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Application of Whole-body Autoradiography to Distribution Studies of Volatile Substances

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INTRODUCTION

Exposure to volatile substances of toxicological significance takes place most frequently in occupational settings (e.g. organic solvents, plastics monomers), but volatile substances may be encountered also in e.g. clinical practice (anesthetic agents) or as constituents of tobacco smoke or food.

The volatility of a chemical substance at a certain temperature is expressed by its vapour pressure. Conventional whole-body autoradiographic techniques cannot be used for distribution studies of compounds exerting significant vapour pressures at or above -20°C. The autoradiographic registration of volatile substances can only be accomplished by working at low temperatures. Theoretically, evaporation occurs at all temperatures above absolute zero, but a negligible evaporation can be said to occur when the vapour pressure is less than 0.5 mm Hg (3). Today, most low-temperature autoradiography is performed at temperatures around -80°C, which are sufficient to lower the vapour pressure of e.g. most organic solvents to 0.5 mm Hg or less.

LOW-TEMPERATURE AUTORADIOGRAPHY OF VOLATILE SUBSTANCES

The preparation of a whole-body autoradiogram requires a flat surface to be pressed against X-ray film and, since thin sections warm up more easily than thick ones, all low-temperature autoradiographic methods found in the literature describe techniques to flatten the frozen animal resulting in thick slabs or slices (3). After rapid freezing, usually in liquid nitrogen, the animal is milled down or sawn sagittally to a body level suitable for autoradiography. Improved sawing equipment has allowed the preparation of 5-6 sections (2-3 mm thick) from a mouse, both sides of which can be pressed against X-ray film (3). Film exposure must take place at a low temperature, e.g. over solid carbon dioxide or in a low-temperature freezer.

CONVENTIONAL AUTORADIOGRAPHY OF VOLATILE SUBSTANCES

Whole-body autoradiography, like any other tracer technique, does not supply any information about the nature of the registered radioactivity, which may belong both to the administered substance and/or to its metabolites. However, when doing autoradiography of volatile substances it is possible to take advantage of their volatility to distinguish between the volatile substance itself and its usually non-volatile metabolites. Low-temperature autoradiography registers the total radioactivity, i.e. both volatile and non-volatile radioactivity (AUTORADIOGRAM A). If conventional autoradiography with tape-fastened and freeze-dried 20 µm sections is used, the volatile parent substance will evaporate and only the non-volatile metabolites are registered (AUTORADIOGRAM B). The tape-fastened sections may be taken from the thick sections, after their film exposure at low temperature, or from a separate series of animals. To ensure a complete evaporation of volatile radioactivity the thin sections may be warmed carefully prior to film exposure (3).

Low-temperature autoradiography in combination with conventional autoradiography has been successfully applied to distribution studies of several organic solvents (2,3,9,11,12), anesthetic agents (6,7,8,10), ethanol (1), dimethyl mercury (14) and volatile N-nitroso compounds (5,13).

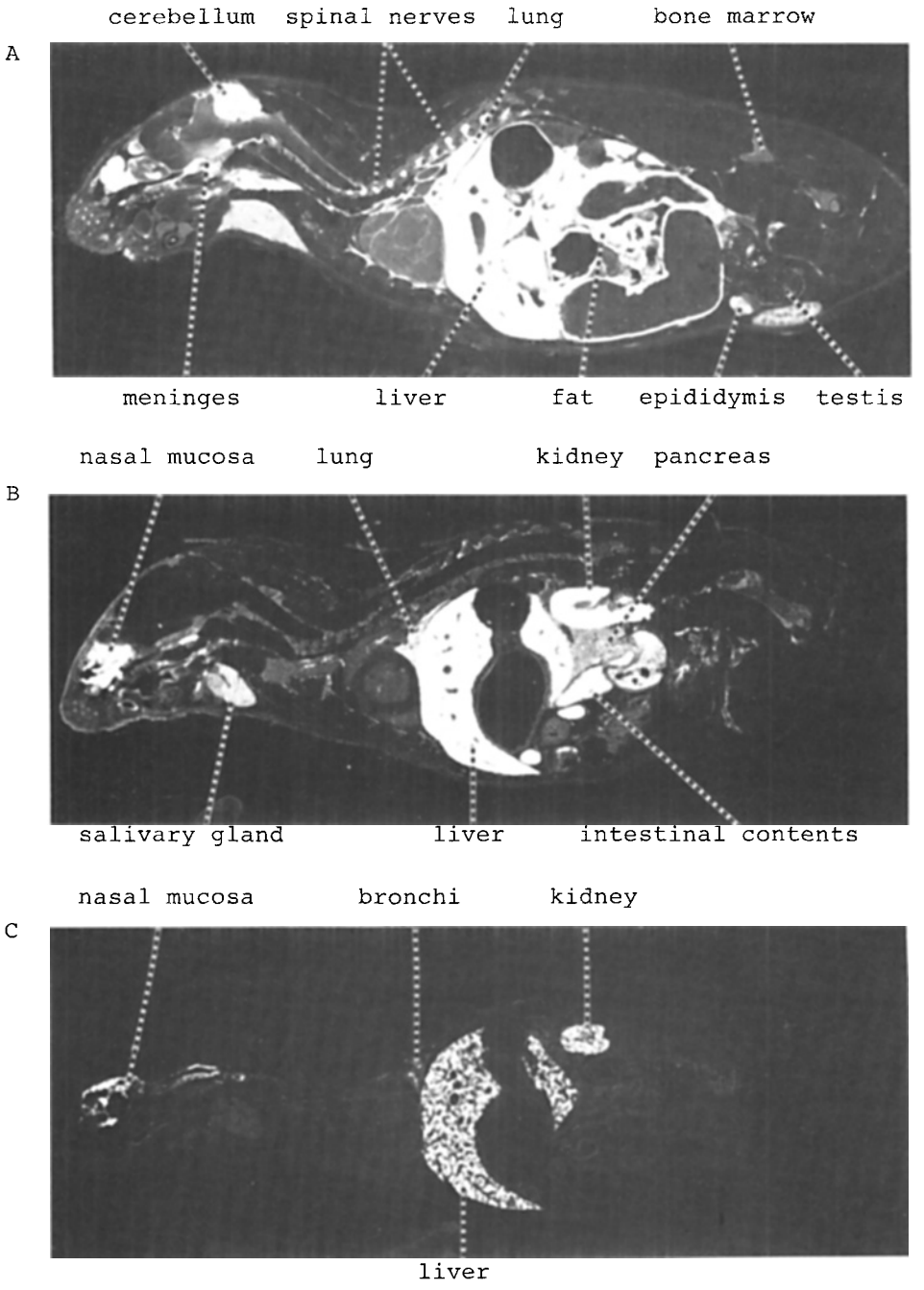
WHOLE-BODY AUTORADIOGRAPHY OF NON-EXTRACTABLE METABOLITES

Some low-temperature autoradiographic investigations have included the study of covalently bound metabolites, which are associated with tissue injury (2,3,4,9,10,11,12,13). An extraction procedure for tape-fastened sections, which removes non-bound radioactivity, has been developed (3). Usually, pairs of one extracted and one non-extracted section are exposed and developed together in order to enable an estimate of the amount of bound radioactivity in relation to total non-volatile radioactivity (AUTORADIOGRAM C).

CONCLUSIONS

The technique of low-temperature autoradiography has been successfully applied to distribution studies of several important groups of volatile substances, e.g. organic solvents, anesthetic agents and N-nitroso compounds.

It has been possible to take advantage of the volatility of these substances in order to separate between the substances themselves and their non-volatile metabolites, which can be registered with conventional whole-



WHOLE-BODY AUTORADIOGRAPHY OF ^{14}C-CHLOROFORM (survival time 2 hours)

A. *LOW-TEMPERATURE (-80°C) AUTORADIOGRAPHY:* Registration of total radioactivity (chloroform and non-volatile metabolites)
B. *CONVENTIONAL AUTORADIOGRAPHY:* Registration of non-volatile metabolites.
C. *AUTORADIOGRAPHY OF AN EXTRACTED SECTION:* Registration of covalently bound metabolites.

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body autoradiography.

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