In summary, this work presents evidence of a protective effect of taurine on viability of lymphoblastoids in conditions of cell damage produced by a model of lipid peroxidation. Taurine effects seem to occur through an action on calcium permeability through the injured membranes.

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Metabolism of mandelonitrile in the rat

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Mandelonitrile, $C_6H_5CH(OH)CN$, arises from the hydrolysis of amygdalin, found in apricot pits and bitter almonds, and of laetrile, a drug controversially used in cancer chemotherapy [1]. Mandelonitrile is also used for the treatment of urinary tract infection [2]. Amygdalin has been shown to release hydrogen cyanide *in vivo* in rodents [3] and dogs [4], and it has been postulated that mandelonitrile may be an intermediate in this conversion. These reactions are catalyzed by the "emulsin" complex of enzymes in plants [5] and by the gut microflora [6], but may also be non-enzymic [7].

The present studies were designed to elucidate the possible metabolic pathways of mandelonitrile in the rat. Metabolites were identified and quantitatively determined to establish the relevant importance of these pathways with reference to the toxicity of mandelonitrile.

Materials and methods

Chemicals. *dl*-Mandelonitrile, b.p. 170°, density 1.24 g/ml, was obtained from Albright & Wilson Ltd., Warley, West Midlands, U.K.

Animal preparation. Male and female Wistar rats (Birmingham Wistar strain), weighing 200–350 g, were maintained on a diet as previously described [8]. The animals were housed in metabolism cages which allowed the separate collection of urine and faeces. Mandelonitrile was administered to adult rats as a solution in corn oil ("Mazola") at doses of 30 mg/kg (0.2 ml) orally by stomach tube. Urine was collected for 24 hr before dosing and for successive 24-hr periods up to 4 days.

Metabolite identification. Metabolites were identified in 10-μl aliquots of urine and acidic ethereal extracts by paper chromatography (Whatman 3MM) using butan-1-ol/ethanol/aq. NH_3 (sp. g. 0.88)/water (10:10:1:4, by vol.) as solvent [9] and by TLC on plates coated with silica gel G (0.25 mm thick) containing a fluorescent indicator (E Merck AG, Darmstadt, West Germany) using propan-2-ol/aq. NH_3 (sp. g. 0.88) (4:1, v/v) [10]. Mandelic acid and benzoic acid were detected by 8-hydroxyquinoline sulphate [11] and hippuric acid by *p*-dimethylamino-benzaldehyde in acetic anhydride (10%, w/v) [12]. Cyanide (CN^-) and thiocyanate (SCN^-) were detected as violet spots against a

red background when the chromatogram was sprayed with neutral red in ethanol (pH 6.8 to 8.0).

Metabolite determination. CN^- and SCN^- were estimated in samples of dilute urine by the method of Pettigrew and Fell [13]. Absorbances were read at an isobestic wavelength of 510 nm. SCN^- was separated from CN^- by bubbling off hydrogen cyanide from acidified urine with nitrogen [14]. Mandelic acid and hippuric acid were estimated by gas–liquid chormatography (GLC) as their methyl esters in samples of the ethereal extracts of acidified urine after their methylation with diazomethane. A Pye series 104 dual ionization chromatograph (W. G. Pye & Co. Ltd., Cambridge, U.K.) fitted with a column packed with silanized "Supasorb" and coated with 5% OV-17 was used [15]. The temperature of the column was held at 130° for 3 min and then raised to 240° at a rate of 12°/min; the argon flow rate was 45 ml/min. The recovery of mandelic acid and hippuric acid added to normal urine were 98 ± 3% and 100 ± 2% respectively. When statistical comparisons were made, Student's *t*-test was used, with $P < 0.05$ to indicate a significant difference between mean values [16].

Results and discussion

Identification of metabolites. CN^-, SCN^-, hippuric acid, benzoic acid and mandelic acid were identified as metabolites of mandelonitrile in the urine of dosed rats (Table 1). Mandelic acid (R_f 1.30 min), as the methyl ester, was readily detected by GLC in the methylated ethereal extracts of acidified urine. Trace amounts of two other metabolites (R_f 2.5 and 2.8 min) were also detected but not characterized.

Estimation of metabolites. There was substantial excretion of CN^-, SCN^-, and hippuric acid within the first 24 hr after dosing with mandelonitrile; from 24 hr to 96 hr significant further excretion occurred (Table 2). Smaller amounts of mandelic acid (13% of dose) were present in urine collected within 72 hr after dosing but were not present in the 72–96 hr urine or in normal urine.

From these results a scheme for the metabolism of mandelonitrile in the rat is proposed (Fig. 1). The cyanohydrin, mandelonitrile, is converted to cyanide and benzaldehyde. This reaction is catalyzed by a hydroxynitrile-lyase in plants [5] and may also be catalyzed by enzymes present in the gut microflora [7]; some non-enzymic decomposition also occurs [5, 7]. Cyanohydrins (α-hydroxynitriles) are intermediates in the metabolism of organonitriles which

Table 1. Chromatographic parameters of the metabolites of mandelonitrile excreted in urine

Metabolites	R_f*	R_f†	R_t‡ (min)
Cyanide	0.52	0.10	
Thiocyanate	0.64	0.77	
Benzoic acid	0.91	0.53	0.54
Hippuric acid	0.77	0.48	10.6
Mandelic acid	0.86	0.50	1.30

* Descending paper (Whatman 3MM) chromatography in butan-1-ol/ethanol/aq. NH_3 (sp. g. 0.88)/H_2O (10:10:1:4, v/v), 16 hr run.

† TLC in propan-2-ol/aq. NH_3 (sp. g. 0.88) (4:1, v/v), 3 hr run.

‡ GLC of methyl esters on a 5% "OV-17" column. Methyl *p*-chlorobenzoate was used as the internal standard (R_t 7.2 min).

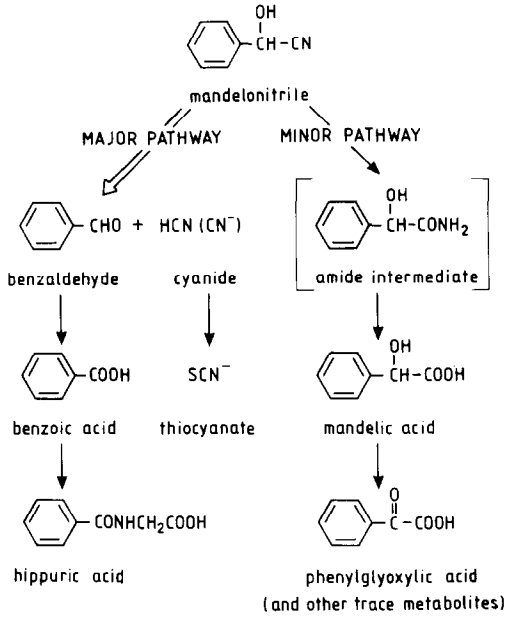

Fig. 1. Proposed metabolic pathways for mandelonitrile in the rat. Cyanide, thiocyanate, benzoic acid, hippuric acid and mandelic acid were identified in urine. Other hypothetical metabolites are shown.

Table 2. Excretion of metabolites in the urine of rats dosed with mandelonitrile (225 μmoles/kg, 30 mg/kg*)

Metabolite	0–24 Hours Amount (μmoles)	0–24 Hours % Dose	24–96 Hours Amount (μmoles)	24–96 Hours % Dose
Cyanide	23.2 ± 0.70	43.5 ± 1.2	1.1 ± 0.06	2.1 ± 0.08
Thiocyanate	11.0 ± 0.60	20.6 ± 1.0	10.6 ± 0.24	19.8 ± 0.55
Hippuric acid†	34.9 ± 1.8	56.1 ± 2.0	9.4 ± 0.50	15.1 ± 0.43
Mandelic acid	6.3 ± 0.62	8.6 ± 0.71	3.4 ± 0.24	0.45 ± 0.30

* Values are mean ± S.E.M. obtained from six rats. Mandelic acid was absent from control urine and in samples of urine collected between 72 and 96 hr after dosing. The combined recoveries of hippuric acid and mandelic acids account for 84.3% of the dose.

† Results are corrected for hippuric acid present in normal urine.

are hydroxylated in the α-methylene group by microsomal liver enzymes; mandelonitrile, for example, is produced as a metabolite of benzylcyanide [7, 14]. Benzaldehyde is then oxidized to benzoic acid which is conjugated with glycine to form hippuric acid. Cyanide, the other major urinary metabolite, is also partly detoxified to thiocyanate, a reaction catalysed by thiosulphate-cyanide-sulphur transferase (EC 2.1.1.1.) or rhodanese [17]. The excretion of CN^- and SCN^- together, corresponding to 86% of the dose, indicated that scission was the major metabolic pathway of mandelonitrile *in vivo*. The benzaldehyde formed concomitantly would be oxidized to benzoic acid and mainly converted to hippuric acid; 71.2% of the dose was excreted in this form. Conversion of mandelonitrile to mandelic acid occurred to the extent of 13.1% of the dose which constitutes a relatively minor but significant metabolic pathway. In Fig. 1, it is proposed that mandelonitrile is oxidized via an amide intermediate to mandelic acid, a reaction known to occur with other aromatic cyanides and nitriles [18]. Trace amounts of other metabolites detected by GLC but not characterized possibly include *p*-hydroxy-mandelic acid and phenylglyoxylic acid, which are known to be formed from the metabolism of mandelic acid in man [19].

The significant high levels of CN^- relative to SCN^- within the first 24 hr after dosing but not afterwards ($P < 0.05$) may indicate an initial saturation of the rhodanese enzyme, perhaps as a result of limitation of sulphur availability. This is in agreement with the pharmacokinetics of administered potassium cyanide in the rat [20] and dog [21]. Moreover, toxicity of laetrile to dogs occurred before CN^- could be converted to SCN^- [4]. Thus, the failure, rapidly and effectively to detoxify this initial large amount of CN^- formed from mandelonitrile accounts for the acute toxicity of this compound in the rat.

In summary, male and female Wistar rats were dosed orally with mandelonitrile (30 mg/kg), and metabolites were identified and estimated in urine. The cyanide plus thiocyanate excreted represented 86% of the dose, hippuric acid formed via the same metabolic pathway corresponded to 71% of the dose, while 13% of the dose was excreted as mandelic acid. A metabolic scheme was proposed for mandelonitrile (Fig. 1), involving a major toxication pathway yielding cyanide and a minor detoxication pathway to mandelic acid.

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Silymarin protection against hepatic lipid peroxidation induced by acute ethanol intoxication in the rat

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From the metabolic viewpoint, the liver is one of the organs primarily affected by ethanol consumption [1]. Some of the metabolic alterations induced by ethanol have been implicated in the production of liver damage after prolonged and excessive alcohol ingestion [1–4]. Among these, the hypothesis of ethanol-induced lipid peroxidative injury in the liver has been re-evaluated recently [5]. In experimental animals, it has been demonstrated that acute and chronic [5–8] ethanol administration produce a drastic increase in the hepatic content of reduced glutathione (GSH), the most important protective biomolecule against chemically-induced cytotoxicity [5, 9]. In fact, GSH can participate in the elimination of either reactive xenobiotics by conjugation [10], hydroperoxides by reduction [11], or free radicals by direct quenching [9]. Ethanol-induced liver GSH depletion has been observed concomitantly with an enhancement in hepatic lipid peroxidation, measured by different experimental procedures [5, 6, 12, 13]. Liver GSH depletion [14] and increased malondialdehyde levels [15] have also been reported in liver biopsy specimens from chronic alcoholic patients, when compared to basal values. These observations indicate that hepatic lipid peroxidation