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Hypothesis Paper

Ketone Bodies, Potential Therapeutic Uses

Richard L. Veech,[1] Britton Chance,[2] Yoshihiro Kashiwaya,[3] Henry A. Lardy,[4] and George F. Cahill, Jr.[5]

[1] *Unit on Metabolic Control, LMMB/NIAAA, Rockville, Maryland, U.S.A.*
[2] *Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pennsylvania, U.S.A.*
[3] *Division of Neurology, Tottori University, Faculty of Medicine, Yanago, Japan*
[4] *Enzyme Institute, University of Wisconsin, Madison, Wisconsin, U.S.A.*
[5] *Harvard Medical School, Boston, Massachusetts, U.S.A.*

Summary

Ketosis, meaning elevation of D-β-hydroxybutyrate (R-3-hydroxybutyrate) and acetoacetate, has been central to starving man's survival by providing nonglucose substrate to his evolutionarily hypertrophied brain, sparing muscle from destruction for glucose synthesis. Surprisingly, D-β-hydroxybutyrate (abbreviated "βOHB") may also provide a more efficient source of energy for brain per unit oxygen, supported by the same phenomenon noted in the isolated working perfused rat heart and in sperm. It has also been shown to decrease cell death in two human neuronal cultures, one a model of Alzheimer's and the other of Parkinson's disease. These observations raise the possibility that a number of neurologic disorders, genetic and acquired, might benefit by ketosis. Other beneficial effects from βOHB include an increased energy of ATP hydrolysis ($\Delta G'$) and its linked ionic gradients. This may be significant in drug-resistant epilepsy and in injury and anoxic states. The ability of βOHB to oxidize co-enzyme Q and reduce $NADP^+$ may also be important in decreasing free radical damage. Clinical maneuvers for increasing blood levels of βOHB to 2–5 mmol may require synthetic esters or polymers of βOHB taken orally, probably 100 to 150 g or more daily. This necessitates advances in food-science technology to provide at least enough orally acceptable synthetic material for animal and possibly subsequent clinical testing. The other major need is to bring the technology for the analysis of multiple metabolic "phenotypes" up to the level of sophistication of the instrumentation used, for example, in gene science or in structural biology. This technical strategy will be critical to the characterization of polygenic disorders by enhancing the knowledge gained from gene analysis and from the subsequent steps and modifications of the protein products themselves.

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Address correspondence to Dr. George F. Cahill, Jr., P. O. Box 367, Stoddard, NH 03464. Fax: 603-446-7750. E-mail: cahill@cheshire.net

HISTORY

Fear of Ketosis

Physicians have long been taught to fear ketosis; the hallmark of potentially fatal diabetic ketoacidosis (*1*) where severe insulin deficiency causes free fatty acids to pour out of adipose tissue and undergo conversion in liver to the ketone bodies, D-β-hydroxybutyrate (R-3-hydroxybutyrate) and acetoacetate.[1] In this condition, ketone bodies can reach 25 mM in blood, causing blood bicarbonate to fall to near zero, with resultant severe acidosis. This and the accompanying hypovolemia due to urinary loss of water from the hyperglycemia and glycosuria, plus loss of sodium and potassium from the ketonuria, result in death if untreated.

Ketosis is the Physiological Response to Fasting in Homo sapiens

This fear of ketosis may be exaggerated. Mild ketosis can have therapeutic potential in a variety of disparate disease states. Blood ketone bodies reach 5–7 mM in fasting man (*2*) and are essential to preserve muscle mass from conversion to glucose for brain consumption. Episodic starvation was a normal state during the evolution of the hunter-gatherer. Mild ketosis (2–7 mM) remains today peculiar to man as a species (except in some ruminants, particularly during exuberant lactation or during twinning). Man is distinguished from other animals by his large brain/body weight ratio and brain's very high energy requirements. At rest, 20% of oxygen consumption is needed to

[1] Acetone is historically the third "ketone body" and its metabolism provides daily about 3–5 g of glucose production in fasting man (*2*, *4*). The remaining acetone is lost via other pathways including lungs and urine. We will use "ketone bodies" to mean D-β-hydroxybutyrate plus acetoacetate. Levels of both in postabsorptive man approximate 0.010–0.015 mM, and after one week of fasting, 4–5 mM and 1–1.5 mM, respectively.

support the 1.5 kg of brain, which is 2% of body weight. It can be argued that since ketone bodies are the only available alternative to glucose for brain's energy, ketosis was a critical evolutionary development to provision man's hypertrophied brain while sparing muscle mass (*3*). The survival benefit is obvious; about 2 months for an average weight starving adult compared to a calculated 2–3 weeks were ketone bodies not available.

Ketosis as Treatment for Epilepsy

The ability of brain to use ketone bodies, with one notable exception, the treatment of epilepsy by prolonged fastings, has not been utilized therapeutically. In the early 20th century, French neurologists, including Pierre Marie, proposed fasting as a treatment for epilepsy on the grounds that it was the result of intestinal intoxication. This theory was furthered by a Wisconsin osteopath, Hugh Conklin, who successfully treated a proportion of epileptic children with a diet of only water for 30 days. Russell Wilder, of the Mayo Clinic, proposed that the beneficial effects of starvation in epilepsy could be achieved by feeding a high fat/low carbohydrate diet, thus creating the "ketogenic diet." The history of this therapy has been recently reviewed by Freeman and Vining (*5*). In a study of 150 consecutive, difficult to control, epileptic children, averaging 400 seizures per month on a mean of 6.2 antiepileptic medications, 30% had a greater than 90% decrease in seizures and 3 were seizure free on the "ketogenic diet." The "ketogenic diet" is comprised of 4 parts fat to 1 part protein, with almost no carbohydrate. The principal fat components are whipping cream, cheese, and fatty meats.

Two problems are present in ketosis therapy. First, ingestion of even small amounts of carbohydrate cause insulin release and an immediate drop in ketone body levels followed by seizures. Second, the mean plasma cholesterol in these children increased from 168 to 220 mg/100 ml, with a decrease in HDL, an increase in LDL, and elevation of total triglycerides, putting them at slightly greater risk of atherosclerosis. In practice, therefore, this diet is rarely used in patients over 17 years of age. Other groups have used the "ketogenic diet" for treatment of specific forms of epilepsy resulting from a genetic decrease in GLUT1 (*6*). Simpson et al. (*7*) showed that GLUT1 was the major glucose transporter across the blood/brain barrier. In these cases, ketone bodies provide an alternative energy substrate, compensating for decreased glucose transport into brain. The mechanism for the efficacy in other forms of epilepsy remains unknown (*8*).

The "ketogenic diet" has been used extensively in the treatment of obesity, and, like most other therapies, is only transiently effective at best, thanks to a decrease in caloric intake due to the unsavory qualities of the diet.

THE EFFECTS OF KETONE BODY METABOLISM

Improvement in Metabolic Efficiency

In the 1940's it was observed that β-hydroxybutyrate and acetoacetate were unique among the 16 carbohydrates, lipids, and intermediary metabolites tested on sperm in their ability to *decrease* oxygen consumption while *increasing* mobility (*9*, *10*). The reasons for this apparent increase in metabolic efficiency remained a mystery for 50 years. Recently, detailed studies by Veech and colleagues of the metabolism of ketone bodies in the working perfused rat heart showed that 5 mM ketone bodies added to the glucose-containing perfusate resulted in a 25% increase in hydraulic work with a significant decrease in oxygen consumption (*11*, *12*). It also resulted in a reduction of the mitochondrial NAD couple and an oxidation of the mitochondrial co-enzyme Q couple, increasing the energy of the redox span between site I and site II of the electron transport system (Fig. 1). An increase in the redox span between two sites will result in an increased energy release by the electron traveling across that span because:

$$\Delta G' = -nF\Delta E_{\text{SiteII/SiteI}}$$

where $\Delta G'$ is the free energy, n the number of electrons, F the Faraday constant, and ΔE the difference in redox potential between sites I and II. With an increase in redox energy of the respiratory chain, there is a corresponding increase in the energy of the protons ejected from the mitochondria at the energy-conserving sites, which is then reflected in an increase in the energy of ATP hydrolysis. It is often not emphasized in biochemical textbooks that while the standard free energy of ATP, $\Delta G^{\circ}_{\text{ATP}}$, is a constant, the actual free energy of ATP hydrolysis, $\Delta G'_{\text{ATP}}$, depends on both the standard free energy and the ratio of products over reactants. Put formally:

$$\Delta G'_{\text{ATP}} = \Delta G^{\circ}_{\text{ATP}} + RT\ln\frac{[\text{ADP}][\text{Pi}]}{[\text{ATP}]}$$

where R is the gas constant and T the temperature in degrees Kelvin.

Additionally, ketone bodies caused a 16-fold elevation in acetyl CoA content and increases in TCA cycle metabolites from citrate to α-ketoglutarate and its aminated partner, L-glutamate. Precisely similar effects were obtained from the addition of saturating doses of insulin, which in addition to increasing glucose transport into heart (*12*) through translocation of insulin sensitive GLUT4 to the plasma membrane, also stimulated the activity of the pyruvate dehydrogenase multienzyme complex (*11*). It was thus apparent that the acute metabolic effects of insulin in working heart could be mimicked by ketone bodies. The implication was that ketosis, which is the physiological response to insulin deprivation during starvation, was equivalent in metabolic effects to the actions of insulin. By providing an alternative substrate which is transported into cells on the monocarboxylate carrier, ketones by-passed the block in glucose transport caused by lack of insulin, even stimulating glycogen synthesis (*12*). Ketones also by-passed the blockade of pyruvate dehydrogenase induced by insulin deficiency by providing an alternative source of mitochondrial acetyl CoA.

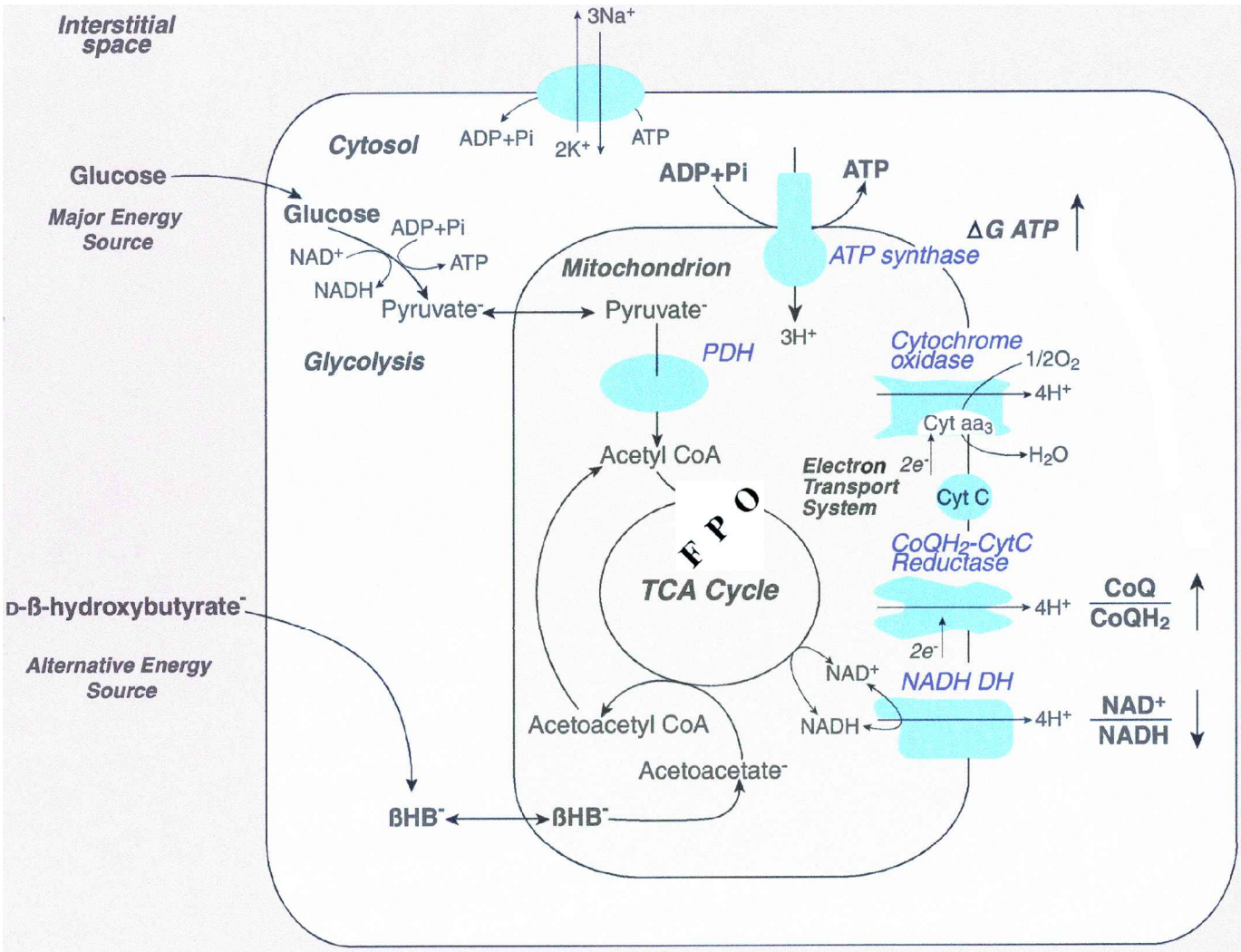

Figure 1. Ketone bodies added to glucose fundamentally alter mitochondrial metabolism. When added to glucose, a physiological level of ketone bodies reduces the mitochondrial NAD couple, oxidizes the co-enzyme Q couple, increases the $\Delta G'$ of ATP hydrolysis, and increases metabolic efficiency. These changes are shown on the right side of the figure. The arrows illustrate the effects of ketone bodies compared to glucose alone. Ketone bodies provide an alternative metabolic fuel which can act during blockade of glycolysis, as occurs in diabetes or insulin resistance, or during inhibition of pyruvate dehydrogenase complex (PDH) as occurs in the presence of amyloid β_{1-42}. Oxidation of the co-enzyme Q couple decreases the major source of mitochondrial oxygen radical generation. Increases in the $\Delta G'$ of ATP hydrolysis widen the extra/intracellular ionic gradients leading to hyperpolarization of cells, which may play a role in treating certain forms of epilepsy. The actions of ketone bodies mimic the acute effects of insulin in insulin-sensitive tissue. They are particularly important in brain, which, because of the blood–brain barrier, lacks insulin in most areas.

Increased Energy of Ion Gradients

In addition to increasing metabolic efficiency, the greater energy ($\Delta G'$) produced by ATP hydrolysis has another important consequence: it increases the extent and energy of the gradients of the major inorganic ions between the extra and intracellular phase of the cell (*13*). This is because the energy of the Na^+ and K^+ gradients are in near-equilibrium with the resting electrical potential between the phases and also with the $\Delta G'$ of ATP hydrolysis through the action of the Na^+ pump (EC 3.6.1.37). Increasing the energy of the K^+ gradient in muscle and nervous tissue necessarily must increase the potential between the extra and intracellular phases which is equal to the electric potential of $[K^+]_{out}/[K^+]_{in}$. Conversely, injury of the cell by a variety of means leads to a stereotypic reaction with the loss of cellular K^+ a gain of intracellular Na^+ and Ca^{2+} accompanied by swelling of the cell and loss of electrical potential. The relationship of cellular ATP energy and ionic gradients is of clinical importance in the treatment of acute trauma, and, probably through increased

inhibitory postsynaptic potentials, in the treatment of unwanted discharge of excitable high voltage tissue such as heart, muscle and nerve.

Human Cerebral Metabolism in Ketosis

Parallel to the rat heart studies reported above (*11*), Kety et al. (*14*) showed diminished cerebral blood flow in diabetic ketoacidosis (45 ml/min/100 g) and diminished oxygen consumption (2.7 ml O_2/min/100 g). These returned to the normal range after therapy (54 and 3.3, respectively). Owen et al. (*3*) found flow and oxygen consumption to be 45 and 2.96 in 1-month-fasted obese subjects, well below the normal levels reported for adult humans of 57 and 3.6 by McHenry (*15*). These data suggest a similar increase in metabolic efficiency in human brain using ketoacids as the principal source of energy in place of glucose.

Earlier studies by Schneider and Droller (*16*) showed acetoacetate infusion induced coma in rabbits whereas β-hydroxybutyrate had no effect. More recently, Kirsch and D'Alecy found ketosis induced tolerance to hypoxia in rodent models in vivo and in vitro (*17*, *18*).

Decreased Free Radical Damage

Another aspect of the effects of metabolizing ketone bodies is their ability to oxidize co-enzyme Q. The major source of mitochondrial free radicals is the half-reduced semiquinone of co-enzyme Q (*19*). Q semiquinone reacts directly with O_2 to form the superoxide radical $O_2^{-\cdot}$. By decreasing the reduced form of co-enzyme Q, the mitochondrial production of free radical can be decreased. In a second action of ketone body metabolism, in addition to reducing the mitochondrial NAD couple, there is also a reduction of the cytoplasmic free NADP couple. This favors the reduction of glutathione, which is in near equilibrium through the action of glutathione reductase (EC 1.6.4.2) (*20*). This in turn favors the destruction of H_2O_2 by the glutathione peroxidase reaction (EC 1.11.1.9).

KETOSIS FOR NEURODEGENERATIVE AND OTHER DISEASES

Alzheimer's Disease

Approximately one fifth of Alzheimer's disease can be related to 5 different genes, all of which lead in one way or another to the accumulation of amyloid proteins. The remaining cases have no apparent genetic cause for the increase in amyloid production. The development of metabolic treatments, therefore, offers novel alternatives to what appears to be a difficult problem for genetic manipulations. Several recently published articles give a rationale for the use of mild ketosis as a treatment. As discussed earlier, a major metabolic effect of the metabolism of ketone bodies is to by-pass a blockade of the pyruvate dehydrogenase multienzyme complex (*11*). It is also recognized that accumulation of amyloid peptides, (*21*) both intra (*22*) and extracellularly is a hallmark of Alzheimer's disease. Hoshi et al. (*23*) have shown that a fragment of the beta chain of amyloid stimulates the activity of glycogen synthase kinase 3β, leading to the phosphorylation and thus the inhibition of pyruvate dehydrogenase in primary cultures of hippocampal neurons. They also reported that the 1–42 fragment of the beta chain of amyloid, Aβ_{1-42}, can inhibit acetyl choline synthesis in cultures of septal neurons (*24*). A block in pyruvate dehydrogenase decreases citrate concentrations, the precursor of acetyl choline, through the action of citrate cleavage enzyme (EC 4.1.3.8) and choline acetyl transferase (EC 2.1.3.8). Metabolism of ketones overcomes the decrease in citrate content. It has now been shown that when cultured hippocampal neurons are exposed to Aβ_{1-42}, they die. Addition of 4 mM D-B-hydroxybutyrate protected these neurons from Aβ_{1-42} induced death (*25*). This finding and the recent identification of the aspartate protease responsible for the cleavage of the 1–42 fragment (*26*, *27*) and the possibility of immunization against amyloid (*28*) give hope that new therapies may soon be available for this currently untreatable disease.

Parkinson's Disease

Except for rare exceptions such as maternal mitochondrial inheritance (*29*), the disease appears to result from an acquired defect in the mitochondrial NADH multienzyme complex of the dopaminergic cells of the mesencephalon. The search for non-genetic therapies is therefore essential. Fortunately it is treatable for a period by administration of dopamine, but eventually continued free radical damage lessens the effectiveness of this therapy. The meperidine analogue MPP^+, when taken by humans causes increased oxygen radical formation (*30*) and inhibition of NADH dehydrogenase (*31*). Recent reports show that primary cultures of mesencephalic dopaminergic neurons exposed to MPP^+ could be protected from death by addition of 4 mM D-β-hydroxybutyrate (*25*). Although the mode of ketone body action has not been thoroughly investigated, it would be reasonable to suppose that they act by decreasing the source of mitochondrial oxygen radical formation by oxidizing the co-enzyme Q couple while at the same time reducing the redox potential of the NADP couple which, through glutathione, is the final detoxification step for H_2O_2. Trials of dopamine therapy in combination with ketone bodies, might be expected to prolong the useful therapeutic life of dopamine.

Friedreich's Ataxia

Friedreich's ataxia is a genetic disease resulting from decreased translation of the message for frataxin, a mitochondrial protein thought to be involved in iron export from mitochondria. As reviewed by Wilson et al. (*32*, *33*) this defect leads to mitochondrial iron overload with oxidative damage to a number of electron transport enzymes containing iron sulfur clusters as well as a decrease in aconitase, and to increased expression of the cytoplasmic iron uptake protein, transferrin. Therapy with coenzyme Q analogues appears to hold some promise in decreasing

myocardial damage, but to have no effects on the ataxia, probably due to poor penetration into CNS. Clearly, ketone body therapy might also have a role in this disease, first by increasing the substrate level for aconitase in brain and secondly by decreasing free radical damage by the mechanisms described above.

A recent study (*34*) reported that overexpression of frataxin in mammalian cells results in increased TCA cycle flux, increased mitochondrial membrane potential, and elevated ATP content. Precisely the same effects are achieved by administration of ketone bodies to heart (*12*). Ketone bodies, therefore, may provide an immediate approach in Friedreich's ataxia which does not require the insertion of a gene into brain, heart, or muscle.

Leprechaunisms and Lesser Forms of Insulin Resistance

An extreme form of insulin resistance is found in the rare diseases known as Leprechaunism and Rabson-Mendenhall syndromes, where a mutant insulin receptor is unable to bind insulin (*35*, *36*). The current treatment of these children, as in most cases of less severe insulin resistance, consists of administering increasing doses of insulin, up to several thousand units per day. Because insulin does not bind to the mutant receptor and since these cases are almost uniformly fatal, the rationale for this therapy is questionable. Because of the ability of ketone bodies to mimic the acute effects of insulin, their administration as an alternative substrate emerges as a clear therapeutic alternative. Another therapeutic possibility is diabetic lipodystrophy, where there are multiple defects in the complex pathway of insulin signaling. Similar conditions of presumed defects in insulin signaling include LaFora body disease (*37*). This dementia is a uniformly fatal genetic disease associated with a protein with analogy to a mutant tyrosine phosphatase. These patients accumulate LaFora bodies comprised of glycogen in brain and muscle and show insulin resistance. They develop myoclonic epilepsy and "drop" attacks during late puberty associated with progressive dementia. In one case of this disease being treated with the standard ketogenic diet, improvement in cognition, gait and awareness were noted after 1 month of the diet, but myclonus was still present (*37*).

Cognitive Disorders

Other possibilities include the use of ketone bodies in the treatment of cognitive disorders associated with tight glucose control in the treatment of type I diabetes. It has earlier been shown in experimental subjects (*38*) that in the presence of mild ketosis of 5–7 mM achieved during prolonged fasting, blood glucose could be lowered to below 1 mM without either convulsions or any impairment of cognitive function. It may therefore be worthwhile to explore the use of ketosis in the treatment of cognitive disorders associated with hypoglycemia as well as other disorders of cognition with no obvious relationship to blood sugar levels.

Ketone Bodies in Fluid Therapy

The uses of the substitution of D-β-hydroxybutyrate for racemic lactate in resuscitative fluid therapy has been discussed by Alam et al. (*39*). Using a rat hemorrhage model, resuscitation with standard Ringer's lactate solution led to apoptosis of lung parenchymal cells. If D-β-hydroxybutyrate was substituted for lactate, no apoptosis occurred. An explanation of this observation is the ability of lactate to reduce the cytosolic NAD couple causing the glyceraldehyde-3-phosphate dehydrogenase reaction to become rate limiting in glycolysis (*40*). Without glycolytic ATP production during hypoxia, activation of the NFκB signaling pathway led to profound consequences suggestive of the initiation of adult respiratory distress syndrome and multiple organ failure.

WHAT NEEDS TO BE DONE

Further laboratory experiments are required using transgenic animal models of Alzheimer's disease (*41*) or cell culture models substituting mitochondria from the platelets of patient's with maternally inherited Parkinson's disease (*29*) in order to broaden our understanding of the pathophysiological processes involved in these diseases. However, given the body of evidence already existing showing the therapeutic efficacy of ketone bodies in a variety of conditions (*5*, *42*), a direct study involving ketone therapy seems warranted. Such a study with human patients requires collaboration with the biotechnology industry. Firstly, large quantities, perhaps up to 100 to 150 g per day per patient (*43*), of a nutritionally acceptable form of ketone bodies would be required to induce a physiological ketosis of between 2 to 7 mM in humans without the untoward hypercholesterolemia associated with the very unappetizing, high-fat ketogenic diet. Fortunately the biotechnology industry has already developed methods for the large scale production of poly D-β-hydroxybutyrate by fermentation (*44*) or by insertion of the genes for its production in plants (*45*). Although this biodegradable plastic failed to be competitive with polyethylene as a source of plastic (*46*), the effort provides a means of producing cheaply large quantities of material. Although poly D-β-hydroxybutyrate exists in humans (*47*), the enzyme activity for its synthesis and degradation must be very low and the polymer, as produced by *Alcaligenes eutrophus* containing 5,000 to 2,500 monomeric units, is not readily absorbed after oral ingestion by rats. This, therefore, will require the partial hydrolysis of the natural material to smaller subunits and probably its esterification to produce a dietarily acceptable form of ketone bodies. Neither route should be particularly problematic. Desrochers et al. (*48*) have studied the (*R*-*S*)-1,3-butanediol acetoacetate ester given enterally and parenterally to conscious pigs, producing total ketone levels of 5 mM without deleterious side effects.

A second major task for the biotechnology industry is the production of suitable analytical equipment capable of performing the detailed metabolic analysis of the effects of ketone bodies that have led to the realization of their potential usefulness in

medicine (*11*, *12*). Just the detailed *metabolic control analysis* (*11*) required over 5 senior scientists and 3 years to determine the concentrations of the substrates and products, the kinetic constants forward and backward and the equilibrium constants and the flux through each of the enzymes of the pathways involved. Given existing analytical technology, this was a Quixotic task. Better microanalytical tools, including improved HPLC-mass spectrophotometric equipment capable of dealing with water soluble substrates and automated analysis are required.

A major conclusion is that a biotechnology dedicated to measurements "closer to the phenotype" is required if progress is to be made in understanding the dynamic changes in metabolism, particularly in multigenic interactions that most surely impact on human disease states. If these methods were at least as routine as those developed for analysis of molecular structure or for gene sequencing or other recent technologic developments, one could anticipate a more rapid application of the identification of metabolic "phenotypes" associated with disease and hence a more rapid implementation of the knowledge derived from the sequencing of the human genome (see recent reports from Fell and Wagner (*49*) and from Jeong et al. (*50*). Recent studies in yeast by Raamsdonk et al. (*51*) and reviewed by Cornish-Bowden and Luz Cárdenas (*52*) underscore this need and its usefulness in revealing otherwise silent mutations.

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