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WHOLE-BODY AUTORADIOGRAPHY

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Whole-body Autoradiography

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Foreword

There are many definitions of an expert. For some of us, the practical ones, it is sufficient to have made all the known mistakes, some several times. For the theoretically inclined, great facility at explaining where others have gone wrong is enough, without need of practical skill. Some achieve the rank of expert by being in the right place or knowing the right people.

By any sensible definition, the authors of this book deserve the title. In many years of practical autoradiography, they have produced material of the highest quality. Their understanding of the theoretical basis of the technique is excellent, and they have contributed significantly to the evolution of the method, particularly in the quantitative interpretation of whole-body autoradiographs. Finally, and this is the crucial test of expertise, they have described in this book all aspects of the technique with a readable clarity that makes it all seem remarkably simple.

Making an autoradiograph in fact, is simple. Difficulties emerge only when one wishes to make consistent and reliable autoradiographs, and to interpret the images in terms of the functioning of the living body. Here, the technical steps are described so fully and clearly that anyone with the usual quota of brains, hands and equipment should be able to produce satisfactory autoradiographs. Many books that describe techniques are content to stop at that point, and still achieve and deserve success. Nearly half this book, however, is a careful and thorough review of the process of interpreting autoradiographic results, and of the types of information that can be extracted from autoradiographic experiments. This discussion is invaluable, and I know of nowhere else where it can be found.

It is a pleasure to introduce a well-written and useful book. It is an added pleasure when one knows the authors, and can look back on working with them on the early training courses in autoradiography held in Oxford. Whole-body autoradiography has grown from its origins with Dr Sven Ullberg to become probably the most widely used group of autoradiographic techniques today. It is high time the subject had a full and clear description. This book will be hard to beat.

September, 1980
Bedford Park
South Australia

A. W. Rogers

Preface

It is our intention that this text should be used as a practical manual for the technique of whole-body autoradiography.

By bridging the gap between what is administered to animals and what is excreted, the technique embraces a number of arbitrary sub-divisions of biology including chemistry, biochemistry, physiology, anatomy, pharmacology, toxicology and environmental science. In this book we emphasize the interdisciplinary nature of autoradiography which must be appreciated when designing experiments, so that full advantage can be gained from the interpretation of autoradiographs.

In essence this book is a recommendation for the technique of whole-body autoradiography and many examples of the usefulness of the method are cited in support. However, the inherent limitations of the technique are well recognized and enthusiasm for the method must be tempered with caution. Fortunately the disadvantages are relatively few and if properly appreciated they do not seriously reduce the value of the technique. Nevertheless, the research worker should be aware of them and they are therefore accorded due prominence in the text.

In addition to the methodology we have included a separate section on the interpretation of autoradiographs. Using selected examples we have attempted to show how the technique can be manipulated to provide information for a wide variety of research interests concerned with the movement, fate and effects of labelled molecules *in vivo*.

The decision to write this book arose from the interest shown in the autoradiography courses held every two years under the auspices of the British Anatomical Society.

September, 1980
Oxford

C. G. Curtis
S. A. M. Cross
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G. M. Powell

Acknowledgements

Autoradiographs, particularly whole-body autoradiographs, are always impressive even if they mean nothing to the viewer. In bridging the gap between the picturesque and what is scientifically meaningful the authors are grateful to the memorable teachings of Professor A. W. Rogers, the founder of the "Autoradiography Courses". These began in Oxford in 1968 and have continued to be held every two years. As former pupils on the early courses the authors are delighted that Professor Rogers has agreed to write the foreword to this book. We are also indebted to the other teaching staff on the courses, particularly Mr M. Tarbit, for useful discussions on the interpretation of whole-body autoradiographs.

The illustrations are a particularly important feature of this book and we wish to thank Smith Kline and French for permission to use Figs 2, 3, 4, 8, 9, 10, 11, 18, 22, 23, 29, 30, 31, 32, 33, 34, 36, 37, 39, 40 and 43 which were prepared by S. A. M. Cross at Welwyn Garden City. In the same way we are grateful to Glaxo Group Research for permission to use Figs 1, 17, 19, 26, 33, 35, 38, 41 and 42 which were prepared by R. J. McCulloch at Harefield and to Imperial Chemical Industries for permission to use Fig. 24 prepared by S. Longshaw at Alderley Edge.

We are particularly indebted to Mr David Kedgley for the preparation of the illustrations: to Mrs Carolyn Thomas for typing and retyping the manuscript and to Miss Barbara Card for proof reading the manuscript.

During the preparation of this book we have been fortunate in having full access to all the facilities of the Biochemistry Department at University College, Cardiff and for this we are most grateful to Professor K. S. Dodgson.

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1

Introduction

When a compound is administered to an animal, two fundamental questions should be considered. First, how is the structure of the compound modified by the animal and secondly, how may the physiological and pharmacological effects of the compound or its metabolites be explained at the molecular level? These questions are deceptively simple and for the majority of compounds the answers cannot be given because they require a detailed knowledge of both the distribution and metabolism of each compound *in vivo*, and also a knowledge of the mechanisms by which a compound and its metabolites influence normal metabolic pathways. Such information is not usually available.

Although a great deal is known about the possible interactions of different chemicals in the body, much of the information has been gained using standard techniques of biochemical analysis. Unfortunately, the conditions under which some information has been obtained, either with cell organelles or with purified enzyme systems, may severely limit interpretation in terms of tissues and whole animals. Indeed, information gained at the molecular level often cannot be extrapolated to whole cells with any degree of certainty. Extrapolation to mixed populations of cells is even more difficult. The general appreciation of this problem is reflected in the perceptible increase of emphasis on whole-organ and whole-animal studies.

Whole-body autoradiography is a technique for studying the tissue distribution of radiolabelled molecules in the whole animal. The results obtained contribute significantly to our understanding of the movement of substances and their metabolites *in vivo*. Moreover, the sites of accumulation of radioactivity may be related to the metabolic fate of a compound or to physiological response, pharmacological parameters, therapeutic and toxic effects. Consequently, the technique is used extensively in attempts to produce safe drugs and to investigate potentially toxic substances present

in the environment. Although other methods are available for determining the distribution of radioisotopes at all levels of biological organization, autoradiography is often superior to more conventional methods using electronically amplified instruments. Small concentrations of a radioisotope which are barely detected by using a Geiger-Müller tube or a well-type scintillation counter can yield useful information when autoradiography is used as the recording method. This is because the nuclear emulsion is able to integrate the incident radiation over a much longer time than is practicable with counting methods. Furthermore, accurate counting of radioisotopes in tissues requires the complete destruction of the tissues. This is not necessary with autoradiography when the tissues are sliced or sectioned and structures can be correlated directly with the autoradiographic pattern.

Autoradiographic phenomena were described in the late nineteenth century when it was demonstrated that uranium salts produced images of their own outlines when placed in contact with photographic film (Niepce de St. Victor, 1867; Becquerel, 1896a,b,c,d). According to Boyd (1955) the first biological application of autoradiography was carried out in 1904 by London who produced a whole-body autoradiograph of a frog which had been exposed to radium emanations. At about the same time Bouchard *et al.* (1904) were carrying out investigations on the microscopic distribution of inhaled radium in the guinea-pig. Between 1904 and 1930 most of the published autoradiographs described the distribution of isotopes at the microscopic level (Kotzareff, 1922; Lacassagne and Lattes, 1924; for reviews, see Boyd, 1955; Rogers, 1967). In 1930 Lomholt devised a technique for preparing sections of whole animals for autoradiography to study the deposition of lead in mouse tissues and in new born rats. By the 1940s the use of photographic emulsions for localizing radioactivity in tissues and cells was well established and Leblond (1943) demonstrated the value of this recording medium by correlating the distribution of radioiodine with the metabolism of the thyroid gland.

Much of the impetus for studying the macroscopic distribution of soluble compounds was provided by the elegant studies of Ullberg and his associates from 1954 onwards. Mice injected with radioactive compounds were rapidly frozen in order to "fix" *in situ* both soluble and insoluble isotopically-labelled substances. Sections of the frozen mice were cut with a heavy-duty sledge microtome and translocation of the isotope was prevented by freeze-drying the sections, which were supported on adhesive tape. Each section, still attached to the tape, was then pressed against X-ray film, which after exposure was separated from the section and developed. The success of this method is illustrated by the variety of reports by Ullberg and his co-workers describing the distribution of numerous compounds. In some studies, attempts were made to relate the localization of the

isotope to the physiological or toxicological effects of the administered compounds, and the information obtained was occasionally used as a guide for more detailed autoradiography of selected organs.

Several groups of workers have been engaged in producing frozen specimens for whole-body autoradiography by alternative techniques. Pellerin (1961) described a technique which consisted of freezing the animal in liquid nitrogen following the administration of radio-elements. The carcass was milled to expose the surface of the organs of interest; the tissue surface was subsequently exposed to X-ray film at low temperature, producing autoradiographs showing the distribution of the radio-elements. Pellerin claimed that his low temperature method "eliminated the chemical diffusion of the radio-elements", that there were no pseudophotographic effects and that it enabled the fastest metabolic steps to be "grasped". The anatomical localization of the radio-elements was simplified by direct colour photography of the prepared animal surfaces. This technique was used by Chaidot *et al.* (1964) to study the fate *in vivo* of a chelating agent labelled with ^{59}Fe and the distribution of radioactivity was correlated with the metabolism of the injected material.

The technique of whole-body autoradiography was modified by Martin *et al.* (1962). The distribution of radio-elements was studied by rapidly freezing the injected animals in a mixture of acetone and solid carbon dioxide. The machining was carried out using a drum-cutter attached to an electric drill, a method suitable for small animals only. A technique which permitted longitudinal and transverse sectioning of larger animals, up to a weight of 500 g or more, was subsequently developed by Kalberer (1966). The animals were frozen in liquid air and a special circular saw-blade was used which cut hard materials such as bones and teeth as cleanly as the soft tissues.

The development of alternative methods for preparing autoradiographs has been accompanied by an increasing awareness of the potential of the technique as an investigative tool for a variety of purposes. Whole-body autoradiographs obtained by each method can provide a picture of the distribution of isotopically-labelled compounds *in vivo* extending from the principal organs down, even to the cellular level in some cases. The picture is an essentially static one providing information on the distribution at a precise moment after the isotope is administered. However, a series of distribution patterns obtained at various times after administration can be interpreted to provide a dynamic view of the movement of labelled molecules. Visual scanning of autoradiographs obtained at different times after administration can provide useful preliminary information on absorption, general distribution and routes of excretion. In some instances the method reveals deposition in tissues which would remain undetected by other

methods and on those frequent occasions when quantitative analysis of excreta shows incomplete recovery of radioactivity, the method will usually reveal the site of the "lost" radioactivity remaining in the animal.

The technique pinpoints those biological membranes which are permeable to new pharmaceutical compounds and toxic agents. The ability of certain compounds to penetrate the placental barrier, the central nervous system or the retina of the eye, can be of importance is assessing their therapeutic activity or potential toxicity. The method can also be used to guide the direction of subsequent research. For example, accumulation of a radioisotope in the liver and evidence of biliary excretion could initiate experiments to investigate hepatic metabolism, the nature of biliary metabolites and the possibility of an enterohepatic circulation of the original compound or its metabolites. Another avenue for further study would be the effect of the compound and its metabolites on the biochemistry of the liver and bile production. The retention of a high concentration of a radiolabelled compound in a particular tissue might suggest that this site should be given special attention during chronic toxicity experiments.

Whole-body autoradiography whilst providing extremely valuable information, cannot and should not be regarded as an end in itself. Quite clearly, for a complete understanding of molecule movement *in vivo*, the results of many other techniques, for example, biochemical, physiological and pharmacological, are required. However, these can be more clearly understood when viewed together with whole-body distribution patterns.

2

Experimental Design

I. Radioisotopes and Radiochemicals

A. Measurement of Radioactivity

The unit of radioactivity in the International System of Units (SI) is the becquerel, which is equal to one nuclear transformation per second. This is intended to replace the curie as the measure of radioactivity. The prefixes to be used with SI units and the relationship between the becquerel and curie are shown in Table 1.

Table 1
Prefixes for SI units

Factor	Prefix	Symbol
10^{18}	exa	E
10^{15}	peta	P
10^{12}	tera	T
10^{9}	giga	G
10^{6}	mega	M
10^{3}	kilo	k
10^{-3}	milli	m
10^{-6}	micro	μ
10^{-9}	nano	n
10^{-12}	pico	p
10^{-15}	femto	f
10^{-18}	atto	a

Relationship between becquerel and curie
SI unit: becquerel (Bq). 1 Bq = $2{\cdot}70 \times 10^{-11}$Ci = 27 pCi
Non-SI unit: curie (Ci). 1 Ci = $3{\cdot}7 \times 10^{10}$ Bq = 37 GBq

B. Choice of Radionuclide

Whole-body autoradiographs have been obtained with many radioisotopes, but those most commonly used are ^{3}H, ^{14}C, ^{35}S and ^{125}I (Table 2).

Table 2
Physical data of isotopes in common use for whole-body autoradiography

Isotope	Half-life	Particle	Particle energy (keV) E_{max}	Particle energy (keV) E_{mean}	Approximate resolution (μm)
Tritium (Hydrogen-3)	12·3 years	β^-	18	5·5	2
Carbon-14	5760 years	β^-	159	50·0	30
Sulphur-35	87·2 days	β^-	167	49·2	30
Iodine-125	60 days	Internal conversion	35	—	5

The β particles emitted by ^{14}C can be efficiently recorded on X-ray emulsions. The isotope has a long half-life and it can be introduced into most organic molecules. The major disadvantages are the high cost of labelling molecules and the problems of disposing of an isotope with such a long half-life into the environment.

The radiation properties of ^{35}S are similar to ^{14}C making it a convenient isotope for whole-body autoradiography following the administration of sulphur-containing compounds. The relatively short half-life can be a disadvantage when long exposure periods are needed to locate small amounts of residual radioactivity in the animal. However, considerable advantages accrue from the ease of labelling and the lower costs compared with other isotopes.

Tritiated compounds are usually cheap and easy to prepare. Tritium emits a very low energy particle and large amounts of the isotope are usually required to produce satisfactory autoradiographs.

Iodine-125 is a useful isotope for tagging proteins, peptides and iodine-contrast agents and is usually preferred to ^{131}I because it has a longer half-life, and because of its lower energy better resolution is achieved. Other isotopes which have been used are listed in Table 3.

C. Synthesis of Radiochemicals

Methods for isotopic labelling vary considerably, depending on the nature