Measurements of Arteriovenous Differences in the Study of Substrate Supply to the Brain

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Less than a decade ago it was accepted biochemical dogma that the only energy-yielding substrate utilized by the brain was glucose. This belief was based on (a) the impairment of cerebral function in hypoglycaemic states, (b) the failure of potential energy-yielding substrates other than glucose to overcome the deleterious effects of hypoglycaemia and (c) measurements of arteriovenous differences across the head which indicated that glucose was the only circulating substrate taken up by the brain. Experiments which necessitated a reappraisal of the accepted view that the brain had an obligatory requirement for glucose were reported in 1967 by Cahill and his collaborators (Owen et al., 1967; Cahill et al., 1968). These experiments stemmed from calculations which suggested that in prolonged starvation sufficient glucose could not be synthesized from endogenous precursors to supply the metabolic needs of the brain and therefore other substrates must be utilized by this tissue. Measurements of arteriovenous differences in obese humans undergoing therapeutic starvation showed that the alternative substrates were ketone bodies, acetoacetate and 3-hydroxybutyrate (Owen et al., 1967). In this situation, ketone-body utilization accounted for about 60% of the oxygen consumed by the brain. This elegant experiment, with its teleological approach, renewed interest in the question of substrate supply to the brain and indicated the value of measurements of arteriovenous differences in such studies. An interesting question is why was this discovery not made earlier, because the technique of blood sampling from the internal jugular vein and an arterial site (femoral) was by no means new. At least two factors may have influenced the timing of the discovery, one being the somewhat earlier formulation of the role of free fatty acids as alternative substrates to glucose in many tissues (Fredrickson & Gordon, 1958) and the other the development of sensitive enzymic methods for the measurement of ketone bodies (Williamson et al., 1962).

The discovery that ketone bodies could be utilized by brain in prolonged starvation has stimulated further work on the question whether this is due to an adaptive change within the brain or simply to the increased availability of circulating ketone bodies.

One approach to this question is to measure the activities of the enzymes of ketone-body utilization in brains from animals in various physiological states. The results of such measurements in rat brain indicate that, in the adult, starvation, high-fat diets or
diabetes do not appreciably change the activities of the three relevant enzymes (3-hydroxybutyrate dehydrogenase, EC 1.1.1.30; 3-oxo acid CoA-transferase, EC 2.8.3.5; acetoacetyl-CoA acetyltransferase, EC 2.3.1.9), although the concentrations of blood ketone bodies vary over 20-fold in the situations studied (Williamson et al., 1971; Dierks-Ventling & Cone, 1971; Tildon et al., 1971). In contrast, during the suckling period the brain content of these enzymes was found to be 2-3-fold higher than in the adult (Klee & Sokoloff, 1967; Page et al., 1971; Tildon et al., 1971).

The presence of the requisite enzymes and the availability of circulating ketone bodies is not unequivocal proof that the brain uses these substrates in situations other than prolonged starvation. The final proof has been provided by measurements of arteriovenous differences for glucose and ketone bodies across the head of experimental animals and humans. The results indicate that in adult man and rat there is a direct relationship between the arterial concentration of ketone bodies and the arteriovenous difference in a variety of situations (Hawkins et al., 1971; Daniel et al., 1971; Gottstein et al., 1971; Persson et al., 1972). This means that availability of ketone bodies is the major factor controlling their utilization by the brain. In the suckling rat, for any given arterial concentration the arteriovenous difference for ketone bodies is at least 2-fold higher than in the adult. It seems reasonable to assume that the higher activities of the enzymes of ketone-body utilization are responsible for this difference (Page et al., 1971; Klee & Sokoloff, 1967; Tildon et al., 1971). In the neonatal human brain there appears to be a similar situation, but the increase in arteriovenous difference is less than in the suckling rat (Persson et al., 1972). Whether this more effective uptake of ketone bodies by human neonatal brain is due to increased enzyme activity or increased permeability is an open question.

Availability of ketone bodies results, not only in their increased uptake by the brain, but also in a decreased uptake of glucose (Owen et al., 1967). In addition, a larger proportion of the glucose taken up is released as lactate and pyruvate. For example, in the fed state about 15% of the glucose removed is converted into lactate+pyruvate; this increases to 44% after 6 weeks' starvation (Owen et al., 1967). In shorter-term starvation (15-120h), Wicklmayr & Dietze (1975) have demonstrated a significant correlation between ketone-body extraction by human brain and the proportion of glucose extracted that is released as lactate+pyruvate. Similar findings have been reported with rats (Hawkins et al., 1971; Ruderman et al., 1974). The explanation is presumably a decrease in the proportion of active pyruvate dehydrogenase. The net result of this 'sparing' of glucose is, of course, a decrease in the animal's requirement to synthesize 'new' glucose from endogenous precursors.

The key enzyme (3-oxo acid CoA-transferase) in the utilization of ketone bodies is virtually absent from the brain of sheep (Williamson et al., 1971), dog, calf and pig (Tildon & Sevdalian, 1972). The other enzyme concerned in the utilization of acetoacetate, acetoacetyl-CoA synthetase, is present in low activity in brain cytosolic fractions and is considered to have a purely biosynthetic function (Buckley & Williamson, 1973). This suggests that ketone bodies do not play a major role as alternative substrates for the nervous tissue of these species. Confirmation of this view, for ovine brain, has recently been provided by arteriovenous-difference measurements which show no significant uptake of hydroxybutyrate (and a small but significant output of acetoacetate) by brains of adult sheep, lambs or foetuses (Jones et al., 1975). Admittedly, the concentrations of ketone bodies in fed sheep are low (about 0.3 mmol), but a significant uptake of ketone bodies would have been detectable in the human. In contrast, there is an uptake of acetate by ovine brain (about 25% of the arterial concentration), but it makes only a minor contribution to brain metabolism in the fed state and this decreases after 7 days' starvation (Kammula & Fong, 1973). This period of starvation in sheep is not accompanied by any decrease in cerebral utilization of glucose or in its oxidation to CO₂ (Kammula & Fong, 1973), which is in direct contrast with the findings in man described above. This raises the question of how the sheep manages to maintain the same supply of glucose to the brain despite absence of caloric intake. One answer may be the large reserve of substrate provided by the rumen. Alternatively ovine brain may adapt to starvation by 'induction' of
the enzymes of ketone-body utilization; if so, it is surprising that glucose uptake and oxidation is unchanged.

A similar situation appears to exist for adult dog brain, namely low activity of 3-oxo acid CoA-transferase (Tildon & Sevdalian, 1972) is correlated with no significant cerebral uptake of ketone bodies and a resistance to starvation ketosis (Wiener et al., 1971). However, brains of puppies appear to be able to utilize ketone bodies (Spitzer & Weng, 1972).

In summary, measurements of arteriovenous differences, in conjunction with studies in vitro, have provided new information on cerebral metabolism, in particular the role of ketone bodies in decreasing glucose uptake and pyruvate oxidation and acting as alternative substrates. From present evidence it would appear that rat brain provides the best model for the energy metabolism of human brain.

For information on the techniques of measuring arteriovenous differences, the reader is referred to the following publications: rat (Hawkins et al., 1971); sheep (Jones et al., 1975; Kammula & Fong, 1973); human (Owen et al., 1967; Gottstein et al., 1971; McIlwain & Bachelard, 1971).

Note added in proof. The inability of sheep brain to utilize appreciable amounts of ketone bodies has been confirmed by infusion of 3-hydroxy[U-14C]butyrate (Lindsay & Setchell, 1974; D. B. Lindsay, personal communication). Even after infusion of ketone bodies to increase their concentration above 5 mM, less than 5% of the radioactivity appeared in venous blood CO2.

Kammula, R. G. & Fong, B. C. (1973) Am. J. Physiol. 225, 110-113