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Nitrosamine-Induced Carcinogenesis

THE ALKYLATION OF N-7 OF GUANINE OF NUCLEIC ACIDS OF THE RAT BY DIETHYLNITROSAMINE, *N*-ETHYL-*N*-NITROSOUREA AND ETHYL METHANESULPHONATE

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1. The extent of ethylation of N-7 of guanine in the nucleic acids of rat tissue *in vivo* by diethylnitrosamine, *N*-ethyl-*N*-nitrosourea and ethyl methanesulphonate was measured. 2. All compounds produced measurable amounts of 7-ethylguanine. 3. A single dose of diethylnitrosamine or *N*-ethyl-*N*-nitrosourea produced tumours of the kidney in the rat. Three doses of ethyl methanesulphonate produced kidney tumours, but a single dose did not. 4. A single dose of diethylnitrosamine produced twice as much ethylation of N-7 of guanine in DNA of kidney as did *N*-ethyl-*N*-nitrosourea. A single dose of both compounds induced kidney tumours, although of a different histological type. 5. A single dose of ethyl methanesulphonate produced ten times as much ethylation of N-7 of guanine in kidney DNA as did *N*-ethyl-*N*-nitrosourea without producing tumours. 6. The relevance of these findings to the hypothesis that alkylation of a cellular component is the mechanism of induction of tumours by nitroso compounds is discussed.

The *N*-nitroso compounds are highly toxic and carcinogenic. The characteristic toxic lesion produced by the nitrosamines is centrilobular necrosis of the liver, but the damage produced by the nitrosamides is more widespread, with the most severe lesions in organs of rapid cellular proliferation such as the bone marrow, the intestinal epithelium and the lymphoid tissue (Magee & Barnes, 1967). This difference in behaviour of the two classes of nitroso compounds is possibly caused by a difference in their chemical properties: the chemically stable nitrosamines damage only organs such as the liver, which have enzyme systems capable of their breakdown, but the unstable nitrosamides damage cells in any organ that they penetrate (for review see Magee & Swann, 1969). The *N*-nitroso compounds include some of the most powerful known carcinogens, several being capable of producing tumours when given as a single dose in spite of their rapid elimination from the body. They are thus valuable agents for the study of mechanisms of the induction of cancer.

Administration of several *N*-nitroso compounds to animals results in different degrees of alkylation of bases in the nucleic acids of different organs, but the role, if any, of this alkylation in the production of tumours is not known. In a previous attempt to elucidate this role the amounts of methylation of the N-7 position of guanine produced in rat tissues *in vivo* by single doses of *N*-methyl-*N*-nitrosourea, dimethylnitrosamine, methyl methanesulphonate and dimethyl sulphate were compared and contrasted with the carcinogenic activity of the same dose of each compound (Swann & Magee, 1968). The experiments reported here extend this comparison to three ethylating agents, diethylnitrosamine, *N*-ethyl-*N*-nitrosourea and ethyl methanesulphonate.

Diethylnitrosamine is an acute hepatotoxin and is carcinogenic in many species of animals (Druckrey, Preussmann, Ivancovic & Schmähl, 1967). Prolonged feeding to rats at a high dosage in the diet produces liver tumours (Schmähl, Preussmann & Hamperl, 1960), but as the dose is decreased oesophageal tumours are produced (Druckrey, Schildbach, Schmähl, Preussmann & Ivancovic, 1963). In contrast, administration of diethylnitrosamine to hamsters commonly results in tumours of the lung and bronchi (Dontenwill & Mohr, 1961; Herrold, 1964). Druckrey, Steinhoff, Preussmann & Ivancovic (1963) showed that a single large dose of diethylnitrosamine produced kidney tumours in two of four rats of the BD strain. This result has been confirmed in a Wistar-derived stock (P. F. Swann & P. N. Magee, unpublished work). All the kidney tumours induced by diethylnitrosamine were derived from epithelial cells, with no tumours of the mesenchymal type. The major interest in the carcinogenic activity of *N*-ethyl-*N*- nitrosourea has centred on the induction of tumours of brain and

nerve in the offspring of rats treated around the fifteenth day of pregnancy (Ivancovic, Druckrey & Preussmann, 1966; Wechsler *et al.* 1969). Single doses administered to young rats produced mesenchymal tumours of the kidney as well as tumours of the nervous system (Druckrey, Schagen & Ivancovic, 1970). Ethyl methanesulphonate given in three large doses increased the incidence of pulmonary adenomas and induced epithelial-cell tumours of the kidney in mice (Alexander & Connell, 1963) and produced mesenchymal cell tumours of the kidney in rats (Swann & Magee, 1969).

Magee & Lee (1963) reported that administration of diethylnitrosamine to rats resulted in ethylation of N-7 of guanine of nucleic acids of the liver, but Krüger, Ballweg & Maier-Borst (1968) failed to find ethylation of N-7 of guanine when *N*-ethyl-*N*-nitrosourea was given to the same species. In the present paper it is shown that *N*-ethyl-*N*-nitrosourea does produce 7-ethylguanine in nucleic acids of rat tissue and the amount of ethylation produced by this and the two other carcinogenic compounds, diethylnitrosamine and ethyl methanesulphonate, are compared. A similar approach to that used in studies on methylating agents was adopted (Swann & Magee, 1968). The amount of alkylation was measured in rats given each compound in a dose similar to that used in the tests for carcinogenic activity, at a time when the alkylation was expected to have reached its highest value from knowledge of the rate of metabolism of the compound. The rate of metabolism of ethyl methanesulphonate (Roberts & Warwick, 1958) and of diethylnitrosamine (Heath, 1962) were already known. The rate of decomposition of ethyl methanesulphonate in the rat was approximately exponential, the half-life of a dose of 300mg/kg body wt. being about 6.5h. The rate of metabolism of diethylnitrosamine was linear through the greater part of its course, 4.3% of a dose of 204mg/kg body wt. being metabolized each hour.

In the work described in the present paper, the rate of decomposition of *N*-ethyl-*N*-nitrosourea *in vivo* was measured; *N*-[^{14}C]ethyl-*N*-nitrosourea, [^{14}C]diethylnitrosamine and [^{14}C]ethyl methanesulphonate were prepared; and the amount of ethylation of *N*-7 of guanine in nucleic acids of tissues of the rat that each produced *in vivo* was measured and compared with the carcinogenic activity of each compound. A preliminary report of this work has been given (Swann & Magee, 1970).

METHODS

Animals. Male or female Wistar-derived albino rats from the Courtauld Institute stock were maintained in cages with floors covered in wood-chips or sawdust. The rats were fed on Rowett Research Institute diet 86. Syrian golden hamsters were obtained from the Laboratory Animals Centre, Carshalton, Surrey, U.K.

Chemicals. *N*-Ethyl-*N*-nitrosourea was obtained from Fluka A. G., Buchs, Switzerland. Ethyl methanesulphonate and diethylnitrosamine were obtained from Eastman–Kodak, Rochester, N.Y., U.S.A. Both were redistilled before use.

Radioactive chemicals. *N*-[2-^{14}C]Ethyl-*N*-nitrosourea (Sp. radioactivity 0.325mCi/mmol) was synthesized from [2-^{14}C]acetonitrile by reduction of the nitrile to [^{14}C]-ethylamine with the complex of lithium aluminium hydride and aluminium chloride (Nystrom, 1955). *N*-[^{14}C]Ethylurea was formed by reaction of the [2-^{14}C]-ethylamine and sodium isocyanate and nitrosated with nitrous acid (Cox & Warne, 1951; Jones & Skraba, 1953). The *N*-[2-^{14}C]ethyl-*N*-nitrosourea was extracted from the nitrosation mixture with diethyl ether and recovered by evaporation of the ether (yield by polarographic analysis, 34% of theoretical). [1-^{14}C]Ethyl methanesulphonate (sp. radioactivity 0.412mCi/mmol) was prepared by reaction of [1-^{14}C]ethyl iodide and silver methanesulphonate *in vacuo* without the use of solvent (Swann & Magee, 1968). [1-^{14}C]Diethylnitrosamine was prepared from [1-^{14}C]ethyl iodide by Dr D. F. Heath (Dutton & Heath, 1956) or from sodium [1-^{14}C]acetate through intermediate production of ethyl acetamide and diethylamine by Dr A. R. Mattocks (A. R. Mattocks, unpublished work). All radioactive precursors were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Measurement of radioactivity. All radioactivity measurements were made in a scintillation counter (Packard Instrument Co., LaGrange, Ill., U.S.A.) by conventional methods. Corrections from c.p.m. to d.p.m. were normally made by addition of standard [^{14}C]toluene (Packard Instrument Co.) but where the solutions were highly radioactive the method of pulse-height shift was used (Baillie, 1960). Two scintillation solutions were used: 0.6% 2,5-diphenyloxazole in toluene for samples that did not contain water, and 1% 2,5-diphenyloxazole, 0.05% 1,4-bis-(4-methyl-5-phenyloxazole-2-yl)benzene and 9% naphthalene dissolved in dioxan–cellosolve–toluene (3:3:1, by vol.) for aqueous samples (Bruno & Christian, 1961).

Conduct of animal experiments. (*a*) General procedure. The animals were allowed access to water but were not given food for 20h before being killed by bleeding from the abdominal aorta after anaesthesia with sodium pentobarbital (Vetinary Nembutal, Abbott, Queenborough, Kent, U.K.). The organs were removed and frozen in liquid N_2. In the experiment in which the rate of decomposition of *N*-ethyl-*N*-nitrosourea was measured the rats (150g) were allowed free access to food until immediately before the experiment. *N*-Ethyl-*N*-nitrosourea was dissolved in 0.01M-sodium citrate buffer, pH6, containing 8.5g of NaCl/l to give a final solution of 20mg/ml. This solution (1.5ml) was injected into the tail vein of each rat. After various time-intervals the rats were bled from the abdominal aorta and the amount of *N*-ethyl-*N*-nitrosourea in the blood was determined by polarography (Heath & Jarvis, 1955; Swann, 1968). Rats treated for a short period of time remained under sodium pentobarbital anaesthesia throughout, but those left for longer

periods were injected under light ether anaesthesia and anaesthetized again before being bled.

(*b*) Ethylation by diethylnitrosamine. A solution of [1-^{14}C]diethylnitrosamine in NaCl was prepared (40mg; 250μCi/ml of 0.9% NaCl) and 1.125ml was injected intraperitoneally into each of eight female rats (180g) (i.e. 250mg, 1.56mCi/kg of body wt.). The rats were treated as detailed in the 'general procedure' above and killed 24h later. Nucleic acids were prepared from liver, kidney, lung and small intestine. In other experiments rats were given 200mg (242μCi/kg of body wt.) or Syrian golden hamsters (70g) were given 166mg (330μCi/kg of body wt.).

(*c*) Ethylation by ethyl methanesulphonate. [1-^{14}C]-Ethyl methanesulphonate (283mg; 970μCi) was dissolved in 0.9% NaCl (10.5ml) and 1ml of this solution given by intraperitoneal injection to each of ten female rats (90–110g) (i.e. 270mg; 920μCi/kg of body wt.). These animals were starved for 17h after injection, then killed, and nucleic acids were prepared from the liver, kidney, small intestine, lung and brain.

(*d*) Ethylation by *N*-ethyl-*N*-nitrosourea. *N*-[2-^{14}C]-Ethyl-*N*-nitrosourea (229mg; 330μCi) was dissolved in 0.9% NaCl–0.01M-sodium citrate, pH6 (12ml) and 1.2ml was given to each of eight male rats (150g) that had been starved as in the other experiments (i.e. 150mg; 220μCi/kg of body wt.) by intravenous injection. These were killed 1.5–2h later and nucleic acids were prepared from liver, kidney, small intestine, large intestine, lung and brain.

Preparation of nucleic acids. Both RNA and DNA were prepared from the same sample of frozen tissue by the method of Kidson, Kirby & Ralph (1963) except that phenol–*m*-cresol–8-hydroxyquinoline–water (100:14:0.1:11, by wt.) was used in place of 90% (w/v) phenol. The DNA was freed of RNA by ribonuclease treatment and carbohydrates were removed from both RNA and DNA by the methoxyethanol–phosphate procedure (Kirby, 1956). The nucleic acids were finally precipitated by addition of an equal volume of cetyltrimethylammonium bromide (1%, w/v). The cetyltrimethylammonium salt of the nucleic acid was washed with water and converted into the sodium salt by treatment with 2% (w/v) sodium acetate in 70% (v/v) ethanol. The sodium salt of the nucleic acid was washed in ethanol, ethanol–diethyl ether (1:1, v/v), and diethyl ether and dried *in vacuo*. In one experiment where diethylnitrosamine was given, DNA, microsomal, soluble and nuclear RNA from the liver and kidneys of the rats were prepared by the method of Villa-Treviño, Shull & Farber (1966).

Determination of the proportion of guanine residues ethylated on N-7. The nucleic acids were hydrolysed in 1M-HCl by heating to 100°C for 1h. The hydrolysate was chromatographed on a Dowex 50W (X12; H^+ form) column (10cm × 1cm) by using an exponential gradient of 1–4M-HCl (Magee & Farber, 1962). In all cases a radioactive peak was eluted between guanine and adenine, in the same fractions as an authentic sample of 7-ethylguanine generously given to us by Dr P. D. Lawley. The amount of 7-ethylguanine was calculated from the amount of radioactivity in the peak by assuming that the specific radioactivity was the same as that of the ethyl group of the injected ethylating agent. The amount of guanine was calculated from the extinction of the guanine peak by taking ϵ_{260} in acid to be 8000.

RESULTS

Polarographic determination of the amount of *N*-ethyl-*N*-nitrosourea remaining in the blood of a rat given a dose of 200mg/kg of body wt. by intravenous injection showed that the concentration fell rapidly, with a half-life of about 5–6min (Fig. 1). Thus it appears slightly more stable *in vivo* than the methyl homologue, which seemed to have a half-life of approx. 1min (Swann, 1968).

A peak of radioactivity that eluted from the Dowex 50 column in a similar position to 7-ethylguanine was found in the acid hydrolysate of nucleic acids of tissues of rats given [1-^{14}C]ethyl methanesulphonate, [1-^{14}C]diethylnitrosamine or *N*-[2-^{14}C]ethyl-*N*-nitrosourea (Fig. 2). The amount of 7-ethylguanine is given in Table 1.

The first experiments with [1-^{14}C]diethylnitrosamine were done with a sample synthesized from [1-^{14}C]ethyl iodide by the method of Dutton & Heath (1956). Hamsters were used because it was hoped to discover why the respiratory tract of the hamster is so highly susceptible to the carcinogenic effects of diethylnitrosamine (Dontenwill, Mohr & Zagel, 1962). When an acid hydrolysate of nucleic acid from the livers of these animals was chromatographed a peak of radioactivity that eluted in a position similar to that of 7-methylguanine was found, as well as a peak corresponding to 7-ethylguanine (Fig. 3). By contrast, when a sample

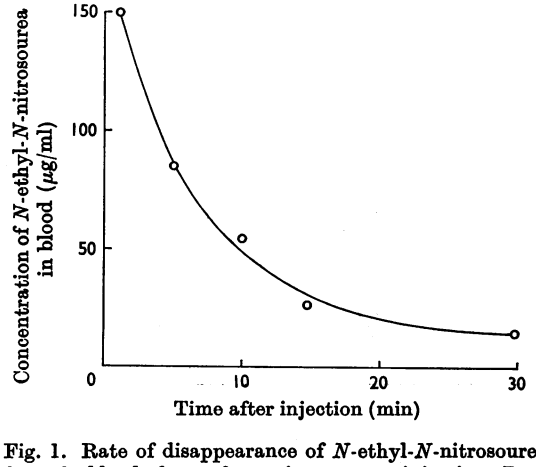

Fig. 1. Rate of disappearance of *N*-ethyl-*N*-nitrosourea from the blood of rats after an intravenous injection. Rats (each 150g) were each given 200mg/kg of body wt. The blood proteins were precipitated with sulphosalicylic acid and the amount of *N*-ethyl-*N*-nitrosourea in the supernatant was determined by polarography.

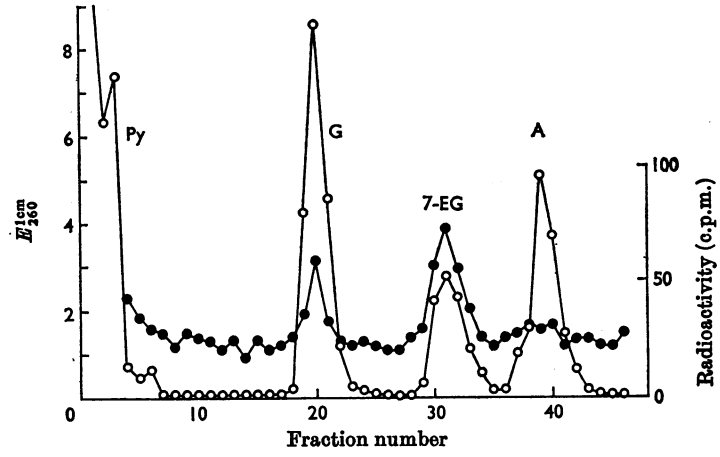

Fig. 2. Ion-exchange chromatography of an acid hydrolysate of lung RNA from rats given *N*-[2-^{14}C]ethyl-*N*-nitrosourea (150mg; 220μCi/kg). The isolated nucleic acid was hydrolysed in 1M-HCl at 100°C for 1h and chromatographed on a column (10cm×1cm) of Dowex 50 (X12; H^+ form) with exponential 1–4M-HCl gradient elution. Fractions (8.6ml) were collected ○, E_{260}; ●, radioactivity. Py, pyrimidine nucleotides; G, guanine; A, adenine; 7-EG, added authentic 7-ethylguanine.

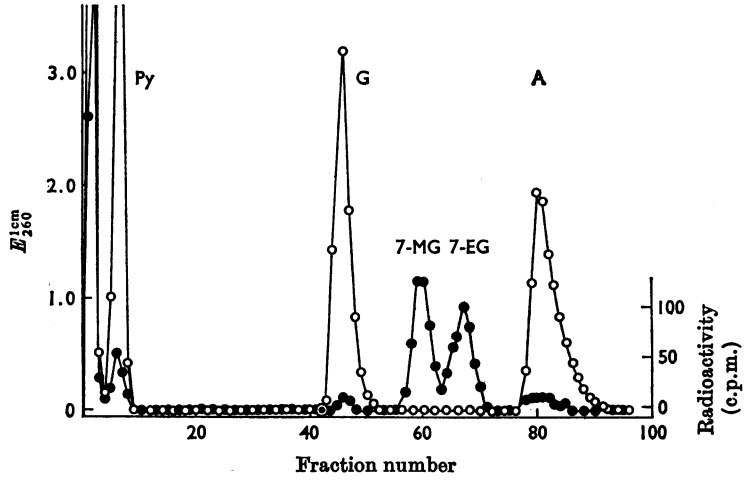

Fig. 3. Ion-exchange chromatography of an acid hydrolysate of liver RNA from hamsters given [1-^{14}C]-diethylnitrosamine synthesized from [1-^{14}C]ethyl iodide. The isolated nucleic acid was hydrolysed in 1M-HCl at 100°C for 1h and chromatographed on Dowex 50 (X12; H^+ form) with exponential 0.1–4M-HCl gradient elution. Fractions (10ml) were collected. ○, E_{260}; ● radioactivity. Py, pyrimidine nucleotides; G, guanine; A, adenine; 7-EG, 7-ethylguanine; 7-MG, 7-methylguanine. The 7-methylguanine co-chromatographed with a known specimen of 7-methylguanine.

of di[1-^{14}C]ethylnitrosamine synthesized from sodium [1-^{14}C]acetate was given to rats no 7-[^{14}C]methylguanine, but only 7-[^{14}C]ethylguanine, was detected in their nucleic acids. This perplexing result was at first thought to be caused by contamination of the [^{14}C]ethyl iodide. No detectable impurities were found in the first sample of diethylnitrosamine and the reason for the appearance of 7-methylguanine is still unknown.

As part of this investigation tests for carcinogenic activity were done with diethylnitrosamine given as a single dose (P. F. Swann & P. N. Magee, un-

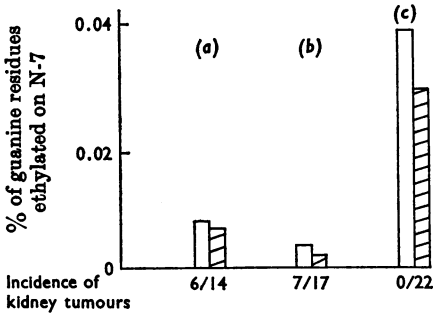

Fig. 4. Comparison between the amount of 7-ethylguanine produced in nucleic acids of rat kidney by diethylnitrosamine (*a*) (280 mg/kg), *N*-ethyl-*N*-nitrosourea (*b*) (150 mg/kg) and ethyl methanesulphonate (*c*) (270 mg/kg) and the carcinogenic activity of a similar dose of each compound. Although a single dose of ethyl methanesulphonate did not produce kidney tumours, three doses did. RNA, ▨; DNA □.

published work) and with ethyl methanesulphonate given as three separate doses (Swann & Magee, 1969). The results of these and previous investigations are given in Table 2.

DISCUSSION

In experiments with methylating agents (Swann & Magee, 1968) a single dose of methyl methanesulphonate produced greater methylation of N-7 of guanine in rat kidney DNA than that by dimethylnitrosamine, and roughly equivalent methylation of N-7 of guanine in rat kidney DNA to that by *N*-methyl-*N*-nitrosourea, but methyl methanesulphonate did not induce kidney tumours whereas both the nitroso compounds did. Methyl methanesulphonate was not entirely without carcinogenic activity, however, because it induced a significant number of tumours of the nervous system (Swann & Magee, 1969). This lack of correlation between the amount of alkylation of N-7 of guanine in nucleic acids by each compound and their carcinogenic activity cast some doubt on the importance of alkylation of N-7 of guanine for the initiation and development of cancer. The experiments reported here add to that doubt. It has been found that each of the compounds studied ethylates N-7 of guanine of nucleic acids *in vivo*. The amount of ethylation produced is less than the amount of methylation that the analogous methylating agent produced in the same organ. In kidney, diethylnitrosamine produces approximately one-tenth the alkylation of N-7 of guanine produced by dimethylnitrosamine; and *N*-ethyl-*N*-nitrosourea one-twentieth that produced by *N*-methyl-*N*-nitrosourea; and ethyl methanesulphonate one-

Table 1. *Ethylation of N-7 of guanine in nucleic acids of the rat by diethylnitrosamine, ethyl methanesulphonate and N-ethyl-N-nitrosourea*

The amount of 7-ethylguanine is expressed as a % of guanine. The method of calculation is given in the Methods section. Microsomal RNA (mic RNA), soluble RNA (S RNA) and nuclear RNA (Nuc RNA) were prepared by the method of Villa-Trevino, Shull, and Farber (1966); other nucleic acids were prepared as outlined in the Methods section.

Compounds	Dose (mg/kg) and route	Time after dose (h)	Content of 7-ethylguanine															
			Liver				Kidney				Lung		Ileum		Brain			
			DNA	Mic RNA	S RNA	Nuc RNA	DNA	Mic RNA	S RNA	Nuc RNA	DNA	RNA	DNA	RNA	DNA	RNA		
Diethylnitrosamine	250 (intraperitoneal)	24	0.034	0.025	0.06	0.015	0.008	0.0065	*	0.0078	0.007	0.0015	None detected		*	*		
			0.031															
Ethyl methanesulphonate	200 (intraperitoneal)	24			0.033		0.007		0.0073									
	270 (intraperitoneal)	17	0.045		0.024		0.041		0.031		0.047	0.028	0.053	0.024		0.038		
N-Ethyl-*N*-nitrosourea	152 (intravenous)	2	0.004		0.002		0.0034		0.002		0.0032	0.0015	*	0.002	0.003	0.002		

* Indicates that no measurement was made.

Table 2. *Carcinogenic activity in the rat of compounds producing 7-ethylguanine in nucleic acids* in vivo

This is not an exhaustive report on the carcinogenic activity of these compounds but shows those experiments where the dose used was similar to that used in the experiments reported here for determination of the amount of ethylation.

Compound	Dose and route	No. of rats	Tumours found	References
Diethylnitrosamine	280 mg/kg (intravenous)	4	Kidney (2)	Druckrey *et al.* (1964)
	280 mg/kg (intravenous)	14	Kidney* (6)	P. F. Swann & P. N. Magee (unpublished work)
N-Ethyl-*N*-nitrosourea	60–80 mg/kg (intravenous)	9 (pregnant)	Uterus (2), ovaries (1)	Alexandrov (1969)
	80 mg/kg (oral)	16 (10 days old)	Kidney† (9), nervous system (16)	Druckrey *et al.* (1970)
	80 mg/kg (oral)	17 (30 days old)	Kidney† (7), nervous system (16)	Druckrey *et al.* (1970)
Ethyl methanesulphonate	350 mg/kg (intraperitoneal)	22	Brain (1)	Swann & Magee (1969)
	3 × 275 mg/kg (intraperitoneal)	24	Kidney† (12)	Swann & Magee (1969)

*Epithelial cell tumours.
† Mesenchymal tumours.

fifth that produced by methyl methanesulphonate. These results imply that if alkylation of N-7 of guanine is responsible for the induction of tumours then ethylation is much more potent than methylation.

The correlation between the amount of ethylation of N-7 of guanine produced by these compounds *in vivo* and their carcinogenic activity can best be examined in the case of kidney, since they all can produce kidney tumours in the rat under suitable conditions. In comparing the incidence of tumours with the amount of ethylation it should be emphasized that the tumours that each compound induced were not of the same histological type. However, the proportion of mesenchymal to epithelial tumours may be influenced by the age (Murphy, Mirand, Johnston, Schmidt & Scott, 1966) or sex of the animals (Jasmin & Cha, 1969). Therefore it is impossible to decide whether the production of epithelial tumours with diethylnitrosamine and mesenchymal tumours with both ethyl methanesulphonate and *N*-ethyl-*N*-nitrosourea represents a real difference in the action of these chemicals, and the significance of the twofold difference between the amount of ethylation of N-7 of guanine by diethylnitrosamine and *N*-ethyl-*N*-nitrosourea (Fig. 4) is difficult to assess. However, a single dose of 270 mg of ethyl methanesulphonate/kg of body wt. produced five times more 7-ethylguanine in rat kidney DNA than the carcinogenic dose of diethylnitrosamine, and ten times more than a carcinogenic dose of *N*-ethyl-*N*-nitrosourea, yet a dose even larger than this (350 mg/kg body wt.) failed to induce kidney tumours. It is not clear why this single dose of ethyl methanesulphonate failed to induce kidney tumours in the rat when three separate injections of a smaller amount did (Swann & Magee, 1969).

The lack of quantitative correlation between the amount of 7-alkylguanine produced in the nucleic acids of the kidneys of rats by both these ethylating agents and by the methylating agents previously studied (Swann & Magee, 1968) might be taken to imply that the alkylation of N-7 of guanine plays no major role in the initiation of cancer by these compounds. This may be so, but it cannot be conclusively proved by evidence such as that reported here, for these measurements give no information about the distribution of alkylation between cell populations in the kidney, and it is possible that the distribution may vary with the various compounds; neither do the measurements describe the distribution of alkyl groups within a nucleic acid molecule. Singer & Fraenkel-Conrat (1969*a*,*b*) have suggested that an affinity by nitroso compounds for parts of the nucleic acid in suitable conformation, an affinity not shared by other alkylating agents, is the basis for the difference in the mutagenic action of the two

classes of compound. Similar considerations may apply to their carcinogenic activity. It is also possible that the different agents may have different effects on metabolic and immunological processes, which determine whether tumours do or do not follow the initial interactions with cellular components. Also, it must be emphasized that these experiments compare only the extent of alkylation of N-7 of guanine by each compound. This is the greatest product of the reaction of methylating agents *in vivo* and possibly also of ethylating agents but reaction at other sites in the nucleic acids such as the O–6 position of guanine (Loveless, 1969) which may be the cause of mutation in DNA containing bacteriophage or the 3-position of cytosine which appears to cause miscoding *in vitro* (Ludlum, 1970*a*,*b*) may be of greater importance in the induction of cancer.

In these experiments it was decided to study alkylation rather than any other reaction of the nitroso compounds, and to study nucleic acids rather than other components of the cell. The carcinogenic activity of ethyl methanesulphonate, though not a proof, would seem sufficient justification for a belief that alkylation can be a sufficient initiator of the growth of tumours. The importance of alkylation of some cellular component other than nucleic acids cannot be excluded, but there is evidence from viral oncology (see Dulbecco, 1969) and from the work of Cleaver (1969) on xeroderma pigmentosum that nucleic acids do play a central role in carcinogenesis.

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