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Methyl Mercaptan in Relation to Foetor Hepaticus

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The presence of a characteristic odour (foetor hepaticus) in the breath of patients with severe liver disease is a well-recognized finding. This has been described variously as 'a musty smell' (Schiff, 1946), 'the smell of a freshly opened corpse' (Himsworth, 1947) and 'a mixture of rotten eggs and garlic' (Davidson, 1949). Reviewing the literature Lichtman (1949) referred to the smell as 'amin breath' but added that there was no evidence as to the chemical nature of the compound responsible. Davidson suggested that it was a mercaptan derived from cystine or methionine, but showed that its presence was not dependent upon methionine therapy. What is probably less well recognized is that the same or a very similar odour is sometimes present in the urine of patients manifesting foetor hepaticus (Lichtman, 1949). When present in the urine the smell may be so strong as to be highly offensive. Recently a case of massive hepatic necrosis has been studied in University College Hospital, London, W.C. 1, in which the smell was very marked in the urine and opportunity was taken to isolate and identify the compound producing the odour.

Case report

E.N. a housewife aged 45 years was admitted to University College Hospital jaundiced and in light coma. The following history was obtained from her relatives and from her physician. The illness started in December 1953 with diarrhoea, anorexia and nausea; a few days later she was noticed to be lightly jaundiced. However, she did not take to her bed and after a few days her jaundice faded and she felt better. In mid-January 1954 she again felt unwell and jaundice returned together with severe vomiting. Despite rest in bed her condition did not improve and she was admitted to another hospital on 4 February 1954. There she was treated with aureomycin but her condition continued to deteriorate and after a few days she became delirious; at the same time it was observed that the size of her liver was decreasing and that she had developed a strong foetor hepaticus and neurological signs characteristic of acute hepatic failure. She was therefore transferred to University College Hospital for further treatment. This consisted of continuous infusion of 25% glucose into the superior vena cava together with careful control of the fluid and electrolyte balance, intravenous sodium glutamate and prophylactic penicillin. Paper-chromatographic examination of the plasma repeatedly showed a moderate increase in the concentration of the commonly found amino acids, but a very marked increase of methionine, as has been observed

previously in cases of severe hepatitis (Walshe, 1953). Death occurred after the patient had been in coma for 10 days. Post-mortem examination (Dr R. A. B. Drury) showed that the liver weighed only 840 g. and that this reduction in size was due to almost complete destruction of the parenchymal cells.

Whilst the patient was in University College Hospital all urine was collected over thymol and stored at 4°. It was observed that an odour very similar to the characteristic foetor hepaticus in the breath was present in the urine as soon as it was passed. It seemed probable that this odourous substance in the urine was either an alkyl mercaptan $C_nH_{2n+1}SH$, a dialkyl sulphide $C_nH_{2n+1}S.C_nH_{2n+1}$ or a dialkyl disulphide $C_nH_{2n+1}S.S.C_nH_{2n+1}$ or a mixture of all three. It was decided to employ the method developed by Challenger & Rawlings (1937) and Blackburn & Challenger (1938) for identification.

EXPERIMENTAL

Examination of the urine. A slow stream of nitrogen was passed through approx. 5–6 l. of the urine and then successively through tubes containing dilute H_2SO_4 (to remove NH_3) and then through (a) aqueous $Hg(CN)_2$ and (b) aqueous $HgCl_2$. The volume of liquid in the tubes was about 5 ml. in each. A deposit formed in (a) which had a very faint yellow tint, gave an odour of methyl mercaptan (CH_3SH) on acidification and melted unsharply and decomposed from 150 to 180°. It was heated for some time with water, to remove $Hg(CN)_2$ from small quantities of the double compound $(CH_3S)_2Hg$, $Hg(CN)_2$, filtered and recrystallized from much hot ethanol, when it separated in shining leaflets closely resembling in appearance $Hg(SCH_3)_2$. These melted and decomposed at 170–174° and at 168° in admixture with an authentic sample of m.p. 167–168° (decomposed). $Hg(SCH_3)_2$ melts and decomposes at 174–175°. The authentic sample evidently contained traces of $Hg(CN)_2$, hence the lower m.p.

Similar m.p.'s and mixed m.p.'s were obtained with the $Hg(CN)_2$ ppt. which had been heated with water, but not recrystallized. When the m.p. tube containing the $Hg(SCH_3)_2$ from the urine was removed from the apparatus, the characteristic odour of $(CH_3)_2S$ was readily detected. This arises from the thermal decomposition:

Its formation affords further evidence of the presence of CH_3SH in the urine. The deposit in tube (b) in the original experiment was much smaller in amount than that in (a). It gave a scarcely perceptible odour with $NaOH$, indicating the absence of the double compound $2(CH_3)_2S$, $3HgCl_2$ and hence of $(CH_3)_2S$ from the urine (see below and Challenger & Rawlings, 1937). With dilute acid the odour of CH_3SH was readily detected. The solid sintered from 150° and decomposed at 180° without completely melting, a behaviour consistent with that of a mixture of CH_3SHgCl , $HgCl_2$ and CH_3SHgCl . The deposit in the $HgCl_2$ arose either from traces of CH_3SH , which escaped precipitation in the cyanide or, more likely, by fission of $CH_3.S.S.CH_3$ produced by atmospheric oxidation of the CH_3SH in the urine. Disulphides pass unchanged through $Hg(CN)_2$.

DISCUSSION

The high concentration of methionine observed in the blood of the patient suggests that the methyl mercaptan may have arisen from this amino acid by hydrolytic or reductive fission of the bond between the CH_3S- group and the adjoining carbon atom, giving rise to homoserine or α -aminobutyric acid respectively as the second product. Such a fission has been observed with methionine and *S*-methylcysteine giving methyl mercaptan, both in cultures of the mould *Scopulariopsis brevicaulis* (Challenger & Charlton, 1947) and with the enzyme thionase occurring in liver tissue (Binkley, 1950).

It is well known that, in addition to acting as a source of cysteine and of other amino acids, methionine can supply a methyl group for the biological synthesis of choline, creatine, carnosine, adrenaline and *N*-methylnicotinamide. The methionine is then re-formed and is maintained at a fairly constant level. Many of these transmethyations presumably take place in the liver and in cases of severe damage to that organ, methyl transfer is possibly inhibited, with the result that methionine may accumulate; such an accumulation has actually been observed in the case of this and other patients with severe parenchymal liver disease (Walshe, 1953). The organism may then employ another mechanism, namely fission of the other carbon-sulphur link with elimination of methyl mercaptan, in order to deal with the excess of methionine. Evidence for this fission was obtained by Dent (1947), who found that, in normal subjects and especially in a case of the Fanconi syndrome, increased excretion of α -aminobutyric acid followed the administration of methionine 'as if some direct desulphurization had taken place'. The fate of the CH_3S- group of the methionine was not investigated.

The oxidation of the methyl group of methionine labelled with ^{14}C giving $^{14}CO_2$ has been studied by du Vigneaud, Mackenzie and their colleagues (for review see du Vigneaud, 1952) in intact rats and with liver and kidney-cortex slices. Mercaptan production may, of course, be preceded by oxidative deamination of methionine and formation of the corresponding α -keto acid, which has been shown by Challenger & Liu (1950) to yield methyl mercaptan and, by methylation, dimethyl sulphide in cultures of *S. brevicaulis*. Amination of the keto acid to methionine followed by fission, was, however, not excluded.

The absence of dimethyl sulphide in the urine is noteworthy and may be due to impairment of the methylation processes of the liver. In man, methyl mercaptan may be expected to be methylated, to some extent at least, to dimethyl sulphide. This sulphide is almost certainly exhaled on administration to man of thiourea, a type of mercaptan

(Challenger & Liu, 1950; Astwood, 1943*a, b*; Himsworth, 1943), and also on injection into dogs (Pohl, 1904). Man also converts sodium tellurite Na_2TeO_3 into dimethyl telluride, $(\text{CH}_3)_2\text{Te}$ (Bird & Challenger, 1939). The odour of the breath of the patient E.N. was less unpleasant than that of the urine. This may have been due to the oxidation of the methyl mercaptan to dimethyl disulphide, $\text{CH}_3\cdot\text{S}\cdot\text{S}\cdot\text{CH}_3$, which has a less obnoxious odour. The breath was not examined chemically, so the presence of some dimethyl sulphide, $\text{CH}_3\cdot\text{S}\cdot\text{CH}_3$, is not excluded.

The accumulation of methionine in the blood may possibly have aggravated the toxic symptoms in the case of this patient E.N.; we know of no work on the pharmacological properties of methyl mercaptan. However, Roberts & Eckstein (1944) state that dimethyl disulphide is toxic to rats, the fatal intra-peritoneal dose ranging from 90 to 100 mg./kg. body weight. Dimethyl disulphide appears to yield methyl mercaptan under these conditions and, in common with

methylisothiurea, $\text{HN}=\text{C}(\text{SCH}_3)\cdot\text{NH}_2$

and methylxanthate, $\text{S}=\text{C}(\text{SCH}_3)\cdot\text{OC}_2\text{H}_5$,

to exert a lipotropic action in rats, which is also exhibited by dimethyl sulphide (Roberts & Eckstein, 1944). Methyl mercaptan is readily produced from dimethyl disulphide by reduction and from the other two substances by hydrolysis. Some observations indicate a toxic action of methionine, when present in the diet in excess. It has been shown by Barclay, Kenney & Cooke (1945) that large doses of methionine produce ketosis in man, acetone and β -hydroxybutyric acid being excreted. Raised blood keto acid levels have been reported in patients with severe liver damage by Seligson, McCormick & Sborov (1952) and in patients in hepatic coma by Amatuzio & Nesbitt (1950). Methionine may also produce acidosis as the sulphur is metabolized principally to inorganic sulphates. Furthermore, Watson (1949) has described a patient with chronic liver disease who lapsed into coma on each occasion that methionine was given. Earle, Smull & Victor (1942) found that when methionine was fed to rats as 6.4–12% of the McCollum stock diet, or of a low-protein and low-fat diet, severe atrophy of the liver cells, but no cirrhosis, was produced. All the rats lost weight and considerable mortality occurred in about 1 week. Roth & Allison (1950) showed that rats on a diet containing 4.8% of methionine and 0.1% of choline chloride lost weight, mainly owing to loss of fat. With 7% methionine, loss of weight and excessive destruction of body tissue occurred. These authors refer to the still obscure relation between methionine and fat metabolism and cite the work of Barclay *et al.* (1945) already mentioned. An observation by Challenger, Lisle & Dransfield (1954) may have some

relevance to this discussion. Cultures of *S. brevicaulis* and of *Aspergillus niger* convert sodium selenate Na_2SeO_4 into volatile dimethyl selenide, $(\text{CH}_3)_2\text{Se}$. When

was added as a methyl donor to the cultures, the yield of dimethyl selenide was considerably reduced, although 90% of its methyl groups were found to be labelled. Presumably any substantial increase over the amount of endogenous methionine which the mould normally has at its disposal diminishes its capacity for methylation.

SUMMARY

1. A case of massive hepatic necrosis exhibited foetor hepaticus in the breath and a similar odour in the urine.

2. Passage of nitrogen through the urine into mercuric cyanide yielded mercury dimethyl mercaptide $\text{Hg}(\text{SCH}_3)_2$.

3. The probable source of the methyl mercaptan is the CH_3S — group of methionine, the normal demethylating processes being inhibited by liver damage.

4. Both methyl mercaptan and dimethyl disulphide may be exhaled in the breath.

5. Analogous reactions in certain micro-organisms, and with the enzyme thionase, and the toxicity of methionine to man and animals are considered.

Note added in proof: Sherlock *et al.* (1954) have also commented on the toxicity of methionine in cases of liver disease. It is to be inferred that they attribute this effect to ammonia formation.

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A Glucuronic-decomposing Enzyme from Rumen Micro-organisms

3. MODE OF ACTION, SPECIFICITY AND INHIBITION BY ACIDIC SUGAR DERIVATIVES*

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Other papers in this series (Karunairatnam & Levvy, 1951; Marsh, 1954) have described the discovery of a glucuronide-decomposing enzyme in micro-organisms normally present in the sheep rumen, and the partial purification of the enzyme and the kinetics of its action upon some biosynthetic glucosiduronic acids. No attempts have hitherto been made to demonstrate the hydrolytic action of the so-called β -glucuronidases of *Escherichia coli* and other micro-organisms, and while in the previous work on the rumen enzyme there was no evidence of any alternative mode of action, classification of it has been delayed until the sugar residue after enzyme action could be identified with certainty. The action upon simple α - and β -glycosides and glycosiduronic acids of the three common hexose series was examined. For this specificity study the general method, adopted previously for the study of tissue β -glucuronidase (Levy & Marsh, 1952), whereby possible alternative substrates were tested as competitive inhibitors for the enzymic decomposition of a known standard substrate, was used throughout.

Inhibition of the glucuronide-decomposing rumen enzyme by D-glucosaccharo-1 $\rightarrow$ 4-lactone was noted by Levvy (1952); the negligible lactonization at the nearly neutral pH of optimum activity of the enzyme explained the negative results previously obtained by Karunairatnam & Levvy (1951) with saccharate solutions. The inhibitory effects of this and other hexaric acids and derivatives, and of the common hexuronic acids, were investigated.

The results presented in this paper show that the enzyme isolated from the sheep rumen has the same *in vitro* action and specificity as tissue β -glucuronidase, and may therefore be classified as a true β -glucuronidase.

* Part 2, Marsh (1954).

MATERIALS AND METHODS

Materials. The following compounds were synthesized: phenyl α -D-glucopyranoside (Fischer & v. Mechel, 1916), phenyl β -D-glucopyranoside (Fischer & Armstrong, 1901), (-)-menthyl α -D-glucopyranoside (Fischer & Bergmann, 1917), (-)-menthyl β -D-glucopyranoside (Treibs & Franke, 1950), methyl α -D-glucopyranoside (Helferich & Schäfer, 1948), methyl α - and β -D-galactopyranosides (Dale & Hudson, 1930), methyl α -D-mannopyranoside (Fischer, 1895), methyl β -D-mannopyranoside isopropanol solvate (Isbell & Frush, 1940).

From the above glycosides the following glycosiduronic acids were prepared by catalytic oxidation (Marsh, 1952): (-)-menthyl α -D-glucosiduronic acid, methyl α - and β -D-galactosiduronic acids, methyl α - and β -D-mannosiduronic acids; 3-O-methyl D-glucuronic acid was similarly prepared from 1:2-O-isopropylidene 3-O-methyl D-glucose (Vischer & Reichstein, 1944). Hydrolysis of methyl α -D-mannosiduronic acid by the method of Ault, Haworth & Hirst (1935) yielded D-mannuronic acid, which was converted into mannuronate by treatment with dil. NaOH.

Methyl α -D-xyloside was donated by Professor E. L. Hirst to Dr G. A. Levvy, and the di-O-isopropylidene derivative of 2-keto L-gulonic acid by Dr F. Bergel; to remove the acetone groups of the latter compound, a 10% (w/v) aqueous soln. was boiled for 30 min. (Reichstein & Grüssner, 1934). The following compounds were presented by Professor F. Smith to Dr G. A. Levvy: D-glucosaccharo-1 $\rightarrow$ 4- and 6 $\rightarrow$ 3-monolactones and 1 $\rightarrow$ 5-6 $\rightarrow$ 3-dilactone (Smith, 1944), D-mannosaccharo-1 $\rightarrow$ 4-6 $\rightarrow$ 3-dilactone (Haworth, Heslop, Salt & Smith, 1944), methyl β -D-glucofuranosiduronate 6 $\rightarrow$ 3-lactone (Owen, Peat & Jones, 1941) and methyl 4-O-methyl D-glucosiduronic acid amide (mixture of α - and β -forms) (Smith, 1951). By treating the corresponding lactones with dil. NaOH, solutions of D-mannosaccharate and methyl β -D-glucofuranosiduronate were prepared; the latter compound unlike phenolic glucofuranosiduronate solutions (see Tsou & Seligman, 1952), is stable at neutral pH (Owen *et al.* 1941). Methyl 4-O-methyl D-glucosiduronate was prepared by heating an aqueous solution of the amide with dil. NaOH (Smith, 1951), and the glycosidic linkage