The encephalopathic action of five-carbon-atom fatty acids in the rabbit

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Summary

1. Five-carbon-atom organic acids (C-5 acids) have been administered intravenously to rabbits with ventriculocisternal perfusion and continuous electroencephalographic recording (EEG). The concentration of the acids in the cerebrospinal fluid (CSF) perfusate have been compared with changes in integrated low-frequency activity in the EEG.

2. The C-5 acids investigated were those accumulating in inborn errors of metabolism, i.e. isovaleric acid, β-methylcrotonic acid, tiglic acid and α-keto- and α-hydroxy-isovaleric acid. Their activity was compared with that of valeric acid.

3. Valeric acid and isovaleric acid produced coma and pronounced increase in slow-wave electrical activity and these changes paralleled the increase in concentration of the acids in the CSF perfusate.

4. The concentration of β-methylcrotonic acid and tiglic acid in the CSF perfusate reached values comparable with valeric acid and isovaleric acid but showed less encephalopathic activity. An interaction between β-methylcrotonic acid and isovaleric acid was observed.

5. Although the concentrations of α-ketoisovaleric acid and α-hydroxyisovaleric acid rose to the lesser extent in the CSF perfusate, changes in rousability of the animal and in the EEG recording were demonstrated.

6. It is concluded that all the C-5 acids tested have encephalopathic activity although this is lessened by the presence of either a double bond or an oxygenated functional group.

Key words: brain perfusion, cerebrospinal fluid, coma, electroencephalography, inborn errors of metabolism, perfusion, volatile fatty acids.

Introduction

Volatile fatty acids containing three to eight carbon atoms have been shown to produce coma and electroencephalographic changes in rats and rabbits (Samson, Dahl & Dahl, 1956; White & Samson, 1956). The concentrations of these volatile acids are raised in the blood of patients with hepatic coma, and it has been suggested that elevation is at least in part responsible for the production of coma seen in liver failure (Muto & Takahashi, 1965; Zieve, 1966). In children with inborn errors of organic acid metabolism, high concentrations of organic acids in the blood are associated with coma. A parallel has been drawn between the production of coma in animals with straight-chain volatile fatty acids, and the coma occurring in these children. However, several of the volatile fatty acids accumulating in the organic acidemias are branched-chain acids and may also be unsaturated (Gompertz, 1974). In this study the effect of some branched-chain saturated, unsaturated and oxygenated acids that occur in these inherited
conditions has been investigated by intravenous administration to conscious rabbits during ventriculocisternal perfusion with artificial cerebrospinal fluid. The concentrations of these acids have been measured in the CSF perfusate throughout the experiments and simultaneous electroencephalographic recording has been subjected to computer-assisted analysis.

Previous experimental studies have shown that the effectiveness of volatile fatty acids in producing coma is related to chain-length. In this study, therefore, only C-5 acids have been used and have been restricted to those accumulating in children with inborn errors of organic metabolism. In view of earlier investigations involving straight-chain acids, valeric acid has been included to act as an internal standard and to allow comparison with earlier work.

The organic acids investigated and the conditions in which they accumulate are: isovaleric acid (isovaleric acidaemia); β-methylcrotonic acid (β-methylcrotonyl CoA carboxylase deficiency); tiglic acid (propionic acidaemia, methylmalonic acidaemia and β-ketothiolase deficiency); α-keto- and α-hydroxyisovaleric acid (maple-syrup urine disease) (Fig. 1) (Gompertz, 1974).

**Methods**

**Preparation of rabbits for ventriculocisternal perfusion and continuous EEG recording**

*Preparation.* The preparation of the rabbits for ventriculocisternal perfusion was essentially by the method of Moir & Dow (1970). The ventricular guide tubes were shortened to 3 mm as the longer tubes used by Moir & Dow produced epileptiform activity in the EEG tracing. The cisternal guide was modified to give a better seating on the skull. The EEG electrodes were constructed and implanted as described by Dow, McQueen & Townsend (1972) for their studies with rats. Four electrodes were used and were of sufficient length to abut on to the dura mater without penetrating it. Two of the electrodes were placed 11 mm posterior to the coronal suture and two were inserted 3 mm anterior to this suture. All four electrodes were placed 5 mm laterally from the sagittal suture. A template was used to position both the ventricular guide tubes and the EEG electrodes.

*Perfusion technique.* Artificial CSF was prepared as described by Moir & Dow (1970), except that inulin was replaced by Blue Dextran (2 mg/ml). The artificial CSF was perfused at a rate of 40–50

![Fig. 1. Structure of five-carbon-atom (C-5) acids administered in this study.](image-url)
$\mu l$/min; a peristaltic pump was used. Technicon Auto-analyzer tubing was employed to prevent diffusion of CO$_2$ from the artificial CSF through the walls of tubing and thus any appreciable change in the pH of the perfusate. Intracranial pressure was monitored continually with an open manometer.

The CSF perfusate from the cisternal cannula was collected with a fraction collector during consecutive 10 min periods. The flow rate of CSF perfusate of 40–50 $\mu l$/min, and the length of the tubing from the cisternal cannula to the fraction collector, resulted in a delay of approximately 15 min between the emergence of perfusate from the cisternal cannula and the collection of the timed specimen by the fraction collector. This delay time has been allowed for in correlating the EEG changes and the measurement of metabolite concentration in the perfusate.

**EEG recording.** Fine wire plugs (Amphenol 220-PO1) were inserted into the electrodes and a common contact was established with the cisternal cannula by using a probe clip. Electrical signals were amplified and recorded on paper and magnetic tape with a Grass 7B polygraph and a Tandberg 1000 instrumentation recorder. The signal from the right posterior electrode was subjected to computer analysis.

**Computer analysis of electroencephalographic activity**

The tape-recorded EEG signal was digitized at 64 Hz and divided into 4 s epochs for Fourier analysis. Each epoch was multiplied by a cosine bell window before being Fourier-transformed with a hardware Fast Fourier Transform device. A 4 s epoch gives 0.25 Hz resolution in the frequency domain and the coefficients from 0.25 to 1.75 Hz inclusive were added to give a low-frequency filter band.

These filter values for groups of five consecutive epochs were averaged to give a 20 s resolution. The plots of these 20 s epochs against time, smoothed with a twenty-point moving average filter, constitute the graphs in the Figures. The vertical scale is in arbitrary units of integrated amplitude and shows changes from the resting state of the amount of low-frequency activity in the original EEG trace.

**Administration and measurement of organic acids**

**Administration** The organic acids, $n$-valeric acid, isovaleric acid, $\beta$-methylcrotonic acid (3-methyl-2-butenoic acid), tiglic acid (2-methyl-2-butenoic acid), $\alpha$-ketoisovaleric acid (3-methyl-2-oxobutanoic acid) and $\alpha$-hydroxyisovaleric acid (3-methyl-2-hydroxybutanoic acid) were administered as their sodium salts in aqueous solution, pH 7–4. The final concentration of the fatty acid anion was 1 mol/l. The neutralized solutions were prepared freshly and given intravenously via an ear vessel at a dosage of 4 mmol/kg body weight, over a period of 12–15 min (White & Samson, 1956).

**Measurement in the CSF perfusate.** All acids except $\alpha$-hydroxyisovaleric acid were measured directly by gas chromatography of the free acid on a 20% neopentylglycoladipate–2% phosphoric acid column (1.5 m) at 130°C. The acids were quantified by using either one of the five acids not involved in the particular experiment or $n$-butyric acid as the internal standard. In a typical experiment 10 $\mu l$ of internal standard was added to 50 $\mu l$ of perfusate, the mixture was acidified with 1 drop of H$_3$PO$_4$ (2 mol/l) and 3 $\mu l$ was injected directly on to the column. In order to quantify $\alpha$-ketoisovaleric acid with this method, no injection heater was used and no evidence of spontaneous decarboxylation on the column was noted (Gompertz & Draffan, 1972). Standard mixtures of the volatile fatty acids were analysed daily to establish the relative response ratio of the gas chromatograph for the individual acids.

$\alpha$-Hydroxyisovaleric acid was measured as its methyl ester after ether extraction of the CSF perfusate. A sample of the perfusate was diluted with distilled water, acidified with H$_2$SO$_4$ (5 mol/l) and saturated with solid NaCl. The acidified solution was extracted three times with diethyl ether, and the pooled ether extracts were evaporated to dryness under N$_2$ at room temperature. Decanoic acid was added in methanol as internal standard and the mixture was methylated with a minimal quantity of an ethereal solution of diazomethane. $\alpha$-Hydroxyisovaleric acid methyl ester was measured by gas chromatography on a 12% diethylene-glycol–suc-cinate column (1.5 m) at 94°C.

**Experimental design**

The rabbits were placed in a restraining box; ventricular and cisternal cannulae were connected and perfusion with artificial CSF was started. EEG electrode leads were attached and ear veins cannulated. When all preparations were completed an assessment of the efficiency of perfusion and the quality of the EEG tracing was made once the rabbit had been alerted with auditory and tactile stimuli.
Only those animals with free CSF perfusion and an EEG without appreciable movement artifacts were selected for intravenous perfusion with the volatile fatty acids.

Three series of experiments were performed. In the first the reproducibility of effect of an individual fatty acid and the effect of the sodium cation were tested. In the second series of experiments the four non-oxygenated acids were used, i.e. valeric acid, isovaleric acid, $\beta$-methylcrotonic acid and tiglic acid. Each acid was presented in turn, first to a newly prepared animal; this initial infusion was followed by various numbers of the other acids. The initial experimental design was to perform experiments not only to test each acid in a newly prepared animal but also to test interaction between four different acids. As this series of experiments developed, and interaction became apparent, fewer acids were used in subsequent perfusions. In the third series, the two oxygenated acids, $\alpha$-keto- and $\alpha$-hydroxy-isovaleric acid, were compared with valeric acid.

During the perfusions, the animals were exposed to auditory and tactile stimuli to prevent drowsiness and the typical slow-wave electrical activity associated with sleep.

**Results**

Preliminary experiments showed that satisfactory EEG recording could be obtained during ventriculocisternal perfusion, and that valeric acid administered at a dose of 4 mmol/kg produced unconsciousness in rabbits with EEG changes of a comparable magnitude to those described previously. Computer-assisted analysis of these initial recordings showed that the maximal changes occurred in the lower-frequency range; this finding is comparable with the well-recognized changes in the EEG in liver and other metabolic disturbances (Kennedy, Parbhoo, MacGillivary & Sherlock, 1973; MacGillivary & Kennedy, 1969).

**Experiments 1a and 1b**

These experiments were designed to test reproducibility of effect and to exclude the influence of the sodium cation. In the first experiment (1a, Fig. 2), isovaleric acid was administered twice, the two intravenous infusions being separated by 1 h. The concentration of isovaleric acid in the CSF reached a maximum of 3–3.5 mmol/l, 15–20 min after the end of each infusion. The rise and fall of isovaleric acid concentrations were comparable during these infusions and the integrated EEG changes were similar. In experiment 1b two infusions of NaCl (1 mol/l; 4 mmol/kg) were administered 35 min apart; the first was given without stimulating the animal and the second with auditory and tactile stimulation. There was no change in the EEG tracing or level of consciousness during these two periods, but a subsequent administration of valeric acid produced an elevated concentration of valeric acid in the CSF with associated changes in the EEG analysis and level of consciousness. The animal was not rousable. This experiment indicated that the sodium cation at this concentration has no effect either on the EEG and level of consciousness of the animal or on its subsequent response to valeric acid.

**Experiments 2a–2e**

In this series of experiments each non-oxygenated acid was presented first to newly prepared animals, and followed by the other acids.

**Experiment 2a.** The four acids were perfused in the order, valeric acid, isovaleric acid, $\beta$-methylcrotonic acid and tiglic acid (Fig. 3). An additional adminis-
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Tiglic acid Valeric acid

EEG record

Conc. of acid (mmol/l)

0 60 120 180 240 300

Time (min)

FIG. 3. Effects of non-oxygenated C-5 acids on the integrated low-frequency EEG activity (upper trace) and the concentration of these acids in the CSF perfusate. •, Valeric acid; ■, isovaleric acid; ○, β-methylcrotonic acid (βMCA); □, tiglic acid. Black horizontal bars represent the periods of C-5 acid infusion. Breaks in the EEG record are due to changing magnetic tapes.

dration of valeric acid was performed at the end of the experiment to investigate whether there had been any changes in response to this acid due to the four previous perfusions. All four acids appeared in the CSF perfusate with maximum concentrations 5–10 min after the end of the infusion. Valeric acid and isovaleric acid produced appreciable changes in the EEG tracing and in the corresponding integrated low-frequency EEG analysis (Fig. 3). β-Methylcrotonic acid reached a higher concentration than the two previous acids but had a smaller effect on the EEG recordings. Tiglic acid had no obvious effect in this experiment, but the second administration of valeric acid at the end of the experiment showed that the previous acids perfused had not altered the sensitivity of the animal to valeric acid.

Experiment 2b. Here the four acids were perfused in the reverse order from experiment 2a (Fig. 4).

Tiglic acid βMCA

EEG record

Conc. of acid (mmol/l)

0 60 120 180 240 300

Time (min)

FIG. 4. Effect of perfusing unsaturated C-5 acids before the saturated acids. Symbols are as identified in Fig. 3.
Tiglic acid reached a concentration in the CSF perfusate comparable to that in the previous experiments and produced a change in consciousness and EEG record. The change in the integrated low-frequency EEG activity correlated with the rise and fall in tiglic acid concentrations in the CSF but was less than that seen with valeric acid and isovaleric acid. β-Methylcrotonic acid produced a similar effect. The rate of change in isovaleric acid concentration differed from the previous experiment. The initial rise in isovaleric acid concentration was not followed by a similar rate of fall; the concentration of this acid remained elevated in the CSF perfusate until the end of the experiment, i.e. for over 90 min after the maximum isovaleric acid concentration. However, valeric acid concentration rose to a maximum and fell in the same manner as in the previous experiment; this was in spite of isovaleric acid and β-methylcrotonic acid still being present in the perfusate. The prolonged elevation of isovaleric acid concentrations was paralleled by an extended EEG change; the integrated record was still showing increased slow-wave activity when the valeric acid perfusion produced a new peak of activity. This prolongation of raised isovaleric acid concentrations in the CSF was ascribed to an interaction between isovaleric acid and β-methylcrotonic acid.

Experiment 2c. In this experiment isovaleric acid was administered first and then again after β-methylcrotonic acid (Fig. 5). This experiment was designed to confirm the effect of β-methylcrotonic acid seen in the previous experiment: i.e. to test for the interaction between isovaleric acid and β-methylcrotonic acid suggested by the results of experiment 2b.

The first infusion of isovaleric acid produced changes in the concentrations of this acid in the CSF perfusate and changes in the EEG of the same type as seen in experiments 1a and 2a. β-Methylcrotonic acid had minimal effect on the EEG record, but showed the slow disappearance from the perfusate as seen in experiments 2a and 2b. The second isovaleric acid perfusion confirmed the interaction between isovaleric acid and β-methylcrotonic acid. The isovaleric acid concentration in the CSF perfusate remained at a higher value for an extended period of time, and the EEG changes were similarly prolonged.

Experiment 2d. Here β-methylcrotonic acid was perfused first, followed by tiglic acid and valeric acid (Fig. 6). Isovaleric acid was not included because of the interaction between it and β-methylcrotonic acid demonstrated in the previous experiments. All three acids produced appreciable EEG changes, β-methylcrotonic acid having an effect comparable with that seen in experiments 2a and 2b. Tiglic acid produced a similar small response to that in experiment 2b. Both unsaturated acids, β-methylcrotonic acid and tiglic acid, reached higher concentrations in the CSF perfusate than valeric acid but had a proportionally less effect on the EEG recording in relation to their concentration in the perfusate.

Experiment 2e. In all previous experiments in this series, the disappearance rate of β-methylcrotonic acid from the perfusate was prolonged compared with the saturated acids. However, in all these experiments a second perfusion had been started before β-methylcrotonic acid had been cleared from the CSF. This experiment was designed to exclude the

![Figure 5](image-url)  
Fig. 5. Interaction between β-methylcrotonic acid and isovaleric acid. Symbols are as identified in Fig. 3.
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possibility that the perfusion of a second acid might inhibit the clearance of \( \beta \)-methylcrotonic acid. The shape of the curve for \( \beta \)-methylcrotonic acid concentrations was the same in this experiment as in the previous experiments in which a perfusion with another acid was started while concentrations of \( \beta \)-methylcrotonic acid were still elevated.

Experiments 3a and 3b

In this series of experiments \( \alpha \)-ketoisovaleric acid and \( \alpha \)-hydroxyisovaleric acid were compared with valeric acid in four animals.

Experiment 3a. \( \alpha \)-Ketoisovaleric acid was infused into two rabbits, followed by valeric acid, introduced to give a standardized response. In one animal the maximum concentration of \( \alpha \)-ketoisovaleric acid in the CSF perfusate was 0.6 mmol/l and this produced appreciable EEG changes (Fig. 7). Although there was good correlation between the time of maximum valeric acid concentration and EEG changes, the alteration in EEG after the administration of \( \alpha \)-ketoisovaleric acid appeared to be maximal 15 min after the peak concentration of acid was reached in the CSF perfusate. During the period of maximum EEG change the animal was not rousable by painful stimuli. The reason for this late effect was not apparent. In the other animal the maximum concentra-

Fig. 6. Perfusion of \( \beta \)-methylcrotonic acid, followed by tiglic acid and valeric acid. Symbols are as identified in Fig. 3.

Fig. 7. Effect of \( \alpha \)-ketoisovaleric acid (\( \text{aKIVA} \)) on the integrated low-frequency EEG record. \( \text{v} \), \( \alpha \)-Ketoisovaleric acid; \( \text{o} \), valeric acid.

tion of \( \alpha \)-ketoisovaleric acid reached only 0.2 mmol/l but minimal EEG changes that might have been observed were masked owing to technical problems with the EEG recording. In both animals the response to valeric acid was the same as in previous experiments.

Blood and CSF were taken before and during \( \alpha \)-ketoisovaleric acid perfusion for measurement of valine concentrations as an index of the amount of \( \alpha \)-ketoisovaleric acid removed during the period of infusion by reverse transamination. During the study shown in Fig. 7, the plasma valine increased from 0.16 to 0.71 mmol/l during the infusion.

The valine concentration in the CSF perfusate rose from less than 0.02 to 0.10 mmol/l. Samples of CSF perfusate were also analysed by gas chromatography (see the Methods section) for \( \alpha \)-hydroxyisovaleric acid to see whether the low concentrations of \( \alpha \)-ketoisovaleric acid in the CSF were due to reduction of the keto acid to \( \alpha \)-hydroxyisovaleric acid. No \( \alpha \)-hydroxyisovaleric acid was detected in these samples.

Experiment 3b. \( \alpha \)-Hydroxyisovaleric acid was perfused into two rabbits, and in both experiments valeric acid was also used as standard. In both animals low concentrations of \( \alpha \)-hydroxyisovaleric acid were detected in the perfusate up to 3 h after administration. In the animal with the higher concentrations of \( \alpha \)-hydroxyisovaleric acid, 0.6 mmol/l
FIG. 8. Effect of $\alpha$-hydroxyisovaleric acid ($\alpha$HIVA) on the integrated low-frequency EEG record. $\uparrow$, $\alpha$-Hydroxyisovaleric acid; $\bullet$, valeric acid.

(Fig. 8), there was a pronounced change in the integrated low-frequency EEG response. In the other animal, the rise in $\alpha$-hydroxyisovaleric acid concentration was less, but still an appreciable EEG change was noted. The magnitude of change in this second animal was of the same order as that seen during spontaneous drowsiness, but the animal was not rousable and showed no response to painful stimuli. These stimuli would have immediately modified the EEG in any animals investigated before perfusion with fatty acid had been started.

Discussion

We have found that simultaneous recording of the EEG with ventriculocisternal perfusion is a useful method for studying the encephalopathic action of various organic anions in conscious animals. Using this technique we have confirmed that valeric acid produces coma when administered intravenously to rabbits (White & Samson, 1956) and analysis of the electrical activity in parallel with the measurement of the concentration of valeric acid in the CSF has shown a close correlation between the concentration of this acid in the CSF perfusate and changes in low-frequency electrical activity.

The action of valeric acid in experimental animals has been used by analogy to explain the coma observed in children with isovaleric acidaemia (Tanaka, 1973). Our findings show that intravenous administration of isovaleric acid does produce coma, and its effect is directly comparable with that of valeric acid. The preliminary experiments demonstrated the reproducibility of this effect and have excluded the sodium cation as the cause of the changes in level of consciousness and electrical activity.

$\beta$-Methylcrotonic acid and tiglic acid are also branched-chain acids but are unsaturated, unlike valeric acid and isovaleric acid. $\beta$-Methylcrotonic acid accumulates in $\beta$-methylcrotonyl CoA carboxylase deficiency, but its accumulation is limited by conversion into $\beta$-hydroxyisovaleric acid and by conjugation with glycine. However, the concentration of unconjugated $\beta$-methylcrotonic acid can reach 2 mmol/l in the urine of affected children during metabolic crises (Gompertz, Bartlett, Blair & Stern, 1973). Tiglic acid has been demonstrated in the urine of children with propionyl CoA carboxylase deficiency, and in $\beta$-ketothiolase deficiency (Nyhan, Ando, Rasmussen, Wallington, Kilroy, Cotton & Hull, 1972; Hillman & Keating, 1974). However, the effect of these two acids on the level of consciousness in experimental animals has not been investigated before. In the studies presented here the concentrations of $\beta$-methylcrotonic acid and tiglic acid in the CSF perfusate were comparable with the concentrations of the saturated acids, valeric acid and isovaleric acid. However, $\beta$-methylcrotonic acid and tiglic acid produced lesser changes in the integrated low-frequency EEG recording, but the slowing still correlated with the changes in the concentration of the acid in the CSF perfusate. The lack of response of the animal to painful stimuli differentiated these EEG changes from spontaneous sleep. Minor auditory and tactile stimuli in animals not receiving an acid alerted the animal, and this was associated with an immediate loss in low-frequency activity. During perfusion with $\beta$-methylcrotonic acid and tiglic acid even painful stimuli produce no change in the level of consciousness or in the EEG record.

The effect of $\beta$-methylcrotonic acid appeared to vary with the order in which the different acids were perfused. A greater effect was obtained when this acid was administered first than when it followed the administration of other acids. Tiglic acid was infused in three experiments; in two experiments the rousability of the animal was greatly reduced at the time of maximal EEG change. However, in the third experiment no effect was observed but on this occasion it was the fourth acid to be administered.

In the initial design of these experiments, each of the four acids was to be presented first followed by the other three. The lack of suitably prepared animals
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 prevented the testing of all the twenty-four possible combinations of four acids, but one interaction soon became apparent. The administration of \( \beta \)-methylcrotonic acid modified the subsequent response of the animals to isovaleric acid. The rate of clearance of isovaleric acid from the CSF perfusate was prolonged after the administration of \( \beta \)-methylcrotonic acid and this slow clearance of isovaleric acid was paralleled by an extended period of abnormal EEG activity. These results confirm the relationship between the concentration of an organic acid in the perfusate and the effect of the acid on the electrical activity of the brain.

The mechanism by which \( \beta \)-methylcrotonic acid might impair the clearance of isovaleric acid is not clear. Isovaleric acid and \( \beta \)-methylcrotonic acid occur intracellularly as their coenzyme A esters and are intermediary metabolites in the degradation of leucine. Isovaleryl coenzyme A is oxidized directly to \( \beta \)-methylcrotonyl coenzyme A. Conversion of either of these two acids into ketogenic intermediates would require activation to their coenzyme A derivatives. It could be suggested that \( \beta \)-methylcrotonic acid may compete with isovaleric acid for this initial enzyme-activation step. However, if this were so similar interactions should have been seen with other pairs of acids. Another possibility is that \( \beta \)-methylcrotonic acid (or its coenzyme A ester) inhibits isovaleryl CoA dehydrogenase. Differences in solubility of these acids are unlikely to account for the interaction seen here, as a similar interaction is not seen with valeric acid and \( \beta \)-methylcrotonic acid.

\( \alpha \)-Keto- and \( \alpha \)-hydroxy-isovaleric acid accumulate in branched-chain ketoaciduria (maple-syrup urine disease) together with six-carbon-atom keto acids. The oxygenated isovaleric acids are interconvertible, the \( \alpha \)-hydroxy acid predominating in blood and urine of children with this disease. In normal animals \( \alpha \)-ketoisovaleric acid is rapidly removed peripherally by reverse transamination to valine and by reduction to the hydroxy acid. Measurement of plasma valine concentration showed over a four-fold increase during perfusion of \( \alpha \)-ketoisovaleric acid. Thus transamination may have been a major factor in the low concentration of \( \alpha \)-ketoisovaleric acid (maximum 0.6 mmol/l) achieved in the CSF perfusate during these experiments. Although the concentration of \( \alpha \)-ketoisovaleric acid was much less than that obtained with non-oxygenated acids, there was an appreciable change in rousability and EEG response. The change in electrical activity was maximal 15 min after the peak concentration of \( \alpha \)-ketoisovaleric acid in the perfusate. This suggests that a metabolite of \( \alpha \)-ketoisovaleric acid might have been responsible for the change in EEG activity. However, analysis of \( \alpha \)-hydroxyisovaleric acid concentrations in the CSF during this period showed no conversion of the keto acid into the hydroxy acid. An alternative explanation is that \( \alpha \)-ketoisovaleric acid acts at some other site than the non-oxygenated acids, and the metabolites in this compartment do not equilibrate with the CSF freely.

The response of rabbits to \( \alpha \)-hydroxyisovaleric acid was similar to the response to \( \alpha \)-ketoisovaleric acid. Only a low concentration of the hydroxy acid was achieved in the CSF but this acid was present (in minimal amounts) for a very much longer period. The change in the integrated EEG record and in rousability were similarly prolonged. During these experiments no rise in plasma valine was detected, suggesting that minimal conversion of the hydroxy acid into the keto acid had occurred.

The low concentration of \( \alpha \)-hydroxyisovaleric acid found in the CSF perfusate during these experiments can be explained by the increased polarity of the molecule conferred by the hydroxy group, producing a lowered lipid and membrane solubility. Such a mechanism could also be in part responsible for the low concentrations of \( \alpha \)-ketoisovaleric acid achieved in the CSF when this acid is perfused. White & Samson (1956) compared lactate with propionate and \( \beta \)-hydroxybutyrate with butyrate to investigate the effect of introducing a hydroxyl group on the effectiveness of these three- and four-carbon-atom acids in producing coma in rabbits. They found that the hydroxylated acids had no effect at comparable dosage. A similar lack of response was found in rats by Samson et al. (1956): the difference in the results obtained here with \( \alpha \)-hydroxyisovaleric acid can be ascribed to the longer carbon chain increasing the lipid solubility of hydroxylated acid and/or to the more detailed assessment of EEG changes used in this present study.

This series of experiments support the hypothesis that the coma in isovaleric acidemia can be directly ascribed to raised isovaleric acid concentration in body fluids. The lesser response of the rabbits to \( \beta \)-methylcrotonic acid and tiglic acid suggests that the accumulation of these acids alone may not be the sole cause of coma seen in propionic acidaemia, methylmalonic acidaemia, \( \beta \)-methylcrotonyl CoA carboxylase deficiency and \( \beta \)-ketothiolase deficiency.
In rats and rabbits propionate is the short-chain acid least effective in producing coma (White & Samson, 1956; Samson et al., 1956). In propionic acidaemia it was considered possible that the increased tiglic acid production might be responsible for coma during acute episodes. Although there could be synergism between these two abnormal acids, the coma may be due to the accompanying increase in other metabolites such as lactic acid and ammonia.

In maple-syrup urine disease, the coma associated with acute attacks is associated with raised concentrations of \( \alpha \)-keto acids and \( \alpha \)-hydroxy acids in the plasma. The results presented here suggest that both \( \alpha \)-keto acids and \( \alpha \)-hydroxy acids can produce appreciable changes in consciousness. The observation that the hydroxy acid is also effective indicates that both hydroxy and keto branched-chain acids should be monitored in this disease (Gompertz & Draffan, 1972; Lancaster, Mamer & Scriver, 1974).

The technique of ventriculocisternal perfusion used in combination with computer-assisted analysis of EEG recordings has been used here to investigate the relative encephalopathic effects of different fatty acids accumulating in inherited metabolic diseases. This technique has great potential for investigating other metabolites that might be involved in the genesis of coma due to metabolic disease and could well be extended to a study of those compounds implicated in hepatic encephalopathy.

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References


Gompertz, D., Bartlett, K., Blair, D. & Stern, C.M.M. (1973) Child with a defect in leucine metabolism associated with \( \beta \)-hydroxyisovaleric aciduria and \( \beta \)-methylcrotonylglycinuria. Archives of Diseases in Childhood, 48, 975–977.


Muto, Y. & Takahashi, Y. (1965) Cited in Medicine from Abroad. Postgraduate Medicine, 37, A158.


