

Short Communication

**INDUCTION OF TUMOURS IN INTACT AND PARTIALLY
HEPATECTOMIZED RATS WITH ETHYL METHANESULPHONATE**

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SINCE the suggestion was made that replicating liver cells may be especially sensitive to chemical carcinogens (Pound, 1968), much evidence has accumulated which supports this idea. As an instance of this, certain carcinogenic alkylating agents, dimethylnitrosamine (DMN) and nitrosomethylurea (NMU), which do not usually induce liver cancer by a single treatment, are hepatocarcinogens if given during the period of restorative hyperplasia following partial hepatectomy (Craddock, 1971; Craddock and Frei, 1974). These results suggest that replication of alkylated DNA is an initial event in carcinogenesis. Another methylating agent, methyl methanesulphonate (MMS), on the other hand, was not found to be a hepatocarcinogen, even when given after partial hepatectomy (Craddock, 1973a). Evidence suggests that this difference may be due to a difference in the nature of the reaction products formed in DNA. It appears likely that O⁶-alkylguanine rather than 7-alkylguanine is relevant in carcinogenesis (Loveless, 1969). DMN and NMU give rise to both these methylated bases, whereas no O⁶-methylguanine was detectable in rat liver after treatment with MMS (Craddock, 1973b). A small amount was measured after treatment of DNA with MMS *in vitro* (Lawley and Shah, 1972), and in mice treated with MMS *in vivo* (Frei and Lawley, 1976). In the case of ethyl

methanesulphonate (EMS), on the other hand, O⁶-ethylguanine forms a larger proportion of the products of reaction with nucleic acids (Shooter *et al.*, 1974; Lawley, Orr and Jarman, 1975; Singer and Fraenkel-Conrat, 1975; Sun and Singer, 1975). To investigate further whether replication of DNA containing O⁶-alkylated guanine residues could be responsible for carcinogenesis, it was of interest to determine whether EMS induced liver-cell cancer when given to animals in which liver cells had been stimulated to proliferate by partial hepatectomy.

Treatment with EMS had previously been shown to be carcinogenic in rats and mice (IARC Monograph, 1974). In rats, EMS induced tumours in kidney and brain (Swann and Magee, 1969; Montesano *et al.*, 1974), abdominal wall and lung (Hrushesky, Sampson and Murphy, 1972), heart (Haas, Hilfrich and Mohr, 1974), and in mammary gland (Williams *et al.*, 1974). In newborn (Walters *et al.*, 1967) and adult mice (Frei, 1971), EMS induced pulmonary adenomas. While the experiments to be described did not induce hepatocellular carcinoma, the previous evidence for cancer of brain and kidney was substantiated, and in addition tumours were found in the small intestine and the genital tract.

Female albino rats weighing 195–205 g or 99–105 g were used. Freshly

TABLE.—*Effect of a Single Treatment with EMS on Intact and Partially Hepatectomized (PH) Rats*

	Body wt	Dose mg/kg	Number of animals	Number surviving 3 days	Survival (weeks)	Tumours induced
PH	200 g	300-350	6	3	51-117	—
		250-265	6	5	51-86	1GT
		100-200	4	4	91-118	—
Intact	200 g	265-300	9	9	75-115	1SI, 3GT, 1B
PH	100 g	244-300	8	8	52-97	1GT, 1K, 1B
Intact	100 g	300	9	9	67-112	—

K, mesenchymal kidney tumour; B, glioma of brain; SI, adenocarcinoma of small intestine; GT, malignant mesenchymal tumour of genital tract.

prepared solutions of EMS were administered by i.p. injection to animals either 24 h after partial hepatectomy, or to intact control animals. Partial hepatectomies were carried out between 9 a.m. and 12.30 p.m. by the method of Higgins and Anderson (1931), using light ether anaesthesia. Animals were kept without further treatment until they appeared to be ill, when they were killed. The liver and any organ showing macroscopic lesions were examined histologically.

A few animals given the higher doses of EMS died after a few days, with no apparent cause of death. Other animals became ill and were killed before the end of their normal life span (Table). Although the number of animals used was small, the results show that EMS is not a potent carcinogen in regenerating liver. No hepatocellular neoplasms were induced. Nine animals developed malignant tumours at other sites. One rat had a kidney sarcoma, and two animals had astrocytomas, these tumours being similar to those found previously after treatment with EMS (Swann and Magee, 1969). In addition, tumours were found at sites not previously reported to be susceptible to EMS. One animal had an adenocarcinoma of the small intestine, and five animals had malignant mesenchymal tumours originating in the genital tract.

It appears therefore that EMS can induce cancer in a variety of tissues. It is known that EMS forms 7-ethylguanine in DNA to similar extents in liver, kidney, lung, ileum and brain (Swann

and Magee, 1971). It is likely that O⁶-ethylguanine is formed in the same amount relative to 7-ethylguanine in different tissues. Whether cancer is induced or not, possibly depends on the rate of excision of O⁶-ethylguanine, and on the rate of cell replication, in the tissue concerned. Liver may be protected by the apparently high activity of excision repair enzymes (Goth and Rajewsky, 1974; Kleihuis and Margison, 1974; Nicoll, Swann and Pegg, 1975). In spite of the increased rate of cell replication in liver following partial hepatectomy, the high rate of repair may bring about excision of the O⁶-ethylguanine before "fixation" by miscoding at replication can take place. The rate of repair in tissues which do develop tumours (*i.e.* brain, kidney, small intestine and genital tract) may be less rapid. There is in fact evidence that excision of O⁶-alkylguanine occurs less rapidly in kidney and brain than in liver (Goth and Rajewsky, 1974; Kleihuis and Margison, 1974; Nicoll *et al.*, 1975). In this connection it would be of interest to know the extent of formation and rate of disappearance of O⁶-ethylguanine in liver DNA, and the timing and extent of DNA replication, in the regenerating liver of rats treated with EMS.

In view of the widespread use of EMS as a mutagen, the fact that a single treatment can induce cancer should be stressed.

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