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11. Phadebas β_2-Micro Test Kit (Pharmacia Diagnostics AB, Uppsala, Sweden).
12. Using all 20 subjects as a single group, all permutations of the liver, kidney, blood, urine, and β_2-microglobulin data were tested for possible correlations. Analysis of variance was performed using the SPSS computer package [N. H. Nie, Ed., *Statistical Package for the Social Sciences* (McGraw-Hill, New York, ed. 2, 1975)], in which the level of significance was set at $P < .05$. Since cadmium is considered to be a nonessential trace element, its accumulation in the body may follow a lognormal distribution [J. Aitchison and J. A. C. Brown, *The Lognormal Distribution* (Cambridge Univ. Press, Cambridge, 1976)]. Therefore, normal versus normal, normal versus lognormal, and lognormal versus lognormal correlations were performed. For statistical comparisons between smokers and nonsmokers, the t-test was used on either normal or lognormal distributions whenever appropriate. The level for a significant difference was set at $P < .05$. The results of the t-test are presented in Table 1.
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15. The rate of change of cadmium in the whole body is described by the equation $dC/dt = -\lambda C + R$, where λ = percentage loss per year, C = whole body burden of cadmium (milligrams), and R = yearly exposure (milligrams per year). The loss per year is λC where $\lambda = \ln 2/t_{1/2}$, $t_{1/2}$ = biological half-time for the whole body. Since urinary excretion represents the only significant means of loss from the total body, λC = urinary loss per day $\times$ 365 day/year. In the present study, urinary loss was 2.3 μg/liter $\times$ 1.5 liter/day $\times$ 365 day/year = 1.26 mg/year. Solving for $t_{1/2}$ gives $t_{1/2} = (\ln 2 \times 28.7)/1.26 = 15.7$ years. The variation of $\pm$ 1.2 μg/liter per day of urinary cadmium gives a range of 11 to 33 years for $t_{1/2}$. For nonsmokers, the calculation is $\lambda = (1.66$ μg/liter $\times$ 1.5 liter/day $\times$ 365 day/year)/19,300 μg = 0.047 per year or $t_{1/2} = 14.7$ years. For smokers, $\lambda = (2.72$ μg/liter $\times$ 1.5 liter/day $\times$ 365 day/year)/35,500 μg = 0.042 per year or $t_{1/2} =$ 16.5 years.
16. The rate of change of cadmium is $dC/dt = -\lambda C + R$. Solving the equation gives $R = (\lambda C)(1 - e^{-\lambda t})^{-1}$. In the case of the nonsmokers, R represents the dietary intake, $\lambda = 0.047$ per year, $C =$ 19.3 mg, and $t = 52$ years. Substitutions of these values gives $R = 993$ μg/year = 2.7 μg/day.
17. For smokers, C = additional body burden of 16.2 mg = 35.5 mg $-$ 19.3 mg. R is then the exposure rate due to cigarette smoking. For the present study, $\lambda = 0.042$ per year, $C = 16.2$ mg, and $t = 27$ years (average number of years smoking). Substitution of these values gives $R =$ 1003 μg/year = 2.75 μg/day. The present smokers, however, averaged 1.4 packs per day, thus the absorbed dose is 1.9 μg per pack.
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19. Assuming 2 μg per cigarette (*13*) leads to 40 μg per pack. If only 2/3 to 3/4 of any cigarette is smoked and 70 percent of the cadmium is in the smoke, then 19 to 20 μg per pack may be inhaled. The mainstream smoke represents 10 to 20 percent of the total smoke. Thus, the inhaled smoke from one pack of cigarettes may contain 2 to 4 μg of cadmium.
20. We thank A. LoMonte for performing the atomic absorption measurements, J. Rothmann for assisting in the activation procedures, and D. Pion for preparing the manuscript. Supported by U.S. Department of Energy contract EY-76-C-02-0016.

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Ketone Bodies Are Selectively Used by Individual Brain Regions

Abstract. *Close study of 3-hydroxybutyrate uptake by brain suggests that its metabolism is limited by permeability. Furthermore, the permeability characteristics vary from region to region; areas known to have no blood-brain barrier show the highest rate of utilization. The results imply that rather than substitute fuels, ketone bodies should be considered supplements which partially supply specific areas but are incapable of supporting the entire energy requirement of all brain regions.*

Ketone bodies have been shown to be an alternative fuel of brain energy metabolism. Unlike glucose, they do not appear to be metabolized freely by all cerebral areas. Instead, their metabolism appears to be restricted by transport across the blood-brain barrier. This phenomenon may explain several observations and alter some of our current concepts.

In normal, well-nourished sedentary mammals the ketone bodies 3-hydroxybutyrate and acetoacetate are present in low circulating concentrations. Yet there are physiological and pathological circumstances under which their concentrations rise appreciably and they become an important respiratory fuel. In addition to elevation during fasting and diabetes, increased concentrations of ketone bodies occur during pregnancy, during prolonged exercise, in persons eating high-fat diets, in uremia, during the perinatal period, and during infancy (*1, 2*). A novel situation is the recent popularity of diets consisting almost entirely of fats and proteins, where progress is monitored not only by weight loss but by the presence of ketone bodies in the urine (*3*).

Late in the 19th century the detection of ketone bodies in blood and urine of diabetic patients, coupled with the discovery that ketosis occurred when fatty acids were oxidized, led to the belief that ketone bodies were undesirable side products of fatty acid degradation (*4*). The discovery by Lynen and co-workers (*5*) in the 1950's that acetoacetate and 3-hydroxybutyrate were not direct products of fatty acid degradation, but were synthesized by a separate pathway under close biochemical control, led to a reevaluation of the physiological role of these metabolites. In 1961 Krebs (*6*) presented the view that "the finding that ketone bodies are ready substrates of respiration suggests that their presence in the circulating blood serves to supply tissues with a fuel of respiration; their function is analogous to that of glucose and the nonesterified fatty acids." Many laboratories confirmed this statement, and the concept of "physiological ketosis," whereby acetoacetate and 3-hydroxybutyrate are considered to be normal and useful metabolites (*7*), is now generally accepted.

Consumption of ketone bodies diminishes the demand for glucose, reducing the necessity for gluconeogenesis and concomitant degradation of protein. The most notable example of this is the situation in the human brain. Originally it was thought that brain relied only on glucose as a fuel of respiration. However, Owen *et al.* (*2*) pointed out that humans can starve for more than 4 to 6 weeks. They reasoned that if brain used only glucose during this period, all of the protein in the body would be consumed, since body carbohydrate stores are meager and the primary substrate for gluconeogenesis is protein. By determining arteriovenous differences across brains of humans starved for 40 days, Owen *et al.* (*2*) proved that ketone bodies provided a large additional source of energy. In fact, under these circumstances, ketone bodies became the major fuel for brain metabolism, accounting for about 60 percent of the energy requirement. Subsequently, it was shown not to be necessary to postulate that this was an enzymatic adaptation by brain. Williamson *et al.* (*8*) demonstrated conclusively that the necessary enzymes were always present in amounts greater than required (*9, 10*). Furthermore, these enzyme activities were not changed by starvation or diabetes. Physiological experiments showed that ketone bodies could be and were used as soon as they appeared in the circulation, the most important determinant being their plasma concentrations (*11-13*).

Although the data show conclusively that ketone bodies are at least a partial substitute for glucose, it is an open ques-

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tion whether they are used by all regions of brain in the same proportions. Recently it has become feasible to use autoradiography to examine regional use of various fuels. For instance, [2-^{14}C]glucose can be injected intravenously and its rate of metabolism quantified from autoradiographs of tissue sections. The key to this procedure is the fact that ^{14}C is trapped by equilibration with large intracellular metabolite pools (*14*). By a modification of this procedure, 50 μCi of [2-^{14}C]glucose, 3-[1-^{14}C]hydroxybutyrate, or [1-^{14}C]butyrate were injected intravenously into male Sprague-Dawley rats weighing about 250 g. At 10 minutes the rats were decapitated and the brains were removed and frozen in Freon-12 at −30°C. Thin sections (20 μm) were cut at about −20°C, mounted on glass slides, and exposed to DuPont Lo-Dose mammography film for 2 weeks. The films were developed and photographed. Quantitative densitometric readings are possible but were not necessary for the interpretation of the patterns. Background radioactivity for all regions was considered to be negligible in comparison to the total, since by 10 minutes the blood content of 3-[1-^{14}C]hydroxybutyrate was reduced to about 10 to 15 percent of that present at 1 minute. The results clearly show that glucose and ketone bodies do not nourish the same areas proportionately (Fig. 1). Especially striking is the fact that lower cortical layers consume much more 3-hydroxybutyrate than do upper layers. The striatum and globus pallidus manifest low uptake while the area of the hypothalamic arcuate nucleus–median eminence took up large amounts of label. The greatest amount of radioisotope by far accumulated in the pituitary (and the pineal, not shown).

There exists, in rat brain, a stereospecific saturable carrier mechanism that facilitates the entry of lactate, pyruvate, 3-hydroxybutyrate, acetoacetate, propionate, and butyrate (*15*). We believe that the rate-limiting step for 3-hydroxybutyrate metabolism is its transport into brain. In support of this hypothesis are the following observations.

1) The enzymes of ketone body metabolism are present at much higher activities than necessary for measured rates of utilization (*8, 9, 12, 13*).

2) The brain content of ketone bodies is very low. In fact, when correction is made for blood, cerebrospinal fluid, and extracellular fluid contamination, brain content is almost nil (*12, 13, 16*).

3) The median eminence has fenestrated capillaries, which offer less resistance to solute passage. This area, where transport is not expected to be limiting, shows a high rate of accumulation. Accordingly, both the pituitary and the pineal body, which lack a blood-brain barrier (*17*), accumulated the largest amount of label.

4) Short-chain fatty acids such as butyrate, which share the same transport system as ketone bodies (*15*), show almost identical patterns of utilization (Fig. 1), but these substrates (3-hydroxybutyrate and butyrate) are metabolized by different enzymes (*18*). It seems unlikely that distinct initiating enzymes would fortuitously occur with the same ratio of activities throughout the brain and cause the distribution of label found.

Together, these data indicate that passage of 3-hydroxybutyrate across the blood-brain barrier is limiting to metabolism. Because of the relatively low extraction, 3-hydroxybutyrate supply will not depend primarily on blood flow but on regional transport characteristics (*19*). Most important, the autoradiographs demonstrate that permeability is not homogeneous; rather it is region-specific.

The finding that metabolism of 3-hydroxybutyrate proceeds only as fast as transport permits is in accord with earlier data showing that ketone body utilization by brain occurred approximately in proportion to the blood concentration of these substrates (*11–13*). Thus, arteriovenous differences were comparable when circulating concentrations were raised either by starvation or by infusion of acetoacetate into fed rats. However, by using more sensitive methods of measuring uptake and by prolonging starvation in rats to 4 or 5 days, it has been demonstrated that adaptive changes in the transport system can occur that permit 3-hydroxybutyrate to enter 35 to 45 percent more rapidly (*20, 21*). Whether this is a general adaptation in the trans-

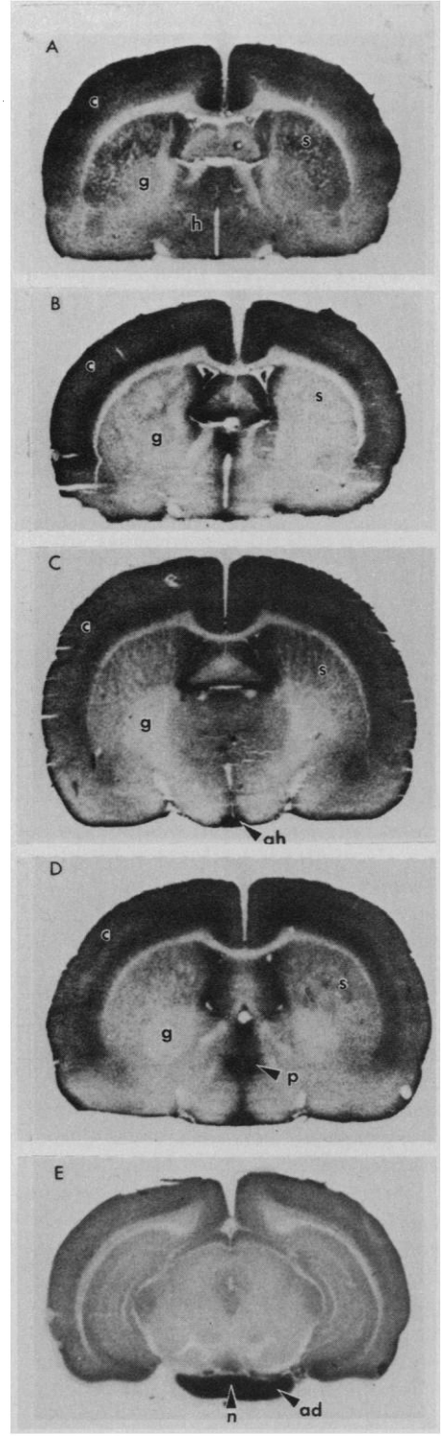

Fig. 1. Autoradiographs of [2-^{14}C]glucose, 3-[1-^{14}C]hydroxybutyrate, and [1-^{14}C]butyrate incorporated by rat brain. All autoradiographs are of coronal sections through the rat forebrain area and demonstrate relative rates of metabolite utilization. Darker areas indicate greater utilization. Key: *ad*, adenohypophysis; *ah*, hypothalamic arcuate nucleus; *c*, cortex; *g*, globus pallidus; *h*, hypothalamus; *n*, neurohypophysis; *p*, paraventricular nucleus; and *s*, striatum. (A) [2-^{14}C]Glucose incorporation into a normal fed rat. Note the high metabolic rate of cortex and the gradation from greatest utilization in the superficial layers to lesser rates in the deepest layers. (B) 3-[1-^{14}C]Hydroxybutyrate incorporation into a rat starved for 48 hours. Although 3-hydroxybutyrate uptake is considerably less than that of glucose, the autoradiographs were exposed for longer periods to attain similar densities. Especially notable is 3-hydroxybutyrate utilization in the lower cortical layers and decreased accumulation of label in the basal ganglia. (C) Incorporation of [1-^{14}C]butyrate, which is metabolized by a different pathway but carried by the same blood-brain transport carrier. Note the similarity to 3-hydroxybutyrate. (D) [1-^{14}C]Butyrate incorporation slightly rostral to other sections. Of particular interest is the high density of the paraventricular nuclei. (E) 3-[1-^{14}C]Hydroxybutyrate incorporation showing the sharp demarcation between pituitary and brain. The lack of blood-brain barrier apparently allows 3-hydroxybutyrate to be metabolized rapidly.

port process or is specific to regions remains open to investigation.

Another factor of possible importance that may modify the rate of ketone body utilization is decreased competition for passage across the blood-brain barrier. Since several monocarboxylic acids share the same transport mechanism, there is competition for entry into brain. Thus lactate, pyruvate, acetoacetate, and 3-hydroxybutyrate all compete on a relatively equal footing. During fasting or when carbohydrates are restricted, lactate and pyruvate concentrations decrease, allowing acetoacetate and 3-hydroxybutyrate to enter more rapidly. We calculate that after 48 hours of starvation influx will be increased by about 15 percent by this mechanism alone.

The markedly different uptake patterns of glucose and 3-hydroxybutyrate may account for the observation that ketone bodies cannot substitute entirely for glucose as a fuel of respiration. For instance, in isolated perfused rat brain, electroencephalographic activity ceases when the circulating glucose concentration is lower than 1 to 2 m*M*, despite high circulating concentrations of 3-hydroxybutyrate (*21, 22*). This can be understood when it is considered that many areas of brain are relatively impermeable to 3-hydroxybutyrate and must rely wholly or almost so on glucose.

Under a variety of conditions, whole or regional brain metabolism has been found to increase. This can be a consequence of normal conditions requiring redistribution of blood flow and metabolism or can result from stress, pharmacological agents, or seizures (*23*). In the event that oxidative metabolism is increased and ketone body metabolism restricted by permeability, the increased fuel requirement must be met by glucose. If this cannot be done—for instance, if the blood glucose concentration is low or cannot be raised sufficiently to meet demand—changes in neural function may occur. Superficially it would appear that some areas of brain may be more susceptible than others.

Some years ago biochemical compartmentation was demonstrated in brain. It was found that the specific activity of glutamine was higher than that of its precursor glutamate when the label originated from short-chain fatty acids such as acetate or butyrate (*24*). If glucose or ketone bodies were the precursors, the specific activity of glutamate was higher than that of glutamine, as would be predicted from the normal precursor-product relationship. At first it may be thought that this is a result of anatomic compartmentation in different areas of brain and that our results are a visual presentation of this phenomenon. This view, however, must be rejected. 3-Hydroxybutyrate and glucose show identical biochemical patterns of labeling glutamate and glutamine; that is, the expected precursor-product relationship is obtained, in contrast to the entirely different morphological patterns of metabolism (*25*). On the other hand, butyrate and 3-hydroxybutyrate share similar regional patterns but different metabolic compartments.

In conclusion, ketone bodies are used by brain as a respiratory fuel, but their metabolism seems to be limited to specific regions by permeability. Therefore it may be necessary to revise the concept of ketone bodies as a substitute fuel of cerebral respiration. Instead, ketone bodies may be considered supplementary, partially supplying specific areas of brain but incapable of supporting the entire energy requirement of all portions.

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