β-Hydroxybutyrate transport in rat brain: developmental and dietary modulations

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MOORE, THOMAS J., ARMAND P. LIONE, MARY C. SUGDEN, AND DAVID M. REGEN. β-Hydroxybutyrate transport in rat brain: developmental and dietary modulations. Am. J. Physiol. 230(3): 619-630. 1976. Transport of β-hydroxybutyrate (βHB) into rat brain was estimated from the early rise in brain/serum 14C ratio after subcutaneous injection of [14C]βHB. Permeability of the D isomer exceeded that of the L isomer. Permeability of either isomer rose throughout suckling (sevenfold) and declined after weaning to the low, newborn values. This age dependence differed markedly from those of cerebral blood flow and cerebral permeabilities of urea, glucose, valine, leucine, and DMO (5,5-dimethyloxazolidine-2,4-dione). Fat-feeding more than doubled cerebral βHB permeability without significantly affecting cerebral blood flow or the permeabilities of urea, glucose, and DMO. Temperature dependence of βHB permeability was similar to that of glucose transport. The age and diet dependence of βHB permeability were not accounted for in terms of body temperature, capillary surface, capillary porosity, or plasma proton concentration. A modulable βHB carrier seemed indicated. Utilization of βHB by the brain was significantly governed by permeability, hence the increased permeability in ketotic states should contribute to glucose sparing and eventually to protein sparing.

THE BRAIN OF THE INTACT RAT OBTAINS SUBSTANTIAL PORTIONS OF ITS ENERGY FROM OXIDATION OF βHB AND ACETOACETATE UNDER ANY CIRCUMSTANCES WHERE THESE FUELS ARE SUFFICIENTLY ABUNDANT IN THE BLOOD. THESE CIRCUMSTANCES INCLUDE SUCKLING (8, 12), STARVATION (8, 12, 23), DIABETES (23), AND KETONE BODY INFUSION (8, 12, 23). SIMILAR OBSERVATIONS HAVE BEEN MADE WITH HUMANS (5, 11, 18, 21) AND OTHER ANIMALS (26). THE ENZYMES OF KETONE BODY CATABOLISM INCREASE GRADUALLY DURING SUCKLING THEN DECLINE GRADUALLY AFTER WEANING (10, 12, 14, 19). THIS PICTURE SUGGESTS SUBSTRATE INDUCTION (14, 25); HOWEVER, THESE ENZYMES HAVE BEEN REPORTED NOT TO RISE SIGNIFICANTLY DURING ADULT KETOTIC STATES (12, 14, 25, 27). IT HAS BEEN PROPOSED THAT TRANSPORT OF βHB FROM CAPILLARY TO BRAIN PARENCHYMA ACROSS THE BLOOD-BRAIN BARRIER IS THE PRINCIPAL RATE-LIMITING STEP IN βHB UTILIZATION (2, 4, 8, 23, 25). YET LITTLE EFFORT HAS BEEN DEVOTED TO βHB TRANSPORT IN BRAIN. WE HAVE INVESTIGATED THIS PROCESS BY METHODS THAT PROVED SUCCESSFUL IN CHARACTERIZING CEREBRAL GLUCOSE TRANSPORT IN RATS AND MICE (3, 6, 15, 16).

METHODS

Sprague Dawley rats (pregnant females and males of various ages) were obtained from Harlan Industries and fed Purina laboratory chow. Procedures were generally as described earlier (6). Pups at various ages and older animals were injected in the subcutaneous space of the back with small volumes (10-40 μl, depending on animal size) of D-[3-14C]β-hydroxybutyrate (30 μCi/ml, 32 Ci/mmol; New England Nuclear) or L-[3-14C]β-hydroxybutyrate (30 μCi/ml, 4 Ci/mmol; prepared as described below). These were usually accompanied by a tritiated compound at 5 times higher radioactivity: n-[5-3H]glucose (1 Ci/mmol, Amersham/Searle); L-[4,5-3H]leucine (5 Ci/mmol, New England Nuclear); L-2,3-3Hvaline (31 Ci/mmol, Amersham/Searle); or L-[3-O-methyl-3H]glucose (4 Ci/mmol, New England Nuclear). At various times after injection, the head was immersed in Freon-12 at −150°C for 10-15 s and then cut off, dropping into liquid N2. (Heads of furry animals were shaven at least 1 h prior to the experiment). Blood was sampled from the neck stump. Perchloric acid extracts of serum and cerebral cortex were prepared and neutralized as described earlier (6). These were assayed for 14C and/or 3H by liquid scintillation spectrometry and for D-βHB enzymically (28) in an Aminco fluorimeter.

In another series of experiments, animals received a subcutaneous dose of [14C]urea (6 Ci/mmol, New England Nuclear) or DMO ([2,4-dione-2-14C]5,5-dimethyloxazolidine 9 Ci/mmol, New England Nuclear) and then at various times later received an intraperitoneal dose of 3H2O (New England Nuclear) and were sacrificed 40 s after the latter injection by decapitation (the head falling into liquid N2). The body was immediately turned erect and the skin about the neck pulled up to form a cup into which blood pooled. A heparinized, glass 1-ml syringe with an 18-gauge needle was used to collect 0.2-0.5 ml blood from the bottom of this pool as it flowed in from below. This sample—a mixture of arterial and venous blood essentially unexposed to air—was used for the pH determination. After removal of this sample, another portion of blood was collected for analyses of 3H and 14C as described above. In this series, decapitation preceded head freezing because a) no metabolizable substances were measured, hence there was no need to stop brain metabolism quickly, b) sharp time discrimination was needed for the 40-s 3H2O data, and c) a period of asphyxia prior to blood sampling would alter blood pH.
The L-[14C]βHB was prepared from DL-[3-14C]βHB (7.1 mg, 0.25 μCi; New England Nuclear, NEC-326). The crystalline solid was dissolved in 1 ml H2O. On separate occasions 200 μl of this were added at room temperature to a reaction mixture based on the βHB assay described by Williamson and Mellanby (28). The reaction mixture contained 0.8 ml of fresh 0.85 M hydrazine Cl buffer, pH 8.5; 0.1 ml of 0.1 M Tris-Cl buffer, pH 8.5; 0.1 ml of 0.5 M NAD+ (Sigma grade 3). The reaction was started by addition of 0.3 mg β-hydroxybutyrate dehydrogenase (Boehringer-Mannheim). At 15-min intervals, 10-μl samples were transferred to 3 ml water in an optical cuvette, and absorbancy was read at 340 mμ in a Gilford spectrophotometer. When the reaction was complete (about 1 h), the mixture was acidified by addition of 500 μl of 1 M HCl and passed through a 0.9 cm x 10 cm Dowex 50W-X8 column equilibrated and eluted with 0.01 M HCl. The column usually failed to delay all the acetooacetate (hydrazone) as hoped, but it did separate the radioactive product and the unreacted L-βHB from other nonvolatile substances. The fractions containing L-[14C]βHB were kept at room temperature in an exhaust hood for 1 wk, during which [14C]acetooacetate decomposed to its volatile products, leaving almost exactly half of the eluted radioactivity. (When the procedure was carried out on D-βHB, virtually all the radioactivity disappeared during this period indicating the effectiveness of the enzymic and spontaneous reactions.) The product was lyophilized to remove HCl and then dissolved in water to give a stock solution of 30 μCi/ml.

Expression of results. Most of our conclusions are drawn from brain:serum ratios of injected or endogenous substances. This is the amount measured in a gram of brain divided by the amount measured in a milliliter of serum from the central circulation. In this work H2O is the only substance that equilibrates across the blood-brain barrier quickly relative to its exchange between central circulation and cerebral capillary. For all other substances, exchange between capillary and brain is slow relative to that between central circulation and capillary. For those substances, a brain:serum ratio of 0.02 ml/g is assumed to be achieved instantly (this is the serum or mannitol space of brain), and the brain:serum ratio above 0.02 ml/g is considered to be the parenchyma:serum ratio, representing permeation across the blood-brain barrier. Hence the zero-time brain:serum ratios of Figs. 1 and 2 are taken to be 0.02 ml/g, and the mannitol space (0.020–0.025 ml/g) is shown in other figures to permit subtraction of this “background” by eye. The symbols Ws,Wo in Figs. 1 and 5 and Ws,Wo in Fig. 2 stand for this 0.02 ml/g serum space.

RESULTS

Time course of L-[14C]βHB distribution in serum and brain. Figure 1 shows the time course of serum [14C] concentration and the brain:serum [14C] ratio in 2-day-old, 15-day-old, and adult injected with L-[14C]βHB (the abnormal isomer). At all ages, the serum [14C] rose to a maximum within 10–15 min, indicating a rather consistent absorption from the subcutaneous injection site. The subsequent decline was very slow in sucklings (also in 4-day-old and 8-day-old rats) and rather slow in adults (cf. Fig. 2), indicating slow metabolism and/or excretion. Based on the area of the serum curve, clearance by metabolism and excretion (6) was roughly 8 ml/min·kg in 2-day-old animals, 5 ml/min·kg in 15-day-old animals and 12 ml/min·kg in 300-g animals. The clearance of glucose in newborns (16) was about 19 ml/min·kg, and clearances of D-βHB label (next section) were also about twice those of L-βHB. Since the serum time courses at the three ages were similar in shape, we may estimate the age dependency of brain permeability simply by inspection of the early brain:serum ratios. Those clearly rose about 3 times faster in 15-day-old rats than in 2-day-old or 380-g rats (the latter two being similar), indicating approximately a 3 times faster influx coefficient. The lines drawn through the brain:serum ratios are based on the assumptions that the radioactivity in serum was one compound (L-βHB) and that the brain parenchyma (all extravascular spaces) behaved as a single compartment with respect to L-βHB distribution. The slow L-βHB clearance and poor L-βHB metabolism by brain mitochondria (10) and muscle (7) provide some basis for these assumptions. The data show no systematic deviations from the lines, hence, no evidence for membranes beyond the blood-brain barrier (capillary membrane) impeding L-βHB distribution in the brain parenchyma and no evidence for slow-turnover metabolite pools in the brain. Thus an influx coefficient (k in) and fractional efflux coefficient (k out/Wi) attributable to the blood-brain barrier suffice to describe the data. These reflect the threefold higher permeability in 15-day brains (as compared to 2-day-old and adult) suggested by inspection of the data.

The ratio of influx coefficient to fractional efflux coefficient, Ws,Wo/Wo, was about 0.30 ml/g in 2-day-old rats, 1

Sera from several [14C]βHB-injected adult rats were subjected to anion-exchange chromatography—0.9 cm x 10 cm Dowex 1 formate, eluted with an upward-curving gradient of 0–1.5 M formic acid (150 ml), followed by a linear gradient of 1.5 M formic acid to 6 M ammonium formate, pH 2.7 (120 ml). The procedure (which separates βHB from its usual metabolites including acetooacetate) failed to detect [14C]acetooacetate presumably because of long storage of the sera prior to chromatography. Ten minutes after L-[14C]βHB injection, 90% of eluted radioactivity was in the βHB peak (10% being a polyatomic contaminant of the preparation). At 30 min 82% was in the βHB peak (8% being the contaminant and 10% being a non-anionic metabolite). Four times after D-[14C]βHB injection, 90% was eluted with βHB and 10% was non-anionic metabolites. At 30 min only 28% was in the βHB peak (72% being metabolites). By 90 min after L-[14C]βHB injection, the serum radioactivity was 60% βHB in chow-fed animals but 82% βHB in fat-fed animals. This confirms the poor metabolic reactivity of L-βHB as compared to D-βHB. The polyatomic contaminant probably does not enter the brain and causes about 10% underestimation of L-βHB influx and equilibrium brain:serum ratio. The non-anionic metabolites likely have an opposite effect on the apparent L-βHB equilibrium, on the assumption that glucose is a significant component (6). Thus the equilibrium ratios, though tentative, may not be seriously in error. We present and discuss these ratios because they undergo interesting modulations which are directly related to the much better defined influx coefficients, and because it is doubtful that more accurate data will be forthcoming in the foreseeable future. Fat-feeding not only slowed by 70% the fall in L-[14C]βHB (total [14C] but also slowed by 50% the decline in total serum [14C], so that [14C] clearance was only 6 ml/min·kg in fat-fed adults (cf. Fig. 1C). The errors due to metabolites are probably least in this group.
0.58 ml/g in 15-day-old rats, and 0.23 ml/g in 380-g rats. These are the apparent equilibrium values of $S_i / [S_o]$ (parenchyma:serum L-βHB ratio). If the $d$ isomer enters by the same mechanism, its equilibrium ratios (equilibrium $G_i / [G_o] = W_{d}k_{d\rightarrow s} / k_{s\rightarrow d}$) should have similar values. The fact that equilibrium L-βHB ratios are no higher than those of other nonmetabolized substances (3, 6, 15, 16) especially DMO (Fig. 8) suggests that L-βHB is not significantly converted to metabolites in the brain and supports the view that this unnatural isomer is poorly metabolized, as contrasted to $d$-βHB in 15-day-old rats (Fig. 2), in which the apparent influx to eflux ratio was about 1.6 ml/g.

**Time course of $d$-[14C]βHB distribution in serum and brain.** In animals injected with $d$-[14C]βHB (Fig. 2), the serum radioactivity rose quickly as with the $l$ isomer, then fell more rapidly giving clearances about twice those of L-βHB. Though these clearances are only incidental in the context of this work, it should be mentioned that the modest difference between Fig. 1 and Fig. 2 clearances is compatible with the view that L-βHB is very poorly metabolized relative to $d$-βHB because a) the fraction of clearance due to excretion is independent of metabolic reactivity; b) the natural isomer, $d$-[14C]βHB, quickly gives rise to labeled products, particularly acetoacetate (1), which contribute to the curve area and, therefore, diminish 14C clearance; c) D-βHB metabolism is not limited by the first reaction (dehydrogenase), hence its unidirectional dehydrogenation flux is many times faster than its clearance (1). Thus $β$-hydroxybutyrate dehydrogenase activity would have to be reduced to a tiny fraction of normal to make 14C clearance from $d$-[14C]βHB resemble that from L-[14C]βHB.

From the early rise in brain:serum ratios, it is quite clear that 14C from $d$-βHB gains access to the brain several times more readily in 15-day-old than in 2-day-old animals. This correspondence with L-βHB permeability (which holds for other conditions presented below) argues that the natural ketone bodies enter by the same mechanisms as does the L-βHB. At each age, the brain:serum 14C ratios increased faster when $d$-[14C]βHB was injected (Fig. 2) than when L-[14C]βHB was injected (Fig. 1). This suggests carrier mediated transport with preference for the natural isomer. Alternatively, the two βHB isomers may be equally permeable, while acetoacetate is several times more permeable and accounted for the majority of 14C entry when $d$-[14C]βHB was injected. In the latter case, however, we
would expect to see a delayed rise in the brain:serum $^{14}$C ratio corresponding to the time for conversion of label from $\beta$-HB to the (presumably) more permeable acetoacetate. Such a delay was not obvious. Possibly the conversion occurred during absorption. However, Daniel et al. (4) reported no large difference between $\beta$HB and acetoacetate with respect to brain permeability. It seems that whatever the chemical form of the influxing label, it is subject to the influences responsible for the age dependence of $L$-$\beta$ HB entry (see also Fig. 3, A and

![Graph A](image-url)

**FIG. 2.** Time dependence of $^{14}$C distribution in serum (○) and cerebrum (●) of 2-day-old and 15-day-old rats after injection of $D$-$[^{14}$C]$\beta$HB. Symbol $G_0$ is injected dose, $[G_0^*]$ is serum $^{14}$C concentration, and $[G_0^*]$ is cerebrum $^{14}$C content. Theoretical treatment was as in Fig. 1, but significance of influx and fractional efflux coefficients ($k_{in}$ and $k_{e}/W_0$) is unclear because of mixture of chemical species labeled.

![Graph B](image-url)

**FIG. 3.** Age dependence of (A) $L$-$\beta$HB permeability and (B) $D$-$\beta$HB permeability. Time between injection and sampling was demonstrated to be ideal for permeability estimation in that brain:serum ratios were essentially independent of variations in serum time course or efflux coefficients and dependent almost exclusively on influx coefficients. Various symbols represent various series of experiments. In B, open circles are controls for closed circles, latter being obtained in animals injected intraperitoneally with unlabeled $L$-$\beta$HB (2 mmol/kg body wt) 2 min prior to $D$-$[^{14}$C]$\beta$HB injection. Entry was mediated by a carrier which was significantly saturated by concentrations achieved, we would expect competitive reduction in $^{14}$C entry. This was not seen.
The high brain:serum ratios seen late in the time course in 15-day-old rats were presumably due to the accumulation of labeled intermediates such as those of the Krebs cycle and related amino acids as well as to the fact that the denominator of the ratio, serum $^{14}C$, was declining rapidly. The lines through the brain:serum ratios were generated with the same assumptions as in Fig. 1, though we knew them to be untrue when D-$^{14}C$PHB was injected. The influx coefficients cannot be attributed to D-βHB, but do measure the ability of D-βHB to provide fuel to the brain both directly (as such) and indirectly (as acetooacetate).

Age dependence of cerebral βHB transport. The experiments of Figs. 1 and 2 are examples of many time courses which we have obtained, all of which demonstrate that the brain:serum ratio taken 4 min after D-$^{14}C$βHB injection or 10 min after L-$^{14}C$βHB injection are on the steeply rising portions of the curves, hence are much more sensitive to influx coefficients than to efflux coefficients. The shapes of the serum-$^{14}C$ time-course curves did not differ enough with age to influence the brain:serum ratios significantly at these early times. Thus the brain:serum $^{14}C$ ratios in Fig. 3, A and B, may be taken as measures of permeability. A glance at Fig. 3, A and B, will show that the entry of L-βHB and that of D-βHB are subject to the same modulating influences and are, therefore, likely to occur by the same mechanism. Since this is the case, the poorly metabolized L-βHB is probably the more reliable isomer for transport studies. From either figure, we see that βHB permeation of the brain was slow at birth, increased steadily from the 1st or 2nd day until weaning, declined rapidly during the 2 or 3 wk after weaning, and then declined slowly for as many weeks as it was followed. Both the rise and the fall were about sevenfold (permeation of the blood-brain barrier is the brain:serum ratio in excess of the mannitol space). The physiological appropriateness of this age dependence should be emphasized. During suckling, ketone bodies are abundant (8, 12, 13, 19); they provide a major fraction of brain fuel (8) at a time when cerebral energy requirements increase about fourfold (16). After weaning, ketone bodies are rarely elevated enough to fuel the brain significantly, and glucose becomes the dominant and to some degree essential fuel. Thus high βHB permeability is valuable throughout suckling but only intermittently so after weaning. The extent of the modulation, especially the rapid fall during the 2 or 3 wk following weaning, is most readily explained in terms of a carrier, the abundance of which can be adjusted. The sudden switch, from rising permeability before weaning to falling permeability after weaning, suggests substrate induction, inasmuch as serum βHB falls to adult levels within a day or so of weaning (12, 13, 19).

Effects of high-fat diet on ketone body permeability. In relation to the question of inducibility, it was of interest to determine whether a ketogenic diet would prevent the fall in ketone body permeability after weaning and/or increase permeability in older animals. Figure 4 shows that 1 wk of fat-feeding approximately tripled permeability in older animals. Ketosis was apparent after 2 days and fully developed after 7 days. This suggests that permeability depends not on the blood chemistry of the moment but on its recent history, i.e., that ketone body permeability is adaptive. Further evidence of this is presented in Table 1. The first two lines (1a and 1b) of this table simply show that fat feeding increased L-βHB permeability (P < 0.005) in large animals to about the same degree as it did D-βHB permeability (P < 0.005). Glucose permeability was also increased (P < 0.005), but to a much lesser degree. On the assumption of a glucose transport $K_m$ of 7 mM (3, 6, 16), only 15% of this increase could be accounted for in terms of reduced competition with endogenous glucose. Lines 2b and 3b compared, respectively, to 2a and 3a show the rapid decline in cerebral βHB permeability (but not glucose permeability) during the period of rapid growth shortly after weaning (P < 0.005 for each isomer). Lines 2c and 3c compared, respectively, to 2b and 3b show that a high-carbohydrate diet exaggerated the fall slightly (P < 0.01 for D-βHB; P < 0.15 for L-βHB). Lines 2d and 3d compared, respectively, to 2b and 3b show that a high-fat diet retarded the fall in βHB permeability slightly (P < 0.005) and even increased L-βHB permeability above the initial value in lines 2a and 3a (P < 0.005). It appears in this case that L-βHB permeability was more influenced by the high-fat diet than was D-βHB permeability, but it should be noted that the two isomers were tested in different animals. Moreover, metabolic events during absorption of the n-$^{14}C$βHB and prior to entry into the brain may influence its apparent permeability. As already indicated, L-$^{14}C$βHB is the more reliable transport probe. Line 3e compared to 3d and 3b shows that fat-fed animals allowed access to sodium acetate in addition to water responded as other fat-fed animals; this suggests that acidosis may not have been responsible for the increased permeability (see also Fig. 8). Line 3f compared to 3d and 3b shows that complete abolition of the hyperketonemia (by a high-carbohydrate diet during the 24 h prior to testing) did not alter the effects of 6 days’ fat-feeding. This again suggests that the high-fat diet promoted βHB transport by some means other than by providing...
Values are means ± SE. Numbers in parentheses are number of animals. *High CH₂O is a high-carbohydrate diet which consisted of 65 parts sucrose, 25 parts sodium caseinate, 5 parts salt mixture number 3 of Sure, 2 parts vitamin diet fortification mixture in dextrose, and 3 parts Alphacel (all components from Nutritional Biochemicals Corporation). These animals were provided with both water and 10% sodium acetate as indicated. Controls received Purina laboratory chow ad libitum. Since all the diets were calorie and protein rich, the poor growth on artificial diets must be attributed to poor

a higher proton concentration in the blood (thereby increasing the fraction of βHB which is protonated and hence able to permeate membranes (9, 22)).

Some of the data of Table 2, however, are difficult to reconcile with this view. This table is a summary of several time-course studies including those of Figs. 1 and 2. It is seen that both the influx and fractional efflux coefficients1 for L-βHB (k₀ and kᵢ/W₀) as well as the n-βHH influx coefficient (kᵢ) rose during suckling; the natural isomer is about 3 times as permeable as the unnatural one throughout suckling. This confirms some of the conclusions of Fig. 3. The L- and L-βHB influx coefficients shown for 19- to 23-day-old rats are estimates from the single-time data of Fig. 3, and arc displayed here simply to recall the fact that this is the age of maximal βHB permeability. The last four lines show two more examples of enhanced L-βHB transport in fat-fed animals. The interesting point gained from these time courses is that fat-feeding did not enhance efflux in either case, hence the equilibrium parenchyma:serum ratio, W₀/kᵢ/W₀, was increased by fat-feeding.1 This is the result expected if fat-feeding promoted influx simply by lowering blood pH, thereby increasing the fraction of βHB in the protonated (presumably more permeable) form—auto-regulation of blood flow would defend the brain against a fall in pH during acidosis, and efflux would not be enhanced by this mechanism. If a fall in blood pH was responsible for the enhanced βHB permeability, however, the abolition of ketosis (Table 1, line 3f) should have reduced permeability immediately. There seems to be a paradox. In this connection it should be noticed that the equilibrium space also rose during suckling, suggesting that low serum pH may be partly responsible for the high permeability during suckling. Studies of serum and brain pH will be presented in Fig. 8.

**Table 1. Effects of diet on cerebral βHB permeability**

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal Wt. g</th>
<th>Conditions*</th>
<th>Serum Conc. mM</th>
<th>Brain:Serum Ratio, ml/g</th>
<th>10 min L-[¹⁴C]βHB</th>
<th>4 min n-[¹⁴C]βHB</th>
<th>4 min [¹⁴C]Gluc</th>
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<tbody>
<tr>
<td>1a</td>
<td>342 ± 4</td>
<td>Control</td>
<td>0.25 ± 0.01</td>
<td>8.92 ± 0.38</td>
<td>0.076 ± 0.011</td>
<td>0.065 ± 0.010</td>
<td>0.123 ± 0.005</td>
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<tr>
<td>1b</td>
<td>305 ± 5</td>
<td>Fat-fed 14 days</td>
<td>1.50 ± 0.14</td>
<td>8.06 ± 0.25</td>
<td>0.148 ± 0.005</td>
<td>0.149 ± 0.010</td>
<td>0.171 ± 0.008</td>
</tr>
<tr>
<td>2a†</td>
<td>65 ± 2</td>
<td>Control initial</td>
<td>0.80 ± 0.12</td>
<td>7.80 ± 0.20</td>
<td>0.260 ± 0.028</td>
<td>0.235 ± 0.028</td>
<td>0.123 ± 0.017</td>
</tr>
<tr>
<td>2b†</td>
<td>99 ± 4</td>
<td>Control final</td>
<td>0.38 ± 0.03</td>
<td>7.99 ± 0.14</td>
<td>0.197 ± 0.015</td>
<td>0.217 ± 0.006</td>
<td>0.139 ± 0.010</td>
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<tr>
<td>2c†</td>
<td>71 ± 2</td>
<td>High CH₂O, 7 days</td>
<td>0.28 ± 0.05</td>
<td>7.16 ± 0.15</td>
<td>0.175 ± 0.006</td>
<td>0.131 ± 0.012</td>
<td>0.131 ± 0.013</td>
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<tr>
<td>2d†</td>
<td>82 ± 3</td>
<td>High fat, 7 days</td>
<td>2.80 ± 0.28</td>
<td>6.33 ± 0.30</td>
<td>0.315 ± 0.008</td>
<td>0.240 ± 0.022</td>
<td>0.155 ± 0.010</td>
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<tr>
<td>3a†</td>
<td>76 ± 3</td>
<td>Control initial</td>
<td>0.21 ± 0.02</td>
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</tr>
<tr>
<td>3b†</td>
<td>111 ± 4</td>
<td>Control final</td>
<td>0.29 ± 0.02</td>
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<tr>
<td>3c†</td>
<td>64 ± 3</td>
<td>High CH₂O, 7 days</td>
<td>0.13 ± 0.03</td>
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<tr>
<td>3d†</td>
<td>79 ± 3</td>
<td>High fat, 7 days</td>
<td>3.45 ± 0.06</td>
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<tr>
<td>3e</td>
<td>85 ± 5</td>
<td>High fat + ad lib</td>
<td>3.31 ± 0.6</td>
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<tr>
<td>3f</td>
<td>77 ± 5</td>
<td>High fat, 6 days + high CH₂O, 1 day</td>
<td>0.10 ± 0.03</td>
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**Table 2. Summary of time-course data**

Parameters were derived from experiments carried out and analyzed as illustrated in Figs. 1 and 2.

<table>
<thead>
<tr>
<th>Age, Wt., or Condition</th>
<th>Influx coeff, kᵢ, ml/min/g</th>
<th>Flux eff. coeff, kᵢ/₀, W₀, min⁻¹</th>
<th>Equilibrium ratio, W₀/kᵢ, ml/g</th>
<th>n-[¹⁴C]βHB influx coeff, kᵢ, ml/min/g</th>
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<tr>
<td>1-2 days</td>
<td>0.007</td>
<td>0.023</td>
<td>0.30</td>
<td>0.021</td>
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<td>8 days</td>
<td>0.015</td>
<td>0.029</td>
<td>0.52</td>
<td>0.056</td>
</tr>
<tr>
<td>14-15 days</td>
<td>0.033</td>
<td>0.057</td>
<td>0.58</td>
<td>0.11</td>
</tr>
<tr>
<td>19-23 days</td>
<td>0.04</td>
<td>0.09</td>
<td>0.23</td>
<td>0.14</td>
</tr>
<tr>
<td>100 g</td>
<td>0.021</td>
<td>0.062</td>
<td>0.34</td>
<td>0.036</td>
</tr>
<tr>
<td>Fat-fed 7 days (60 g)</td>
<td>0.004</td>
<td>0.065</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>100 g</td>
<td>0.007</td>
<td>0.030</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Fat-fed 14 days (310 g)</td>
<td>0.014</td>
<td>0.028</td>
<td>0.50</td>
<td></td>
</tr>
</tbody>
</table>

W₀/kᵢ/W₀ is the amount of L-βHB in the parenchyma of 1 g of brain at equilibrium divided by the amount in 1 ml of serum, i.e., equilibrium Sᵢ/[S₀] (see footnote 1).

**Brain βHB content.** Enzymic assays of βHB were carried out on extracts of sera and some brains, and the results are presented in Fig. 5. The serum concentrations are essentially as expected from other reports (and the data of Fig. 4 and Table 1) — somewhat elevated in sucklings and markedly elevated by fat-feeding. The brain βHB levels are presented as brain:serum ratios, the symbols with standard errors below the dashed line. The corresponding symbols above this line are the apparent equilibrium brain:serum ratios, as judged from the L-βHB time courses summarized in Table 2 (see footnote 1). In both fat-fed groups and in the 15-day sucklings, the brain:serum ratios exceeded the mannitol space less than one-fifth as much as did the corresponding equilibrium ratios. In these cases, transport was clearly the major limiting step of βHB clearance. In the remaining groups with lower serum concentrations, the equilibrium values were lower, and the discrepancies

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**Footnote:** For Table 2. Summary of time-course data: The influx coefficient, kᵢ, represents the amount of substance entering the brain per unit time. The flux efflux coefficient, kᵢ/₀, W₀, represents the rate at which the substance is extracted from the brain. The equilibrium ratio, W₀/kᵢ, represents the equilibrium concentration of the substance in the brain relative to the concentration in the serum. The n-[¹⁴C]βHB influx coefficient, kᵢ, represents the rate at which the substance enters the brain. The brain:serum ratio is used to compare the concentration of the substance in the brain relative to the concentration in the serum.
between steady-state brain:serum βHB ratios and equilibrium ratios were not so great. Still, the steady-state ratios exceeded the mannitol space less than half as much as did the equilibrium ratios, showing transport to be an important limiting step in βHB clearance by the brain in these animals as well. Thus cerebral βHB clearance is very sensitive to the permeability modulations of maturation and fat-feeding. These conclusions are essentially the same if one disregards the apparent L-βHB equilibrium ratios and accepts the DMO data (Fig. 8) as representing the equilibrium sought by weak acids.

Temperature dependence. As seen in Fig. 6, the temperature dependence of cerebral βHB entry was as great as that of glucose entry, which is known to be carrier mediated. This suggests that βHB entry may be mediated also. With either permeant, the increased entry with higher temperature may involve not only the activation energies, but also increased capillary dimensions incident to increased blood flow. Although the slopes of these dependences were not well defined, it seems likely that increasing temperature contributes significantly to the increasing entries of both permeants with age during suckling. But temperature could play no role in the falling βHB entry after weaning. Fat-feeding did not increase body temperature.

Blood flow. According to earlier studies (6, 15, 16) the brain:serum "H₂O ratio 40 s after intraperitoneal injection is approximately proportional to cerebral blood flow. A 40-s ratio of 0.3 ml/g corresponds to a blood flow of about 1 ml/min-g or a plasma flow of about 0.6 ml/min-g (cf. refs. 6, 15, 16). As seen in Fig. 7, blood flow increased about four- to fivefold between birth and adulthood; the major increase is between the 10th and 20th days, after which plasma flows were typically in the range of 0.6 ml/min-g. This compares to the 20-day-old L-βHB influx coefficient (k₁) of about 0.04 ml/min-g and the 20-day D-βHB influx coefficient (k₂) of about 0.14 ml/min-g. These are the volumes of serum containing the amounts of "C fluxing unidirectionally into the parenchyma of a gram of brain each minute. Dividing them by plasma flow gives the fractions of delivered molecules that leave the capillary, 7% and 23%, respectively. The mean capillary concentrations would then be reduced, respectively, about 4% and 12% below arterial concentrations. These relations hold approximately.
do show that l-βHB entry is not significantly flow
limited and that D-βHB entry is only slightly flow limited,
hence the age and diet dependences (Figs. 3 and 4, Table
1) are properly attributed to permeability rather than
blood flow alterations. However, passive and adaptive
increases in capillary surface might accompany the in-
crease in flow between the 10th and 20th days and
contribute to the permeability rise during suckling. The
threefold permeability increase during the first 10 days,
the sixfold permeability decline after weaning, and the
threefold permeability increase with fat-feeding were
not accompanied by like changes in blood flow and
presumably were due to factors other than capillary
surface changes.

Plasma and brain pH. To examine the possibility that
variations in plasma pH might contribute to modula-
tions of βHB permeability, we measured the blood pH of
animals between 9 days of age and 400 g, some of which
were fat-fed. As seen in Fig. 8, the blood pHi's of suck-
lings, weanlings, and adults were essentially the same.
This argues against the view that a low serum pH
contributes to the high βHB permeability of sucklings
and weanlings as compared to adults. Fat-feeding usu-
ally lowered blood pH, but the fall was always less than
0.1 U, which corresponds to a less than 26% increase in
proton concentration. This could increase the fraction of
βHB protonated by less than 26%, which could increase
permeability by only less than 26%. Since fat-feeding
increased βHB permeability by about 200%, most of the
increase must have been due to factors other than se-
rum pH.

It is conceivable that the serum pH of the cerebral
capillary was lower in sucklings and weanlings than in
adults, even though the pH of the general circulation
was not. Likewise, fat-feeding may have lowered cere-
bral capillary pH more than that of the general circula-
tion. To test for these possibilities, we measured DMO
entry and equilibrium distribution in the brains of ani-
mals under these conditions. A lower cerebral capillary
pH should enhance DMO influx, and, if this is not
associated with lower cerebral pH, efflux from brain to
capillary will not be enhanced and the equilibrium
brain:serum DMO ratio will be increased (22). Of
course, these measurements are subject to other influ-
ces—capillary surface will affect both fluxes, and
brain water content will affect equilibrium space. In
Fig. 8, the 7-min brain:serum DMO ratios reflect influx,
and the 80-min ratios are equilibrium values. It is seen
that fat-feeding enhanced neither the influx nor the equi-
librium brain:serum ratio, despite the fact that se-
rum pH was slightly lower in fat-fed animals. The en-
hancement of βHB entry in fat-fed animals is, there-
fore, probably not due to cerebral capillary acidosis. The
age dependency, on the other hand, suggests that the
cerebral-capillary proton concentration of sucklings
may be twice that of adults, despite our failure to detect
this in the central circulation. The change seems to
occur between weaning and 150 g body wt, the period of
rapid decline in βHB permeability. If we assume that
the influences (capillary pH or other) which lower DMO
permeability during this period also lower βHB permea-
bility, then a factor of 1/2 is accounted for. A considera-
ble portion of the decline in βHB permeability (to one-
sixth its weaning value) must be due to factors not
affecting DMO permeability.

After 14C-DMO injection, serum 14C levels are incred-
ibly stable for hours (data not shown), indicating meta-
bolic inertness. If one doubts the accuracy of l-βHB
equilibrium ratios, then the DMO data provide an al-
ternate estimate of the equilibrium sought by weak
acids. The two kinds of data agree very well in control
animals between 8 days of age and 100 g body wt (Table
2 vs. Fig. 8). In newborn and adults, apparent l-βHB
equilibria are about 60% of DMO equilibria, which is
still rather good agreement. The major discrepancy was
seen in fat-fed animals where DMO equilibrium was
unaffected but apparent l-βHB equilibrium increased
twofold. We are inclined to believe the discrepancy be-
cause l-βHB was very poorly metabolized in fat-fed
animals, hence the tendency for metabolites to cause
overestimation of l-βHB equilibrium should be less in
fat-fed than in control animals. Moreover, the varia-
tions in apparent l-βHB equilibrium correlate very well
with the variations in l-βHB influx coefficient.

Other permeants. To test further the specificity of
βHB permeability modulations, we have studied the
permeabilities of several substances as these depend on
age (and in some cases on diet). The permeability of no
CEREBRAL \( \beta \)HB TRANSPORT

substance (except \( \beta \)HB) increased during the first 10 days of life. These include: a) DMO, as just presented; b) urea (Fig. 9A), which should enter via aqueous pores; c) glucose (Fig. 9B), which is very temperature dependent; d) leucine and valine (Fig. 9C), which presumably also enter by a carrier. The fact that only \( \beta \)HB permeability rose during this period suggests that its rise may be a specific regulation, at least in part, depending on factors other than proton concentration, capillary surface, porosity, and temperature. Likewise, none of the substances tested (DMO, urea, glucose, valine, leucine) showed a permeability decline after weaning comparable to that of \( \beta \)HB, again suggesting that the latter was due in part to a specific regulation. Urea permeability like DMO permeability failed to increase with fat-feeding which argues against some kinds of nonspecific mechanisms of increasing \( \beta \)HB permeability.

DISCUSSION

Kinetics of \( \beta \)HB uptake. Daniel et al. (4) found the influx of \( \beta \)HB into the adult rat brain to be proportional to blood \( \beta \)HB concentration throughout the range of concentrations tested (0.5–30 mM). Thus the transport rate coefficient \( (k_m) \) appeared to be independent of substrate concentration, i.e., entry was neither saturable nor cooperative. They calculated an influx coefficient of 0.022 ml/min·g (volume of blood containing amount undergoing influx in 1 g of brain per min). On the assumption that blood concentration is about 80% of the serum concentration, this would correspond to \( k_m = 0.018 \) ml/min·g (the volume of serum containing the amount undergoing influx in 1 g of brain per min). This agrees rather well with the value of \( k_m \) which one would deduce from Figs. 1 and 2, which suggest that \( k_m = 3 k_{so} \), hence that \( k_{so} \) in adults is about 0.021 ml/min·g. Comparing these estimates with serum flow (~0.6 ml/min·g) indicates that only 0.03 of serum-delivered \( \beta \)HB undergoes influx, hence that blood flow does not significantly limit influx in adults. Hawkins et al. (8) and Daniel et al. (4) found that about 0.04 of \( \beta \)HB delivered to the brain by the flood was metabolized (anesthetized rats). This would be roughly 0.05 of that delivered in serum. The serum flow in whole brain of anesthetized rats is about 0.3 ml/min·g (24). Thus, cerebral \( \beta \)HB clearance was about 0.015 ml/min·g (volume of serum containing the amount metabolized by 1 g of brain per min). Comparing this clearance estimate to the estimated influx coefficients suggests that the majority of influxing molecules undergo metabolism rather than efflux, hence that transport is a major rate-limiting step in \( \beta \)HB uptake (4).

The measurements of steady-state brain:serum \( \beta \)HB ratios in 15-day sucklings and fat-fed rats (Fig. 5) lead to a similar conclusion (see also refs. 2, 8, 23). In these animals the parenchyma:serum ratio, \( G_i/G_s \), appeared to be about 20% of the transport equilibrium ratio, \( W_{so}k_{so}/k_s \) (assumed to be the same as \( W_{so}k_{so}/k_{si} \), see footnote 1. Accordingly, \( G_i/G_s \)/\( (W_{so}k_{so}/k_s) = 0.2 \), or \( G_i/k_s = 0.2 \) [\( G_s/k_{so} \), i.e., efflux ~ 0.2 influx, and metabolism ~ 0.8 influx. Entry appears, then, to be the major rate-limiting step even in animals with high permeability (2–5 times that of chow-fed adults, see Table 2).

It should be appreciated that the flux from serum
\(\beta\text{HB}\) to end products is even more limited and controlled by transport than indicated in the above considerations, for there are modest backward fluxes from glycolytically generated acetyl CoA and from serum acetoacetate to \(\beta\text{HB}\). These fluxes increase \(G_i/G_o\) and reduce \(\beta\text{HB}\) clearance. They are more important relative to forward flux in the less ketotic animals, where they raise steady-state \(G_i/G_o\) ratios close to the apparent equilibrium ratios (Fig. 5) or higher (2, 8, 23). The backward fluxes can exceed forward flux in rats (8, 23, 29), dogs (26), and humans (5, 21) with sufficiently low blood \(\beta\text{HB}\) concentrations. It is, therefore, necessary to judge the rate-limiting role of transport in hyperketonemic animals and to appreciate that one underestimates this role.

In view of the rate-limiting role of transport, one may predict that the age and diet-dependent transport modulations produce nearly proportional alterations in the \(\beta\text{HB}\) to products flux coefficient if the enzymes of ketone body respiration are poorly saturated with substrates. This condition appears to obtain, as judged from the rectilinear dependence of A-V difference on arterial concentration up to high physiological concentrations (5, 8, 11, 21, 23, 26, 29).

Since transport is the major limiting step in \(\beta\text{HB}\) uptake and since acetoacetate clearance is usually about 3 times \(\beta\text{HB}\) clearance, we may conclude that acetoacetate permeability is at least 3 times \(\beta\text{HB}\) permeability. Steady-state brain:serum acetoacetate ratios are just as low as the \(\beta\text{HB}\) ratios (2, 8, 23), suggesting that entry limits uptake of this fuel as well, and hence, that its permeability is not significantly greater than 3 times that of \(\beta\text{HB}\). Daniel et al. (4) failed to see this permeability difference in their studies, but we have recently demonstrated it (unpublished observation) by Oldendorf's (17) technique.

**Mechanism of \(\beta\text{HB}\) entry.** Oldendorf (17) has recently proposed carrier-mediated entry of short-chain carboxylic acids into the brain. His evidence included saturation kinetics, competition for entry, and preferential entry of \(L\) over \(D\)-lactate. Entry of \(\beta\text{HB}\) does not seem saturable by concentrations up to 30 mM (4). Nevertheless, several observations are suggestive of a carrier mechanism: a) \(L\)-\(\beta\text{HB}\) permeability was nearly 3 times the urea permeability at weaning, suggesting that pores could not contribute significantly to \(\beta\text{HB}\) entry. b) The appropriateness, extent, and distinctiveness of the age dependency and dietary modulations are most readily explained in terms of an adjustable \(\beta\text{HB}\) carrier. c) The temperature dependence was much greater than expected of a simple diffusion process, being comparable to the temperature dependence of glucose transport, which we know to be carrier mediated (3, 6, 16). d) Finally, there is the apparent preferential entry of the natural \(D\)-\(\beta\text{HB}\) as compared with that of \(L\)-\(\beta\text{HB}\), which we have recently confirmed (unpublished observations) by the Oldendorf technique (17). Each of these considerations must be considered weak evidence for a carrier mechanism since other interpretations are possible. Together they give us some conviction.

**Mechanism of \(\beta\text{HB}\) permeability modulations.** In principle, cerebral \(\beta\text{HB}\) permeability should be an increasing function of temperature, capillary, or blood-brain barrier surface area, the fraction of that area occupied by pores or defects large enough to admit \(\beta\text{HB}\), the plasma proton concentration, and the density or activity of \(\beta\text{HB}\) carriers in the barrier surface. All but the last are nonspecific factors which should influence permeabilities of other substances.

Rising body temperature prior to weaning is certainly responsible for some of the increase in \(\beta\text{HB}\) permeability. Yet glucose, valine, and leucine permeabilities (which are carrier mediated and should be temperature sensitive), and cerebral blood flow (which along with respiration should be temperature sensitive) did not increase significantly for the first 10 days. Body temperatures measured with a thermistor seemed to depend more on the vicissitudes of nesting than on age during this period. There is no basis for postulating a major increase in capillary surface prior to the increase in blood flow. This seems to be confirmed by the lack of increase in any permeability other than that of \(\beta\text{HB}\) for the first 10 days—especially those of DMO and urea which should be sensitive to surface area and porosity. The fact that DMO permeability did not increase with age argues also against a falling plasma pH contributing to the early rise in \(\beta\text{HB}\) transport; indeed, the plasma pH of 10- to 20-day-old rats was not low. It appears that the only nonspecific factor contributing significantly to the increasing \(\beta\text{HB}\) permeability prior to weaning is the rising body temperature and that this factor probably does not account entirely for the increased permeability during the first 10 days. There is reason, then, to suspect that a \(\beta\text{HB}\) carrier is enhanced in amount or activated during this period.

The postweaning decline in \(\beta\text{HB}\) permeability presumably has nothing to do with temperature. Blood flow did not decline during this period, and provides no basis for supposing that capillary surface declined. Urea permeability should be sensitive to capillary surface as well as its porosity, and this declined by a factor of about 0.8 between weaning and 400 g body wt. Glucose permeability remained high for this entire period, while valine and leucine permeabilities showed no significant decline during the period around 100 g body wt, when \(\beta\text{HB}\) transport was declining rapidly. Since these carrier-mediated processes are presumably selectively adjustable, they cannot argue strongly for or against a fall in capillary surface. DMO permeability (influx) declined by a factor of 0.47 after weaning, and this corresponded in time to the period of rapid decline in \(\beta\text{HB}\) permeability. Presumably this reflects the combined effects of decreasing capillary surface and decreasing capillary proton concentration, both of which might influence \(\beta\text{HB}\) permeability similarly. At the same time the equilibrium brain:serum DMO ratio fell by a factor of 0.63. Since the equilibrium represents the ratio of influx coefficient to fractional efflux coefficient, we can calculate the factor by which the fractional efflux coefficient fell: 0.47/0.63 = 0.75. This is similar to the fall in urea permeability and may represent a decline in capillary surface, since brain pH (the other factor in DMO efflux) is probably guarded in weanlings as in adults. This argument implies that the capillary proton concentration of adults was less than 0.63 that of sucklings, or
that the pH was 0.2 U higher. Acidosis was not seen in the pH measurements on blood from the central circulation of sucklings. In any case, the decline in capillary surface and capillary proton concentration might affect βHB permeability as much as they do DMO influx, namely by a factor of 0.47. The overall decline in βHB influx was by a factor of 0.19, which contains a factor of about 0.40 (i.e., 0.47 × 0.40 = 0.19) not accounted for in terms of surface area and proton concentration combined. Lacking any other nonspecific factors to account for this, we suggest that the density or activity of βHB carriers (per unit capillary surface) fell by a factor of about 0.4.

Fat-feeding did not increase urea permeability, DMO permeability or the equilibrium brain:serum DMO ratio as would be expected if capillary surface or capillary proton concentration were increased. Moreover, abolition of ketosis did not reverse the effects of the high-fat diet on βHB permeability. The tripling of βHB permeability with fat-feeding is, therefore, most readily explained in terms of enhanced density or activity of a βHB carrier.

In summary, then, each of the βHB permeability modulations is difficult to explain entirely by nonspecific factors, and in view of other evidence of a βHB carrier and the appropriateness of the modulations, we propose specific regulation of such a carrier to account for the otherwise unexplained effects.

Aside from this argument, there are two other points which might be made at the risk of over-interpreting the data. First, there was the discrepancy between the pH measurements on blood from the neck stump and the age-dependent pH changes of the cerebral capillary as suggested from DMO permeability and equilibrium brain:serum DMO ratios. If this discrepancy is real, then we must assume that the ratio of cerebral acid output to blood buffering power falls during the post-weaning period. Second, the apparent r-βHB equilibrium space1 rose during suckling and fat feeding while the DMO equilibrium space did not. Thus far we have assumed that both of these compounds cross membranes (by whatever mechanism) only if protonated, i.e., that anion movements are coupled to proton movements stoichiometrically. If the discrepancies between βHB and DMO equilibria are real, then we must suspect that βHB movement is coupled through its carrier to an event other than or in addition to proton movement. Moreover, the chemical potential for this event seems highest when it is dissipated fastest by βHB entry. Indeed, it seems that the chemical potential for this hypothetical event may be the factor responsible for those modulations attributed to regulation of the βHB carrier.

Age and cerebral βHB clearance. The present studies demonstrated a sevenfold increase in cerebral βHB permeability during the suckling period followed by a nearly equal decline during the months following weaning. This age dependence parallels closely the brain levels of every enzyme unique to ketone body respiration: β-hydroxybutyrate dehydrogenase (10, 12, 19), 3-oxo-acid CoA transferase (12, 19), and mitochondrial acetoacetyl-CoA thiolase (14). Each of these increases fivefold during suckling and then falls fivefold after weaning. The correspondence is so close as to suggest coordinated control of all four steps including transport. Hawkins et al. (8) observed the βHB extraction (clearance/blood flow) by the brain to be nearly 5 times greater in rats near the end of suckling than in adults. Since, as already discussed, transport was the rate-limiting step in adults, we may conclude that the much higher clearances in the suckling animals (compared to adults) could not occur were it not for their much greater permeability. With this in mind, we may also conclude that βHB clearance in newborns is about as low as in adults (i.e., about one-fifth that of 3rd-wk sucklings); for βHB permeability is as low in newborns as in adults. According to Lockwood and Bailey (13), blood βHB is typically about 25% higher in the 1st wk than in the 3rd wk of life, hence βHB uptake (Ig.) clearance in the 1st wk would be about one-fourth that in the 3rd wk. Cerebral blood flow and presumably respiration in the 1st wk are also about one-fourth their values in the 3rd wk (16). Thus βHB seems to make about the same fractional contribution to respiration through most of the suckling period. Cerebral ketone body extraction is about 5 times higher in the newborn than adult human (11) and declines throughout infancy (11) and childhood (21). This is most likely due to declining permeability, since the enzymes of ketone body respiration in human brain undergo little change between birth and adulthood (20).

Diet and βHB clearance. A high-fat diet approximately tripled βHB permeability in adults (Fig. 4 and Table 1) and opposed the permeability decline in weanlings. We have recently confirmed these observations (unpublished observations) by the Oldendorf technique (17). Presumably the permeability rise during suckling and the fall after weaning are in part manifestations of this dietary responsiveness. As already indicated, the tripling of permeability with fat-feeding should nearly triple βHB clearance. According to the literature (12, 14, 25, 27) the cerebral enzymes of ketone body respiration do not increase during adult ketogenic states. Hence the permeability modulation is the only adaptation yet demonstrated which would promote ketone body utilization by the brain during ketogenic states.

We are unaware of evidence for such adaptations in humans. Gottstein et al. (5) found cerebral ketone body extraction in diabetic patients to be no higher than in overnight-fasted controls of comparable ketosis. Moreover, the 40-day-fasted obese patients studied by Owen et al. (18) showed cerebral βHB extractions somewhat less than predicted by extrapolation of the regression line calculated by Gottstein et al. (5). At present, then, we must suppose that cerebral βHB metabolism in the adult human is controlled simply by the blood concentration.

Judging from the studies of Hawkins et al. (8) and Ruderman et al. (23), 2–4 days of ketosis (starvation or streptozotocin) fails to produce an adaptive increase in the cerebral βHB extraction of rats. However, Zivin and Snarr (29) found that 3–5 days of fasting increased by 60% the slope of the substrate dependence line (uptake vs. blood βHB concentration) in perfused rat brain.
Since physiological ketotic states are ones of carbohydrate deprivation, the enhanced βHB permeability and clearance would have two benefits. First, the brain will be better nourished in case serum glucose falls to inadequate levels. Second, the need for gluconeogenesis from amino acids will be reduced to the extent that the brain glucose is spared from glycolysis and/or pyruvate spared from oxidation. This should slow protein depletion significantly, since the brain accounts for a surprisingly large fraction of glucose consumption in such conditions (18).

We thank Dr. Mildred T. Stahlman for advice and help in the measurements of blood pH.

This work was supported by Public Health Service Grants 5 P01 AM07467, 1 P17 17026, and 04527.

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Received for publication 9 April 1975.

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