Short Communication

**THE INFLUENCE OF AMMONIUM CHLORIDE ON THE
INDUCTION OF BLADDER TUMOURS BY
4-ETHYLSULPHONYLNAPHTHALENE-1-SULPHONAMIDE**

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IT HAS BEEN established that the administration of 4-ethylsulphonylnaphthalene-1-sulphonamide (ENS, 0.01% in the diet) to mice results in epithelial hyperplasia and tumours of the bladder (Clayson and Bonser, 1965; Clayson, Pringle and Bonser, 1967; Dzhoiev, Wood and Cowen, 1969). In addition, ENS causes the production of urine and bladder calculi (Levi *et al.*, 1971).

Flaks, Hamilton and Clayson (1973) confirmed these findings in a preliminary report and in an experiment of 52 weeks' duration noted that if the animals received ammonium chloride (NH_4Cl , 1% in the drinking water) in addition to ENS, urinary pH remained normal while calculi and tumours of the bladder did not develop, but mild hyperplasia of the bladder epithelium was present. This report presents a final assessment of the effect of NH_4Cl on the action of ENS during the life span of the animals.

MATERIALS AND METHODS

IF $\times$ C57 F1 hybrid female mice were bred in our laboratory and fed Oxoid diet No. 41B and water *ad libitum* and started on treatment when 10–12 weeks old. ENS was prepared by the method of Brimelow and Vasey (1958) and incorporated into the diet at a level of 0.01% as described by Clayson *et al.* (1967). NH_4Cl (1.0% in aqueous

solution) was substituted for drinking water.

The experiment consisted of 4 groups of 52 animals, treated as follows: (1) ENS (0.01% in diet); (2) NH_4Cl (1.0% in drinking water); (3) ENS (0.01% in diet) and NH_4Cl (1.0% in drinking water); (4) untreated controls.

All the animals were examined for urinary pH. They were inspected daily and any which were moribund were killed and autopsied. Bladders and all major organs were taken for histological examination. The pathological results were interpreted by the criteria described by Bonser and Jull (1956). Carcinoma I is the tumour which had not invaded and carcinoma II is the tumour which had invaded the muscle of the bladder.

RESULTS AND DISCUSSION

It emerged that the addition of NH_4Cl (1.0% in the drinking water) prevented the formation of an alkaline urine, bladder calculi and bladder tumours in ENS (0.01% in the diet) treated animals (Table I). Epithelial hyperplasia, marked with ENS alone, was much less severe and no neoplasms were detected during the life span of the mice. Because of the absence of calculi, hydronephrosis was absent from the ENS/ NH_4Cl treated group, together with a marked reduction in the incidence of nephritis when compared with ENS treated mice. Untreated

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TABLE I.—*Effect of NH_4Cl (1.0% in Drinking Water) on Bladder Tumour Formation by ENS (0.01% in diet) in IF $\times$ C57 F1 Female Mice*

Group	No. of mice	Treatment	pH of urine	Average survival	Bladder		Epithelial		Kidney
			average range	in days range	Tumour	Stone	hyperplasia		
1	50	0.01% ENS	7.2 6.9–9.0	417 96–648	19 ^b 7 stage I 12 stage II	22	Marked	41	29 Hydronephrosis 15 Nephritis
2	50	NH_4Cl	6.2 5.8–6.7	492 365–652	—	—	Mild	1	1 Nephritis
3	48	ENS 0.01% and NH_4Cl	6.5 5.6–7.2	465 365–652	—	1	Mild	40	4 Nephritis
4	49	1.0% None	6.2 5.5–6.7	485 365–652	—	—	—	—	—

^b Carcinoma I, Carcinoma II.TABLE II.—*Variation of the Incidence of Gross and Microscopic Lesions of the Urinary Bladder in IF $\times$ C57 F1 Mice Fed ENS (0.01%) in their Diet*

Days on treatment	No. of mice	Stone at autopsy	Epithelial hyperplasia	Tumours
1–150	3	0 (0)	0 (0)	0 (0)
151–300	9	7 (78)	8 (89)	3 (33)
301–450	22	10 (44)	20 (90)	7 (33)
450–600	8	3 (38)	8 (100)	4 (50)
601–648	8	2 (25)	8 (100)	5 (60)
Totals	50	22 (46)	44 (88)	19 (38)

Numbers in parentheses are percentages.

control animals and those given NH_4Cl alone were free from bladder tumours, epithelial hyperplasia (with the exception of one of 50 mice) and calculi, while a few cases of nephritis were noted.

ENS inhibits carbonic anhydrase (Clayson, 1973) and thus leads to alkalineuria and the formation of stones of calcium and ammonium phosphate and oxalate in the urinary bladder (Levi *et al.*, 1971). The present experiment indicates that either the alkalineuria or the stone is concerned in the genesis of the bladder tumours, but does not distinguish between these possibilities. The progressive effect of ENS with time on the mouse bladder is outlined in Table II. Whereas the macroscopically observed gross thickening of the whole viscus, the bladder epithelial hyperplasia and the formation of tumours increase with time after the start of ENS feeding, the incidence of bladder stones rises to a maximum and then tends to decline.

The absence of stone in the 3 mice

dying before 150 days is unusual; as in other experiments involving female IF $\times$ C57 mice, ENS (0.01% in the diet) induced stones in the bladder of the majority by 80 days (unpublished observations). At death, these mice showed loss of weight but histologically no cause of death could be determined.

The fall in the incidence of bladder stones in the mice from 78% at 151–300 days to 25% at 601–648 days demonstrates clearly the need for sequential killing studies if the influence of bladder stones in the development of vesical neoplasms is to be elucidated (Clayson, 1974).

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