The resemblance to phenylketonuria is so marked that a similar genetic mechanism suggests itself, especially as the four cases of Menkes *et al.* were in one family. This aspect is being pursued.

After this paper was written we heard that Cusworth, Dancis, Menkes, Miller, and Westall had independently found keto-acids in the urine of their cases and identified them as the α -keto-acids corresponding to valine, leucine, and isoleucine (Westall, private communication). A possible explanation of the presence of keto- and hydroxy-acids in the urine in their cases, while the urinary concentrations of the parent amino-acids were not always raised, would be that the blood and tissue keto-acid concentration has to build up to a critical level before enough amino-acid is formed by transamination to exceed the renal threshold. The reverse situation holds in phenylketonuria, where the blood phenylalanine concentration has to build up to a critical level before phenylpyruvic acid appears in the urine. The alternative explanation, that the metabolic error is becoming more severe and more extensive with time, seems unlikely; it would suggest an acquired rather than an inborn error of metabolism.

This condition could easily be mistaken for phenylketonuria. Clinically they are very similar, they give exactly similar reactions with 2:4-dinitrophenylhydrazine reagent and identical urinary indole patterns. However, the ferric chloride reaction, while somewhat similar, is not identical in the two conditions. The smell of the urine is easily missed, and can be masked by preservatives; in this case the smell was not noticed until we deliberately examined the urine for it, having been led by the chemical reactions to consider "maple syrup urine disease" as a possible diagnosis. Acidification with dilute sulphuric acid in the cold enhances the smell considerably; it may be worth investigating, in this way, every case diagnosed as having phenylketonuria. Of course, amino-acid chromatography can differentiate the conditions conclusively.

"Maple syrup urine disease" is not a satisfactory name for the condition. Although one could use for example "leucic aciduria," there is no logical reason for picking one rather than another substance in the urine to provide a name for the disease. If it is confirmed that the metabolic defect suggested above is the cause of the condition, "carboxylase deficiency disease" might best meet the need.

Further work on the identification of the abnormal substances present in the body fluids and on determining the site and extent of the metabolic defect is continuing.

Summary

A grossly mentally retarded infant was found to excrete large amounts of keto-acids; these were identified as the α -keto-acids corresponding to valine, leucine, and isoleucine. The free amino-acids were also found in great excess in the urine, blood, and C.S.F. The urine had a characteristic smell resembling maple syrup; this seems to be largely due to the α -hydroxy-acids corresponding to valine, leucine, and isoleucine. It seems probable that this is an inborn error of the metabolism of the three amino-acids with a block at the oxidative decarboxylation stage. Tryptophan metabolism is also interfered with, as in phenylketonuria. Differentiation of this condition from phenylketonuria needs special care.

We thank Professor Alan Moncrieff for permission to study and report this case and for his interest in the work; the Research Committee of the Hospital for Sick Children for a Research Fellowship awarded to one of us (L. I. W.); Professor Sir George Pickering for kindly providing laboratory facilities; Dr. G. Pampiglione for the E.E.G. report; Dr. W. W. Payne for determining the fasting blood sugar; the nursing staff for their untiring co-operation; and Mrs. S. Phillips, Miss J. Wise, and Mr. P. Cousins for their expert technical assistance.

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"MAPLE SYRUP URINE DISEASE"

BY

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Menkes, Hurst, and Craig (1954) described a familial syndrome characterized by early onset of central nervous system symptoms, a maple syrup odour to the urine, and a fatal outcome. We have more recently studied a similar case, that of a child, in which the characteristic odour of maple syrup was first noted in the urine when he was 4 months old.

The patient was obviously mentally retarded and died at the age of 20 months, the longest survival on record. Samples of urine and plasma collected shortly before death and analysed by the Moore and Stein (1954) method revealed elevations of the branched chain amino-acids—leucine, isoleucine, and valine—strongly indicating a block in the further metabolism of these amino-acids. Other abnormalities were a high methionine and low cystine level, and reduced levels of several other amino-acids, notably alanine, serine, and threonine (see Fig.). Transaminase activity (Awapara and Seale, 1952) for the branched chain amino-acids was demonstrated in the tissues of the patient obtained at necropsy, indicating that the block lay somewhere below the level of the formation of the respective keto-acids. These observations have been previously presented (Westall, Dancis, and Miller, 1957; Westall, Dancis, Miller, and Levitz, 1958). Menkes (personal communication) later succeeded in demonstrating the keto-acids of leucine, isoleucine, and valine in the urine of this patient, adding significantly to the evidence that the metabolic block lay below the level of transamination.

Because all of the observations so far had been made on specimens collected shortly before the death of the patient, it was not clear whether the metabolic block was the primary factor of the patient's disease or a pre-terminal complication. Fortunately a urine from this patient was found that had been collected by the paediatrician a year before death and had been kept in deep freeze ever since. Analysis of this urine failed to reveal an elevation of the branched chain amino-acids, adding temporarily to the confusion. However, it was

The organic acids in the urine are also being investigated with gas phase chromatography. A preliminary analysis has demonstrated eight major peaks, three of which probably represent the keto-acids. It remains to investigate the rest of the peaks as soon as suitable standards are available. The presence of the next lower branched acids—isovaleric, isobutyric, and methylbutyric—must be investigated to locate more exactly the point of metabolic block. If the block lies below the level of oxidative decarboxylation we would expect large

excesses of these acids. The possibility that some of these peaks may be attributed to abnormal metabolites, particularly the respective hydroxy-acids of the branched amino-acids, has been entertained and is to be investigated. This was suggested by the recent report that hydroxybutyric acid could impart a "burnt-sugar odour" to the urine of a baby with a disease which may be related to "maple syrup disease" (Smith and Strang, 1958). Also a similar conversion is accomplished by the phenylketonuric in reducing phenylpyruvic acid to phenyllactic acid. We were greatly interested to hear recently that Mackenzie and Woolf (personal communication) have been studying a similar case where they have shown an elevation of the hydroxy-acids as well as the branched chain keto-acids.

Plasma amino-acid pattern determined by column chromatography (Moore and Stein, 1954). The patient's levels (broken line) are compared with a normal (continuous line).

possible to demonstrate and identify the corresponding keto-acids in the urine (Meister and Abendschein, 1956). It then became evident that the metabolic block was of long standing and probably the primary factor in the patient's disease.

Quantitative analysis of the urines for keto-acids, using keto-isocaproic acid as standard (Natelson, 1957), demonstrated that the patient excreted 252 mg./24 hours before death and 49 mg./24 hours in the urine collected a year before. This contrasts with figures from three normal infants of 4, 2, and 3 mg./24 hours. The reason for this variation in the amount excreted by the patient is not clear; it may have been due to partial degradation on storage, but it is of interest that it was much higher in the urine where the block "backed up" to involve the amino-acids.

The normal degradative pathway for the branched chain amino-acids is believed to involve oxidative decarboxylation of the keto-acids to the next lower acids—*isobutyric*, *isovaleric*, and *methylbutyric* (Coon and Gurin, 1949). Attempts were made to define more clearly the metabolic block in the patient's tissues. Using leucine-1-¹⁴C it was possible to demonstrate that fresh rat tissue (liver, brain, and kidney) was capable of cleaving the carboxyl-carbon from this amino-acid. Unfortunately, tissues obtained from an infant who died of trauma and maintained under identical conditions of refrigeration as those of our patient could not do this. Further study of the patient's tissues, therefore, was not profitable.

After exchanging information we decided to arrange simultaneous publication.

At this point it is a matter for speculation whether Menkes's original cases represent examples of the same metabolic disease. From the experience with this patient, it is possible that the normal amino-acid pattern (as distinct from keto-acids) found in the urine of one of their cases does not exclude the diagnosis. However, it will take further experience to decide how pathognomonic a criterion for diagnosis is the maple syrup odour in the urine, since this does seem to have been constantly present.

Summary

The case of a patient with "maple syrup urine disease" is described in which there was an increased excretion in the urine of the branched chain amino-acids leucine, isoleucine, and valine, and also their keto-acid analogues. It is thought that there is a metabolic block of the degradative pathway of these keto-acids in this patient.

It is a pleasure to acknowledge the help of Dr. James Dick, the child's paediatrician, who made the original diagnosis, and Dr. David Cusworth, who identified amino-acids regenerated from the isolated keto-acids. The gas phase chromatography was carried out by Dr. S. Lipsky and Dr. Robert Landowne. The research was aided by grants from the Association for Aid to Crippled Children, and P.H.S. grant 4024, National Institutes of Health.

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TREATMENT OF TRICHURIASIS WITH DITHIAZANINE

A PRELIMINARY REPORT

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Human infestation with whipworm (*Trichuris trichiura*) is said to be world-wide, and both Stoll (1947) and Faust (1949) have estimated that about 355 million persons are affected. Faust stated that the worm is most prevalent in the warm moist regions of the world, where ascariasis may coexist with trichuriasis in the same host, but he gives the incidence of whipworm infestation in Europe as 34 million.

Despite the known prevalence of human infestation, there was no satisfactory treatment until Swartzwelder *et al.* (1957) described the use of dithiazanine against trichuriasis and other worm infestations. Faust refers to the use of oil of chenopodium, carbon tetrachloride, latex of the fig tree ("higueronia"), and emetine hydrochloride, but none of these preparations is considered satisfactory. Jung (1954) described the use of hexylresorcinol enemata; and Dunn (1955) claimed success in 28 out of 31 children treated with piperazine adipate. With the same compound Hoekenga (1956) reported a 50% cure rate from single-dose treatment, but other workers, including Swartzwelder *et al.* (1956), failed to confirm these findings. Hucker and Schofield (1956) observed no therapeutic effect in a trial of four piperazine compounds, used orally, on patients heavily infected with whipworm.

The following report describes the preliminary findings in six mentally defective patients, treated with dithiazanine at Coldharbour Hospital, who were found to be carriers of a moderate whipworm load as judged by faecal egg counts. A more extensive trial is being conducted as larger quantities of dithiazanine become available.

The adult whipworm is usually found in the human caecum, but may also occur in the colon and rectum and

posterior part of the ileum. The female worm may measure up to 5 cm.; the male rather less. *Trichuris* eggs are barrel-shaped, having an outer and inner shell and transparent polar prominences. The eggs measure about 50 μ in the long axis. Infection can occur only by the ingestion of fully embryonated eggs, a process which requires three weeks in a warm moist habitat. After the egg is ingested a further three months must elapse before the worm can complete its development and begin egg-laying. Migration of the larva to other parts of the body does not occur.

The anthelmintic effects of the cyanine dyes, and of dithiazanine in particular, have been investigated by Peters *et al.* (1949) and by Swartzwelder *et al.* (1957). It seems that the action of the cyanines is due to their interference with oxygen uptake and carbohydrate metabolism, and the fact that certain parasitic worms make use of enzyme systems different from those required by mammalian tissues. Bueding and Swartzwelder (1957) have shown that the "amidinium" ion system is responsible for inhibiting oxygen uptake in the filarial worm of the cotton-rat and that cyanine dyes cause an irreversible inhibition of anaerobic carbohydrate metabolism in *Trichuris vulpis*, the whipworm of dogs. In human trichuriasis the cyanines probably affect metabolism in the worm by inhibiting oxidative processes and also anaerobic reactions. Bueding and Swartzwelder state:

"The amidinium ion system in which a quaternary nitrogen is separated from a tertiary nitrogen by a resonating or 'conjugated' carbon chain of alternating double and single bonds" is illustrated thus:

"If both nitrogens are incorporated in two heterocyclic rings, such compounds are known as cyanines." The formula for dithiazanine (3,3'-diethylthiadiazocyanine iodide) is as follows:

Trichuriasis was found by one of us (T.V.C.) at Coldharbour Hospital in January, 1955, when a patient under investigation for loss of weight, anorexia, and episodal diarrhoea showed a large number of eggs in the stools. Other patients subsequently examined also showed unusually high trichuris egg counts, which were in the region of 30,000 eggs per g. of faeces. Further tests revealed that about half the 160 patients in the hospital were infected. Measures to prevent the spread of whipworm have been largely successful, but various therapeutic trials up to December, 1957, gave negative or equivocal results. Following the papers by Swartzwelder *et al.* (1956, 1957), a small quantity of dithiazanine was made available at Coldharbour Hospital, and six patients, known to be carriers of whipworm, were selected for treatment.

Method

In view of the findings of Swartzwelder *et al.* (1957) the trial consisted of a five-day course of treatment with dithiazanine, given orally as 100-mg. "telmid" tablets. These tablets contain dithiazanine covered with a coating of cellulose acetate phthalate and an outer sugar coating.