Biochem. J. (1974) **138**, 1–10
Printed in Great Britain

Regulation of Glucose and Ketone-Body Metabolism in Brain of Anaesthetized Rats

By NEIL B. RUDERMAN,* PETER S. ROSS, MICHAEL BERGER and MICHAEL N. GOODMAN
Elliott P. Joslin Research Laboratory and the Department of Medicine, Harvard Medical School and the Peter Bent Brigham Hospital, Boston, Mass. 02215, U.S.A.

(*Received* 22 *June* 1973)

1. The effects of starvation and diabetes on brain fuel metabolism were examined by measuring arteriovenous differences for glucose, lactate, acetoacetate and 3-hydroxybutyrate across the brains of anaesthetized fed, starved and diabetic rats. 2. In fed animals glucose represented the sole oxidative fuel of the brain. 3. After 48h of starvation, ketone-body concentrations were about 2mM and ketone-body uptake accounted for 25% of the calculated O_2 consumption: the arteriovenous difference for glucose was not diminished, but lactate release was increased, suggesting inhibition of pyruvate oxidation. 4. In severe diabetic ketosis, induced by either streptozotocin or phlorrhizin (total blood ketone bodies >7mM), the uptake of ketone bodies was further increased and accounted for 45% of the brain's oxidative metabolism, and the arteriovenous difference for glucose was decreased by one-third. The arteriovenous difference for lactate was increased significantly in the phlorrhizin-treated rats. 5. Infusion of 3-hydroxybutyrate into starved rats caused marked increases in the arteriovenous differences for lactate and both ketone bodies. 6. To study the mechanisms of these changes, steady-state concentrations of intermediates and co-factors of the glycolytic pathway were determined in freeze-blown brain. 7. Starved rats had increased concentrations of acetyl-CoA. 8. Rats with diabetic ketosis had increased concentrations of fructose 6-phosphate and decreased concentrations of fructose 1,6-diphosphate, indicating an inhibition of phosphofructokinase. 9. The concentrations of acetyl-CoA, glycogen and citrate, a potent inhibitor of phosphofructokinase, were increased in the streptozotocin-treated rats. 10. The data suggest that cerebral glucose uptake is decreased in diabetic ketoacidosis owing to inhibition of phosphofructokinase as a result of the increase in brain citrate. 11. The inhibition of brain pyruvate oxidation in starvation and diabetes can be related to the accelerated rate of ketone-body metabolism; however, we found no correlation between the decrease in glucose uptake in the diabetic state and the arteriovenous difference for ketone bodies. 12. The data also suggest that the rates of acetoacetate and 3-hydroxybutyrate utilization by brain are governed by their concentrations in plasma. 13. The finding of very low concentrations of acetoacetate and 3-hydroxybutyrate in brain compared with plasma suggests that diffusion across the blood–brain barrier may be the rate-limiting step in their metabolism.

The studies of Owen *et al.* (1967) established that the human brain can utilize ketone bodies as a fuel in place of glucose. In obese patients starved for 40 days, they observed that ketone bodies account for approx. 60% of the fuel needs of brain and that glucose oxidation is decreased to less than one-third of the value in the post-absorptive state. Hawkins *et al.* (1971) observed that acetoacetate and 3-hydroxybutyrate are also utilized by the brain of the rat during starvation. In these experiments, however, ketone-body metabolism accounted for less than 22% of the brain's fuel needs, and glucose utilization was not significantly diminished.

* To whom reprint requests should be addressed.

The present study was designed to investigate some of the factors that determine the relative magnitude of glucose and ketone-body metabolism in brain. For this purpose arteriovenous differences for glucose, lactate, acetoacetate and 3-hydroxybutyrate were determined across the brains of fed and starved rats, rats with diabetic ketoacidosis induced by either phlorrhizin or streptozotocin and rats infused with 3-hydroxybutyrate. To determine the sites at which the metabolism of glucose and ketone bodies is regulated in these situations, brain metabolites were measured in rapidly frozen tissue obtained with the 'freeze-blowing apparatus' devised by Veech *et al.* (1973). A preliminary report of this work has appeared (Berger *et al.*, 1973).

Experimental procedure

Materials

Phlorrhizin was obtained from K & K Laboratories Inc., Plainview, N.Y., U.S.A., and streptozotocin (lot no. 9681-665-118FI) was a gift from Dr. W. E. Dulin, Upjohn Co., Kalamazoo, Mich., U.S.A. Sodium acetoacetate, free of ethanol, was prepared from ethyl acetoacetate as described by Krebs & Eggleston (1941). Sodium pentobarbital powder, obtained from K & K Laboratories Inc., was prepared as an aqueous solution shortly before use. All enzymes and other reagents were obtained from either Boehringer (Mannheim) Corp., New York, N.Y., U.S.A., or Sigma Chemical Co., St. Louis, Mo., U.S.A.

Animals

Female rats of the Sprague–Dawley strain weighing 180–210g were used. They were maintained on Purina Laboratory Chow for at least 3 days before use. Rats made diabetic with streptozotocin received 150mg of the drug/kg by tail-vein injection. They were allowed free access to food and were killed after 2–3 days. None of the animals was in coma. Phlorrhizin (40mg in 0.1ml of propylene glycol) was administered subcutaneously to rats starved for 24h. The animals received four doses of phlorrhizin 6h apart, and were killed 3–5h after the final dose. The degree of hyperketonaemia produced by this regimen was comparable with that caused by streptozotocin (see Table 1); however, the phlorrhizin-treated animals did not appear ill.

Collection of blood samples

Rats were anaesthetized with an intraperitoneal injection of pentobarbital. A dose of 40–50mg/kg was used for all animals except those with streptozotocin-diabetes, which required only 30–40mg/kg. The procedure used for collecting arterial and cerebral venous blood was essentially that described by Hawkins *et al.* (1971). Blood samples were deproteinized in 10% (w/v) $HClO_4$, centrifuged, and the supernatant was neutralized with $KHCO_3$.

Infusion studies

In some experiments 3-hydroxybutyrate was infused through a polyethylene catheter (internal diam. 0.58mm; external diam. 0.965mm; type PE50; Clay–Adams) that had been passed into the inferior vena cava through an incision in a femoral vein. Sodium DL-3-hydroxybutyrate (3M), pH8.9, was infused for 10min at a rate of 120μmol/min (2.4ml/h) and then for an additional 20min at a rate of 60μmol/min (1.2ml/h). A stable arterial concentration of 3-hydroxybutyrate was attained by 20min of infusion. In another series of experiments sodium acetoacetate was infused into the rats for 60–90min in the same manner as DL-3-hydroxybutyrate, but at half the rate.

Tissue studies

The brains of a group of rats were freeze-blown by using the apparatus devised by Veech *et al.* (1973). Arteriovenous differences across the brain were not determined in these rats; however, the rats were anaesthetized with pentobarbital for approx. 20min before the tissue was obtained, in order to mimic the conditions of the other animals. Samples of blood were obtained from the tail vein or from the abdominal aorta immediately before freeze-blowing, in order to determine the blood/brain ratio of certain metabolites.

Analyses

Frozen brain tissue was powdered under liquid N_2 in a mortar and deproteinized and neutralized by a modification of the procedure described by Nelson *et al.* (1971). The extract was analysed spectrophotometrically for glucose (Slein, 1963), acetoacetate and 3-hydroxybutyrate (Williamson *et al.*, 1962), glucose 6-phosphate and fructose 6-phosphate (Hohorst, 1963*a*), ATP (Lamprecht & Trautschold, 1963), ADP and AMP (Adam, 1963), creatine phosphate (Lamprecht & Stein, 1963) and citrate (Start & Newsholme, 1968) by standard enzymic methods. Fructose 1,6-diphosphate was assayed fluorimetrically as described by Williamson & Corkey (1969). Acetyl-CoA was assayed fluorimetrically by coupling acetyl-CoA disappearance during citrate synthesis to the malate dehydrogenase reaction (Williamson & Corkey, 1969). The results were corrected for conversion of oxaloacetate into malate. Tissue was prepared for glycogen determination as described by Good *et al.* (1933). Glucose present as glycogen was determined in the neutralized acid hydrolysate by the glucose oxidase method (Huggett & Nixon, 1957) as modified by Krebs *et al.* (1964). Neutralized blood extracts were analysed for glucose, acetoacetate and 3-hydroxybutyrate, and lactate (Hohorst, 1963*b*).

Enzyme nomenclature

The following enzymes are referred to in the text: hexokinase (EC 2.7.1.1); phosphofructokinase (EC 2.7.1.11); pyruvate dehydrogenase (EC 1.2.4.1); glycogen synthetase (glycogen–UDP glucosyltransferase) (EC 2.4.1.11); creatine kinase (EC 2.7.3.2); 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30); 3-oxo acid CoA-transferase (EC 2.8.3.5).

Results

The fuel metabolism of brain was assessed on the basis of arteriovenous differences for glucose, acetoacetate, 3-hydroxybutyrate and lactate. In order to calculate the arteriovenous difference for O_2, it was assumed that all the ketone bodies taken up were completely oxidized, as was all the glucose not released as lactate. The validity of this assumption is borne out by studies in both man (Owen *et al.*, 1967; Gottstein *et al.*, 1971) and rat (Hawkins *et al.*, 1971), in which it was demonstrated that the arteriovenous difference for O_2, calculated in this way, is very close to the measured arteriovenous difference across the brain.

Fuel metabolism in fed and starved rats

The arterial concentrations of glucose, lactate, acetoacetate and 3-hydroxybutyrate in all of the groups studied are presented in Table 1 and the arteriovenous differences for these substances in Table 2. In agreement with earlier studies in man (Himwich, 1951) and in the rat (Hawkins *et al.*, 1971), glucose was essentially the sole fuel of brain in the fed state and lactate release was negligible (Table 2). In rats starved for 24 and 48h the concentrations of acetoacetate and 3-hydroxybutyrate in blood were substantially higher and there were significant arteriovenous differences for these ketone bodies across the brain. Here, ketone bodies accounted for 20–25% of the calculated O_2 consumption of the brain. The arteriovenous differences for glucose and for O_2 consumption attributable to glucose oxidation were somewhat less than in fed rats; however, the differences were not statistically significant (see the Discussion section).

Fuel metabolism in diabetic rats

In rats with streptozotocin-diabetes, the concentrations of acetoacetate+3-hydroxybutyrate and of glucose were increased to 8.5 and 23mM respectively (Table 1). The arteriovenous difference for acetoacetate was approximately 3 times that of the two groups of starved rats and the difference for 3-hydroxybutyrate was 1.5 times as much (Table 2). Together, the two ketone bodies accounted for 52% of the calculated O_2 consumed by brain. In comparison with fed rats, the contribution of glucose to the calculated O_2 consumption was significantly diminished.

Rats treated with phlorrhizin achieved the same degree of hyperketonaemia and had approximately the same arteriovenous differences for acetoacetate and 3-hydroxybutyrate as did the rats treated with streptozotocin. The arteriovenous differences for glucose were also comparable in the two groups despite the 10-fold greater glucose concentration in the streptozotocin-treated animals. Likewise, the contribution of glucose to the calculated O_2 consumption was significantly depressed in the phlorrhizin-treated group, although here it was due to a combination of a decreased arteriovenous difference for glucose and an increased glucose conversion into lactate.

Infusion of 3-hydroxybutyrate

When sodium DL-3-hydroxybutyrate was infused into rats starved for 24 h, the arterial concentrations of 3-hydroxybutyrate and acetoacetate increased to 5.23 and 1.01mM respectively (Table 1) and the 3-hydroxybutyrate/acetoacetate ratio increased to 4.8 (see Table 1). The arteriovenous difference for 3-hydroxybutyrate of 0.29mM (Table 2) was 3 times that observed in starved rats and twice that in rats with streptozotocin-diabetes. Glucose uptake was not significantly diminished by 3-hydroxybutyrate; however, the arteriovenous difference for lactate increased markedly from 0.12 to 0.37mM. As a result, the calculated value for O_2 required for glucose oxidation decreased to 1.2mM, about half the value found in 24h-starved animals, and glucose oxidation accounted for only 40% of calculated O_2 consumption.

In another series of experiments sodium acetoacetate was infused in place of DL-3-hydroxybutyrate

Table 1. *Effect of nutritional status, diabetes and infusion of 3-hydroxybutyrate on the concentrations of metabolites in arterial blood*

Results are means ±S.E.M. with numbers of observations in parentheses. Further details are given in the Experimental Procedures section.

State of animal	Concn. of metabolite (mM)			
	Glucose	Lactate	3-Hydroxybutyrate	Acetoacetate
Fed (6)	7.46±0.12	1.03±0.24	0.06±0.02	0.09±0.02
Starved 24h (12)	5.11±0.15	0.70±0.08	0.83±0.07	0.37±0.04
Starved 48h (13)	4.77±0.10	0.70±0.11	2.05±0.14	0.53±0.03
Streptozotocin-diabetes (9)	23 ±2.9	1.60±0.25	6.55±0.93	1.95±0.19
Phlorrhizin-diabetes (7)	2.37±0.05	0.68±0.06	5.54±0.37	2.30±0.19
Starved 24h, then infused with 3-hydroxybutyrate	5.33±0.31	2.79±0.27	5.52±0.19	1.13±0.12

Table 2. *Effect of nutritional status, diabetes and ketone-body infusion on arteriovenous differences of metabolites across the rat brain*

Results are means ± S.E.M. of arteriovenous differences and are derived from the data in Table 1. The symbols + and − indicate appearance and removal of the metabolite. Values significantly different from those of the fed group at 5%, 1% and 0.1% levels are indicated by the symbols *, ** and *** respectively. The amount of O_2 required for glucose oxidation was calculated by using the formula (glucose − lactate/2) × 6. Conversion of glucose into pyruvate was negligible and was therefore omitted. The amount of O_2 used for ketone-body oxidation was calculated by using the formula [3-hydroxybutyrate] × 4.5 + [acetoacetate] × 4.

	Arteriovenous difference (mM)					Calculated O_2 consumption (mM)			Percentage of calculated O_2 consumption due to ketone bodies
State of animal	Glucose	Lactate	3-Hydroxy-butyrate	Acetoacetate	Total ketone bodies	Glucose	Ketone bodies	Total	
Fed	−0.48±0.05	+0.02±0.04	+0.008±0.04	−0.014±0.04	−0.005±0.02	2.78±0.43	0.02±0.10	2.80±0.29	0.6
Starved 24 h	−0.42±0.09	+0.12±0.02*	−0.08 ±0.03	−0.07 ±0.01*	−0.14 ±0.04**	2.19±0.53	0.61±0.17**	2.81±0.64	22
Starved 48 h	−0.40±0.05*	+0.18±0.04*	−0.09 ±0.02	−0.06 ±0.01	−0.15 ±0.02***	1.85±0.27	0.64±0.10***	2.55±0.31	25
Streptozotocin-diabetes	−0.25±0.09†*	+0.12±0.04	−0.13 ±0.02***	−0.16 ±0.02***	−0.29 ±0.04***	1.15±0.59*	1.24±0.19***	2.39±0.68	52
Phlorrhizin-diabetes	−0.34±0.05*	+0.24±0.05**	−0.11 ±0.02**	−0.13 ±0.03**	−0.24 ±0.04***	1.32±0.22*	1.02±0.15***	2.32±0.26	44
Starved 24 h, then infused with 3-hydroxybutyrate	−0.38±0.06	+0.37±0.09**	−0.29 ±0.03***	−0.13 ±0.03**	−0.41 ±0.05***	1.19±0.18**	1.79±0.18***	2.99±0.36	60

† The arteriovenous difference for glucose was also measured in an additional 12 rats with streptozotocin-diabetes. The mean arteriovenous difference ± S.E.M. for the entire group ($n = 21$) was 0.25 ± 0.06 ($P < 0.01$ versus fed rats). Lactate was not measured in the additional rats; therefore these values were not used for calculating O_2 consumption.

Table 3. *Effect of nutritional status, diabetes and infusion of acetoacetate on the concentrations of hexose phosphates, citrate, acetyl-CoA and glycogen in freeze-blown rat brain*

Results are mean ± S.E.M. and are not corrected for contamination by blood (see Table 1) or cerebrospinal fluid. Numbers of observations are given in parentheses. Values significantly different from those of the fed rat at the 5, 1 and 0.1% levels are indicated by the symbols *, ** and *** respectively. Absolute concentrations of ATP and ADP are listed in Table 4. Further details are given in the Experimental Procedure section.

	Concentration (nmol/g wet wt.)					Concn. of glycogen	$\frac{[\text{Fructose diphosphate}][ADP]}{[\text{Fructose 6-phosphate}][ATP]} \times 10^3$
State of animal	Glucose 6-phosphate	Fructose 6-phosphate	Fructose 1,6-diphosphate	Citrate	Acetyl-CoA	(μmol/g)	
Fed	159±5 (10)	51±4 (10)	9.1±0.5 (11)	241±10 (11)	3.0±0.5 (4)	4.1±0.3 (11)	44±4 (9)
Starved 48 h	159±7 (10)	49±4 (10)	9.4±0.4 (9)	267±15 (10)	4.8, 7.5 (2)	3.7±0.4 (10)	51±5 (9)
Streptozotocin-diabetes	229±14 (8)***	74±4 (9)***	5.2±0.5 (8)***	389±30 (9)***	6.5±0.6 (6)**	6.7±0.4 (8)***	16±2 (8)***
Phlorrhizin-diabetes	176±8 (6)†	61±2 (6)†‡	6.7±0.5 (6)**	300±15 (6)**	4.5, 3.6 (2)	3.7±0.4 (6)	28±3 (6)**

† Difference from fed group: $0.1 > P > 0.05$.
‡ Difference from starved 48 h group: $P < 0.05$.

(for 60–90min rather than 30min, however). The findings were very similar to those of Hawkins *et al.* (1971), namely, a very large increase in the arteriovenous difference for acetoacetate with no significant change in the arteriovenous difference for either glucose or lactate. Evaluation of the creatine kinase equilibrium in brain (see below) indicated that sodium acetoacetate probably caused an increase in brain pH. Since this would increase the activity of phosphofructokinase (Trivedi & Danforth, 1966) and since the effect of acetoacetate on the redox state of brain was not evaluated the results are not presented.

Lactate release and ketone-body uptake

In general the arteriovenous difference for lactate was greater when the brain obtained a greater percentage of its fuel from ketone bodies (Table 2). Thus, fed animals, which used very little acetoacetate and 3-hydroxybutyrate, had a small net release of lactate (0.02mM), whereas rats starved for 24 and 48h and rats treated with phlorrhizin had arteriovenous differences for lactate of 0.12, 0.18 and 0.24mM respectively. Rats infused with 3-hydroxybutyrate derived a larger percentage of their calculated O_2 consumption from ketone-body oxidation than did any other group and also demonstrated the largest arteriovenous difference for lactate (0.37mM). The possibility that part of this increment in lactate release was due to a change in the redox state of the brain was not evaluated; however, Miller and co-workers have not found an appreciable increase in the lactate/pyruvate ratio in the brain of rats infused with 3-hydroxybutyrate (A. Miller, R. A. Hawkins & R. L. Veech, personal communication).

Rats with streptozotocin-diabetes were the exception to the trend. The arteriovenous difference for lactate in these animals was only 0.12mM despite the very large arteriovenous difference for ketone bodies in this group. Although the arteriovenous difference for lactate was 6-fold greater than that of the fed rats the difference was not significant ($0.1 > P > 0.05$).

Glucose and ketone-body uptake

Although the arteriovenous difference for glucose tended to be greater and the difference for ketone-bodies smaller in fed and starved rats than in the diabetic groups, the negative correlation was not statistically significant when all the data were pooled ($r = -0.002$).

Concentration and uptake of ketone bodies

In agreement with earlier studies (Daniel *et al.*, 1971; Hawkins *et al.*, 1971), the arteriovenous difference for acetoacetate across the rat brain was approximately proportional to its arterial concentration ($r = 0.71$, $P < 0.001$, $n = 54$). The relationship between the arterial concentration of 3-hydroxybutyrate and its removal by brain was more complex. Although the arteriovenous difference for 3-hydroxybutyrate was also proportional to its concentration, the correlation was not as close as for acetoacetate ($r = 0.52$, $P < 0.001$, $n = 54$) because of the relatively small arteriovenous differences in the two groups of diabetic rats. For instance, the arteriovenous difference for 3-hydroxybutyrate was only slightly greater in the streptozotocin- and phlorrhizin-treated rats than in starved rats, even though their arterial concentrations of 3-hydroxybutyrate were 3–8-fold higher. Likewise, the rats infused with 3-hydroxybutyrate had a lower arterial concentration of 3-hydroxybutyrate than did the diabetic animals, but had a more than 2-fold greater arteriovenous difference.

Brain metabolites

Brains were obtained from separate groups of fed, 48h-starved and diabetic rats for the assay of metabolites. The blood concentrations of glucose, acetoacetate and 3-hydroxybutyrate in these rats and the rats used for determining arteriovenous differences were comparable (see Tables 5 and 6).

Hexose phosphates. As shown in Table 3, the concentrations of the hexose monophosphates and fructose 1,6-diphosphate in brain were not altered by 48h of starvation; however, glucose 6-phosphate and fructose 6-phosphate were significantly increased and fructose 1,6-diphosphate was decreased in rats with diabetic ketoacidosis induced by streptozotocin. The fact that fructose-6-phosphate is increased indicates that the block in cerebral glycolysis in these animals is at the level of phosphofructokinase. Similar changes in the concentrations of hexose phosphates were noted in the brains of rats with phlorrhizin-diabetes. Here, the concentrations of both glucose 6-phosphate and fructose 6-phosphate, although elevated, were not significantly higher than in fed rats ($P < 0.1$). Fructose 6-phosphate was significantly higher than in starved animals, and the concentration of fructose 1,6-diphosphate and the mass-action ratio of phosphofructokinase (see Table 3) were both significantly diminished.

Citrate. The regulation of phosphofructokinase is very complex. In addition to the fact that the enzyme has several molecular forms, it is strongly inhibited by one of its substrates, ATP, by citrate and by an increase in $[H^+]$: this inhibition can be counteracted by a variety of substances including fructose 6-phosphate, fructose 1,6-diphosphate, 5′-AMP, P_i and 3′:5′-cyclic AMP, and by a decrease in $[H^+]$ (reviewed by Mansour, 1972). Of the factors measured, only the concentration of citrate was altered in the

brains of rats with diabetic ketoacidosis. The concentration of this intermediate increased by more than 60% in rats treated with streptozotocin and by approx. 25% in phlorrhizin-treated rats (Table 3). These increases in citrate are smaller than those associated with inhibition of phosphofructokinase in heart muscle perfused with free fatty acids or ketone bodies; however, phosphofructokinase was probably less completely inhibited in the present study (see Randle *et al.*, 1966).

Acetyl-CoA. Increases in the concentrations of citrate in heart muscle of diabetic rats have been attributed to an increase in the acetyl-CoA/CoA ratio due to an accelerated rate of ketone-body and free fatty acid oxidation (Randle *et al.*, 1966). In the present study, the concentration of acetyl-CoA was increased in the brains of animals with streptozotocin-diabetes when compared with fed animals (Table 3). The numbers of determinations of acetyl-CoA in the other groups were too few to allow statistical comparison; nevertheless, it is noteworthy that the individual values for acetyl-CoA in starved rats were comparable with those of animals treated with streptozotocin and were 2S.D. or more greater than the mean value in the fed group. The concentration of free CoA was not determined.

Adenine nucleotides (*Table* 3). In general the concentrations of adenine nucleotides differed very little from one group to another, suggesting that they played little or no role in the decrease in phosphofructokinase activity seen in diabetes. The concentration of AMP was slightly higher in starved than in fed rats: the changes were small, however, and the fed and starved rats did not differ with respect to arteriovenous difference for glucose uptake or the mass-action ratio of phosphofructokinase (Table 3). A small but significant increase in ATP was also noted in rats with streptozotocin-diabetes.

Creatine phosphate. As shown in Table 4, the concentrations of creatine phosphate and values for the expression [ATP][creatine]/[ADP][creatine phosphate] were very similar in all groups. Creatine itself was not measured; however, the equilibrium value was calculated on the assumption that the total creatine concentration in brain is 8.9mM and that it varies very little from one animal to another (Lowry & Passoneau, 1964; Siesjö *et al.*, 1972). The fact that the equilibrium ratio did not vary is noteworthy, since the complete equilibrium expression for creatine kinase (Kuby & Noltmann, 1962) is:

$$K_{eq.} = \frac{[ATP][Creatine]}{[ADP][Creatine\ phosphate]} \times \frac{1}{[H^+]}$$

Thus, if the $[H^+]$ of the brain cell were increased, the ratio [ATP][Creatine]/[ADP][Creatine phosphate] would also increase if equilibrium were maintained (see Siesjö *et al.*, 1972). The fact that it did not indicates that brain pH was not decreased in rats with diabetic ketoacidosis and that the inhibition of phosphofructokinase in these animals cannot be explained on this basis.

Infusion of sodium acetoacetate into 48h-starved rats caused an increase in the concentration of creatine phosphate to 4517±257nmol/g and of ADP to 675±20nmol/g. The equilibrium ratio was decreased to 3.6±0.5, suggesting intracellular alkalosis. If the assumption is correct that the sum of creatine+creatine phosphate is constant, the increment in pH would have been approx. 0.2 unit.

Glucose in blood and brain. A change in the distribution of glucose between blood and brain could theoretically be due to alterations in either the blood–brain barrier for glucose, the permeability of brain cells to glucose, or the activity of hexokinase. A small but significant increase in the brain/blood ratio for glucose was noted after 48h of starvation (Table 5). The meaning of this change is not clear, since the arteriovenous difference for glucose across the brain was not significantly decreased. An increase in the brain/blood ratio for glucose, of a comparable magnitude, was also noted in rats with diabetes induced by streptozotocin. Here a decrease in hexokinase activity caused by inhibition by glucose 6-phosphate may have been a factor, although it remains to be determined

Table 4. *Effect of nutritional status and diabetes on the concentrations of adenine nucleotides and creatine phosphate in freeze-blown brain*

Results are mean±S.E.M. with numbers of experiments in parentheses. See legend to Table 3 for further details.

State of animal	Concn. (nmol/g wet wt.) ATP	ADP	AMP	Creatine phosphate	[Creatine][ATP]/[Creatine phosphate][ADP]
Fed	2363±110 (12)	602±20 (12)	86±4 (12)	3788±147 (12)	5.6±0.4 (8)
Starved 48h	2307±128 (10)	600±24 (10)	104±5 (10)**	3808±319 (10)	6.0±1.0 (10)
Streptozotocin-diabetes	2649± 34 (9)*	591±10 (9)	96±4 (9)	4044±178 (9)	5.8±0.5 (9)
Phlorrhizin-diabetes	2609± 87 (6)	645±21 (6)	93±6 (6)	3781± 33 (6)	5.9±1.0 (6)

Table 5. *Concentrations of glucose in blood and brain*

Results are means±S.E.M. with numbers of observations in parentheses. Blood specimens of fed, starved and phlorrhizin-diabetic rats were from tail veins; blood specimens of other animals were from the femoral artery. In calculating brain glucose and the brain/blood ratio, no correction was made for that portion of brain glucose present in the vascular space. Values for blood glucose are for whole blood. See legend to Table 3 for further details.

State of animal	Concn. (μmol/g wet wt.)		
	Blood glucose	Brain glucose	Brain/blood ratio
Fed (10)	5.6±0.2	2.9±0.2	0.52±0.02
Starved 48h (8)	4.2±0.2	2.4±0.1	0.64±0.04*
Streptozotocin-diabetes (8)	19 ±2	11.4±0.9	0.60±0.03*
Phlorrhizin-diabetes (6)	2.1±0.1	1.5±0.1	0.74±0.05***

whether hexokinase activity was decreased for other reasons or whether glucose permeability was altered. The highest brain/blood ratio for glucose was observed in phlorrhizin-treated rats; indeed, the ratio was significantly higher than that of the streptozotocin-treated group, suggesting that phlorrhizin may have had an independent effect on glucose permeability beyond the blood–brain barrier or on hexokinase activity. The relatively high brain/blood ratios for glucose reported in this study are probably due to the use of anaesthetized rats (Nelson *et al.*, 1971; Passoneau *et al.*, 1971).

Glycogen. The concentration of glycogen in brain was not affected by starvation or by phlorrhizin-diabetes (Table 3). On the other hand, in rats with streptozotocin-diabetes, glycogen was substantially increased. This could have been due to the elevated concentration of glucose 6-phosphate in these animals (0.23mM), as the K_a (glucose 6-phosphate) for brain glycogen synthetase is 0.26mM (Goldberg & O'Toole, 1969). Another possibility is that glycogen synthetase was secondarily activated by the high concentration of glucose itself (Hers *et al.*, 1970). If the latter mechanism were operative, it could explain why no increase in brain glycogen was observed in the phlorrhizin-treated animals.

Acetoacetate in blood and brain. The concentrations of ketone bodies in blood and brain were determined in starved and diabetic rats (Table 6). In agreement with earlier findings (Hawkins *et al.*, 1971), the brain/blood ratios for acetoacetate and 3-hydroxybutyrate were extremely low. When the concentration of acetoacetate in whole blood ranged between 2.4 and 2.7mM, the calculated concentration of acetoacetate in the extravascular space of brain was less than 0.1mM. Thus the concentration of acetoacetate in brain was less than 4% of that in whole blood and 2.6% of that in plasma. Since the interstitial space of brain, as determined with inulin, comprises approx. 15% (Tschirgi, 1960) of its volume, the fact that acetoacetate is distributed in a volume smaller than this suggests that entrance into the interstitial space may be rate-limiting for acetoacetate utilization in brain.

The brain/blood ratio for acetoacetate was highest in starved rats for reasons not understood. Despite this, the calculated concentration of acetoacetate in the extravascular space of brain was higher in rats with diabetes because of their markedly increased blood concentration of acetoacetate.

3-Hydroxybutyrate in blood and brain. The brain/blood ratio for 3-hydroxybutyrate was about 5–10-fold greater than that for acetoacetate (Table 6). This could occur if the blood–brain barrier was more permeable to 3-hydroxybutyrate than to acetoacetate, if the 3-hydroxybutyrate/acetoacetate ratio of the brain cell, as determined by the redox state of brain mitochondria, is lower than the ratio in plasma, or if 3-hydroxybutyrate is utilized at a much slower rate than acetoacetate. In support of the latter possibility is the finding that the activity of 3-hydroxybutyrate dehydrogenase in brain is less than one-quarter that of 3-oxo acid CoA-transferase (Williamson *et al.*, 1971).

In contrast to acetoacetate, the calculated concentration of 3-hydroxybutyrate in the extravascular space of brain bore little relationship to the concentration in blood; in fact, the calculated value for 3-hydroxybutyrate in the brain of starved rats was equal to or higher than that in diabetic animals (Table 6). Although the basis for this finding is not known, it may bear on the observation that the arteriovenous difference across the brain for 3-hydroxybutyrate was not significantly greater in rats with diabetic ketoacidosis than in starved rats (see Table 2).

Discussion

The data indicate that in diabetic ketoacidosis the uptake of glucose by brain of an anaesthetized rat is decreased, glycolysis is inhibited at phosphofructokinase and the uptake of ketone bodies is increased. Although it seems likely that the increase in brain ketone-body utilization is related to the elevated concentrations of acetoacetate and 3-hydroxybutyrate in blood (Hawkins *et al.*, 1971; Daniel *et al.*, 1971; the present study), the basis for the decrease in glucose metabolism is less clear. A possible explanation is

Table 6. *Concentrations of acetoacetate and 3-hydroxybutyrate in blood and brain*

Results are means ± S.E.M. with numbers of observations in parentheses. For calculation of 3-hydroxybutyrate and acetoacetate in extravascular space, it was assumed that blood comprises 3% of brain mass (Hindfeldt & Siesjö, 1970; Mark *et al.*, 1968). Values for brain/blood ratio significantly different from those of the 48 h-starved rat at the 5%, 1% and 0.1% levels are indicated by the symbols *, ** and ***. The 3-hydroxybutyrate/acetoacetate ratio of brain was derived from the calculated values for 3-hydroxybutyrate and acetoacetate in the extravascular space.

State of animal	Blood acetoacetate (mM)	Brain acetoacetate (μmol/g)	Brain/blood ratio	Calculated acetoacetate in extravascular space (μmol/g)	Blood 3-hydroxybutyrate (mM)	Brain 3-hydroxybutyrate (μmol/g)	Brain/blood ratio	Calculated 3-hydroxybutyrate in extravascular space (μmol/g)	Ratio 3-hydroxybutyrate / acetoacetate: Blood	Ratio 3-hydroxybutyrate / acetoacetate: Brain
Starved 48 h	0.42±0.05 (9)	0.05±0.01 (9)	0.14±0.02 (9)	0.037	1.46±0.11 (7)	0.36±0.06 (7)	0.25±0.03 (7)	0.316	3.5	8.6
Streptozotocin-diabetes	2.67±0.19 (9)	0.16±0.02 (9)	0.06±0.01 (9)**	0.080	6.78±1.19 (9)	0.54±0.06 (9)	0.09±0.01 (7)***	0.336	2.5	4.2
Phlorrhizin-diabetes	2.41±0.14 (5)	0.12±0.01 (5)	0.05±0.01 (5)*	0.048	4.72±0.43 (3)	0.39±0.06 (3)	0.08±0.01 (3)*	0.249	2.0	5.2

suggested by the observation of Randle *et al.* (1966) that the isolated rat heart uses less glucose when it is perfused with a high concentration of free fatty acids or ketone bodies (either of which can replace glucose as a fuel) or when it is obtained from a diabetic rat. It was noted that this decrease in glucose uptake was associated with a decreased rate of glycolysis, increases in glycogen and citrate concentrations and a tissue-metabolite pattern which indicated inhibition of phosphofructokinase. A similar switch in fuels, from glucose to ketone bodies, occurs in the brains of rats in diabetic ketoacidosis. This and the fact that the metabolite changes found in the brains of these rats were strikingly similar to that found by Randle *et al.* (1966) in the heart suggest that the basis for the decrease in glucose uptake in the two tissues may have been the same. In other words, in brain, the decrease in glucose uptake and glycolysis may have been a consequence of the increased rate of acetoacetate and 3-hydroxybutyrate metabolism. Although this hypothesis is attractive certain findings do not support it. For instance, when the data from all the groups were pooled, we did not find a significant negative correlation between the arteriovenous differences for glucose and ketone bodies, although there appeared to be a rough correlation when the fed and starved rats as a group were compared with the diabetic rats (Table 2). In addition, the infusion of 3-hydroxybutyrate had no effect on the arteriovenous difference for glucose despite a substantial increase in the uptake of ketone bodies. These findings may be explained eventually by methodological difficulties in precisely determining the arteriovenous difference for glucose and/or by an artifact introduced by infusing DL-3-hydroxybutyrate; nevertheless, it must be stated at the present time that the evidence that an accelerated rate of ketone-body oxidation can by itself initiate inhibition of cerebral glycolysis at phosphofructokinase is inconclusive.

The evidence that an accelerated rate of ketone-body metabolism can lead to inhibition of oxidation of pyruvate and hence of glucose by brain is more substantial. In addition to the fact that there was a significant positive correlation between the arteriovenous differences for ketone bodies and lactate, infusion with 3-hydroxybutyrate caused a marked increase in lactate release, indicating inhibition of pyruvate oxidation. Once again, the analogy to the perfused heart is relevant, since Randle *et al.* (1966) found that pyruvate oxidation in this preparation is inhibited when ketone bodies or free fatty acids are added to the perfusate.

The conclusions of the present study are substantially in agreement with those of earlier investigations in which the effects of ketone bodies on cerebral glucose and pyruvate metabolism were studied in slices of cerebral cortex (Ide *et al.*, 1969; Itoh & Quastel, 1970; Rolleston & Newsholme, 1967) and in minced

brain (Openshaw & Bortz, 1968). In general, neither acetoacetate (Itoh & Quastel, 1970; Rolleston & Newsholme, 1967) nor 3-hydroxybutyrate (Rolleston & Newsholme, 1967) were found to diminish glucose uptake, although in one investigation, 3-hydroxybutyrate seemed to decrease the disappearance of [U-^{14}C]glucose (Ide *et al.*, 1969). In contrast, ketone bodies invariably inhibited glucose oxidation, a finding which was explained by inhibition of pyruvate oxidation, rather than a block higher in the glycolytic pathway (Ide *et al.*, 1969; Itoh & Quastel, 1970). Itoh & Quastel (1970) proposed that this inhibition, like that found in heart muscle perfused with ketone bodies (Randle *et al.*, 1966), results from a competition between acetyl-CoA, derived from acetoacetate metabolism, and free CoA for the pyruvate-oxidation system. More recently, such an inhibition of pyruvate dehydrogenase, by acetyl-CoA in competition with CoA, has been demonstrated with a purified enzyme obtained from pig brain (Siess *et al.*, 1971).

A change in the acetyl-CoA/CoA ratio could have accounted for the decrease in pyruvate oxidation observed in the present study, since the concentration of acetyl-CoA was elevated in both starved and streptozotocin-treated rats, although in the latter group, the 6-fold increase in the arteriovenous difference for lactate was not statistically significant. Changes in the concentration of pyruvate dehydrogenase, or in the proportion of active and inactive enzyme, may also have been a factor, since Siess *et al.* (1971) have noted a 27% decrease in the activity of pyruvate dehydrogenase in rat brain after 48h of starvation. Measurements of pyruvate dehydrogenase activity in the brains of diabetic rats have not been reported.

During starvation ketone bodies accounted for 20–25% of the calculated O_2 consumption of brain, and the arteriovenous difference for lactate was increased. Glucose oxidation was diminished by 21% at 24h and by 33% at 48h of starvation. Although this compensated completely for the increment in calculated O_2 consumption caused by ketone-body oxidation, these decreases in calculated glucose oxidation were not statistically significant. In all likelihood this was due to the variability of the measurements needed to calculate values for glucose oxidation, since the increase in the arteriovenous difference for ketone bodies was highly significant and the calculated arteriovenous difference for O_2 was unchanged. The fact that the arteriovenous difference for lactate was also significantly increased lends further support to this view.

The enzymic apparatus for ketone-body utilization in muscle and brain appear to be the same (Tildon & Sevdalian, 1972; Williamson *et al.*, 1971) and in both tissues the magnitude of acetoacetate and 3-hydroxybutyrate uptake is a function primarily of their concentration in plasma (Hawkins *et al.*, 1971; Daniel *et al.*, 1971; Ruderman & Goodman, 1973). On the other hand, in a 48h-starved rat the oxidation of ketone bodies can account for 77% of the O_2 consumption of muscle (Ruderman *et al.*, 1971), whereas it provides only 25% of the fuel needs of brain (Hawkins *et al.*, 1971; the present paper). It is only in the diabetic rat, in which the concentrations of ketone bodies are 2–3-fold greater than those of the starved animal, that acetoacetate and 3-hydroxybutyrate comprise more than 40% of brain fuel. These findings probably reflect the fact that ketone bodies are able to diffuse freely into muscle and are therefore able to saturate 3-oxo acid CoA-transferase when their concentration in blood is relatively low (Ruderman & Goodman, 1973), whereas the entrance of ketone bodies into brain is restricted (Table 6), and, as a result, the concentrations of acetoacetate and 3-hydroxybutyrate in brain, at a given blood concentration, are much lower than in muscle. The high rates of ketone-body utilization reported for human brain (Owen *et al.*, 1967) can be accounted for by the fact that during prolonged starvation man achieves much higher concentrations of ketone bodies in his circulation (Cahill *et al.*, 1966; Owen *et al.*, 1967) than does the rat (Hawkins *et al.*, 1971).

We most gratefully acknowledge the assistance and advice of Dr. Richard Hawkins throughout this study. We also thank Dr. George F. Cahill, Jr., for reviewing the manuscript and Mrs. Z. Skulte and Mrs. L. Klavins for their technical assistance. These studies were supported in part by grants AM-05077, AM-15272 and AM-15191 from the U.S. Public Health Service. M. B. was the recipient of grants from the German Research Association and the German Diabetes Association and N. B. R. was the recipient of a Career Research and Development Award from the U.S. Public Health Service (K04-AM-15729-02).

References

Adam, H. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 573–577, Academic Press, New York and London

Berger, M., Ross, P. S., Goodman, M. N. & Ruderman, N. B. (1973) *Clin. Res.* **21**, 616

Cahill, G. F., Jr., Herrera, M. G., Morgan, A. P., Soeldner, J. S., Steinke, J., Levy, P. L., Reichard, G. A. & Kipnis, D. M. (1966) *J. Clin. Invest.* **45**, 1751–1769

Daniel, P. M., Love, E. R., Moorhouse, S. R., Pratt, O. E. & Wilson, P. (1971) *Lancet* **ii**, 637–638

Goldberg, N. D. & O'Toole, A. G. (1969) *J. Biol. Chem.* **244**, 3053–3061

Good, C. A., Kramer, H. & Somogyi, M. (1933) *J. Biol. Chem.* **100**, 485–491

Gottstein, U., Müller, W., Berghoff, W., Gärtner, H. & Held, K. (1971) *Klin. Wochenschr.* **49**, 406–411

Hawkins, R. A., Williamson, D. H. & Krebs, H. A. (1971) *Biochem. J.* **122**, 13–18

Hers, H. G., DeWulf, H. & Stalmans, W. (1970) *FEBS Lett.* **12**, 73–82
Himwich, H. E. (1951) *Brain Metabolism and Cerebral Disorders*, pp. 1–25, Williams and Wilkins, Baltimore
Hindfelt, B. & Siesjö, B. K. (1970) *Life Sci.* **9**, 1021–1028
Hohorst, H. J. (1963*a*) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 134–142, Academic Press, New York and London
Hohorst, H. J. (1963*b*) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 266–270, Academic Press, New York and London
Huggett, A. St.G. & Nixon, D. A. (1957) *Lancet* **ii**, 368–370
Ide, T., Steinke, J. & Cahill, G. F., Jr. (1969) *Amer. J. Physiol.* **217**, 784–792
Itoh, T. & Quastel, J. H. (1970) *Biochem. J.* **116**, 641–655
Krebs, H. A. & Eggleston, L. V. (1941) *Biochem. J.* **39**, 408–419
Krebs, H. A., Dierks, C. & Gascoyne, T. (1964) *Biochem. J.* **93**, 112–121
Kuby, S. A. & Noltmann, E. A. (1962) *Enzymes*, 2nd edn., **6**, 515
Lamprecht, W. & Stein, P. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 610–616, Academic Press, New York and London
Lamprecht, W. & Trautschold, I. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 543–551, Academic Press, New York and London
Lowry, O. H. & Passoneau, J. V. (1964) *J. Biol. Chem.* **239**, 31–42
Mansour, T. E. (1972) *Curr. Top. Cell Regul.* **6**, 1–46
Mark, J., Godin, Y. & Mander, P. (1968) *J. Neurochem.* **15**, 141–143
Nelson, S. R., Schulz, D. W., Passoneau, J. V. & Lowry, O. H. (1971) *J. Neurochem.* **18**, 1271–1279
Openshaw, H. & Bortz, W. M. (1968) *Diabetes* **17**, 90–95
Owen, O. E., Morgan, A. P., Kemp, H. G., Sullivan, J. M., Herrera, M. G. & Cahill, G. F., Jr. (1967) *J. Clin. Invest.* **46**, 1589–1595
Passoneau, J. V., Brunner, E. A., Molstad, C. & Passoneau, R. (1971) *J. Neurochem.* **18**, 2317–2328
Randle, P. J., Garland, P. B., Hales, C. N., Newsholme, E. A., Denton, R. M. & Pogson, C. I. (1966) *Recent Progr. Horm. Res.* **21**, 1–48
Rolleston, F. S. & Newsholme, E. A. (1967) *Biochem. J.* **104**, 524–533
Ruderman, N. B. & Goodman, M. N. (1973) *Amer. J. Physiol.* **224**, 1391–1397
Ruderman, N. B., Houghton, C. R. S. & Hems, R. (1971) *Biochem. J.* **124**, 639–651
Siess, E., Wittman, J. & Wieland, O. (1971) *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 447–452
Siesjö, B. K., Folbergrova, J. & MacMillan, V. (1972) *J. Neurochem.* **19**, 2483–2495
Slein, M. W. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 117–123, Academic Press, New York and London
Start, C. & Newsholme, E. A. (1968) *Biochem. J.* **107**, 411–415
Tildon, J. T. & Sevdalian, D. A. (1972) *Arch. Biochem. Biophys.* **148**, 382–390
Trivedi, B. & Danforth, W. H. (1966) *J. Biol. Chem.* **241**, 4110–4114
Tschirgi, R. D. (1960) *Handb. Physiol., Neurophysiol. Sect.* 3 1865–1890
Veech, R. L., Harris, R. L., Veloso, D. & Veech, E. H. (1973) *J. Neurochem.* **20**, 183–188
Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **82**, 90–96
Williamson, D. H., Bates, M. W., Page, M. A. & Krebs, H. A. (1971) *Biochem. J.* **121**, 41–47
Williamson, J. R. & Corkey, B. (1969) *Methods Enzymol.* **13**, 434–513