Ciba Foundation Symposium

CELLULAR INJURY

Edited by A. V. S. DE REUCK, M.Sc., D.I.C., A.R.C.S.
and
JULIE KNIGHT, B.A.

With 81 illustrations

J. & A. CHURCHILL, LTD.
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CELLULAR INJURY

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Printed in Great Britain

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Under the guidance of its distinguished Trustees, the Foundation offers accommodation to scientists from all over the world at its home in Portland Place. Foremost in its activities is the organization of small conferences, the proceedings of which are published in book form in the manner of the present volume. The Foundation convenes many other informal discussions between research workers of different disciplines and different nationalities and each year invites an outstanding authority to deliver a special lecture. An exchange programme between French and British postgraduates is conducted and a library service is available. Furthermore, the Ciba Foundation attempts in every other way possible to aid scientists, whether they be Nobel Laureates or young graduates making their first original contribution to research.

The purpose of the Ciba Foundation, which is to promote international co-operation in medical and chemical research, is symbolized in the armorial bearings by five interlaced rings representing the continents, a black sacrificial cock (emblem of Aesculapius) holding a medical caduceus, and three regular hexagons for chemistry. Its domicile in London is indicated by the red sword of St. Paul and the British lion; the wyvern and the crozier, symbols associated with Basle, refer to the sponsoring firm located in this ancient Swiss town.

The Ciba Foundation

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Preface

THIS symposium arose out of a suggestion made by Dr. J. D. Judah to the Deputy Director of the Ciba Foundation at a time when plans were already being laid for a similar small international conference on "Lysosomes". No further persuasion was needed to grasp the opportunity so presented for the Foundation to arrange a meeting on the related topic of "Cellular Injury". It is indeed hoped that although each symposium was conceived as an independent entity in its own right—so that Professor de Duve, for example, opens the general discussion towards the end of the present meeting by reviewing the lysosome concept in relation to cell damage (p. 369)—nevertheless these proceedings will be found to be to some extent complementary to those of the earlier symposium ([1963]. *Ciba Foundation Symposium on Lysosomes*. London: Churchill).

Sir Roy Cameron generously consented to act as Chairman on this occasion and it is a sincere pleasure to record here how much the symposium owed to his sure light touch, and also how greatly the Foundation is indebted to his wise counsel during the planning stages in considering the scope and membership of this conference.

The programme of "Cellular Injury" was expressly designed, with the invaluable collaboration of Dr. Jack Judah and Professor John Biggers, to exclude consideration of radiation damage, but to include sections on injury by exogenous, endogenous and humoral agents, on the mechanisms of protection by drugs, on organogenesis and necrosis, and on the fine structure of damaged cells and the lesions produced by viruses. Ample time was allowed for informal discussion of the papers offered. Such free and intimate exchanges of ideas in depth are made possible only by limiting the number of those taking part. It is hoped that the complete record of the proceedings here presented will afford the pleasure of vicarious participation to many of those working in the field who could not be invited to attend the meeting.

Contents

Membership

Symposium on Cellular Injury held 2nd–4th July 1963

Sir Roy Cameron (*Chairman*)	Dept. of Morbid Anatomy, University College Hospital Medical School, London
A. D. Bangham	A.R.C. Institute of Animal Physiology, Babraham, Cambridge
W. Bernhard	Institut de Recherches sur le Cancer, Villejuif (Seine)
M. Bessis	Centre National de Transfusion Sanguine, Paris
J. D. Biggers	King Ranch Laboratory of Reproductive Physiology, University of Pennsylvania, Philadelphia
M. J. R. Dawkins	Nuffield Institute for Medical Research, Oxford
C. de Duve	Laboratoire de Chimie Physiologique, Louvain, and The Rockefeller Institute, New York
L. Ernster	The Wenner-Gren Institute, University of Stockholm
Dame Honor Fell	Strangeways Research Laboratory, Cambridge
Sir Howard Florey	The Queen's College, Oxford
A. Glücksmann	Strangeways Research Laboratory, Cambridge
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J. W. Harman	Dept. of Pathology, University College, Dublin
W. S. Hartroft	Research Institute, Hospital for Sick Children, Toronto
W. Jacobson	Strangeways Research Laboratory, Cambridge
J. D. Judah	Institute of Medical Research, Chicago Medical School, Chicago
P. N. Magee	M.R.C. Toxicology Research Unit, Carshalton
G. Majno	Dept. of Pathology, Harvard Medical School, Boston
K. R. Rees	Dept. of Chemical Pathology, University College Hospital Medical School, London
E. Reid	Chester Beatty Research Institute, London
W. G. Spector	Dept of Pathology, St. Bartholomew's Hospital, London
B. Sylvén	Cancer Research Division of Radiumhemmet, Karolinska Sjukhuset, Stockholm
A. M. Woodin	Sir William Dunn School of Pathology, Oxford
E. Zwilling	Dept. of Biology, Brandeis University, Waltham, Mass.

CELLULAR INJURY AND ALKYLATION OF CELL COMPONENTS

P. N. MAGEE

Medical Research Council Toxicology Research Unit, Carshalton

AFTER very extensive studies during the second world war, the idea emerged that mustard gas (di-2-chloroethyl sulphide) owed its tissue damaging, vesicant and other biological activities to reaction of the chloroethyl groups with essential tissue components, that is, to alkylation of receptor sites in these components (Peters, 1947). Since then this alkylation concept has been extended to include several other groups of compounds, such as the nitrogen mustards (2-chloroethylamines), epoxides, ethyleneimines, sulphonic acid esters, β-lactones and several others. These compounds are known under the general term of biological alkylating agents and their chemistry, pharmacology, chemotherapeutic activities and general biological action have been discussed in several comprehensive reviews (Philips, 1950; Mandel, 1959; Wheeler, 1962; Ross, 1962). The chemistry of these alkylation reactions is discussed in detail by Ross (1962). Essentially, alkylation is the replacement of a hydrogen atom in a molecule by an alkyl group or the addition of the alkyl group to a molecule containing an atom in a lower valency state, as in the formation of quaternary ammonium ions from tertiary amines and in the formation of esters from negatively charged carboxyl ions. The definition includes compound radicals as well as simple alkyl groups. The alkylating agent can thus be regarded as an electrophilic reactant which will combine with electron-rich centres, and the biological activity of the agents is attributed to this capacity to combine with such centres in the cell.

The biological actions of the alkylating agents are extremely well known and have been classified under the general description "radiomimetic" (Dustin, 1947; Boyland, 1954; Clayson, 1962). The properties now grouped under this heading are: vesicant action, and, with lethal doses, haemoconcentration and diarrhoea; production of chromosomal aberrations; mutagenicity; bone marrow damage; leucopenia; bleaching of hair in experimental animals at the site of application; enzyme inhibition; effect on malignant disease; carcinogenicity and damage to sperm cells and precursors.

In spite of all this work on the alkylating agents, both chemical and biological, it was not until quite recently that the occurrence of alkylation of cell components *in vivo* was demonstrated, and the suggested mechanism of action was based on studies *in vitro*. Thus Mandel (1959) states: "Although these carcinostatic drugs have been generally referred to as 'alkylating agents', only one example of biological alkylation has been demonstrated so far...". He was referring to the work of Roberts and Warwick (1957) who showed that *S*-ethylcysteine was excreted in the urine of rats treated with the drug known as "half myeleran", that is, ethyl methane sulphonate, and this was still regarded as the most complete study of the site of reaction of the alkylating agents *in vivo* by Ross (1962). To this example must be added the demonstration by Brookes and Lawley (1960) that alkylation on the 7-position of guanine moieties in nucleic acids followed the administration of sulphur mustard gas to rats carrying ascites tumour cells. Mandel (1959) went on to say "the non-specific *in vitro* reactions of the agents with amino acids, purines, pyrimidines, proteins, RNA, DNA and many other compounds have been most controversial in the elucidation of the *in vivo* action, largely because such enormous doses were usually used in order to provoke the desired response and physiological conditions were not duplicated. This lack of correlation was recognised even in 1946."

In this paper some biological and biochemical aspects of another group of compounds will be discussed, some of which are alkylating agents in the accepted sense but others are not (Table I).

Table I

SOME KNOWN AND PROBABLE BIOLOGICAL ALKYLATING AGENTS

Dialkylnitrosamines, e.g. Dimethylnitrosamine	$(CH_3)_2NNO$
Alkylnitrosamides, e.g. Nitrosomethylurethane	$CH_3(NNO)COOC_2H_5$
Diazoalkanes, e.g. Diazomethane	CH_2N_2
Alkyl sulphates, e.g. Dimethyl sulphate	$(CH_3)_2SO_4$
Ethionine—ethyl analogue of methionine	$C_2H_5S(CH_2)_2CHNH_2COOH$
Methylazoxymethanol derivatives, e.g. Cycasin	$CH_3—N{=}N—CH_2O—Glucose$ (O)

However, among these compounds some certainly and some very probably alkylate cell components *in vivo*. The evidence for this and the possible implications of such alkylation in the pathological processes induced by these compounds will be discussed.

The dialkylnitrosamines are not alkylating agents as such but there is considerable evidence that they may become so by metabolic conversion. The alkylnitrosamides have rather similar chemical structure but are less stable than the nitrosamines and decompose more or less readily to give the corresponding diazoalkanes. The diazoalkanes are extremely reactive compounds and powerful alkylating agents, as are the alkyl sulphates. An important point of difference relevant to the present discussion is that the dialkyl sulphates are not nitrosamines and are not derived from them. Ethionine is the ethyl analogue of the amino acid methionine. It is not an alkylating agent in the chemical sense, but possibly may behave as one *in vivo*. The methylazoxymethanol derivatives, of which the glycoside cycasin is an

example, are naturally occurring substances found in Cycad nuts which grow on *Cycas circinalis* and other trees of *Cycas* type. Extracts of these nuts have been known to be hepatotoxic for many years and have recently been shown to be carcinogenic by Laqueur and co-workers (1963). The lesions produced in the rat, both acute and chronic, resemble those induced by dimethylnitrosamine very closely indeed.

DIALKYLNITROSAMINES

These compounds are the *N*-nitroso derivatives of secondary amines. Aspects of the cellular injury and carcinogenesis induced by the nitrosamines and of their metabolism have been discussed recently (Heath and Magee, 1962; Magee, 1962, 1963).

When administered in lethal or sublethal doses many of the nitrosamines, of which the dimethyl compound is typical, induce very severe liver damage in all the common laboratory mammals (Barnes and Magee, 1954; Schmähl and Preussmann, 1959; Schmähl, Preussmann and Hamperl, 1960; Druckrey *et al.*, 1961). The lesion is a very haemorrhagic zonal centrilobular necrosis (Fig. 1) which may be accompanied by haemorrhages in the gastro-intestinal tract and lungs and by haemorrhagic ascites. In rats surviving the initial acute illness the liver recovers almost completely so that there is little evidence of abnormality after a year or longer. Feeding dimethylnitrosamine to rats at a level of about 50 p.p.m. in the normal diet gives rise to malignant tumours of the liver (Magee and Barnes, 1956; Schmähl and Preussmann, 1959). Histologically these are usually hepatocellular carcinomas with marked cystic hyperplasia and occasional sarcomas. Diethylnitrosamine has been more widely studied as a carcinogen and found to be active in the rat (Schmähl, Preussmann and Hamperl, 1960; Argus and Hoch-Ligeti, 1961), hamster (Dontenwill and Mohr, 1961; Herrold and Dunham, 1963), mouse (Schmähl, Thomas and Konig, 1963) and guinea pig (Druckrey and Steinhoff, 1962; Argus and Hoch-Ligeti, 1963).

FIG. 1. Rat liver after one oral administration of dimethylnitrosamine, 30 mg./kg. Animal killed 48 hr. later. Centrilobular haemorrhagic necrosis is seen. Haematoxylin and eosin. ×94.

Tumours have been induced in several organs including liver, kidney and lung. The carcinogenic activity in the guinea pig is noteworthy since this species has hitherto proved remarkably resistant to the experimental induction of liver tumours (Argus and Hoch-Ligeti, 1963). In very extensive studies of the relation of chemical structure to carcinogenic activity in a large series of

nitrosamines, Druckrey and co-workers (1961) have shown that many other nitrosamines are carcinogenic. These findings will be discussed below in relation to theories of the mechanism of action of the nitrosamines. In our experience, feeding diets containing hepatocarcinogenic levels of dimethylnitrosamine to rats for periods of a few weeks, followed by return to normal diet, did not produce liver tumours but a high incidence of kidney tumours (Magee and Barnes, 1962). Of special interest was the observation that a small proportion of the survivors of a single dose at about the median lethal level developed renal tumours which, in some cases, did not become apparent until the death of the animal more than a year later. Since the compound is known to be very rapidly metabolized (Magee, 1956; Dutton and Heath, 1956; Heath and Dutton, 1958; Heath, 1961, 1962) and none of the compound as such can be detected in the rat 24 hours after such a single dose, it appears that the carcinogenic transformation may have started in the kidney during this initial period. There is now considerable experimental evidence that the acute tissue-damaging and carcinogenic action of these compounds may be dependent on their metabolism in the cells of target organs, with the intracellular production of alkylating agents which are the active molecules and which produce their effects by combination with essential sites in the cell. A possible metabolic scheme is shown in Fig. 2, taking dimethylnitrosamine as an example. The essential metabolic step is the oxidative removal of one of the *N*-alkyl groups by the enzyme *N*-demethylase (Magee and Vandekar, 1958; Brouwers and Emmelot, 1960) which occurs in the microsome fraction of the liver and probably, in considerably lower amount, in some other organs, depending on the species (Lee, Lijinsky and Magee, 1964). The resulting monoalkylnitrosamines are extremely unstable and are thought to break down very rapidly to give the active alkylating agents. The exact nature of the agent is not yet known but some possibilities are the corresponding diazoalkane, a carbonium ion or the

monoalkylnitrosamine itself (R. Schoental, personal communication; Rose, 1958; Hultin *et al.*, 1960; Druckrey *et al.*, 1961; Heath, 1961, 1962; Magee and Farber, 1962; Mizrahi and Emmelot, 1962).

The evidence for this alkylation hypothesis of the biological action of the nitrosamines is of two kinds, biological and biochemical. The biological evidence is derived from studies of the relationship of chemical structure and biological action in a series of nitrosamines in which the alkyl groups are varied. If the scheme shown in Fig. 2, in which the metabolic production of a diazoalkane occurs, is correct, then only those nitrosamines capable of such a decomposition should be biologically active, while those from which the diazoalkane could not be derived should not produce the typical response. We have studied the capacity to induce acute liver necrosis in a series of dialkyl, heterocyclic and aromatic nitrosamines and have found a good correlation between activity and a chemical structure allowing the production of a diazoalkane. The observation of Heath (1961) that *n*-butylmethylnitrosamine is typically necrogenic while *tert.*-butylmethylnitrosamine is not is particularly interesting

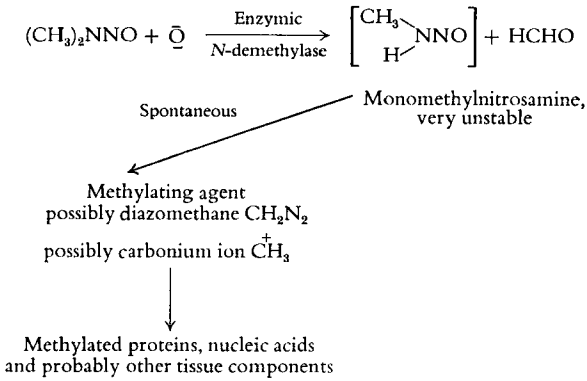

FIG. 2. A possible scheme for the metabolism of dimethylnitrosamine *in vivo*.

since the tertiary alkyl group could not give rise to a diazoalkane. Studies by Druckrey and co-workers (1961) on the carcinogenic action of a considerably larger group of nitrosamines are also essentially consistent with the alkylation hypothesis. This work and that of others has been recently reviewed and tabulated (Magee, 1963).

The biochemical evidence for the alkylation hypothesis depends on the demonstration of the presence of alkylated cell components in liver and some other organs of animals treated with the dialkyl nitrosamines (Magee and Farber, 1962; Craddock and Magee, 1963; Magee and Lee, 1963) and in components of liver slices incubated with the nitrosamine *in vitro* (Magee and Hultin, 1962). Most of these experiments have been done with isotopically-labelled nitrosamines. In a typical experiment the labelled nitrosamine is injected and the animal killed after a suitable interval, depending on the rate of metabolism of the compound. The cellular component most often studied has been RNA since this can be readily isolated and its reaction with alkylating agents *in vitro* has been very thoroughly studied (Lawley and Wallick, 1957; Reiner and Zamenhof, 1957; Brookes and Lawley, 1961; Ross, 1962). It is now well established that when the alkylating agent is present in low concentration in relation to the nucleic acid the alkylation occurs almost entirely on the 7-position of the guanine moieties of both RNA and DNA. If, therefore, alkylation of the nucleic acid has occurred *in vivo* in animals treated with labelled nitrosamines it should be possible to isolate a radioactive sample in which much of the radioactivity is present in the form of the 7-alkylguanine. This has been done with dimethylnitrosamine in the rat, mouse, hamster and guinea pig (Magee and Farber, 1962; Lee, Lijinsky and Magee, 1964) and with diethylnitrosamine and *n*-butylmethylnitrosamine in the rat (Magee and Lee, 1963). It is interesting that there was no detectable alkylation of rat liver RNA with *tert*-butylmethylnitrosamine. In these experiments the isolated

nucleic acid, RNA and in some cases, DNA, was hydrolysed with acid and the presence of radioactive 7-methylguanine demonstrated by ion-exchange and paper chromatography of the hydrolysate and comparison of the ultraviolet spectra of the radioactive material with authentic samples of the 7-alkyguanine. The structure of 7-methylguanine is shown in Fig. 3.

Guanine 7-Methylguanine

FIG. 3. Structure of guanine and 7-methylguanine.

It must be emphasized that even in rat liver after dimethylnitrosamine in necrotizing doses, the extent of methylation of RNA is only such that 1–2 per cent of the guanine moieties are converted to the methylated base and that methylation of DNA is rather less. With the other nitrosamines and in other organs the extent of alkylation is considerably smaller still. There does, however, appear to be some correlation between the degree of methylation of the nucleic acid and the susceptibility of an organ to carcinogenesis by dimethylnitrosamine in rat and mouse (Lee, Lijinsky and Magee, 1964). Methylation of RNA and DNA in rat liver and kidney rapidly reaches a maximum at about 6 hours after injection of labelled dimethylnitrosamine and then quite quickly falls off, so that most of the label has disappeared at 48 to 72 hours. It is difficult to be certain whether minute amounts of methylated nucleic acid persist in the liver and kidney for longer periods (Craddock and Magee, 1963). It is apparent that these changes in the nucleic acids are chemically identical with those known to occur in the nucleic acids of organisms treated with methylating mutagenic agents such as dimethyl

sulphate (Fraenkel-Conrat, 1961), which is discussed further below. The possible significance of these changes in RNA and DNA for the carcinogenic action of the nitrosamines has been discussed (Magee, 1962, 1963) with special emphasis on the instability of alkylated DNA (Brookes and Lawley, 1961).

ALKYLNITROSAMIDES

These compounds, for example, *N*-nitrosomethylurethane, are quite similar in chemical structure to the dialkylnitrosamines. In this example, one of the methyl groups of dimethylnitrosamine is replaced by the amide grouping. They are very irritant materials and induce extremely severe local lesions at the site of application. When given to rats or mice by stomach tube they produce a very violent local reaction, including massive necrosis and destruction of the stomach wall extending down to the serous coat in the squamous part and more superficial damage in the glandular part with oedema and cellular infiltration of the wall (Fig. 4). The degree and site of the damage appears to depend on the amount of material given and its local distribution. Acute lung lesions, with severe congestion and intra-alveolar oedema, follow the subcutaneous injection of nitrosomethylurethane, and are accompanied by a severe local lesion at the injection site. If rats and mice which survive a small number or, in some cases, even single doses of nitrosomethylurethane are allowed to live out their life-span, squamous carcinomas of the stomach and oesophagus may appear (Schoental, 1960; Druckrey *et al.*, 1961; Schoental and Magee, 1962). The site of appearance of the tumour appears to be dependent to some extent on the length of the cannula used to administer the carcinogen. The point to be emphasized here is that with this group of compounds as with the dialkylnitrosamines, cancer can be induced with single doses or only very brief exposure periods and that the tumours may not become clinically apparent for many months after the exposure. Although no data are available at the moment on the fate of

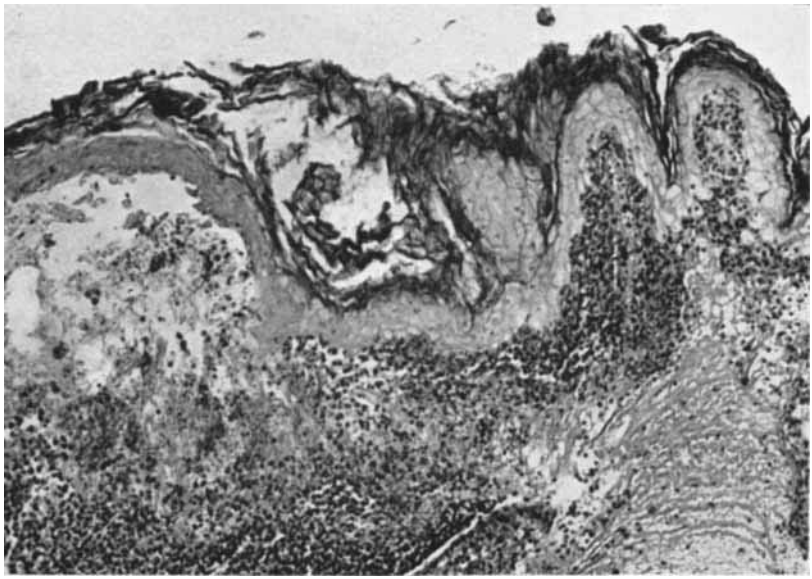

FIG. 4. Rat fore-stomach after one oral administration of nitrosomethylurethane, 10 mg./kg. Animal killed 48 hr. later. The squamous epithelium is partly preserved. Oedema, inflammation and haemorrhages are seen in the submucosa. Haematoxylin and eosin. × 120.

nitrosomethylurethane in the animal body it seems highly probable that it is rapidly broken down. A possible scheme for the decomposition of the compound is shown in Fig. 5. The compound has been used for many years by organic chemists in the preparation of diazomethane which is itself widely used as a methylating agent in synthetic chemistry. This mechanism (Fig. 5) was suggested by Sidgwick (1942) and postulates the intermediate formation of the very unstable monomethylnitrosamine. The reaction is usually carried out under mildly alkaline conditions, but very recently, Dr. Regina Schoental, in our laboratory, has shown that decomposition occurs at physiological pH in the presence of certain normal cellular constituents, notably sulphydryl compounds (Schoental, 1961). The important

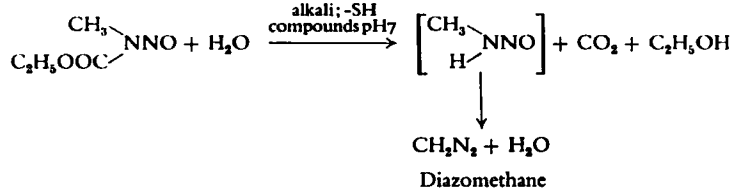

FIG. 5. Possible decomposition of nitrosomethylurethane.

point here is that the decomposition is not enzymic, but chemical, and thus the alkylating agent would be expected to be released at the site of application or in the region of immediate venous or lymphatic drainage. Evidence of actual methylation of cell components is not yet available but this seems to be very likely.

DIAZOALKANES

The lower members of this group are highly irritant gases and diazomethane, the simplest, is mutagenic (Boyland, 1954). Diazomethane is also very prone to induce sensitization reactions which makes it troublesome to handle in inhalation experiments. Nevertheless, Schoental succeeded in obtaining enough survivors of groups of rats and mice exposed to the gas, in spite of its great toxicity, to show that the treatment had increased the incidence of pulmonary adenomas. In one of the rats there was a very extensive squamous carcinoma of the lung (Schoental, 1960; Schoental and Magee, 1962).

DIALKYLSULPHATES

All the agents mentioned already have been nitroso compounds or derived from them. Although there is good evidence of alkylation of cell components, including nucleic acids, with some of them (Magee and Hultin, 1962; Magee and Farber, 1962; Craddock and Magee, 1963; Magee and Lee, 1963), the fact remains that there is no convincing evidence that the alkylation

is related to the carcinogenesis. Since the nitroso compounds appear to be more powerfully carcinogenic than the common biological alkylating agents mentioned earlier this might suggest that they owe this remarkable activity to being nitroso compounds, and that the alkylation is either irrelevant, or at most contributory to the induction of cancer. For this reason we have started experiments with the dialkyl sulphates, of which dimethyl sulphate is the simplest. Dimethyl sulphate was used in chemical warfare in the first world war and is an alkylating agent *per se* without the necessity of activation. It is obviously highly poisonous and very irritating to the skin but it does not seem to have been tested for carcinogenicity. It is mutagenic in tobacco mosaic virus and other organisms and has been shown to methylate the viral nucleic acid on the 7-position of guanine bases (Fraenkel-Conrat, 1961). In exerting its mutagenic effect, therefore, it appears to make a change in the nucleic acid which is very similar to that which occurs in organs susceptible to carcinogenesis by dimethylnitrosamine (Magee and Farber, 1962; Lee, Lijinsky and Magee, 1964; Magee and Lee, 1963). It is clearly of considerable interest, therefore, to know whether this compound would alkylate the nucleic acids of tissues and organs *in vivo* and whether the same organs would be susceptible to carcinogenesis by it. Unfortunately our carcinogenesis experiments on skin and stomach of rat and mouse are still at an early stage and no conclusions can yet be drawn. In metabolic experiments, however, using [^{14}C] dimethyl sulphate, we have recently demonstrated the probable presence of radioactive 7-methylguanine in acid hydrolysates of stomach RNA from rats treated with doses of the compound adequate to cause a severe acute lesion (Fig. 6). It appears, therefore, that the typical alkylation reaction does occur *in vivo* with this compound. Since our rats are known to be highly susceptible to the induction of squamous cancer of the stomach (Schoental and Magee, 1962) failure to induce tumours with repeated oral doses of dimethyl sulphate

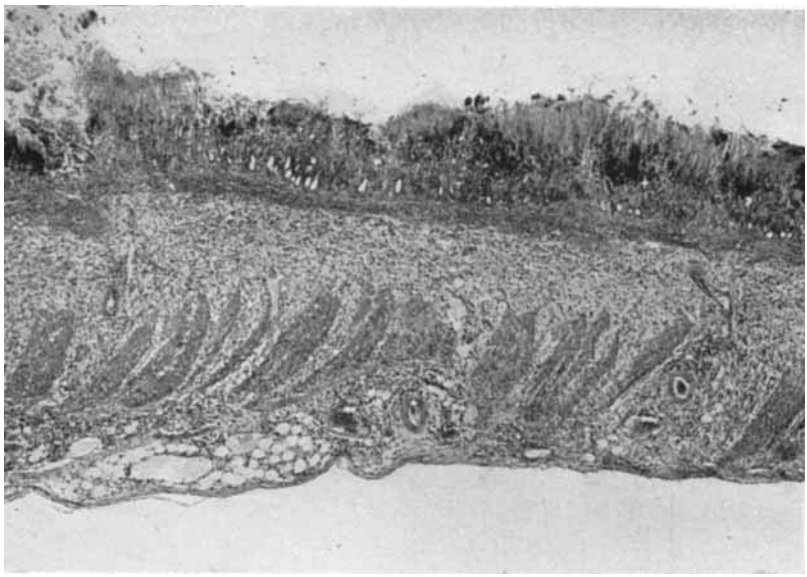

FIG. 6. Rat stomach after one oral administration of dimethyl sulphate, 25 mg./kg. Animal killed after three days. A grossly recognizable ulcer is present in the glandular mucosa. Most of the glandular mucosa is necrotic and haemorrhagic with marked oedema and inflammation in the submucosa and in the muscular layer. Haematoxylin and eosin. × 37·5.

would indicate the need for revision of the simple alkylation idea.

ETHIONINE

This compound is the ethyl analogue of the amino acid methionine, and has been studied very extensively from the pathological and the biochemical point of view (Farber, 1963). In acute experiments it induces hepatic fatty change in female rats and is carcinogenic in the liver after chronic administration in both sexes. Chemically it is not an alkylating agent and biochemically it seems that it can replace methionine in many of its metabolic pathways. It can be activated in the same way as

methionine and other amino acids and can be incorporated into tissue proteins in place of methionine, but only to a rather small extent. It can also undergo another type of activation which is peculiar to methionine among the normally occurring amino acids, that is, conversion to *S*-adenosylmethionine (Fig. 7).

S-adenosylmethionine *S*-adenosylethionine

FIG. 7. "Active" methionine and ethionine.

S-adenosylmethionine is the active source of the so-called "labile methyl groups" and in this sense can be regarded as a physiological methylating agent. Ethionine can also be activated in the same way, with the formation of *S*-adenosylethionine (Shapiro and Schlenk, 1960) and this may perhaps be regarded as a physiological or perhaps pathological ethylating agent. Some of the normal metabolic methylations from *S*-adenosylmethionine are shown in Fig. 8. Ethionine is known to be able to replace methionine in several of these (Shapiro and Schlenk, 1960) but the pathway relevant to the present discussion is that leading to the minor methylated base components of RNA. Until quite recently, the nucleic acids were thought to be macromolecular long chain polynucleotides of which the component bases were adenine, guanine and cytosine in both RNA and DNA, with

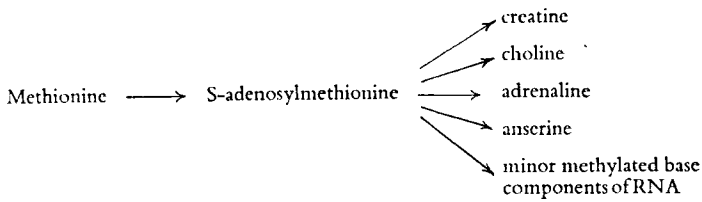

FIG. 8. Some physiological methylations.

thymine in DNA being replaced by uracil in RNA. Largely through the work of D. B. Dunn and his colleagues at Cambridge (Dunn and Smith, 1955, 1958; Littlefield and Dunn, 1958*a* and *b*; Dunn, 1961; Dunn, 1963) this is now known not to be correct and a number of minor methylated base components of the nucleic acids have been discovered, particularly in the case of RNA (Table II). It is interesting that the concentration of the

Table II

MINOR METHYLATED BASE COMPONENTS OF NUCLEIC ACIDS

RNA	DNA
6-Methylaminopurine	6-Methylaminopurine
6-Dimethylaminopurine	5-Methylcytosine
1-Methyladenine	
2-Methyladenine	
2-Methylamino-6-hydroxypurine	
2-Dimethylamino-6-hydroxypurine	
1-Methylguanine	
7-Methylguanine	
Thymine (5-Methyluracil)	
5-Methylcytosine	

methylated bases is much higher in the soluble RNA (s-RNA) than in the other subcellular fractions and Bergquist and Matthews (1962) have suggested that they may occur there only and may be present in the other fractions as s-RNA not removed from these particles during their isolation. This is in contrast with our observation that the microsomal RNA is methylated to a greater extent than the others in livers of rats treated with dimethylnitrosamine (Craddock and Magee, 1963). Evidence that methionine is the source of the methyl groups of these minor base components, with *S*-adenosylmethionine as the active intermediate, has come from the work of Borek and his colleagues with micro-organisms (Srinivasan and Borek, 1963) and of Biswas, Edmonds and Abrams (1961) with mammalian cells in tissue culture. Borek has shown the existence of an enzyme, RNA methylase, which is present in microbial and mammalian cells,

and which catalyses the transfer of methyl groups from *S*-adenosylmethionine to all the bases of RNA. It is very interesting that this enzyme mediates methylation of the RNA macromolecule rather than the biosynthetic incorporation of a small molecular precursor. In this sense, the mechanism of physiological methylation of RNA appears to be very similar to that which we have postulated for the methylation of the nucleic acids by dimethylnitrosamine *in vivo* (Magee and Farber, 1962). In fact, in comparing their observations with ours, Srinivasan and Borek (1963) suggest that under certain conditions, RNA methylase may be a natural carcinogen. Returning to ethionine and its conversion to *S*-adenosylethionine, it seems reasonable to expect that some of the activated ethyl groups might be transferred to RNA and thus form the ethyl analogues of the minor base components, and we have obtained some evidence which suggests that this may be the case in rat liver (Farber and Magee, 1960). RNA isolated from the livers of rats treated with [^{14}C]ethionine was found to be radioactive and to have higher specific radioactivity than the proteins. This radioactivity appeared to be chemically bound, and similar results have been reported by Stekol, Mody and Perry (1960). Acid hydrolysis of the labelled RNA followed by ion-exchange chromatography gave an elution pattern which was quite different from the corresponding elution pattern of RNA labelled with [^{14}C]methionine *in vivo*, where most of the radioactivity was in the major base components. With [^{14}C] ethionine there are several radioactive peaks which probably correspond to minor ethylated base components, but this is not yet certain. Since ethionine is carcinogenic in rat liver these findings support the idea that under certain conditions, for example, when provided with an abnormal substrate, RNA methylase could be regarded as a natural carcinogen, if the ethylation of the nucleic acid is related to the induction of cancer. There is, of course, the additional possibility that excessive or aberrant function of the enzyme might lead to wrong placement of