\text{oxoglutarate} + \text{aspartate} \quad (\text{Eq. 1})
\]
\[
2[3,3,4,4,2H4]\text{Oxoglutarate} + \text{aspartate} \rightarrow [3,3,4,4,2H4]\text{glutamate} + \text{oxaloacetate} \quad (\text{Eq. 2})
\]

It can be seen that in the pellet by 2 min there was essentially no difference in the labeling of glutamate+5 versus glutamate+4 (Fig. 2A). At 5 min and later, labeling in glutamate+4 exceeded that in glutamate+5, reflecting the fact that transamination to aspartate is faster than the glutaminase reaction.

The omission of glucose had essentially no effect on the rate of formation of glutamate+5, but it enhanced generation of glutamate+4 (Fig. 2B). This confirms our earlier studies (1) that transamination of glutamate to aspartate is faster in the absence of the carbohydrate source.

Labeling of Tricarboxylic Acid Cycle Intermediates with 2H—Also shown in Fig. 2, A and B, is the transfer of the deuterium label in malate, succinate, and citrate after incubation with [2,3,3,4,4,2H5]glutamine. In either the presence or absence of glucose, enrichment of each of these tricarboxylic acid cycle intermediates was increased in the absence of the carbohydrate source.

\footnote{The designation "n" refer to the number of atoms that are labeled in excess of the base-line value.}
constituents was apparent by 2 min. The labeling in succinate+4 and malate+2 was almost identical and about half that in glutamate+4. The label in citrate consistently was less than that of any other acid, particularly in the absence of glucose (Fig. 2B). Of note is the fact that there was substantial enrichment in malate+1, which at all time points was almost identical to that in aspartate+1.

Release of Labeled Metabolites into the Supernatant—Labeled glutamate and aspartate were released into the external environment (Fig. 2, C and D). Enrichment in medium aspartate was comparable with that of the pellet, and the same was true for glutamate+4 in the absence of glucose. With the carbohydrate present, labeling of glutamate+4 was somewhat higher in the medium than in the internal compartment.

Similarly, the synaptosomes appeared to release malate, succinate, and citrate into the supernatant, where labeled species of each of these acids could be detected (Fig. 2, C and D).

Net Formation of Deuterated Amino Acids—Incubation of synaptosomes with glutamine caused an increase in the concentrations of glutamate and aspartate in both pellet (Fig. 3A) and supernatant (Fig. 3C). The internal content of glutamate rose from 40–45 nmol/mg protein to >60 nmol/mg protein in the presence of glucose and to >50 nmol/mg protein without glucose. In the supernatant, total glutamate increased to about 25 \( \mu \text{M} \) in 30 min.

Changes in intracellular aspartate were less than those of glutamate, rising to ~35 nmol/mg protein in the absence of glucose and remaining <30 nmol/mg protein when glucose was included in the incubation medium (Fig. 3A). The aspartate concentration of the supernatant increased in a linear manner and reached values 5–7 \( \mu \text{M} \) after 30 min (Fig. 3C).

The absolute concentrations (nmol \(^{3}H\) metabolite/mg protein or \( \mu \text{M} \) of medium) of deuterated glutamate+4 and aspartate+1 are shown in Fig. 3, B and D. Production of labeled glutamate steadily rose during the incubation and appeared to be somewhat greater in the absence of glucose. Formation of labeled aspartate was considerably slower than that of glutamate, particularly in the medium where the changes were negligible until about 30 min. Rises in the pellets were significantly greater when glucose was omitted from the incubation.

Metabolism of \([3^{13}C]\)Aspartate in Synaptosomes—Fig. 4 shows the labeling of aspartate and glutamate and of tricarboxylic acid cycle intermediates, malate and citrate, with \(^{13}C\) following 75 min of incubation with \([3^{13}C]\)aspartate. Internal labeling with aspartate itself was extremely rapid (Fig. 4A), as might be anticipated in a system with very active excitatory amino acid transporters. There also occurred a very active transfer of label to malate (Fig. 4A), enrichment in which markedly exceeded that in either citrate or glutamate (Fig. 4B). After 75 min of incubation, the labeling in internal and external aspartate was equal; the same was true for glutamate (Fig. 4, A and C). It also is of interest that the label in either glutamate or citrate appeared after an initial lag period (Fig. 4B).

The enrichment in succinate was very small and appeared at incubations longer than 15–30 min (data not shown).

Fig. 5 shows the levels of internal (A) and external (C) aspartate and glutamate and the amounts of \(^{13}C\) substrate (aspartate) and product (glutamate) present in the pellets and supernatants during 30 min of incubation with 50 \( \mu \text{M} \) \([3^{13}C]\)aspartate in the presence and absence of glucose. The internal content of aspartate increased, whereas that of glutamate fell; the opposite was true in the supernatant. These results are consistent with our previous study (9). Accumulation of labeled aspartate in the pellet was very rapid and reached a plateau within 15 min (Fig. 5B), whereas generation of \(^{13}C\)glutamate was slow and showed a lag. The concentration of \(^{13}C\)aspartate in the medium steadily declined, while the appearance of labeled glutamate was very slow and small (Fig. 5D).

Pathways of Label Distribution from \([2,3,3,4,4^{-2}\text{H}_{5}]\)Glutamine and \([13\text{C}]\)Aspartate—The pathway of \([2,3,3,4,4^{-2}\text{H}_{5}]\)glu-
Fig. 5. Amino acid levels and absolute amounts of glutamate and aspartate formed in the pellets and supernatants after incubation with [3-13C]-aspartate. Results are from the same experiments that are shown in Fig. 3. Absolute amounts of labeled amino acids formed were calculated by multiplying the content found in the pellet (A) and supernatant (C) by the appropriate values for enrichment displayed in Fig. 3. Data are means ± S.E. for 3 experiments. S.E. values are not shown if their magnitude is less than the size of the symbol.

Fig. 6. Pathways of the label transfer from [2,3,3,4,4-2H5]glutamine (A) and [3-13C]aspartate (B). The outline of the diagrams follows the scheme in Fig. 1.

tamine metabolism, based on the results above, is shown in Fig. 6A. When the labeled amino acid is added to the synaptosomal suspension, it is transported to the mitochondrial where it is hydrolyzed to [2,3,3,4,4-2H5]glutamate by phosphate-dependent glutaminase. The latter is converted to [2,3,4,4-2H4]oxoglutarate either via aspartate aminotransferase, which is very rapid (1) or other pathways (glutamate dehydrogenase, other 2-oxoglutarate-linked aminotransferases, glutamine transaminase-ω-amidase pathway), which in brain are very slow (1, 10, 11). [2,3,4,4-2H4]Oxoglutarate is then either transformed by transamination with aspartate to [3,3,4,4-2H4]glutamate or successively metabolized to [2,3,3-2H4]su-
cinate, [2,3-2H]fumarate, [2,3-2H]malate, and [3-2H]oxaloacetate. The latter is converted to [3-2H]aspartate through aspartate aminotransferase. The deuterium is irretrievably lost in the aconitase reaction, thus there is no recycling of the label.

[3-2H]Aspartate leaves the mitochondrion on the aspartate/glutamate exchanger and is transaminated in the cytosol to [3-2H]oxaloacetate. The latter is reduced to [3-2H]malate by malate dehydrogenase.

Distribution of the 13C label after incubation of synaptosomes with [3-13C]aspartate is shown in Fig. 6B. The [3-13C]aspartate is taken up to the cytosol, where it is converted to [3-13C]oxaloacetate and then to [3-13C]malate; the latter is transported into the mitochondrion on the dicarboxylate carrier, which is also a part of the malate/aspartate shuttle. The [3-13C]malate then enters the tricarboxylic acid cycle, which successively converts it to [2-13C]citrate (in the presence of 2 carbon units supplied from the metabolism of glucose) and 2-[13C]oxoglutarate. Transamination of the latter with aspartate forms [2-13C]glutamate.

Flux through the Tricarboxylic Acid Cycle—The rate of formation of [3-13C]aspartate (i.e. the absolute rate) calculated from results in Figs. 2 and 3 was 0.21 ± 0.02 nmol/min/mg of protein in the presence and absence of glucose, respectively (Table I). This measurement is a product of a number of reactions: glutaminase, the formation of 2-oxoglutarate from aspartate, the latter being inferred from labeling in glutamate+4 (Fig. 2). Thus, if the keto-enol transition occurs in synaptosomes, it does not seem to affect significantly the reactions evaluated in the present study.

Table I

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Atom percent excess in precursor</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>A [2,3,4,4-2H2]Glutamine as precursor</td>
<td>2.14 ± 0.02</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>[2,3,4,4-2H2]Glutamate + glucose</td>
<td>2.38 ± 0.04</td>
<td>0.38 ± 0.04</td>
</tr>
<tr>
<td>[2,3,4,4-2H2]8-Oxoglutarate + glucose</td>
<td>6.8 ± 0.6</td>
<td>3.14 ± 0.39</td>
</tr>
<tr>
<td>[2,3,3-2H]Succinate + glucose</td>
<td>7.7 ± 0.5</td>
<td>4.94 ± 0.66</td>
</tr>
<tr>
<td>[2,3,3-2H]Malate + glucose</td>
<td>6.8 ± 0.6</td>
<td>3.14 ± 0.39</td>
</tr>
<tr>
<td>[2,3-2H]Citrate + glucose</td>
<td>2.6 ± 0.2</td>
<td>8.21 ± 0.88</td>
</tr>
<tr>
<td>[2,3-2H]Malate + glucose</td>
<td>4.5 ± 0.5</td>
<td>8.55 ± 1.41</td>
</tr>
</tbody>
</table>

DISCUSSION

The results presented in this paper lead to the following conclusions. (i) The flux through two segments of the tricarboxylic acid cycle, i.e. that between 2-oxoglutarate and oxaloacetate and the portion between oxaloacetate and 2-oxoglutarate, in the presence of glucose differs by a factor of 3. The slowest step is between oxaloacetate and citrate, which reflects either the activity of citrate synthase or the supply of 2 carbon units by pyruvate dehydrogenase. (ii) The fastest reaction that supplies metabolites to the tricarboxylic acid cycle is aspartate aminotransferase. (iii) The malate/aspartate shuttle is closely linked to the mitochondrial combustion of 2 carbon units and operates at a rate that seems to be faster than the cycle itself but considerably slower than the aspartate aminotransferase reaction. (iv) Cytosolic aspartate enters the mitochondrion as malate, an observation consistent with the evidence that in well-coupled mitochondria the glutamate/aspartate exchanger operates in the direction of glutamate entry and aspartate exit. The rate of flux through the tricarboxylic acid cycle in the nervous tissue has been previously estimated with two types of
approaches. The first used computer simulation (14, 15) of a model based on the time courses of incorporation of tracers ($^{13}$NH$_4$ and $^{14}$C]glucose and acetate) into glucose, glutamine, and aspartate. Most of the substances and pathways were divided between a large (which contains neurons) and a small (which corresponds to glial cells) compartment. The calculated flux through the tricarboxylic acid cycle in the large compartment, which corresponded to the slowest reaction, was 1.25 $\mu$mol/min/g, wet weight. An interesting feature of the fit to the model was a requirement for a rapid exchange between 2-oxoglutarate and glutamate, the rate of which was 10–20-fold greater than the cycle flux. More recently, a mathematical model of cerebral glucose metabolism was developed (16) to analyze the isotopic labeling of carbon atoms C4 and C3 of glutamate obtained by NMR after an intravenous infusion of [1-13C]glucose (17). The tricarboxylic acid cycle rate in rat brain obtained from labeling of glutamate C4 was calculated to be 1.58 $\mu$mol/min/g of tissue, whereas the 2-oxoglutarate/glutamate exchange, assessed from the difference between the rates of isotopic enrichment of glutamate C4 and C3, was estimated to be 82–126 times faster. The cerebral rate of oxygen consumption derived on the basis of the measured tricarboxylic acid cycle flux was 4.4–4.7 $\mu$mol/min/g, a value very similar to that measured directly by independent methods (18). It is important to mention that in the latter study, brain was treated as a single compartment system.

In the present work, the flux through the tricarboxylic acid cycle of isolated nerve ending particles was determined by measuring with gas chromatography-mass spectrometry the isotopic enrichment in the cycle intermediates after incubation with labeled precursors. The same assumption was used as in the study of Mason et al. (16), and no attempt was made to distinguish between the cytosolic and the mitochondrial compartments. Synaptosomes as a model are, however, simpler than the whole brain because they contain predominantly neuronal elements and are relatively free of glial cells. The calculated rate of the slowest step of the cycle was 0.92 $\mu$mol/min/mg of protein (Table I), which is about 3-fold slower than the mitochondrial rate of oxygen consumption at the same temperature (1, 19). Thus, by synaptosomes, the relation between the flux through the tricarboxylic acid cycle and the rate of oxygen consumption is the same as in whole brain.

In synaptosomes, the slowest step of the tricarboxylic acid cycle (0.92 $\mu$mol/min/mg of protein) (Table I) was found to be between oxaloacetate and citrate and thus indicates the involvement of either citrate synthase or pyruvate dehydrogenase reactions. If the former is the rate-determining step, our observations mean that the rate of condensation of acetyl-CoA with oxaloacetate must be tightly controlled because activity of citrate synthase in whole brain (29 $\mu$mol/min/g of tissue) (20) as well as in synaptosomes (32 $\mu$mol/mg of protein/h) (21) is high. Although our results do not allow us to distinguish which of the two key enzymes determines the flux in this segment of the tricarboxylic acid cycle (and most likely the overall rate), it is worth pointing out that the activity of pyruvate dehydrogenase both in whole brain (2 $\mu$mol/min/g of tissue) (20) and in isolated synaptosomes (3.5 $\mu$mol/mg of protein/h) (21) is 10 times lower than that of citrate synthase.

The flux in the segment between 2-oxoglutarate and oxaloacetate was 5–5-fold in the absence of glucose) faster than in the portion between oxaloacetate and 2-oxoglutarate (Table I). This difference suggests that in brain the tricarboxylic acid cycle may not function as a single unified entity but that under some conditions amino acids might serve as an alternate energy source, which helps maintain tissue ATP level. This is consistent with our earlier postulate of the existence of a “mini-cycle” based on experiments with $^{15}$N-glutamate and glutamine (1, 3). Recent studies with fluorocitrate on cultured astrocytes (22) support this contention.

In addition to serving as an energy buffer, the mini-cycle might, to a varying degree, spare consumption of the mitochondrial pool of acetyl-CoA in the citrate synthase reaction. This could be important when there is a need to utilize acetyl-CoA to produce acetylcholine or N-acetylaspartate. Conversely, if a need arose for citrate in the cytoplasm (e.g. for the purpose of providing acetyl-CoA), flux through the mini-cycle might become depressed, and the oxaloacetate thus made available could be used by the citrate synthase reaction.

As illustrated in Fig. 1, oxaloacetate represents a “meeting ground” of the malate-aspartate shuttle, the aspartate aminotransferase reaction, the tricarboxylic acid cycle, and the mini-cycle and thus occupies a key position in cellular energy metabolism. Thus, the importance of the amino acid-driven mini-cycle also might be in its ability to replenish rapidly and maintain intramitochondrial level of this oxoacid, the pool of which is smaller than that of any other tricarboxylic acid cycle intermediate. A similar role for amino acids has been postulated in the heart based on their protective effects on cardiac function after ischemia and cardioplegia (Ref. 23 and references therein).

The pattern of enrichment in the different intermediates also provides information on relative activities of some of the reactions within the synaptosomal tricarboxylic acid cycle and on the location of putative rate-determining steps. The much greater labeling in glutamate+4 (i.e. 2-oxoglutarate) than either in succinate or malate (which were almost equal) suggests that 2-oxoglutarate dehydrogenase operates at a slower rate than the reactions between succinate and oxaloacetate. Such an observation is consistent with the postulated flux-controlling function of 2-oxoglutarate dehydrogenase in a number of mitochondrial types (24) as well as with computer modeling of Van den Berg and Garfinkel (15), which showed that the flux between succinate and oxaloacetate was 4-fold faster than between 2-oxoglutarate and succinate. Similarly, an almost identical enrichment in citrate and glutamate from [3-13C]aspartate indicates that isocitrate dehydrogenase is unlikely to be an important rate-determining step of the flux through the tricarboxylic acid cycle in synaptosomes.

Two current observations indicate that transamination between aspartate and glutamate is very rapid. First, with glutamine as precursor, the enrichment in glutamate+4 (which is derived from glutamate+5 via the aspartate aminotransferase reaction) is as high (or higher) as that in glutamate+5 (Fig. 2A) even at the earliest time point studied. Second, during incubation with [3-13C]aspartate, enrichment in citrate and glutamate occurs after a considerable lag period. This can be explained by a fast equilibration of labeled intramitochondrial oxaloacetate with a much greater pool of intramitochondrial aspartate. Such a conclusion is not unexpected because aspartate aminotransferase, one of the most active brain enzymes (see Refs. 11 and 25 for review), catalyzes a near equilibrium reaction (9, 26, 27). It is tempting to speculate that because of its high activity and ready reversibility, this reaction serves as a buffer that maintains a proper balance of the intermediates in the tricarboxylic acid cycle. The fact that aspartate and glutamate are present in severalfold higher concentrations than other relevant metabolites is consistent with this prediction.

The rapid generation of labeled malate from exogenous aspartate (Fig. 4A) and the appearance of malate+1 after incubation with glutamine+5 (which can only occur via the malate/aspartate shuttle) indicate that the malate/aspartate shuttle in synaptosomes is quite active. The importance of this pathway
for cerebral transfer of reducing equivalents from the cytosol to the mitochondrion and the maintenance of ATP levels has been previously inferred from experiments either with inhibitors of transamination (28–30) or with 3-nitropropionate, a blocker of succinate dehydrogenase (31). However, the existence of a substantial lag in the labeling of citrate from labeled aspartate (and rapidly generated labeled malate) suggests that the shuttle operates at a rate considerably slower than that of aspartate aminotransferase reaction. This is consistent with the physiological role of the shuttle, which is designed to maintain and not to equilibrate the cytosolic and mitochondrial redox states (32).

Our final comment concerns aspartate entry into the mitochondrion. It has been postulated from studies on isolated mitochondria that an electrogenic glutamate/aspartate exchanger exhibits polarity (33). Entry of aspartate in exchange for glutamate is very slow and cannot be shown in energized mitochondria (34, 35). Little is known about this mechanism in brain mitochondria, although the existence of the exchanger has been demonstrated (36–38). The results reported here show that in intact synaptosomes, in which mitochondria are likely to be tightly coupled, the rate of entry of aspartate into the organelle is very slow and that the bulk of it is transported as malate after transamination to oxaloacetate. The mitochondrial aspartate aminotransferase then rapidly regenerates aspartate from oxaloacetate produced by oxidation of malate.

REFERENCES

34. LaNoue, K. F., and Williamson, J. R. (1971) Metabolism 20, 119-140
35. Palmieri, F., Gerch, G., and Quagliariello, E. (1971) Experientia (Basel) 17, 139-140