Biochem. J. (1970) **119**, 95–102
Printed in Great Britain

Selective Inhibition of Glucose Oxidation by Triethyltin in Rat Brain *in vivo*

By J. E. CREMER
Biochemical Mechanisms Section, Medical Research Council Toxicology Unit, Woodmansterne Road, Carshalton, Surrey, U.K.

(*Received* 20 *April* 1970)

Results are reported of a comparative study *in vivo* of the metabolism of [2-^{14}C]-glucose and [1-^{14}C]acetate in brains of rats intoxicated with triethyltin sulphate. The incorporation of ^{14}C from glucose into glutamate, glutamine, γ-aminobutyrate and aspartate was greatly decreased. The incorporation of ^{14}C from acetate into these amino acids was unaffected. The experimental data indicated that the main action of triethyltin was to decrease the rate at which pyruvate formed from glucose is oxidized. Glycolysis was not inhibited. Changes in glucose metabolism in the brain are shown not to be directly due to hypothermia. Some of the advantages of measuring the labelling of intermediates at very short time intervals after the injection of the labelled glucose are demonstrated.

An interesting feature of metabolism in the adult brain is the different proportional distribution of ^{14}C in those amino acids closely associated with the tricarboxylate cycle, depending on whether the radioactive precursor is glucose or one of several other compounds known to be metabolized via acetyl-CoA, which include acetate, butyrate and leucine. The last-named compounds all give rise to high labelling of glutamine relative to that of glutamate (see Berl & Clarke, 1970). When [^{14}C]-glucose is the precursor glutamate is more highly labelled than glutamine (Cremer, 1964; O'Neal & Koeppe, 1966). A review of the literature, together with further evidence for the existence of two different tricarboxylate cycles in brain has been presented by Van den Berg, Kržalić, Mela & Waelsch (1969).

To date, quite a wide variety of drugs and toxic compounds have been shown to alter the distribution in brain metabolites of ^{14}C from [^{14}C]glucose (Bachelard & Lindsay, 1966; Minard & Mushahwar, 1966; Godin, Mark & Mandel, 1968; Flock, Tyce & Owen, 1969). However, far fewer studies have been made to test whether such agents alter the metabolism of those precursors that give rise to a high labelling of glutamine, of which acetate is an example (Berl & Frigyesi, 1969). The present paper reports results of a comparative study *in vivo* of the metabolism of [2-^{14}C]glucose and [1-^{14}C]acetate in the brains of rats intoxicated with triethyltin sulphate. From earlier work *in vitro* with slices of rat cerebral cortex, it is known that triethyltin inhibits the oxidation of glucose but not that of added pyruvate or glutamate (Cremer, 1962, 1967). Since administration of triethyltin sulphate to rats at an environmental temperature of 20°C causes a fall in body temperature (Rose & Aldridge, 1966) some features of brain metabolism in relation to hypothermia were examined. A preliminary report of this work has been published (Cremer, 1969).

METHODS

Animals. Male albino rats (7–8 weeks old, 190–210g body wt.) of the Porton strain were fed *ad libitum* on diet 41B (Bruce & Parkes, 1949). At 10 a.m. animals were given triethyltin (10μmol/kg body wt., i.e. 10mg of triethyltin sulphate/kg body wt.) by intraperitoneal injection. At 12 noon animals that had received triethyltin 2h earlier and control animals were injected with radioactive material into a tail vein. Each animal received either [2-^{14}C]glucose (5μCi/100g body wt.) or [1-^{14}C]acetate (7.5μCi/100g body wt.) and 0.5mg of sodium acetate. The animals were guillotined such that the heads fell directly into liquid nitrogen. The frozen brains were homogenized in 6% (w/v) $HClO_4$, the protein was removed by centrifugation and the acid-soluble fraction neutralized to pH8.0 with KOH. The $KClO_4$ precipitate was removed by filtration, the filtrate was evaporated to dryness at 35°C and the dried brain extract dissolved in water. In some experiments, indicated in the text, the heads were not frozen but the brains were rapidly removed within 20s at room temperature, dropped into ice-cold 10% (w/v) trichloroacetic acid and homogenized. After removal of the protein the supernatant was washed three times with twice its volume of ether and evaporated to dryness.

Blood was collected from the trunk into beakers containing heparin (approx. 50 units per ml of blood).

Assay of metabolites. The concentrations of metabolites in blood and brain were determined by enzymic assay as described by Bergmeyer (1963) for L-lactate, L-glutamate and D-glucose by using hexokinase and glucose 6-phosphate dehydrogenase.

Separation of metabolites. Procedure 1. This procedure, for the separation of amino acids, was essentially the paper electrophoretic method of Biserte, Plaquet-Schoonaert, Boulanger & Paysant (1960). Electrophoresis was carried out on Whatman no. 1 paper in pyridine–acetic acid–water (1:3:750) buffer, pH 3.5, for 90 min at 30 V/cm of paper. Tissue samples were run in duplicate, together with a standard solution containing glutamate, aspartate and γ-aminobutyrate. After electrophoresis the papers were dried; a portion containing both a tissue sample and the standard amino acids was heated to 40°C, dipped in a ninhydrin solution for quantitative measurement of amino acids and the colour was developed at 60°C for 20 min (Gaitonde, 1962).

For the separation of glutamine, the fraction containing neutral amino acids from a column of AG1 X 10 (CO_3^{2-} form) (see procedure 2) was hydrolysed overnight in 2.5 M-HCl at 104°C to convert glutamine into glutamic acid. The hydrolysate was freed from HCl by repeated evaporation with additions of water and the glutamic acid was separated by paper electrophoresis.

Procedure 2. A preliminary separation of metabolites in brain extracts was made as described by Cremer (1964) by using AG1 X10 (200–400 mesh; CO_3^{2-} form) as described by Vrba, Gaitonde & Richter (1962). The water washings contained glucose. The column was eluted with 0.1 M-HCl and a neutral fraction was collected containing the neutral amino acids. Further acidification eluted glutamic acid, aspartic acid and lactic acid. These were separated on a column (0.9 cm × 2.5 cm) of AG1 X 8 (200–400 mesh, acetate form) as described by Minard & Mushahwar (1966), except that a Technicon Autograd (Technicon Instruments Ltd., Chertsey, Surrey, U.K.) was used for the gradient elution. Glutamine was converted into glutamic acid as described above and the glutamic acid was separated on an AG1 X8 (acetate form) column.

Measurement of radioactivity. Liquid-scintillation counting was used. The efficiency of counting was determined by the addition of standard [^{14}C]toluene and all values were corrected to d.p.m.

Spot areas corresponding to each amino acid separated by procedure 1, but without being stained by ninhydrin were eluted with water into counting vials. Samples from columns of AG1 X 8 (acetate form) were evaporated to near dryness, neutralized and equal amounts were taken for enzymic assay and measurement of radioactivity. Specific radioactivities of metabolites are expressed as d.p.m./μmol.

Results are expressed as the arithmetical mean ± standard deviation (S.D.) and were subjected to a test of significance using Student's '*t*' test with $n = n_1 - 2$. Results were considered not significant when $P > 0.05$.

Chemicals. D-[2-^{14}C]glucose (sp. radioactivity 3.24 mCi/mmol) and sodium [1-^{14}C]acetate (sp. radioactivity 55 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Enzymes were obtained from the Boehringer Corp. (London) Ltd., London, W.5, U.K. The resins AG1 X10 and AG1 X8 were obtained from Bio-Rad Laboratories, Richmond, Calif., U.S.A.

RESULTS

Labelling of amino acids. When [2-^{14}C]glucose was the radioactive precursor the specific radioactivities of glutamate, aspartate, γ-aminobutyrate and glutamine were markedly lowered in the brains of animals that had received triethyltin (Table 1). This was most apparent when animals were killed within a few minutes after injection of [2-^{14}C]-glucose.

The series of assays was made on brains that had been rapidly removed at room temperature. A few experiments were made in which the method used for analyses of the amino acids was the same but in which the heads from the guillotined rats fell directly into liquid nitrogen and the brains were

Table 1. *Specific radioactivity of amino acids in the brains of rats at various time-intervals after the intravenous injection of* [2-^{14}C]*glucose*

Rats (200 g body wt.) received 10 μCi of [2-^{14}C]glucose by intravenous injection. Where indicated, rats were given triethyltin sulphate (10 mg/kg body wt.) by intraperitoneal injection 2 h before the radioactive glucose. All the animals were kept at an ambient temperature of 18°C. They were guillotined, the brains rapidly removed at room temperature and the amino acids separated according to Procedure 1 as described in the Methods section. Mean values ±S.D. are given with the number of determinations in parentheses.

Time (min)	Animals	Total radioactivity (d.p.m./g of brain)	Specific radioactivity (d.p.m./μmol) Glutamate	Aspartate	γ-Aminobutyrate	Glutamine
3	Control	143700±14950 (6)	5480±830 (5)	3910±1050 (5)	3210±750 (5)	1810±390 (3)
3	Triethyltin	98700±10830 (4)	2070±520 (4)	1840±490 (4)	860±210 (4)	620 (2)
10	Control	201170±18910 (5)	8490±1550 (3)	7260±300 (3)	6000±360 (3)	5210 (2)
10	Triethyltin	135125±13180 (4)	5990±1045 (3)	4660±1250 (3)	2870±970 (3)	2120 (2)
20	Control	244175 (2)	10370 (2)	8660 (2)	6720	
20	Triethyltin	166060±19480 (3)	9200±650 (3)	7400±560 (3)	4990±1190 (3)	
30	Control	174625 (2)	8670 (2)	6010 (2)	6240 (2)	
30	Triethyltin	147350 (2)	8820 (2)	—	4500 (2)	

Table 2. *Labelling of amino acids from* [2-^{14}C]*glucose (frozen brain)*

The conditions were as described in the legend to Table 1 except that the guillotined heads fell into liquid N_2 and the frozen brains were removed for amino acid analyses. All animals were killed at 3min after receiving [2-^{14}C]glucose. The relative specific radioactivity is the specific radioactivity of the amino acid/the specific radioactivity of glutamate. The probabilities that values are significantly different from the non-frozen brain control means of Table 1 and Table 6 group (*b*) are * $P<0.025$.

	Control			Triethyltin		
	μmol/g of tissue	Specific radioactivity (d.p.m./μmol)	Relative sp. radioactivity	μmol/g of tissue	Specific radioactivity (d.p.m./μmol)	Relative sp. radioactivity
Glutamate	10.07±1.76 (4)	4070±470*	1.0	8.0 (2)	1800	1.0
Aspartate	2.25±0.21 (3)	2940±150	0.73	2.3 (2)	—	—
γ-Aminobutyrate	1.67±0.25 (4)	2480±420	0.61	1.39 (2)	730	0.41
Glutamine	4.30±1.06 (3)	1410±310	0.35	5.30 (2)	530	0.30

Table 3. *Specific radioactivity of amino acids in the brains of rats after the intravenous injection of* [1-^{14}C]*acetate*

Rats (200g body wt.) received 15μCi of [1-^{14}C]acetate by intravenous injection. Where indicated, rats were given triethyltin sulphate (10mg/kg body wt.) by intraperitoneal injection 2h before the radioactive acetate. Animals were guillotined and the head fell into liquid N_2. Analyses of amino acids in the frozen brains were made by procedure 1 as described in the Methods section. Mean values ±S.D. are given with the number of determinations in parentheses. The probabilities that values for the animals given triethyltin were significantly different from the control means were tested and found to be not significant except where marked * $P<0.05$.

Time (min)	Animals	Total radioactivity (d.p.m./g of brain)	Specific radioactivity (d.p.m./μmol) Glutamate	Aspartate	γ-Aminobutyrate	Glutamine
3	Control	28340±4020 (4)	830±79 (4)	525±99 (4)	408±114 (3)	2920±383 (4)
3	Triethyltin	35390±1950 *(3)	949±146 (3)	573±158 (3)	321±59 (3)	3540±354 (3)
10	Control	28920±1800 (4)	1149±118 (4)	814±98 (4)	663±75 (4)	2430±663 (4)
10	Tritheyltin	34110±3920 (4)	1048±108 (4)	758±101 (4)	638±29 (4)	3210±582 (4)

removed in the frozen state. The results are given in Table 2. The only significant difference in the amino acids between the frozen and non-frozen brains was a smaller specific radioactivity of glutamate in the frozen brains.

Similar experiments were made in which [1-^{14}C]-acetate was the radioactive precursor. Rats were killed at either 3 or 10min. The total amount of radioactivity in the brains of rats given triethyltin tended to be higher than in the control animals. At neither time-interval did the specific radioactivities of the amino acids differ significantly between the control and triethyltin-treated animals (Table 3). The pattern of ^{14}C distribution, namely the high specific radioactivity of glutamine relative to that of glutamate, was typical for [1-^{14}C]acetate as described by others (O'Neal & Koeppe, 1966; Van den Berg, Mela & Waelsch, 1966; Van den Berg *et al.* 1969).

Since triethyltin appeared to inhibit selectively the incorporation of ^{14}C into amino acids when [2-^{14}C]glucose was the precursor, the metabolism of glucose was investigated more fully. Measurements were made of the amount and specific radioactivity of blood glucose and brain glucose, lactate and glutamate in control and triethyltin-treated rats killed at 30, 90 and 180s after injection with [2-^{14}C]glucose. These time intervals are much shorter than those used by other workers, particularly in studies on the effects of drugs on glucose metabolism by brain. They were chosen for the study of the initial rates of labelling of some of the intermediate metabolites. Results are given in Table 4.

Triethyltin caused a moderate hyperglycaemia in most animals. In all animals there was a marked increase in the amount of glucose in the brain. The amount of lactate was the same in the brains of both groups of animals, but the amount of glutamate was about 20% lower in the brains of rats given triethyltin.

At 30s after injection of [2-^{14}C]glucose, although the total amounts of ^{14}C per ml of blood and per g of brain were the same in the control and triethyltin-treated animals, in the latter the specific radioactivities of blood glucose and brain glucose were

Table 4. [2-^{14}C]*Glucose metabolism in the blood and brain of fed rats with and without triethyltin sulphate*

Rats (200g body wt.) received 10μCi of [2-^{14}C]glucose by intravenous injection. Where indicated, rats were given triethyltin sulphate (10mg/kg body wt.) by intraperitoneal injection 2h before the radioactive glucose. Animals were guillotined and the heads fell into liquid N_2. Analyses of the metabolites in the frozen brains was as given in procedure 2 of the Methods section. Mean values ±S.D. are given with the number of determinations in parentheses. The probabilities that values for the animals given triethyltin were significantly different from the control means at a particular time are given as test of significance; n.s. is not significant with $P>0.05$.

				Blood glucose		Brain glucose		Brain lactate		Brain glutamate	
Animals	Time (s)	Blood (d.p.m./ml)	Brain (d.p.m./g)	(μmol/ml)	(d.p.m./ μmol)	(μmol/g)	(d.p.m./ μmol)	(μmol/g)	(d.p.m./ μmol)	(μmol/g)	(d.p.m./ μmol)
Control	30	363700± 43440 (8)	68020± 17950 (8)	6.10±0.39 (8)	59700± 7320 (8)	0.242± 0.048 (8)	52160± 10500 (8)	3.67±0.63 (8)	10400± 3490 (8)	7.79±0.39 (7)	410±140 (7)
Triethyltin	30	431600± 60555 (5)	56710± 6395 (5)	8.79±1.07 (5)	49150± 4840 (5)	0.746± 0.204 (5)	33370± 4970 (5)	3.37±0.71 (5)	7430± 1495 (5)	5.41±0.36 (4)	203±50 (4)
Test of significance		n.s.	n.s.	$P<0.001$	$P<0.01$	$P<0.001$	$P<0.005$	n.s.	n.s.	$P<0.001$	$P<0.005$
Control	90	321475± 47720 (8)	98240± 17250 (8)	6.21±0.59 (8)	51880± 6650 (8)	0.323± 0.056 (7)	33740± 10770 (5)	4.03±0.26 (5)	9350± 1790 (7)	8.63±1.17 (8)	1870±330 (7)
Triethyltin	90	341260± 17520 (6)	87610± 15300 (6)	7.41±1.32 (6)	47410± 10630 (6)	0.714± 0.206 (5)	22870± 5300 (5)	3.67±0.71 (6)	9210± 1750 (6)	7.26±1.65 (6)	1070±198 (6)
Test of significance		n.s.	n.s.	n.s.	n.s.	$P<0.005$	n.s.	n.s.	n.s.	n.s.	$P<0.001$
Control	180	277640± 49700 (5)	142530± 6995 (9)	5.65±0.54 (9)	47100± 7730 (5)	0.319± 0.109 (16)	33820± 7540 (9)	3.88±0.80 (16)	12480± 2600 (9)	8.76±1.18 (16)	4660±850 (9)
Triethyltin	180	297500± 34850 (5)	107910± 9870 (9)	7.63±1.43 (9)	44795± 11760 (5)	0.949± 0.306 (13)	29290± 5535 (9)	3.59±0.52 (13)	9810± 2955 (7)	6.66±0.69 (12)	2400±725 (7)
Test of significance		n.s.	$P<0.001$	$P<0.005$	n.s.	$P<0.001$	n.s.	n.s.	n.s.	$P<0.001$	$P<0.001$

Table 5. *Concentrations of metabolites in brains of rats with and without triethyltin sulphate and maintained at an ambient temperature of* 18°C *or* 33°C

Where indicated, rats (200g body wt.) were given triethyltin sulphate (10mg/kg body wt.) by intraperitoneal injection. For the next 2h the animals were kept either at room temperature (approx. 18°C) or in an air-oven maintained at 33°C. Control animals were kept at 18°C. All animals were guillotined and the heads of group (*a*) were frozen in liquid N_2 and the brains of group (*b*) were removed at 18°C. Brain metabolites of group (*a*) were measured by enzymic assays; those in group (*b*) were measured by high-voltage paper electrophoresis as described in procedure 1 of the Methods section. Mean values ±S.D. are given as μmol/g with the number of determinations in parentheses. The probabilities that values for the animals given triethyltin are significantly different from the control means are: * $P<0.01$; † $P<0.001$.

		(*a*)			(*b*)			
Animal	Ambient temp. (°C)	Glucose	Lactate	Glutamate	Glutamate	Aspartate	γ-Amino-butyrate	Glutamine
Control	18	0.358±0.088 (7)	3.60±0.63 (7)	8.13±0.32 (7)	9.26±0.77 (15)	2.48±0.22 (15)	1.72±0.19 (15)	4.15±0.82 (7)
Triethyltin	18	0.986±0.357† (8)	3.75±0.41 (8)	6.42±0.45† (8)	7.07±0.69† (14)	2.29±0.32 (14)	1.70±0.17 (14)	4.5 ±0.44 (7)
Triethyltin	33	0.905±0.367* (5)	3.81±0.63 (5)	6.58±0.46† (5)	7.35±0.29† (6)	2.03±0.21† (6)	1.53±0.25 (6)	3.81±0.48 (5)

lower. This was partially due to the increased amounts of glucose. However, the specific radioactivity of lactate was not significantly different from the control animals, but that of glutamate was. At the two later time-intervals essentially similar results were obtained, except that the specific radioactivities of blood glucose and brain glucose in the animals given triethyltin were not significantly different from the control animals. It would seem therefore that the conversion of glucose into lactate was unimpaired in the brains of rats given triethyltin. The amount of radioactivity in amino acids other than glutamate was too low in animals killed at 30s to allow an accurate measurement of their specific radioactivity. The average values for the specific radioactivities obtained at 90s in two control and two triethyltin-treated animals were, respectively: glutamate 1800 and 1170 d.p.m./μmol; aspartate 1540 and 740; glutamine 510 and 225 in addition to the values given for glutamate in Table 4. Similarly, the values given in Table 2 for animals killed at 180s show that all the amino acids measured had a lower specific radioactivity in the brains of animals given triethyltin. Therefore, all the experimental evidence points to a block in the conversion of glucose carbon into amino acids at a stage after glycolysis.

Effects of hypothermia. Rats that were left at room temperature (18–20°C) after receiving triethyltin became hypothermic. The drop in body temperature of 4–5°C could be prevented by maintaining the animals at an ambient temperature of 33°C (Rose & Aldridge, 1966). Measurements of the amounts of several metabolites in the brains of hypothermic (at 18°C) and coenothermic animals (at 33°C) showed that the increase in glucose and decrease in glutamate still persisted when a decrease in body temperature was prevented (Table 5). Likewise, the decreased labelling of amino acids from [2-^{14}C]glucose persisted. In a group of three rats injected with triethyltin and kept at an ambient temperature of 33°C and killed 3min after an injection of [2-^{14}C]glucose, the mean specific radioactivities (d.p.m./μmol) of the amino acids were 2460 for glutamate, 2330 for aspartate, 1140 for γ-aminobutyrate and 920 for glutamine. These values should be compared with those given in Table 1 for non-frozen brains. Prevention of hypothermia appeared slightly to raise the specific radioactivities of the amino acids but they were still all significantly less than the values for normal rats kept at approx. 18°C.

Once ^{14}C from glucose has entered glutamate, this amino acid can be considered the radioactive precursor of γ-aminobutyrate, glutamine and aspartate. When the specific radioactivities of each of the three amino acids are compared with that of glutamate in the brain of the same animals, there were several differences between the control animals and those given triethyltin (Table 6). Table 6 also shows that whereas in control animals the relative specific radioactivities of aspartate and γ-aminobutyrate were not significantly different from each other, they were in the brains of rats given triethyltin. This difference still persisted when hypothermia was prevented.

Approximate rate of flux of pyruvate to glutamate. In both the normal rats and those given triethyltin, the specific radioactivity of lactate remained relatively constant between 30 and 180s (Table 4).

Table 6. *Specific radioactivity of aspartate, γ-aminobutyrate and glutamine relative to the specific radioactivity of glutamate in the brain after the injection of* [2-^{14}C]*glucose*

Animals were as described in the legends to Tables 1 and 5 group (*b*). Exactly 3 min before a rat was guillotined it received 10 μCi of [2-^{14}C]glucose by intravenous injection. Mean values (d.p.m./μmol) ±S.D. are given with the number of determinations in parentheses. The specific radioactivities of the amino acids in the brains of animals given triethyltin and kept at an ambient temperature of 33°C were not significantly different from the values given in Table 1 for animals given triethyltin and kept at an ambient temperature of 18°C. The probabilities that values are significantly different from the control means are * $P<0.05$; † $P<0.01$.

Animal	Ambient temp.	Aspartate	γ-Aminobutyrate	Glutamine	Test of significance Aspartate/ γ-aminobutyrate
Control	18°C	0.71±0.09 (5)	0.59±0.13 (5)	0.36±0.1 (3)	not significant
Triethyltin	18°C	0.89±0.03 (4)†	0.42±0.03 (4)*	0.25 (2)	$P<0.001$
Triethyltin	33°C	0.74 ; 1.16 (2)	0.46±0.02 (3)	0.36±0.01 (3)	$P<0.025$

During the same time interval the specific radioactivities of glutamate increased linearly in both groups of animals (Table 4). The total amount of glutamate in the brain is large and ^{14}C from [^{14}C]-glucose enters it rapidly due to the small amount of 2-oxoglutarate and high transaminase activity (Balazs & Haslam, 1965). For a small time-interval after an injection of [2-^{14}C]glucose loss of label from glutamate by further metabolism can be considered negligible for purposes of calculation of the rate of flux to glutamate. The activity of lactate dehydrogenase in rat brain is high (about 60 μmol/min per g of brain) and therefore pyruvate can be assumed to have the same specific radioactivity as the lactate that was measured. The rate of flux from pyruvate (or lactate) to glutamate is given by:

$$\frac{\Delta q/\text{min}}{\text{sp. radioactivity of lactate}}$$

where q = total d.p.m. in glutamate/g of brain.

In control animals the rate was 1.3 μmol/min per g of brain, whereas in animals given triethyltin the rate was 0.66 μmol/min per g of brain.

For a comparison of the rates in the two groups of animals this calculation is valid. However, the accuracy of the absolute values is questionable, mainly because of evidence that brain glucose breaks down to lactate after decapitation (Mark, Godin & Mandel, 1968).

DISCUSSION

Some studies on glucose metabolism in the brains of rats given triethyltin sulphate were reported several years ago (Cremer, 1964). The similarities between the results of the earlier and the present work on the effects of triethyltin on the brain are (1) the decrease in the amount of glutamate and (2) the increase in the amount of glucose that was inferred in the earlier work and confirmed by direct assay in the present study. Although the metabolism of [^{14}C]glucose was followed in the earlier study, there were several features about the experimental design that can now be considered as unsatisfactory. These are as follows.

(1) The results of the specific radioactivities of the amino acids labelled from [U-^{14}C]glucose were unreliable because radioactivity measurements were made after reaction with ninhydrin. Correction for the loss of carbon was made on the incorrect assumption that the distribution of label in the carbon atoms of each brain amino acid was equal.

(2) Animals were killed at 7 and 30 min after injection of [U-^{14}C]glucose. No significant differences were found between the control and triethyltin-treated animals in the specific radioactivities of the amino acids. The present work shows that differences in rates of interconverions of metabolites are best detected within the first few minutes after the administration of a pulse label of radioactive glucose.

(3) Some of the complexities in the distribution of ^{14}C with time, inherent in the use of [U-^{14}C]-glucose, can be simplified by using glucose specifically labelled in the C-2 position.

Compartmentation of tricarboxylate cycles in brain. Detailed studies by Van den Berg *et al.* (1969) on the time-course of labelling of amino acids from variously labelled [^{14}C]glucose and [^{14}C]acetate has led these authors to conclude that there are at least two metabolically distinct tricarboxylate cycles in brain. This could mean either that different types of mitochondria exist, for which there is some experimental evidence (Salganicoff & De Robertis, 1965; Salganicoff & Koeppe, 1968; Neidle, Van den Berg & Grynbaum, 1969) or that similar mitochondria exist in different micro-environments which determine the metabolic sequences closely associated with the tricarboxylate cycle.

If compounds can be found which preferentially

inhibit one or other of the cycles they should be of use in helping to decide between these two alternatives.

Berl & Clarke (1970) from the work of Lahiri & Quastel (1963), Gonda & Quastel (1966) and Nicklas, Clarke & Berl (1968) have suggested that fluoroacetate acts mainly on a tricarboxylate cycle that contains the smaller pools of intermediates and from which most of the glutamine is formed.

Triethyltin would seem to be an example of an inhibitor that preferentially acts on that tricarboxylate cycle which contains the larger pools of intermediates (see Berl & Clarke, 1970 for a discussion of the size of pools of intermediates). In particular, it inhibits the labelling of the 'large' pool of glutamate from which most of the γ-aminobutyrate is formed.

Triethyltin is an inhibitor of processes involved in the conservation of energy both in mitochondria from mammalian sources (Aldridge, 1958; Aldridge & Street, 1968; Aldridge & Rose, 1969) and in chloroplasts (Lynn & Straub, 1969). It inhibits State 3 respiration. No other enzyme systems have been found to be as sensitive to triethyltin. It can be supposed that if triethyltin comes into contact with brain mitochondria it will inhibit their metabolism. The results with [1-^{14}C]acetate did not reveal any impairment of the incorporation of ^{14}C into amino acids in the brains of rats injected with triethyltin (Table 3). On the basis that all mitochondria are equally sensitive to triethyltin it might be argued that triethyltin did not come into contact with those mitochondria involved in the oxidation of acetate.

Rose & Aldridge (1968) showed that within 1h after a dose of 40μmol of triethyltin/kg body wt. (the same as used in the present study) the brain contained 20nmol of triethyltin/g. On subfractionation of brain homogenates 40% of the triethyltin was recovered from the mitochondrial fraction. However, whether triethyltin is predominantly located in a particular cell type (i.e. neurons or glia) is not yet known. The only morphological change consistently observed in the central nervous system is frequent intramyelinic splitting that creates enlarged fluid-filled spaces low in protein but high in Na^+ and Cl^- (Magee, Stoner & Barnes, 1957; Lee & Bakay, 1965). No changes have been observed in other structures and organelles including mitochondria. Recovery from grossly altered myelin lamellae can be complete. It should be made clear that in the present experiments on brain metabolism [^{14}C]glucose and [^{14}C]-acetate were injected into rats that had received triethyltin 2h previously. There is no measurable increase in fluid content in the brains at this time.

Hypothermia. Rose & Aldridge (1966) showed that the decrease of $^{32}P_i$ incorporation into brain phospholipids, seen in rats given triethyltin, was due to the hypothermia it caused. In contrast, the increased content of brain glucose, diminished concentration of glutamate and decreased amino acid labelling from [2-^{14}C]glucose were not overcome by preventing hypothermia. Elevated cerebral glucose contents in ether and pentobarbitone anaesthesia also persist when hypothermia is prevented (Hegab & Miller, 1968; Chowdhury & Spector, 1969).

Rates of glucose utilization. Glycolysis in brain is of major importance, but the rate at which it occurs *in vivo* is difficult to determine. In man and larger animals the rate can be equated with the rate of uptake of glucose from the blood as measured by arterio-venous differences. An average value of 0.3μmol/min per g of brain has been found (see Gaitonde, 1965). The rate in smaller animals, such as the rat and the mouse, is probably higher. Gaitonde (1965) calculated from his data with [U-^{14}C]glucose an approximate rate of glucose utilization in rat brain of 0.96μmol/min per g. If [2-^{14}C]glucose is used, then for a short time after intravenous injection the loss of radioactivity as $^{14}CO_2$ from the brain can be considered negligible.

Since the change in specific radioactivity of blood glucose in the time-interval 30–90s was small, an approximate rate of glucose utilization by the rat brain can be calculated from the increase in d.p.m./g of brain between 30 and 90s divided by the average specific radioactivity of blood glucose during this period. For the control animals this gives a rate of 0.54μmol of glucose/min per g of brain and for animals given triethyltin a rate of 0.64μmol/min per g of brain. The rate of flux of pyruvate to glutamate (see the Results section) was calculated to be 1.3μmol/min per g of brain (equivalent to 0.65μmol of glucose) for control rats, and for animals given triethyltin the rate was 0.66μmol/min per g of brain (equivalent to 0.33μmol of glucose). In the control animals therefore, there is quite good agreement between the two sets of calculated rates, but there is a considerable discrepancy in the rats given triethyltin. When glucose utilization is calculated as described above for the time-period between 90 and 180s, the rate for the control animals is 0.6μmol of glucose/min per g of brain, whereas in the animals given triethyltin the rate is only 0.29μmol/min per g of brain. In the control animals killed at 30s 75% of the total radioactivity in the brain is present as glucose plus lactate and only 5% is present as glutamate (Table 4). By 180s 30% is present as glutamate and 42% as glucose plus lactate. The rate of glucose utilization calculated from the time-interval between 30 and 90s will represent mainly glycolysis; the rate calculated from the 90–180s interval will include metabolism via the tricarboxylate cycle. In the control animals, as is to be

expected in a steady state, the rate is virtually the same during the two time-intervals. In the animals given triethyltin the rate during the 90–180s interval is only half that between 30 and 90s. This implies that the rate of glycolysis was faster than the functioning of the tricarboxylate cycle and therefore lactate would be expected to accumulate. Since the amount of lactate present in the brain was the same in both groups of animals, the possibility is raised that lactate was leaving the brains of the animals given triethyltin.

The indication that the main action of triethyltin in the brain *in vivo* is to decrease the rate at which the pyruvate formed from glucose is oxidized is in agreement with the conclusion reached from studies *in vitro* with cerebral cortex slices (Cremer, 1967). One of the main points of interest of the present findings is the lack of effect of triethyltin on [1-^{14}C]-acetate metabolism. Some of the anomalies in the results obtained with slices, such as lack of inhibition of the oxidation of added pyruvate and glutamate (Cremer, 1967), might be explained by the existence of metabolically distinct tricarboxylate cycles and selective inhibition by triethyltin of one of these. This particular tricarboxylate cycle is concerned with metabolism of the major portion of the pyruvate formed from glycolysis.

The author thanks Mr P. Matthews and Miss H. Lucas for their valuable technical assistance during different stages of this work.

REFERENCES

Aldridge, W. N. (1958). *Biochem. J.* **69**, 367.
Aldridge, W. N. & Rose, M. S. (1969). *FEBS Lett.* **4**, 61.
Aldridge, W. N. & Street, B. W. (1968). *Biochem. J.* **107**, 315.
Bachelard, H. S. & Lindsay, J. R. (1966). *Biochem. Pharmac.* **15**, 1053.
Balázs, R. & Haslam, R. J. (1965). *Biochem. J.* **94**, 131
Bergmeyer, H. (Ed.) (1963). *Methods of Enzymatic Analysis.* New York: Academic Press Inc.
Berl, S. & Clarke, D. D. (1970). In *Handbook of Neurochemistry*, vol. 2, p. 447. Ed. by Lajtha, A. New York: Plenum Publishing Co.
Berl, S. & Frigyesi, T. L. (1969). *Brain Res., Amsterdam*, **14**, 683.
Biserte, G., Plaquet-Schoonaert, T., Boulanger, R. & Paysant, P. (1960). *J. Chromat.* **3**, 25.
Bruce, H. M. & Parkes, A. S. (1949). *J. Hyg., Camb.*, **47**, 202.
Chowdhury, A. K. & Spector, R. G. (1969). *Biochem. Pharmac.* **18**, 1248.
Cremer, J. E. (1962). *J. Neurochem.* **9**, 289.
Cremer, J. E. (1964). *J. Neurochem.* **11**, 165.
Cremer, J. E. (1967). *Biochem. J.* **104**, 212.
Cremer, J. E. (1969). *Proc. 2nd int. Meet. int. Soc. Neurochem., Milan*, p. 138.
Flock, E. V., Tyce, G. M. & Owen, C. A. (1969). *Endocrinology*, **85**, 428.
Gaitonde, M. K. (1962). *J. Neurochem.* **8**, 234.
Gaitonde, M. K. (1965). *Biochem. J.* **95**, 803.
Godin, Y., Mark, J. & Mandel, P. (1968). *J. Neurochem.* **15**, 1085.
Gonda, O. & Quastel, J. H. (1966). *Biochem. J.* **100**, 83.
Hegab, El-S. H. H. & Miller, J. A. (1968). *J. appl. Physiol.* **25**, 130.
Lahiri, S. & Quastel, J. H. (1963). *Biochem. J.* **89**, 157.
Lee, J. C. & Bakay, L. (1965). *Archs Neurol., Chicago*, **13**, 48.
Lynn, W. S. & Straub, K. D. (1969). *Proc. natn. Acad. Sci. U.S.A.*, **63**, 540.
Magee, P. N., Stoner, H. B. & Barnes, J. M. (1957). *J. Path. Bact.* **73**, 107.
Mark, J., Godin, Y. & Mandel, P. (1968). *J. Neurochem.* **15**, 141.
Minard, F. N. & Mushahwar, I. K. (1966). *J. Neurochem.* **13**, 1.
Neidle, A., Van den Berg, C. J. & Grynbaum, A. (1969). *J. Neurochem.* **16**, 225.
Nicklas, W. J., Clarke, D. D. & Berl, S. (1968). *Fedn Proc. Fedn Am. Socs exp. Biol.* **27**, 463.
O'Neal, R. M. & Koeppe, R. E. (1966). *J. Neurochem.* **13**, 835.
Rose, M. S. & Aldridge, W. N. (1966). *J. Neurochem.* **13**, 103.
Rose, M. S. & Aldridge, W. N. (1968). *Biochem. J.* **106**, 821.
Salganicoff, L. & De Robertis, E. (1965). *J. Neurochem.* **12**, 287.
Salganicoff, L. & Koeppe, R. E. (1968). *J. biol. Chem.* **243**, 3416.
Van den Berg, C. J., Kržalić, L. T., Mela, P. & Waelsch, H. (1969). *Biochem. J.* **113**, 281.
Van den Berg, C. J., Mela, P. & Waelsch, H. (1966). *Biochem. biophys. Res. Commun.* **23**, 479.
Vrba, R., Gaitonde, M. K. & Richter, D. (1962). *J. Neurochem.* **9**, 465.