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METABOLIC STUDIES WITH CERTAIN ETHYLENEIMINE DERIVATIVES IN RELATION TO DIURESIS

BY

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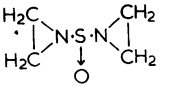
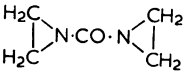
Ethyleneimine (I) and a number of its derivatives have been shown to produce a water diuresis in non-hydrated rats (Jackson & James, 1963). A detailed study of one of the compounds, di(aziridin-1-yl)sulphoxide (II), showed it to be metabolized to ethyleneimine and sulphate (Craig, Jackson & James, 1963). This suggested that the diuretic effects might be correlated with the liberation of ethyleneimine *in vivo*. To further investigate this possibility the excretion of ethyleneimine was examined after administration of di(aziridin-1-yl)sulphoxide (II), di(aziridin-1-yl)formaldehyde (*NNN'N'*-diethyleneurea; III) and tri(aziridin-1-yl)phosphine oxide (triethylenephosphoramide; TEPA; IV).

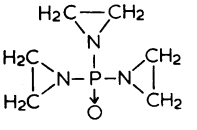
I



II



III



IV

Studies were carried out on rat and mouse because earlier work with triethylenephosphoramide indicated considerable species differences in ability to metabolize this compound (Craig & Jackson, 1955; Nadkarni, Goldenthal & Smith, 1957). A comparison was also made of the diuretic effects of the compounds in both species.

METHODS

Materials. *NNN'N'*-Diethyleneurea and triethylenephosphoramide were prepared using the method described by Bestian (1950). The preparation of di(aziridin-1-yl)sulphoxide has been described previously (Jackson & James, 1963).

Animal techniques. Adult male Wistar rats and adult male albino mice were used throughout. The LD50 values were determined in rats and mice, deaths being recorded up to 28 days. Groups of twelve mice or five rats were used in the diuretic and ethyleneimine excretion studies. In the latter experiments,

food was withheld for 6 hr after injection during which urine was collected at 2-hr intervals in containers cooled with solid carbon dioxide. Before sampling, the metabolic cage and funnel were rinsed with distilled water (up to 20 ml.), which was added to the excreted urine.

Methods of analysis. The ethyleneimine content of urine was determined in duplicate using a modification of the method of Rosenblatt, Hlinka & Epstein (1955) described previously (Craig *et al.*, 1963). Small blank values were obtained by applying the same method to urine from untreated rats and mice for which due allowance was made in estimating the ethyleneimine present. On adding ethyleneimine to rat and mouse urine (up to 25 and 40 mg/100 ml. respectively) the recovery ranged from 80 to 100%. A correction factor was applied assuming an average recovery of 90%.

RESULTS

Toxicity. The LD50 values of the compounds are shown in Table 1. All compounds were better tolerated by the mouse.

Diuresis and ethyleneimine excretion. Ethyleneimine, *NNN'N'*-diethyleneurea and di-aziridin-1-yl)sulphoxide produced a marked diuresis in both species in contrast to triethyl-

TABLE 1

THE LD50/28-DAY VALUES FOR ETHYLENEIMINE, *NNN'N'*-DIETHYLENEUREA, DI-(AZIRIDIN-1-YL)SULPHOXIDE AND TRIETHYLENEPHOSPHORAMIDE IN THE RAT AND MOUSE

Compounds were given intraperitoneally by single injections in water

Compound	LD50 (mg/kg) for Rat	Mouse
Ethyleneimine	3·5	4·0
NNN'N'-Diethyleneurea	5·8	8·5
Di(aziridin-1-yl)sulphoxide	7·1	13·0
Triethylenephosphoramide	8·8	25·5

TABLE 2

VOLUME AND ETHYLENEIMINE CONTENT OF URINE OBTAINED FROM GROUPS OF FIVE RATS GIVEN ETHYLENEIMINE, *NNN'N'*-DIETHYLENEUREA, DI(AZIRIDIN-1-YL)SULPHOXIDE AND TRIETHYLENEPHOSPHORAMIDE

Compound	Dose (mg/kg)	Ethyleneimine "content" (mg/kg)	Ethyleneimine excreted in 0–6 hr: mg/kg	% of base injected	Urine excreted in 0–3 days (ml./kg)
None (control)	—	—	—	—	82
Ethyleneimine	1·0	1·0	0·20	20	206
	1·8	1·8	0·38	21	200
	2·9	2·9	1·10	38	250
NNN'N'-Diethyleneurea	2·7	2·1	0·33	16	273
	3·8	2·9	1·04	36	307
	6·0	4·7	0·80	17	327
Di(aziridin-1-yl)sulphoxide	1·3	0·9	0·20	22	133
	2·3	1·5	0·38	25	268
	2·7	1·8	0·29	16	249
	4·4	2·9	0·88	30	270
Triethylenephosphoramide	2·1	1·6	0·12	8	54
	3·8	2·8	0·03	1	74
	7·5	5·6	0·17	3	57

enephosphoramide which was ineffective (Tables 2 and 3). One group of mice given triethylenephosphoramide (10 mg/kg) produced 155 ml./kg of urine in 3 days, which was greater than the mean control (120 ml./kg). There was, however, no consistent diuretic effect as higher doses failed to induce polyuria (Table 3). Results using ethyleneimine, *NNN'N'*-diethyleneurea and di(aziridin-1-yl)sulphoxide showed that the major proportion

TABLE 3

VOLUME AND ETHYLENEIMINE CONTENT OF URINE OBTAINED FROM GROUPS OF TEN MICE GIVEN ETHYLENEIMINE, *NNN'N'*-DIETHYLENEUREA, DI(AZIRIDIN-1-YL)-SULPHOXIDE AND TRIETHYLENEPHOSPHORAMIDE

Compound	Dose (mg/kg)	Ethyleneimine "content" (mg/kg)	Ethyleneimine excreted in 0–6 hr		Urine excreted in 0–3 days (ml./kg)
			mg/kg	% of base injected	
None (control)	—	—	—	—	120
Ethyleneimine	1·0	1·0	0·07	7	163
	1·4	1·4	0·39	28	178
	2·0	2·0	0·51	26	215
	2·5	2·5	0·64	26	275
	2·8	2·8	0·69	25	319
	5·0	5·0	0·96	19	392
NNN'N'-Diethyleneurea	2·7	2·1	0·74	35	233
	4·0	3·1	0·54	17	321
	6·0	4·6	1·54	33	309
	8·0	6·1	2·16	35	305
Di(aziridin-1-yl)sulphoxide	2·5	1·6	0·17	11	132
	5·0	3·3	0·82	25	278
	6·5	4·2	0·55	13	260
	10·0	6·5	2·48	38	500
Triethylenephosphoramide	3·8	2·8	0·09	3	103
	10·0	7·5	0·11	1	155
	12·5	9·4	0·27	3	100
	20·0	15·0	0·54	4	110
	25·0	18·8	0·83	4	133

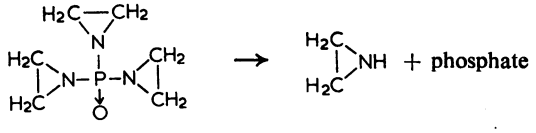
of the ethyleneimine was eliminated within 4 hr of administration. In both species the amount of ethyleneimine excreted within 6 hr and the 3-day urine volume generally increased as the dose of compound was increased. In the rat the maximum amount of ethyleneimine excreted was 36 and 30% for *NNN'N'*-diethyleneurea (3.8 mg/kg) and di(aziridin-1-yl)-sulphoxide (4.4 mg/kg) respectively; for the mouse the corresponding figures were 35 and 38% (8 and 10 mg/kg). Mice tolerated larger doses of the phosphoramide than the rat and excreted substantially more ethyleneimine at these higher levels. At doses of 12.5 mg/kg or more, mice cleared amounts of ethyleneimine similar to those observed after diuretic doses of the other compounds.

DISCUSSION

The results clearly show that rats and mice are able to metabolize the difunctional alkylating agents, *NNN'N'*-diethyleneurea and di(aziridin-1-yl)sulphoxide with the formation of ethyleneimine. With the exception of triethylenephosphoramide in the rat, a correlation generally exists between the amount of compound administered and the quantity of ethyleneimine excreted. The maximum degree of metabolism to ethyleneimine ranged from 25 to 35% (Tables 2 and 3). The fate of the remaining ethyleneimine residues has not been

established. It is known that the sulphoxide is completely metabolized by a number of species including the rat and mouse (Craig *et al.*, 1963). In the rat, the phosphoramide (7.5 mg/kg) failed to raise the level of ethyleneimine excretion above 0.2 mg/kg/6 hr, which is seemingly a threshold diuretic level in this species. In the mouse, however, triethylenephosphoramide did not induce polyuria although higher doses brought about ethyleneimine excretion at rates similar to effective diuretic doses of the other compounds (Table 3).

In both species, ethyleneimine is apparently only a minor metabolite of triethylenephosphoramide. In the rat this correlates with the knowledge that most of the compound (80% of [^{32}P]-labelled compound) appears unchanged in the urine (Craig & Jackson, 1955). In contrast, the phosphoramide is completely metabolized by the mouse (Nadkarni *et al.*, 1957) so that this species might be expected to excrete considerably more ethyleneimine than was found to be the case—assuming that simple hydrolytic cleavage of the molecule occurred, viz:



As relatively small amounts of ethyleneimine were excreted, the metabolism of triethylenephosphoramide must, in the main, proceed via some other pathway, perhaps by opening of the ethyleneimine rings. This would also explain the failure of the drug to produce diuresis in the mouse. Indirect evidence of renal metabolism of the phosphoramide in the mouse was obtained by Craig, Fox & Jackson (1959), who found that much more [^{32}P]-labelled phosphate (90% in 18 hr) was excreted after triethylenephosphoramide than after an equivalent dose of labelled phosphate (14% in 18 hr). Ethyleneimine may therefore be produced in the kidney without causing diuresis, which suggests that its mechanism of action could be extrarenal, perhaps at the pituitary level. The failure of ethyleneimine to affect proliferating cell systems (Hendry, Homer, Rose & Walpole, 1951; Jackson, 1964) indicates that its alkylating capacity is small. Thus the latter property is presumably not the explanation of the diuretic action. Pharmacologically potent alkylating agents of the ethyleneimine type, as well as those of other categories (sulphonic esters and nitrogen mustards) do not produce polyuria. Since much of the triethylenephosphoramide administered can be excreted unmetabolized (for example in the rat) its chemical reactivity as an alkylating agent is small under these circumstances. The question of mode of action of ethyleneimine and its diuretic derivatives as well as the possibility of indirect action via the pituitary requires further investigation.

SUMMARY

1. Various ethyleneimines cause a water diuresis in the rat. The present study sought to correlate the diuresis with the excretion of ethyleneimine—itself highly active in this respect.

2. After administration of ethyleneimine, *NNN'N'*-diethyleneurea, di(aziridin-1-yl)-sulphoxide and triethylenephosphoramide, ethyleneimine excretion was followed colorimetrically.

3. Ethyleneimine produced from *NNN'N'*-diethyleneurea and di(aziridin-1-yl)sulphoxide appeared to correlate with the severity of the diuresis. No polyuria followed administration of the phosphoramide although high doses to the mouse resulted in ethyleneimine excretion comparable to that following diuretic doses of the other compounds. The evidence suggests the possibility of an extrarenal site of action of ethyleneimine.

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