Further Observations on the Biochemical Lesion in Maple Syrup Urine Disease

MAPLE syrup urine disease is an inherited disorder of amino-acid metabolism characterized clinically by irritability, rigidity, convulsive seizures and coma commonly manifest in the first weeks of life. Menkes[1] found that patients afflicted with this neurological disorder excrete large amounts of branched-chain keto-acids in their urine, and Dancis *et al.*[2] demonstrated a deficiency in branched-chain keto-acid decarboxylase in leucocytes. This enzyme defect results in the accumulation of branched-chain keto- and amino-acids in the plasma and tissues of these patients.

It has not been possible to explain why increased concentrations of these normal metabolites in the plasma and tissues can cause a disorder of brain function. Tashian[3] reported that branched-chain keto-acids inhibited the activity of glutamic acid decarboxylase of rat brain homogenates, and suggested that this could limit the production of γ-aminobutyric acid. Howell and Lee[4] noted that whereas branched-chain keto-acids depressed the oxygen consumption of rat brain slices, homogenates were unaffected. Both of these investigations were based on the manometric measurement of either total oxygen consumption or carbon dioxide production rather than a specific enzyme assay.

The present investigation was undertaken to ascertain whether branched-chain keto-acids are capable of inhibiting two specific decarboxylation reactions: that of glutamic acid leading to the formation of γ-aminobutyric acid and that of pyruvate resulting in two carbon fragments. We chose to use α-ketoisocaproic acid in our experiments because it results from the transamination of leucine. This amino-acid appears to be responsible, either directly or through its keto-acid derivative, for the cerebral symptoms[5]. Adult Sprague–Dawley rats were killed by decapitation. Small samples of cerebral cortex and liver were removed and immediately homogenized in ice cold Krebs–Ringer buffer (*p*H 7·4) (5 ml. of buffer/g of tissue). Special decarboxylation vessels, described previously[6], were used in these experiments. For the estimation of glutamic acid decarboxylase, the incubation medium consisted of potassium phosphate buffer (0·1 molar *p*H 6·3), 0·1 molar L-glutamic acid (of which most was unlabelled L-glutamic acid and 0·04 μmoles/sample was [1-^{14}C] L-glutamic acid with a specific activity of 5 mc./mmole) and 5×10^{-4} molar pyridoxal phosphate. Pyruvate decarboxylase determinations were carried out as before[7]. Varying concentrations of α-ketoisocaproic acid (0 to 0·015 molar) were added to the vessels. All the samples were incubated at 37° C for 1 h and the reaction was stopped with two drops of 5 normal sulphuric acid added to the centre of the vessel. After an equilibration period of 1 h, the labelled carbon dioxide which was collected in 'Hyamine' was counted in a scintillation spectrophotometer. Blanks consisted of heat-inactivated homogenate and the degree of quenching was established by adding toluene labelled with carbon-14 to each vial as an internal standard. All determinations were carried out in triplicate and the results were expressed as μmoles $^{14}CO_2$ produced/g of protein/h.

The results of our preliminary investigations (Table 1) show that the *in vitro* addition of α-ketoisocaproic acid to fresh rat brain homogenates has no significant effect on the activity of glutamic acid decarboxylase, an enzyme present exclusively in brain. Investigations have demonstrated that the concentration of γ-aminobutyric acid in the brains of patients with maple syrup urine disease is about one quarter of the normal control values[8]. Glutamic acid is commensurately reduced, resulting in a normal glutamic acid/γ-aminobutyric acid ratio. These findings further suggest that the activity of glutamic acid decarboxylase is probably normal.

Table 1

Concentrations of ketoisocaproic acid	Cerebral cortex PD	Cerebral cortex GAD	Liver PD
No acid added	296	114	111
0·001 molar	203	108	–
0·005 molar	192	124	27
0·0075 molar	172	115	28
0·015 molar	149	–	39

The results are expressed as μmoles of carbon dioxide produced/g of homogenate protein/h. PD, Pyruvate decarboxylase; GAD, glutamic acid decarboxylase.

We noted, however, that the decarboxylation of pyruvate is reduced in the presence of very small concentrations of α-ketoisocaproic acid (0·001 molar). The inhibition was more pronounced with liver than with brain homogenates. Swaiman and Milstein[9] found that large concentrations of this acid did not block uptake of oxygen or oxidation of the carbon skeleton of (U-^{14}C)-glucose by brain slices of immature rabbits. This could reflect a species difference or the fact that a partial block at the level of pyruvate does not restrict the utilization of glucose by alternate metabolic pathways.

No information has been published about the branched-chain keto-acid levels in the brains of patients afflicted with maple syrup urine disease. During this investigation, we measured the branched-chain keto-acids in the grey matter of a 25 day old infant who died of this disease. The concentration was 0·0008 molar as estimated by the method described by Dancis *et al.*[10]. The keto-acid concentration in the plasma was 0·002 molar and the concentration of plasma leucine was 0·0046 molar immediately before death (personal communication from J. Dancis). This represents the highest concentration of leucine yet recorded in human plasma. It would therefore seem that in *in vitro* experiments, keto-acid concentrations of 0·002 molar or less approach the values fourd in patients affected with this disease. Even a 0·001 molar concentration of α-ketoisocaproic acid added to brain homogenates partially inhibits the decarboxylation of pyruvate, which suggests that the reduced concentrations of γ-aminobutyric acid and glutamic acid in the brain of patients with maple syrup urine disease can be the result of a failure of the Krebs cycle which would affect the production of α-ketoglutaric acid and the operation of the so-called "γ-aminobutyric acid shunt".

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Suppression of Plasma Immunoreactive Insulin in Rats given D-Mannoheptulose

SIMON and co-workers[1–3] produced marked hyperglycaemia in the fasting rat with a single injection of D-mannoheptulose, a seven carbon sugar found in avocados. Their work suggests that heptulose blocks normal pancreatic