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Ketone-Body Utilization by Adult and Suckling Rat Brain *in vivo*

By R. A. HAWKINS, D. H. WILLIAMSON AND H. A. KREBS
Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford OX2 6HE, U.K.

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1. Ketone-body utilization in fed and starved adult and suckling rats has been investigated by measuring arterio-venous differences across the brain. Venous blood was collected from the confluence of sinuses and arterial blood from the femoral artery in adult rats and by cardiac puncture in suckling rats. 2. During starvation the arterio-venous difference of ketone bodies increased in proportion to their concentrations in the blood and reached a value of 0.16mM at 48h. At a given concentration of the respective ketone bodies the arterio-venous differences of acetoacetate were about twice those of 3-hydroxybutyrate. 3. Fed rats in which the concentrations of ketone bodies were raised by intravenous infusion of sodium acetoacetate had the same arterio-venous differences as starved rats at corresponding ketone-body concentrations. Thus the ability of the rat brain to utilize ketone bodies is independent of the nutritional state. 4. The concentrations of glucose, acetoacetate and 3-hydroxybutyrate were much lower in the brain than in the arterial blood. The measured (blood concentration)/(brain concentration) ratio was 4.4 for glucose, 4.5 for acetoacetate and 8.1 for 3-hydroxybutyrate in 48h-starved rats. 5. The mean arterio-venous difference of glucose across the brain was 0.51mM in fed rats and 0.43mM in 96h-starved rats. 6. Conversion of glucose into lactate rose from negligible values in the fed state to 0.2mM after 48h starvation and decreased to zero after 96h starvation. 7. In 16–22-day-old suckling rats the arterio-venous differences of ketone bodies across the brain were also proportional to the ketone-body concentration, but they were about 3–4 times greater than in adult rats at the same blood ketone-body concentration. 8. Arterio-venous differences of glucose were about the same in adult and suckling rats. 9. The brain of fed suckling rats formed more lactate from glucose than fed adult rats. 10. The results indicate that ketone bodies are major metabolic fuels of the brain of the suckling rat under normal conditions.

Ketone bodies are known to be oxidized by rat and guinea-pig brain slices (Drahota, Hahn, Mourek & Trojanová, 1965; Rolleston & Newsholme 1967; Ide, Steinke & Cahill, 1969; Itoh & Quastel, 1970). Measurements of the arterio-venous differences in obese human patients undergoing prolonged starvation (Owen *et al.* 1967) indicate that the human brain can also utilize ketone bodies *in vivo*. However, the work of Owen *et al.* (1967) leaves it open whether the utilization of ketone bodies by brain is an adaptation of this tissue to prolonged fasting or whether it is a consequence of the high concentrations of ketone bodies in the blood. In rats the necessary enzymes for ketone-body utilization have been shown to be present in the brain irrespective of the nutritional state (Williamson, Bates, Page & Krebs, 1971).

To determine the importance of blood concentration as a factor in the regulation of ketone-body utilization, acetoacetate was infused into the femoral vein of fed rats and arterio-venous differences across the brain were measured.

MATERIALS AND METHODS

Rats. Female rats of the Wistar strain, weighing about 200g and suckling rats, 16–22 days old were used. All rats were allowed free access to food unless otherwise indicated.

Reagents and analytical methods. The reagents and analytical methods were the same as those described by Page, Krebs & Williamson (1971) except where indicated.

Sodium acetoacetate for infusion was prepared from ethyl acetoacetate as described by Krebs & Eggleston (1941). The final solution was concentrated to 4.5M under vacuum. It contained no ethanol when analysed by the method of Dickinson & Dalziel (1967). Oxygen in blood was determined by the method of Van Slyke & Neill (1924).

Collection of blood samples. Adult rats were anaesthetized by intraperitoneal injection of a freshly prepared aqueous solution of Nembutal (50 mg/kg body wt.). A catheter (Portex Nylon Intravenous Cannula, size 12FG 3, external diameter 0.75 mm with a bevelled end; Portex Ltd., Hythe, Kent, U.K.) was placed in the femoral artery after surgical exposure and through it a heparin solution (1000 units per kg body wt.) was injected. Then a small hole was drilled through the skull over the confluence of the sagittal and transverse sinuses. The dura was penetrated with a needle and 0.2 ml of blood was collected. The sinuses drain primarily the cerebral hemispheres and measurements of sinus blood therefore reflect metabolism of this area. Arterial blood (0.2 ml) was sampled from the femoral artery immediately before and after the sinus sample.

Arterial blood (0.1 ml) from suckling rats was taken from the left ventricle of the heart. Sinus blood (0.1 ml) was obtained within 30 s by insertion of a 25 gauge needle into the confluence of the sinuses. Blood samples were treated with 3 ml of 2% (w/v) $HClO_4$. A neutral deproteinized solution was obtained by the standard method.

Infusion of acetoacetate. Adult rats were infused through a catheter introduced into the femoral vein and positioned into the inferior vena cava. Acetoacetate (4.5 M) was infused with a motor-driven syringe at a rate of 15 μmol/min (0.2 ml/h) for 35 min to attain concentrations of ketone bodies approximating to those of starvation. Higher concentrations were attained by infusing acetoacetate at a rate of 65 μmol/min (0.86 ml/h) for 15 min and the concentration was maintained by infusion at a rate of 30 μmol/min (0.4 ml/h) for an additional 20 min. The rate of infusions maintained a steady concentration of ketone bodies and blood samples were taken at 35 min.

Brain metabolites. Anaesthetized rats were frozen in liquid N_2 and the brains removed as described by Mark, Godin & Mander (1968). Further treatment was as described by Williamson, Lund & Krebs (1967) for liver. Glycogen was measured by the method of Lowry, Passonneau, Hasselberger & Schulz (1964).

RESULTS

Arterio-venous differences of ketone bodies and glucose in the brain of adult rats. The mean arterio-venous difference of glucose across the brain of fed animals was found to be 0.51 mM whereas the differences for lactate and pyruvate were negligible (Table 2). Although the concentration of acetoacetate was low in the fed state (Table 1) small but significant arterio-venous differences (mean 0.022 mM) existed (Table 2). The oxygen required for the complete oxidation of glucose and ketone bodies removed (taking into account the lactate and pyruvate produced) during passage through the brain was 3.08 mM, a value in approximate agreement with the measured arterio-venous difference of 2.84 mM (Table 2). In rats starved for 48 h the arterio-venous difference of ketone bodies rose about sevenfold to 0.16 mM. The arterio-venous difference of ketone bodies did not increase when the starvation period was extended to 96 h. After 96 h

Table 1. *Concentration of metabolites in arterial and venous blood across the brain of the adult rat*

The results are mean values (± S.E.M.) with the number of observations in each group in parentheses. For other experimental details see the Materials and Methods section.

State of rats	Blood sample	Lactate (mM)	Pyruvate (mM)	[Lactate]/[Pyruvate]	3-Hydroxybutyrate (mM)	Acetoacetate (mM))	[3-Hydroxybutyrate]/[Acetoacetate]	Sum of ketone bodies (mM)	Glucose (mM)
Fed (9)	Arterial	1.10 ±0.18	0.141 ±0.014	7.8	0.094 ±0.01	0.134 ±0.009	0.70	0.228	6.34 ±0.15
	Venous	1.13 ±0.19	0.138 ±0.014	8.2	0.090 ±0.01	0.112 ±0.100	0.80	0.202	5.82 ±0.15
Fasted, 48 h (10)	Arterial	0.539 ±0.045	0.059 ±0.002	9.1	2.00 ±0.15	0.811 ±0.047	2.47	2.81	4.76 ±0.28
	Venous	0.738 ±0.069	0.080 ±0.004	9.2	1.92 ±0.14	0.732 ±0.054	2.62	2.65	4.27 ±0.29
Fasted, 72 h (4)	Arterial	0.360 ±0.037	0.057 ±0.006	6.3	2.12 ±0.21	0.914 ±0.078	2.32	3.03	4.36 ±0.22
	Venous	0.500 ±0.071	0.066 ±0.004	7.6	2.07 ±0.22	0.834 ±0.068	2.49	2.90	3.90 ±0.21
Fasted, 96 h (7)	Arterial	0.566 ±0.023	0.043 ±0.004	13.2	2.62 ±0.34	0.726 ±0.094	3.61	3.35	4.28 ±0.34
	Venous	0.598 ±0.029	0.069 ±0.004	7.8	2.52 ±0.36	0.668 ±0.092	3.77	3.19	3.86 ±0.29
Fed, acetoacetate infused at low rate (10)	Arterial	1.03 ±0.19	0.146 ±0.022	7.1	0.898 ±0.093	1.54 ±0.18	0.58	2.44	5.34 ±0.12
	Venous	1.08 ±0.18	0.151 ±0.017	7.2	0.869 ±0.089	1.43 ±0.17	0.61	2.30	4.75 ±0.12
Fed, acetoacetate infused at high rate (9)	Arterial	1.61 ±0.18	0.162 ±0.020	9.9	1.92 ±0.11	5.36 ±0.36	0.36	7.28	3.97 ±0.13
	Venous	1.56 ±0.19	0.195 ±0.024	8.0	1.83 ±0.13	5.17 ±0.38	0.35	7.00	3.50 ±0.12

Table 2. *Arterio-venous differences of metabolites across the brain of adult rats*

The values are means (±S.E.M.) of the arterio-venous differences. The symbols + and − indicate appearance or removal of a metabolite and the symbols * and ** indicate the statistical significance of the arterio-venous differences at the 5 and 1% levels respectively. All results are derived from the experiments contained in Table 1 except for O_2 measurements which were done on four to six different rats treated as indicated. The amount of O_2 necessary for glucose oxidation was calculated by using the formula $\left(\text{glucose} - \frac{\text{lactate} + \text{pyruvate}}{2}\right)6$, and for ketone bodies by using [(3-hydroxybutyrate)4.5 + (acetoacetate)4]. For other experimental details see the Materials and Methods section.

State of rats	Lactate (mM)	Pyruvate (mM)	3-Hydroxybutyrate (mM)	Acetoacetate (mM)	Glucose (mM)	Calculated O_2: Glucose (mM)	Calculated O_2: Ketone bodies (mM)	Calculated O_2: Total (mM)	Measured O_2 (mM)	Calculated O_2 due to ketone bodies (%)
Fed	+0.04±0.03	+0.003±0.005	−0.004±0.007	−0.022±0.007**	−0.51±0.05**	2.98±0.33	0.10±0.04	3.08±0.34	2.84±0.10	3
Starved, 48h	+0.20±0.06**	+0.022±0.003**	−0.075±0.021**	−0.079±0.013**	−0.49±0.07**	2.29±0.47	0.65±0.12	2.94±0.50	3.43±0.14	22
Starved, 72h	+0.14±0.05*	+0.009±0.004	−0.048±0.028	−0.079±0.010**	−0.46±0.03**	2.31±0.16	0.53±0.09	2.84±0.23	—	19
Starved, 96h	+0.03±0.01*	+0.025±0.002**	−0.100±0.028**	−0.057±0.003**	−0.43±0.08**	2.41±0.48	0.68±0.12	3.08±0.58	—	22
Fed, acetoacetate infused at low rate	+0.05±0.03	+0.005±0.005	−0.029±0.008**	−0.106±0.022**	−0.58±0.06**	3.33±0.41	0.56±0.12	3.89±0.47	—	14
Fed, acetoacetate infused at high rate	−0.03±0.07	+0.033±0.009**	−0.087±0.054	−0.193±0.070*	−0.47±0.05**	2.80±0.29	1.16±0.23	3.96±0.30	3.13±0.12	29

starvation the arterio-venous difference of glucose decreased about 16%. In contrast with the brains of fed rats, those of animals starved for 48h, converted about 20% of the glucose taken up into lactate. On prolonged starvation (96h) the lactate production returned to almost zero. The calculated arterio-venous difference of oxygen remained constant throughout starvation indicating that ketone bodies partially replaced glucose as a fuel.

When ketone-body concentrations were raised to starvation values by infusion of acetoacetate into fed rats the arterio-venous differences were comparable with those of starved rats (Tables 1 and 2). Rats infused with acetoacetate to attain abnormally high arterial concentrations, showed larger arterio-venous differences of ketone bodies. Acetoacetate and 3-hydroxybutyrate were removed from blood approximately in proportion to their concentrations in the physiological range by both starved and acetoacetate-infused fed rats (Fig. 1 and 2). However, at a given concentration the acetoacetate arterio-venous concentration difference was twice that of 3-hydroxybutyrate. This caused the 3-hydroxybutyrate/acetoacetate concentration ratio to increase after passage of the blood through the brain (Table 1).

Fed rats, infused with acetoacetate at a high rate did not produce lactate and the calculated arterio-venous concentration difference of oxygen (3.96mM) was not significantly different ($P<0.49$) from the measured value (3.13mM) obtained on different rats.

Concentration gradients of metabolites between brain and arterial blood. The concentrations of glucose, acetoacetate and 3-hydroxybutyrate were strikingly lower in the brain than in blood. The measured (concentration in blood)/(concentration

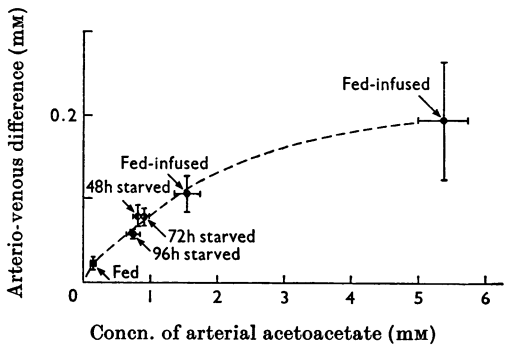

Fig. 1. Concentration dependence of acetoacetate uptake by brain of adult rats. The arterio-venous differences and arterial concentrations are mean values (±S.E.M.) expressed as μmol/ml. For other experimental details see the text.

in brain) ratio was 4.4 for glucose, 4.5 for acetoacetate and 8.1 for 3-hydroxybutyrate in 48h-starved rats (Table 3). Since brain-tissue concentrations were not corrected for blood content (about 3%; Mark *et al.* 1968; Hindfelt & Siesjö, 1970) or for the content of cerebrospinal fluid (about 12%; Hindfelt & Siesjö, 1970), the actual concentration gradients must be much greater. Similar concentrations of glucose have been found in brain (Gey, 1956; Lowry *et al.* 1964; Mark *et al.* 1968). Large concentration gradients for ketone bodies have also been observed in muscle (Harrison & Long, 1940). Thus penetration of ketone bodies into brain may be a factor limiting the rate of their metabolism.

Arterio-venous differences of ketone bodies and glucose in the brain of suckling rats. The results with suckling rats (Tables 4 and 5) show that ketone bodies account for a high proportion of the calculated oxygen consumption of the brain. Although there was considerable variation between litters a linear relationship between arterial concentrations of acetoacetate and arterio-venous concentration differences and an approximately linear relationship for 3-hydroxybutyrate existed (Figs. 3 and 4). As in adult rats the rate of acetoacetate uptake was twice as great as that of 3-hydroxybutyrate at a given concentration. However, 3-hydroxybutyrate contributed more to total ketone-body uptake due to its higher circulating concentrations (Tables 4 and 5). At a given concentration the arterio-venous differences of both acetoacetate and 3-hydroxybutyrate were about 3–4 times those observed in adult rats. Also as was observed in adult rats, starvation did not enhance ketone-body uptake (Figs. 3 and 4). Glucose uptake by brain of suckling rats was comparable to, or less than, adult values but in contrast with adult rats a considerable portion was converted into lactate (Table 5).

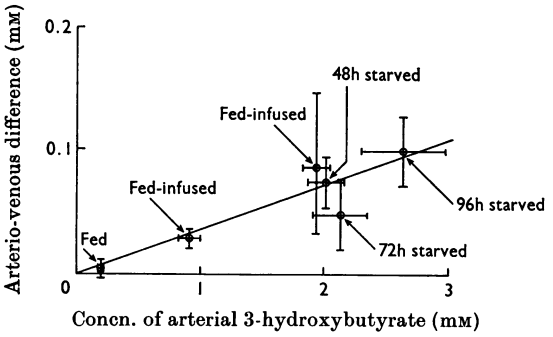

Fig. 2. Concentration dependence of 3-hydroxybutyrate uptake by brain of adult rats. The arterio-venous differences and arterial concentrations are mean values (±S.E.M.) expressed as μmol/ml. For other experimental details see the text.

DISCUSSION

The present experiments indicate that ketone-body utilization by brain becomes important whenever these substances are present at a sufficiently high circulating concentration. A critical experiment is the demonstration that the arterio-venous difference of ketone bodies across the brain is independent of the nutritional state. Thus the arterio-venous differences are the same whether circulating ketone-body concentrations are raised by starvation or by infusion of acetoacetate into fed rats. This is in agreement with the finding that the enzymes of ketone-body utilization are not altered by the nutritional state (Williamson *et al.* 1971). Whether this is also true for the human brain (see Owen *et al.* 1967) remains to be tested, but it seems unnecessary to postulate an adaptive increase in the enzymes necessary for ketone-body

Table 3. *Metabolite concentrations in brain and arterial blood*

The values are means (±S.E.M.) of four observations and are expressed in μmol/g of brain or μmol/ml of arterial blood. Fed–infused rats were infused with acetoacetate at the high rate as described in the Materials and Methods section. The values for brain were not corrected for contamination by blood or cerebrospinal fluid.

	Fed rats	Fed–infused rats			48h-starved rats		
Metabolite	Brain	Brain	Blood	[Blood]/[Brain]	Brain	Blood	[Blood]/[Brain]
Glucose	1.21 ±0.16	1.00 ±0.11	4.43 ±0.27	4.4	1.01 ±0.04	4.14 ±0.19	4.3
Glycogen	4.96 ±0.22	5.11 ±0.35	—	—	4.97 ±0.07	—	—
Lactate	1.89 ±0.34	2.02 ±0.15	0.304±0.022	0.15	1.48 ±0.23	0.415±0.017	0.28
Pyruvate	0.077±0.008	0.047±0.002	—	—	0.046±0.005	—	—
3-Hydroxybutyrate	0.094±0.046	0.117±0.042	1.55 ±0.31	13.1	0.200±0.010	1.62 ±0.17	8.1
Acetoacetate	0.041±0.005	0.409±0.062	7.60 ±1.26	18.6	0.181±0.028	0.808±0.068	4.5

Table 4. *Arterial and venous metabolites across the brain of suckling rats*

The results are mean values (±S.E.M.) with the number of observations given in parentheses. For other experimental details see the Materials and Methods section.

State and age of animal	Blood sample	Lactate (mM)	Pyruvate (mM)	[Lactate]/[Pyruvate]	3-Hydroxybutyrate (mM)	Acetoacetate (mM)	[3-Hydroxybutyrate]/[Acetoacetate]	Sum of ketone bodies (mM)	Glucose (mM)
Fed, 16 days old (6)	Arterial	1.07±0.10	0.115±0.009	9.3	1.25 ±0.19	0.296±0.030	4.22	1.55	6.97±0.16
	Sinus	1.61±0.20	0.220±0.017	7.3	1.05 ±0.11	0.180±0.028	5.83	1.23	6.40±0.15
Fed, 18 days old (10)	Arterial	1.23±0.09	0.164±0.008	7.5	1.69 ±0.18	0.508±0.049	3.32	2.20	5.62±0.15
	Sinus	1.48±0.13	0.203±0.010	7.3	1.38 ±0.14	0.369±0.046	3.77	1.75	5.37±0.13
Fed, 20 days old (5)	Arterial	1.34±0.13	0.104±0.010	12.9	0.906±0.225	0.224±0.052	4.03	1.13	6.97±0.22
	Sinus	1.56±0.15	0.148±0.010	10.5	0.740±0.184	0.162±0.035	4.57	0.90	6.35±0.19
Starved, 24h, 17 days old (6)	Arterial	1.02±0.18	0.094±0.008	10.8	2.30 ±0.23	0.528±0.061	4.36	2.83	5.02±0.10
	Sinus	1.18±0.13	0.140±0.010	8.4	2.11 ±0.20	0.396±0.064	5.33	2.51	4.80±0.13
Starved, 16h, 22 days old (11)	Arterial	1.12±0.17	0.124±0.010	9.0	1.05 ±0.10	0.361±0.046	2.91	1.41	4.84±0.11
	Sinus	1.39±0.27	0.159±0.014	8.7	0.91 ±0.08	0.274±0.042	3.32	1.18	4.39±0.17
Starved, 40h, 22 days old (6)	Arterial	1.15±0.08	0.128±0.019	9.0	2.58 ±0.21	0.665±0.056	3.88	3.24	4.59±0.23
	Sinus	1.19±0.01	0.209±0.016	5.7	2.20 ±0.14	0.491±0.045	4.48	2.69	4.12±0.24

Table 5. *Arterio-venous differences across the brain of suckling rats*

The values are means (±S.E.M.) of the arterio-venous differences and are derived from the experiments contained in Table 3. The symbols + and − indicate appearance or removal of a metabolite and the symbols * and ** indicate the statistical significance of the arterio-venous difference at the 5 and 1% levels respectively. The amount of O_2 necessary to oxidize glucose and ketone bodies was calculated as indicated in Table 2. For other experimental details see the Materials and Methods section.

State and age of rats	Lactate (mM)	Pyruvate (mM)	3-Hydroxybutyrate (mM)	Acetoacetate (mM)	Glucose (mM)	Calculated O_2: Glucose (mM)	Calculated O_2: Ketone bodies (mM)	Calculated O_2: Total (mM)	Calculated O_2 due to ketone bodies (%)
Fed, 16 days old	+0.54±0.17*	+0.105±0.018**	−0.203±0.126	−0.117±0.024**	−0.58±0.12**	1.51±0.77	1.38±0.55	2.89±0.57	48
Fed, 18 days old	+0.26±0.08**	+0.040±0.017*	−0.305±0.068**	−0.139±0.010**	−0.25±0.06**	0.61±0.57	1.93±0.33	2.54±0.57	76
Fed, 20 days old	+0.22±0.06*	+0.043±0.017*	−0.165±0.050*	−0.062±0.020*	−0.63±0.09**	2.96±0.71	0.99±0.31	3.95±0.59	25
Starved, 24h, 17 days old	+0.16±0.09	+0.045±0.005**	−0.195±0.049**	−0.132±0.028**	−0.22±0.10*	0.71±0.64	1.41±0.21	2.12±0.84	67
Starved, 16h, 22 days old	+0.27±0.11*	+0.034±0.011**	−0.139±0.016**	−0.086±0.014**	−0.44±0.08**	1.73±0.31	0.97±0.11	2.70±0.39	36
Starved, 40h	+0.05±0.07	+0.071±0.017**	−0.380±0.105**	−0.174±0.029**	−0.47±0.14*	2.51±0.74	2.40±0.53	4.92±1.09	49

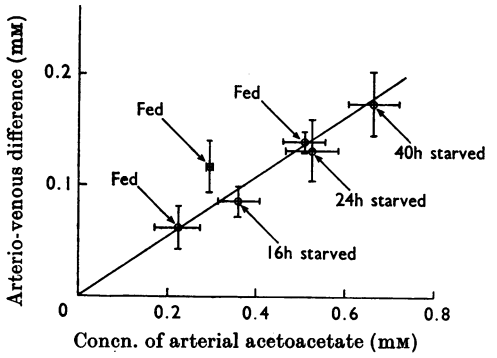

Fig. 3. Concentration dependence of acetoacetate uptake by brain of suckling rats. The arterio-venous differences and arterial concentrations are mean values (±S.E.M.) expressed as μmol/ml. For other experimental details see the text.

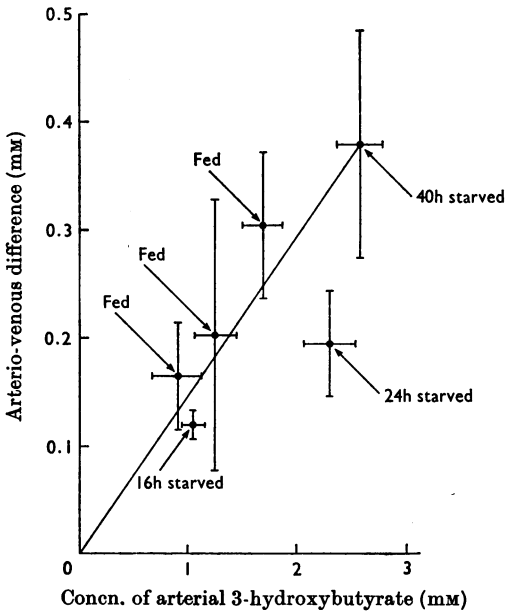

Fig. 4. Concentration dependence of 3-hydroxybutyrate uptake by brain of suckling rats. The arterio-venous differences and arterial concentrations are mean values (±S.E.M.) expressed as μmol/ml. For other experimental details see the text.

utilization to explain the observations on human brain in prolonged starvation.

In the brain of suckling rats ketone bodies appear to be at least as important as glucose as a source of metabolic fuel. The larger arterio-venous differences observed across the brain of suckling rat is paralleled by the greater enzymic capacity for ketone-body utilization observed at this age (Page *et al.* 1971) and are in agreement with a higher rate of ketone-body oxidation shown for brain slices by Itoh & Quastel (1970).

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