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THE EFFECT OF CERTAIN ETHYLENEIMINES ON RENAL FUNCTION

BY

H. JACKSON AND R. M. V. JAMES

From the Christie Hospital and Holt Radium Institute, Manchester 20

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Certain simple derivatives of ethyleneimine cause an intense and prolonged diuresis in nonhydrated rats. Sodium, potassium and chloride excretion is unaffected by these compounds. Ethyleneimine also causes a diuresis in the rat, but the polyfunctional compound thiotepa is ineffective. The relationship between structure and activity of a number of compounds is discussed with particular reference to the possibility that the diuresis is due to the *in vivo* liberation of ethyleneimine.

Ethyleneimines, some of which have been used with success in the chemotherapy of malignant disease, are a subgroup of the compounds known as alkylating agents. The effects of some of these agents have been investigated on the normal proliferating cells of the haemopoietic system (Fox, Craig & Jackson, 1960) and the testis (Jackson, Fox & Craig, 1959). During such investigations it was observed that *NN*-ethyleneurea (II, Table 1) caused an intense diuresis in the rat. This paper is concerned with a more detailed examination of this phenomenon.

METHODS

The compounds used (Table 1) were prepared according to the methods described by Bestian (1950). Di(aziridin-1-yl) sulphoxide (diethyleneiminosulphoxide: VI) and *NN*-ethylene-*N'*-isopropylurea (V) have not been previously described and their preparation is detailed below. All compounds were stored at −20° C.

Preparation of di(aziridin-1-yl) sulphoxide (VI)

Thionyl chloride (1 mole) in dry ether was slowly added, with continuous stirring, to a mixture of ethyleneimine (2 moles) and triethylamine (2 moles) in dry ether at −20° C. The triethylamine hydrochloride which precipitated was removed by filtration, and the product was isolated by fractional distillation. It had the following properties:

Boiling point 60° C at 0.1 mm Hg. $C_4H_8N_2OS$ requires C 36.4%, H 6.1%. Found C 36.3%, H 6.1%. Yield 35%.

Preparation of NN-ethylene-N'-isopropylurea (V)

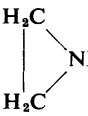
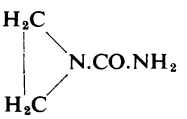
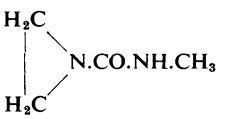
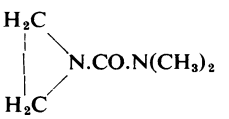
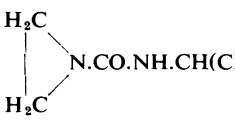
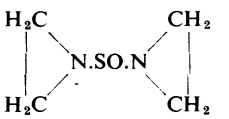
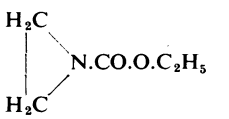
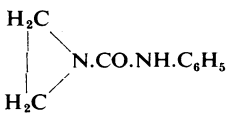
Isobutyryl chloride was first prepared by refluxing thionyl chloride (1 mole) with isobutyric acid (1 mole). The acid chloride was then converted to isopropyl isocyanate by refluxing with sodium azide using di-isopentyl ether as the solvent. The freshly distilled isocyanate was then allowed to react with an equivalent amount of ethyleneimine in dry ether at 0° C. Removal of the solvent yielded a white solid which was recrystallized from heptane. It had the following properties:

Melting point 53° C. $C_6H_{12}N_2O$ requires C 56.3%, H 9.4%. Found C 56.3%, H 9.3%.

TABLE 1

LIST OF COMPOUNDS EXAMINED TOGETHER WITH THEIR LD50 IN RATS AND EQUIVALENT CONTENTS OF ETHYLENEIMINE

The compounds were injected intraperitoneally in water, except for (VIII) which was in arachis oil. LD50 are based on a period of 28 days

Compound	Formula	LD50 (mg/kg)	Ethyleneimine equivalent to LD50
(I) Ethyleneimine		3·5	3·5
(II) *NN*-ethyleneurea		30	15
(III) *NN*-ethylene-*N'*-methylurea		90	39
(IV) *NN*-ethylene-*N'N'*-dimethylurea		200	76
(V) *NN*-ethylene-*N'*-isopropylurea		90	30
(VI) Di(aziridin-1-yl) sulphoxide		6·0	3·9
(VII) *NN*-ethyleneurethane		8·6	3·2
(VIII) *NN*-ethylene-*N'*-phenylurea		25	6·6

Experimental methods

Freshly prepared solutions of the compounds were administered intraperitoneally to groups of male Wistar rats. Toxicity results were obtained by injecting groups of five to ten animals with graded doses of the compounds, deaths being observed up to 4 weeks. Diuretic potency was investigated, using groups of five male rats (260 to 320 g) in metabolism cages. When biochemical estimations were to be carried out, urine was collected in glass vessels, cooled in powdered solid carbon dioxide. Urine sodium and potassium levels were determined by flame photometry, chloride by the Volhard method, and urea by the urease method. Total and nondialysable nitrogen were estimated, using the Kjeldahl technique, the nondialysable urinary nitrogen representing protein content.

RESULTS

Diuresis

An increase in the output of urine became apparent about 2 hr after administration of the drugs, and the duration of the polyuria depended upon the dose of compound used. Small doses caused a reversible effect, normal levels of excretion being resumed within a few days. At the other extreme, animals surviving large doses of compounds (for example, *NN*-ethyleneurea, II, 25 mg/kg) exhibited a polyuria of at least 3 months duration, so that the effect appeared permanent. The cumulative urine excretions by groups of five rats given graded doses of the compounds are shown in Figs. 1 and 2.

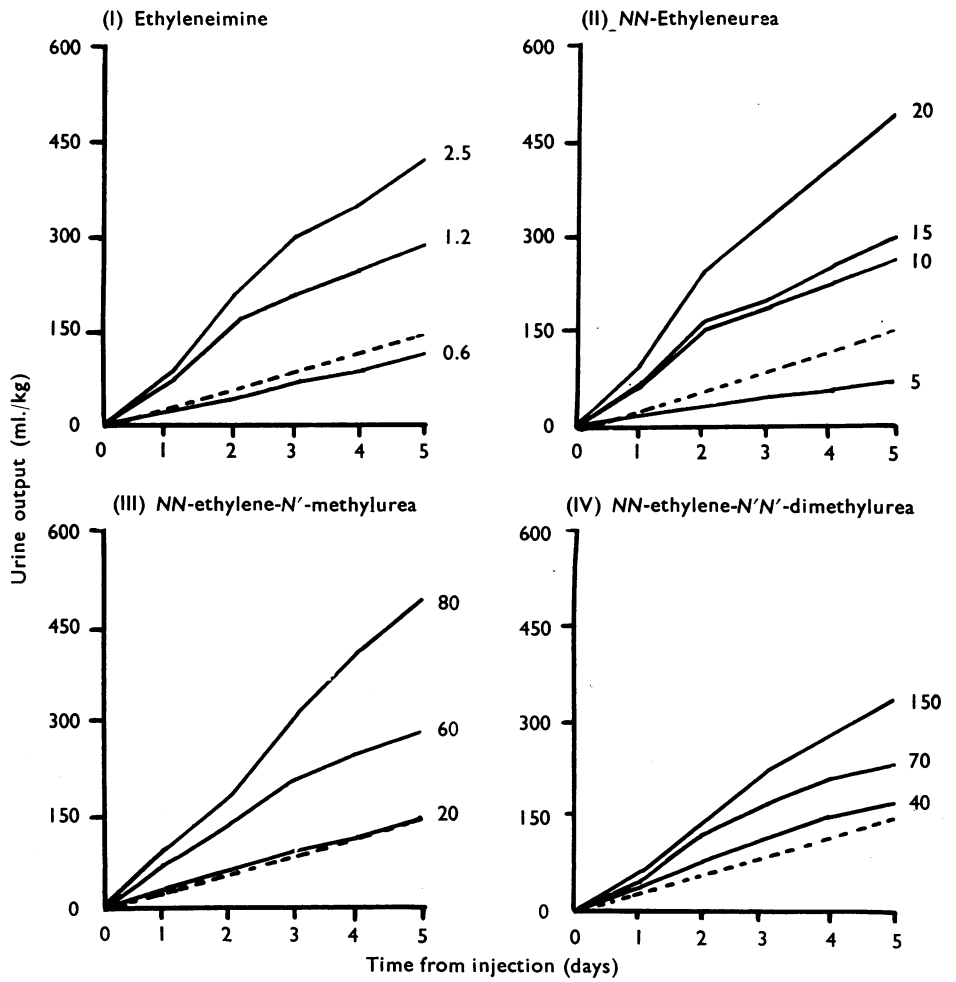


Fig. 1. The effect of graded doses of various ethyleneimines on the cumulative excretion of urine (ordinate) by groups of five male rats. Numbers on the right of each curve indicate the dose in mg/kg. Interrupted lines are control responses.

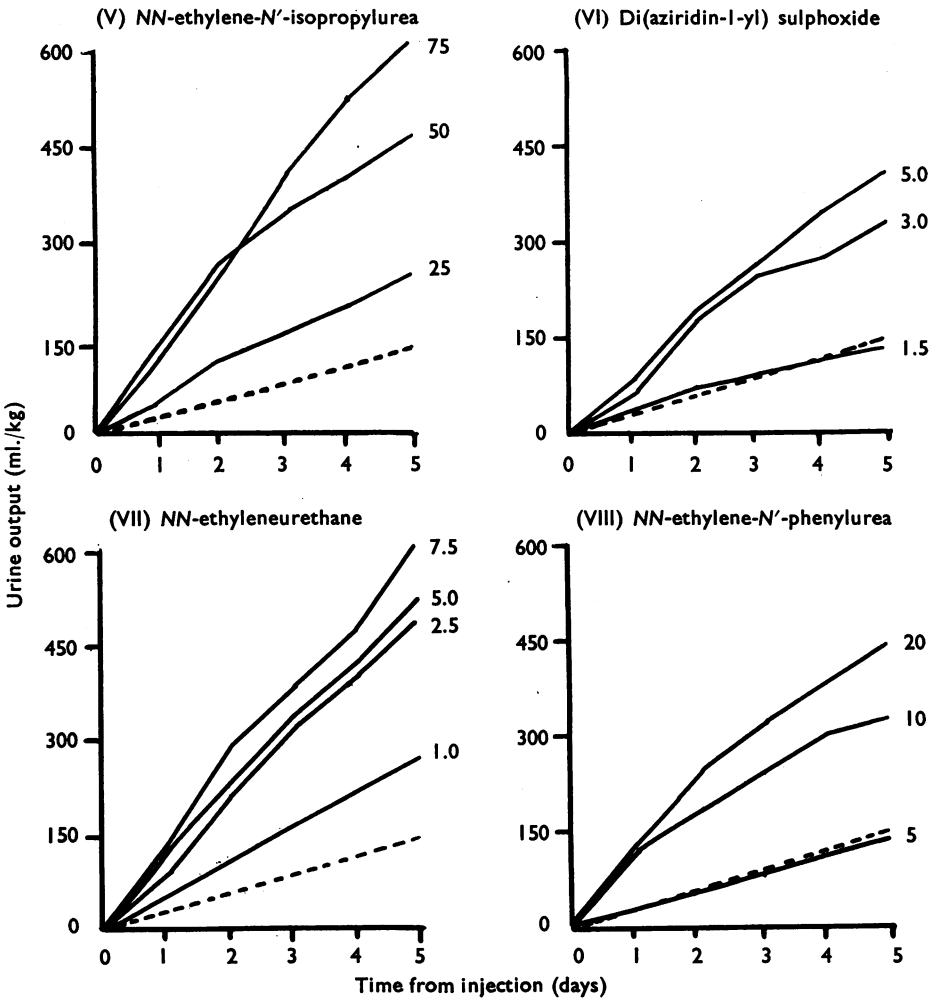


Fig. 2. As Fig. 1, for further ethyleneimines.

The diuresis could be separated into two stages. Initially, there was an acute phase lasting 1 to 2 days (Fig. 3), which was followed by a chronic phase during which urine excretion remained elevated although at a lower level than before.

From Figs. 1 and 2, an order of effectiveness was obtained by comparing the 0 to 3 and 0 to 5 day cumulative excretions at the 0.5 LD50 level. Thus, for the urine output for 0 to 3 days the descending order of activity is:

V, VII	VIII	I, II, IV	III, VI
300	275	200	175 ml. of urine in 3 days,

with a control value of 85 ml.,

and for 0 to 5 days:

VII	V	VIII	I, II, VI	IV	III
475	400	350	300	250	200 ml. of urine in 5 days,

with a control value of 140 ml.

Using this method of evaluation, *NN*-ethyleneurethane (ethyl aziridin-1-ylcarbamate; VII) and *NN*-ethylene-*N'*-isopropylurea (V) are the most effective compounds. *NN*-ethyleneurea (II) is about as effective as ethyleneimine and *N'*-methylation results in the less active compounds *NN*-ethylene-*N'*-methylurea

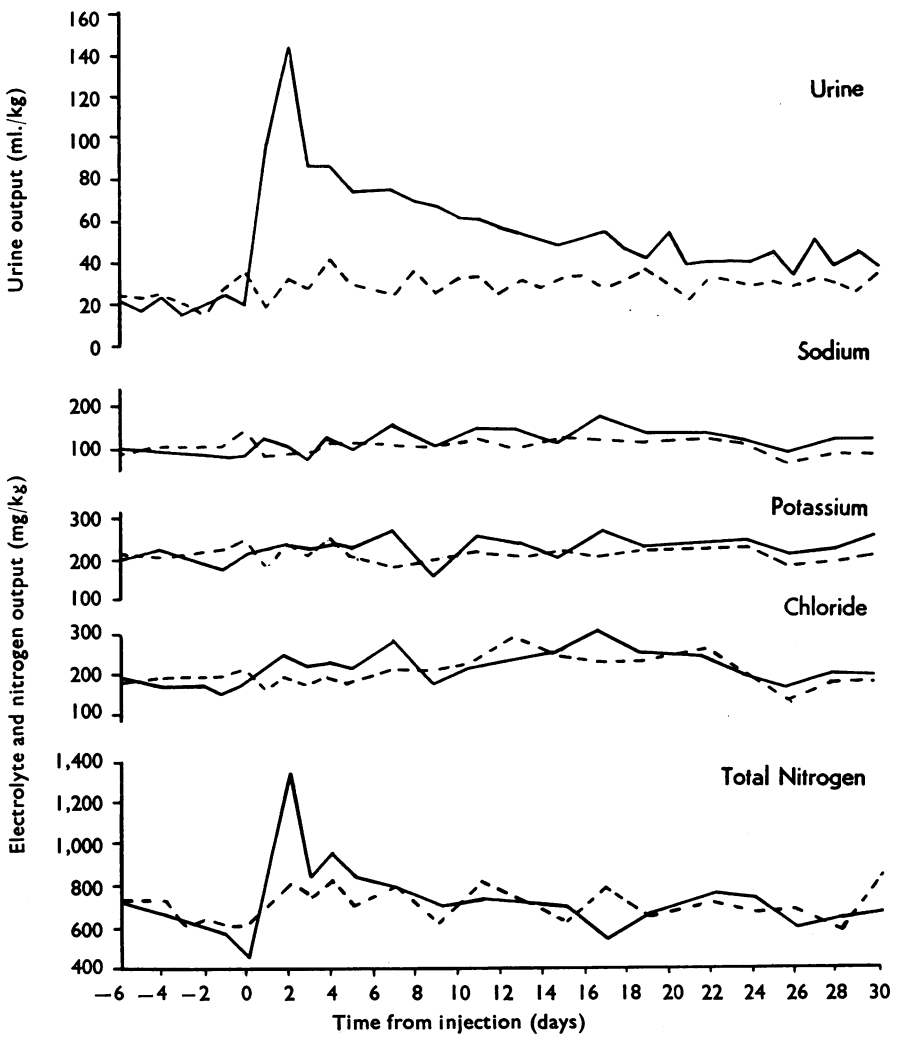


Fig. 3. The effect of *NN*-ethyleneurea (20 mg/kg) on the urine and electrolyte excretion by a group of five male rats. The solid line represents the response of treated animals and the broken line that of the controls.

(III) and *NN*-ethylene-*N'N'*-dimethylurea (IV). The polyfunctional ethyleneimine derivatives, thiotepa and tretamine, failed to evoke a diuresis, even when given in maximal tolerated doses (7.5 and 1 mg/kg respectively).

Toxicity

The toxicities of the compounds studied are shown in Table 1. Ethyleneimine (I), di(aziridin-1-yl) sulphoxide (VI) and *NN*-ethyleneurethane (VII) were of similar toxicity when calculated in terms of the ethyleneimine moiety injected. However, such a relationship did not apply to *NN*-ethyleneurea (II) and its *N'*alkyl homologues *NN*-ethylene-*N'*-methylurea (III), *NN*-ethylene-*N'N'*-dimethylurea (IV) and *NN*-ethylene-*N'*-isopropylurea (V).

Composition of urine during diuresis

The daily excretion of urine, total nitrogen, sodium, potassium and chloride after administration of *NN*-ethyleneurea (II, 20 mg/kg, intraperitoneally) are shown in

TABLE 2

THE CUMULATIVE EXCRETION OF URINE CONSTITUENTS AFTER ADMINISTRATION OF VARIOUS ETHYLENEIMINES

Results were obtained from groups of five male rats and are expressed as total output for 4 days after intraperitoneal injection

Drug	Dose (mg/kg)	Total output of: Urine (ml./kg)	Sodium (g/kg)	Potassium (g/kg)	Chloride (g/kg)	Total nitrogen (g/kg)	Urea nitrogen (g/kg)
II	12·5	382	0·41	0·82	0·78	3·58	3·11
II	20·0	419	0·47	0·96	0·91	3·99	—
II	25·0	509	—	—	0·94	—	—
III	40·0	252	0·45	0·92	0·77	4·33	3·77
V	40·0	228	0·47	0·95	0·56	4·50	3·83
VII	4·0	370	0·50	1·11	0·96	4·44	3·71
Thiotepa	6·0	131	0·54	1·00	0·71	4·32	—
Mean control	—	101	0·49	0·94	0·83	3·47	3·10

Fig. 3. Whilst electrolyte excretion was unaffected, the daily total nitrogen excretion was considerably elevated during the second day of the experiment. Data showing the cumulative 4-day excretion of Na^+, K^+, Cl^-, total and urea nitrogen at several dose levels of *NN*-ethyleneurea (II) and for single doses of *NN*-ethylene-*N'*-methylurea (III), *NN*-ethylene-*N'*-isopropylurea (V) and *NN*-ethyleneurethane (VII) are presented in Table 2. In spite of an intense polyuria the daily output of sodium, potassium and chloride was not significantly affected. Increased total nitrogen excretion was associated with enhanced urea output after administration of the three last-mentioned drugs (40, 40 and 4 mg/kg respectively). Over the 4 day collection period, the enhanced excretion of total and urea nitrogen seemed to bear no relationship to the severity of the diuresis. Heat-coagulation tests showed protein to be present in urine from treated and control rats, and in view of the possibility of severe renal damage the rate of protein excretion was measured (Table 3). Protein excretion was significantly increased after injection of *NN*-ethyleneurea (II) (12.5 and 17.5 mg/kg) and *NN*-ethyleneurethane (VII) (4 mg/kg);

TABLE 3

THE DAILY URINE AND NONDIALYSABLE NITROGEN (NDN) EXCRETED BY A GROUP OF FIVE MALE RATS GIVEN VARIOUS ETHYLENEIMINES

Average control values: urine 28 ml./kg/day; NDN 12 mg/kg/day. * 0·33LD50; † 0·5LD50

Time after injection (days)	Outputs after administration of: *NN*-ethyleneurea (II) (12·5 mg/kg)*		*NN*-ethyleneurea (II) (17·5 mg/kg)†		*NN*-ethylene-*N'*-methylurea (III) (40 mg/kg)†		*NN*-ethylene-*N'*-isopropylurea (IV) (40 mg/kg)†		*NN*-ethylene-urethane (VII) (4 mg/kg)†	
	NDN (mg/kg)	Urine (ml./kg)	NDN (mg/kg)	Urine (ml./kg)	NDN (mg/kg)	Urine (ml./kg)	NDN (mg/kg)	Urine (ml./kg)	NDN (mg/kg)	Urine (ml./kg)
0–1	25	129	45	94	12	81	16	87	35	107
1–2	25	148	70	162	16	82	15	60	39	96
2–3	12	48	44	93	17	50	12	42	17	73
3–4	13	57	38	89	8	39	11	39	23	94
4–5	12	59	47	88	11	39	12	41	17	67

however, *NN*-ethylene-*N'*-methylurea (III) and *NN*-ethylene-*N'*-isopropylurea (V) (40 mg/kg) had no definite effect.

DISCUSSION

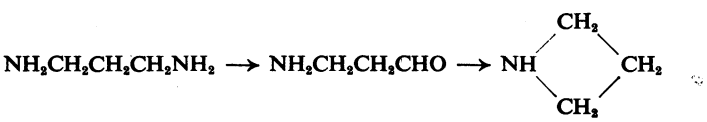
Ehrlich (1906) found that lethal doses of ethyleneimine produced necrosis of the renal papillae in the rat, but the diuretic effect in this species appears to have escaped observation until the present work. Mandel & Popper (1951) used ethyleneimine, in doses of approximately 2 to 8 mg/kg, to induce experimental necrosis of the rabbit kidney. They observed proteinuria and azotaemia; polyuria is referred to, but the evidence for it is not convincing. *NN*-ethylene-*N*-phenylurea (VIII) has been shown to increase tubular mitosis in the rat (British Empire Cancer Campaign, 29th Annual Report, p. 29, 1951), but no diuresis was recorded. As whole-body X-irradiation of rats also produced diuresis (Mefferd & Martens, 1955; Pentz & Hasterlik, 1957), the water diuresis produced by ethyleneimine and some of its derivatives suggested that the effect might be a "radiomimetic" action. However, the failure of thiotepa and tretamine to cause diuresis is inconsistent with such a view.

The selective action shown by the ethyleneimine derivatives is indicated by the ability of the rat kidney to conserve sodium, potassium and chloride in spite of the intense water diuresis. The results show that the diuresis bears no resemblance to that induced by conventional diuretics in clinical use, particularly because the activity of drugs such as acetazolamide and chlorothiazide has been observed to be complete in 6 hr (Nielsen, 1961). Also, Friedman, Nakashima & Friedman (1960), examining the relation of the saluretic and hypotensive effects of hydrochlorothiazide in the rat, collected urine for 3 hr from animals which were not water-loaded, and observed sodium and water excretion to be proportional in the log of the dose administered. The water diuresis which occurred in the present experiments is similar to that in diabetes insipidus, and the possibility that the action of the compounds is concerned with the production or action of the antidiuretic hormone has not been discounted.

Treatment with the diuretic ethyleneimines and with thiotepa caused an increase in total-nitrogen excretion, which also occurs after whole-body irradiation (Nims

& Thurber, 1962) and stress (Ingle, Ward & Kuizenga, 1947). The results of administration of *NN*-ethyleneurea (II) at several dose levels (Table 2) are consistent with the view that effects on nitrogen metabolism are due to systemic toxicity and are not directly associated with the diuretic action. Dicker (1946) observed enhanced proteinuria in water-loaded rats and a similar result might also be anticipated in response to any mechanism evoking polyuria in this species. Daily urine analyses showed that the larger doses of diuretic ethyleneimines could increase protein excretion (Table 3). It is curious however, that *NN*-ethylene-*N'*methylurea (III) and *NN*-ethylene-*N'*-isopropylurea (V) failed to cause an increase in protein excretion (Table 3).

The amine, spermine, has been described by Tabor & Rosenthal (1956) to be diuretic in the rat when using a water-loading assay procedure (Lipschitz, Hadidian & Kerpcsar, 1943). According to this report severe proteinuria was observed which reached a maximal concentration of 2.5% within 1 to 4 days after treatment. In contrast, the maximal proteinuria in the present series occurred after treatment with *NN*-ethyleneurea (II), with a concentration of only 0.23% in urine collected 24 to 48 hr after injection. Tabor & Rosenthal (1956) have also recorded similarities in the nephrotoxic action of ethyleneimine, ethylenediamine and propylenediamine; higher polyethylenediamines were inactive. It was thought that the diamines could be converted to cyclic imines *in vivo*:



Propylenediamine Propyleneimine

According to these authors spermine is " 20 to 70-times more toxic to the kidney than either the short-chain diamines or ethyleneimine," and they suggested that the responsible metabolite is an amino-aldehyde.

In the present series the diuresis is similar to that produced by ethyleneimine (I), and the question arises whether the action of the other compounds is due to the *in vivo* liberation of ethyleneimine. In the case of di(aziridin-1-yl) sulphoxide (VI), the rapid liberation of ethyleneimine (I) has been established (Craig, Jackson & James, 1963) and the *in vivo* production of the base from its derivatives is being investigated. Although the toxicity and diuretic activity of di(aziridin-1-yl) sulphoxide (VI) and *NN*-ethyleneurethane (VII) are related to their ethylenimine content, the urea derivatives showed no such correlation. In the absence of knowledge of the metabolic fate of these compounds it is difficult to compare directly the results from experiments using ethyleneimine and other derivatives. The latter might not only liberate the free base at varying rates but perhaps at different metabolic sites; for example, the local production of ethyleneimine in the kidney could result in an enhanced diuretic effect.

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