Short Communication

Methylation of Ribonucleic Acid and Deoxyribonucleic Acid and Tumour Induction in Livers of Hypophysectomized Rats Treated with Dimethylnitrosamine

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(Received 6 November 1967)

With the discovery of the carcinogenicity of DMN* by Magee & Barnes (1956), a powerful new group of chemical carcinogens, the *N*-nitrosamines, has contributed to investigation of the mechanism of cancer induction. DMN is a potent carcinogen for liver, kidney and other organs in several species; its metabolism and the products of its interaction with cells have been extensively studied (Heath & Dutton, 1958; Heath & Magee, 1962; Heath & Jarvis, 1955; Magee & Hultin, 1962; Magee & Farber, 1962; Lee, Lijinsky & Magee, 1964; Lee & Spencer, 1964). These workers showed that methylation of liver proteins, RNA and DNA occurs shortly after treatment with DMN, and it has been postulated that this chemical damage, particularly of the genetic material, may provide the basis for a molecular mechanism underlying the biological change to malignancy (Magee, 1968).

It has been known for several years that hypophysectomized rats are resistant to the action of several powerful liver carcinogens, including the aminoazobenzenes, 2-aminofluorene and other fluorenylamines (Goodall, 1968). Hypophysectomy also blocked liver carcinogenesis in rats treated with aflatoxin (Goodall & Butler, 1966), one of the most powerful carcinogens known. It seemed possible that examination of nucleic acids from livers of hypophysectomized rats treated with DMN might help to test whether alkylation of nucleic acids was an essential part of the mechanism of liver carcinogenesis. In the present communication we report the occurrence of methylation of liver DNA as well as RNA (Lee & Goodall, 1966) in hypophysectomized rats treated with ³H-labelled DMN. In corresponding long-term experiments we have found a high yield of malignant liver tumours in hypophysectomized rats receiving DMN (C. M. Goodall & K. Y. Lee, unpublished work).

DMN was obtained from Eastman Organic Chemicals (Rochester, N.Y., U.S.A.) and purified by distillation. The ³H-labelled DMN was prepared by Dr W. Lijinsky by the method of Lee *et al.* (1964). The authentic 7-methylguanine was a gift

* Abbreviation: DMN, dimethylnitrosamine.

from Dr C. H. Hitchings of Wellcome Research Laboratories (Tuckahoe, N.Y., U.S.A.).

Wistar albino Porton (M.R.C.) strain rats of both sexes weighing 80–100 g. each were hypophysectomized at 8 weeks of age and were then kept under observation for 5 weeks before treatment. The operated animals were fed on powdered Rockland chow and given 5% sucrose in their drinking water, and were housed at 75°F in plastic cages.

Three hypophysectomized male rats weighing 80 g. each were injected intraperitoneally with 4.6 mg. of ³H-labelled DMN containing 138 μ C and were killed 3.5 hr. afterwards. Nucleic acids and protein were prepared by the method of Lee *et al.* (1964) and the nucleic acids were hydrolysed by the method of Vischer & Chargaff (1948). Dowex 50 ion-exchange resin (H⁺ form) column chromatography was carried out by gradient elution with 1-4N-HCl. Paper chromatography, spectrophotometry and radioassay procedures were as described by Lee *et al.* (1964). Two female hypophysectomized rats weighing 118 g. each were injected intraperitoneally with 4 mg. of ³H-labelled DMN containing 97 μ C for study of liver DNA.

Further groups of 20 male and 20 female rats together with control groups of intact animals were given 0.0025% DMN in their drinking water 5 days/week during 30 weeks, and the animals were then kept without further treatment until tumours or death occurred.

Incorporation of radioactivity from ³H-labelled DMN into nucleic acids and proteins of liver, kidney, spleen and pancreas is shown in Table 1. After allowance for weight differences between male and female rats the incorporation of radioactivity into liver RNA of male rats was considerably higher than that into liver RNA of females. The labelling pattern in other organs was similar to that of intact rats previously studied (Lee *et al.* 1964). After acid hydrolysis and column chromatography of RNA and DNA, as in previous experiments (Lee *et al.* 1964), a major radioactive peak containing nearly all the radioactivity appeared between

Table 1. *Incorporation of radioactivity from ^3H -labelled DMN into RNA, DNA and protein of liver, kidney, spleen and pancreas of hypophysectomized male and female rats*

Average body weights were 80 g. for females and 118 g. for males. Rats for the RNA experiment were injected with 4.6 mg. of ^3H -labelled DMN containing $138\mu\text{C}$ and rats for the DNA experiment were injected with 4.02 mg. of ^3H -labelled DMN containing $97\mu\text{C}$. DMN was given intraperitoneally and rats were killed 3.5 hr. afterwards.

Organ	Radioactivity incorporated (disintegrations/min./mg.)				
	RNA		DNA	Protein	
	Male	Female		Male	Female
Liver	22950	12615	4501	1960	2085
Kidney	2960	2760	*	1210	1450
Spleen	2485	1820	*	580	975
Pancreas	285	185	*	1660	1745

* Insufficient material for radioassay.

guanine and adenine; this is the position in which 7-methylguanine is known to appear under these experimental conditions.

The fractions containing the radioactivity from RNA and DNA were concentrated and applied to Whatman no. 1 paper and developed by descending chromatography with propan-2-ol-water (17:3, v/v) saturated with conc. NH_3 as solvent. Authentic 7-methylguanine was applied on the paper as a marker. The spot corresponding to 7-methylguanine when viewed in ultraviolet light was cut out and eluted with 0.5N-HCl, and it had an ultraviolet spectrum identical with that of 7-methylguanine. Although no further attempts to identify the compound could be made because of the small amount available, this material with high specific radioactivity was considered to be 7-methylguanine on the basis of previous studies (Magee & Lee, 1964; Lee *et al.* 1964) and the coincidence of chromatographic behaviour and ultraviolet spectra. Apparently identical material was also previously identified positively as 7-methylguanine by mass spectrometry (Lee & Lijinsky, 1966).

In the experiments with chronic administration of DMN, metastasizing malignant liver tumours occurred in both hypophysectomized and intact animals. The incidences of liver tumours in intact rats surviving 27 weeks (the latency of the first tumour) were 16/17 in females and 14/14 in males, and the corresponding incidences in the hypo-

physectomized rats were 10/12 in females and 8/13 in males. Additional primary tumours were found in lung, ovary and other organs besides liver. Tumour latencies were not obviously affected by hypophysectomy.

Hypophysectomy completely blocks carcinogenesis in rat liver with every chemical carcinogens so far tested (Goodall, 1968). Our present results show that in contrast DMN is a powerful carcinogen for the liver of hypophysectomized rats, and that in experiments with acute administration of DMN the alkylation of nucleic acids is not greatly affected in these abnormal animals. This represents apparently the first successful induction of malignant liver tumours in hypophysectomized animals with an efficiency equal to that in intact rats. As discussed more extensively elsewhere (Goodall, 1968), it now appears that liver of hypophysectomized rats possesses the same potential for neoplasia as that of normal rats, and that the failure of aflatoxins, fluorenylamines and azo-dyes to be carcinogenic in hypophysectomized rats probably is due to differences in the metabolism and activation of these carcinogens. The present biochemical results show the normal pattern of alkylation of RNA and DNA by treatment with DMN (Lee *et al.* 1964). Thus our results support the view that there is some correlation between interaction of this carcinogenic nitrosamine with nucleic acids and the eventual appearance of neoplasms.

This work was supported by U.S. Public Health Service Contract no. PH-43-65-67 and by grants from the Cancer Society of New Zealand. We are grateful for the technical assistance of Miss Irene Ham and Mr B. Paliulis.

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