THE METABOLIC ROUTES OF PYRUVATE IN RAT LIVER, MUSCLE AND BRAIN

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CHAPTER I

INTRODUCTION

A. Some Aspects of Pyruvate Metabolism

Pyruvate, a branch point in the catabolic sequence of glucose (1), can enter the tricarboxylic acid cycle in two different ways, by oxidative decarboxylation to acetyl-CoA (2, 3), or by condensation with carbon dioxide to form a dicarboxylic acid (4, 5, 6). Pyruvate entering the TCA cycle as a dicarboxylic acid yields a net increase in the mass of cycle intermediates, and permits their use in synthesis. On the other hand, pyruvate entering as acetyl-CoA provides no net increase in intermediates but permits the use of the Krebs cycle for energy purposes only. The relative proportion of pyruvate entering the TCA cycle by these routes has been estimated by administration of pyruvate-2-C^{14} (7) or of DL-alanine-2-C^{14} (8) to rats and the determination of the relative radioactivity of the individual carbon atoms of tissue L-glutamic acid. An abbreviated diagram of the TCA cycle is shown in Figure 1.

Labeling patterns in glutamic acid have been used to indicate the relative amounts of pyruvate converted to acetate and to dicarboxylic acids (7, 8). When pyruvate is metabolized by oxidative decarboxylation, the resulting acetyl-CoA will be a precursor of carbons 4 and 5 of glutamate, and when pyruvate is metabolized via a dicarboxylic acid, oxalacetic acid will be a precursor of carbons 1, 2 and 3 of glutamate.

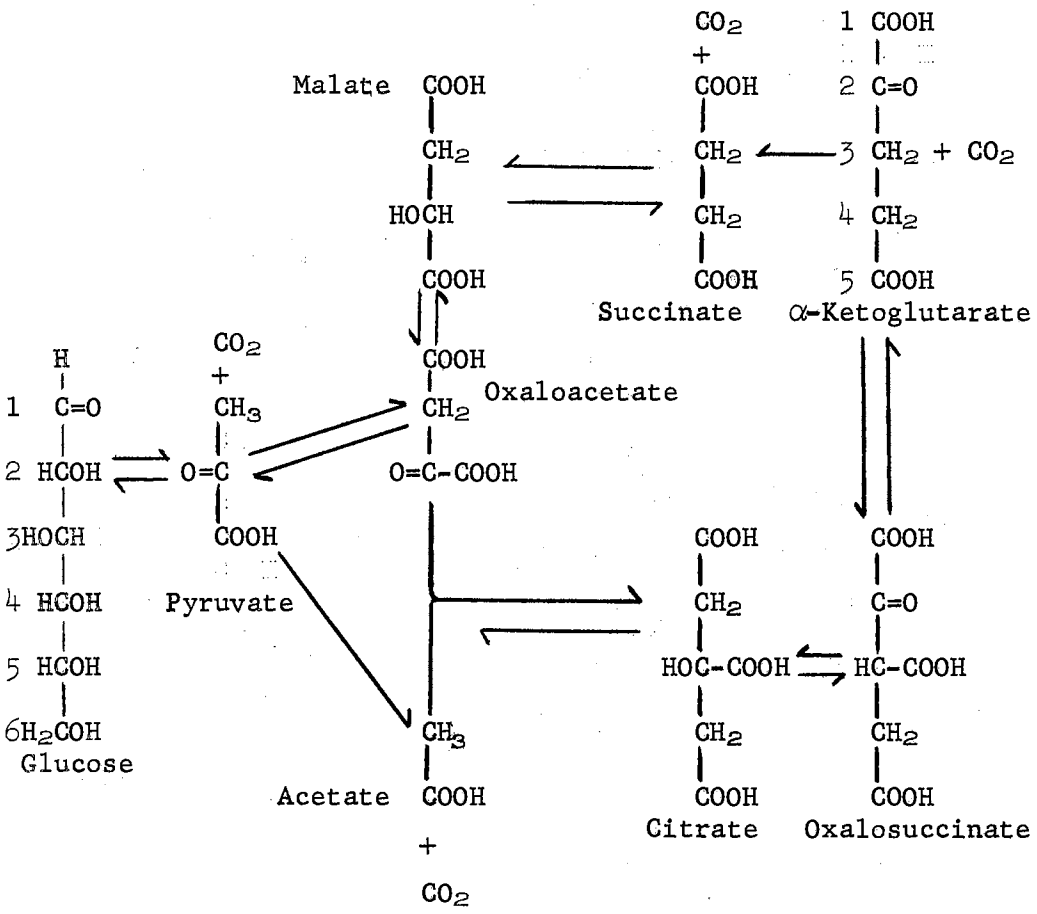

Figure 1. The Krebs Cycle

If pyruvate-2-C^{14} is metabolized via acetyl-CoA, the carboxyl carbon of acetyl-CoA will be labeled. Consequently carbon 5 in glutamate will be labeled (7,8) by the conversion of pyruvate-2-C^{14} to acetyl-1-C^{14}-CoA. Labeling in carbon 3 of glutamic acid will result from the conversion of pyruvate-2-C^{14} to a dicarboxylic acid via carbon dioxide fixation (7, 8). The presence of isotope in carbon 2 indicates the randomization of pyruvate-2-C^{14} through fumarate during the formation of noncarboxyl-labeled dicarboxylic acid. Carbon 1 of glutamate can be labeled from carbon 5 and carbon 2 only after the decarboxylation of ketoglutarate to succinate, leading to carboxyl-labeled oxalacetate. This results in labeling in carbon 1 to an extent not greater than one-half that of carbon 5 plus carbon 2 (9, 10). Carbon 1 of glutamate may also be labeled by carbon dioxide fixation of bicarbonate-C^{14} to yield carboxyl-labeled dicarboxylic acid (11, 12).

The presence of radioactivity in carbon 4 of glutamate after administration of pyruvate-2-C^{14} or alanine-2-C^{14} can be explained in at least two ways. The first is the formation of acetyl-1,2-C^{14}-CoA as a product of two sequential decarboxylations of noncarboxyl-labeled dicarboxylic acid (7, 8) formed during the equilibration of pyruvate-2-C^{14} with fumarate. Recently, Potanos, Freedman and Graff (13) have observed an unusually high labeling in carbon 4 of brain glutamate after the administration of alanine-2-C^{14} to rats. Because of this observation these workers proposed that brain has an unusually active "carbon metabolism" which caused the high labeling in carbon 4 of brain glutamate. They suggested that the rapidity of the serine-glycine interconversion permits pyruvate-2-C^{14} to act as a source of glycine-2-C^{14}, serine-2-C^{14},

formate-C^{14} and acetate-2-C^{14}, all potential sources of acetyl-2-C^{14}CoA. The results to be reported here do not support the hypothesis of Potanos et al. (13).

The second mechanism involves the formation of glucose-1,2,5,6-C^{14} from pyruvate-2-C^{14} prior to its utilization by some organ(s). During the synthesis of glycogen from labeled lactic acid (14), pyruvic acid (15) and propionic acid (16) extensive randomization of carbons 2 and 3 of these acids has been observed. An interesting measurement was made by Topper and Hastings (17), with the help of C^{14}-labeled substrates, of the relative proportion of pyruvate molecules which enter "the shuttle" before phosphorylation compared with those that are phosphorylated directly. They concluded that "the dicarboxylic acid shuttle is quantitatively a very important intermediate pathway." About 4 pyruvate molecules out of 5 are phosphorylated only after "shuttling." In other words the majority of the pyruvate molecules that are converted into liver glycogen pass through the C_4-dicarboxylic acid stage. Lorber and his associates have shown that analogous considerations also apply to the conversion of lactate (14) and propionate (16) into liver glycogen in the rat. These authors fed various isotopic forms of lactate to rats and studied the isotopic distribution in glucose prepared from liver glycogen. They concluded that less than one-sixth of the lactate could have been converted to glycogen by a direct reversal of the reactions of glycolysis. The data suggest that the bulk of the lactate passes through reactions which cause randomization of the isotope between the α and β carbon atoms. A pathway in which such randomization is accomplished would be conversion to pyruvic acid, carboxylation to oxalacetic

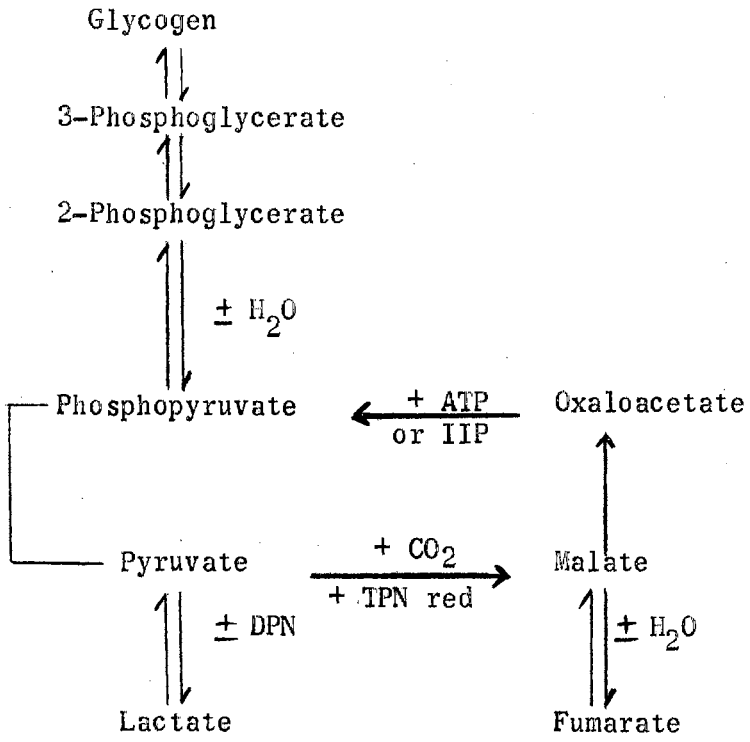

Figure 2. Pathway of synthesis of phosphopyruvate.

acid and reduction of the latter to fumaric acid.

It should be emphasized, however, that the formation of the dicarboxylic acid is probably not the result of a shuttle but of a one-directional pathway involving the stages shown in Figure 2. Krebs proposed that when the thermodynamic properties of the system are considered, the thermodynamic equilibrium of carbohydrate fermentation is very unfavorable for a reversal (18). An important contribution was made by Utter and Kurahashi (19) who showed conclusively that pigeon liver possesses 2 different mechanisms for carbon dioxide fixation. One involves the malic enzyme (20) and requires TPN but not ATP. The other involves the phosphoenolpyruvic-carboxykinase and requires ATP but not TPN.

Thus glycogen formed from pyruvate-2-C^{14} should produce blood glucose-1,2,5,6-C^{14} which, if it is metabolized extensively by other tissues, will yield pyruvate-2,3-C^{14}, acetyl-1,2-C^{14}, and a high labeling in carbon 4 of glutamate (21).

B. The Effect of Ethionine on Lipid Metabolism

In 1938, Dyer (22) reported that ethionine (S-ethyl homocysteine). when administered in a 5 per cent casein diet to young rats, induced a rapid loss in animal weight and death. The simultaneous presence of equivalent amounts of ethionine and methionine in the diet prevented this loss in weight and no death occurred during the experimental period. She concluded that ethionine could not be utilized by the rat in lieu of methionine because no metabolic deethylation of ethionine to homocysteine took place.

In 1941, Harris and Kohn (23) observed in E. coli a growth inhibition effect produced by ethionine. This was completely reversed by

methionine at one tenth the concentration of ethionine in the medium. These experiments furnished the basis for the concept that ethionine is an antimetabolite of methionine.

In 1950, when Farber, Simpson and Tarver administered ethionine intraperitoneally to fasted female rats, this analogue of methionine was found to produce a moderate rise in the lipid content of the liver within 12 hours and a more marked rise in 24 to 48 hours (24). It has been reported by several workers (25, 26) that the fatty infiltration caused by ethionine could be prevented by methionine or choline. Salmon and Copeland reported that the antagonistic action of methionine or choline against ethionine was synergized by vitamine B_{12} (26). According to these workers methionine acted as a methyl donor for the synthesis of choline. However, choline has been found to be ineffective in preventing the rise in liver lipid caused by an intraperitoneal injection of ethionine to fasted rats (24, 27). Artom has found that in livers of fasted rats injected with a large dose of DL-ethionine, the increases in neutral fat were not accompanied by any decrease in the level of choline-containing or total phospholipides. This led to the conclusion that the fatty infiltration of the liver is not due to the rapid development of a choline deficiency. However, after prolonged administration of ethionine in the diet, the production of a chronic choline deficiency has been demonstrated (25, 26).

The influence of hormones upon the development of fatty livers has been studied by several workers (24, 28, 29, 30). Farber et al. (24) reported that excess fat accumulation was observed only in female rats. Castration made the male animal susceptible. Administration of testos-

terone protected the castrated males while adrenalectomy prevented fatty livers in females.

Stekol and Weiss have investigated the possibility of deethylation of ethionine in the intact rat by administering ethionine which was labeled with either S^{35} of with C^{14} in the methylene carbon of the ethyl group (31). They isolated radioactive choline and creatinine from the tissue of rats which were given C^{14} ethionine. The activity from the methylene carbon was found only in the trimethylamine moiety of the choline molecule. These workers isolated radioactive p-bromophenyl mercapturic acid from the urine of rats which received S^{35}-ethionine and bromobenzene. This indicated that ethionine sulfur was available to the rat for cysteine synthesis.

Recently Artom (27) has demonstrated the effect of ethionine on fatty acid metabolism. He observed that homogenates of livers from ethionine-treated female rats exhibited a very considerable decrease in their ability to oxidize stearate-1-C^{14} as compared to homogenates of normal liver. These homogenates also had a decreased ability to oxidize shorter fatty acids, acetate and pyruvate.

C. Scope of the Problem

In order to observe how the disturbance of lipid metabolism in ethionine-treated rats affects the routes of pyruvate metabolism, pyruvate-2-C^{14} was administered to ethionine-treated animals and the radioactivity of carbon 5 of the muscle and liver glutamic acid was determined. The radioactivity of carbon 5 of glutamate reflects the relative amounts of pyruvate converted to acetyl-CoA and oxalacetic acid. Assuming no variation in pool sizes (7) the results indicate that the abnormal lipid

metabolism caused by ethionine does not greatly alter the routes of pyruvate metabolism.

The metabolism of pyruvate in rat brain has been studied in order to determine whether the reported (13) unusual metabolism of this compound by brain results from the blood-brain barrier (32, 33) or whether this is due to a peculiarity of brain tissue. The labeling patterns in brain glutamate have been determined after the administration of pyruvate-2-C^{14} and glucose-2-C^{14} to rats. A finding of high activity in carbon 4 of brain glutamate of adult rats given pyruvate-2-C^{14} would be consistent with the results observed by Potanos et al. (13).

If the administered pyruvate-2-C^{14} was metabolized directly in the brain without prior conversion to other radioactive compounds, the labeling in carbon 4 of brain glutamate of rats receiving glucose-2-C^{14} should be similar to that of rats receiving pyruvate-2-C^{14}. However, the finding of a very low activity in carbon 4 of the brain glutamate obtained from the animals given glucose-2-C^{14} would suggest that glucose-2-C^{14} was absorbed directly by brain whereas pyruvate-2-C^{14} was converted to glucose-1,2,5,6-C^{14} prior to its entry into brain. The data to be reported support the latter proposal.

In order to further study the effect of the blood-brain barrier on glutamate labeling patterns in brain, young rats were given pyruvate-2-C^{14}.

CHAPTER II

EXPERIMENTAL

In the experiments on fatty liver, young female rats weighing 100 to 150 gm. were used. In the experiments on brain, male rats weighing 300 to 380 gm. were used. Rats 154 and 163 were eight days old. The radioactive metabolite to be given was dissolved in about 1 ml. of 0.9 per cent saline and injected intraperitoneally. After the injection, the animals were placed in a glass metabolism cage which was swept with air. The exhaled carbon dioxide was trapped in 1N sodium hydroxide for half an hour in the fatty liver experiments and for one hour in the brain experiments. All the animals were sacrificed by a blow on the head. Rats 130, 137 and 150 were anaesthesized with ether prior to sacrifice. The liver and muscle were isolated from the fatty liver animals. Brain was isolated by opening the top of the skull with the aid of a forceps and was blotted to remove most of the blood. Table I summarizes the individual experiments which were performed.

Development of the Fatty Liver by DL-Ethionine

Fatty liver was induced by intraperitoneal injection of ethionine as described by Chaikoff and Tarver (34).

Young female rats (34) weighing 100 to 150 gm. received four intraperitoneal injections, at 2.5 hours intervals, of 50 mg. (0.33 m moles) of DL-ethionine in 2 ml. of warm saline solution per 100 gm. of

TABLE I

A SUMMARY OF THE EXPERIMENTS

Rat No.	Rat Weight	Pretreatment of Animal	Compound Injected	uc of C^{14} Injected	% Exhaled as $C^{14}O_2$
117	110	Normal	Pyruvate -2-C^{14}	69.7	25
118	145	"	"	43.5	34
119	138	Ethionine	"	51.8	10
126	116	"	"	47.4	8
123*	100	**E + Glucose	"	63	20
124*	114	"	"	50.2	8
130	125	**F, 24 Hours	"	55.3	5
137*	122	F + Glucose	"	59.8	28
145°	301	F, 48 Hours	"	88.1	13.5
146°	341	"	"	128.5	16
154°	11.5	Normal	"	32	23.1
163°	19.2	"	"	18.2	19.9
148°	313	"	Glucose -2-C^{14}	74.2	17
150°	347	"	"	37	19
158°	122	"	Acetate -1-C^{14}	128	61.5
152°	380	"	DL-Glutamic Acid-2-C^{14}	49.5	6.5

*Rats 123, 124 and 137 were given 2 gm. of glucose by stomach tube 30 minutes before the injection of pyruvate-2-C^{14}.

°Brain was isolated one hour after the injection of labeled compounds and liver and muscle were isolated from the Rats 117 through 137 30 minutes after the injection of pyruvate-2-C^{14}.

**E = Ethionine F = Fasted

body weight. After the first injection food was removed for the duration of the experiment. The animals seemed nervous and sensitive to environmental noise after the second injection and chose to hide at the corner of the cage. The animals seemed to be even more nervous after the fourth injection. At the end of 24 hours the animals, which had lost about 10 to 15 gm. of their body weight, were given sodium pyruvate-2-C^{14}. Without exception the livers of the animals which received ethionine were yellow.

Isolation of Glutamic Acid from the Muscle and Liver of Rats 117 Through 137

After the removal of the liver, head, feet, viscera, lungs, heart and skin, a protein powder was prepared (11, 35) from the remaining muscle and skeleton. This will be referred to as muscle protein hereafter.

Glutamic and aspartic acids were isolated from this protein according to the methods previously described (35).

Isolation of Glutamic Acid and Alanine from the Brain of Rats 145 and 146

Brains were removed immediately after sacrifice and weighed. The brain protein was obtained by the method previously described for muscle and liver (11).

Five hundred mg. of L-glutamic acid, 250 mg. of alanine and 50 mg. of cycteine were added as carrier before the brain protein of Rat 146 was hydrolyzed. This hydrolysate was combined with that of Rat 145 for the isolation of glutamic acid and alanine as previously described (11, 35).

The specific activity in the brain protein glutamate was estimated by assuming that 1 mmole (15 per cent) of glutamic acid was present in

a gram of brain protein. The estimated specific activity and dilution factors are shown below:

Rat No.	Weight of Brain (gm.)	Weight of Protein (gm.)	mmoles of Carrier	Dilution Factor	Specific Activity mμc/mmole (Diluted)	Specific Activity Mμc/mmole (Undiluted)
145	1.9	0.267	3.4	8.1	10.5	85
146	1.77	0.213				

Isolation of Glutamic Acid and Alanine from Whole Brain Tissue of Rat 148

Whole brain tissue hydrolysate was prepared as described by Freedman and Graff (8). Whole brain tissue was homogenized with 5 ml. of 1 N hydrochloric acid for 2 minutes and diluted with 10 ml. of 5 N hydrochloric acid and 5 ml. of concentrated hydrochloric acid to make a volume of 5 N hydrochloric acid 20 times the original weight of tissue. The solution was transferred to a flask to which 500 mg. of L-glutamic acid, 250 mg. of alanine, and 20 mg. of cysteine were added as carrier. The cysteine was added to make the sharp band on the IR-120 column which was used as an indicator of amino acid movement.

The solution was then heated under a reflux for 24 hours. The humin was removed by filtration with 1 to 2 gm. of Celite. The filtrate was concentrated to 25 ml. in vaccuo and extracted 3 times with 20 ml. portions of amyl alcohol to remove lipids. The residual amyl alcohol was removed with 20 ml. of ethyl ether and glutamic acid and alanine were isolated as previously described (11, 35).

The specific activity in the whole brain glutamate was estimated as follows: it was assumed that one gram of brain contained about 0.13

gm. of protein (estimated from Rats 145 and 146) and 10 μmoles of free glutamate (33). The calculated data are as follows:

Rat No.	Weight of Brain (gm.)	mμmoles of Protein Glutamic	μmoles of Free Glutamic	mmoles of Carrier	Dilution Factor	Specific Activity mμc/mmole (Diluted)	Specific Activity mμc/mmole (Undiluted)
148	1.37	178	13.7	3.4	18.7	133	2490

Isolation of Free Glutamate from the Brain of Rats 150, 154, 163, 158 and 152

Brain tissue was homogenized with 1.3 ml. of 0.6 N perchloric acid per gram net weight of tissue (36). The homogenate was centrifuged for 10 minutes. The protein precipitate was rehomogenized with a volume of 0.33 N perchloric acid equal to that of the first supernatant solution and centrifuged. The supernatants were combined, neutralized with 2 N potassium hydroxide and allowed to stand overnight in the refrigerator. The solution, whose volume was about 5 ml., was centrifuged to remove the precipitate ($KClO_4$). Three millimoles of carrier L-glutamic acid were added, the pH of the solution dropping to about 4. The solution was warmed for a few minutes and then allowed to cool at room temperature. An equal volume of 95 per cent ethanol was then added to the solution and glutamic acid was allowed to precipitate overnight in the refrigerator. Glutamic acid was removed by filtration and washed with 5 ml. of absolute ethanol and ether. The yields were usually about 90 to 100 per cent.

Glutamic acid was dissolved in 5 ml. of water and a few drops of 2 N hydrochloric acid, and the solution was warmed on the steam bath

for a few minutes. Aqueous pyridine (25 per cent) was added dropwise to the warm solution to bring the pH up to 3.5 and the solution was allowed to cool for 10 minutes at room temperature. Then an equal volume of 95 per cent ethanol was added, and glutamic acid was allowed to crystallize overnight in the refrigerator. Constant specific acitvity was usually obtained by the second recrystallization. The yields after the second recrystallization were approximately 70 per cent.

This procedure is an elegant one for obtaining free glutamic acid from brain.

The specific activity in the free glutamate of brain was estimated according to the data given by Tower (33).

Rat No.	Weight of Brain (gm.)	μmoles of Free Glutamate	mmoles of Carrier	Dilution Factor	Specific Activity mμc/mmole (Diluted)	Specific Activity mμc/mmole (Undiluted)
150	1.68	16.8	3.4	202	45.5	9200
154	0.52	5.2	1.0	192.5	42.6	8200
163	0.71	7.1	2.0	282	44.7	12605
158	1.52	15.2	1.5	98.6	2.2	217
152	1.26	12.6	1.0	79.5	1.1	825

Decarboxylation of Glutamic Acid by the Schmidt Reaction

Carbon 5 of glutamic acid was determined directly by treating this amino acid with hydrazoic acid to give carbon 5 as carbon dioxide (39), and α,γ-diaminobutric acid. The latter was isolated as the dipicrate by a method similar to that described by Adamson (37). The values obtained for carbon 5 by this method were fairly consistent with those

obtained from Schmidt reactions on butyric acid during the complete degradation of glutamic acid (39, 40). Figure 3 represents the Schmidt reaction apparatus.

0.68 mmoles (100 mg.) of glutamic acid were placed in a 15 ml. pear shaped flask (A), and 0.75 ml. of 100 per cent (37) sulfuric acid was carefully added. The flask was then stoppered with a glass stopper and warmed over a small flame to dissolve the amino acid. The solution was chilled in an ice bath for 15 minutes. Three mmoles (about 200 mg.) of sodium azide were added to the chilled solution and the reaction flash was quickly connected to a distillation head (B) through which ran a long air inlet tube (C) equipped with a stopcock. The air inlet tube was attached to the head by means of a short length of tygon tubing (D). A side arm from the head was coupled by means of a semi-ball joint to a fritted glass bubbler containing 5 ml. of 5 per cent acid permanganate (E) to trap sulfur dioxide formed by the reduction of sulfuric acid by hydrazoic acid. The permanganate bubbler (E) was attached, by means of semi-ball joints (F) to a second fritted glass bubbler (G) containing 5 ml. of carbon dioxide-free 1 N sodium hydroxide. The alkali bubbler was attached to a water pump by which the system was evacuated. When a fine stream of bubbles was observed in the sodium hydroxide trap the reaction flask was slowly warmed by immersing in a water bath which was heated so that the temperature of water reached 50° C. in 30 minutes. If the reaction became too vigorous at the beginning the water bath was removed temporarily. After the evolution of carbon dioxide ceased the water bath was raised to boiling and kept there for an hour. At the end of this hour, the solution was clear. The stopcock of the air inlet tube was opened and carbon dioxide-free air (I) was swept through the

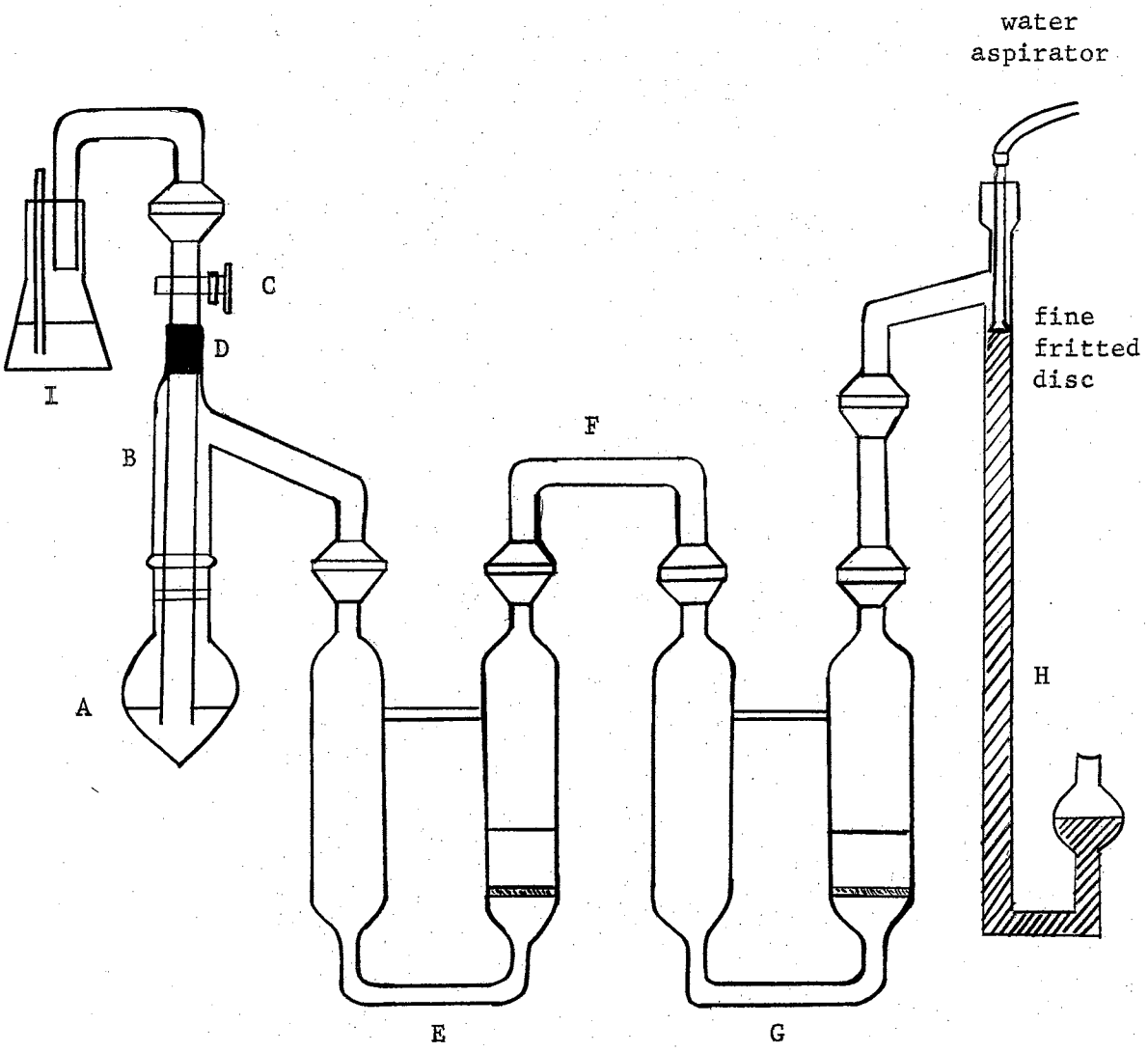

Figure 3. The Apparatus Used in Degrading Glutamic Acid

system for a period of an hour. During this time the reaction flask was heated in the boiling water bath. At the end of this period, the contents of the alkali bubbler were transferred to a Van Slyke apparatus for the manometric determination of CO_2 and subsequent C^{14} assay (38). The yields of carbon dioxide were 80 to 90 per cent.

Preparation of α,γ-Diaminobutyric Acid Dipicrate

The solution remaining after the Schmidt reaction on glutamic acid was dissolved in 3 to 4 ml. of water and transferred to a 50 ml. beaker. The solution was then neutralized to pH 6 with hot saturated barium hydroxide solution and the barium sulfate was removed by centrifugation. The supernatant was filtered and concentrated to 7 to 10 ml. on the steam bath. After the addition of 300 mg. (1.33 mmoles) of picric acid the solution was warmed until all the picric acid was dissolved. Some of solutions were allowed to cool at room temperature to crystallize the dipicrate of α,γ-diaminobutyric acid. When no crystals formed at room temperature, the solutions were left overnight in the refrigerator. The dipicrates were removed by filtration, washed with 2 to 3 ml. of water, and recrystallized from 5 to 8 ml. of hot water. The dipicrates were recrystallized until their melting points reached 180° to 183°.

Degradation of Glutamic Acid

The degradation of glutamic acid was accomplished by the method of Mosbach, Phares and Carson (39,40,41) as modified by Koeppe and Hill (12). Usually 2 mmoles of glutamic acid were used. The series of reactions is summarized in Figure 4.

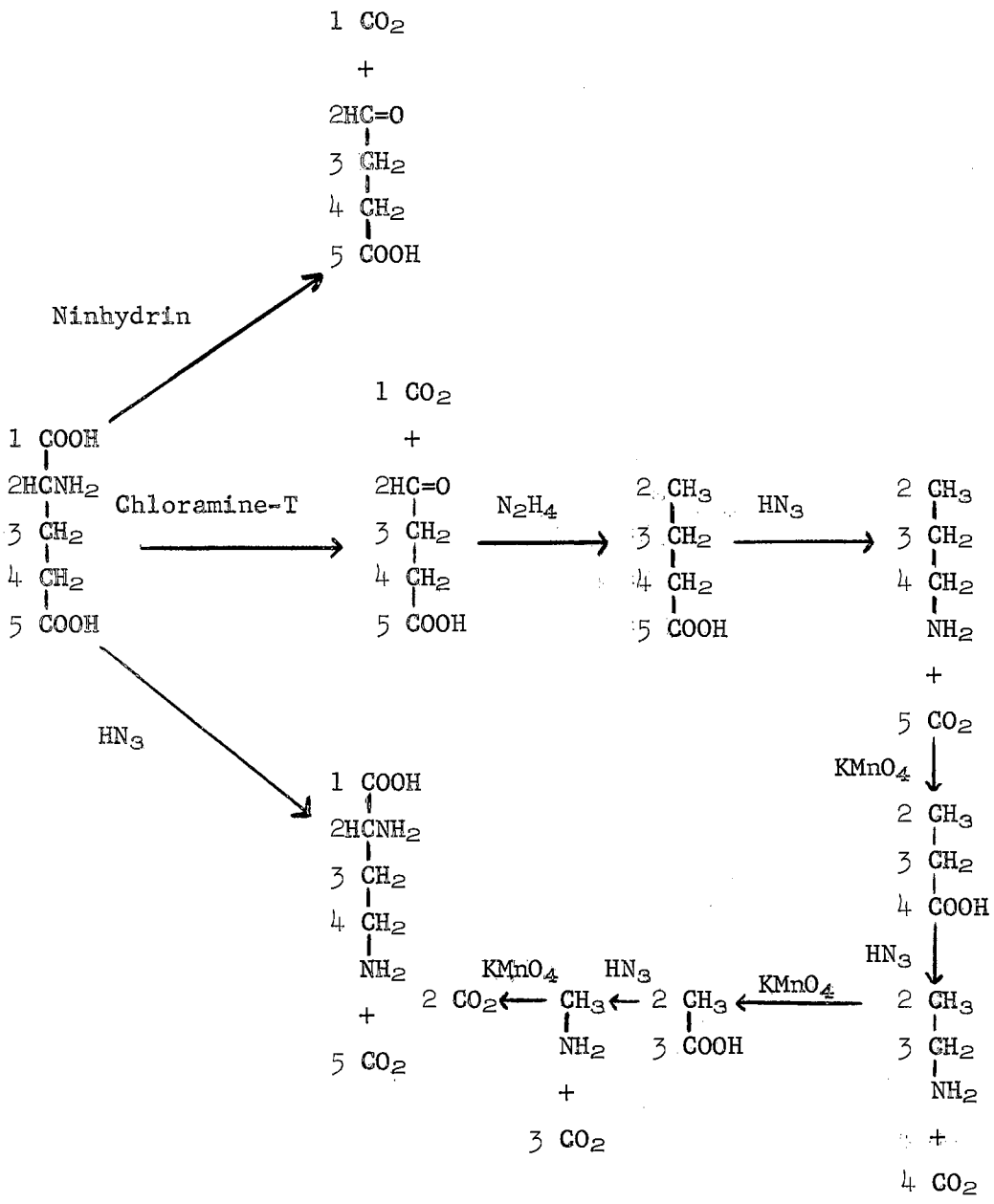

Figure 4. The Degradation of Glutamic Acid

Degradation of Alanine

The degradation of alanine was carried out as described by Koeppe, Minthorn and Hill (42).

Carbon Analysis and Radioactivity Assay

The total carbon content of the various compounds was determined by the macrocombustion method of Van Slyke and his coworkers (43). Carbon dioxide liberated during degradation procedures was also measured manometrically.

The carbon dioxide from the wet combustion of samples and from the various degradations was swept into a 280 ml. Borkowsky (44) ionization chamber and assayed with a vibrating reed electrometer (45).

CHAPTER III

RESULTS

A summary of the experiments is presented in Table I. The glutamic acid labeling patterns are presented in Tables II, III and IV. In these tables, the specific activities are expressed as millimicrocuries per millimole. The specific activities reported for the liver and brain amino acids have been corrected for dilution with carrier during their isolation (see Experimental).

It is of interest to notice that the rate of $C^{14}O_2$ excretion by the normal rats is much faster than by the ethionine-treated and fasted animals. Feeding glucose to both animals (ethionine-treated and fasted) enhanced the $C^{14}O_2$ excretion.

Labeling Patterns in Glutamic Acid of Muscle after Administration of Pyruvate-2-C^{14}

As shown in Table II, approximately 50 per cent of the total radioactivity is located in carbon 5 of muscle glutamate of normal rats. The labeling in carbon 5 of muscle glutamate is very similar to that of skeletal muscle glutamate of normal rats which had received alanine-2-C^{14} (46). In the glutamate of ethionine-treated animals, about 12 to 25 per cent of total activity was located in carbon 5 (Rats 119 and 126). The labeling in carbon 5 of muscle glutamate of fasted animal is about 22 per cent of the total activity (Rat 130). This is slightly greater than that of carcass glutamate of male rats which were fasted for 48 hours prior to the injection of pyruvate-2-C^{14}, as previously reported by Koeppe, Mourkides and Hill (7).

As shown in Table II, the injection of glucose by stomach tube prior to the administration of pyruvate-2-C^{14} to fasted and ethionine-treated rats markedly increased the radioactivity of carbon 5 of glutamate. The effect of prior treatment with glucose seems to be greater in the fasted rats than in the ethionine-treated animals.

Labeling Patterns in Glutamic Acid of Liver after Administration of Pyruvate-2-C^{14}

Table III summarizes the results obtained from liver glutamate 30 minutes after administration of pyruvate-2-C^{14}. In the liver of normal animals, about 20 per cent of the total radioactivity was located in carbon 5. The percentages of activity in carbon 5 of liver glutamate from both fasted and ethionine-treated animals are less than 7. As in the case of muscle, administration of glucose by stomach tube increased the activity in carbon 5 of glutamate isolated from both fasted and ethionine-treated rats.

Labeling Patterns in Glutamic Acid of Brain after Administration of Labeled Compounds

The labeling patterns of brain glutamate are summarized in Table IV. The results obtained from Rats 145 and 146 are quite consistent with those of Potanos, Freedman and Graff (13), who used alanine-2-C^{14}. About 50 per cent of the total radioactivity is located in carbons 5 and 4 of Rats 145 and 146. It should be noted that more than 15 per cent of the activity is located in carbon 4. The ratio of the activity between the two carbons is approximately 2. In the brain glutamates of Rats 148 and 150, which were given glucose—2-C^{14}, more than 80 per cent of total activity was found in carbons 5 and 1. It is of importance to

point out that less than 3 per cent of the total activity was located in carbon 4, while 55 per cent was in carbon 5.

It should be noted that the radioactivity in carbon 4 of the brain glutamate isolated from young animals (Rats 154 and 163) given pyruvate-2-C^{14} is much less than that of the adult brain glutamate. Also the radioactivity in carbon 1 of both young brain glutamates is greater than in carbon 5. About 60 to 70 per cent of total activity was found in carbons 1 and 5 of the brain glutamates.

In the animals which were given acetate-1-C^{14} and glutamic acid-2-C^{14}, brain glutamate had a very low specific activity (Rats 158 and 152). As shown in Table IV the activity in carbon 1 is much greater than in carbon 5 (Rat 15[illegible]). These results are different from those obtained by other workers (12, 47, 48, 49), who have shown that the administration of carboxyl-labeled acetate to rats results in ratios of radioactivity of carbon 5 to carbon 1 in liver and carcass glutamate which approach a theoretical value of 2.

The finding of 45 per cent of the total activity in carbon 1 of brain glutamate (Rat 152), upon administration of glutamic acid-2-C^{14} is in sharp contrast with the results of Wilson and Koeppe who reported that about 20 per cent of the activity of liver and carcass glutamates was located in carbon 1 (50).

TABLE II

LABELING PATTERNS IN MUSCLE GLUTAMATE AFTER ADMINISTRATION OF PYRUVATE-2-C^{14}

Rat No.	Pretreatment of animal	Total mμc/mmole	Per cent of total in carbons 1 through 4	5
117	Normal	55	48	49
118	Normal	28	49	47
119	Ethionine	11	79	26
126	Ethionine	10	83	12
123	Ethionine + Glucose	34	70	36
124	Ethionine + Glucose	21	64	30
130	Faster, 24 hours	12	74	22
137	Fasted + Glucose	38	49	48

TABLE III

LABELING PATTERNS IN LIVER GLUTAMATE AFTER ADMINISTRATION OF PYRUVATE-2-C^{14}

Rat No.	Pretreatment of animal	Total mμc/mmole	Per cent total in carbons 1 through 4	5
117	Normal	414	79	20
118	Normal	215	79	20
119	Ethionine	204	95	7
126	Ethionine	202	97	3
123	Ethionine + Glucose	120	82	18
124	Ethionine + Glucose	241	90	11
130	Fasted, 24 hours	341	90	2
137	Fasted + Glucose	309	65	24

TABLE IV

LABELING PATTERNS IN BRAIN GLUTAMATE*

Rat No.	Compound injected	Total mμc/mmole	Per cent of total in carbons 1	2	3	4	5
145[a]	Pyruvate-2-C^{14}	85	18.8	15.7	15.9	15.7	33.0
146[a]	Pyruvate-2-C^{14}						
154[b]	Pyruvate-2-C^{14}	8200	30.5	19.3		6.6	29.6
163[b]	Pyruvate-2-C^{14}	12605	37.8	lost	8.8	6.7	33.2
148[c]	Glucose-2-C^{14}	2490	28.0	4.4	5.8	2.4	55.0
150[c]	Glucose-2-C^{14}	9200	25.7	2.9	5.9	2.4	55.5
158[c]	Acetate-1-C^{14}	217	51.0				32.8
152[c]	Glutamic Acid-2-C^{14}	825	45.5				

*Protein glutamate was pooled from Rats 145 and 146, whole (protein plus free) glutamate from Rat 148, and free glutamate from Rats 154, 163, 158 and 152.

[a]Fasted 48 hours before administration of pyruvate-2-C^{14}.

[b]8-day-old rats were used.

[c]Normal adult rats were used.

CHAPTER IV

DISCUSSION

The presence of a larger amount of radioactivity in carbon 5 of muscle glutamate of normal rats than that of liver glutamate, an observation similar to those made by Koeppe and his coworkers (7), and Freedman et al. (8), may indicate that pyruvate is more extensively converted to acetate by muscle than by liver tissues. However, this explanation may be incorrect since the acetyl-CoA and the dicarboxylic acid pool sizes of the individual tissues are not known.

The effect of ethionine treatment on the activity of carbon 5 of muscle and liver glutamates appears to be the same as that of fasting. If the acetyl-CoA and dicarboxylic acid pool sizes do not vary extensively the presence of low activity in carbon 5 of the muscle and liver glutamates of fasted and ethionine-treated rats indicates that under these conditions of treatment, pyruvate is metabolized less extensively to acetate than in the normal animal. Since the development of fatty liver with ethionine required fasting it was necessary to examine the effect of glucose on the pyruvate metabolism in fasted and ethionine-treated rats. As shown in Tables II and III, the administration of glucose raised the activity in carbon 5 of both muscle and liver glutamates of fasted and ethionine-treated rats.

If the assumption that glutamate labeling patterns reflect pyruvate

metabolism is a correct one and if one ignores the possibility of marked variation in pool sizes, the results presented here suggest that the gross disturbance in lipid metabolism of the ethionine-treated rats does not greatly alter the routes of pyruvate metabolism.

The finding of a high activity in carbon 4 of the brain glutamate isolated from Rats 145 and 146, which were fasted prior to the administration of pyruvate-2-C^{14}, is consistent with the observation by Potanos, Freedman and Graff (13), who used alanine-2-C^{14}. As discussed in the Introduction, labeling in carbon 4 may be explained in at least two ways. One mechanism involves the formation of acetyl-1,2-CoA by the double decarboxylation of noncarboxyl-labeled oxalacetate after equilibration of pyruvate-2-C^{14} with a symmetrical dicarboxylic acid (fumaric). Another plausible mechanism involves the formation of glucose-1,2,5,6-C^{14} from pyruvate-2-C^{14} prior to its entry into brain, presumably as a result of the peculiarity of the blood-brain barrier. Subsequently the labeled glucose will yield pyruvate-2,3-C^{14} in the brain which, in turn will give a considerable labeling in carbon 4 of brain glutamate. The finding of a very small amount of radioactivity in carbon 4 of the brain glutamate of animals given glucose-2-C^{14} strongly suggests that this glucose was absorbed directly by the brain and converted to pyruvate-2-C^{14} which gives a low activity in carbon 4. The results obtained with glucose-2-C^{14} clearly support the second explanation (above) for the labeling patterns observed in brain glutamate after giving pyruvate-2-C^{14} or alanine-2-C^{14} (13). Potanos, Freedman and Graff proposed that the brain has an unusually active "carbon metabolism" (presumably they meant this to read "one carbon metabolism") which caused the high labeling in carbon 4 of

brain glutamate. These authors suggested that a rapid serine-glycine interconversion permits pyruvate-2-C^{14} to act as a source of glycine-2-C^{14}, serine-2-C^{14}, formate-C^{14} and acetate-2-C^{14}, all capable of forming methyl-labeled acetyl-CoA, which will label carbon 4 of glutamate. The proposal of a highly active "one carbon metabolism" in rat brain does not explain the very low activity we have found in carbon 4 of brain glutamate isolated from the rats which received glucose-2-C^{14}. Therefore, the metabolism of pyruvate in rat brain appears to be similar to its metabolism in other tissues. Blood glucose-1,2,5,6-C^{14} is probably formed from pyruvate-2-C^{14} in the liver and transported across the blood-brain barrier to be utilized by brain as an energy source. Thus the unusual labeling patterns observed in brain glutamate after the administration of alanine-2-C^{14} (13) or pyruvate-2-C^{14} are probably due to the metabolism of pyruvate prior to its entry into the brain.

An interesting observation is that about 55 per cent of the total activity was located in carbon 1 of brain glutamate of the rats given glucose-2-C^{14} (Rats 148 and 150). Thus brain, like muscle (46), appears to convert a large proportion of its pyruvate to acetate.

The presence of lower activity in carbon 4 of young rat brain (Rats 154 and 163) may indicate that the young brain is more permeable to pyruvate. It has been shown by Tower (33) that the blood-brain barrier is absent or rudimentary in new-born mammals and becomes active during the first few weeks of life.

The high activity of carbon 1 relative to carbon 2 plus carbon 5 of young brain glutamates is also of interest. Koeppe, Hill and Mourkides (7), and Freedman and Graff (8) have demonstrated that carbon 1

of glutamates isolated from rats given pyruvate-2-C^{14} is never more than one half that of carbon 2 plus carbon 5. Since theoretically (11) carbon 1 should not exceed 50 per cent of carbon 2 plus carbon 5, the labeling in carbon 1 of the young brain glutamates is difficult to explain. One possibility is that carbon dioxide fixation in the brain from a bicarbonate-C^{14} pool of high specific activity resulted in a substantial synthesis of carboxyl-labeled oxalacetate, which in turn would label carbon 1 of glutamic acid (12, 51, 52, 53). Recently Berl et al. demonstrated the extensive participation of carbon dioxide fixation in brain metabolism (54).

In order to further demonstrate the effect of the blood-brain barrier on glutamate labeling patterns in brain, acetate-1-C^{14} and glutamic acid-2-C^{14} were administered to normal adult rats. The high activity of carbon 1 of the brain glutamates from these animals is unusual. If acetate-1-C^{14} or glutamic acid-2-C^{14} were taken up directly by brain (Rats 152 and 158), the activity in carbon 1 would be expected to be about 30 per cent from acetate-1-C^{14} (12) and 20 per cent from glutamic acid-2-C^{14} (50, 55). However, the finding of about 50 per cent of the total activity in carbon 1 and of a very low total specific activity in the brain glutamate (Rats 152 and 158) suggests that much of the radioactivity from acetate-1-C^{14} (56) and glutamate-2-C^{14} entered the brain only after prior conversion in the liver to glucose-3,4-C^{14}.

CHAPTER V

SUMMARY

Pyruvate-2-C^{14} was given to normal, ethionine-treated and fasted female rats. Glutamic acid was isolated from muscle and liver. Degradation of glutamic acid demonstrated that in normal animals pyruvate was more extensively converted to acetate by muscle than by liver tissue. The degree to which pyruvate is converted to acetate is reflected in a direct manner by the extent to which radioactivity is incorporated into carbon 5 of tissue glutamate. The animals which were either fasted or pretreated with ethionine exhibited a markedly lower activity in carbon 5 of muscle and liver glutamate. The effect of ethionine on the incorporation of activity into carbon 5 of glutamic acid appears to be the same as that of fasting since feeding glucose to the fasted and ethionine-treated rats markedly raised the activity in carbon 5 of both muscle and liver glutamates.

After administration of pyruvate-2-C^{14} to adult and to eight-day old rats, and of glucose-2-C^{14} to normal adult rats, brain glutamate was isolated and completely degraded. The presence of approximately 15 per cent of the total radioactivity in carbon 4 of adult brain glutamate of rats receiving pyruvate-2-C^{14}, and the presence of less than 3 per cent of the total activity in carbon 4 of the brain glutamate of rats receiving glucose-2-C^{14}, suggests that, in adult rats, pyruvate-2-C^{14}

was converted to glucose-1,2,5,6-C^{14} prior to utilization by the brain. On the other hand, the finding about 6.7 per cent of total activity in carbon 4 of the brain glutamate of young rats receiving pyruvate-2-C^{14} may indicate that in the young rat more pyruvate was taken up directly by the brain.

Since the above results may be explained on the basis of a blood-brain barrier, acetate-1-C^{14} and glutamic acid-2-C^{14} were given to adult rats, and the brain glutamates were isolated and degraded. The finding of a high amount of activity in carbon 1 of the brain glutamates from these animals suggests that much of the acetate-1-C^{14} and the glutamic acid-2-C^{14} was taken up by the brain only after prior conversion to blood glucose-3,4-C^{14} by the liver.

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