Utilization *in vivo* of Glucose and Volatile Fatty Acids by Sheep Brain for the Synthesis of Acidic Amino Acids

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1. Free glutamic acid, aspartic acid, glutamic acid from glutamine and, in some instances, the glutamic acid from glutathione and the aspartic acid from *N*-acetyl-aspartic acid were isolated from the brains of sheep and assayed for radioactivity after intravenous injection of [2-¹⁴C]glucose, [1-¹⁴C]acetate, [1-¹⁴C]butyrate or [2-¹⁴C]propionate. These brain components were also isolated and analysed from rats that had been given [2-¹⁴C]propionate. The results indicate that, as in rat brain, glucose is by far the best precursor of the free amino acids of sheep brain. 2. Degradation of the glutamate of brain yielded labelling patterns consistent with the proposal that the major route of pyruvate metabolism in brain is via acetyl-CoA, and that the short-chain fatty acids enter the brain without prior metabolism by other tissue and are metabolized in brain via the tricarboxylic acid cycle. 3. When labelled glucose was used as a precursor, glutamate always had a higher specific activity than glutamine; when labelled fatty acids were used, the reverse was true. These findings add support and complexity to the concept of the metabolic 'compartmentation' of the free amino acids of brain. 4. The results from experiments with labelled propionate strongly suggest that brain metabolizes propionate via succinate and that this metabolic route may be a limited but important source of dicarboxylic acids in the brain.

Glucose is the main source of energy utilized by the brain of man and various laboratory animals (White, Handler & Smith, 1964). During the last few years it has been shown, by the use of ¹⁴C-labelled glucose, that part of the glucose carbon is metabolized directly to carbon dioxide but that an appreciable portion is retained in the free amino acids of brain (Beloff-Chain, Catanzaro, Chain, Masi & Pocchiari, 1955; Flexner, Flexner & Roberts, 1958; Allweis & Magnes, 1958; Geiger, Kawakita & Barkulis, 1960; Barkulis, Geiger, Kawakita & Aguilar, 1960; Vrba, Gaitonde & Richter, 1962; Koeppe & Hahn, 1962; Cremer, 1964; O'Neal & Koeppe, 1966). These components of brain, especially glutamate, aspartate and glutamine, are found in considerable and consistent quantities and have been observed to incorporate ¹⁴C after the injection of other labelled compounds (Busch, 1955; Potanos, Freedman & Graff, 1960; Koeppe & Hahn, 1962; McMillan & Mortensen, 1963; O'Neal & Koeppe, 1966).

The metabolism of some ¹⁴C-labelled compounds by brain has been studied by measuring the amount of incorporation and the distribution of ¹⁴C in the

free amino acids of rat brain (Koeppe & Hahn, 1962; McMillan & Mortensen, 1963; Koeppe, O'Neal & Hahn, 1964; O'Neal & Koeppe, 1966). The results indicate that glucose is by far the best precursor of the amino acids of rat brain, but that some lactate, acetate and butyrate are utilized without prior metabolism by other tissues.

Ruminants are known to derive as much as 70% of their total energy requirements from the metabolism of volatile fatty acids and have lower concentrations of glucose in the circulating blood than do non-ruminants. Significant arterial-venous differences in acetate concentration of the blood circulating through the head of sheep have been observed (Reid, 1950). However, data on the arterial-venous differences of acetate and glucose circulating through the brain of sheep indicate that, as with non-ruminants, glucose is the main source of energy utilized by ruminant brain (McClymont & Setchell, 1956).

In an effort to gain further insight into the energy metabolism of ruminant brain, the utilization of labelled volatile fatty acids and glucose by sheep brain was investigated. We report results on the

incorporation and distribution of ^{14}C in free amino acids isolated from the brain of sheep after the intravenous administration of $[2\text{-}^{14}\text{C}]\text{glucose}$, $[1\text{-}^{14}\text{C}]\text{acetate}$, $[1\text{-}^{14}\text{C}]\text{butyrate}$ or $[2\text{-}^{14}\text{C}]\text{propionate}$. The results are comparable with those found in rats (O'Neal & Koeppe, 1966).

MATERIALS AND METHODS

Experimental animals. Hampshire wethers, except sheep 11, which was a western type, weighing 50–75 lb. were obtained from the Animal Science Department, Oklahoma State University. These animals were kept in a dry lot and allowed to eat and drink *ad libitum* before the surgery and the labelling experiments. The ration provided during this period was a commercially prepared sheep pellet composed of alfalfa, Milo, soya-bean oil meal, vitamins and minerals. It contained 12% of crude protein. Male albino rats, obtained from the Holtzman Rat Co., Madison, Wis., U.S.A., were allowed to consume a 25%-protein commercial rat diet and water *ad libitum* before the experiments.

Radioactive compounds. The D- $[2\text{-}^{14}\text{C}]\text{glucose}$ was obtained from Merck and Co. Ltd., Montreal, Quebec, Canada. Sodium salts of the labelled fatty acids were obtained from the California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A. These compounds were dissolved in 0.9% NaCl. The saline used for the fatty acids was made slightly basic by adding a drop or two of 2N-NaOH. Samples of labelled glucose were chromatographed on Whatman no. 1 paper in butan-1-ol-methylCellosolve-water (2:1:1, by vol.) solvent system. The chromatograms were scanned with a strip counter and found to have only one radioactive peak whose R_f value (0.44) was similar to that of a glucose standard. One sample of D- $[2\text{-}^{14}\text{C}]\text{glucose}$ was degraded (Gunsalus & Gibbs, 1952), 99% of the activity being found in C-2. The ^{14}C -labelled fatty acids were degraded by Schmidt reactions (Phares, 1951). The carboxyl carbon atoms of $[1\text{-}^{14}\text{C}]\text{acetate}$ and $[1\text{-}^{14}\text{C}]\text{butyrate}$ and the ethylamine from $[2\text{-}^{14}\text{C}]\text{propionate}$ contained over 99% of the radioactivity of the parent molecule in each case.

Experimental procedure. A short time before the precursors were to be administered the carotid arteries of sheep 2, 4 and 5 were surgically exposed by blunt dissection. Procaine hydrochloride (4%, w/v) was used as a local anaesthetic. Although this procedure allowed for very convenient injections into the carotid artery it was discontinued because of the high blood-sugar concentrations observed in all animals treated in this manner.

The surgical technique employed with the other sheep is a modification of the method described by Butler (1962) for calves. The sheep were restrained and procaine hydrochloride (15–20 ml. of a 2% solution) was injected subcutaneously along the midline of the neck and sterile drapes were applied. A longitudinal incision was made in the skin, commencing at the angle of the mandible and extending 12–14 cm. caudally along the midline of the neck. The left carotid artery, enclosed in the carotid sheath, was exposed by reflecting the sternocephalicus and omohyoideus muscles by blunt dissection. The carotid sheath was incised along a distance of approx. 6–8 cm. and the carotid artery was separated from the vagus and sympathetic nerves at this point. The exposed artery was brought to the surface. A piece of polyethylene tubing 5 cm. long was split longitudinally

and half the circumference of the cannula, except for 1 cm. at each end, was removed. This cannula was placed around the artery. The cannulated artery was immobilized by means of two silk sutures that were placed around the polyethylene tubing near each end, brought through the skin and secured externally. This surgical procedure was repeated on the right carotid artery; circulation through both arteries was checked. Subcutaneous tissue and skin incisions were closed in a conventional manner. The relocated arteries (lying within the cannulae) were easily located by palpating the skin in the region between the silk fixation sutures. The labelling experiments were conducted 1–2 weeks after this surgery.

Samples of ^{14}C -labelled precursor were injected with heparin-treated syringes into the relocated carotid arteries of the sheep, except sheep 6 where $[1\text{-}^{14}\text{C}]\text{acetate}$ was injected into the jugular vein. It was found that connecting two syringes to one needle by use of a three-way valve eliminated contamination of the labelled precursors by venous blood. A syringe that contained 0.9% NaCl was used to ascertain that the needle was in the carotid artery. Then the valve was turned to allow the labelled compounds to be injected. The prevention of clotting in the needles and maintenance of a constant rate of injection was difficult in the first two experiments, where injections were prolonged over 4 min. Thereafter these problems were eliminated by reducing the injection periods to 1–2 min. Information about kind, amount and rate of injection of compounds administered and animal size is given in Table 1.

At 20 min. after injection of the labelled precursor, the sheep were decapitated. Samples of blood were collected in beakers wetted with 10% (w/v) potassium oxalate. These were analysed for blood sugar, volatile fatty acids and radioactivity. The blood sugar determinations were made by the Nelson-Somogyi method (Hawk, Oser & Summerson, 1954), the volatile fatty acid estimations were made by the use of gas-liquid chromatography (Baumgardt, 1964) and blood radioactivity was determined by wet combustion of dried whole blood (Koeppe & Hill, 1955). The cerebrum and cerebellum were removed, blotted with filter paper and frozen in liquid nitrogen. A 3–5 min. period was required to decapitate the sheep, remove the brain and place it in liquid nitrogen.

The rats were injected subcutaneously with Nembutal (60 mg./kg. body wt.) 1 hr. before injection of the labelled propionate. At 10 min. after the injection of sodium $[2\text{-}^{14}\text{C}]\text{propionate}$ into the femoral vein the rats were decapitated and the brain was removed, blotted and frozen in liquid nitrogen.

Isolation and assay of amino acids. The amino acids were isolated from the frozen brain tissue by the method described by O'Neal & Koeppe (1966). To isolate the large amounts of amino acids found in sheep brain, Dowex 1 (X8; 100–200 mesh; acetate form) columns (2.4 cm. $\times$ 30 cm.) were used. The cold protein-free supernatants were passed over columns, held at 4°, at a rate of 40–50 ml./hr. The neutralized hydrolysates of glutamine were made up to a volume of 250 ml. before being passed over the Dowex 1 (acetate form) columns. The amount of aspartate and glutamate in the various peaks was quantitatively determined by the method of Rosen (1957), as modified by Grant (1963).

Degradation procedures and radioactivity assays. Portions of the glutamate and aspartate samples were dried, diluted with carriers, degraded (Hill, Hobbs & Koeppe, 1958) and

Table 1. *Animal sizes, amounts and rates of administration of labelled precursors and blood sugar concentrations and radioactivity contents*

The blood sugar concentrations and radioactivity contents were determined at the end of the labelling experiments. Experimental details are given in the text.

Animal	Body wt. (kg. for sheep, g. for rats)	Compound administered	Labelled precursor administered				Blood	
			Amount (μ C)	Sp. activity of precursor (mc/m-mole)	Volume (ml.)	Rate (ml./min.)	Sugar (mg./100ml.)	Total 14 C (μ C/ml.)
Sheep 1	28.6	D-[2- 14 C] Glucose	40	2.2	10.0	2.5	73	3.6
Sheep 5	28.2	D-[2- 14 C] Glucose	67	3.0	10.0	5.0	84*	3.4
Sheep 9	24.2	D-[2- 14 C] Glucose	67	3.0	5.5	5.5	77	1.6
Sheep 2	28.2	Sodium [1- 14 C] acetate	100	23.5	5.0	1.5	156	0.6
Sheep 4	23.6	Sodium [1- 14 C] acetate	100	23.5	5.0	2.5	112	1.4
Sheep 6	34.6	Sodium [1- 14 C] acetate	106	22.3	5.0		90	0.6
Sheep 7	34.6	Sodium [1- 14 C] butyrate	105	11.9	5.0	5.0	60	3.0
Sheep 10	26.4	Sodium [1- 14 C] butyrate	105	11.9	5.0	5.0	52	1.2
Sheep 8	23.2	Sodium [2- 14 C] propionate	100	3.7	5.5	5.5	177	1.5
Sheep 11	27.2	Sodium [2- 14 C] propionate	100	3.7	5.5	5.5	112	2.6
Rat 254	200	Sodium [2- 14 C] propionate	20	3.7	0.2			42.0
Rat 256	275	Sodium [2- 14 C] propionate	20	3.7	0.2			47.0
Rat 260	240	Sodium [2- 14 C] propionate	37	3.7	0.4			50.0

* This value was obtained before injection of the [2- 14 C] glucose.

assayed for 14 C (Koepppe & Hill, 1955). This involved manometric measurement of CO_2 and determination of radioactivity with a vibrating-reed electrometer. Most of the glutamate samples were recrystallized to constant specific activity before degradation. When the radioactivity in C-5 of glutamate was determined by the Schmidt reaction, radioactivity in C-1 to C-4 was determined by combustion of the dipicrate of diaminobutyric acid formed from the glutamate (Adamson, 1939; Koepppe & Hill, 1955). Some of the glutamate and aspartate samples were counted in the liquid scintillator described by Bray (1960) with a liquid-scintillation spectrometer equipped with an automatic external standard (Packard Instrument Co. Inc., Downers Grove, Ill., U.S.A.). A 72% counting efficiency was attained and it was not necessary to make corrections for quenching by the amino acids or acetic acid.

RESULTS

The labelled fatty acids were rapidly removed from the blood of both sheep and rats. Determinations of the total radioactivity remaining in the whole blood show that less than 10% of the injected 14 C remained in the blood of these animals at the time of death, assuming that the blood volume was 6% of body weight (Table 1). The radioactivity remaining in the blood of two of the sheep given [2- 14 C] glucose was appreciably greater than that in any of the animals given labelled fatty acids. Whether or not rapid distribution in extracellular fluid accounts for much of the removal of isotope from blood was not determined.

Concentrations of brain components. Concentra-

tions of brain glutamate, glutamine and aspartate (Table 2) isolated from the brain of rats treated with [2- 14 C] propionate are in general agreement with values reported for rats by Tallan (1962). The concentrations of glutamate and glutamine found in the brain of sheep (Table 2) agree very well with values previously reported, though the aspartate concentration observed was higher than the reported value (Tallan, 1962). The average concentration of glutathione found in the brain of sheep 1 and 6 was 1.6 μ moles/g. The concentration of *N*-acetylaspargate found in the brain of these two animals was 6.0 μ moles/g. These concentrations are similar to those found in the brain of rats (Tallan, 1962).

Concentration of blood sugar and volatile fatty acids. The concentration of blood sugar found at the end of the labelling experiments with sheep (Table 1) was always higher than the normal values reported (Reid, 1950; Long, 1961). Extremely high blood-sugar values were observed in all the sheep where the labelled precursor was injected immediately after the carotid artery had been surgically exposed. These high values are believed to have been caused by the trauma of restraint, injection and surgery. In some instances samples of blood were taken before surgery or injection of precursor and the sugar concentrations were 60–80 mg./100 ml. The concentrations of the volatile fatty acids in the blood of sheep were in general agreement with reported values (Long, 1961), acetate ranging from 4 to

Table 2. *Incorporation of ^{14}C from $[2\text{-}^{14}\text{C}]\text{glucose}$, $[1\text{-}^{14}\text{C}]\text{acetate}$, $[1\text{-}^{14}\text{C}]\text{butyrate}$ or $[2\text{-}^{14}\text{C}]\text{propionate}$ into free amino acids isolated from brain of sheep and rats*

The specific activities and concentrations of compounds isolated from the brain of rats and sheep were measured 10 and 20 min. respectively after intravenous administration of the labelled precursor. Experimental details are given in the text. The 'corrected' specific activities were determined by dividing the observed specific activity ($\mu\text{C}/\text{m-mole}$ for sheep, $\mu\text{C}/\text{m-mole}$ for rats) by the estimated specific activity ($\text{mC}/\text{m-mole}$) of the precursor in the blood during administration (see the text for method of calculating this quantity). The results in parentheses are μmoles of compound/g. of tissue.

Animal	Compound administered	Observed sp. activity ($\mu\text{C}/\text{m-mole}$ for sheep, $\mu\text{C}/\text{m-mole}$ for rats)			'Corrected' sp. activity ($\mu\text{C}/\text{m-mole}$)	
		Glutamic acid	Aspartic acid	Glutamine	Glutamic acid	Glutamine
Sheep 1	D- $[2\text{-}^{14}\text{C}]\text{Glucose}$	267 (8.3)	248 (2.0)	176 (2.0)		
Sheep 5	D- $[2\text{-}^{14}\text{C}]\text{Glucose}$	104 (9.3)	101 (2.4)	60 (3.1)	3.0	1.7
Sheep 9	D- $[2\text{-}^{14}\text{C}]\text{Glucose}$	113 (9.2)	83 (2.0)	72 (2.0)	1.6	1.0
Sheep 2	Sodium $[1\text{-}^{14}\text{C}]\text{acetate}$	29 (8.9)	26 (2.1)	61 (2.4)	0.20	0.41
Sheep 4	Sodium $[1\text{-}^{14}\text{C}]\text{acetate}$	21 (9.3)	26 (2.2)	32 (2.6)	0.09	0.13
Sheep 6	Sodium $[1\text{-}^{14}\text{C}]\text{acetate}$	16 (9.4)	12 (2.2)	35 (2.3)		
Sheep 7	Sodium $[1\text{-}^{14}\text{C}]\text{butyrate}$	149 (9.6)	109 (2.2)	336 (3.7)	0.03	0.06
Sheep 10	Sodium $[1\text{-}^{14}\text{C}]\text{butyrate}$	82 (8.3)	52 (1.9)	177 (2.3)	0.02	0.03
Sheep 8	Sodium $[2\text{-}^{14}\text{C}]\text{propionate}$	312 (9.0)	258 (2.2)	554 (2.7)	0.17	0.30
Sheep 11	Sodium $[2\text{-}^{14}\text{C}]\text{propionate}$	147 (8.1)	95 (1.8)	188 (2.2)	0.08	0.10
Rat 254	Sodium $[2\text{-}^{14}\text{C}]\text{propionate}$	1.8 (7.6)	1.1 (2.8)	3.4 (2.8)	0.5	0.9
Rat 256	Sodium $[2\text{-}^{14}\text{C}]\text{propionate}$	2.6 (6.4)	1.8 (1.5)		0.7	
Rat 260	Sodium $[2\text{-}^{14}\text{C}]\text{propionate}$	2.2 (9.5)	1.6 (2.8)	2.2 (3.4)	0.6	0.6

Table 3. *Distribution of radioactivity in glutamate isolated from brain of sheep and rats after administration of specifically ^{14}C -labelled glucose, acetate, butyrate or propionate*

The glutamate was isolated from brain of rats and sheep 10 and 20 min. respectively after intravenous injection of the precursor. Experimental details are given in the text.

Animal	Compound administered	Total radioactivity ($\mu\text{C}/\text{m-mole}$ for sheep, $\mu\text{C}/\text{m-mole}$ for rats)	Percentage of total radioactivity in carbon atoms				
			C-1	C-2 + C-3	C-4	C-1 + C-2 + C-3 + C-4	C-5
Sheep 1	D- $[2\text{-}^{14}\text{C}]\text{Glucose}$	267	20	6	2		74
Sheep 5	D- $[2\text{-}^{14}\text{C}]\text{Glucose}$	104	18			29	71
Sheep 9	D- $[2\text{-}^{14}\text{C}]\text{Glucose}$	113	20			32	70
Sheep 2	Sodium $[1\text{-}^{14}\text{C}]\text{acetate}$	29					65
Sheep 4	Sodium $[1\text{-}^{14}\text{C}]\text{acetate}$	21				29	62
Sheep 6	Sodium $[1\text{-}^{14}\text{C}]\text{acetate}$	16				29	68
Sheep 7	Sodium $[1\text{-}^{14}\text{C}]\text{butyrate}$	149	26	0	0.1		74
Sheep 10	Sodium $[1\text{-}^{14}\text{C}]\text{butyrate}$	82				23	74
Sheep 8	Sodium $[2\text{-}^{14}\text{C}]\text{propionate}$	312	11	77	7		7
Sheep 11	Sodium $[2\text{-}^{14}\text{C}]\text{propionate}$	147	11	55	16		17
Rat 254	Sodium $[2\text{-}^{14}\text{C}]\text{propionate}$	1.8					8
Rat 256	Sodium $[2\text{-}^{14}\text{C}]\text{propionate}$	2.6					14
Rat 260	Sodium $[2\text{-}^{14}\text{C}]\text{propionate}$	2.2				84	17

7 mg./100 ml. and propionate and butyrate, so low they were difficult to determine quantitatively, in no case exceeding 1.0 and 0.5 mg./100 ml. respectively.

Incorporation of labelled precursors into amino acids of brain. In single experiments the amounts of

radioactivity incorporated into glutamate of glutathione and aspartate of *N*-acetylaspartate of brain were very small when labelled glucose, acetate or propionate was used in sheep. As observed with rats (Lajtha, Berl & Waelsch, 1959; Jacobson, 1959; O'Neal & Koeppe, 1966) these results show that

glutathione and *N*-acetylaspatic acid of sheep brain turn over relatively slowly. Because of this the isolation of glutamate and aspartate from these two brain components was omitted in subsequent experiments. Measurable amounts of ^{14}C were incorporated into the glutamate, aspartate and glutamine of sheep brain from all the labelled precursors tested (Table 2). The amounts of ^{14}C incorporated into the amino acids of rat brain when $[2-^{14}\text{C}]$ propionate was administered (Table 2) were comparable with the incorporation observed when $[2-^{14}\text{C}]$ glucose or $[2-^{14}\text{C}]$ lactate was used (O'Neal & Koeppe, 1966). In agreement with the observations made in rats (O'Neal & Koeppe, 1966; Table 2), glutamate of sheep brain had a higher specific activity than did aspartate regardless of the precursor used, and aspartate had a higher specific activity than glutamine when $[2-^{14}\text{C}]$ glucose was the precursor. When the labelled volatile fatty acids were used as precursors of the amino acids of sheep or rat (O'Neal & Koeppe, 1966) brain, glutamine always had a higher specific activity than glutamate.

Labelling pattern of glutamate of brain. Degradation of the glutamic acid isolated from the brain of sheep given $[2-^{14}\text{C}]$ glucose, $[1-^{14}\text{C}]$ acetate and $[1-^{14}\text{C}]$ butyrate showed that most of the ^{14}C was incorporated into C-5 (Table 3). Comparable distribution of radioactivity was observed in the glutamate from the brain of rats given these same precursors (O'Neal & Koeppe, 1966). When $[2-^{14}\text{C}]$ propionate was used as the precursor over 50% of the ^{14}C was found in C-2 and C-3 of glutamate from sheep brain, and partial degradation indicated that C-2 and C-3 of glutamate from rat brain also contained high percentages of the ^{14}C .

DISCUSSION

The fact that more radioactivity was found in the blood of sheep at the end of the labelling experiments when $[2-^{14}\text{C}]$ glucose was the precursor than when labelled fatty acids were used is consistent with the well-established fact that a large portion of the total energy requirement of ruminants is derived from the metabolism of the volatile fatty acids. However, these results are not very useful for comparing the utilization of glucose and the volatile fatty acids by a particular organ or the whole body because they may be a reflection of the rates of distribution of these compounds in the extracellular fluids. They were used only as an indication of successful injection of the labelled compounds.

To attack the problem of the comparative utilization of glucose and the volatile fatty acids by the brain of ruminants, the incorporation of precursors labelled with ^{14}C in specific carbon atoms into the free amino acids of brain was examined.

The precursors were injected into the carotid artery to eliminate the possibility of having much of the labelled propionate and butyrate removed from the blood by the liver (Annison, Hill & Lewis, 1957) and of having the labelled acetate and glucose diluted extensively, by the large quantities in the blood, before they could be utilized by the brain. The use of specifically labelled precursors allowed us to determine, from the distribution of radioactivity in glutamate of brain, whether the precursor was metabolized initially by brain or was converted into labelled blood glucose before entering the brain (Koeppe & Hahn, 1962).

Determination of the amounts of ^{14}C incorporated into the free amino acids of sheep brain from the labelled precursor used shows that comparable amounts of incorporation occurred after the injection of $[2-^{14}\text{C}]$ glucose, $[2-^{14}\text{C}]$ propionate and $[1-^{14}\text{C}]$ butyrate and that a considerably smaller amount of incorporation occurred after injection of $[1-^{14}\text{C}]$ acetate. However, direct comparison of these results is not a valid assessment of the comparative utilization of these precursors by brain. If ^{14}C data are to be used for comparing precursors, the specific activities of the isolated components in question should be 'corrected' for differences in specific activities of the precursors presented to the brain. This 'correction' was made (Table 2) by dividing the observed specific activities ($\text{m}\mu\text{C}/\text{m-mole}$) of the isolated glutamate of brain by the estimated specific activity ($\text{mC}/\text{m-mole}$) of the precursor in blood of the carotid artery during the injection of the precursor. The estimated specific activity of the precursor was determined by use of the dilution equation:

$$\text{Estimated sp. activity} = \frac{\text{Sp. activity of compound injected}}{1 + r_d c_d / r_i c_i}$$

where r_d is the rate of blood flow, c_d is the concentration of diluting compound in the blood, r_i is the rate of injection of labelled precursors and c_i is the concentration of the labelled precursor. A value of 200 ml./min. was used as the rate of blood flow in the carotid artery (Baldwin & Bell, 1963). The concentrations of acetate, propionate and butyrate of carotid blood used were 6.0, 1.0, 0.5 mg./100 ml. respectively. The other information used for these estimations is presented in Table 1. The 'correction' factor includes dilution by components in the blood and the variation of specific activity of the compound injected. Admittedly there is considerable error in this 'correction'; however, the 'corrected' values presented in Table 2 certainly give a more realistic picture of the relative abilities of the various compounds studied to act as precursors than do the observed values.

Glucose. The 'corrected' values indicate that

glucose rapidly enters the brain and is a superior precursor of the acidic amino acids of brain. This confirms the conclusion of McClymont & Setchell (1956) and indicates that, even though the volatile fatty acids have a unique role in the metabolism of ruminants, the major energy source of sheep brain is glucose.

The labelling patterns of glutamate isolated from the brain of sheep (Table 3) and rats (O'Neal & Koeppe, 1966) after the injection of $[2-^{14}\text{C}]$ glucose are comparable. The high percentage of labelling observed in C-5 of glutamate indicates that the glucose was metabolized via pyruvate and that the major route of pyruvate metabolism was conversion into acetyl-CoA (Koeppe & Hahn, 1962; McMillan & Mortensen, 1963). These results suggest a similar type of glucose metabolism in the brain of both species and emphasize the unique ability of brain to metabolize very high proportions of pyruvate via acetyl-CoA (Koeppe, Mourkides & Hill, 1959; Freedman, Rumsey & Graff, 1960).

Acetate and butyrate. The 'corrected' values indicate that acetate was as good a precursor of the brain amino acids as the other volatile fatty acids tested and suggest that, even though acetate is not as good a source of energy for ruminant brain as is glucose, a small amount may be utilized. The fact that labelled butyrate appeared to be a better precursor than acetate before the results were 'corrected' is misleading and is explained by the difference in dilution of the two precursors during injection into the arterial blood. The 'corrected' values show that butyrate is not a major source of energy for the ruminant brain. These results with sheep agree with those showing that that rat brain *in vivo* metabolizes small amounts of acetate and butyrate (O'Neal & Koeppe, 1966).

The percentage of labelling found in C-5 of glutamate of sheep brain after administration of $[1-^{14}\text{C}]$ acetate and $[1-^{14}\text{C}]$ butyrate always exceeded 60%. Such high percentage of labelling in C-5 after injection of these precursors is consistent with their metabolism via the tricarboxylic acid cycle and indicates that the labelled butyrate and acetate entered the brain without prior metabolism by liver, because such metabolism yields $[3,4-^{14}\text{C}_2]$ glucose (Lifson, Lorber, Sakami & Wood, 1948; Annison, Leng, Lindsay & White, 1963), which in turn would label C-1 of glutamate of brain (Koeppe & Hahn, 1962). In experiments of longer duration Koeppe & Hahn (1962) observed a much lower percentage of label in C-5 of glutamate after administration of $[1-^{14}\text{C}]$ acetate. Using different experimental conditions, McMillan & Mortensen (1963) showed that intracisternally administered acetate was metabolized via the tricarboxylic acid cycle, and Beattie & Basford (1965) showed that mitochondria isolated from ox brain can oxidize low concentrations of

butyrate at a rate comparable with that obtained with mitochondria from rat liver and ox heart. Although the present results suggest that the brain can and does utilize acetate and butyrate as an energy source, they do not prove a net uptake of these precursors from the blood by the brain because they can be explained by a rapid exchange between the pools of blood and brain.

Propionate. The 'corrected' incorporation values for amino acids isolated from the brain of both sheep and rats given $[2-^{14}\text{C}]$ propionate indicate that propionate is metabolized by brain but to a smaller extent than is glucose. The labelling pattern of the glutamate isolated from the $[2-^{14}\text{C}]$ propionate-treated animals is consistent with the established mammalian metabolic pathway (Flavin & Ochoa, 1957): propionate $\rightarrow$ methylmalonate $\rightarrow$ succinate $\rightarrow$ glucose or citrate. The fact that a major portion of the labelling was found in C-2 and C-3 of the glutamate of brain indicates that most of the propionate entered the brain without prior metabolism by liver, since such metabolism by the liver gives $[1,2,5,6-^{14}\text{C}_4]$ glucose (Lorber, Lifson, Sakami & Wood, 1950; Annison *et al.* 1963), which in turn would label C-4 and C-5 of glutamate equally and to a greater extent than C-2 and C-3. Glutamine of brain isolated from the $[2-^{14}\text{C}]$ propionate-treated rats and sheep had equal or higher specific activities than the glutamate. The opposite was observed in animals given labelled glucose, thus further supporting the concept that the propionate was metabolized initially by brain. Noble, Stjernholm & Clayton (1965) have demonstrated that $[3-^{14}\text{C}]$ -propionate labels the glutamate of brain of 1-day-old rats. Metabolism of propionate by brain has a special significance relative to the other volatile fatty acids in that it may be a source of dicarboxylic acids of the tricarboxylic acid cycle. This could be important during conditions such as ammonia toxicity, where the depletion of the tricarboxylic acid-cycle intermediates has been proposed as a mechanism (Bessman, 1958). Metabolism of propionate by brain also indicates the existence of a carbon dioxide-fixation mechanism such as propionyl-CoA carboxylase in addition to the mechanism previously observed (Berl, Takagaki, Clarke & Waelsch, 1962b; McMillan & Mortensen, 1963). Again, as with the other volatile fatty acids tested, these results do not prove a net utilization of propionate by brain, but can be explained by a rapid exchange between the blood and brain propionate pools.

Metabolic compartments in brain. Administration of labelled glutamic acid, glutamine, bicarbonate, ammonium acetate, acetate and butyrate (Berl, Lajtha & Waelsch, 1961; Berl *et al.* 1962a,b; O'Neal & Koeppe, 1966) to experimental animals has yielded glutamine containing higher specific activity

or atoms per cent excess in the α -amino group than glutamate of brain, which has led to the postulate that in brain glutamine is rapidly formed from a small pool of glutamate that is not in rapid equilibrium with the total tissue glutamate. However, when labelled glucose, lactate or glycerol is administered to rats the specific activity of glutamate of brain exceeds that of glutamine (Cremer, 1964; Gaitonde, Dahl & Elliott, 1965; O'Neal & Koeppe, 1966). The results in Table 2 show that the same phenomenon occurs in sheep brain and that labelled propionate is another of the precursors that yield glutamine of brain with a higher specific activity than glutamate in both rats and sheep. The propionate metabolism of brain fits very well into the pool hypothesis, proposed by O'Neal & Koeppe (1966), which invokes two pools of tricarboxylic acid-cycle intermediates. The pool accessible to propionate is the same as that accessible to bicarbonate, acetate and butyrate and is closely associated with glutamate dehydrogenase and glutamine synthetase. The fact that the glutamine/glutamate specific activity ratio after administration of labelled propionate is not always as great as that observed with acetate or butyrate may be explained by assuming that in those cases some of the label may have entered the brain as glucose and thus been accessible to both tricarboxylic acid-cycle pools. The fact that propionate is known to be glucogenic, and that an appreciable amount of labelling was observed in C-5 or C-4 or both of glutamate isolated from the brain of animals that had lower glutamine/glutamate ratios, supports such an explanation.

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