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The Technique of Whole Body Autoradiography—Some Examples of Applications

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INTRODUCTION

When Sven Ullberg in the beginning of the 1950's started his Ph.D. thesis work all determinations of the penicillin concentration in organs of the living organism were done by microbiological techniques. In his attempt to investigate the fate of penicillin in vivo Ullberg tried different, by then relatively new methods. He produced radiolabelled penicillin by growing the penicillin producing fungi in a radioactive (^{35}S) growth medium. After administraion of this labelled penicillin to experimental animals measurements of penicillin concentrations in different organs were then performed with Geiger-Müller technique and autoradiography of sections from excised organs. A problem with traditional autoradiography is the fact that penicillin, which is a water soluble substance, may be dissolved when preparing the histological sections used. To be able to make a direct comparison of the radioactivity in different organs Sven Ullberg elaborated a method (3) which makes it possible to simultaneously localize a radioactive substance in practically all the organs in the body without risking leakage of water soluble substances. The difficult preselection of organs which - for practical reasons - usually has to be done is thus needless.

DESCRIPTION OF THE TECHNIQUE

The technique can briefly be described as follows (4): After administration of the radioactive substance the animals are sacrificed after different survival times by an anaesthetizing agent. Their bodies are immediately frozen in a mixture of hexane and solid carbon dioxide and then sectioned in a special microtome at ≈ -20°C. To be able to handle the large tissue sections a piece of Scotch tape is pressed onto the block before sectioning. It is possible to take sections from animals up to the size of e.g. a newborn pig by this technique. After freeze-drying the sections they are pressed against a photographic emulsion. After exposure the sections are separated from the films and the films are developed to visualize the blackening caused by the radioactive

substance in the different organs. Examples of whole body autoradiograms are shown in Fig. 1.

Sven Ullberg's solution to the problem is as ingenious as simple. The whole body autoradiography as introduced by Ullberg has been increasingly used in our departments but also in many other laboratories in practically all parts of the world. The pioneer work included sectioning the specimens in a freeze-room (-20°C) which meant that fur coats and gloves were necessary equipment. Some improvements of the technical details when handling the embedding of the specimens and the sectioning has been introduced during the years. The introduction of a cryostate with a specially designed, heavy microtome has facilitated the work considerably. The developmental work for an automatic sectioning machine has lately been successful and a prototype for this purpose is now tested at the department of Toxicology, Uppsala University.

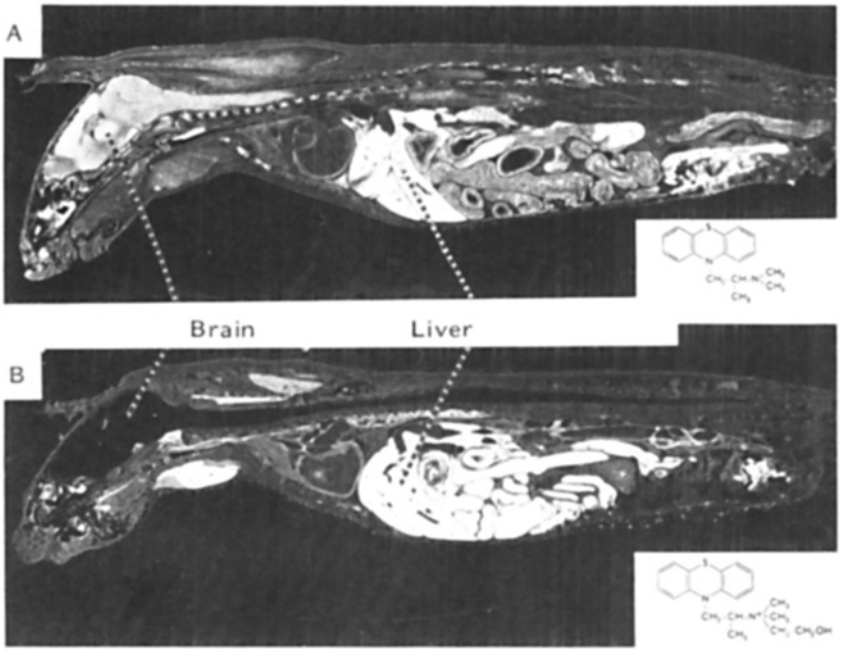

Fig. 1 Whole body autoradiograms from mice injected with ^{35}S-promethazine (A) or ^{35}S-hydroxyethyl promethazine (B). White areas correspond to high amounts of radioactivity. Note the different uptake of the compounds in the brain, which explains why substance (B) did not cause drowsiness when used for e.g. allergic rhinitis (2).

USE OF THE TECHNIQUE

For references exemplifying the use of the technique the reader is referred to (4), where an extensive bibliography of applications is published. The most apparent advantage of the whole body autoradiography technique as a scientific tool is that it offers a detailed, but also complete, view of the distribution of an injected compound in all the tissues of the body. This fact favours the

possibility to make unexpected discoveries and also contributes to suggestions for further research. The method is also extensively used for routine screening work in the pharmaceutical industry. The combination of Ullberg's method with other techniques has to large extent contributed to elucidating the mechanisms of actions for many endogenous substances as well as the principle for certain pharmacological and toxicological effects. As an example can be mentioned the autoradiographic studies which gave the explanation why two antihistamines (promethazine and hydroxyethyl promethazine) influenced the sleeping period after barbiturate administration in different ways (Fig. 1). An important question when studying the distribution of antibiotics is the concentration in the site of the infection, e.g. the ability to penetrate the membrane of an encapsulated abscess. The condition for ^{35}S-penicillin in this context is shown in Fig. 2.

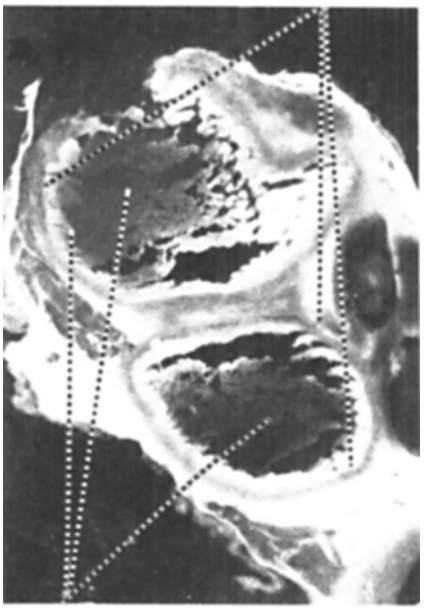

Fig. 2 Autoradiogram showing the distribution of radioactivity in two 30 day old abscesses after intravenous injection of ^{35}S-penicillin into a mouse. White areas represent high amounts of radioactivity. Penicillin penetrates the abscess membrane and a relatively high concentration is seen immediately inside the membrane. The concentration is then diminished towards the center of the abscess (3).

From the toxicological point of view retention of a substance in a certain tissue may be of interest. The previously not known, strong accumulation of tetracyclines in bone and teeth, which was observed by whole body autoradiographic studies in 1956 has contributed to the understanding of the side effects reported regarding foetal growth and malformations of the enamel in the deciduous teeth when using these antibiotics in pregnant individuals.

To be able to evaluate the autoradiographic image in a quantitative way different methods have been tried. Simple systems using 'isotope staircases', tiny photo cells and advanced systems with computerized image analysis have

been used. Whole body sections have also been used for parallel investigations such as quantitation and radiochemical analysis of pieces punched out from the different tissues in the sections. Histochemical methods have been adjusted to whole body sections in attempts to further explain the mechanism of action in relation to an autoradiographically found localization, e.g. enzyme inhibition. Substances with fluorescence of their own (e.g. tetracyclines and certain mycotoxins) have been identified directly in whole body sections by ultra violet radiation. By using a special dry mounting technique whole body sections and photographic emulsions can be permanently mounted to enable a more precise localization of the radioactivity to special types of cells ('semi micro technique') (Fig. 3). When interpreting the autoradiograms it is important to remember that the radioactivity can represent the original substance as well as radiolabelled metabolites. As an example can be mentioned that the radioactivity in Fig. 3 in the adrenal cortex represents only 10 % of unchanged cholesterol according to parallel radiochromatographic studies. The majority of ^{14}C represents different cholesterol esters which are the storage forms for the starting material for synthesis of cortical steroid hormones.

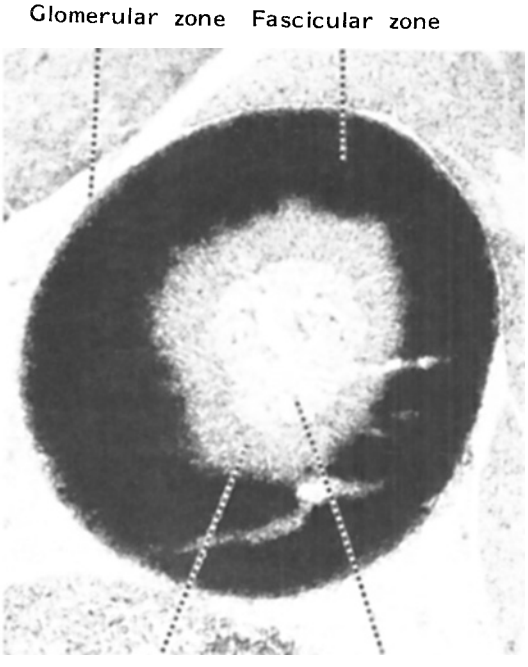

Fig. 3 Detail of 'semi microautoradiogram' (whole body section and photographic emulsion permanently mounted together) from a mouse injected intravenously with ^{14}C-cholesterol and sacrificed after four days. Black areas correspond to high amounts of radioactivity. The radioactivivty is confined to only one cell type layer of the cortex (Zona fasciculata) (1).

Whole body autoradiography implies that radiolabelled substances are at hand. In the late 1950' there were not too many labelled pharmacolgical or toxicological substances available. Beside the biosynthesis of labelled sub-

stances Ullberg and his coworkers spent much time for 'chemical' labelling of interesting compounds by exchange reactions (tritium, iodine exchange etc.). To possibly improve the autoradiographic resolution, an inventory of radionuclides with suitable low energy radiation was made in collaboration with the Royal Technical College in Stockholm. Several of these radionuclides were successfully applied in biological experiments. Also very short-lived radionuclides such as ^{18}F (half-life 110 min.) have been used for both whole body autoradiography and the above mentioned 'semi micro technique'. Interesting are also the possibilities to use the very same section for localization of ^{45}Ca as well as ^{18}F by using their differences in half-lives.

Several volatile substances have been studied by a sofisticated method. After freezing the whole animal, the specimens are kept at -80°C and after sawing the block into two parts at dry ice temperature the 'halves' are placed against X-ray films and exposed while kept at the same low temperature. By this method it is possible to locate quite many volatile substances as well as their labelled metabolites. When sections prepared in the traditional way are then exposed, only the non-volatile labelled material is visible in the autoradiograms. After extraction with different solvents re-exposure of the sections can reveal if firmly (covalently?) bound metabolites are present.

In connection with drug use during pregnancy and toxicology evaluation of compounds possibly effecting pregnant women many contributions have been achieved through the good resolution of whole body autoradiography making it possible to trace substances in organs of the growing foetus.

The whole body autoradiography technique has also been very useful for finding suitable substances with selective localization for diagnostic and/or therapeutic uses in connection with neoplastic diseases. Substances with specific localization to certain tissues may be labelled with γ-emitting isotopes for such purposes. Lately the use of short-lived positron emitting nuclides in connection with positron emission tomography (PET) has also increased the need for finding substances with selective localizations.

CONCLUSION

That the whole body autoradiography technique is still relevant and up to date within biomedical research is shown by its extensive use (alone and in combination with other methods) in the field of pharmacology and toxicology. The introduction of PET has also given current interest to the possibilities to find distinct, specific localizations for which the whole body autoradiography is still the unrivalled method.

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