METABOLISM OF GLUTAMIC ACID-1-^{14}C AND ASPARTIC ACID-4-^{14}C IN RAT BRAIN AND KIDNEY HOMOGENATES*

by

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This study attempts to gain insight into the overall dissimilation of glutamic acid-1-^{14}C and aspartic acid-4-^{14}C by rat tissue homogenates. Whole homogenates contain all of the enzymes and hence present data often difficult to interpret; but they are excellent systems for evaluating physiological factors such as growth and hormonal action. Brain tissue was chosen because contradictory results were reported in the literature as to the role of this tissue in amino acid dissimilation, and kidney tissue because of its high metabolic activity. Some clarification of the contradictory reports on the difference in the behaviour of the D- and L-forms of these amino acids is also undertaken.

EXPERIMENTAL

Male rats of the Sprague-Dawley strain, weighing 160 to 200 g, were used. Immediately after decapitation, 1.5 g of tissue per 2 ml of 0.1 *M* phosphate buffer (pH 7.4) was minced in a ground glass homogenizer. 2 ml aliquots of the homogenate were placed in a Warburg flask containing either 0.20 mg ($1.1 \cdot 10^{-3}$ mc) DL-glutamate-1-^{14}C or 0.33 mg ($1.8 \cdot 10^{-3}$ mc) of DL-aspartate-4-^{14}C dissolved in 0.1 ml distilled water. A filter paper square was inserted into the center well containing 0.2 ml of 5% KOH. Each flask was oxygenated, then incubated at 37° C in the Warburg apparatus for 45 minutes. At the end of the incubation, the filter paper was removed from the center well, spread out on a watch glass and counted.

In determining the effect of various factors on $^{14}CO_2$ production, the particular compound was dissolved in 0.1 *M* phosphate buffer and placed in the flask prior to the addition of the homogenate. A corresponding volume of buffer was added to each control flask.

The resolution of DL-glutamic acid was carried out as follows: 4 mg of DL-glutamic-1-^{14}C was dissolved in 0.5 ml sodium acetate buffer (pH 4.9) and placed in a Warburg flask containing 20 mg of *Clostridium welchii* suspended in 1.7 ml of the same buffer. The flask was oxygenated, then incubated at 37° C. At approximately 3 hour intervals, the filter paper in the center well was removed and counted in order to determine the end-point of the reaction. Each time the reaction was interrupted for counting, the center well was prepared with fresh KOH and filter paper. When the reaction reached completion, indicated by the decrease in $^{14}CO_2$ produced, the solution was concentrated and purified by elution from a Dowex 50 column with 1.5 *N* HCl.

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DISCUSSION

When an inert isomer is added to the radioactive racemic mixture of an amino acid prior to incubation with the tissue, the isomer should decrease by isotope dilution the amount of radioactive CO_2 produced, if the isomer is identical with that member of the racemic mixture which the homogenate selects for dissimilation. If there is no decrease in $^{14}CO_2$ when an inert isomer is added, then that isomer is not utilized. If the radioactivity is diminished, however, it may either be due to dilution of the form dissimilated, or to inhibition of the reaction by the optical antipode. Our results (Tables I–IV) suggest that both whole rat kidney and rat brain homogenates utilize the L-form of aspartic acid or glutamic acid only. If the homogenate is centrifuged at high speed and the supernatant incubated, the same conclusion holds, except in the case of the brain tissue supernatant (Table IV), where the addition of 10 mg of inert D-glutamate causes a 25% inhibition, thus suggesting that the D-form might be utilized somewhat. This latter observation is in agreement with the finding of WEIL-MALHERBE[1], that only the L-form was oxidized in brain slices and the specificity was reversed in extract of brain and that this difference was more pronounced as the extract was diluted. In order to check this conclusion by a different procedure D-glutamate-1-^{14}C was prepared from DL-glutamate-1-^{14}C by exposure to *Clostridium welchii*. On incubation with the purified D-glutamate-1-^{14}C, both whole brain and whole kidney homogenates showed no utilization.

TABLE I

INFLUENCE OF VARIOUS SUBSTANCES ON $^{14}CO_2$ PRODUCTION FROM DL-ASPARTATE-4-^{14}C BY KIDNEY AND BRAIN HOMOGENATES

Substance added	*Relative radioactivity of CO_2 produced*	
	Kidney	*Brain*
Control	100*	100*
Nitrogen atmosphere to replace oxygen atmosphere	14	5.2
10 mg inert L-aspartate	35	26
10 mg inert D-aspartate	111	112
10 mg inert D-aspartate incubated with rabbit instead of rat homogenate	58	—
1 mg pyruvate	173	187
3 mg pyruvate	248	198
1 mg α-ketoglutarate	107	160
3 mg α-ketoglutarate	138	199
6 mg ATP	72	88
3 mg AMP	68	134
1 mg DPN	117	164
1 mg DPN and 6 mg ATP	126	136
2 mg TPN	122	160
0.5 mg pyridoxal phosphate	117	109
Supernatant of homogenate after spinning at high speed in phosphate buffer	80	15

* This activity averaged 0.3% of administered counts eliminated as $^{14}CO_2$ for kidney homogenates and 0.1% of administered counts eliminated as $^{14}CO_2$ for brain homogenates. Each incubation flask contained 1.5 g of tissue suspended in 2.0 ml of 0.1 phosphate buffer (pH 7.4) and 0.33 mg ($1.8 \cdot 10^{-3}$ mc) of DL-aspartate. The flasks were incubated at 37° C for 45 minutes in an oxygen atmosphere.

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TABLE II

INFLUENCE OF VARIOUS SUBSTANCES ON $^{14}CO_2$ PRODUCTION FROM DL-GLUTAMATE-1-^{14}C BY KIDNEY AND BRAIN HOMOGENATES

Substance added	*Relative radioactivity of CO_2 produced*	
	Kidney	*Brain*
Control	100*	100*
Nitrogen atmosphere to replace oxygen atmosphere	39	46
10 mg inert L-glutamate	22	39
10 mg inert D-glutamate	106	110
1 mg pyruvate	101	97
3 mg pyruvate	101	58
1 mg oxalacetate	182	119
3 mg oxalacetate	156	109
6 mg ATP	137	116
3 mg AMP	112	98
1 mg DPN	212	134
1 mg DPN and 6 mg ATP	128	172
2 mg TPN	136	132
Supernatant of homogenate after spinning at high speed in phosphate buffer	55	72

* This activity averaged 0.8% of administered counts eliminated as $^{14}CO_2$ for kidney homogenates and 0.7% of administered counts eliminated as $^{14}CO_2$ for brain homogenates. Each incubation flask contained 1.5 g of tissue suspended in 2.0 ml of 0.1 *M* phosphate buffer (pH 7.4) and 0.2 mg ($1.1 \cdot 10^{-3}$ mc) of DL-glutamate. The flasks were incubated at 37° C for 45 minutes in an oxygen atmosphere.

TABLE III

$^{14}CO_2$ PRODUCTION FROM LABELLED DL-ASPARTATE-4-^{14}C BY SUPERNATANT OF KIDNEY AND BRAIN HOMOGENATES

Substance added	*Relative radioactivity of CO_2 produced*	
	Kidney	*Brain*
Control	100*	100*
10 mg inert L-aspartate	35	58
10 mg inert D-aspartate	102	123

* This activity averaged 0.24% of administered counts eliminated as $^{14}CO_2$ for kidney and 0.02% of administered counts eliminated as $^{14}CO_2$ for brain. Each incubation flask contained the supernatant (obtained by spinning at high speed) of a homogenate made from 1.5 g of tissue and 2.0 ml of 0.1 phosphate buffer (pH 7.4) and 0.33 mg ($1.8 \cdot 10^{-3}$ mc) of DL-aspartate. The flasks were incubated at 37° C for 45 minutes in an oxygen atmosphere.

TABLE IV

$^{14}CO_2$ PRODUCTION FROM LABELLED DL-GLUTAMATE-1-^{14}C BY SUPERNATANT OF KIDNEY AND BRAIN HOMOGENATES

Substance added	*Relative radioactivity of CO_2 produced*	
	Kidney	*Brain*
Control	100*	100*
10 mg inert L-glutamate	23	36
10 mg inert D-glutamate	96	71

* This activity averaged 0.45% of administered counts eliminated as $^{14}CO_2$ for kidney and 0.5% of administered counts eliminated as $^{14}CO_2$ for brain. Each incubation flask contained the supernatant (obtained by spinning at high speed) of a homogenate made from 1.5 g of tissue and 2.0 ml of 0.1 *M* phosphate buffer (pH 7.4) and 0.2 mg ($1.1 \cdot 10^{-3}$ mc) of DL-glutamate. The flasks were incubated at 37° C for 45 minutes in an oxygen atmosphere.

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NAKADA AND WEINHOUSE[2], from studies with washed homogenates of *rat* liver (in our experiments rat kidney was used), concluded that D-aspartic acid oxidase was either absent from the tissue or inactivated during its preparation. Apparently, the first alternative is more reasonable. The importance of animal specificity cannot be overemphasized. For example, STILL[3] observed that D-aspartic acid oxidase exists in rabbit liver or kidney (for confirmatory results see Table I, where it is shown that in rabbit kidney addition of 10 mg of inert D-aspartic acid depresses the rate of utilization of the radioactive DL-mixture almost 50%, indicating that the D-form is utilized); and that D-glutamic acid exerts an inhibitory effect on the oxidation of L-glutamate in rabbit kidney[4]. According to our results, neither observation holds for *rat* kidney or brain.

WEIL-MALHERBE[1] stated that brain slices oxidize only L-glutamic acid and no other amino acid. EDLBACHER AND WISS[5], working with "hack brei" of brain, measured the ammonia produced by incubating the tissue with the amino acid and reported that both forms of alanine, valine, leucine, aspartic acid, isoleucine, phenylalanine, the L-isomer of arginine, lysine, tryptophan and the D-form of histidine can be deaminated, while glycine and L-histidine produce no ammonia. These contradictions can be explained by assuming that the "blood brain barrier" prevents entrance of the amino acid into the intact cell, but that the substrate reaches the enzyme in the broken cell readily. When glycine-1-^{14}C is incubated with brain homogenates, it is metabolized as evidenced by the production of $^{14}CO_2$, even though the amount of glycine dissimilation is very small[6], and whole rat kidney homogenate utilizes both L- and D-alanine while the brain utilizes only the L-form[7]. These recently observed discrepancies with the results of EDLBACHER AND WISS[5] are now supplemented with the experiments reported here (Table I), that the brain (or kidney) does not utilize D-aspartate. According to EDLBACHER AND WISS[5], the D-forms were deaminated more rapidly than their optical antipodes.

According to NAKADA AND WEINHOUSE[2], washed particles of rat *liver* must be fortified with adenine nucleotides to oxidize L-aspartic acid (adenylic acid not being as effective as ATP). It is to be noted that in our study (Table I), AMP and ATP inhibit the reaction in the kidney, but AMP (not ATP) stimulates the reaction in brain. Addition of DPN, TPN, pyruvate or α-ketoglutarate enhances the oxidation of aspartic acid by rat kidney or brain homogenates. The reaction described is dependent upon the presence of oxygen. Addition of pyridoxal phosphate is without effect. In the case of the kidney, but not the brain, the enzymes responsible for the oxidation are present in the supernatant obtained by centrifuging the homogenate at high speed in phosphate buffer.

The oxidation of aspartic acid in the various tissues studied, as shown in Table V, decreases in the following order: liver, kidney, brain, spleen and blood. The spleen and blood have practically no activity.

When a similar study was done on glutamic acid (Table II) ATP, AMP, DPN, TPN, oxalacetate, but not pyruvate, had a stimulatory effect in both rat kidney and brain (exception AMP in the case of brain). Again the reaction was dependent upon the presence of oxygen and the responsible enzymes could be extracted into the supernatant at least to some degree for each one of these two tissues. The oxidation of glutamic acid in the various tissues decreased in the following order: liver, kidney, brain, spleen and blood. Activity in the blood was nil (Table VI).

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TABLE V

$^{14}CO_2$ PRODUCTION FROM LABELLED DL-ASPARTATE-4-^{14}C BY VARIOUS RAT TISSUE HOMOGENATES

Tissue	*Counts of $^{14}CO_2$ produced × 100 / Counts of administered dose*
Brain	0.14
Kidney	0.30
Liver	0.40
Spleen	0.05
Blood	0.02

Each incubation flask contained 1.5 g of tissue suspended in 2.0 ml of 0.1 *M* phosphate buffer (pH 7.4) (except flask which contained 2.0 ml of fresh blood, drawn from the heart, in presence of substrate only) and 0.33 mg ($1.8 \cdot 10^{-3}$ mc) of DL-aspartate. The flask were incubated at 37° C for 45 minutes.

TABLE VI

$^{14}CO_2$ PRODUCTION FROM LABELLED DL-GLUTAMATE-1-^{14}C BY VARIOUS RAT TISSUE HOMOGENATES

Tissue	*Counts of $^{14}CO_2$ produced × 100 / Counts of administered dose*
Brain	0.74
Kidney	0.82
Liver	2.08
Spleen	0.32
Blood	0.003

Each incubation flask contained 1.5 g of tissue suspended in 2.0 ml of 0.1 *M* phosphate buffer (pH 7.4) (except flask which contained 2.0 ml of fresh blood, drawn from the heart, in presence of substrate only) and 0.2 mg ($1.1 \cdot 10^{-3}$ mc) of DL-glutamate. The flasks were incubated at 37° C for 45 minutes.

Since whole homogenates present excellent systems for evaluating physiological factors, the effect of normal and pathological growth on the oxidation of glutamic acid and aspartic acid was studied. COHEN AND HEKHUIS[8] report that the activity of the glutamic-oxalacetic acid transaminase is low in early fetal life and increases with age, and COHEN[9] states that tumors also show a low transaminase activity. This does not apply to lymphosarcoma when compared to normal lymphatic tissues[10].

TABLE VII

$^{14}CO_2$ PRODUCTION FROM LABELLED DL-ASPARTATE-4-^{14}C AND DL-GLUTAMATE-1-^{14}C BY RAT EMBRYO LIVER

	Relative radioactivity of CO_2 produced	
	DL-*aspartic acid-4-^{14}C*	DL-*glutamic acid-1-^{14}C*
Early embryo liver	66	40
Advanced embryo liver	51	62
Liver of non-pregnant female	106	86

Maternal liver was taken as 100. Experimental conditions are identical to those given for the other tables.

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We observed that the rate of oxidation of glutamic acid or aspartic acid in the *liver* of the embryo was low, compared to that in the liver of its own mother, or to that of the non-pregnant female (Table VII). The liver of a Walker Tumor bearing host was less active than that of a normal rat, and the tumor tissue itself showed poor oxidative capacity (Table VIII). It may well be that tumor growth is possible because amino acid catabolism is at a minimum. The literature has not shown that tumors have an exceedingly high protein synthetic activity[11], but has demonstrated that embryonic tissue displays an extremely rapid incorporation of amino acids into the protein[12].

TABLE VIII

$^{14}CO_2$ PRODUCTION FROM LABELLED DL-ASPARTATE-4-^{14}C AND DL-GLUTAMATE-1-^{14}C BY NORMAL AND TUMOR LIVER AND BY WALKER SARCOMA

	Counts of $^{14}CO_2$ produced × 100 / *Counts of administered dose*	
	DL-*aspartic acid-4-*^{14}C	DL-*glutamic acid-1-*^{14}C
Walker sarcoma	0.073	0.029
Tumor liver	0.39	0.19
Normal liver	0.71	0.39

Experimental conditions are identical to those given for the other tables.

SUMMARY

Whole rat kidney and rat brain homogenates utilize the L-form of aspartic acid or glutamic acid only. If the supernatant is incubated, the same conclusion holds, except in the case of brain tissue supernatant where the D-form might be utilized somewhat. Whole rabbit kidney homogenate dissimilates D-aspartic acid.

Both AMP and ATP inhibit the oxidation of aspartic acid by kidney homogenate. But AMP stimulates this reaction in brain. Addition of DPN, TPN, pyruvate or α-ketoglutarate enhances dissimilation of this amino acid by either whole kidney or brain homogenates. In the case of the kidney (but not the brain) the enzymes responsible for the oxidation are present in the supernatant obtained by centrifuging.

ATP, AMP, DPN, TPN, oxalacetate have a stimulatory effect on the catabolism of glutamic acid by rat kidney or brain homogenates. The responsible enzymes can be extracted into the supernatant, at least to some degree.

The oxidation of aspartic acid and glutamic acid by various tissues decreases in the following order: liver, kidney, brain, spleen and blood. Activity in the blood is nil.

The rate of oxidation of the two amino acids is low in fetal liver, livers of WALKER tumor bearing hosts, and in the tumor tissue itself.

RÉSUMÉ

Des homogénats totaux de rein et de cerveau de rat utilisent exclusivement la forme L des acides aspartique ou glutamique. Les mêmes résultats sont obtenus avec le surnageant, sauf dans le cas du tissu cérébral où la forme D peut être utilisée dans une certaine mesure. Un homogénat total de rein de lapin catabolise l'acide D-aspartique.

L'AMP et l'ATP inhibent l'oxydation de l'acide aspartique par l'homogénat de rein. Mais l'AMP la stimule dans le cerveau. L'addition de DPN, de TPN, de pyruvate ou d'α-cétoglutarate accroît le métabolisme de cet aminoacide par les homogénats totaux de rein ou de cerveau. Dans le cas du rein (mais non du cerveau) les enzymes responsables de l'oxydation sont présents dans le surnageant obtenu après centrifugation.

L'ATP, l'AMP, le DPN, le TPN, l'oxalacétate stimulent le catabolisme de l'acide glutamique par les homogénats de rein ou de cerveau de rat. Les enzymes responsables peuvent être, au moins partiellement, extraits dans le surnageant.

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Les divers tissus peuvent être classés dans l'ordre suivant relativement à leur capacité d'oxyder les acides aspartique et glutamique: foie, rein, cerveau, rate et sang. L'activité du sang est nulle. La vitesse d'oxydation des deux aminoacides est faible dans le foie foetal, dans les foies d'animaux porteurs de tumeurs de WALKER et dans le tissu tumoral lui-même.

ZUSAMMENFASSUNG

Homogenate von der ganzen Rattenniere und dem ganzen Rattengehirn verwerten nur die L-Form der Asparagin- oder Glutaminsäure. Inkubation des Überstandes liefert das gleiche Ergebnis, ausgenommen der Überstand von Gehirngewebe, der auch die D-Form ein wenig angreift. Gesamthomogenat der Rattenniere dissimiliert D-Asparaginsäure.

AMP wie ATP hemmen die Oxydation der Asparaginsäure durch Nieren-Homogenat. AMP jedoch stimuliert diese Reaktion im Gehirn. Die Dissimilation dieser Aminosäure durch das Gesamthomogenat der Niere wie des Gehirns wird durch Hinzufügen von DPN, TPN, Pyruvate oder α-Ketoglurat vergrössert. Im Fall der Niere (nicht des Gehirnes) sind die für die Oxydation notwendigen Enzyme im Überstand vorhanden, der durch Zentrifugieren erhalten wird.

ATP, AMP, DPN, TPN, Oxalacetat stimulieren den Abbau der Glutaminsäure durch Homogenat aus Rattenniere oder Gehirn. Die fraglichen Enzyme können, wenigstens in einem gewissen Umfang, im Überstand extrahiert werden. Die Oxydation der Asparagin- und der Glutaminsäure durch die verschiedenen Gewebe nimmt in folgender Reihenfolge ab: Leber > Niere > Gehirn > Milz > Blut, wobei im Blut die Aktivität gleich Null ist.

Die Oxydationsrate der beiden Aminosäuren ist klein in der fötalen Leber, in Lebern bei WALKER-Tumor tragenden Tieren und in dem Gewebe dieser Tumoren selbst.

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