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

THE CONVERSION TO THIOCYANATE

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Abstract—N-methylpyridinium-2-aldoxime methanesulphonate (P2S) was administered to rats and the urinary excretion of thiocyanate was determined by different methods. In addition to a small increase in thiocyanate excretion a metabolite was found which interfered with one of the test methods. It was concluded that formation of cyanide could not account for the toxicity of the oxime. Similar experiments on human beings showed no significant increase in thiocyanate excretion, but small amounts of an unknown metabolite were formed.

THE value of certain oximes as antidotes against nerve gases or organophosphorus insecticides is well established.[1, 2] One of the most efficient of these oximes is N-methylpyridinium-2-aldoxime, used either as the iodide (PAM) or the more soluble methanesulphonate (P2S). Although little is known about the metabolic fate of this oxime, conversion of some oximes to cyanide (for instance di*iso*nitrosoacetone (DINA)) has been demonstrated and is presumably responsible for their toxicity.[3] Similar experiments have apparently not been carried out on N-methylpyridinium-2-aldoxime but it is known that this compound slowly decomposes to cyanide at neutral pH.[4] Cyanide and P2S poisoning have certain features in common, i.e. they show the same symptoms (respiratory stimulation followed by arrest and convulsions) and the dog is the most sensitive animal for both.[5, 6] It has also been observed in this laboratory (unpublished) that a combination of sodium nitrite and sodium thiosulphate, a well-known antidote combination against cyanide, decreases the toxicity of N-methylpyridinium-2-aldoxime, indicating that formation of cyanide could contribute to the toxicity of the oxime.

To test conversion of N-methylpyridinium-2-aldoxime to cyanide in the body, the urinary excretion of thiocyanate was followed after administration of the oxime. This technique is based on the fact that cyanide is rapidly converted *in vivo* to thiocyanate by enzymatic processes.[7, 8]

MATERIALS AND METHODS

N-*methylpyridinium*-2-*aldoxime methanesulphonate* (P2S) was prepared according to Creasy and Green.[9] *Di*iso*nitrosoacetone* (DINA) was prepared according to Koessler and Hanke.[10] All other compounds used were commercial products of analytical purity.

Rats of either sex weighing 200–300 g were kept in metabolism cages and fed a diet of low thiocyanate content.[11] The human volunteers were male students (non-smokers) who were allowed to eat a free diet. Thiocyanate analysis was carried out by three different methods. *Method I* was a modification[12] of the procedure of Boxer and Richards[13] based upon oxidation of the thiocyanate to hydrogen cyanide by permanganate in acid solution. The hydrogen cyanide is transfered into sodium hydroxide and cyanide is then determined colorimetrically by Zincke–Königs reaction. Although this method is claimed to be specific for thiocyanate, experiments described in the following section demonstrated that a metabolite from P2S gave a positive interference in this method. *Method II* was developed from the observation that the procedure could be made more specific by a preliminary precipitation of thiocyanate with silver ions. The difference between the value obtained by method I applied directly to the sample and that obtained after silver precipitation was then taken to represent the thiocyanate content of the sample. Cyanide (hydrocyanic acid) would interfere in this method, but it was verified (by acidification and aeration of the samples as in method I, but with the oxidation step omitted) that the urine did not contain any cyanide.

It was also verified that neither metabolites from P2S nor the oxime itself interfered in the silver precipitation step. ^{14}C-labelled thiocyanate, when added to urine samples obtained from P2S-treated animals, was completely removed from the urine by this treatment. The silver precipitation step was carried out as follows: 5 ml of urine (acidified with acetic acid) and 0·9 ml 1 M $AgNO_3$ were mixed and the precipitate removed by centrifugation. To 5 ml of the supernatant was added 1·0 ml 1 M NaCl and the silver chloride removed by centrifugation. *Method III*[14] was based on the red colour given by ferric ions with thiocyanate. This method is considerably less specific for thiocyanate as certain urinary pigments also give coloured products with ferric ions.

RESULTS

In the first part of the investigation the urinary excretion of thiocyanate after intramuscular and oral administration of P2S was determined in rats. When thiocyanate was determined with method I the results shown in Figs. 1 and 2 were obtained. A large increase in thiocyanate excretion was observed, amounting to about 5 and 3 per cent, respectively, of the P2S administered, calculated on an equimolar conversion of P2S to the thiocyanate. However, when the analysis was made by other methods the increase in thiocyanate was much less (Table 1). This indicated the formation of a compound easily converted to cyanide but not identical with thiocyanate. The increase in true thiocyanate (as determined by method II) after P2S administration is shown in Fig. 3. It amounted to about 1 per cent of the P2S injected. It was verified that when P2S was incubated with rat urine for 24 hr neither a formation of thiocyanate nor of the unknown metabolite could be detected.

Attempts to purify the unknown cyanide-forming metabolite (metabolite X) were undertaken. It was observed that it was not adsorbed on the hydrogen form of strong cation exchangers but was completely removed by treatment of solutions with strong anion exchangers (acetate and hydroxide form). However, the metabolite could not

be eluted from the latter. It was possible to adsorb the metabolite on active carbon and a partial elution (to about 30 per cent) could be accomplished with aqueous pyridine. The possibility was considered that metabolite X was 2-imino-4-thiazolidine carboxylic acid (a detoxification product of cyanide),[15] but tests with an authentic specimen of this substance showed that it was not oxidized to cyanide under the test conditions used in method I.

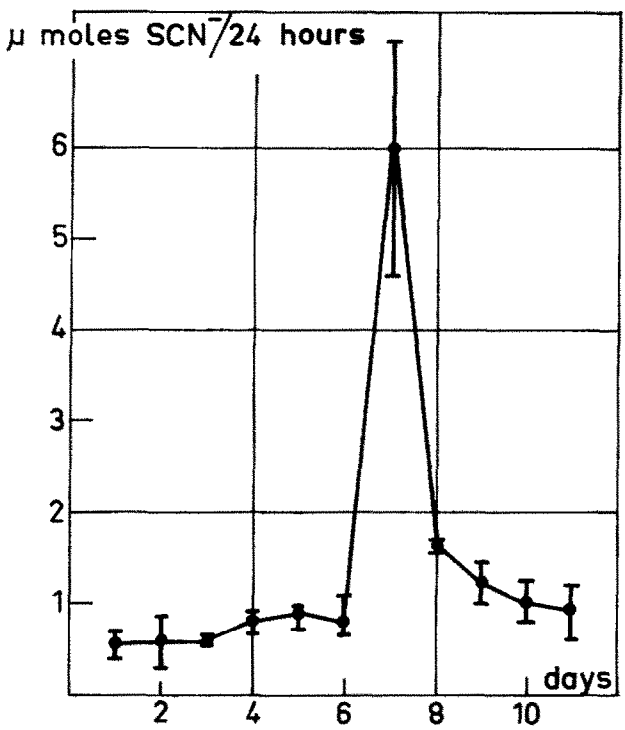

FIG. 1. Thiocyanate excretion (according to method I) after intramuscular injection of P2S. Five rats were injected with P2S (100 mg/kg) on day 6. The dots indicate means and the bars extreme values.

The possibility that metabolite X could be formed from other oximes was also considered. Rats were injected with DINA and their urine analysed as above. About 60 per cent of the injected dose (10 mg/kg body weight intramuscularly, twice a day) was excreted as thiocyanate but no formation of metabolite X could be detected.

To determine whether metabolite X was formed from cyanide, rats were given a sublethal dose of KCN (1 mg KCN per kg body weight intramuscularly, twice a day) and the following 24-hr urine analysed for thiocyanate and metabolite X. No formation of the latter could be detected, but about 50 per cent of the cyanide was excreted as thiocyanate in confirmation of earlier reports in the literature.[16]

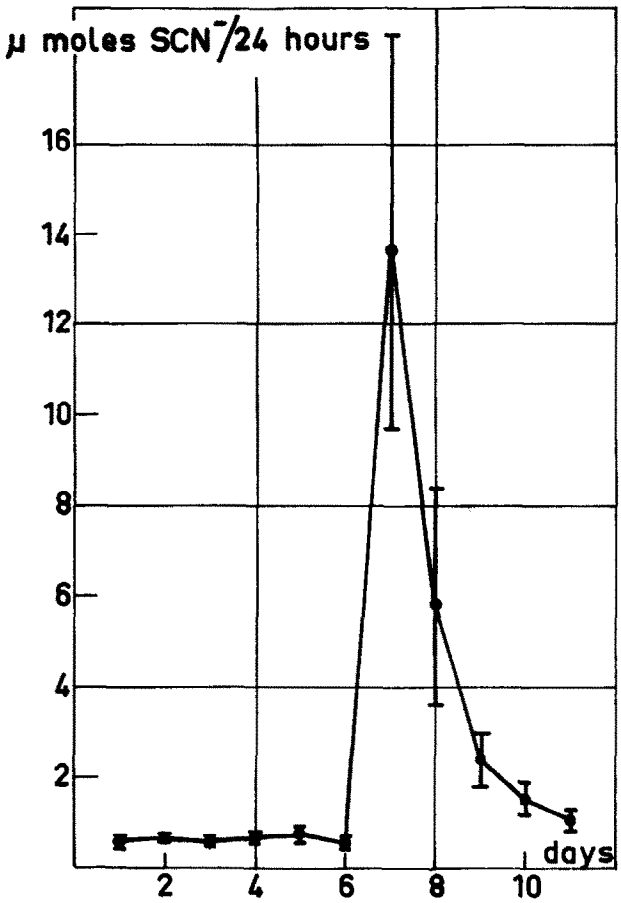

FIG. 2. Thiocyanate excretion (according to method I) after oral administration of P2S. Five rats received 500 mg/kg of P2S on day 6. Other conditions as in Fig. 1.

TABLE 1. THIOCYANATE DETERMINATION ON URINE FROM RATS BEFORE AND AFTER TREATMENT WITH P2S

A rat received 120 μmoles P2S i.m. (corresponding to 100 mg/kg). The 24 hr urine was collected and assayed for thiocyanate by the different methods described in text.

Method	μMoles SCN^-/24 hr	
	Control	After P2S
I	0·85	4·69
II	0·58	0·82
III	5·0	5·2

In the second part of the investigation P2S was administered to human volunteers and the excretion of true thiocyanate and the metabolite X were determined. The results summarized in Table 2, show no significant increase in thiocyanate excretion but a small formation of the unknown metabolite.

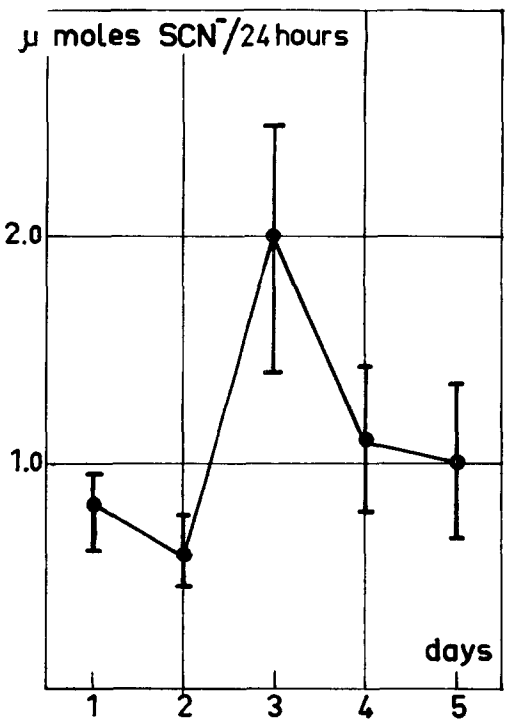

FIG. 3. Thiocyanate excretion (according to method II) after intramuscular injection of P2S. Conditions as in Fig. 1. but P2S was given on day 2.

TABLE 2. THIOCYANATE DETERMINATIONS ON URINE FROM NORMAL AND P2S TREATED HUMAN VOLUNTEERS

The 24-hr urine from four male students was collected and analysed for SCN^- before and after P2S administration (3 g orally in gelatin capsules). The amount of metabolite X excreted was obtained from the difference between the values given by method I and method II. Numbers in brackets indicate extreme values.

Day before treatment	μMoles SCN^-/24 hr		
	Method I	Method II	Metabolite X
1	67·5 (57·0–84·5)	62·7 (52·1–80·0)	4·8 (4·2–5·5)
2	75·7 (49·3–102)	70·8 (43·3–97·0)	4·9 (4·0–6·0)
3	78·9 (47·2–150)	73·3 (43·5–141)	5·6 (2·8–9·0)
Day after treatment			
1	123 (89·5–183)	87·6 (61·1–139)	35 (28·4–44)
2	92·6 (65·2–133)	79·4 (58·5–117)	13·2 (6·0–16·2)
3	73·8 (52·7–113)	66·6 (45·8–108)	7·2 (6·2–9·3)
4	71·8 (54·1–115)	65·5 (48·2–108)	6·3 (5·6–7·4)

DISCUSSION

The present investigation has shown that very little thiocyanate is formed from P2S in rats. The increase in thiocyanate excretion found corresponded to 0·1 mg hydrogen cyanide per kg body weight when 0·5 LD_{50} of P2S was administered.[5] It is reasonable to assume that if one LD_{50} dose of P2S had been given not more than twice as much cyanide (0·2 mg/kg) could be formed. The LD_{50} of cyanide (HCN) after slow poisoning (intraperitoneal injection) is 3·1 mg/kg in rats.[17] Consequently the amounts of cyanide formed from P2S cannot account for the toxicity of the latter.

The increase in thiocyanate (plus metabolite X) excretion found by method I in human beings after P2S *per os* was about 50 μmoles (the increase was in fact not statistically significant) which is equivalent to a 0·4 per cent conversion. The corresponding figure for rat is 3 per cent. Consequently P2S is more extensively metabolized to thiocyanate in rats than in human beings.

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