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METABOLIC STUDIES ON N-METHYLPYRIDINIUM-2-ALDOXIME—II

THE CONVERSION TO N-METHYLPYRIDINIUM-2-NITRILE

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Abstract—N-methylpyridinium-2-nitrile has been identified in urine from rats and human beings treated with N-methylpyridinium-2-aldoxime. When injected into rats the nitrile is partially converted to inorganic thiocyanate and a metabolite possibly identical with metabolite X found in urine from oxime-treated animals. The nitrile was somewhat more toxic than the oxime.

EVIDENCE has been reported in the literature[1] for the *in vitro* conversion of N-methylpyridinium-2-aldoxime to N-methylpyridinium-2-nitrile. The aim of the present investigation was to see if the same reaction occurs *in vivo*. An analytical procedure for N-methylpyridinium-2-nitrile (MPN) in biological material has been developed and applied to urine from rats and human beings which had received N-methylpyridinium-2-aldoxime methanesulphonate (P2S). In a previous paper in the series it was reported that small amounts of thiocyanate are formed in rats treated with P2S.[2] As it is known that many nitriles are converted to thiocyanate in the body experiments were now performed to see if this also happens with MPN.

MATERIALS AND METHODS

N-methylpyridinium-2-nitrile methanesulphonate (*MPN*) was synthesized as follows: 5·2 g pyridine-2-nitrile and 5·7 g methyl methanesulphonate were solved in benzene and heated under reflux for 2 hr. The solid mass obtained after cooling was washed with dry ether and the product recrystallized from absolute ethanol. A weight of 5·2 g (yield: 50 per cent) of a white, very hygroscopic substance was obtained. The melting point (determined on a Kofler heating stage) was 128 °C. (Found: C, 44·8; H, 4·61. Calc. for $C_8H_{10}N_2O_3S$: C, 44·8; H, 4·67).

MPN was measured by a procedure based on the determination of cyanide by Zinke-Königs reaction following the rapid hydrolysis of the nitrile to cyanide in alkaline solution. Thiocyanate, which interferes in the colorimetric step, was removed by silver precipitation before hydrolysis of the sample. The analysis was carried out as follows: Urine was treated with $AgNO_3$ and NaCl as described in the previous paper.[2] To 0·4 ml of the supernatant was added 3·2 ml water and 0·4 ml 1 M NaOH. Ten minutes later 0·8 ml 1 M NaH_2PO_4 and 0·2 ml 1% Chloramine T solution were added, followed after 1 min by 1 ml of barbituric acid pyridine reagent (3 g barbituric acid + 1·6 ml HCl (s.g. 1·19) and 16 ml pyridine diluted with water to 50 ml final volume*). The absorbancy at 580 mμ was determined from 7 to 15 min later. The

* This is the reagent used in Ref. 3, but the composition of the reagent is incorrectly described in this reference.

MPN content of the sample was obtained from a standard graph. It was verified that the hydrolysis of MPN is complete within 1 min under these conditions and that MPN added to normal urine was quantitatively recovered by the analytical procedure. Thiocyanate and the cyanide-forming metabolite were determined as described previously.

The identity of MPN in the urine from P2S treated animals was established by paper chromatography. Ascending development on Whatman no. 1 was used. The spots were revealed by a colour reaction based on the hydrolysis of MPN to cyanide and the immediate reaction of the latter with a modified copper–phenolphthalin reagent[4]* The colour, red spots on white background, was developed by spraying the paper with a mixture of equal volumes of 0·1 M KOH and a solution prepared by mixing 2·5 ml 1% phenolphthalin in ethanol and 50 ml 0·1% $CuSO_4 \cdot 5H_2O$ in water and diluting this mixture to 100 ml with water.

The LD_{50} determinations were performed on inbred mice of both sexes weighing 20 ±1 g. The calculations were made according to Miller and Tainter.[5] Solutions were freshly prepared in distilled water and the injected volume was 10 ml/kg body weight.

RESULTS

Rats were given P2S and the urinary excretion of MPN was then determined. The results (Table 1) shows that about 8 per cent of the orally administered and 4 per cent

TABLE 1. MPN DETERMINATIONS ON URINE FROM NORMAL AND P2S TREATED RATS

Two rats received 120 μmoles P2S i.m. and two 400 μmoles orally The 24-hr urine was collected and assayed for MPN.

Dose of P2S	μmoles MPN/24 hr	
	Control	After P2S
400 μmoles *per os*	<0·2	31·5
	<0·2	30·8
120 μmoles i.m.	<0·2	4·8
	<0·2	4·6

of the intramuscularly injected P2S was excreted as MPN during the first 24-hr interval. Paper chromatography of the urine from P2S treated rats demonstrated the presence of a nitrile with the following R_f-values: acetone–water (3:1) R_f 0·65; *n*-butanol–acetic acid–water (4:1:1) R_f 0·43 and phenol–water (4:1) R_f 0·91. The R_f values were found to be identical with those of an authentic specimen of MPN.

P2S was also administered orally to human volunteers and the urinary excretion of MPN determined. As shown in Fig. 1, a significant excretion of MPN was detected but the amount found during the first 2 days corresponded only to about 0·3 per cent of the P2S administered. Thus, considerably less MPN was excreted in human beings than in rats.

Of interest in this connection is the toxicity of MPN and P2S. The LD_{50} ±s.e. (after i.p. injection in mice) was found to be 75 ±3 mg/kg and 132 ± 4 mg/kg, respectively.

* Thiocyanate did not respond to this test. It was not possible to detect cyanide on paper chromatograms.

As many nitriles are converted to thiocyanate *in vivo*, rats were injected with MPN and the urine analysed for thiocyanate by the two methods used previously.[2] The results are shown in Fig. 2.

From these results it is evident that thiocyanate and a compound which behaves as metabolite X formed in P2S treated animals[2] are found in the urine from MPN

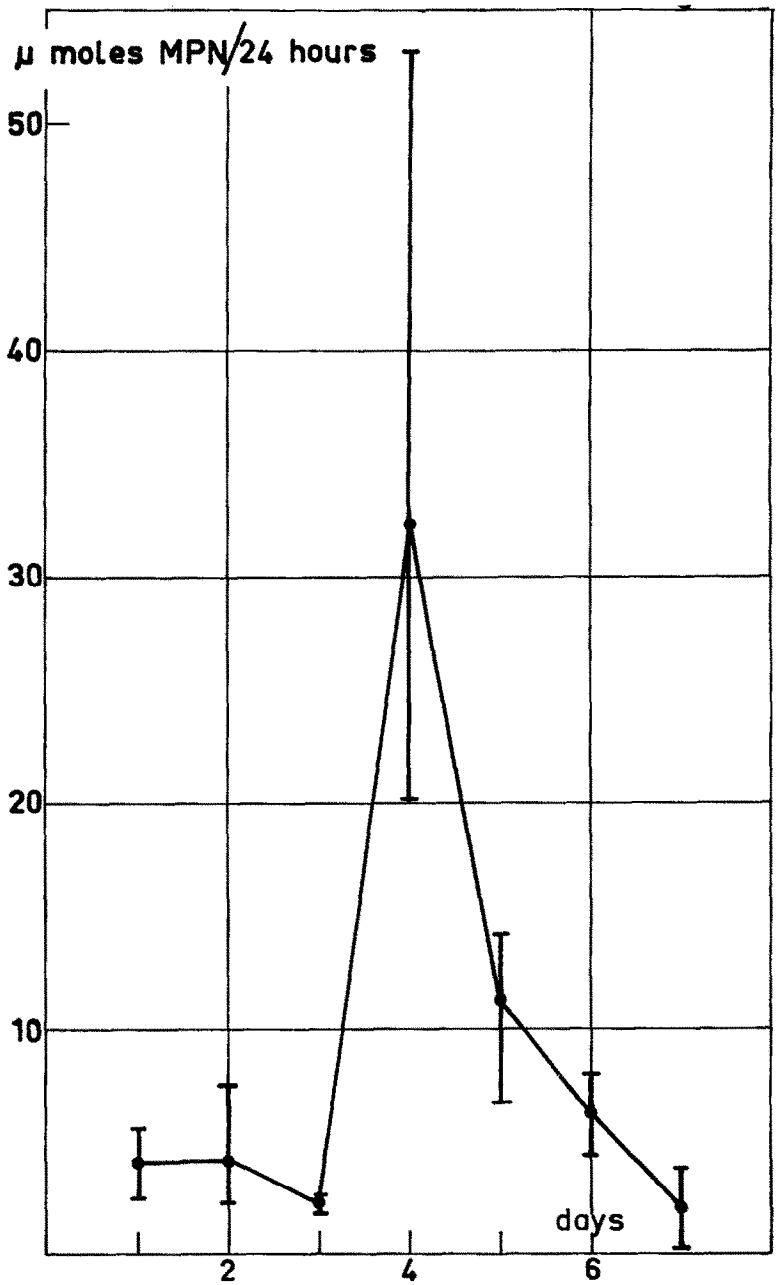

FIG. 1. MPN determinations on urine from P2S treated human beings. Four human volunteers were given 3 g of P2S orally on day 3.

treated animals.* The amounts found corresponded to from 6 to 7 per cent each of thiocyanate and metabolite X.

With a tenfold smaller dose of nitrile the amounts of thiocyanate and metabolite X found in the first 24-hr period were 5 and 10 per cent, respectively.

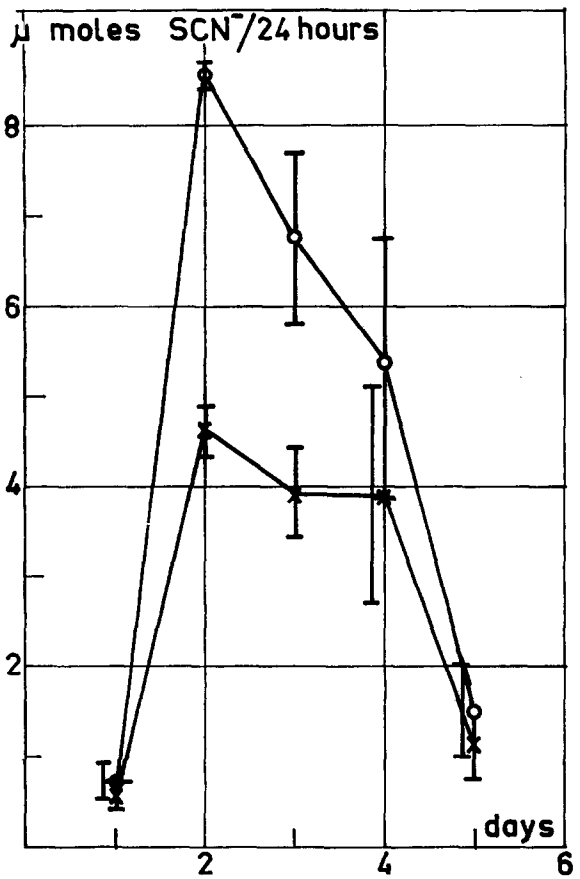

FIG. 2. Thiocyanate determinations on urine from MPN treated rats. Two rats received an intramuscular injection of 120 μmoles of MPN (corresponding to 90 mg/kg) on day 1. ○——○ Method I (thiocyanate plus unknown metabolite). +——+ Method II true thiocyanate.

DISCUSSION

The results presented in this paper show that thiocyanate and possibly metabolite X are formed from MPN. Since it has also been demonstrated that MPN is formed from P2S *in vivo* it is very likely that the nitrile is the precursor of both thiocyanate and metabolite X. Agreeing with this hypothesis is the fact that man excretes less nitrile and forms less thiocyanate and metabolite X after administration of P2S than do rats.

Although MPN is slightly more toxic than P2S it cannot significantly contribute to the toxicity of P2S as only a small fraction of P2S is converted to MPN.

* Traces of cyanide (less than 0·5 μmoles/24 hr) were also found in these urine samples.

The conversion of MPN to thiocyanate is of interest, as no heterocyclic nitriles have been studied from this point of view. It may be mentioned that aliphatic, but not aromatic, nitriles are converted to thiocyanate in the body.[6]

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