PATHOLOGY

Interaction of the Carcinogenic *N*-Methyl-Nitrosourethane with Sulphydryl Groups

In a preliminary communication I recently reported the production of lung adenoma in mice and squamous lung carcinoma in a rat, which was exposed to gaseous diazomethane. Among other rats given *N*-methyl-*N*-nitrosourethane (NMU) by stomach tube, a carcinoma of the stomach developed after a single dose of this compound¹.

Since this communication was published, more stomach tumours have been observed in the rats given NMU by stomach tube². Stomach tumours have also been reported in rats given NMU in drinking water continuously until death³. Moreover, when NMU was administered to adult rats by a short mouse-stomach tube, the animals developed oesophageal squamous carcinoma⁴. Subcutaneous injections into mice gave rise to local sarcomata. It is evident that NMU acts at the site of application and that it is able to produce tumours in various tissues. This would qualify it to be considered a 'local' carcinogen.

However, the evidence described here indicates that the effective entity is not NMU but diazomethane, which is formed when NMU interacts with tissues. In 1895 von Pechmann⁵ suggested that the toxic effects of NMU, which he observed on himself, might be due to diazomethane, formed from NMU in contact with tissues.

That this is really the case was qualitatively confirmed in the following way: NMU was added to tissue homogenates at 37° C. and the mixture was covered with a layer of ethyl ether. Rapid evolution of gas occurred; the gas bubbled through the layer of ether. This indicated that the gas is not soluble in ether, hence it is not diazomethane. Evidently, the diazomethane formed according to reaction (1) reacts immediately with tissue constituents, with evolution of nitrogen according to reaction (2):

Expecting this to be an enzymatic reaction, it appeared convenient to study it in the Warburg apparatus, the likely final product being nitrogen. The other gaseous product of the reaction, carbon dioxide, is conveniently absorbed by 10 per cent potassium hydroxide, placed in the usual way in the middle well of the vessel. Tissue homogenates were prepared in Sørensen's phosphate buffer, pH 7.2 (1:10), and introduced into the main compartment of the Warburg flask (3 ml.). Solutions of NMU (1:100) were placed in the side-arm (0.5 ml.). (The solutions of NMU were freshly prepared from commercial *N*-methylnitroso-urethane by dissolving it in 50 per cent ethyl alcohol, 1:20; this gave a clear solution which was then diluted further with distilled water 1:5.) The effect of the buffer on NMU and of 10 per cent alcohol (0.5 ml.) on tissue homogenates was tested concurrently each time. The contents of the side-arms were added to the main compartment after 15 min. equilibration at 38.5° C. With tissue homogenates rapid gas evolution immediately followed the addition of NMU and was almost complete in 15 min.; the

decomposition of NMU in buffer solution was very slow and slight gas evolution persisted for many hours.

When heparinized rat blood and plasma were tested in this way, rapid evolution of gas from NMU occurred only in the presence of blood corpuscles. The specific agent responsible for the decomposition of NMU is evidently cellular. The reaction was not affected by cyanide, azide or fluoride; it was, however, abolished by copper, indicating that SH-groups may be involved.

Indeed, when 1 per cent solutions of cysteine or glutathione in phosphate buffer at pH 7.2 were substituted for blood or tissue homogenates, the evolution of gas from NMU took place in the same way immediately after mixing. This proved that the reaction is chemical rather than enzymatic. Biotin, having sulphur in the ring, cystine, thioacetamide, or some representative cell constituents, such as glycine, tyrosine, guanine, pantothenic acid, etc., had no effect on NMU under the conditions tested.

The question arises: Are some of the SH-cellular compounds directly involved in the carcinogenic action of NMU, diazomethane and possibly of certain other alkylating agents? Diazomethane is known to be a very versatile reagent; it can methylate not only SH-groups but also any acidic or phenolic hydroxyls, amino- and imino-groups, it can add to double bonds, enlarge ring and carbon chains, form methyl branchings. It can conceivably form intra- and intermolecular methylene-bridges.

Which of the multiplicity of possible reactions is related to the carcinogenic action of this compound? The fact that decomposition of the strongly carcinogenic NMU takes place at the SH-groups supports the already existing evidence⁶ that these may be involved in the carcinogenic process. However, if carcinogens combine with certain SH-compounds *in vivo*, the type of their binding is likely to differ fundamentally from that of non-carcinogenic agents, many of which are excreted as mercapturates. The action of carcinogens is irreversible and compatible with the survival of the affected cells; the interaction products possibly remain irreversibly *in situ*, and prevent the physiological replacement of some specific SH-compounds, the action of which they block.

Since this communication was submitted for publication, Emmelot and Mizrahi⁷ reported that cysteine can counteract the action of dimethyl-nitrosamine. As the latter can act as a methylating agent (probably through the release of diazomethane after enzymatic oxidation)⁷, these results confirm the view based on the more direct evidence here reported, that certain sulphydryl compounds are involved in the action of diazomethane and probably also of other carcinogens.

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¹ Schoental, R., *Nature*, **188**, 420 (1960).

² Schoental, R., and Magee, P. N. (to be published).

³ Druckrey, H., Preussman, R., Schmähl, D., and Müller, M., *Naturwiss.*, **48**, 165 (1961).

⁴ von Pechmann, H., *Ber.*, **28**, 856 (1895).

⁵ Crabtree, H. G., *Brit. Med. Bull.*, **4**, 345 (1947).

⁶ Emmelot, P., and Mizrahi, I. J., *Nature*, **192**, 42 (1961).

⁷ Hultin, T., Arrhenius, E., Löw, H., and Magee, P. N., *Biochem. J.*, **78**, 109 (1960).