AUTORADIOGRAPHIC STUDIES
IN CONNECTION WITH THE CLINICAL
USE OF RADIOACTIVE ISOTOPES

by

A. F. Phillips.

A THESIS
submitted for the degree of
DOCTOR OF MEDICINE

in the

UNIVERSITY OF EDINBURGH.

Department of Radiotherapeutics
University of Cambridge.

MCMXV
ACKNOWLEDGEMENTS

I wish to express my gratitude to Professor J.S. Mitchell for suggesting this line of work in general, and measurement of gamma-ray doses in particular, and for his continued interest and advice. And I wish to thank the surgeons concerned, Mr. B. McN. Truscott, Mr. J.E. Rowlands, Mr. P.H.R. Ghey and Mr. C. Parish, for permission to obtain specimens and to report on cases under their care, Dr. R. Braams for a specimen, and Dr. C.H. Whittle for photographs and for permission to use his notes on one case.

I am grateful to the medical staff and the physicists of the Radiotherapeutic Centre, Addenbrooke's Hospital, for their co-operation, and in particular to Mr. J.L. Haybittle, Senior Hospital Physicist, for many valuable discussions on dosimetry.

I am grateful to Mr. E.W. Mitchell for assistance in preparing photographs and photomicrographs, to Miss S. Lowman for photographic reproductions, to Mr. E.A. King for teaching me histological techniques, and to Miss R.G.M. Pole for technical assistance in the later part of the work.
# CONTENTS

<table>
<thead>
<tr>
<th>I</th>
<th>Introduction ... ... ... ...</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>Autoradiographic Methods ... ...</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Quantitative estimations of radioactivity from autoradiographs ... ...</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Sensitivity of autoradiographic emulsions</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Radiation dose to the tissue ...</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Resolution in autoradiography ...</td>
<td>27</td>
</tr>
<tr>
<td>III</td>
<td>Clinical Applications of Autoradiography with Radioactive Iodine ... ...</td>
<td>31</td>
</tr>
<tr>
<td>IV</td>
<td>Clinical Applications of Autoradiography with Other Radioactive Isotopes ...</td>
<td>39</td>
</tr>
<tr>
<td>V</td>
<td>The Radiation Dose in Radio-Isotope Therapy</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Dosage in a uniformly active sphere</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Absorption of β-rays in tissue</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Dosimetry with I(^{131})</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Selective concentration of radioactive isotopes ... ... ... ...</td>
<td>53</td>
</tr>
<tr>
<td>VI</td>
<td>Scope of the Present Studies ... ...</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Material ... ... ... ...</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Techniques used ... ... ...</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Stripping film autoradiographs ...</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Electron-track autoradiographs ...</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>Beta-ray autoradiography using X-ray film ... ... ...</td>
<td>74</td>
</tr>
<tr>
<td>VII</td>
<td>Gamma-Ray Dose Measurements ... ...</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>Preparation of the specimen ...</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>Shrinkage of the specimen ... ...</td>
<td>81</td>
</tr>
</tbody>
</table>
Necessary absorber thickness... ... 81
Relation between dose and optical density ... ... ... ... 83
Measurement of optical density ... 88
Calibration and standardization ... 88
Rate of uptake and excretion ... 91
Extrapolation to zero distance ... 92

VIII Cases Investigated with Radio-Iodine ... 96
Case 1. Recurrent carcinoma of the thyroid, following radio-iodine therapy ... ... 97
Case 2. Carcinoma of the thyroid with distant metastases ... 107
Case 3. Carcinoma of the thyroid ... 114
Case 4. Nodular colloid goitre ... 121
Case 5. Carcinoma of oesophagus (post-cricoid); secondary extension to thyroid ... 125

IX Cases Investigated with Radioactive Phosphorus and with Radioactive Colloidal Gold ... 127
Case 6. Malignant melanoma ... ... 129
Case 7. ?? Eosinophil granuloma ... 135
Case 8. Malignant haemangio-endothelioma with multiple secondaries ... 137
Case 9. Carcinoma of the ovary; secondary carcinomatosis of peritoneum and skin ... ... ... ... 140
Case 10. Squamous cell carcinoma of chest 142
Case 11. Mycosis fungoides ... ... 145
Case 12. Carcinoma of the bronchus ... 148
X The Choice of Radioactive Isotopes for Clinical Use ... ... ... ... 151

Appendix. Reduction of Autoradiographic Sensitivity for Isolated Objects ... 163

References ... ... ... ... ... 167

List of Tables

Table I. Physical Constants for I\textsuperscript{131} ... following 50

Table II. Effect of Excretion on Radiation Dose from I\textsuperscript{131} ... ... facing 51

Table III. A. Specimens with I\textsuperscript{131} 65
B. Specimens with P\textsuperscript{32} 66
C. Specimen with Au\textsuperscript{198} 66

Table IV. Percentages for Extrapolation 95

Table V. Differential Absorption Ratios for I\textsuperscript{131} 96

Table VI. Case 1. Radio-iodine Therapy, with Estimated Tissue Dose 100

Table VII. Case 2. Radio-iodine Therapy and Uptake Measurements 108

Table VIII. Carcinoma of Thyroid. Calculated $\beta$- and $\gamma$-ray Doses, and Measured $\gamma$-ray Doses 113

Table IX. Six Specimens containing Radio-Phosphorus, and Differential Absorption Ratios 127

Table X. Isotopes of Iodine. X-ray, $\gamma$-ray, and $\beta$-ray Doses ... ... following 152

Table XI. Isotopes of Phosphorus ... facing 158

Table XII. Isotopes which Emit $\beta$-rays of Low Energy, or $\alpha$-particles. ... following 160
CHAPTER I.

INTRODUCTION

Autoradiography is the use of photographic material to demonstrate the presence and situation of a radioactive substance. The invisible rays from radioactive substances cause a change in photographic emulsion which is similar to the latent image produced by exposure to light or ultra-violet rays. It was this property of the rays which led Becquerel in 1895 to the discovery of the radioactivity of Uranium, and he subsequently made photographic images of some crystals of a uranium salt. The much greater photographic activity of radium led to the discovery of this element by the Curies in 1898. I have traced the word "auto-radiograph" back to Sir William Crookes (1914) who obtained images of diamonds, made superficially radioactive by exposure to radium, by placing them on photographic film in the dark.

Photographic emulsions were much used in the early research on radioactivity, but gave way to methods which lent themselves more readily to quantitative measurements. The more recent development of emulsions suitable for showing tracks of individual particles and distinguishing different types of particles, has restored photographic methods to a very important place in physical research on radioactivity.
With regard to biological applications of autoradiography, recent reviews (Gross & Leblond, 1946; Gross, Borogoch, Nadler & Leblond, 1951; Bourne, 1952) state that London, in 1904, was the first to make autoradiographs of animal tissues. The radioactive element he used was radium. Kotzoroff & Weyl also studied the distribution of radium in animal tissues in 1923. At this time, and until 1934, the only radioactive elements were those which occur naturally. These did not include isotopes of any elements known to be important in normal physiology. A radioactive isotope of lead was available in Radium D, which could possibly have had some application in the toxicology of lead; and the distribution in the body of the commercially exploited radioactive elements themselves, notably radium, was of interest because of the harmful effects of their radiations on the tissues. The volume of autoradiographic work published on these subjects was small, but its quality was high.

Lacassagne & Lattès (1924) in a number of papers published the method and results of their researches with polonium injected into rats and rabbits, including pregnant animals and animals bearing transplanted tumours. Polonium emits only α-particles, which have very short range in tissue and in emulsion and therefore give clear images. Some autoradiographs are reproduced
in these papers which show considerable detail. These authors were evidently aware of the therapeutic possibilities of radioactive elements if the activity became concentrated in tumour, but in fact in their experiments tumour took up less than many other tissues. Placental villi, spleen, and renal cortex, took up the most. The method they used was to embed the tissues in paraffin wax by ordinary histological techniques, face the blocks on the microtome (saving and staining the last few sections), and place the faced blocks on photographic film. A good deal of the experimental work reported in this thesis has been done by the same method. These authors also mention attempts by Lazarus in 1912 to obtain autoradiographs in vivo, through the skin of experimental animals, using the β- and γ-rays from Actinium X. Lacassagne continued these researches with various collaborators, using other natural radioactive elements as well as Polonium. In particular, he studied the distribution of Bismuth, by autoradiography with its isotope RaE, in animals with spontaneous and implanted tumours (Lacassagne, 1931). He noted the inferior definition obtained with the β-rays from RaE, and preferred to allow this element to decay in situ (half-life 5 days) to Polonium (RaF, half-life 140 days) and obtain an autoradiograph with the α-particles as before. The results
obtained by autoradiography were confirmed by chemical analysis (Lacassagne & Loiseleur, 1931) of the organs and tumours; they did not show any high concentration of bismuth in tumours, such as had been reported by Hevesy & Wagner (1930), Kahn (1930), and other workers.

Hevesy & Wagner (1930) assayed tissues by means of the electroscope for the heavy metals Thorium, Radium D (Lead), and Radium E (Bismuth). They found selective uptake of bismuth in a mouse tumour, from 5 times to 55 times the amount in muscle. Kahn (1930) repeated this work by injecting "Bi-diasporal" labelled with RaE into mice carrying Ehrlich adenocarcinoma, and as well as assay by electroscope he embedded the tissues in paraffin and made autoradiographs from 50μ sections. These clearly showed concentration of the activity in the tumours. He then injected the radioactive bismuth preparation into 3 human patients with carcinoma of the cervix uteri, 18 hours before operation, and found the activity in the tumour to be about 10 times that in the unaffected uterine muscle. He made autoradiographs from these specimens, which showed that the activity was highest in the growing edge of the tumours. These autoradiographs are clearly reproduced side by side with photographs of the sections at the same magnification, and are the earliest autoradiographs from human
tissues which I have found. They seem to have been overlooked by the writers of the reviews cited above.

Lomholt (1930) used Radium D from old radon tubes to investigate the distribution of lead injected as chloride in baby rats and mice. His autoradiographs were made from paraffin sections 10μ and 20μ thick, and in this case the main photographic action is that of the rather penetrating β-rays of the daughter product RaE. A fair amount of detail could be made out. Cartilage in process of ossification was among the several tissues with the highest activity, the animals having been killed at 24 hours after injection. At this time the activity was still as high in liver, renal cortex, spleen and blood, and his researches do not seem to have been pursued to demonstrate by this method the persistence of the deposits in bone.

Gettler & Norris (1933) analysed very thoroughly the tissues of a man who had died from the effects of drinking, over a number of years, a large quantity of tonic water sold on account of its radium content. They estimated that the whole body contained 74 micrograms of radium, of which more than 99% was in the skeleton. As a scientific curiosity they made autoradiographs from the ashed bones and teeth. The bone autoradiographs were not well-defined, but those of the teeth showed a very irregular distribution with
highly active areas not corresponding to obvious anatomical features. Their findings accounted well for two of the lesions present at death, namely necrosis of the jaw, and anaemia. Cerebral abscess and broncho-pneumonia were also present, no doubt associated with leucopenia due to the damage to the bone marrow by radiation.

Artificially produced radioactivity was discovered by I. Curie & Joliot in 1934, and by the end of that decade very small quantities of radioactive isotopes of several elements were available to a few workers. They were produced by artificially accelerated particles in high voltage machines or in the cyclotron, and by the action of neutrons from a radium-beryllium source. Biological work with radioactive phosphorus ($^{30}$P and $^{32}$P) included that of Bulliard, Grundland & Moussa (1938) who made use of an unusual autoradiographic technique with $^{32}$P in their investigations of the suprarenal glands. They cut frozen sections of unfixed tissue, which they then made radioactive by immersion in an aqueous solution containing radioactive phosphorus, for 24 hours. They found by means of autoradiographs that the activity due to exchange between tissue and solution was greatest in the cortex of the suprarenal, and corresponded to the areas of the sections which afterwards took up stains for fat.
During the following decade, radioactive isotopes became widely available in rapidly increasing variety, and in large amounts in a number of cases. By now, a number of different radioactive isotopes are known for every naturally occurring element, and there are several new radioactive elements. Isotopes of a number of biologically important elements can be bought at prices comparable with the most expensive drugs, and are research tools of extraordinary value. Following the wide use of radioactive tracers in biochemistry, mainly in investigations of the metabolism of particular elements and compounds in whole organs, autoradiography is proving of value in localisation of radioactivity in particular tissues and even in localisation within the cell, for research purposes in biology generally. Until very recently, however, success in or improvement of the technique has been the main interest of research workers, and in relatively few biological papers is autoradiography mentioned as a method deliberately chosen to attack a problem suggested independently. The studies of Howard & Pelc (1950, 1951a, b, c) of the synthesis of deoxyribonucleic acid and nucleoprotein in dividing cells form an outstanding example. Leblond and his co-workers have used autoradiography with radioactive iodine in the investigation of iodine metabolism in the thyroid gland. (Leblond, 1943, 1944;

In clinical work, the main applications of autoradiography have been to show the distribution of administered radioactive substances in biopsy or operation specimens. It has been used to give two main types of information: the first, with considerable success, to estimate the degree of function of thyroid tissue by the uptake of radio-iodine; the second, in cases where there is a possibility of therapeutic irradiation by means of radioactive isotopes administered internally, to give evidence on selective uptake into malignant tumours. In this second group, little information of practical value in therapy has been obtained except in the case of radio-iodine in carcinoma of the thyroid. In these applications, autoradiography is supplementary to electronic counter measurements, which give less detailed information, but which are more sensitive and quicker. Clinical studies by autoradiography will be reviewed in Chapters III and IV.

Autoradiography of a very simple type is most valuable in checking radium needles against certain defects, and is used as a routine for this purpose.
CHAPTER II.

AUTORADIOGRAPHIC METHODS

The theory and practice of autoradiography have been reviewed by Gross & Leblond (1946), by Gross, Borogoch, Nadler & Leblond (1951) by Bourne (1952) and by Doniach, Howard & Pelc (1953). As Bourne says: "a section of tissue must be brought into intimate contact with a sensitive photographic film"; and he classifies this process according to four different methods.

(1) A glass slide bearing the section is clamped to the emulsion of a photographic plate. The plate is developed after the slide and section have been removed. (The "contact" method).

(2) The section is mounted directly on the emulsion, and photographic development is carried out without removing the section. ("Mounted autograph" method).

(3) The section, on a slide, is covered by special photographic film ("stripping film") designed for the purpose, which then stays in place throughout photographic development and subsequent microscopical examination.

(4) Melted photographic emulsion is poured or painted over the slide bearing the section. ("Coated autograph" method).
The first of these methods has the great disadvantage, for work on a microscopical scale, that it is most difficult to align the section with the autoradiograph for examination. For most applications this outweighs the advantage which it has in the independence of photographic and histological processing. It was the method used by the first workers in the field of thyroid autoradiography (Hamilton, Soley & Eichorn, 1940). A modification of this "contact" method (not mentioned by Bourne) has been used by Hoecker & Roofe (1949) and by Williams (1951) in which a strip of photographic plate and a long flexible coverslip bearing the section are cemented or clamped together at one end only, and the section can be lifted away from the emulsion by bending the coverslip after exposure. During development, Williams protected the sections by a thin waterproof bag. Hoecker & Roofe report that careful tests showed no evidence of displacement of the sections relative to the emulsion.

The "mounted autograph" method was described independently by Evans (1947) and by Bourne (1948) and overcomes difficulties of alignment. At the same time it introduces the disadvantage of carrying out photographic processing with the section superimposed on the latent image, with the possibility of imperfect development, and of the deposition of silver by chemical
interaction between emulsion and section. Very perfect contact between section and emulsion is ensured, avoiding any loss of resolution due to a gap between them.

Pelc (1947) arranged the two active components the other way up, and described autoradiography with Ilford Stripping Emulsion placed over the slide carrying the section. Later, in collaboration with Kodak, Ltd., this method was improved by the manufacture of a stripping emulsion specially for this purpose, in which a layer, about 4μ thick, of emulsion sensitive to electrons of minimum ionising power, is backed with a layer of gelatine, inactive and permeable to reagents in solution (Doniach & Pelc, 1950; Berriman, Herz & Stevens, 1950). In this arrangement, the difficulty is in obtaining satisfactory staining of the section. Most of the ordinary histological stains are adversely affected by the chemicals necessary for photographic development and fixing; Feulgen staining is one which is unaffected, but has limited application because the acid hydrolysis at 60°C may remove a large proportion of the radioactivity. Hot carbol fuchsin has also been used before autoradiography, but has the same disadvantage. Usually staining has been done after photographic processing, and many of the ordinary stains can be used, with some modification of the usual technique. No process involving heat can be used at this stage.
Results are rather variable, and staining is somewhat less clear than with a simple section. Dilution of the stain and application for a longer time than for ordinary staining is often an advantage, and differentiation (rather than progressive staining) is essential to obtain clear gelatine, as this lies over the emulsion. A red nuclear stain — Feulgen's reagent, Neutral Red, or Carmalum — allows better visibility of the black silver grains than does a blue stain. The variety of stains recommended in the literature, changes in later publications from the same group of authors, and the use of unusual stains and combinations of stains, suggest that many workers in this field are dissatisfied with their staining methods. Phase contrast microscopy has been used with very good effect, for example by Doniach & Pelc, (1949), and by Howard & Pelc (1950, 1951a, 1951b). They use the phase contrast condenser to view the cells, unstained, and then change to a normal condenser which makes the cells disappear and shows up the grains of the autoradiograph. They have published excellent photomicrographs side by side, with the two methods of illumination.

The "coated autograph" technique was described by Belanger & Leblond (1946), and with more detail in the review by Gross et. al., (1951). In the earlier work emulsion scraped off lantern slide plates was used.
More recently a range of emulsions has become available for research in nuclear physics, of which the most sensitive will show the actual track of an electron at the energy (0.5 - 1.0 MeV.) at which its ionising power is a minimum (Berriman, 1948; Boyd & Levi, 1950). If a somewhat thicker layer of this emulsion is used (20-50μ) the individual tracks of the β-particles are shown up as a line of silver grains. With the thicker emulsion, however, it is even more difficult to obtain really clear staining. I have found the same principles to apply as with stripping film, but Belanger & Leblond (1946) used, among other methods, Harris' Haematoxylin as a progressive stain. In the paper in which this is mentioned, however, the autoradiographs reproduced were at a magnification of only x 50.

There are two particular advantages of this technique. First, that the theoretical resolving power should be very good - of the order of the thickness of the section or the diameter of a silver grain (developed), since the exact point at which the electron entered the emulsion should be marked by the first grain of the track. The direction of travel can be ascertained unambiguously in most instances by the increase of grain density along the track or by the slope of the track up through the emulsion. However, although this advantage is very clear in the case of α-particle
tracks, it is almost lost in the case of $\beta$-particles because of the comparatively long distance between grains in the tracks of the faster particles, and the marked tortuosity of the tracks of the slower ones. The second advantage is the ease of discrimination against artifacts, especially chemography, and against background fog (provided it is not too dense) whether it is inherent in the emulsion or due to errors in development or exposure to safe-light. Cosmic ray tracks recorded by the emulsion before spreading on the slide are disrupted by the process of melting and spreading and only add slightly to the background fog; those recorded after spreading are usually easy to distinguish by their straightness.

These four methods are the principal ones which have been used in microscopical autoradiography, though a number of variants of them have been tried by different workers. Smears of blood (Boyd, Casarett, Altman, Noonan & Salomon, 1948) and of cells from ascitic fluid (Fukui, Miwa & Shibahashi, 1951) have been made directly on emulsion, which must be a difficult technique, in the dark. Fixed Paramoecia have been mixed with melted emulsion and then spread on a slide (King, Harris & Tkaczyk, 1951), to give very clear electron tracks from $^3P$.

A "wet-process autoradiography" has been described
by Gomberg (1951) which is claimed to be capable of very good resolution, of the order of 1μ, and to have great flexibility with regard to grain size and sensitivity. The sensitive silver bromide is deposited in a collodion layer directly on the specimen, and photographic exposure is carried out in a bath of silver nitrate solution. Development is by means of ferrous sulphate, in a solution of which the acidity controls the final grain size.

X-ray film, with much larger grain size than the other emulsions mentioned, has been widely used for macroscopic autoradiographs, where the resolution obtained by naked eye examination is sufficient. For recording the β-radiation from I^{131} in thyroid tumours, and for some of my other larger specimens, I have obtained quite satisfactory autoradiographs by laying a slice of tissue flat on the envelope of an X-ray film ("Ilfex" or dental film), with a thin sheet of waterproof material in the case of fresh tissue. This is a technique which requires only a few minutes to set up, exposure can be carried out in daylight, and no equipment is required beyond what is always available in a hospital or laboratory. If the specimen has sufficient activity - no higher than is required for a satisfactory microscopical autoradiograph - the whole process can be carried out in two or three hours. For
a true representation of the relative activities of different areas, it is important to keep the specimen in firm contact with the film envelope all over, and the best type of specimen is a paraffin wax block, faced on the microtome. Large blocks have been faced on a milling machine. A fresh specimen, carefully weighted, is also satisfactory.

Quantitative Estimations of Radioactivity from Autoradiographs.

A number of workers have made more or less successful attempts to obtain quantitative results from microscopical autoradiographs. For accurate results, the number of factors which have to be calibrated or controlled, is considerable. First there is variation of the emulsion, particularly in experimental types. In the tissue, removal of activity by fixation and dehydration must be allowed for, and any shift of activity from one part of the tissue to another would cause error. Then the thickness of tissue sections, flatness on the slide, and perfect contact with the emulsion, are critically important. Conditions of exposure must be uniform, particularly with regard to humidity, and in the case of short exposures the speed of initial drying of the emulsion will influence the time at which it acquires full sensitivity. In the case
of exposures of a number of days, "latent image fading" must be avoided by low temperature and low humidity, and possibly the exclusion of oxygen. In development, the usual photographic routine of brushing the surface of the emulsion can hardly be applied, and vigorous movement of the fluid is undesirable when there is a danger of dislodging stripping film. With the mounted autograph method, development is slightly hindered by the presence of the section, and with the poured emulsion technique the thickness of the emulsion layer is apt to vary.

When the image has been obtained, the intensity of it can be estimated either by counting silver grains or electron tracks, or by densitometry. Counting of silver grains has been used by a number of workers (Gross et al., 1951; Odeblad, 1951; Pelc, personal communication), although it is laborious. Recently Andresen, Chapman-Andresen, Holter & Robinson (1953) have carried out a very thorough research on the accuracy of this method, and, on the basis of many hundreds of counts including repetitions to determine personal errors and variations in the emulsion and in many of the processes involved, conclude that in their work on $^{14}\text{C}$ assimilation by an amoeba they obtained an accuracy of $\pm 5\%$. An apparatus for automatic grain counting, to reduce the labour of this method, is being developed at Hammersmith (Dudley & Pelc, 1953).
With the coated autograph method, King, Harris & Tkaczyk (1951) have counted electron tracks from Paramoecia containing $P^{32}$. Campbell (1951) also makes brief reference to this method.

Densitometer measurements on a microscopic scale require particular cleanliness, and are liable to be affected by a superimposed tissue section even if unstained. A photometer in combination with a low-power microscope has been used by Axelrod & Hamilton (1947), Branson & Hansborough (1948), Dudley & Dobyns (1949) and Clayton (1953). Steinberg & Selverstone (1950) made photometer measurements on optical projections of their autoradiographs on a groundglass screen. These were autoradiographs on X-ray film, of $P^{32}$ in brain sections, and the tissue was not superimposed.

In a thesis on quantitative autoradiography, Odeblad (1952a) made a very thorough study of the factors involved in the absolute determination of radioisotopes in tissues, for the particular application he was concerned with. This was the uptake of $P^{32}$ into the granulosal cells of the rabbit's ovary. He used the contact method of autoradiography, with dental X-ray film, on paraffin sections. A refinement introduced by him, which may be important for quantitative work and also to obtain the highest resolution was the use of methacrylate instead of glass slides.
under the sections. He showed clearly by a separate experiment that backscattering was far less with methacrylate than with glass, but his geometrical arrangement for this experiment was different from that for his autoradiographs, and the importance of the backscattering in practice could not be estimated. Odeblad gave reasons for preferring densitometry to the counting of silver grains; and he used the contact method so as to be able to remove the section and carry out photographic development and densitometry accurately.

Standards for calibration have been made by various methods including $\text{BaS}^{35}\text{O}_4$ suspended in shellac (Axelrod & Hamilton), $\text{AgI}^{131}$ precipitated in melted gelatine which was subsequently hardened and sectioned (Clayton), $\text{Ca}^{45}$ in plaster of paris (Dudley & Dobyns). Steinberg and Selverstone made only relative measurements. Andresen et al. made their measurement absolute by comparison with a Geiger counter of which the geometry and sensitivity were carefully determined.

**Sensitivity of autoradiographic emulsions.**

One microcurie of any radioactive substance is defined as the quantity in which the number of disintegrations occurring per second is $3.7 \times 10^4$.

Taking as a convenient example a section of thickness $5\mu$, from a specimen containing $1 \mu c/ml.$, the
number of β-rays emitted from one side of the section (neglecting a number of small complicating factors) is

\[3.7 \times 10^4 \times \frac{1}{2} \times 5 \times 10^{-4} \times 60 \text{ per cm}^2 \text{ per minute} = 550 \text{ per cm}^2 \text{ per minute.}\]

For microscopical work, a more convenient unit of area is a square whose side is 10μ; the same activity is equivalent to

\[5.5 \times 10^{-4} \beta\text{-rays per 100 } \mu^2 \text{ per minute}\]

or \[0.80 \beta\text{-rays per 100 } \mu^2 \text{ per day.}\]

These numerical values give a convenient basis for discussion of sensitivity and times of exposure. The factors neglected in the calculation so far are:

- β-particles which though emitted in an upward direction, do not emerge because of very low initial energy or a prolonged path within the section itself (with its protective coating, if any); backscattering by the glass behind the section; the photographic action of any γ-rays which are emitted, including that of internal conversion electrons; and loss of emitted electrons by K-capture in the case of positron emitters.

For stripping film, the number of grains rendered developable per incident electron is of the order of unity (Berriman et al., 1950; Lamerton quoted by Herz, 1951a), being somewhat less, perhaps as low as 0.5, for the high energy β-rays of P^{32}, and somewhat higher, perhaps as high as 1.8, for the very low energy
β-rays of $^{35}$S (Doniach et al., 1953). The number of grains required to make a recognisable autoradiograph is a difficult quantity to estimate, and depends greatly on the circumstances, particularly on the distribution of the activity. Pelc (1951) estimates it as $10^7$ grains per cm$^2$. for stripping film, or a minimum of 10 grains for a single object not exceeding 100 $\mu^2$ in area. Under good conditions, with low background, such an autoradiograph is perfectly definite, but by no means obvious to an inexperienced observer.

An effective reduction of sensitivity not mentioned by Pelc, for isolated small objects of this size, is due to the fact that not all the β-particles emitted upwards will strike the emulsion directly over the object. A calculation given in Appendix 1 shows that on certain reasonable assumptions for a 5$\mu$ section and stripping film with emulsion 4$\mu$ thick, this factor reduces the number of useful grains to approximately half the total number rendered developable. Also, doubling the specimen thickness by no means doubles the number of useful grains under these circumstances. This calculation only applied to isolated objects: for the case of a sheet of cells all radioactive, no such correction is required.

In the example I have taken, and on Pelc's calculation, an exposure of 12 days at full activity
would be required. Allowing for radioactive decay, using the isotope $^{131}$I, a specimen containing this initial activity would give only 75% of this number of grains in two half-lives (16 days). With $^{32}$P (still assuming one grain per electron) the necessary exposure would be 24 days, just under two half-lives. With half-lives of this order of magnitude, it is never worth while to prolong the exposure beyond two half-lives, by which time three-quarters of the total activity of the specimen will have been used.

On this calculation, the lower limit of useful activity for this purpose would appear to be in the region of 1-2 μc/ml. In practice, however, the main interest of autoradiographs is when the activity is not uniformly distributed, and in such a case the mean activity may be much less.

Thyroid tissue containing $^{131}$I has given satisfactory autoradiographs with as little as 0.27 μc/gm in 5μ sections (Doniach & Pelc, 1949) or 0.2 μc/gm (Marinelli, Foote, Hill & Hocker, 1947).

With X-ray film, the grain yield for $^{131}$I is also approximately one per electron, but owing to the larger size of the grains, equal optical density is reached with one hundredth of the number of electrons required by stripping film (Berriman et al., 1950). Since X-ray film is normally assessed by optical density, whereas
stripping film is normally used for microscopical examination, a factor of 10 probably represents the useful increase of sensitivity.

With the "coated autograph" technique, and observing electron tracks, fewer electrons are required to produce a definite autoradiograph. Under good conditions, even two tracks from a small object in the microscope field would be convincing, or the repeated appearance of a single track from a certain part. The published photograph (King et al., 1951) of $^{14}$C-ray tracks from Paramoecium illustrates this. On the other hand, owing to the accumulation of cosmic-ray tracks, the exposure can only be prolonged beyond one or two weeks when special precautions are taken, such as exposing at the bottom of a deep well, or inside a thick lead shield (Walters & Thaine, 1954). At sea level, Herz (1951b) gives the figure of $10^4$ cosmic ray tracks per sq. cm. per day in emulsion of thickness 200$\mu$.

However, Walters and Thaine obtained very low backgrounds when their exposures were protected by a 4" thickness of lead, and claim to be able to detect $5 \times 10^{-10}$ parts by weight of $^{14}$C (0.003 $\mu$C/gm) in plant tissues. This activity corresponds to only 100 disintegrations per gram per second.

Radiation dose to the tissue.

It is impossible to administer a radioactive
isotope to an organism without causing irradiation of each tissue into which the isotope finds its way.
For an autoradiograph to be obtained with, for example, stripping film, the activity of the tissue used has to be of the order of 1 μc/gm, or somewhat less in favourable circumstances. At this level, the β-ray dose rate in the case of I¹³¹ or P³² is by no means negligible. This is a most serious disadvantage of autoradiography from the point of view of biological research.

The actual dose necessary has been calculated from first principles by Pelc (1951), who has derived general formulae for a number of types of biological experiment, and has worked out some particular cases for the isotopes Na²⁴, P³², I¹³¹, S³⁵ and C¹⁴. The detailed cases are for stripping film, assuming one grain per electron for all isotopes, a minimum autoradiograph of 10 grains per 100 μ², and an exposure of two half-lives for the shorter-lived isotopes. It makes a great difference whether the organism is killed at the same time as the specimen is fixed for autoradiography, or whether it survives and part or all continues to be irradiated until radioactive decay or excretion is complete. The human case has always to be planned on the second basis, but the first type of calculation is also of interest in showing what dose has been received by the tissue which is actually
examined. From Pelc's curves it appears that in the case of $^1\text{I}_{131}$, if the specimen is removed within 48 hours after maximum uptake, it will have received up to 40 rep, but the remaining thyroid will receive a total of about 250 rep (neglecting excretion). In the case of $^3\text{P}_{32}$, the figures are 60 and 700 rep.

The figures for $^1\text{I}_{131}$ can be compared with direct calculation from the activity mentioned in the preceding section, namely $1 \times \frac{1}{0.75} = 1.33\mu\text{c/gm}$. Now 1 µc destroyed per gram, assuming excretion at a rate to give an effective half-life of 6.3 days, gives a tissue dose of approximately 100 rep (Mitchell, 1951). If the activity is present two days before the specimen is taken, this gives a tissue dose of $100 \times 1.33 \times 1.2 = 160$ rep. This is sufficiently good agreement with the figure from Pelc's curve, which does not allow for excretion.

Thus the dose received by the actual specimen before removal can be small enough to make no difference visible histologically (except possibly a reduction in the number of mitoses). The dose to the remaining thyroid tissue is not large enough to contra-indicate a single diagnostic procedure, provided the percentage uptake by the thyroid has previously been determined using a small tracer dose, so that the correct amount of radio-iodine can be administered. For example, with
25% uptake in a thyroid weighing 50 gm., the activity required to yield a specimen containing approximately 1.3 μc/gm., is 0.32 mc. The final dose to the thyroid would be then of the order of 200 rep. As the differential absorption ratio* is high for thyroid and not for any other tissue, the dose to other tissues of the body would be negligible: an approximate calculation for the epithelium of the bladder, if the excreted portion occupies 1.5 litres of urine and has an average stay in the bladder of 4 hours, is 0.3 rep.

In the case of P³² the situation is far less favourable, because of the higher energy of the β-rays and the lower differential absorption ratios which are found. Calculation on the same lines as those for I¹³¹, assuming a D.A.R. of 10, and a tissue activity for autoradiograph of 1 μc/gm, gives 8 mc. as the amount to be administered to a man weighing 70 Kg. Whole body irradiation is of the order of 50 rep. with perhaps twice this dose to the haemopoetic tissues and testis (Mitchell, 1951). This is far too high for a diagnostic procedure, and even as a therapeutic measure would only be considered in malignant disease. The dose to the tissue with D.A.R. 10 is approximately 600 rep (allowing for excretion) (Mitchell, op. cit.) Mitchell estimated 10 mc. to be the largest safe amount of P³² to administer,

* Differential absorption ratio (D.A.R.)
\[ \frac{\text{activity of tissue per gram}}{\text{activity administered per gram of body weight}} \] (See Ch.V.)
on the basis of published results of survival experiments on mice and rats.

Using X-ray film, the sensitivity is increased by a factor of 10, and the minimum tissue dose to obtain an autoradiograph is correspondingly reduced. Useful application to human tissues is, however, limited to gross autoradiographs of operation specimens.

Resolution in autoradiography.

The greatest possibilities for high resolution autoradiography may lie in electron track emulsions. In principle it is possible to follow individual tracks back to their point of origin (Yagoda, 1949; Boyd & Levi, 1950; Herz, 1951 a, b; Bourne, 1952). With a-particle tracks this is satisfactory, but the changes of the direction of the β-ray tracks, particularly in the slower tracks, and in the faster tracks the distance between grains, allows only a statistical approach to exact localisation (Boyd & Levi), and little advantage is gained over other fine-grain emulsion techniques. In any case, the limit of resolution cannot be much less than the thickness of the section plus any protective layer between it and the emulsion. Improvement beyond about 1μ is bound to be extremely difficult, so there is little to be gained over the simpler "stripping film" technique. Pelc & Howard (1952) claim resolution of 2μ using stripping film, even with P32, and better than
this with the less energetic $\beta$-rays of $\text{S}^{35}$ and $\text{C}^{14}$.

Doniach & Pelc (1950) have calculated curves of the relative numbers of exposed grains as a function of the distance from the centre of the autoradiographic image for various small theoretical radioactive sources, over a range of values of parameters corresponding to section thickness, emulsion thickness, and separation between section and emulsion. For example, with section and emulsion of equal thickness, $2\mu$, and separation $0.1\mu$, they calculate a resolving power of $2\mu$. They also tested experimentally Kodak stripping film by a technique developed by Stevens (1948) and, using $\text{I}^{131}$, obtained resolution of pairs of lines $2.5\mu$ apart. In this case both source thickness and separation from the emulsion were negligible, and the emulsion thickness was $4\mu$. This is in satisfactory agreement with the relevant calculated figure. Their calculation is based on an inverse square law relating intensity to distance, and neglects absorption of the $\beta$-rays. The effect of absorption is to improve the resolution for $\beta$-particles of very low energy, such as those from $\text{S}^{35}$ and $\text{C}^{14}$, where the mean energy is $0.05$ MeV, and the total path length of a large proportion of the $\beta$-particles is less than $10\mu$ in emulsion*. $\text{I}^{131}$ emits $\beta$-rays of mean energy $0.2$ MeV. For $\beta$-rays of greater energy, such as those from $\text{P}^{32}$ (mean energy $0.5$ MeV) the resolution is somewhat less good than

* By my calculation from the table of Ross & Zajac (1948).
calculated because the photographic action of the electron is greatest near the end of its track.

A number of methods have been used to improve the resolution. Gomberg (1951) claims resolution of 1μ by the "wet process" already mentioned, using I\(^{131}\). Odeblad (1952b) obtained improvement at times, but not regularly, by incinerating paraffin sections before autoradiography in order to reduce the thickness of the source. He also used mica foil or methacrylate slides, in place of glass, in order to reduce back-scatter of the β-rays. Pink (1951) describes a remarkable method of increasing the size of the specimen as much as 100 diameters by rolling out between lead discs.

Some of the best published autoradiographs from the point of view of resolution are those of Pelc & Spear (1950), of chick fibroblasts in tissue culture, those of Howard & Pelc (1950) of chromosomes in mouse spermatocytes (both of these with P\(^{32}\)), and those of Lajtha (1952) showing concentration of P\(^{32}\) in the nuclei and of S\(^{35}\) in the cytoplasm of cells in cultures of human bone marrow. Fitzgerald, Eidinoff, Knoll & Simmel (1951) have made autoradiographs from protozoa containing tritium. Here the maximum range of the β-rays (maximum energy 0.018 MeV) is only 2μ in emulsion, and 90% of the particles are stopped within 1.2μ. They obtained resolution of 0.5μ using sections of thickness 1μ.
The highest resolution can only be obtained when the specimen has considerably greater activity than that required to give a minimum detectable autoradiograph.
CHAPTER III.

CLINICAL APPLICATIONS OF AUTORADIOGRAPHY WITH
RADIOACTIVE IODINE.

The first clinical application of autoradiography with the new artificial radioactive elements was that of Hamilton, Soley & Eichorn (1940). They used radio-iodine, ¹³¹I, prepared in the cyclotron, which they administered to 9 patients awaiting operation for non-toxic goitre, thyrotoxicosis, or carcinoma of the thyroid. They gave doses quoted as 0.1 to 1.0 millicuries, but their unit, based on the ionization due to β-ray emission compared with that of radium, was several times larger than the millicurie as now defined. The activity was administered by mouth two days before operation. The tissue was embedded in paraffin wax by ordinary techniques, and contact autoradiographs were made on X-ray film from sections of thickness 3-5μ. The resolution was about 10μ.

In non-toxic goitre, they found little or no activity in the large deposits of colloid, but activity in the epithelial cells, and in small follicles. In hyperplastic regions the activity was high. In cancer of the thyroid, the tumour took up very little radioactivity, but adjacent normal thyroid tissue was highly active. Their thyrotoxic patients had been treated pre-operatively with Lugol's iodine, and the uptake and distribution of radio-iodine was similar to that in
normal thyroid tissue, but from their observations on the hyperplastic regions in the other cases, they made the suggestion that the hyperplastic thyroid of an untreated thyrotoxic patient might well be irradiated therapeutically by means of internally administered radio-iodine.

Leblond and his co-workers followed up extensive studies of iodine metabolism in animals, for which they used autoradiography and chemical methods (Leblond, 1943, 1944; Belanger & Leblond, 1946), by similar observations on human goitrous thyroids. Two patients, one with toxic, one with non-toxic goitre, were given, respectively, 24 µc and 1 mc of I¹³¹ the day before operation, and autoradiographic studies were made on the gland after removal (Leblond, Fertman, Puppel & Curtis, 1946). Chemical fractionation of the radioactive iodine was carried out on the same specimens (Leblond, Puppel, Riley, Radike & Curtis, 1946). The autoradiographs showed that in both their cases thyroid adenomas were less active than the surrounding tissue.

Evans (1947) also obtained surgical specimens of thyroid adenomas, 1 mc of I¹³¹ having been administered 24 hours before operation. This paper is concerned entirely with technique. Later, Frantz, Quimby & Evans (1948) published a larger series of cases of carcinoma of the thyroid studied by means of radio-iodine, with histological grading, and the results of follow-up for
at least 5 years. They obtained several autoradiographs from metastases which had taken up radio-iodine, including one from a femoral metastasis recorded in vivo by means of the \( \gamma \)-rays, and a metastasis in a rib which gave a striking picture. Although it was impossible to evaluate the effect of radiotherapy by statistics on their cases, they had concluded on the basis of these studies that in two groups of cases the best treatment was elective total thyroidectomy, followed by radio-iodine therapy as soon as the metastases showed uptake from a tracer dose. The types of cases for which they preferred this scheme of treatment were: (1) Malignant functional adenoma, with metastases chiefly to bone, and (2) Tumours with multiple foci in the neck, with or without involvement of the glands. Surgical removal of the thyroid was preferred to suppression by radio-iodine. Thyrotropic hormone was used to enhance uptake by metastases if necessary. They used therapeutic doses up to 100 mc. of \(^{131}\text{I}\), repeated in some cases; but smaller doses when the active tissue was small in amount. The autoradiographs showed spotty and irregular distribution of radioactivity in the carcinomata, and this was also true of a few cases they had of nodular goitre, but in normal thyroids (removed from cancer cases) they found uniform distribution.

Marinelli, Foote, Hill & Hocker (1947) publish a
number of autoradiographs from 19 cases of thyroid carcinoma of different types. These show very variable uptake, correlated on the whole with follicular arrangement of the cells, and particularly with the presence of colloid; but there were notable exceptions from this correlation. In general the autoradiographs showed a spotty distribution of activity, with gaps of, apparently, several millimetres between active spots (the magnification of their reproductions is not stated, but can be deduced roughly from the size of characteristic structures). The "metastasizing struma" shows by far the greatest uptake, but even this is spotty. They are cautious in recommending attempts at therapy by means of radio-iodine, but would use it in cases of metastasizing struma, and in other cases with proved uptake where no other treatment gave hope of a cure. Fitzgerald & Foote (1949) give results from autoradiography of 100 specimens (operation, biopsy and autopsy) from cases of carcinoma of the thyroid, following administration of tracer doses (0.3 - 5.0 mc) or therapeutic doses up to 250 mc. In papillary carcinoma (the commonest type in their series) 28% showed autoradiograph, and these were all mixed types with some follicle formation. Alveolar and follicular types (including metastasizing struma) showed autoradiographs in 74% of cases, always over areas where colloid was present, and with great variation of intensity. Of
solid carcinomata, 42% showed autoradiographs but nearly all of these were histologically mixed in type.
Of Hürthle cell carcinomata, (cytoplasm eosinophilic) 34% showed autoradiographs. The very malignant spindle-cell and giant-cell type, and anaplastic carcinoma, showed no autoradiographs. Taking all types together, this is a much higher proportion of positive autoradiographs than other authors quote, and even than the previous paper of Foote and co-workers (Marinelli et al., 1947), which may partly be due to selection of cases, and partly to the fact that they could obtain an autoradiograph with less than 0.2 μc/gm average activity provided the distribution was not uniform. This average activity corresponds to only 12 mc uniformly distributed in 60 Kg., and could have been present without a useful degree of concentration in the tissue. In some of their cases, which had received the largest amounts of radio-iodine, even indifferent tissues might have given an autoradiograph. This uncertainty emphasises the advantage which a more quantitative approach would have. They found that radioactivity was on the whole correlated with the presence of colloid, but not invariably. In their series it was commonest for the primary and metastatic lesion to be either both active or both inactive, in presence of functional thyroid tissue. In normal thyroid tissue, they noted the variability of activity of follicles even
of similar size and histological appearance, and discuss the possibility of "phasic" variation of activity in any one follicle, and the significance of this possibility in therapy.

Seidlin, Rossman, Oshry & Siegel (1949) report 30 cases of carcinoma of the thyroid with metastases, of whom 12 were treated with $^{131}$I, repeated doses of 100 mc or more. Some autoradiographic studies were carried out, and they reproduce autoradiographs at low magnification. These are mainly of necrotic tissue, and surprisingly show the spotty distribution characteristic of thyroid follicles. They suggest that this may be due to some effect similar to the iodine staining of amyloid and not to thyroid-like activity. Another possibility which they do not mention is the adsorption of the activity on to colloidal particles before or after injection. Such effects have been observed, usually accidentally, by various workers.

Rall, Keating, Power & Bennett (1949) report on a patient who died 56 hours after a therapeutic dose of $^{131}$I, 63 mc. They traced all the administered iodine, which is of great interest in connection with the question of the practicability of radical therapy by this means, and made autoradiographs. In the primary and a lymph node metastasis which had had previous radiotherapy, the uptake was peripheral and irregular. In a more recent
cranial metastasis which had not been irradiated before, there was greater uptake, and apparently more uniformly distributed.

Dobyns, Skanse & Maloff (1949) publish incidentally an autoradiograph from a case of toxic nodular goitre, which shows a rim of nodules having greater activity than normal thyroid tissue. In a later paper, Dobyns (1951) refers to autoradiographs as part of the routine pathological investigation of surgical thyroid specimens, and notes that among nodules in the thyroid, non-functioning ones are the most likely to be malignant.

Crole (1951) presents autoradiographs showing the non-uniformity of uptake of $^{131}I$, in support of his case for using divided doses in treatment of carcinoma of the thyroid by this isotope. He emphasises the rareness of cases which can best be treated by radio-iodine, not only because of non-uniform uptake but also because a large proportion of functioning carcinomas are operable.

In Britain, Doniach & Pelc (1949) at Hammersmith Hospital have done considerable work on radio-iodine concentration in rat thyroids. They also mention autoradiography of human thyroids, and have obtained autoradiographs using as small a dose as 20 μc. administered by mouth 24-48 hours before thyroidectomy. Taylor (1952) refers to autoradiographs made routinely on surgical specimens at Hammersmith Hospital, and
selected 6 cases of non-toxic goitre for a study of the relationship between follicle size and activity. The "contact" method was used, with ordinary X-ray film. He found that the active follicles had a smaller mean diameter in each case than the inactive follicles, and that the distribution of activity, though always non-uniform, was more irregular in the nodules than in surrounding normal thyroid tissue.

Pochin, Myant, Hilton, Honour & Corbett (1952) in a paper on radio-iodine treatment of carcinoma of the thyroid, mention the use of autoradiography in the measurement of radioactivity. Paterson, Warrington & Gilbert (1952) include autoradiography as part of the routine examination of biopsy specimens of thyroid cancer.

In Germany, Philipp (1950) publishes an autoradiograph from human thyroid tissue in a review article, but does not refer to any extensive work on the subject.
CHAPTER IV.

CLINICAL APPLICATIONS OF AUTORADIOGRAPHY WITH OTHER RADIOACTIVE ISOTOPES.

Autoradiographic work with other isotopes is far less extensive, as there is no case of selective concentration of an element in a tissue comparable with that of iodine in the thyroid gland. Therapeutic applications of other isotopes are rare, and diagnostic dosages can rarely be increased sufficiently to give autoradiographs from tissue removed at operation.

The early work of Kahn (1930) on RaE (bismuth) in carcinoma of the cervix, and of Gettler & Norris (1933) in a case of radium poisoning, have already been mentioned.

Hoecker & Roofe (1951) made autoradiographic studies of the distribution of Radium in human bone, in two cases of death from osteogenic sarcoma. Ingestion of the radium had occurred during the painting of watch dials many years previously. They obtained \( \alpha \)-particle tracks in the photographic emulsion, and were able to calculate the radiation dose which had been received by the bones. Spiers (1953) has made use of Hoecker & Roofe's autoradiographs to compare quantitatively with his calculations of \( \alpha \)-radiation dose to the soft tissue elements of bone. From these calculations he obtained figures for the permissible total burden of radium in the
body, both as regards radiation damage to the soft
tissues and, with less certainty, as regards
carcinogenesis.

Experimental work has been done on human bone
tumours, by administering radioactive bone-seeking
elements before amputation of a limb, in the hope of
finding an isotope which would be concentrated enough
in a tumour to have therapeutic value.

Treadwell, Low-Beer, Friedell & Lawrence (1942)
administered Sr$^{89}$ in 6 cases of osteogenic sarcoma, before
amputation or biopsy, and found in 4 cases an uptake in
tumour of 2-4 times the uptake in skin, which was the next
highest of the tissues they studied. In an autoradiogram
reproduced in this paper, the uptake is very marked
in the tumour and at the epiphyseal line (the patient was
a boy of 13), but in the cancellous bone it was no higher
than in skin. Following this work, some therapeutic
trials were made, but have been given up because of
insufficiently selective uptake, and the serious risk of
carcinogenesis in other bones.

An autoradiograph from Ga$^{72}$ in an osteogenic
sarcoma is reproduced in the review by Doniach et al.,
(1953) which shows uptake correlated in the main with
regions of greatest opacity to X-rays.

Steinberg & Selverstone (1950) have made auto-
radiographs with P$^{32}$ from 18 cases of primary and
secondary brain tumours. Activity ranging from 0.5 to 4 mc was administered 12-72 hours before operation. They made first gross autoradiographs using unfixed frozen slices of the tumour several millimetres thick, and then picked out areas of interest for autoradiographs of sections 20μ thick. They used X-ray film for both types of autoradiography and were not concerned with obtaining high resolution. They compared the autoradiography with adjacent 8μ sections fixed and stained by standard techniques, and found correlation between areas of active growth and high radioactivity. Such areas contained several times the radioactivity of unaffected brain tissue in those cases where the latter was available for autoradiography. Their photometric technique has been mentioned already. The differential absorption ratios are not high enough to give hope of therapeutic application of P32 for such tumours, but these authors hoped to obtain information of value in prognosis.

Various radioactive colloidal particles have been used in therapeutic experiments, and Müller & Rossier (1947) injected Zn63 as a colloidal solution intravenously in a case with pulmonary metastases. Autoradiographs were made from tissue removed at autopsy, and showed that radioactivity had remained localised in the lungs. Practically none escaped in the urine.
CHAPTER V.
THE RADIATION DOSE IN RADIO-ISOTOPE THERAPY

If a radioactive isotope is uniformly distributed throughout a volume of tissue of uniform density, the energy liberated in the forms of α-, β-, and γ-radiation, per unit volume, can be easily calculated from a knowledge of the concentration of the isotope, its rate of decay, and the mode of disintegration and the energy of the radiation emitted. For a β-emitter, the rate of liberation of energy may be written

$$\frac{dQ}{dt} = \frac{dN}{dt} (\bar{E}_\beta + \sum E_\gamma)$$

where $\bar{E}_\beta$ is the mean β-particle energy, and $\sum E_\gamma$ is read as the sum of the separate γ-ray energies each multiplied by the proportion of the disintegrations at which it is emitted. For a positron-emitter the extra term $2mc^2$ must be added within the brackets to represent the annihilation radiation.

The physical constants are sufficiently well known (e.g. the table of Hollander, Perlman & Seaborg, 1953) for isotopes of therapeutic interest at the present time. The concentration of the isotope may or may not be possible to assess accurately in a particular case.

The energy absorbed is equal to the energy emitted (except at the periphery) in the case when the linear dimensions of the active region are much larger than the
range of the radiation emitted. This is usually true for α-emitters, and provides a useful approximation for β-emitters distributed in most human organs or tumours. In the case of γ-radiation, only a small proportion of the energy is usually absorbed within the active region, and this proportion may be calculated from the appropriate absorption coefficient (which is approximately 0.03 per cm. for X- and Y-radiation from 0.06 MeV to 2.0 MeV, in tissue of unit density). Often it is more convenient to make use of a directly determined value for the dose rate in roentgens per millicurie-hour at a certain distance, because the emitted γ-ray spectrum may be complex.

In the case of β-radiation, the biological effects are similar or identical to those of γ-radiation below the threshold for photonuclear effects, since both produce their effects by ionisation by fast-moving electrons. The dose is therefore conveniently measured in a unit proportional to absorbed energy, which is almost exactly proportional to the number of ion-pairs produced. The roentgen-equivalent-physical (rep), 93 ergs per gram in water, corresponds most closely to the roentgen of X- or γ-radiation. A new unit, the "rad", was defined at the International Congress of Radiological Units, 1953, as a unit of absorbed dose equal to 100 ergs per gram. It is fundamentally more satisfactory, as its definition does not depend on any
assumptions of equivalence between radiations of different kinds*.

Dosage in a uniformly active sphere.

A mathematically convenient law of absorption of radiation is an exponential one; that is to say, that the dose rate, or the number of particles, as the case may be, at a distance $x$ from a point source embedded in a homogeneous medium, is proportional to the quantity

$$f(x) = \frac{1}{x} e^{-\mu x}$$

This is a good approximation for the absorption of $\gamma$-radiation. For the $\beta$-ray dose rate due to radioactive isotopes, emitting a continuous spectrum of $\beta$-rays, it is a useful first approximation. For $\alpha$-particles it bears no relation to the truth.

Calculation of dose rates for distributed sources can in principle be made by integrating this "point source function". For the simple case of a uniformly active sphere this integration has been carried out by a number of authors, either precisely for the general case or with appropriate simplifications for the cases when the absorption coefficient $\mu$ is either much larger or much smaller than the reciprocal of the radius.

* The "rep" is used in this thesis, because many of the calculations were done before the definition of the "rad" was accepted.
The $\gamma$-ray case ($\mu < \frac{1}{R}$) was thoroughly dealt with by Marinelli, Quimby & Hine (1948a) and Marinelli (1949), and they also extended the calculations to cover cylindrical distributions of activity. The general case ($\mu$ and $\frac{1}{R}$ are of the same order of magnitude) has been treated by Oddie (1951), by Rossi & Ellis (1952) and by Minder & Schindler (1952). These authors plot curves of dose rate as a function of the distance from the centre of the sphere, both inside and outside the sphere, using $\frac{1}{\mu}$ as the unit of distance. All are concerned mainly with the $\beta$-ray dose in tissue containing $^{131}\text{I}$. Oddie gives full details of his mathematics, and makes use of a tabulated integral. For his numerical results, however, he uses Siri's value for the absorption coefficient, $\mu = 43.5$, which is probably too high and is certainly not known to this accuracy. Minder & Schindler also use a value of approximately 40. Rossi & Ellis do not give their calculations, and plot a curve for the "average dose" for a sphere, which is not fully explained but seems to be inaccurate at any rate for small spheres. For the absorption coefficient they use two values corresponding to the two modes of $\beta$-emission of $^{131}\text{I}$, in their correct proportions, obtaining the actual values by interpolation from measured values for three other isotopes. They also extend their results for a sphere to a pear-shaped
object corresponding to one lobe of a chick's thyroid, approximated by segments of spheres.

The curves published for spheres in these papers, and indeed a simple consideration of the source of the radiation reaching any point, show that the dose at the periphery of a sphere is approximately half of that at the centre, whether the radius is greater or less than $1/\mu$. The biggest difference is actually for the case $\mu R = 2$, when the ratio of surface dose to central dose is 0.44 (Minder & Schindler). For the $\gamma$-ray case, where $\mu$ is small, this fact has been fully appreciated in dosimetry, and was well-known when the radium implantation systems of Patterson & Parker (1938) were worked out. For the $\beta$-ray case, however, the point is apt to be overlooked when the dimensions of the mass to be irradiated are much larger than $1/\mu$, for instance in a carcinoma of the thyroid treated by $\text{I}^{131}$, or in thyroid ablation by $\text{I}^{131}$. Calculation, on the basis of uniform distribution of the isotope, indicates practically uniform dosage over all except a "rind" of thickness about $1/\mu$. The mean dose may well be very little below the central dose, but this does not alter the fact that for a finite thickness, of the order of 0.15 mm. with $\text{I}^{131}$, the dose has little more than half this value. This thickness corresponds to many cells, and even to whole thyroid follicles.
Absorption of Beta-Rays in Tissue.

Precise measurements of the absorption of $\beta$-rays are not easy. The most important source of discrepancy between different measurements is that the geometrical arrangement of source, absorber, and detector, greatly affects the results obtained. For mathematical convenience, most workers have reported their results in terms of exponential absorption coefficients, and the exponential law seems to be a fair approximation under a wide variety of conditions. But the values of $\mu$ quoted by different authors for the same isotope vary over wide ranges, and the empirical relations found for the dependence of $\mu$ upon maximum $\beta$-ray energy also vary (Libby, 1947; Siri, 1949; Rossi & Ellis, 1950, 1952; Minder & Schindler, 1952; Sommermeyer, 1952). Of these, the results of Rossi & Ellis are probably the most appropriate to problems of internal dosimetry. Their collaborator, Bailey, (whose work does not seem to have been published) apparently measured dose rates, rather than the number of $\beta$-particles, by means of an ionisation chamber with a thin flat air-space parallel to the source. From these measurements they calculated $\mu$ for the "point source function". They were aware of the work of Loevinger (1950), who found that the exponential law was not the best approximation to the absorption of $\beta$-rays, but they showed that an exponential absorption co-efficient could be chosen such that the error in dose
was small except at the greater distances from the source, where the dose itself was small compared with that near the source. The geometry in these experiments was well defined, the source being distributed uniformly in a thick slab of the same material as they used for absorption measurements, and the relation between the "point-source function" \(\frac{1}{x^a} e^{-\mu x}\) and the dose rate at different distances from their thick slab, was derived by integration. They quote values of \(\mu\), measured by this method, for several isotopes whose \(\beta\)-spectra are simple. Plotting the values of \(\frac{1}{\mu}\) against maximum \(\beta\)-energy, they obtained a curve, and interpolated to find values of \(\mu\) for \(^{131}\)I, which they treated as having two separate \(\beta\)-spectra with maximum energies 0.6 MeV (85%) and 0.2 MeV (15%) respectively. The corresponding values for \(\mu\) were 18 and 60 cm\(^2\)gm\(^{-1}\). At 0.3 mm. distance, the ratio of the two components is \(\frac{85}{15} e^{1.3} = 20\), and beyond that distance the softer component may be neglected.

Loevinger (1950, 1954) has made careful measurements of \(\beta\)-ray dose by means of an extrapolation chamber, using a thin plane source, with a number of different isotopes. From these measurements, he has deduced an empirical point-source function, which mathematically is in two parts, in terms of one parameter \(\nu\), the "scatter attenuation coefficient" depending on the
maximum $\beta$-energy only. A second parameter corresponding to the maximum range of the $\beta$-particles enters as a correcting term, but this is an almost constant multiple of $\frac{1}{\nu}$. Loevinger's functions, without the correcting term, reduce to:

$$f_1(x) = \frac{1}{(vx)^2} \quad (0 < vx < 1)$$

$$f_2(x) = \frac{1}{vx} e^{-vx} \quad (vx > 1)$$

The value of $\nu$ for the two components of the $^{131}$I $\beta$-spectrum are 35 and 180 cm$^{-1}$. Obviously these cannot be compared directly with values of $\mu$ in a different point source function. In his second paper Loevinger gives graphs comparing the two types of absorption law, and the effect of his correction for maximum range. For maximum ranges he used the ranges measured by Katz & Penfold (1952).

Except for unpublished calculations by Loevinger himself, his functions have not, as far as I know, been used for actual dosage calculations. For some problems at least, the mathematics would be manageable, but it is not worth carrying out at the present time, because of a postscript to his latest paper (Loevinger, 1954) from which it appears that the function $f_1$ will have to be modified for isotopes with maximum $\beta$-ray energy below 1.5 MeV.
Clark, Brar & Marinelli (1954) have made measurements in free air using an ionization chamber defined by very fine wire grids, with the isotopes P$^{32}$, Y$^{91}$, Tl$^{204}$ and RaE. Expressing the absorber thickness in milligrams per square centimeter, for comparison with solid absorbers, their results definitely disagree with the exponential law, and are in better agreement with Loevinger's results. They confirm the form of the point source function suggested by Loevinger for short distances, in the case of the harder β-rays, but there are marked discrepancies for softer β-rays. At the longer distances the agreement is better.

Dosimetry with I$^{131}$.

The relevant constants are given in Table I. In each case the most reliable value available is given, and, with the exception of the absorption coefficient for β-rays, the constants are known with sufficient accuracy for all clinical applications. The total β-ray dose for the case of uniform distribution and complete absorption within the tissue, is given in Table II, for various possible rates of excretion. The rate of loss of the isotope is assumed to be proportional, in any given case, to the total amount of isotope present: this assumption is certainly valid if there is
TABLE I.
Physical Constants for I\textsuperscript{131}.

**Half-life** 8.05 days (1)

**Disintegration scheme** see opposite

**\( \beta \)-rays**
- Maximum energy 0.608 MeV. (87%); also 0.815 (0.7%), 0.335 (9.3%), 0.250 (2.8%).
- Mean energy \( \langle E_\beta \rangle \) 0.20 MeV.

**Absorption coefficient** 18 cm.\(^{-1}\) for the main component, in tissue of unit density (2)

**Half-value layer** 0.34 mm. (3)

**Maximum range** 1.8 mm. (4)

**Dose rate** (uniform distribution in tissue)
- 7.6 rep \( \text{per minute per millicurie per gram.} \) (5)
- 7.1 rad \( \text{per minute per millicurie per gram.} \) (5)

**Total \( \beta \)-ray dose for complete physical decay**
- 127 rep \( \text{per micromicrocurie destroyed (\( \mu \text{c}\)) per gram.} \) (5)
- 118 rad \( \text{per micromicrocurie destroyed (\( \mu \text{c}\)) per gram.} \) (5)

**\( \gamma \)-rays**
- Quantum energies: 0.364 MeV. (80%); also 0.080, 0.284, 0.637, 0.722.

**Absorption coefficient in homogeneous medium (tissue)** 0.03 per cm. (6)

**Half-value layer in tissue** 23 cm. (5).

**Dose rate at 1 cm. from a point source of 1 mc.** (\( K_Y \)) 2.25 roentgens per hour. (7)

**Dose rates in tissue** are given in Diagram 1.

**References**
1. Sinclair & Holloway (1951); Lockett & Thomas (1953).
2. Rossi & Ellis (1950).
3. Calculated for combined absorption coefficients, for the first half-value layer.
5. Calculated from preceding data.

All other values from Hollander, Perlman & Seaborg (1953), who give references to the original papers.
**TABLE II.**

*Effect of Excretion on Radiation Dose from $^{131}$I.*

<table>
<thead>
<tr>
<th>Time to excrete half the isotope (days)</th>
<th>Effective half-life (excretion + decay) (days)</th>
<th>Dose per μCū per gram (rep)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>1.6</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>2.2</td>
<td>35</td>
</tr>
<tr>
<td>5</td>
<td>3.1</td>
<td>49</td>
</tr>
<tr>
<td>10</td>
<td>4.4</td>
<td>70</td>
</tr>
<tr>
<td>20</td>
<td>5.7</td>
<td>91</td>
</tr>
<tr>
<td>∞</td>
<td>8.0</td>
<td>127</td>
</tr>
</tbody>
</table>
Ratio of $\gamma$-ray to $\beta$-ray dose in uniformly active spheres. $^{131}I$: $E_\beta = 0.20$ MeV, $K_\gamma = 2.25$ r/mC-hr at 1 cm, $\mu_\gamma = 0.03$. The numbers on the curves indicate the distance from the centre of the sphere expressed as a fraction of the radius, and the percentage of the total volume which lies within that distance from the centre.
free mixing between $^{131}\text{I}$ and $^{127}\text{I}$ in the same chemical state and if the overall rate of iodine metabolism is constant; and it may be valid without these conditions being satisfied.

$^{131}\text{I}$ emits both $\beta$- and $\gamma$-rays, but inside an active region of moderate size the $\gamma$-ray dose is only a small fraction of the $\beta$-ray dose. The value of the $\gamma$-ray dose expressed as roentgens per $\beta$-ray rep, for spheres large compared with the $\beta$-ray range, is shown in Diagram 1. These curves have been calculated from the exact formula of Oddie (1951), although in fact the approximation used by Marinelli (1949) is quite accurate enough for practical purposes. Seen in this form, it is clear that in the case of uniform distribution of radioactive activity, the $\gamma$-ray contribution to the dose is negligible for therapeutic purposes until a very large size of tumour is reached. In practice, however, it is well known that the distribution of radio-iodine is usually very irregular in the thyroid, particularly in toxic goitre and in functional carcinoma of the thyroid. Autoradiographic studies with human and animal tissue show very intense foci of activity separated by relatively large areas with little or no activity. Examples of this in human tissue are shown in the published work of, among others, Hamilton (1942), Leblond (1943), Marinelli, Foote, Hill & Hocker (1947), Frantz, Quimby & Evans (1948),
Fitzgerald & Foote (1949), Philipp (1950), Rawson & Trunnell (1951), Phillips (1954); and in autoradiographs reproduced in this thesis. Such irregular distribution makes much more difference to the $\beta$-ray dose than to the $\gamma$-ray dose.

Although the exact rate at which $\beta$-ray dose falls off with distance is in some doubt, it is clear that the $\beta$-ray dose would be negligible in many of the inactive areas, however high the activity of the foci of uptake. Regions of low activity would be unimportant in treatment of carcinoma of the thyroid if they consisted entirely of necrotic or non-malignant tissue, but evidence is presented later (Cases 1, 2, Chapter VIII) that this is not always the case. The possible importance of the $\gamma$-ray dose at these situations has been mentioned by Marinelli et al. (1947) and by Rawson & Trunnell (1951), but the very large amount of $^{131}\text{I}$ necessary to give a curative $\gamma$-ray dose would probably not be tolerated because of damage to other organs. In fact it may be impossible to obtain such a dose, because of the rapid excretion of radio-iodine following administration of a large enough amount to cause severe radiation damage to the thyroid in the first day or two (Feller, Chaikoff, Taurog & Jones, 1949). The measured values of $\gamma$-ray doses in three actual tumours, reported in this thesis, give an indication of the amounts of $^{131}\text{I}$ which might be required,
and these are above accepted maxima in all the three cases, and quite out of the question in two of them.

It is not universally accepted that a minimum tumour dose high enough to kill cells is required for a cure by radiotherapy. Jolles (1953) considers a certain calculated departure from uniform dosage to be beneficial. If this view should prove to be correct (which I personally believe to be improbable), it might have a bearing on radio-iodine therapy, in which non-uniformity of dosage seems inevitable.

It is worth mentioning that the amount of iodine in a thyroid gland, say 15 mgm. in a human gland weighing 25 gm., is not sufficient to increase appreciably the absorption of β- or γ-rays.

Selective Concentration of Radio-Active Isotopes.

The possibility of using a radioactive isotope, administered internally, for therapeutic purposes, depends on the concentration of the isotope in the tissue to be irradiated, relative to its concentration in other organs and in the body as a whole. The doses received depend also on the rates of excretion and of radioactive decay, and retention of the isotope by the tissue in question may be as important a factor as the initial concentration. To a lesser extent, the different rates of accumulation
of the isotope by different tissues influences the doses received.

Kenney, Marinelli & Woodard (1941) introduced the concept of "differential absorption ratio" (D.A.R.). Taken alone this is an over-simplification, but used with full awareness of the complexity of the actual situation, it makes a convenient starting point for an attack on the problem. Marinelli, Quimby & Hine (1948b) explain the position very clearly, after defining "differential absorption ratio", in the following words:

"For any tissue, this is the ratio of concentration of an isotope in that tissue to the average concentration in the body (neglecting excretion). ...... In general, single tissues and the whole body have different rates and modes of elimination; hence the tissue D.A.R. will vary with time. The question arises as to the time at which D.A.R.'s have significance in determination of tissue dosage. Evidently the D.A.R. will be too low when taken too soon after the isotope administration because the concentrations have not yet been stabilized. Likewise adoption of the D.A.R. after several half lives will have little or no significance because most of the isotope has disintegrated. One useful index, whenever the isotope reaches the body tissues through the circulatory system, could be
the stabilization of the plasma concentration. No general rule, however, can be given, and good judgment is of paramount importance."

The effect of different rates of excretion on the dose can be calculated, if the rates are known and are approximately exponential, by the simple rule that the total dose to a tissue is inversely proportional to the actual half-life of the tissue radioactivity. If the physical decay of the isotope is represented by

\[ N = N_0 e^{-\lambda_1 t}, \quad \text{where} \quad \lambda_1 = \frac{\log_e 2}{\text{half-life}}, \]

and if the rate of excretion of the element from the tissue is represented by

\[ \frac{dq}{dt} = -\lambda_2 q \quad (\text{where } q \text{ is the total amount present}), \]

then the activity, \( A \), of the tissue at any time, \( t \), after (say) the highest level is reached, is given by

\[ A = A_0 e^{-\lambda t} \]

In this equation, the value of \( \lambda \) is given by

\[ \lambda = \lambda_1 + \lambda_2 = \frac{\log_e 2}{T} \]

where \( T \) is the time at which the activity is halved. The
total radiation dose, D, is given by

\[ D = \int_0^\infty K A_0 g \, dt \]

\[ = \frac{K A_0 g}{\lambda} = 0.693 K A_0 g T \]

where \( K \) is a constant* depending on the physical properties of the isotope, and \( g \) is a geometrical constant depending upon the size and shape of the active tissue, relative to the absorption coefficient for the particular radiation.

For \( \beta \)-emitters in relatively large organs, \( g \) is approximately unity, but falls off to \( \frac{1}{2} \) at the periphery as discussed above. \( A_0 \) is equal to the activity administered (millicuries per Kg. or microcuries per gram) multiplied by the D.A.R. The time, \( T \), in which the activity in the tissue falls to half its original value can be determined in favourable cases with the help of an external counter or ionization chamber; or, for the determination of whole-body irradiation, by measuring urinary and other excretion. \( T \) can never be greater than the half-life of physical decay of the isotope, and is usually less, due to excretion. But in the case of radio-iodine, the value of \( T \) for the thyroid, or carcinoma of thyroid, may be between 4 days and 8 days, whereas for the rest of the body it is in the region of 1 day. The ratio of doses between thyroid and the rest of the body is therefore multiplied by a factor between 4 and 8.

* The derivation of this constant is perfectly straightforward, and is given in most works on radioisotope dosimetry, for example: Low-Beer (1950) p.274, equation (3).
This discussion has so far taken no account of the dose delivered during the period of increasing concentration in the tissue. No general simple treatment of the problem is possible, but the work of Teorell (1937) on the distribution of administered drugs is relevant. In the particular case of $^{131}$I in the treatment of thyroid disease, the time-course of uptake has been thoroughly studied (Pochin, 1950). The tissue and the whole-body dose during this period can be calculated from serial measurements of the activity in tissue, blood and urine (Paterson, Warrington & Gilbert, 1952). In that instance, the greater part of the whole-body irradiation occurs during the phase of uptake into the thyroid, and these authors estimate, from measurements with a tracer dose, the maximum safe activity to be administered, on the basis of a whole-body irradiation of 70 rep during this phase. Excretion of the radio-iodine which is not taken up by thyroid-like tissue is very rapid.

In cases where there is not a favourable difference in the rates of excretion by the tissue to be irradiated and by the body as a whole, the D.A.R. required for effective therapy is very high. If a whole-body dose of 50 rep is permissible, then to achieve a tissue dose of 2,000 rep, the D.A.R. must be 40, if the two excretion rates are the same. Such a value has never been found except for the concentration of iodine
in the thyroid, and the search for elements so concentrated has been disappointing. Incorporation of radioactive isotopes in organic compounds which are concentrated in growing tumours is a field to be explored (Mitchell, 1951). An attempt to make use of radio-iodine incorporated in tetra-iodo-phenolphthalein in treatment of carcinoma of the gall-bladder is reported by Copher, Wallingford, Scott, Zedler, Hayward & Moore (1952), but the dose achieved at the gall-bladder wall was barely twice that to the thyroid and to the bone marrow, and the dose to the tumour was much less. Maxwell (1954) has succeeded in labelling the compound 2-methyl-1:4-naphthohydroquinone (a vitamin K substitute) with radio-bromine and with radiocarbon and finds that it is still preferentially concentrated to a small degree in experimental rat tumours (Walker carcinoma 256). The parent compound is known to be concentrated both in the Walker rat carcinoma and in some human tumours (Mitchell, 1954). In this case, the compound carrying the radioactive isotope is also a radio-sensitizer (Mitchell & Simon-Reuss, 1952), and a selective concentration in tumour is therefore doubly favourable, both increasing the radiation dose and increasing the radiosensitivity relative to normal tissue. Thus a much smaller D.A.R. might allow effective therapy.

As well as undesirable whole body irradiation,
the danger of harmful doses to other organs has to be considered. Some organs are liable to high concentrations of the isotope, such as those concerned with metabolism or excretion of the particular element or compound, or glands producing a concentrated secretion. In this category come kidney and bladder, liver and gall-bladder, pancreas, salivary glands (for radio-iodine), bone (for radio-phosphorus and alkaline earth elements) and lung (radioactive colloids and radio-iodine); though irradiation of bladder, gall-bladder, and salivary glands is probably unimportant in practice. Other tissues have high radio-sensitivity, particularly haemopoietic and lymphoid tissues; and effects on gametogenesis (including mutations) must be considered if there is the possibility of future reproductive function. The risk of long-delayed carcinogenesis has to be remembered, particularly in applications of the longer-lived isotopes.

A list of elements which have been considered for radio-isotope therapy on the grounds of selective concentration in malignant tissue, is reviewed by Mitchell (1951). The list comprises P³² and I¹³¹ as used in practical treatment, I¹³⁰ as having been used in clinical trials, and, in the category of "preliminary studies", the isotopes: Ca⁴⁵, Sr⁸⁹, Ga⁷², As⁷⁶, At²¹¹, and ThC (Bi). He also lists a number of insoluble radioactive colloids, which are taken up by the
reticulo-endothelial system, and which also have in cases been used for "injection implants".

The main therapeutic application of $^{32}P$ is in polycythaemia rubra vera, and its effectiveness is due to a very moderate degree of concentration in the pathological marrow, combined with high radiosensitivity of the tissue. With regard to selective concentration of radio-phosphorus, differential absorption ratios between 1 and 10 have been reported in many malignant tissues, but no higher. In view of the D.A.R.'s for normal red bone marrow (1.5) and for normal lymph nodes (2.5) (Mitchell, 1951), tumour lethal doses cannot be expected with this element without a very favourable time factor for retention, of which there is no evidence.

For $^{131}I$, the D.A.R. calculated from data in the literature is often in the region of 1,000 for normal thyroid tissue, and may be higher in thyrotoxicosis. The concentration in carcinoma of the thyroid is always lower than in normal thyroid tissue, but quantitative information is scanty, and average values may be misleading because of the great variation with time, and from point to point in a tumour. Quantitative autoradiography needs to be developed in a practical form to gain detailed information on tumour dosage. One of the cases reported later (Case 2, Chapter VIII) is of interest in this connection. The D.A.R. measured on the primary tumour was less than 3, and yet when
thyroidectomy had been performed distant metastases showed remarkable retrogression (which is still maintained at the time of writing) under radio-iodine therapy. The retention time factor was favourable in that case.

Astatine, the long-missing higher homologue of the halogens, is now known, as the α-emitting isotope At\(^{211}\), and is taken up by both normal and hyperactive thyroid glands to about one third of the D.A.R. of radio-iodine (data from Hamilton, 1942). Its short half-life (7.5 hours) would make its use technically difficult, and for treatment of thyroid disease the very short range of the radiation would in most cases be a disadvantage.

The bone-seeking isotopes Ca\(^{45}\) and Sr\(^{89}\) are highly concentrated in the growing regions of normal bones and in osteogenic sarcoma. Although the D.A.R. is high, there is not sufficient difference between the tumour and normal bone, and with the relatively long half-lives of these isotopes (152 days and 53 days respectively) there is a danger of carcinogenesis in the normal bone. Radio-gallium (Ga\(^{72}\)) has a half-life of 14 hours, and has been shown to concentrate in a similar way to strontium and calcium (Dudley, Imirie & Istock, 1950).

The distribution of bismuth has been studied for
a much longer time, because it has the two naturally occurring radioactive isotopes RaE (half-life 5 days) and ThC (half-life 1 hour), as well as shorter-lived isotopes. The early autoradiographic work of Lacassagne and co-workers, of Hevesy & Wagner, and of Kahn, has already been mentioned. There was a marked difference of opinion as to whether the bismuth isotopes were or were not concentrated in tumours, perhaps partly due to the use of different types of tumour by the different workers. Hevesy & Wagner (1930) found the ratio of activities in mouse tumour and in muscle to range from 5 to 55, but, from the figures they quote, a D.A.R. can be calculated, and averages only 0.5. Presumably much of the activity was excreted, or remained in kidney and spleen. Kahn (1930) found the activity in human carcinoma of the cervix uteri to be 10 times that of uterine muscle, but again my calculation of the D.A.R. from his data gives a value of approximately unity. Nevertheless, ThC has been considered worth clinical trial in otherwise hopeless cases. Deucher & Leigh-Smith (1943) report its use in 5 cases, in one of which cutaneous metastases from carcinoma of the bronchus disappeared, while the only side effect was a slight lymphopenia. Mitchell (1951) has also given ThC, and observed an effect on tumour. The high relative biological efficiency of the α-particles, and the fact
that the whole radiation effect is produced within the range of a few microns, probably has a bearing on these observations; and the D.A.R. and retention time may have been much more favourable in these cases than in the animal experiments quoted.

The fact that many brain tumours take up substances from the blood stream much faster than normal brain tissue, and in some cases retain them in higher concentration, has not so far had much practical application in radio-isotope therapy, because the tumour does not have a high D.A.R. (relative to the body as a whole). It is, however, the basis for an ingenious experimental treatment reported by Farr, Sweet, Robertson, Foster, Locksley, Sutherland, Mendelsohn & Stickley (1954), who make use of a nuclear reaction within the tumour. The less common stable isotope of boron, B\textsuperscript{10}, has a very large cross-section for slow neutrons, with which it undergoes a reaction as follows:

\[ _{0}^{10}B + _{0}^{1}n \rightarrow _{3}^{7}Li + _{2}^{4}He \]

The resulting \( \alpha \)-particles have a range of 9\( \mu \) in tissue, and the even more heavily ionising recoil nucleus, Li\textsuperscript{7}, also adds to the ionisation, which may take place within a single cell. The principle of the method, which they have used in the treatment of a few cases of glioblastoma multiforme, is to obtain borax made from boron
specially enriched to contain 96% of B\textsuperscript{10}, instead of the natural mixture of B\textsuperscript{11} (80%) and B\textsuperscript{10} (20%), and they inject 20 gm. intravenously. The tumour content of borax follows closely the plasma concentration, while normal brain lags many minutes behind. They therefore irradiate with neutrons as soon as possible after injection of the borax, and owing to the much higher cross-section of the B\textsuperscript{10} than any other element present, the tumour is preferentially irradiated with α-particles liberated in situ.
CHAPTER VI.

SCOPE OF THE PRESENT STUDIES

(i) Material.

The specimens of human tissue which have been available for autoradiographic study over a period of two and a half years, are listed in Table III. They include three operation specimens of carcinoma of the thyroid, containing $^{131}$I, one specimen of nodular colloid goitre, and one of secondary carcinoma involving the thyroid, also containing $^{131}$I; biopsy specimens and one operation specimen containing $^{32}$P from six cases with a variety of malignant conditions, and one operation specimen containing radioactive colloidal gold ($^{198}$Au) from a case of carcinoma of the bronchus. In the cases from the Radiotherapeutic Centre, Addenbrooke’s Hospital, measurements of the total activity of the specimen, or of a weighed piece of the specimen adjacent to that used for autoradiographic study, were made in collaboration with the Hospital Physicists. In addition to information of general interest on the macroscopic and microscopic distribution of the radioactivity, it was hoped to obtain information as to the prospect of successful therapeutic use of the isotope administered in each case.

Cases 1 and 2 were known cases of carcinoma of the thyroid with known metastases which have subsequently
TABLE III.

List of specimens received. All cases, except where otherwise stated, were treated at the Radiotherapeutic Centre, Addenbrooke’s Hospital, Cambridge, under Professor J.S. Mitchell.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>Activity administered</th>
<th>Specimens</th>
<th>Specific activity (µc/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Carcinoma of thyroid (previously irradiated)</td>
<td>25 mc.</td>
<td>(a) Residual primary tumour</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(b) Metastatic lymph nodes</td>
<td>8.5</td>
</tr>
<tr>
<td>2.</td>
<td>Carcinoma of thyroid</td>
<td>25 mc.</td>
<td>Primary tumour and residual thyroid</td>
<td>1.0 to 27</td>
</tr>
<tr>
<td>3.</td>
<td>Carcinoma of thyroid</td>
<td>5.9 mc.</td>
<td>(a) Right lobe of thyroid</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(b) Primary tumour with residual left lobe</td>
<td>&lt; 0.3 to 70</td>
</tr>
<tr>
<td>4.</td>
<td>Nodular colloid goitre</td>
<td>0.6 mc.</td>
<td>operation</td>
<td>5.7</td>
</tr>
<tr>
<td>5.</td>
<td>Carcinoma of oesophagus infiltrating thyroid</td>
<td>25 mc.</td>
<td>biopsy</td>
<td>91</td>
</tr>
</tbody>
</table>
### B - Specimens with Phosphorus - 32.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>Activity administered</th>
<th>Specimens</th>
<th>Specific activity $(\mu\text{c/gm})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.</td>
<td>Malignant melanoma, recurrence in axillary lymph nodes</td>
<td>9.4 mc.</td>
<td>Mass of lymph nodes and fat</td>
<td>0.1 - 0.6</td>
</tr>
<tr>
<td>7.</td>
<td>? Eosinophil granuloma</td>
<td>5 mc.</td>
<td>Biopsy of lymph node</td>
<td>0.42</td>
</tr>
<tr>
<td>8.</td>
<td>Malignant haemangio-endothelioma</td>
<td>10 mc.</td>
<td>(a) cervical lymph node</td>
<td>0.18</td>
</tr>
<tr>
<td>9.</td>
<td>Carcinoma of ovary, recurrence in abdominal wall</td>
<td>7.8 mc.</td>
<td>Biopsy</td>
<td>0.2</td>
</tr>
<tr>
<td>10.</td>
<td>Squamous carcinomatosis of skin and lymph nodes</td>
<td>5 mc.</td>
<td>(a) biopsy - tumour and normal skin</td>
<td>very low</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(b) biopsy - tumour and normal skin</td>
<td>very low</td>
</tr>
<tr>
<td>11.</td>
<td>Mycosis fungoides (from Dr. R. Braams, Utrecht)</td>
<td>2.2 mc.</td>
<td>Biopsy</td>
<td>&lt; 0.2</td>
</tr>
</tbody>
</table>

### C - Specimen with Gold - 198.

| 12.      | Carcinoma of bronchus (from Mr. C. Farrish, Nelson-Langemann Hospital, Papworth). | local infiltration with colloidal gold | Part of tumour removed at operation |
been treated with radioactive iodine. Case 3 had a large carcinoma of the thyroid which appeared by external counting measurements to concentrate iodine, and it was hoped to treat post-operatively with $^{131}$I. Autoradiographs showed, however, that there was little concentration of radio-iodine except in residual normal thyroid tissue.

In these three cases, in addition to other autoradiographic studies, measurements were made by an autoradiographic technique developed for the purpose, of the $\gamma$-ray dose at points within the tumours. By these measurements, the basis of which will be described in the next chapter, it was found that there were regions in the tumours where the $\beta$-ray dose would have been lower than the $\gamma$-ray dose, and that there were malignant cells in such regions. The measured $\gamma$-ray doses agreed approximately with the doses calculated on simplifying assumptions, and were too small to be of curative value.

Of the remaining cases investigated with radioiodine, case 4 was found to be non-malignant and required no more treatment after successful hemithyroidectomy. Case 5 was found to be a squamous carcinoma of the oesophagus infiltrating the thyroid and there was, therefore, no further question of treatment by radio-iodine.

---

* At the suggestion of Professor J.S. Mitchell.
Of the specimens containing $P^{32}$, only one (Case 10) contained sufficient activity to give a satisfactory autoradiograph on a microscopic scale. The operation specimen, from Case 6, gave an autoradiograph on X-ray film which showed activity where there was vascular melanomatous tissue, absence of activity in uninvaded fat, and little activity in necrotic tumour.

In Case 13, the autoradiographic study that was done was merely an item in testing the technique of local infiltration with radioactive colloidal gold. This "injection implant" method is being used occasionally here in treatment of certain accessible tumours, and in some cases of carcinoma of the bronchus which are found at thoracotomy to be inoperable.

(ii) Techniques used.

In the work described in this thesis, four autoradiographic techniques have been used:

(1) "Stripping Film" autoradiographs from microscopical sections.

(2) Electron Track autoradiographs by the "coated autograph" method.

(3) Autoradiographs of slices of large specimens, either embedded in paraffin wax or not, by means of X-ray film ("contact method").
Measurements of \( \gamma \)-rays by means of X-ray film.
The method developed for this measurement will be
described in the next Chapter.

**Stripping Film Autoradiographs.**

The technique was essentially that of Doniach
& Felc (1950).

(i) Fixation.

It was necessary to avoid mercury salts, which
have an adverse effect on the photographic process, and
it was thought advisable to avoid iodine in any form.
The fixative used as a routine was:

- Industrial Spirit .......... 3 parts
- Glacial Acetic Acid .......... 1 part

Occasionally Methanol was used alone. For the third
large specimen of carcinoma of the thyroid (Case 3),
formalin (4% formaldehyde) was used, as this made it
possible to keep the specimen in the fixative for several
days. This choice will be mentioned later in connection
with the \( \gamma \)-ray measurements. In nearly all cases either
an absolute or a relative check was kept upon the amount
of radioactivity extracted by the fixative, and by
subsequent treatment. Extraction of radio-iodine,
though not negligible, was never enough to invalidate
results. Extraction of \( P^{32} \) was sometimes considerable.
(ii) Dehydration and embedding.

These processes were carried out by conventional methods: graded alcohol, xylene or benzene, and 55° paraffin wax.

(iii) Sectioning.

Sections were cut at 5μ, or occasionally at 10μ. They were mounted on slides previously dipped in a solution of gelatine (½%) with chrome alum (0.05%) and dried. Where possible three sections were mounted in a line, to facilitate final checking against artefacts in the autoradiographs.

(iv) Staining.

Wax was removed thoroughly with xylene, followed by graded alcohols, and a wash in water for an hour or more. Some sections were stained by Feulgen's method before autoradiography. More usually, they were stained after autoradiography with Ehrlich's haematoxylin (blued with lithium carbonate), or Carazzi's haematoxylin, and eosin, using longer times than normal at each stage. Other stains have also been tried.

(v) Setting up the autoradiograph.

Diagram 2A shows the arrangement of the Kodak stripping plates for autoradiography. Working with a
Diagram 2A  The arrangement of "stripping film" as supplied by the makers.

Diagram 2B  The arrangement of slide, section, and "stripping film" as set up for autoradiography.

(Not to scale)
Wratten Safelight No.1, the emulsion was cut into pieces approximately 2" x 1", which were stripped off the plate and inverted on to water at 24°C. The water surface was previously cleaned with care. After 3 - 5 minutes, each piece of emulsion was mounted on a slide carrying sections, by raising the slide up to it from underneath and lifting it out of the water, so that the ends wrapped round under the slide. Diagram 2B shows the final position. The slides were then dried in a stream of filtered air at room temperature.

(vi) Autoradiographic exposure.

This was carried out in a light-proof slide box, at 0° - 4°C, for a period from a few hours up to two half-lives (16 days for ¹²³I, 29 days for P³²), according to the activity.

(vii) Photographic processing.

Development was in metol-hydroquinone developer "I.D.19" at 17.5°C ± 0.3°C for 15 minutes, followed by a rinse and fixation, with chrome alum hardener (I.F.9), all at the same temperature. The slide was then washed and dried without heat, and stained if required.

It was usually most convenient to leave the autoradiograph without a coverglass, and examine with the low power of the microscope and with the oil
immersion lens. Some, however, were mounted in "D.P.X." or in glycerine jelly medium.

It was difficult to keep the preparation free from dust throughout this process, as any dust stuck readily to the wet surface of the gelatine. For photometry, and for photomicrography, this was a serious drawback, but for visual examination with the microscope it was not really confusing.

(viii) Controls.

In the majority of cases, enough sections were mounted so that one or two slides could be left for several months, until the activity had decayed to a very low value. These were then put through the whole process, and the absence of autoradiograph confirmed that the images obtained with the original preparations were in fact due to the radioactivity which had been present.
Electron-track autoradiographs.

The technique was adapted from Bourne's summary (1952) of the method of Leblond and his co-workers at McGill University.

The preparatory steps were the same as for the stripping film method. The autoradiograph was set up working with a Wratten Safelight No. 2. A small quantity of NT4 emulsion, a Pasteur pipette, and a fine paint brush, each in a test-tube, were brought to approximately 35°C in a thermostatically controlled bath. Meantime, the slides with their sections were brought to about the same temperature on a level glass plate which had an electric heating element under one end. Then the slides were coated with emulsion, one at a time, 2 - 5 drops of emulsion being spread over the middle third of the slide by the use of the brush and gentle tilting. Each slide was returned to the warm end of the plate for ½ - 1 minute, and then was slid to the cold end. After ½ - 1 hour, the slides were transferred to shelves in a light-proof wooden box inside which the air was kept relatively dry by calcium chloride. At first, considerable trouble was taken to bring the humidity in a sealed vessel to the exact value recommended by the manufacturers of the emulsion, but with doubtful success. The simple method of a box not perfectly airtight with a small area of desiccating agent, was found
to give as good results. Exposure was at 0° - 4°C, for 1 - 14 days in the case of I\(^{131}\), and up to 4 weeks in the case of P\(^{32}\).

Development was for 20 minutes at room temperature in I.D.19 diluted to half concentration. Fixation was for an hour in I.F.9 diluted to half concentration. Both these processes were best done with automatic rocking of the dish.

Staining, if done after autoradiography, was more difficult than with stripping film. A number of stains were tried, but with none were consistently good results obtained. The best was a very dilute toluidine blue left on overnight, and differentiated in 50% alcohol.

This technique has only been used incidentally in my work on human specimens, and merely serves as one control against the remote theoretical possibility that the parallel autoradiographs by means of stripping film were due to an artefact. The appearance of an electron track in emulsion can not be due to anything but the passage of an electron. It cannot be simulated by chemical fogging nor by exposure to light.

Owing to the thickness of the emulsion and the obliquity of the tracks, photomicrographs do not show these autoradiographs well. They are best studied with the oil immersion objective, following the tracks by focussing up and down. One preparation has, however,
Figure 1. Electron-track autoradiographs with $^{131}I$ in normal thyroid tissue (Case 5), unstained.

A. x 120.
B. x 550.
been photographed for figure 1. In the low-power view
the effect is that of an ordinary thyroid autoradiograph,
but at the higher magnification, parts of the individual
tracks are visible.

**Beta-ray Autoradiography Using X-ray Film.**

To find the distribution of radioactivity, on a
macroscopic scale, in large specimens, ordinary X-ray
film ("Ilfex") was used, in its standard black paper
wrapper and envelope. For specimens already embedded
in paraffin wax, the block after cutting sections was
placed face down on the film envelope and weighted with
a few hundred grams. Larger blocks were faced with a
milling machine. Two fresh specimens were also treated
in this way, cut as flat as possible and weighted down.
A sheet of aluminium foil (7 mgm/sq.cm.) was interposed
to avoid wetting the film envelope.

This technique was exceedingly simple, being
carried out in daylight, and the films were placed on
a shelf in the laboratory where exposures could be made
ranging from a few minutes up to many days. With the
most active specimens some care had to be taken to
prevent any one from fogging a neighbouring film. All
Figure 2  Outlines of the specimens shown up by canalization of β-rays along cracks in the wax blocks. Compare fig 5B, after specimens were re-embedded. (Case 1).
Films were marked with calibration spots from a radium source in a manner to be described later. The technique, however, was not suitable for any but the roughest of quantitative work on $\beta$-radiation, for several reasons. Any lack of flatness of the block causing a gap between it and the film would be expected to reduce the autoradiograph appreciably. Filtration by the two layers of paper is difficult to calculate, particularly where it is oblique, and is dependent upon the energy distribution of the $\beta$-rays, as is the photographic action. No satisfactory standard source of I$^{131}$ $\beta$-rays was achieved: a few experiments with I$^{131}$ in gelatine were made, but abandoned because of alterations by evaporation. This was before the publication of Clayton's work (1953). He used microtome sections of hardened gelatine containing Ag$^{131}$, but it is doubtful if the method would be satisfactory for a thicker source than he used. Another factor which discouraged attempts at quantitative estimation of $\beta$-ray intensities by this method, was an effect seen at the edges of many specimens. The edges show up as darker lines on the autoradiograph as is seen in some of the autoradiographs reproduced later, and is very marked in figure 2. This is probably due to canalization of the $\beta$-rays in small cracks in the wax at the edge of the specimen. A similar effect would vitiate results for regions of
the film reached by $\beta$-rays passing obliquely from the paraffin block through two layers of paper to the film. The effect would be greatest at the points of least activity, and in all the work on thyroid tumours, the minimum dose was the main point of interest; there is no doubt that the dose at the most active points can be made large enough to kill all cells.
CHAPTER VII.

GAMMA-RAY DOSE MEASUREMENTS

One of the main purposes of the autoradiographic studies of the three large malignant tumours of the thyroid (Cases 1, 2, 3) was to determine the \( \gamma \)-ray dose from the radio-iodine at points within the tumours, and particularly at points where the uptake, and therefore the \( \beta \)-ray dose, was small or zero. This particular point was of practical importance not in subsequent treatment of these cases, but for the general question of the possibility of treating such cases by radio-iodine alone, without surgery. It was therefore decided to attempt to measure the \( \gamma \)-ray dose alone, without the contribution of any \( \beta \)-rays which might be reaching the regions of lowest activity in these specimens, and which might be absent in other cases. Comparison with calculated values was also easier on this basis. The method employed was changed in details as the work proceeded, to improve its reliability and to reduce the amount of work, but the principles applied in the first case proved satisfactory.

The specimen was first cut into a small number of thick slices. One side of each slice was made flat, taking note of the amount of material lost in the process. Autoradiographs were then taken of the slices, on X-ray film, through an absorber of tissue-like material

The dose at P is the sum of the doses due to the four slices, each at the appropriate distance along XY. These four contributions are shown on the ordinate through P in the lower part of the diagram.

\[
\text{Dose at } P = D_1 + D_2 + D_3 + D_4 + g_{12} + g_{23} + g_{34}.
\]

The small contributions \(g_{12}, g_{23}\) and \(g_{34}\), corresponding to the lost tissue, are calculated according to the thickness lost.
(celluloid or perspex) just thick enough to stop all β-rays, and through a range of thicker absorbers. By measurement of the photographic densities obtained, and calibration of the film by methods which will be described, it was possible to determine the γ-ray dose in roentgens to which the film had been exposed at different points on its surface. Straightforward calculation from the time of beginning and end of the exposure, taking 8.0 days as the half-life of $^{131}I$ (Sinclair & Holloway, 1951; Lockett & Thomas, 1953)* gave the total dose due to the one slice during complete radioactive decay, at these points, starting from any particular time. The time of operation was a convenient fixed time to refer these calculations to.

The dose at a point in the tumour would have been equal to the sum of the doses due to each slice, each at the appropriate distance. The way in which these contributions add up is illustrated in diagram 3. For each slice, graphs were plotted of the dose at increasing distances from the surface of the slice. Each point of the surface of each could have a separate graph, but in practice only one or two easily recognizable points were taken, such as points of minimum or maximum activity. The dose contributions from the different slices at a particular point in the tumour

---

*A value of 8.05 days is in better agreement with the measurements, but the difference turns out to be negligible for the times involved in these experiments.*
could be read off these graphs, with the exception of the contributions from the slices on each side of the cut through the point in question. These two contributions (usually the largest) had to be obtained by extrapolation of the two relevant curves towards zero distance, as illustrated in the diagram. A small addition had also to be made for the tissue lost in facing up the slices.

The details and the validity of the steps of this process will now be discussed.

(1) Preparation of the specimens.

The specimens from Case 1 were sliced, fixed in acetic acid - alcohol mixture, as for ordinary autoradiography, dehydrated, embedded in paraffin wax, and faced on the microtome, sections being kept for histology and microscopical autoradiography. The specimens from Case 2 were found to be too large for the embedding to be done in time to leave sufficient radioactivity for γ-ray measurements. They were therefore taken as far as xylene, and the γ-ray autoradiographs were taken while the slices were allowed to dry, weighted down on the flat celluloid absorber. This was not good histological technique, but no alternative was available which did not risk losing the γ-ray measurements.
(A piece had, of course, been sent for routine pathological examination).

After this, it was decided to treat subsequent specimens differently. Formalin (4% formaldehyde, without saline), seemed to be a fixative in which the specimen could be kept for some days without hardening or shrinking (Baker, 1950). A subsidiary experiment was carried out in which a rat's thyroid, containing radio-iodine, was fixed in formalin and then soaked for 5 days in successive changes of the same solution, the activity extracted by each change being measured. The results are shown in Protocol 1 the total activity extracted by saline and formalin was 21% of the original activity of the thyroid. This amount of extraction was considered to be small enough not to lead to any serious error provided a simple proportional correction was added to the dose measurements. A perspex box was therefore constructed, with a bottom 1.5 mm. thick, in which the specimens could be immersed in formalin, and weighted down, during autoradiographic exposures for the $\gamma$-ray measurements.
Protocol 1  Extraction of Radioactivity by Formalin.

Rat weight 250 gm. Subcutaneous injection $^{131}\text{I}$ 280,000 c.p.m. Killed coal gas after 24 hrs. Thyroid dissected out, halved, each half washed in 0.5 ml saline and fixed as below.

<table>
<thead>
<tr>
<th>Hemithyroid (1) Weight 9 mgm.</th>
<th>Hemithyroid (2) Weight 7.5 mgm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original activity 24,400 c.p.m.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Duration of action</th>
<th>Activity extracted</th>
<th>Fluid</th>
<th>Duration of action</th>
<th>Activity extracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.85% saline</td>
<td>13 min.</td>
<td>741</td>
<td>0.85% saline</td>
<td>13 min.</td>
<td>1725</td>
</tr>
<tr>
<td>4% formaldehyde</td>
<td>1/2 hour</td>
<td>390</td>
<td>acetic acid/alcohol</td>
<td>1/2 hour</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td>1/2 hour</td>
<td>1225</td>
<td></td>
<td>1/2 hour</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>1/2 hour</td>
<td>1117</td>
<td></td>
<td>1/2 hour</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>1 1/2 hours</td>
<td>870</td>
<td></td>
<td>1 1/2 hours</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2 1/2 hours</td>
<td>145</td>
<td>Total fixative</td>
<td>3 hours</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>17 1/2 hours</td>
<td>87</td>
<td>Absolute alcohol</td>
<td>1/2 hour</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3 days</td>
<td>130</td>
<td></td>
<td>1/2 hour</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1 day</td>
<td>40</td>
<td>Xylene</td>
<td>20 min.</td>
<td>0</td>
</tr>
<tr>
<td>Total fixative</td>
<td>5 days</td>
<td>4504</td>
<td></td>
<td>4</td>
<td>1900</td>
</tr>
<tr>
<td>Fluid</td>
<td>Duration of action</td>
<td>Activity extracted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>--------------------</td>
<td>--------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70% alcohol</td>
<td>18 hours</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80% alcohol</td>
<td>3 hours</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>96% alcohol</td>
<td>1½ hours</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>absolute alcohol</td>
<td>4 hours</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>xylene</td>
<td>16½ hours</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: (1) All activities have been reduced to counts per minute on the first day.
(2) Each fluid was 0.5 ml.
(2) Shrinkage of the specimens.

The effect of shrinkage on the γ-ray dose measurements was not thought of until the specimens of Case 1 had been embedded. Such measurements as were still possible (involving density and weight of the slices compared with the original weight) suggested that the shrinkage, if any, had not been serious. With subsequent specimens, a model was made in plaster-of-paris before fixation, using dental moulding compound ("Zelex"). The final shrinkage could then be measured with sufficient accuracy, and a correction applied to the measured γ-ray dose. The effect of shrinking, assumed isotropic, is simply to bring all points within the tumour nearer to each other in the same proportion. The measured dose, correct for the shrunken tumour, has therefore to be reduced in proportion to the inverse square of the change in linear dimensions.

(3) Necessary absorber thickness.

For accuracy of extrapolation it is desirable to make the thinnest absorber used as thin as possible. The thickness required to stop all, or any given proportion, of the β-rays from I\(^{131}\) (maximum energy 0.6 MeV) is not certainly known. Absorption measurements have been discussed in Chapter V; the best value for the maximum
range is probably that of Katz of Penfold (1952), namely 1.8 mm. in tissue of unit density. For cases 1 and 2, I was perhaps too cautious and used a thickness of 2.36 mm. thereby increasing the uncertainty of the extrapolation. For Case 3 the box was made with the bottom only 1.5 mm. thick, and this was the thinnest absorber used. With the value $\mu = 30$, this thickness of material of unit density absorbs 99% of the incident $\beta$-rays.

Some films were exposed with a copper absorber of thickness only 0.4 mm. in the hope that these would give points on the graph nearer to zero distance, but it was found that they gave points lying below the extrapolated part of the curve - sometimes actually below points corresponding to a greater distance. Two effects probably contributed to this: (1) Reduction of the $\gamma$-ray dose by oblique filtration through the copper, at points of low activity; (2) Complete absorption of very soft components, either bremsstrahlung due to scattering of $\beta$-particles in the tissue, or low energy components of the nuclear radiation. The magnitude of these effects being difficult or impossible to calculate, it was decided to accept only measurements with tissue-like absorbers.
(4) Relation between dose and optical density.

In the action of ionizing radiations upon photographic emulsion, certain simple relations hold, which are frequently not true in optical photography. The most important are the reciprocity law and the linear relation between exposure and optical density. Latent image fading and effect of temperature have been investigated experimentally under the conditions of these measurements.

(a) "Reciprocity Law".

In photography it is customary to express the dependence of the time, t, necessary to give a developed image of a certain density, upon the intensity, I, of the light, by a relation of the form:

\[ It^p = \text{constant}. \]

Any departure of \( p \) from unity represents a failure to obey the Reciprocity Law of Bunsen and Roscoe, namely that the time is proportional to the reciprocal of the intensity. In optical photography, \( p \) changes with temperature and other circumstances, and the complex relation between light intensity and exposure time has been explained in terms of the additive effect of successive light quanta impinging upon a silver halide crystal (Webb & Evans, 1938; Berg, 1940; Mees, 1944; Mott & Gurney, 1948). With ionizing radiations, a single ionization within a silver grain is sufficient
to make it developable, and the reciprocity law would be expected to be obeyed. This has been verified experimentally for X-rays and Y-rays by Bell (1936 a, b). Morgan (1944) found it to be untrue for film used with intensifying screens, as would be expected since these produce their effect by fluorescence, but true for the same films used without screens. Wolfe & Wilkins (1935) verified the law for α-rays. The law for β-rays would be expected to be the same as that for Y-rays, because most of the action of Y-rays is due to the secondary electrons which they produce. The experiments of Bell extended up to exposure times of the order of 15 hours.

Recently Ray & Stevens (1953) have verified the reciprocity law for the action of β-rays of I^131 on high-speed non-screen X-ray film for times from 5 seconds to 12 days, and found only a small loss at the longest exposure time (less than 10%), which they attributed to latent image fading. Their material and conditions were very similar to those of my measurements; and I have found no discrepancies which would suggest a departure from reciprocity.
(b) Relationship between exposure and optical density.

Because a single ionization within a silver halide grain makes that grain developable, it follows that for small exposures the number of grains in the developed image is proportional to the product of intensity and time, that is to say, to the dose of radiation, for a given quality of radiation. (This is analogous to a "single-hit" process in the target theory of the action of radiation on living cells). Pelc (1945) starts from this premise in his theoretical and experimental study of the photographic action of X-rays, and goes on to show that, for low optical densities, the density is proportional to the dose for any one quality of radiation. The variation of sensitivity with quality of radiation is more complicated, and his results are only approximate, but the calibration procedure I have adopted requires no assumptions on this matter. Bell (1936 a,b) made careful experimental measurements and found that for low densities the density was proportional to the dose. The limit of density, above which the simple law of proportion is not obeyed, varies for different photographic materials (Pelc, Bell, op. cit.) and with the quality of the radiation (Hirsch, 1935). Kieffer & Seideman (1946), on the other hand, who plot density against the logarithm of the dose following the customary method of Hürter & Driffield for optical
photography, found a non-linear relationship between a density of 0.2 and one of 2.5; and replotting of their observations on a linear dose scale does not give a straight line. Robertson (1943) also states that there is no general linear relation between film density and X-ray dose.

In view of the lack of information about the range of densities over which a linear relation can be assumed, I have calibrated my photographic material, as shown in Diagram 4. The actual dose determinations were made by interpolation, using calibration spots made by radium on the same film for reference (see p. 87). Extrapolation was necessary for a few measurements, and for some of these a correction was necessary for non-linearity at both extremes of the range. The correction was determined by more detailed measurements at high and at very low densities, on separate films.

(c) Latent Image Fading.

A trial with the material used, over a period of 12 days, showed fading to the extent of 9½%. The corresponding correction was negligible in all except two or three of the films used for Y-ray measurement. The method was to make a series of exposures with the radium standard at the beginning of the period, and

Ilfex film. Exposures of 60 seconds to standard radium source.

<table>
<thead>
<tr>
<th></th>
<th>A: exposed 12 days before development</th>
<th>B: exposed 1 hour before development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Densities above background for each exposure.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Film I</th>
<th>Film II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>0.80</td>
<td>0.87</td>
<td>0.74</td>
</tr>
<tr>
<td>0.79</td>
<td>0.89</td>
<td>0.77</td>
</tr>
<tr>
<td>0.79</td>
<td>0.89</td>
<td>0.73</td>
</tr>
<tr>
<td>0.81</td>
<td>0.86</td>
<td>0.80</td>
</tr>
<tr>
<td>0.81</td>
<td>0.89</td>
<td>0.82</td>
</tr>
<tr>
<td>0.80</td>
<td>0.89</td>
<td>0.76</td>
</tr>
<tr>
<td>Mean</td>
<td>0.800</td>
<td>0.882</td>
</tr>
</tbody>
</table>

\[
\frac{A}{B} = 0.908 \quad \frac{A}{B} = 0.903
\]

Latent image fading = 9.5% in 12 days
= 0.8% per day.
again at the end of it, after which the film was
developed, and the densities of the two sets was
compared. The results are shown in Protocol 2, which
also illustrates the consistency obtained in the
measurements. Variations within the groups are mainly
due to unequal development, as is the difference between
the two films.

(d) Effect of Temperature.

A series of exposures with the radium standard
was made first at room temperature (22°C) and then
at 2°C, on the same film. This is a larger range
of temperature than the night-to-day or day-to-day
variation of the laboratory. There was no significant
difference between the measured densities.
DIAGRAM 4  Relationship between density of image, photo-electric cell readings, and exposure time.

Approximate radiation dose is given at the surface of the envelope.
Measurement of optical density.

Towards the end of the work described, a commercial densitometer became available. This was the "EEL Universal Minor Densitometer"* and it was used with a diaphragm 1.8 mm. in diameter. It was simple and quick to use. Previously, for the greater part of the work, a microscope was used with a condenser adapted to give uniform illumination over a square area of side 0.7 mm. A photoelectric cell was placed at the focal plane of a projection eye-piece, and the current in the cell was measured with the help of an amplifier.

The calibration curve of the Ilfex film, as measured on the same three test films by each of the methods, is shown in diagram 4. Over most of the range it is practically a straight line through the origin; more detailed measurements at the two extremes showed small deviations, for which a correction was made in cases where it was necessary.

Calibration of the film and standardization of activity measurements.

Each film was given a range of three calibration spots by means of a radium applicator held at a fixed distance for times of 10, 20 and 40 seconds, or 20, 40 and 80 seconds (Diagram 5). These served as a check

* Evans Electroselenium Ltd., Harlow, Essex.
Distance piece and radium plaque for film calibration.

Monel metal Radium Plaque, diameter 15.5 mm., content 6.14 mgm.

Perspex block $2\frac{1}{8}'' \times 2\frac{1}{8}'' \times 3\frac{3}{8}''$

Lead foil 0.4 mm thick.
of the constancy of development and of the densitometer or photoelectric cell apparatus. All measured densities were referred to the calibration spots on the same film by interpolation, or, outside this range, by comparison with separate calibration films on which the same radium source had been placed for a wider range of times.

The photographic effect of the radium source was compared with that of a known dose of $\gamma$-rays of the same quality as those being measured, namely those of $^{131}$I, by exposure to a known amount of this isotope. A source from A.E.R.E., Harwell, consisting of 10 mc. of $^{131}$I in 2 ml. of water in a sealed tube, was held near the centre of a room, and films were exposed at a distance of 50 cm. in two positions to avoid any systematic error due to scatter from unavoidable objects nearby, for various lengths of time during the radioactive decay of the source. Timed radium exposures were put on to the same films, in a corner shielded with lead during the $\gamma$-ray exposures. It was found that the presence of 3 mm. of perspex either in front of or behind the film or both, made so small a difference to the density as to be negligible for the purposes of these measurements. The following protocol (No. 3) gives the principal measurements made in this calibration.

The $\gamma$-ray dose from the 10 mc. source was
Protocol 3.

**Measurement of a series of films exposed to the 10 mc. $^{131}$I source.**

<table>
<thead>
<tr>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
<th>(5)</th>
<th>(6)</th>
<th>(7)</th>
<th>(8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Film No.</td>
<td>Day started</td>
<td>Exposure time</td>
<td>Exposure factor</td>
<td>Density of radium spots (arbitrary units)</td>
<td>Density of film</td>
<td>Dose (mean)</td>
<td>Dose for complete decay</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>iii</td>
<td>-1</td>
<td>18 hr</td>
<td>15.0</td>
<td>10.7</td>
<td>21.4</td>
<td>43.0</td>
<td>25.4</td>
</tr>
<tr>
<td>vi</td>
<td>0</td>
<td>5d 16.5h</td>
<td>2.56</td>
<td>8.0</td>
<td>16.7</td>
<td>35.2</td>
<td>106</td>
</tr>
<tr>
<td>viii</td>
<td>+6</td>
<td>3d 23h</td>
<td>5.78</td>
<td>7.3</td>
<td>15.2</td>
<td>30.8</td>
<td>45.7</td>
</tr>
<tr>
<td>x</td>
<td>+10</td>
<td>3d 1h</td>
<td>9.84</td>
<td>30 sec = 25.8</td>
<td>26.3</td>
<td>26.7</td>
<td>30.8</td>
</tr>
<tr>
<td>xii</td>
<td>+13</td>
<td>2d 0h</td>
<td>19.4</td>
<td>16.2</td>
<td>24.6</td>
<td>24.6</td>
<td>12.6</td>
</tr>
<tr>
<td>xiv</td>
<td>+15</td>
<td>3d 2.2h</td>
<td>15.0</td>
<td>7.9</td>
<td>16.2</td>
<td>33.0</td>
<td>16.5</td>
</tr>
<tr>
<td>xvi</td>
<td>+21</td>
<td>8d 16h</td>
<td>11.6</td>
<td>8.9</td>
<td>18.0</td>
<td>37.0</td>
<td>32.7</td>
</tr>
</tbody>
</table>

**Mean (omitting xvi)** 319
S.D. = 24.5; S.D. of mean = 10

For explanation of the table see opposite.

The corresponding set of values for films in the second position (subject to more backscatter) was: 330, 341, 366, 364, 344, 378, 378. Mean 357, S.D. = 190; S.D. of mean = 7.

Gamma-ray dose for complete decay of 9.9 mc. at 50 cm. is: $\frac{9.9 \times 2.25}{50 \times 50 \times 0.00361} = 2.47$ roentgens.

$\therefore$ 1 $\gamma$ is equivalent to: $\frac{319}{2.47} = 130$ "rasecs" for this type of film.
Explanation of Protocol 3.

This protocol is illustrative of the method of calculation used for all measurements with the photo-electric cell apparatus, but actually refers to the \( \gamma \)-ray calibration of Ilfex film.

**Column (2):** Day 0 was the day on which the activity was stated by A.E.R.E. to be 10 mc. exactly. Subsequent comparison with the N.P.L. substandard source gave the value 9.9 mc.

**Column (4):** "Exposure Factor" is the number by which the dose actually applied to the film would have been multiplied if exposure had been for an infinite time from Day 0. This was calculated using the value 8.0 days for the half-life of \(^{131}\)I.

**Column (5):** The densities in this and the next column were obtained, in arbitrary units proportional to dose, by reading off graphs which were prepared to avoid the work of looking up logarithms of the photo-electric cell currents. The differences here are due mainly to variations in development.

**Column (6):** Regions (a) and (b) on each film were in positions (relative to a marked corner) corresponding to regions of uniform density on one film (No.viii) which was mapped all over. Region (b) corresponded to minimum density on that film.

**Column (7):** "Rasec" is an abbreviation for the dose, or corresponding blackening of film, due to an exposure of 1 second to the standard radium source at the standard distance.

**Column (8):** These figures are obtained by multiplying the preceding column by the "exposure factor", and should be constant. Film (xvi) has been rejected; the very high value might have been due to an accidental near approach to the source when it was being put in position, or to fogging from some other radioactivity.
calculated from the figure of 2.25 roentgens per millicurie-hour at 1 cm. (Bullard, 1952), and the inverse square law of distance.

The 10 mc. source, when it had decayed to approximately 1 mc. was compared by means of a γ-ray Geiger counter with a standard source of 1 mc. issued with a certificate of accuracy (± 5%) from the National Physical Laboratory. These two were found to agree within the accuracy of measurement (± 1%).

The N.P.L. standard 1 mc. source was also used to calibrate the counting apparatus by which the activity of the specimens, and the activities administered to the patients, were measured.

The dose rate due to the radium plaque at the orifice of the support was measured by Mr. Haybittle using an ionisation chamber, and was found to be 0.0096 rep/sec. due mainly to the β-rays. This value is not required in the calculation of γ-ray dose, but is of interest in comparing the sensitivity of the film (in its envelope) to radium β-rays and to $^{131}\text{I}$ γ-rays. It appears that 0.0096 rep from the radium plaque is equivalent to 0.0076 roentgens of $^{131}\text{I}$ γ-rays under these conditions. Absorption of β-rays in the paper envelope probably accounts for the greater effectiveness of the γ-rays.
(7) Rate of uptake and excretion.

In all calculations, the radioactive decay of I$^{131}$ was taken into account to the nearest whole day. This was permissible because all events involved in such calculations (administration of radio-iodine, operation, measurements of activities, and starts of autoradiographic exposures), with very rare exceptions, took place between the hours of 9.30 a.m. and 7.30 p.m. that is to say within $\pm$ 5 hours of the middle of this period. The radioactive decay of I$^{131}$ in 5 hours amounts to only 1.8%.

Doses were therefore calculated for complete radioactive decay from the arbitrary time of 2.30 p.m. on the day of operation. Two corrections which operate in opposite directions, ought to be made to such a value to obtain the actual dose which would have been received by the tumour if it had not been removed by operation. First, a certain dose was received before operation. Examination of unpublished uptake and excretion curves obtained in the Radiotherapeutic Centre shows that this is likely to amount to between 10% and 20% of the whole dose. Secondly, excretion would have reduced the total dose if the tumour had remained in the body. Taking the figure of 6.3 days for the effective half-life including both decay and excretion (Mitchell, 1951, based on the same curves),
would reduce the total dose by 21%. There is, however, marked variation from case to case in the rate of excretion.

In view of the uncertainties of these factors, it was considered best to quote the results for complete decay from this arbitrary time, and not to attempt any correction.

(8) Extrapolation to zero distance from the face of the slice.

The rate of decrease of γ-ray dose with distance over the first few millimetres depends on the inverse square law, and not on absorption. The mathematics is complicated when the dimensions of the source are comparable with the distance, and the rate of decrease depends very much on the size and shape of the source. A theoretical approach to the problem, however, yielded a lot of useful information. Following Parker (1947), the dose at the central point of the end face of a right circular cylinder was calculated by evaluation of the expression

\[ I = \pi \rho K \gamma \int_0^h \log_e \left( \frac{a^2 + z^2}{x^2} \right) dz \]

where \( \rho \) is the specific activity in millicuries per ml.
and $K_\gamma$ is the dose rate at one centimetre from a point source of 1 mc.; $a$ is the radius, and $h$ the length of the cylinder.

This expression is obtained directly from the inverse square law; it takes no account of absorption, which is small in the distances involved in this work. In the general case taken by Parker, numerical integration was necessary, but the simpler expression here can be integrated exactly*:

$$\int_0^h \log_e \left( \frac{a^2 + z^2}{z^2} \right) dz = h \log_e \left( 1 + \frac{a^2}{h^2} \right) + 2a \tan^{-1} \frac{h}{a}$$

The dose at points at a distance $d$ from the face, on the axis, can be calculated by the difference between the dose from a cylinder of length $d + h$ and one of length $d$. For a hollow cylinder, the values can be obtained by taking the difference of two solid cylinders of radius equal to the external and to the internal radius, respectively.

Some curves obtained by these calculations are shown in Diagram 6. It is clear that satisfactory extrapolation cannot be made for solid cylinders from a point as far out as 2.3 mm., back to zero. However, by plotting the distance on a scale proportional to $\log \left( 1 + kd \right)$ flatter curves are obtained, for which extrapolation is not impossible when some information

* I am indebted to Mr. R. Stearn for this integration.
Diagram 6A
Solid Cylinders - Linear scale
and large thin-walled hollow cylinders.
Length: 10 cm to 11 cm.

Diagram 6B
Solid Cylinders - Logarithmic scale
Hollow: 1 to 1.1 cm.
Diagram 6D
Hollow Cylinders – Logarithmic scale
Length 0-0.1 cm, Outer radius 0.3 to 1.0 cm, Inner radius 0.1 to 1.0 cm.

Distance (cm)

Diagram 6C
Hollow Cylinders - Linear scale
Length 0-0.1 cm, Outer radius 0.3 to 1.0 cm, Inner radius 0.1 to 0.3 cm.

Distance from centre of face (cm)
about the distribution is available from the $\beta$-ray autoradiograph. The choice of $k$ is not at all critical, and after a few trials in the range $1 < k < 100$, the value $k = 5\, \text{cm}^{-1}$ was adopted as most convenient to suit the dimensions of the active areas involved. The curves for hollow cylinders, as one would expect, rise much less steeply, and plotted on the logarithmic scale they are amenable to extrapolation, provided either the internal diameter is at least $4\, \text{mm.}$ or the external diameter at least $2\, \text{cm.}$

For cases 1 and 2, the results for the different slices were plotted against distance on a scale proportional to $\log (1 + 5d)$, and the summation and extrapolation carried out as described. After this it was decided to adopt a simpler approach. By cutting the tumour into two pieces only, the $\gamma$-ray dose can be calculated for any point in the plane of section by adding the contributions from the two parts, each extrapolated to zero distance. And by using a smaller distance (risking the complication of a very small $\beta$-ray contribution) a single measurement for each part will suffice. The extrapolation is done by adding a percentage to the single measurement made at a distance of $1.5\, \text{mm.}$ (In practice a second distance was also used as a check). The percentage to be added has been calculated by the above methods for a number of
about the distribution is available from the β-ray autoradiograph. The choice of $k$ is not at all critical, and after a few trials in the range $1 < k < 100$, the value $k = 5 \text{ cm}^{-1}$. was adopted as most convenient to suit the dimensions of the active areas involved. The curves for hollow cylinders, as one would expect, rise much less steeply, and plotted on the logarithmic scale they are amenable to extrapolation, provided either the internal diameter is at least 4 mm. or the external diameter at least 2 cm.

For cases 1 and 2, the results for the different slices were plotted against distance on a scale proportional to $\log (1 + 5d)$, and the summation and extrapolation carried out as described. After this it was decided to adopt a simpler approach. By cutting the tumour into two pieces only, the Y-ray dose can be calculated for any point in the plane of section by adding the contributions from the two parts, each extrapolated to zero distance. And by using a smaller distance (risking the complication of a very small β-ray contribution) a single measurement for each part will suffice. The extrapolation is done by adding a percentage to the single measurement made at a distance of 1.5 mm. (In practice a second distance was also used as a check). The percentage to be added has been calculated by the above methods for a number of
TABLE IV.

Percentage to be added to observed dose at 1.5 mm.
in order to obtain dose at face.

A. Solid Cylinders

<table>
<thead>
<tr>
<th>Thickness</th>
<th>1.0</th>
<th>1.0</th>
<th>0.5</th>
<th>0.5</th>
<th>0.5</th>
<th>0.5</th>
<th>0.25</th>
<th>0.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>0.4</td>
<td>0.2</td>
<td>2</td>
</tr>
<tr>
<td>Correction</td>
<td>25%</td>
<td>40%</td>
<td>35%</td>
<td>60%</td>
<td>10%</td>
<td>25%</td>
<td>525%</td>
<td>75%</td>
</tr>
</tbody>
</table>

B. Hollow Cylinders

<table>
<thead>
<tr>
<th>Thickness</th>
<th>1.0</th>
<th>1.0</th>
<th>1.0</th>
<th>1.0</th>
<th>0.5</th>
<th>0.5</th>
<th>0.5</th>
<th>0.5</th>
<th>0.5</th>
<th>0.5</th>
<th>0.25</th>
<th>0.25</th>
<th>0.5</th>
<th>0.5</th>
<th>0.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ext. diam.</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>0.8</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Int. diam.</td>
<td>2</td>
<td>0.8</td>
<td>0.8</td>
<td>0.4</td>
<td>4</td>
<td>2</td>
<td>0.2</td>
<td>1</td>
<td>0.4</td>
<td>0.2</td>
<td>0.4</td>
<td>1</td>
<td>0.5</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Correction</td>
<td>7%</td>
<td>14%</td>
<td>19%</td>
<td>50%</td>
<td>2%</td>
<td>3.5%</td>
<td>25%</td>
<td>16%</td>
<td>29%</td>
<td>41%</td>
<td>47%</td>
<td>10%</td>
<td>38%</td>
<td>63%</td>
<td>127%</td>
</tr>
</tbody>
</table>

The figures to the left of the dotted line in each table will allow the correction to be made satisfactorily.

* The effect of closing the back of the hollow cylinder by a solid cylinder of equal thickness, has been shown to be negligible in these two cases.
cylindrical distributions of radioactivity, and the values are shown in Table IV. Table IVB also applies for segments of hollow cylinders, and, therefore, for a region of activity which is not in direct line with the point of measurement. Values for other distributions have been calculated approximately by similar methods. The approximate distribution in an actual case can be determined by a β-ray autoradiograph taken subsequently, and the appropriate correction added to the measured γ-ray dose at 1.5 mm. Except for the case of points in direct line with small active regions, the correction can be determined to give a value within ± 10% to ± 15% for the dose at the face of the slice.
TABLE V

Radio-Iodine Specimens and Differential Absorption Ratios

Differential Absorption Ratios for $^{131}\text{I}$ measured on Thyroidectomy Specimens.

<table>
<thead>
<tr>
<th>Case</th>
<th>Wt. (Kg)</th>
<th>Drink (mC)</th>
<th>Weight (gm)</th>
<th>Mean specific activity (μC/gm)</th>
<th>Mean D. A. R. corrected for decay.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>67</td>
<td>25 mC</td>
<td>5.0</td>
<td>4.1</td>
<td>133</td>
</tr>
<tr>
<td></td>
<td></td>
<td>= 0.37 mC/Kg.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary Carcinoma</td>
<td>207</td>
<td>127</td>
<td>9.4</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Metastasis</td>
<td>207</td>
<td>127</td>
<td>9.4</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>This patient had considerable previous $^{131}\text{I}$ therapy.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 2</td>
<td>51</td>
<td>25 mC</td>
<td>13</td>
<td>26.8</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>= 0.49 mC/Kg.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid tissue (estimated)</td>
<td>349</td>
<td>190</td>
<td>3.5</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Primary Carcinoma</td>
<td>349</td>
<td>190</td>
<td>3.5</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Course of Lugol's Iodine finished 10 days previously. Metastases in this case subsequently showed definite uptake, and clinical improvement with $^{131}\text{I}$ therapy.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 3</td>
<td>60</td>
<td>5-9 mC = 0.10 mC/Kg.</td>
<td>12.3</td>
<td>70.8</td>
<td>880</td>
</tr>
<tr>
<td>Right hemithyroid</td>
<td>870</td>
<td>160</td>
<td>less than 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary Carcinoma</td>
<td>870</td>
<td>160</td>
<td>less than 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 4</td>
<td>54</td>
<td>5.6 mC = 0.010 mC/Kg.</td>
<td>2.4</td>
<td>5.7</td>
<td>600</td>
</tr>
<tr>
<td>Nodular Goitre.</td>
<td>13.7</td>
<td>2.4</td>
<td>5.7</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>Case 5</td>
<td>48</td>
<td>25 mC = 0.52 mC/Kg.</td>
<td>1.0</td>
<td>91</td>
<td>700</td>
</tr>
<tr>
<td>Normal thyroid infiltrated with squamous carcinoma.</td>
<td>91</td>
<td>1.0</td>
<td>91</td>
<td>700</td>
<td></td>
</tr>
</tbody>
</table>

*The highest correction amounts to 24%, based on 2 days' decay with assumed effective half-life, 6.0±3 days.
CHAPTER VIII.

CASES INVESTIGATED WITH RADIO-IODINE

Particulars of the specimens obtained in these five cases are given in Table V, with the activity administered. Radio-iodine was given by mouth in each case, as sodium iodide in water, with approximately 5 micrograms of inactive sodium iodide per millicurie. The activity of each specimen when removed is given, and from that has been calculated the mean differential absorption ratio.
Figure 3 Case 1. Normal thyroid on left; carcinoma on right. Contours of counts per minute.
Case 1. Recurrent carcinoma of the thyroid, following radio-iodine therapy.

Clinical Notes

The patient was a man, a general labourer from Suffolk, aged 26 when first seen at the Radiotherapeutic Centre, in August, 1950. At that time he was complaining of a lump in the right side of his neck, which he had had for 3 years. Otherwise he was well; there was no relevant previous history.

On examination, there was a tumour of the right lobe of the thyroid, clinically malignant, and two palpable lymph nodes of the lower middle deep cervical group, on the same side. Biopsy of one of the lymph nodes showed thyroid tissue and lymphadenoid tissue, but it was not reported as definitely malignant. There was no evidence of distant metastases.

At operation, there was found an enlarged right lobe of thyroid, and glands all along the jugular vein, descending behind the sternum and into the thorax, hard and matted together. Removal was not attempted.

A tracer dose of radio-iodine showed, by Geiger counter measurements, that there was uptake of radioactivity on the right side, as well as in the unaffected left lobe of the thyroid (see Fig. 3). Radiotherapy was
given by means of $I^{131}$, with satisfactory response. The patient returned to work in December, 1950, the course of radio-iodine continuing.

He remained well, and was seen regularly. In November, 1952, the right lobe of the thyroid and the lymph nodes were found to be larger again. He was admitted to hospital, and tracer studies with $I^{131}$ showed $14\%$ uptake in the thyroid region and right side of the neck, and there was no evidence, either from these studies or by conventional methods of examination, of distant metastases. Laboratory investigations showed evidence of hypothyroidism.

The tumours being more mobile than before, operation was decided upon. 25 mc. of $I^{131}$ were given by mouth 48 hours before operation was planned, and radioactivity measurements after 24 hours still gave no evidence of distant metastases or of intra-thoracic activity. At operation on 9.12.52 (Mr. B. McN. Truscott), the right hemi-thyroid and two groups of affected lymph nodes were removed. These were the specimens of which particulars are given in Table V. Two further lymph nodes, one behind the medial end of the clavicle and one in relation to the apex of the pleura, had to be left. The right internal jugular vein was found to be reduced to a fibrous cord for 2 or 3 cm. in relation to the lymph nodes. (No doubt this was an effect of radiation
from the radio-iodine).

Pathological examination showed the presence of carcinoma in both the right lobe of the thyroid and the lymph nodes, reported as acinar large cubical celled carcinoma of the thyroid. The photomicrographs reproduced later illustrate the variation of structure found in the course of the autoradiographic studies.

After operation, a further large therapeutic dose of radio-iodine was given. A month later the patient was clinically myxoedematous. Thyroid, gr. 1/2 b.d. was prescribed, and he returned to work. Thyroid medication was stopped in April, 1953, but he became gradually myxoedematous again and it was restarted in October. He remains well on a maintenance dose, varying between gr. 1/2 twice daily and gr. 1/2 thrice daily.

Owing to the incompleteness of early measurements of uptake of the radioactivity, and to the difficulty of estimating the mass of the residual normal thyroid and of carcinoma, it is impossible to arrive at an accurate estimate of the radiation dose due to each administration of radio-iodine. The average differential absorption ratios were measured for the operation specimens, however, and a very rough value for the dose can be obtained by assuming the differential absorption ratios of primary tumour and metastases to have remained constant throughout the treatment. One would not expect
<table>
<thead>
<tr>
<th>Date</th>
<th>&quot;Drink&quot;</th>
<th>Rep to primary (DAR = 133)</th>
<th>Rep to metastases (DAR = 31)</th>
<th>B.M.R.</th>
<th>Serum cholesterol (mgm/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31.10.50</td>
<td>25 mc.</td>
<td>5,200</td>
<td>1,200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-17.11.50</td>
<td></td>
<td></td>
<td></td>
<td>19%</td>
<td>188</td>
</tr>
<tr>
<td>30.11.50</td>
<td>25 mc.</td>
<td>5,200</td>
<td>1,200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. 1.51</td>
<td>25 mc.</td>
<td>5,200</td>
<td>1,200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. 3.51</td>
<td>20 mc.</td>
<td></td>
<td>1,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total (first course)</strong></td>
<td></td>
<td><strong>19,800</strong></td>
<td><strong>4,600</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>rep in overall time 20 weeks.</td>
</tr>
<tr>
<td>25-26.11.52</td>
<td></td>
<td></td>
<td></td>
<td>31%</td>
<td>347</td>
</tr>
<tr>
<td>2.12.52</td>
<td>5 mc.</td>
<td>1,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.12.52</td>
<td>25 mc.</td>
<td></td>
<td>1,200 (to tumour tissue left after operation)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.12.52</td>
<td>93 mc.</td>
<td></td>
<td>4,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total (second course)</strong></td>
<td></td>
<td><strong>5,900</strong></td>
<td></td>
<td></td>
<td>rep in overall time 4 weeks.</td>
</tr>
</tbody>
</table>
this to be a close approximation to the truth, especially in view of the variability of structure found, but a list calculated on this basis is given in Table VI, together with laboratory investigations bearing on thyroid function.

It is probable that normal thyroid tissue, that is to say the left lobe, would have received a considerably higher dose than this calculation gives for the primary tumour. In that case, it may well have been entirely destroyed by the first course of radio-iodine. Thyroid function during the period up to the second operation would have been carried out entirely by tumour tissue. When this was largely removed, and the residue further irradiated, myxoedema was the natural sequel. Subsequently the patient was maintained in a slightly hypothyroid state, to ensure the optimum condition for any remaining viable tumour tissue to develop the function of concentrating iodine.
**Figure 4**  Case 1. Primary tumour.

A. Paraffin wax block with the slices of the tumour. Scale of cm.

B. Corresponding β-ray autoradiograph. Positive reproduction from the original X-ray film. Calibration spots correspond to exposures in the ratios 1:2:4.
Specimens.

The tissue removed at operation was received in four pieces, after representative wedges of each had been sent for routine pathological examination.

(1) The right hemithyroid, weight 4.9 gm., total activity 190 μc. This was cut into three slices, fixed in acetic acid/alcohol mixture, dehydrated, and embedded in paraffin wax. The blocks, faced on the microtome, are shown in Fig. 4A.

(2) An elongated mass consisting of two infiltrated lymph nodes one of which was cystic. This was cut into 7 slices and similarly treated.

(3) A separate lymph node. This was divided in half, and embedded with the cut faces exposed.

The combined weight of specimens (2) and (3) was 16.1 gm. and the activity 157 μc. The paraffin blocks, faced on the microtome are shown in Fig. 5A.

(4) A subcutaneous nodule. This proved to consist of fibrous tissue around a foreign body, probably a stitch from the previous operation. Its activity was no higher than that of indifferent tissues of the body.
Figure 5  Case 1. Lymph node metastases.

A. Slices of the two metastatic masses, in wax blocks. (A special lighting system has been used for photography giving balanced illumination in front and behind the wax blocks. This conceals cracks and imperfections in the blocks, on the principle of the grease-spot photometer, without reducing detail in the more opaque tissue slices. The photograph is not touched).

B. Corresponding beta-ray autoradiographs. P, Q, are the points selected for y-ray dose determinations.
Figure 6  Case 1, Primary Tumour. A group of tumour cells surrounded by fibrous tissue. H & E.

A. x 60   B. x 320

This group of cells did not show activity on stripping film autoradiograph.
Beta-ray Autoradiography.

Photographs of the primary tumour and of the lymph node metastases, sliced and embedded in paraffin wax, are shown in Figures 4A, 5A. The corresponding β-ray autoradiographs, made by placing these blocks on X-ray film, are shown in Figures 4B, 5B. It is clear at once that the calculation of tissue dose from the mean activity per gram has very little relation to what would have been achieved by therapy. If malignant tissue was confined to active areas, the effective dose would be considerably greater than calculated. On the other hand, if there was malignant tissue at regions of minimum uptake, the effective minimum tumour dose would be many times smaller than calculated; the inactive region on the central slice of the primary tumour measures 12 x 3 mm. which means that no effective amount of β-radiation, but only γ-radiation, could reach the centre. In this case there was clear evidence that malignant tissue was present. Cells which histologically appear malignant and viable were found in several small groups in the inactive central portion, as shown in Figure 6. It must be emphasised that the last previous irradiation of this tumour was 20 months before the present dose, and as these groups of cells had survived so long, they must be regarded as viable.
Figure 7 Case 1. Primary Tumour.

Stripping film autoradiographs from peripheral part of tumour.
Note activity where there is follicle formation.
Stained haematoxylin and eosin.
Microscopical autoradiographs of sections of the primary tumour showed, round the periphery, follicle formation with the usual spotty distribution of activity. On the whole the larger follicles showed less intense radioactivity than the smaller, but there was no uniformity about this. Fig. 7 shows a typical autoradiograph from the periphery. The central part was largely composed of fibrous tissue, but with occasional groups of malignant cells as mentioned. In this part, no radioactivity was detected.

The lymph node metastases showed both follicular adenocarcinoma and anaplastic carcinoma, as well as a great deal of fibrosis due to the previous irradiation. The variability of uptake of radio-iodine is shown by the autoradiographs in Figure 8 which were all taken from the same mass, with almost identical exposures. The well-known correlation between colloid formation and radioactivity is illustrated, as well as exceptions to it. The ratio of activity between the most active and the least active parts is not less than $150 : 1$. 
Figure 8. Case 1. Lymph node metastasis. Stripping film autoradiographs superimposed on sections, showing great variation of histology and of radioactivity within a single tumour. Magnification approx. x 100 in all cases. Haematoxylin and eosin.

A. Follicle formation with good uptake of $^{131}I$.
B. Follicle formation with very variable uptake: note two small follicles (marked by arrows) which contain colloid but give no autoradiograph.
C. Partially differentiated carcinoma with no uptake.
D. Undifferentiated carcinoma invading lymphoid tissue. No uptake.
Figure 9 Case 1, primary tumour.

Gamma-ray autoradiographs through 2.4 mm of celluloid. 2 slices. The exposure was 10 times that of Fig. 4B.

Note that central minimum is still present in the largest slice, but the ratio of density from maximum to minimum is reduced to 1.3:1.
Gamma-ray Measurements.

Autoradiographs were taken through absorbers ranging from 2.4 mm. celluloid to 2.5 cm. perspex. An example of one of these with the thinnest absorber is reproduced in Fig. 9. Here a central minimum is still just observable; with the thicker absorbers there is only a diffuse image, darkest at the centre. The exposure time for Fig. 9 is approximately 10 times that of the β-ray autoradiographs of Fig. 4B, and of course is very much longer still for the thickest absorbers.

From the point of view of radiotherapy, the region of obvious interest is the centre, where the β-ray dose is very small. The γ-ray dose was therefore calculated for this region, taking the central point of the face of slice B. The relevant curves of dose rate against distance for the three slices are reproduced in Diagram 7. The extrapolation to zero distance for slice B has been taken as a straight line from the first two points, in conformity with the shape of the calculated curve for a hollow cylinder of similar dimensions to the active region shown on the β-ray autoradiograph.

Protocol 4 shows the method of obtaining figures for plotting the graphs. Protocol 5 and the associated diagram shows the method of summing the contributions and arriving at the γ-ray dose for the point chosen.

A similar procedure was carried through for the
Diagram 7  Case I. Primary Tumour

The three large crosses indicate the values taken for calculation of the gamma ray dose.
Diagram 7 - Note on Extrapolation for Slice B

The points plotted represent the gamma-ray doses opposite the centre of each slice, and therefore, in the case of "Slice B", opposite the inactive central region.

For the purposes of extrapolation from the nearest measured point (0.23 cm.) back to zero distance, the best estimate of the distribution of activity, as judged from the β-ray autoradiographs, is a hollow cylinder of length 0.5 cm., outer diameter 1.5 cm., inner diameter 0.7 cm.

Reference to Diagram 6D (p.93-1) shows that the curve of dose vs. log(1+5d) should be concave downwards in the relevant region, and intersection with the axis would be some 10% - 15% below the intersection of the tangent at d = 0.23 cm.

Further information can be obtained from Table IV (p.95): by scaling the dimensions in the ratio 0.15:0.23 we obtain a cylinder of length 0.33 cm., O.D. 1.0 cm., I.D. 0.46 cm. The nearest two entries are the columns reading "0.5, 1, 0.4" and "0.25, 1, 0.5", which give for the percentage addition the values 47 and 38% respectively. An addition of just over 40% to the last measured dose gives the value finally taken, marked by the large cross on the graph, and this gives a curve consistent with the theoretical curves of Diagram 6D.
# Protocol 4  Case 1. Primary Tumour Measurement of Y-ray autoradiographs.

<table>
<thead>
<tr>
<th></th>
<th>(1) Film No.</th>
<th>(2) Absorber (cm.)</th>
<th>(3) log (1+5d)</th>
<th>(4) Exposure Duration</th>
<th>(5) Start</th>
<th>(6) Exposure Factor</th>
<th>(7) Film density (raeecs)</th>
<th>(8) Total Rasecs</th>
</tr>
</thead>
<tbody>
<tr>
<td>xxii</td>
<td>0.23</td>
<td>0.332</td>
<td></td>
<td>23.1 h</td>
<td>27/1/53</td>
<td>129</td>
<td>5.2 24 17.6</td>
<td>670 3100 2270</td>
</tr>
<tr>
<td>viii</td>
<td>0.23</td>
<td>0.332</td>
<td></td>
<td>3d 21.5h</td>
<td>10/1/53</td>
<td>8.24</td>
<td>83 black black</td>
<td>680</td>
</tr>
<tr>
<td>xiii</td>
<td>0.63</td>
<td>0.618</td>
<td></td>
<td>1d 22h</td>
<td>14/1/53</td>
<td>22.0</td>
<td>(6.1 63 38)</td>
<td>130 1380 840)</td>
</tr>
<tr>
<td>xiii</td>
<td>0.63</td>
<td>0.618</td>
<td></td>
<td>7d 19h</td>
<td>29/1/53</td>
<td>25.2</td>
<td>(6.0 63 40)</td>
<td>130 1380 880)</td>
</tr>
<tr>
<td>xviii</td>
<td>1.3</td>
<td>0.875</td>
<td></td>
<td>2d 19.5h</td>
<td>23/1/53</td>
<td>33.4</td>
<td>(1.6) 21 11.5</td>
<td>(50) 700 380</td>
</tr>
<tr>
<td>xviii</td>
<td>1.9</td>
<td>1.021</td>
<td></td>
<td>4d 1.5h</td>
<td>19/1/53</td>
<td>17.6</td>
<td>&lt;1 21 11 (&lt;20)</td>
<td>370 190</td>
</tr>
<tr>
<td>xv</td>
<td>2.5</td>
<td>1.130</td>
<td></td>
<td>2d 16h</td>
<td>16/1/53</td>
<td>19.1</td>
<td>- 13.7 4.4</td>
<td>- 260 84</td>
</tr>
</tbody>
</table>

Column 6  "Exposure factor". This is the number by which the actual exposure has to be multiplied to give the exposure which would result from complete decay starting from an arbitrary zero. In this case the zero day was taken as 31/12/52 for convenience, and the final result corrected to the day of operation, as this reduced the arithmetical work.

Column 7  The measured densities are given for the centre of each slice. The unit "rasec" is the density due to exposure for 1 second to the standard Radium source.

Column 8  "Total rasecs". The measured densities of Col.7, multiplied by the "exposure factor". The curves of diagram 7 were plotted from these figures.

Note the agreement between the first two values in Column 8A, derived from films with densities in the ratio 16:1; the good agreement between two photometer measurements of the same film (No.xiii), and the fair agreement between these and the next row derived from a film exposed for very much longer when the radioactivity had decayed one quarter of its original value.
Dose due to B extrapolated to $d = 0$ $\frac{4500}{4} = 1125$

" A " $d = 0.2$ $\frac{800}{4} = 200$

" C at ... ... $d = 0.8$ $\frac{650}{4} = 162.5$

" lost tissue between A and B, say half the mean of A and B each at ... ... ... $d = 0$ $\frac{1700}{4} = 425$

Dose due to lost tissue between B and C, say half the mean of B and C each at ... ... $d = 0.5$ $\frac{750}{4} = 187.5$

Total dose at marked point $8400$ "rasecs"

Taking 1 "rasec" = 0.0076 roentgen of $^{131}I$ γ-radiation, the total dose from the arbitrary zero time (31/12/51) is $8400 \times 0.0076$ r

To this must be added 15% for losses in fixing fluid.

.: Total dose from day of operation (11/12/51) is $8400 \times 0.0076 \times 1.15 \times 2 \times 2 \times 1.414 = 415$ roentgens

No correction has been made for shrinkage as the shrinkage was not measured.
largest of the lymph node masses. In this case, two points were selected for \( \gamma \)-ray dose measurements, corresponding to "P" and "Q" in Fig. 5B. P is a point near the centre of the mass, where there is little or no uptake of radio-iodine, and further than the maximum \( \beta \)-ray range from any region with considerable activity. Q is a point of high \( \beta \)-ray dose, where the therapeutic effect of the \( \gamma \)-rays would not be of importance, and was chosen to give an estimate of the upper levels of \( \gamma \)-ray dose that might be expected. In this case, the alignment of the density measurements for the different slices was a matter of some difficulty. Density measurements along a traverse of each slice were plotted on graphs, which were then aligned by comparison with photographs of the slices on the same scale, and with the \( \beta \)-ray autoradiographs. The points were chosen so that there was little uncertainty about the two nearest slices: the contribution from the more distant ones is not affected by a movement of a few millimetres.

The doses thus calculated are:

\[
\begin{align*}
P & \ldots 100 \text{ roentgens} \\
Q & \ldots 180 \text{ roentgens}.
\end{align*}
\]

Thus the variation between a point of high and a point of low activity is, as would be expected, very much less than the variation of the \( \beta \)-ray dose which is not less than 150:1 between these two points. The
therapeutic value of the \( \gamma \)-ray dose, however, is doubtful even in this metastasis where the average uptake is good. The activity given was 25 mc. which is a quarter of a full therapeutic drink. 100 mc. would have given a minimum tumour dose at the centre of only about 400 r, in an equivalent mean time of 9 days (Mitchell, 1951).

The measured \( \gamma \)-ray doses for this case and the next are collected in Table VIII (p. 113), where they are compared with values calculated on the basis of uniform distribution of radio-activity. The measured and the calculated doses are in fair agreement.
Case 2. Carcinoma of the thyroid with distant metastases.

Clinical Notes.

The patient was a housewife from Suffolk, aged 57. She was first seen at the Radiotherapeutic Centre in February 1953, complaining of a swelling in her neck which had been gradually increasing in size for 15 months. There was no pain, but slight dyspnoea on exertion. She had been treated with Lugol's iodine for two weeks.

On examination there was a large fixed goitre, firm and smooth in outline. There was mild tachycardia with extrasystoles, but no definite thyrotoxicosis. There was definite radiological evidence of metastases in the lungs and in the right os pubis.

A tracer dose of radio-iodine gave only 3% uptake in the neck, and after 10 days without iodine treatment, a second dose (25 mc.) gave 4% uptake at 24 hours. 48 hours after administration of the radio-iodine, the patient was operated on by Mr. J.E. Rowlands at the Newmarket General Hospital. The tumour and the residual thyroid gland were removed together in one mass.

Post-operative recovery was uneventful, and after 5 weeks a further dose of radio-iodine was given for uptake studies. Very little was taken up in the
neck. Readings over the pubis were equivocal because of activity in the urine. There was definite uptake at the upper end of the right tibia, which was the first evidence of a metastasis in that situation. Re-examination of the radiographs showed an area of very slight rarefaction, about $8 \times 3 \times 2$ cm. (corrected for magnification) in the upper end of the tibia, which may have represented the secondary. The appearance was, however, by no means sufficiently abnormal to warrant a diagnosis of tumour, and in fact had not by itself suggested the diagnosis. There was no pain, tenderness, or soft tissue swelling.

Radiotherapy by means of $^{131}$ I has been given as shown in Table VII.

The pulmonary and pubic metastases were reduced in size after the first post-operative course, and were barely detectable radiographically in January 1954. The radiographic appearance of the right tibia, however, has not changed, and the uptake of radio-iodine in that situation has remained higher than on the other side.

Throughout this treatment the patient has remained well, and euthyroid, without replacement therapy. The blood picture has remained normal.
### TABLE VII.

**Case 2 Radio-iodine therapy and uptake measurements.**

<table>
<thead>
<tr>
<th>I$^{131}$ administered (mc.)</th>
<th>Relative activity present</th>
<th>Approx. whole body irradiation (rep)</th>
<th>Total to date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neck</td>
<td>R. Tibia</td>
<td>L. Tibia</td>
</tr>
<tr>
<td>2. 3.53</td>
<td>1.5</td>
<td>3% (24 hrs)</td>
<td></td>
</tr>
<tr>
<td>9. 3.53</td>
<td>25</td>
<td>4% (24 hrs)</td>
<td></td>
</tr>
<tr>
<td>11. 3.53 Operation</td>
<td>2.2% in tumour + thyroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. 4.53</td>
<td>25</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>23. 4.53</td>
<td>25</td>
<td>&gt; 1</td>
<td></td>
</tr>
<tr>
<td>30. 4.53</td>
<td>$\frac{L}{R} = 1.4$</td>
<td>2.0 (still raised at 7 days)</td>
<td></td>
</tr>
<tr>
<td>7. 5.53</td>
<td>$\frac{L}{R} &gt; 1$</td>
<td>&gt; 1</td>
<td></td>
</tr>
<tr>
<td>23. 7.53</td>
<td>25</td>
<td>&gt; 1</td>
<td></td>
</tr>
<tr>
<td>30. 7.53</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. 8.53</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14. 8.53</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative activity present</td>
<td>Neck</td>
<td>R. Tibia</td>
<td>L. Tibia</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Approx. whole body irradiation (%)</td>
<td>35</td>
<td>39</td>
<td>42</td>
</tr>
<tr>
<td>Total to date</td>
<td>1.5</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Administered (mc.)</td>
<td>20</td>
<td>24</td>
<td>18.5</td>
</tr>
<tr>
<td>3.11.53</td>
<td>24.11.53</td>
<td>21.11.53</td>
<td>24.11.53</td>
</tr>
</tbody>
</table>

* Assuming D.A.R. = 1, effective half-life 1.5 days.
Figure 10  Case 2. Model of mass removed from neck, showing method of slicing. The position of the trachea is seen displaced to the right. Further to the right lies the displaced right lobe of the thyroid, infiltrated with carcinoma. The mid-line of the neck would have run near the figure "5" on the centimetre scale.

Figure 11  Case 2. An incidental finding in a section from a region of low radioactivity: mass of tumour occupying the lumen of a large vein.
Specimen.

The whole primary tumour was received as a single mass together with the remaining thyroid gland. Its weight was $16.4 \text{ gm.}$ The right lobe of the thyroid, although infiltrated by carcinoma, was distinguishable on gross examination, and was divided from the rest of the specimen after a model in plaster-of-paris had been made. Residual tissue from the other parts of the thyroid were embedded in the tumour, but were only found when shown up by their more intense autoradiograph. The specimen was divided into 8 slices (Fig. 10), fixed in acetic acid/alcohol mixture, dehydrated and cleared. Autoradiographs were made for $\gamma$-ray measurements before embedding, which was finally done and $\beta$-ray autoradiographs were made as in the previous case.

Shrinkage was measured by comparison of the slices with the plaster model, and was found to have been $27\%$ in linear dimensions.

The routine pathological report by Dr. J.H. Dean was: "small acinar and tubular cubical-celled carcinoma of thyroid." Evidence of invasion of a blood vessel was found in one of the sections prepared for autoradiography (Fig. 11).
Figure 12 Case 2. Slices of the tumour embedded in wax, with corresponding $\beta$-ray autoradiographs. Note uneven distribution of radioactivity. The two arrows indicate the regions selected for measurement of the $\gamma$-ray dose.
Beta-ray Autoradiography.

Beta-ray autoradiographs of the slices of the tumour are reproduced in Fig. 12B, with photographs of the corresponding blocks. The irregular distribution of the radioactivity is clear. There is agreement between some features of the autoradiographs and the photographs. Even in areas which are definitely active, the degree of activity, and therefore the $\beta$-ray dose rate, varies widely. (The round calibration spots represent doses in the ratios $1:2:4$). There are also considerable areas outside the range of $\beta$-rays from any active regions, and the points marked "X" (where there is no detectable radioactivity) and "Y" (where there is very little) have been selected for measurement of the $\gamma$-ray dose. Photomicrographs from these two regions and from a radioactive region are shown in Fig. 14.

Autoradiographs of the portion removed as right lobe of thyroid, which was very much infiltrated by tumour, are shown in Fig. 13. Fig. 13B was made with the same exposure as Fig. 12B and shows the activity of the infiltrating tumour as well as the much higher activity of the thyroid tissue, which is more than enough to blacken the film completely. Fig. 13A was made with one seventeenth of the exposure, and still shows dense, though variable, autoradiography by the thyroid tissue; but the infiltrating tumour gives no
Figure 13. Case 2. $\beta$-ray autoradiographs of right lobe of thyroid and infiltrating carcinoma.

A. Exposure sufficient to show uptake by thyroid but not by carcinoma.

B. Exposure 17 times A, and the same as Fig.12. Thyroid tissue completely blackens film and gamma-rays blur outlines; carcinoma shown up in detail by its own beta-rays.
Figure 14 Case 2.

Photomicrographs of different regions of tumour. H & E.

A. Region X (no radioactivity) Necrotic. x 120.

B. Region Y (slight radioactivity).
   Alveolar carcinoma. x 120.

C. Typical structure in radioactive parts.
   Follicular carcinoma. x 97.
Figure 15 Case 2. Photomicrographs of sections from residual thyroid.

A, B: adjacent fields, with autoradiograph superimposed. Note the high activity of a group of large follicles, which is an unusual feature.

C. Plain section stained H & E. Position in block corresponds to B, but a number of sections away. All x 97.
detectable autoradiograph. It is interesting to note that, if one had only this autoradiograph to study, one might conclude, wrongly but with some justification, that all the activity was concentrated in residual thyroid tissue, and that radio-iodine therapy would not be worth while. The figures for differential absorption ratio (Table V, p. 15) would rather support this conclusion. Yet in this case, metastases in the lungs and bony pelvis responded dramatically to radio-iodine therapy when the thyroid gland had been removed, and an unsuspected metastasis in a limb bone was demonstrated by its uptake of radio-iodine.

Stripping film autoradiographs were made with sections of the thyroid tissue, and photomicrographs are reproduced in Fig. 15. They show the well-known spotty distribution over follicles containing colloid. An unusual feature is the very dense autoradiograph over a few of the largest follicles. Very active small follicles are also seen, as is usual in normal thyroid tissue.
Figure 16  Case 2. Gamma-ray autoradiograph of slice number III. Absorber 2.3 mm celluloid. Exposure 6.2 times the β-ray autoradiograph of Fig. 12B.
Gamma-ray Measurements.

A number of exposures with different absorber thicknesses were taken as before; and the doses calculated for the points "X" and "Y" in Fig. 12B. Small blocks of tissue were taken from these positions after the measurements had been made, and these showed that "X" lay in necrotic tumour. "Y", however, where there was very slight uptake of the radio-iodine, lay in an area of alveolar carcinoma (see Fig. 14B).

The contributions of the five slices were added as before, and yielded the values:

\[
\begin{align*}
X & \quad \ldots \quad 24 \text{ roentgens} \\
Y & \quad \ldots \quad 21 \text{ roentgens}.
\end{align*}
\]

These values are somewhat below the calculated value for a sphere of the same average activity, namely 34 r at the centre. While a difference of this order, after calculations involving the errors inherent in extrapolation and in adding a number of quantities each with some uncertainty, does not demand special explanation, it may be remarked that this tumour contained remnants of very much more active thyroid tissue. These were entirely near one edge, and therefore contributed less \(\gamma\)-radiation to points near the centre of the mass than would have been the case if they had been distributed, even irregularly, throughout it.
TABLE VIII.

Carcinoma of Thyroid. Operation Specimens.
Calculated $\beta$- and $\gamma$-ray doses of radiation and measured $\gamma$-ray doses.

In each case 25mC of $^{131}$I was given by mouth two days before operation. The activities are referred to the day of operation, and all doses are quoted as those due to complete radioactive decay of activity present on that day.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Weight (grams)</th>
<th>Total Activity ($\mu$C/cm)</th>
<th>Specific Activity ($\mu$C/gm)</th>
<th>Calc mean $\gamma$-ray dose (rep)</th>
<th>Measured $\gamma$-ray dose region roentgens</th>
<th>Calc $\gamma$-ray dose at centre of figure of equal volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary tumour</td>
<td>5.0</td>
<td>207</td>
<td>41</td>
<td>5200 centre</td>
<td>400</td>
<td>340 (sphere)</td>
</tr>
<tr>
<td>Metastasis</td>
<td>13.9</td>
<td>190</td>
<td>14.4</td>
<td>1200 centre</td>
<td>100</td>
<td>100 (ellipsoid)</td>
</tr>
<tr>
<td>Case 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larger mass</td>
<td>139</td>
<td>190</td>
<td>14.4</td>
<td>175*</td>
<td>24</td>
<td>34 (sphere)</td>
</tr>
<tr>
<td>(carcinoma)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smaller mass</td>
<td>5.6</td>
<td>366</td>
<td>14.6</td>
<td>500</td>
<td>21</td>
<td>210 (sphere)</td>
</tr>
<tr>
<td>Thyroid tissue</td>
<td>13</td>
<td>349</td>
<td>26.8</td>
<td>3400 maximum</td>
<td>420</td>
<td>280 (ellipsoid)</td>
</tr>
<tr>
<td>(estimated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: In three instances the calculations have been for a spherical mass of the same volume; in the two most elongated of the masses, the calculations have been made for the nearest ellipsoid of revolution, using the formula of Parker (1947). Even in these cases the difference between the ellipsoid and the sphere is only about 10% which shows that the refinement is not necessary for the less elongated specimens.

A.F. Phillips
Sept. 1953
Films were also taken and the γ-ray dose worked out for the smaller mass containing the right lobe of the thyroid, and the value of 420 r was obtained, compared with 3400 rep for the mean β-ray dose. These figures are also included in Table VIII.
Case 3. Carcinoma of the thyroid.

Clinical Notes.

The patient was a housewife from Lincolnshire aged 58. She was first seen at the Radiotherapeutic Centre in September, 1952, complaining of a swelling in her neck. This was first noticed 4 years before, but caused her no trouble until the swelling had increased rapidly and became very tender and inflamed in the last 10 days. She had been admitted to hospital and treated with penicillin, and the inflammation had resolved.

On examination, there was a hard smooth swelling of the left lobe and isthmus of the thyroid gland, which was not tender, and moved on swallowing. The patient was euthyroid. Clinically the tumour was regarded as benign, and a biopsy showed part of a circumscribed edge of tumour consisting of high columnar cells in papillary arrangement, with no evidence of malignancy. By the time investigations were complete, the patient seemed well, and was not troubled by the swelling in her neck.

She was seen regularly during the following year, and the swelling very slowly enlarged, and became less mobile. In September, 1953, a tracer dose of radio-iodine gave a maximum uptake in the neck of 28%.
Figure 17  Case 3. Plaster model of tumour (from behind).

Figure 18  Case 3. Half of the tumour, embedded in wax and faced up. The autoradiograph of this specimen is shown in Figure 21.
At that time it was decided to carry out thyroidectomy, and \(^{131}\text{I} \), 5.9 mc. was given by mouth on 7.10.53. Two days later, she was operated on by Mr. P.H.R. Ghey. The normal right lobe was first removed, and then the greatly enlarged left lobe and isthmus. There was some adhesion to the left carotid sheath, and left side of trachea, and removal of the tumour was probably not quite complete. A small lymph node was also removed from the lateral side of the left external jugular vein. Mild post-operative tetany was easily controlled.

After operation, on 21.10.53 and again on 24.11.53, uptake in the neck from test doses of radio-iodine (23 mc.) was small, of the order of 1%. There was no evidence of selective uptake in other parts of the body. In view of these results, and those of autoradiography of the tumour, post-operative radiotherapy was administered with the radio-iridium unit (Freundlich & Haybittle, 1953), as there was no prospect at that time of achieving a tumour lethal dose by means of radio-iodine.

She has remained well, with no evidence of recurrence (15.10.54) on a maintenance dose of thyroid. A radio-iodine uptake test in September, 1954, showed under 1% uptake in the neck at 24 hours (possibly salivary glands), and on scanning the whole body no abnormal localized uptake was found.
Figure 19  Case 3. The two types of structure seen in the tumour. H & E. x 120.

A. Papillary.
B. Alveolar.
Specimens.

Three masses were removed at operation:

(1) The right hemi-thyroid
(2) The tumour with remnants of the left hemi-thyroid
(3) A small lymph node.

Plaster-of-paris models were made. No radioactivity was found by Geiger counter examination of the lymph node. The right hemithyroid was very active. The tumour showed considerable radioactivity, which on careful examination by means of the Geiger counter, was found to be concentrated in one region. It was possible to dissect out from this region a piece of tissue which proved to be normal thyroid, and this was separated from the main mass. The tumour now showed only a small specific activity. It was divided in half, and the two halves were immersed in formalin in the perspex box for γ-ray autoradiography. The right and left hemithyroids, each cut in half, were fixed in formalin, dehydrated, and embedded in paraffin wax.

Eventually, half of the tumour was also embedded after being divided into three slices parallel to the first cut. Over 99.5% of the activity was found to be in the slice nearest to the first cut.

The activity extracted by the fixative and other fluids was followed in detail for each of the specimens.

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Duration of action</th>
<th>Activity Extracted per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rt. Lobe</td>
</tr>
<tr>
<td>4% formaldehyde</td>
<td>14 hrs</td>
<td>10.7</td>
</tr>
<tr>
<td>4% formaldehyde</td>
<td>14 hrs</td>
<td>8.1</td>
</tr>
<tr>
<td>50% alcohol</td>
<td>24 hrs</td>
<td>1.3</td>
</tr>
<tr>
<td>70% alcohol</td>
<td>24 hrs</td>
<td>0.5</td>
</tr>
<tr>
<td>96% alcohol</td>
<td>24 hrs</td>
<td>0.09</td>
</tr>
<tr>
<td>absolute alcohol</td>
<td>24 hrs</td>
<td>0.03</td>
</tr>
<tr>
<td>absolute alcohol</td>
<td>24 hrs</td>
<td></td>
</tr>
<tr>
<td>xylene</td>
<td>24 hrs</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Total extracted 21.4% 13.7%
The proportions extracted from the two main portions of residual thyroid tissue are shown in protocol 6. Although rather different from each other, these proportions are on the whole similar to those found experimentally for a rat thyroid (protocol 1, p. 36-3).

There was no shrinkage of the tumour during immersion in formalin. During subsequent dehydration and embedding, the shrinkage was about 10% in linear dimensions.

**Beta-ray autoradiography.**

The faced blocks of the two lobes of the thyroid gland gave autoradiographs of the well-known spotty type (Fig. 20). The piece dissected off the tumour, and presumed to be the residue of the original left lobe, gave just as intense an autoradiograph as the normal right lobe. In fact, one of its two pieces gave the most intense autoradiograph of the four (upper right-hand corner of Fig. 20).

Sections from these blocks showed no gross infiltration with tumour, but two tumour emboli in blood vessels were seen. The sections consisted of normal thyroid tissue. The most radioactive one differed very slightly from the others in having more
Figure 20 Case 3. Beta-ray autoradiographs of slices of right lobe of thyroid, and residue of left lobe.
Figure 21  Case 3. Autoradiographs of the tumour, showing only two isolated regions of activity embedded in the mass. Natural size.

A. Beta-ray autoradiograph from faced block.

B. Gamma-ray autoradiograph from half the tumour, at 1.5 mm. Specimen in formalin. Exposure 9 x that of A.
strongly eosinophilic colloid, and when stained with van Gieson's stain, showed more interstitial collagen.

The slices of tumour which were embedded, showed no general uptake of radio-iodine. The only autoradiograph was due to two isolated regions of activity in one of the slices (Fig. 21A). These two regions had approximately the same activity as the thyroid gland itself, and were, no doubt, surviving fragments of the left lobe, separated by tumour. Exposures up to 250 times as much showed nothing else except the faint outline of the block, and gave no indication of selective uptake by tumour tissue. The ratio of activities is of the order of 10,000:1.

The block of the small lymph node was also placed on X-ray film for a very long exposure, and in this case some small regions of activity were seen at points on its periphery (Fig. 22). Comparison with stained sections makes it appear that these regions correspond to more vascular regions and in one case to a small haemorrhage. Near these regions, however, were seen several minute foci of metastatic tumour growth (Fig. 23), and it is not possible to be certain whether the greater vascularity or the presence of tumour foci accounted for the small but definite degree of radioactivity.

The radioactive uptake of the tumour tissue
Figure 22 Case 3. Lymph node containing minute iodine-concentrating metastases.
A. Photograph of section, x 4.
B. X-Ray film autoradiograph (natural size). (See also Figure 23).
Figure 23  Case 3.

A. Photomicrograph from lymph node at a position corresponding to autoradiograph, showing small metastatic deposit. H & E. x 190.

B. The same at higher magnification, showing mitotic figures. x 550.
(both primary and metastatic) was certainly too small at that time to give hope of useful therapeutic effects, and post-operative radiotherapy was therefore given by external radiation. However, the differentiated structure of the tumour, the fact that it had metastasized, and the possibility that slight selective uptake had taken place even in presence of a functioning thyroid gland, were strong indications for periodical tracer doses of radio-iodine as one method for early detection of recurrence in the neck or elsewhere.

**Gamma-ray measurements.**

The autoradiographs for γ-ray measurements were made by the simplified technique described above, in which the specimen was immersed in formalin in a perspex box, and the whole was placed on the X-ray film. Two spacings were actually used: the bottom of the box only, namely 1.5 mm., and the same with the addition of 0.84 mm. of celluloid. Ten films in all were exposed, with various times of exposure so that some gave convenient densities for the most intense spot, and others for the region in between the two active spots. A typical γ-ray autoradiograph of one half of the
tumour is shown in Fig. 21B, compared with the β-ray autoradiograph of the embedded slice taken from the same face. It is obvious that practically all the activity is concentrated at the two small active regions. γ-ray doses have been calculated from the densities found with the two spacings, by extrapolating to zero distance using the simplified method described in Chapter VII. Three points have been taken: (1) the most active region (2) the second active region, and (3) the minimum on a straight line joining (1) and (2). The corresponding total γ-ray doses were:

(1) 190 roentgens  
(2) 12 roentgens  
(3) 5.1 roentgens  

due to 5 mc. administered.

Obviously a radiation dose of 1 roentgen per milli-curie administered is of no therapeutic value, but it is of academic interest to note that the ratio of γ-ray doses between region (1) and region (3) is 35:1, whereas the ratio of activities (and therefore of β-ray doses) is of the order of 10,000:1.
Clinical Notes.

The patient was a housewife from Northamptonshire, aged 43 when first seen at the Radiotherapeutic Centre, in February, 1951. She complained of a swelling in her neck which had been enlarging for one year, and of recent dyspnoea and dysphagia.

She first noticed a lump in her neck during adolescence. It enlarged and caused difficulty in respiration during her only pregnancy, at the age of 31; this was relieved by operation. A diagnosis of simple colloid goitre had been made.

On examination she showed clinical signs of mild thyrotoxicosis, and the thyroid was enlarged and hard, with a rounded swelling 3 cm. in diameter in the midline. A small hard lymph node was palpable in the right lower deep cervical group.

During some delay caused by the necessity of dental extractions, the skin over the midline swelling in the neck broke down, discharged the contents of a cyst, and healed. Her symptoms were relieved, and there was no appearance to suggest malignancy in the thyroid. She remained well until February, 1952, when the left lobe of the thyroid increased in size and caused hoarseness and dyspnoea. The trachea was
markedly compressed from side to side. A tracer dose of radio-iodine showed maximum uptake of 49%, with very slow rate of excretion. \( \text{I}^{131} \), 0.56 mc. was administered 28 hours before operation, and of this 56% was retained in the thyroid.

At operation on 8.4.52, left hemithyroidectomy was carried out. The gland did not appear malignant. The pathological report was nodular colloid goitre, with no evidence of malignancy. A portion of the gland was taken for autoradiographic study. Measurement of the radioactivity of the specimen removed indicated a total dose of approximately 700 rep if it had remained in the body. Measurement of the total uptake in the gland showed a lower average activity (see Table V, p. 96) and the radiation dose in the part left in situ was probably in the region of 350 rep. It is unlikely that this contributed significantly to the clinical improvement which followed the operation. Her symptoms were completely relieved and she put on 10 lb in weight during the next nine months. She remains quite well.
Figure 24  Case 4.  Autoradiograph superimposed on section, stained H. and E, showing different degrees of radioactivity in different parts of the section.

A. x 4½: showing the three main areas.

B. x 55: field includes the dividing septum and portions of the areas of low and intermediate activity.
Autoradiography.

The specimen was fixed in acetic acid/alcohol mixture, dehydrated in absolute alcohol, cleared in benzene, and embedded in paraffin wax. The sections unfortunately were not very good. Fig. 24A is a photograph at low magnification of a whole section with the stripping film autoradiograph superimposed. In spite of dust and other imperfections, it is possible to see from the photograph, what is very clear under the microscope, that there is much more blackening of the film on one side of the section than on the other. There is a thin connective tissue septum between these two main areas. A third small area lying at one end of the junction between the two, has even darker autoradiograph. A rough estimate of the relative uptake was made by densitometer, and gave the ratios 1:4:6 for the three areas.

Seen under the microscope, the autoradiograph shows the usual spotty distribution of activity, with variation of activity partly correlated with size of follicle and partly random. Some typical photomicrographs are shown in Figures 24B, 25.

In a stained section, it is possible to make out histological differences between the three areas. Although there is variation in each, on the whole the follicles are largest in the least radio-active area,
Figure 25  Case 4. Autoradiographs to show the differences of radioactivity between the different regions of the specimen. All photomicrographs from the same slide and therefore identical autoradiographic conditions; the same exposure for negative and print on reproduction; and the same magnification (x 100 approximately)
Figure 26. Case 4. Photomicrographs from section stained H & E (without autoradiograph) to show correlation of histological structure with activity. x 157.

A. Involutional
B. Intermediate
C. Hyperplastic.

The activity increases in this order; compare figure 25A, B, C.
and smallest in the most active. The height of the follicular epithelium is greatest in the most active area, and smallest in the least active. There is also some epithelial hyperplasia in the most active area. This is consistent with accepted views on the association of great functional activity with hyperplasia, and lack of activity with involution (Rundle, 1951). Photomicrographs from representative regions of each area are shown in Figure 26.
Case 5. Carcinoma of oesophagus (post-cricoid); secondary extension to thyroid.

Clinical Notes.

The patient was a housewife aged 64, and was first seen at the Radiotherapeutic Centre in March 1952. She complained of tiredness and swellings in the neck for two months, and recent slight dyspnoea. There was no relevant previous history.

On examination, there were enlarged hard lymph nodes on each side of the neck, and a hard tumour related to the isthmus of the thyroid. Laryngoscopic examination showed no abnormality. X-ray examination showed compression of the trachea both from side to side and antero-posteriorly.

25 mc. of $^{131}I$ was given by mouth on 25.3.52, of which 14% was found to be concentrated in the region of the thyroid after 24 hours. On 27.3.52 a biopsy was taken, and part of this specimen was used for autoradiographic study.

Dyspnoea and dysphagia developed rapidly, and in spite of palliative radiotherapy the patient died on 1.4.52.

Pathological examination of the biopsy specimen showed that the thyroid was infiltrated by squamous carcinoma. Post mortem examination showed that the
carcinoma had origin in the post-cricoid region of the oesophagus, involving secondarily the trachea and thyroid, as well as cervical lymph nodes.

**Autoradiography.**

A number of autoradiographs were made by the "stripping film" technique, and also by the "coated autograph" technique, to show electron tracks. The specific activity of the specimen was high (90 μc/gm) and short exposures of only a few hours were required. As would be expected, radioactivity was found only in the remaining thyroid tissue. The usual picture of variation of activity was found in the areas where thyroid structure was seen. Occasionally a small spot of blackening was seen amongst the carcinoma cells, but always closer examination showed thyroid follicles underlying it. Figures 27, 28, 29, show three different fields with their autoradiographs superimposed, and beside them the corresponding field from the next section, from which no autoradiograph was made.
Figure 27 Case 5. Photomicrographs of corresponding fields in adjacent sections. A. with autoradiograph superimposed. B. plain section. H. and E. x 120. This area is from thyroid tissue not directly affected by carcinoma.

Note  (1) general tendency for smallest follicles to have highest activity.
(2) spread of autoradiograph of darkest follicles due to range of β-rays.

Figure 28 Case 5. Same conditions as Fig. 27. In this field, the thyroid is invaded from the left by carcinoma. The autoradiograph is only present over thyroid follicles.

Figure 29 Case 5. Same conditions as Fig. 27. This field consists mainly of carcinoma, but a small group of follicles near the centre contain colloid and have concentrated the radio-iodine.
**Table IX.**

Six Specimens containing Radio-Phosphorus, and Differential Absorption Ratios

<table>
<thead>
<tr>
<th>Case</th>
<th>Biopsy Type</th>
<th>Body Weight (Kg)</th>
<th>Activity Admin. (mc)</th>
<th>Body Weight (mc/Kg)</th>
<th>Activity Mean activity of specimen (μc/gm)</th>
<th>Mean D.A.R. corrected for decay</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Malignant Melanoma, axillary lymph nodes</td>
<td>84</td>
<td>9.4</td>
<td>0.11</td>
<td>0.62</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.22</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.09</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pieces for autoradiography not included in these measurements</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Biopsy of lymph node</td>
<td>65</td>
<td>5</td>
<td>0.077</td>
<td>0.42</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adjacent piece taken for autoradiography</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Malignant haemangioendothelioma, biopsy of lymph node</td>
<td>60</td>
<td>10</td>
<td>0.17</td>
<td>0.68</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adjacent piece taken for autoradiography</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Metastasis from carcinoma of ovary</td>
<td>52.5</td>
<td>7.8</td>
<td>0.15</td>
<td>&lt;0.3</td>
<td>&lt;2 (in vivo measurements)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Squamous cell carcinoma</td>
<td>72</td>
<td>5</td>
<td>0.069</td>
<td>not measured</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Mycosis fungoides</td>
<td>70</td>
<td>2.2</td>
<td>0.031</td>
<td>&lt;0.15</td>
<td>&lt;5 (in vivo measurements)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER IX.

CASES INVESTIGATED WITH RADIOACTIVE PHOSPHORUS AND WITH RADIOACTIVE COLLOIDAL GOLD.

Particulars of the specimens obtained in the 6 cases treated with radio-phosphorus are given in Table IX, with the activity administered, and the activity of the specimens and differential absorption ratio where known. Owing to self-absorption of the \( \beta \)-rays of \( P^{32} \) in the specimen, measurement of the activity is more difficult and less accurate than in the case of \( I^{131} \), which is measured by means of its \( \gamma \)-rays.

The specific activities of the specimens were all below the level of 1 \( \mu c/gm \), which, as discussed in Chapter II, is required for a satisfactory stripping film autoradiograph. Nevertheless, microscopical autoradiography was attempted in each case by this technique, and sometimes also by the electron track technique, in the hope that some great departure from uniform distribution might give a positive result. In fact, however, none of them had sufficient activity to give an obvious autoradiograph. In several cases, it was possible by counting the silver grains, to demonstrate a slight increase over the natural background of the emulsion, and even to suggest higher activity over tumour than over adjacent normal tissue.
Microscopical autoradiography, therefore, added to what was already known from Geiger counter measurements either on the specimen or in vivo, the information that there was no marked differential uptake of $^{32}P$, by particular cells or groups of cells within the tumour. Selective uptake by the tumours as a whole varied from a D.A.R. of less than 1, to a value of about 5. Geiger counter estimates of the activities of the different specimens are given in the Table.

In Case 6, autoradiography by means of X-ray film gave a definite result. This technique was not applied to the other specimens.
Case 6. Malignant melanoma.

Clinical Notes.

The patient was a man aged 74, a minister of religion. He was first seen at the Radiotherapeutic Centre in July, 1953. 18 months before, a wart had been removed from the right wrist, and 10 months afterwards, the right epitrochlear lymph node had been removed. Sections showed malignant melanoma. When seen at the Radiotherapeutic Centre, there was a mass 5 cm. in diameter deeply situated in the right axilla. There was no evidence of other metastases.

Radical X-ray therapy was given, and the mass reduced greatly in size. Another lump appeared, outside the previous X-ray field, in November, 1953. Surgery, combined with investigation of radio-phosphorus uptake with a view to treatment of possible future recurrences, was decided upon. Accordingly, on 9.12.53, 9.4 mc. of $^{32}$P was given by mouth, and 24 hours later a block dissection of the axilla was made by Mr. V. Pennell. A black malignant mass was removed which consisted of a number of cystic nodules and a cyst 5 cm. in diameter with dark brown contents under tension. Autoradiographic studies of this specimen were made in collaboration with Miss R.D. Saunders, Hospital Physicist, who also made estimates of the differential
absorption ratio by chemical extraction of parts of the specimen. These investigations showed that the D.A.R. varied considerably, and although well above unity in places, was not high enough to give good prospects of success by treatment with $^{32}$P.

Two months after operation the patient was well, with no evidence of recurrence; but a month later he died, without being seen here again.

**Autoradiography.**

The specimen from operation was a mass of fat containing a number of lymph node metastases from the melanoma. Some of the metastases were cystic, the largest cyst being 5 cm. in diameter, and contained dark-coloured fluid under tension. Others were small brown nodules.

Macroscopic autoradiography was carried out on the specimen while fresh. As well as causing delay, fixation might have extracted a large proportion of the activity, while embedding in paraffin wax would have involved great alteration of the geometry of the specimen owing to the large amount of fat. The specimen was therefore sliced (losing some of the cyst contents) and the larger portion laid on X-ray film, protected by
Figure 30 Case 6. Autoradiograph from fresh specimen. Natural size.
a thin sheet of aluminium (7 mgm/sq.cm.). The autoradiograph obtained is reproduced in Figure 30. As far as could be determined by naked eye comparison with the specimen, the regions corresponding to greatest activity were those which were both melanotic and vascular. Most of the areas consisting apparently of fat gave no autoradiograph, though one fatty area gave a diffuse autoradiograph. Blocks were taken afterwards from this and three other marked areas for histological examination. These proved to have suffered much autolysis. However, it was clear that the spots of high activity were melanotic tumour tissue, and the completely inactive areas were normal fat. The marked area of diffuse activity was adipose tissue in which were deposits of brown pigment, but it was not possible to demonstrate tumour infiltration with certainty because of autolysis. The pigment was not haemosiderin.

From the other part of the original specimen, two blocks were taken, and fixed in acetic acid/alcohol mixture about 1½ hours after removal from the patient. These were (1) a wedge from the wall of a large cyst, and (2) the whole of a small cystic nodule, cut open. After fixation, these pieces were dehydrated and embedded in paraffin wax. Sections were cut for stripping film autoradiography, and the faced blocks were used for further autoradiographs on X-ray film.
Figure 31 Case 6.

Sections stained H & E. x 110.

A. Solid tumour, region of high radioactivity.

B. Fat infiltrated with melanoma. Region of intermediate activity.
In the stripping film autoradiographs, the melanin deposits confused the picture, but it was possible to show by counting the silver grains under the oil immersion objective, that there was activity definitely above background over the specimen where tumour cells were present, and there appeared to be most silver grains where the melanin was most abundant. The autoradiograph was still present, but reduced in intensity, after Feulgen staining.

The faced blocks gave satisfactory autoradiographs on X-ray film, and comparison with stained sections confirmed the indications from autoradiography of the fresh specimen, namely that the highest activity was in solid melanomatous tissue, and that there was intermediate activity in infiltrated fat (Fig. 31).
Case 7. Eosinophil granuloma.

Clinical Notes.

This patient, a farmer aged 45, was under treatment at the Radiotherapeutic Centre from 1948 until 1952 when he died. He was also treated at other hospitals before and during this period. The presenting symptom was enlargement of lymph nodes at the back of the neck. During the following year, other nodes enlarged, in the inguinal and epitrochlear groups on each side, and skin lesions began to appear, first in the groin. Then the left upper eyelid became oedematous and indurated, and gradually the skin of the face, especially the nose, became similarly involved. Lymph nodes enlarged further, and oedema of the legs indicated involvement of pelvic nodes. Subcutaneous tumours developed on the dorsum of each hand, and erythema on the trunk and legs. Chest X-ray showed an enlarged right hilar gland, and a right pleural effusion. Considerable enlargement of liver and spleen developed gradually.

Blood count showed a constant eosinophilia of 40% - 45%, with normal or moderately raised total white cell count, and a slight normochromic anaemia. Examination of sternal marrow showed eosinophilia only, with no evidence of leukaemia.
Figure 32 Case 7.

A. Skin lesion, 1950. Low power, showing the thickness of skin and subcutaneous tissue involved. (Dr. Whittle's photomicrograph: Whittle, Lyell & Church, 1950).

B. High power of the same, showing primitive cells with abundant cytoplasm and reticulum fibres. (Dr. Whittle's photomicrograph).

C. Lymph node (1952). (Cytological fixation poor by special fixative used for autoradiography). x 490.
Biopsy of several lymph nodes, at different times, showed infiltration with eosinophils, and marked general and intimal thickening of arterioles. Biopsies of the skin lesions of the eyelid and hand (Fig. 32A,B) showed, in contrast, few eosinophils, but cellular infiltration of the dermis and epidermis, with frequent mitotic figures, and reticulin fibres suggestive of reticulo-sarcoma. However, a later biopsy of a skin lesion showed many eosinophilic cells, more consistent with eosinophil granuloma than with any form of neoplasm. From the appearance of the skin lesions the diagnosis of leprosy was considered at one time, but staining for acid-fast bacilli was negative.

The case was shown by Dr. C.H. Whittle to the Section of Dermatology of the Royal Society of Medicine under the title "? Reticulosarcoma, ? Eosinophilic Granuloma". (Whittle, Lyell & Church, 1950).

The skin lesions and superficial lymph nodes were radiosensitive, and for some time the disease was kept under control by X-ray doses of 500 r (220 kV or 100 kV, with 1 mm. aluminium filtration) to the most affected regions. Irradiation of the trunk and pelvis, with large fields, resulted in reduction of size of liver and spleen, and improvement of the lymphatic drainage of the legs.
Other treatments which gave temporary benefit were antihistamine drugs, ACTH, and cortisone.

Finally in February 1952, when his general condition was deteriorating, a single therapeutic dose of P$_{32}$, 5 mc. was given, followed by biopsy of a lymph node after 24 hours to assess uptake. A photomicrograph is reproduced in Figure 32 C. The differential absorption ratio was determined by Geiger counter measurements on part of the specimen (Mr. J.L. Haybittle), and was found to be 5.7. In view of the high radiosensitivity this was a most encouraging observation, and a fortnight later the glands were definitely reduced in size. A second dose of 5 mc. of P$_{32}$ was administered, but the patient died before this had time to take effect.

**Autoradiography.**

Part of the lymph node containing P$_{32}$ was used for autoradiography. The specimen was fixed in acetic acid/alcohol mixture, dehydrated, and embedded in paraffin wax. The initial activity was 0.4 µc/gm., and no measurement was made of the activity extracted during fixation. Sections were cut at 5µ, 10µ, and 20µ, and autoradiographed by the stripping film
technique. 10μ and 20μ sections exposed for two half-lives showed a slight increase of silver grains over the sections, compared with the background. The increase seemed to be more marked over the stroma than over the solid masses of tumour cells. The activity was not high enough to put these observations beyond question.
Case 8. Malignant haemangio-endothelioma with multiple secondaries.

Clinical Notes.

The patient was a housewife, aged 31 when first seen at the Radiotherapeutic Centre in October, 1948, at which time she was treated for chronic corneal ulceration. The condition was greatly improved by small doses of X-rays, and she was discharged.

In October, 1952, she was again referred with a diagnosis, confirmed by biopsy, of malignant haemangio-endothelioma. There was evidence of deposits in liver, right humerus, brain, skin, lymph nodes of neck and axillae, and possibly adrenals. A trial of radio-phosphorus treatment was made: on 16.10.52, 10 mc. of P32 was given by mouth, and 24 hours later an enlarged lymph node was removed from the right side of the neck. A small nodule was also removed from the skin in the same region. Activity measurement by a Geiger counter on part of the lymph node showed a differential absorption ratio of approximately unity, but unfortunately the node did not show the histological structure characteristic of the general condition. The activity of the much smaller cutaneous nodule was not measured by the Geiger counter, but autoradiographic examination did not show any greater uptake than in the lymph node.
Figure 33 Case 8. Cutaneous nodule.
Section stained H & E. x 110.
The cutaneous nodule was haemangio-endothelioma (Figure 33).

A week after administration of radio-phosphorus, there was a definite reduction in the size of the liver, but no other change. Palliative treatment was continued by conventional radiotherapy. A further treatment with P³², 9.5 mc. was given on 10.12.52.

Ascites developed a month later, which after a time had to be relieved by paracentesis. No malignant cells were found in the ascitic fluid, which was not blood-stained. During the next few months the ascites became the most distressing symptom, and repeated paracentesis was necessary. Mercurial diuretics had little effect on the rate of collection of fluid, and injections of radioactive colloidal gold into the peritoneal cavity did not improve the ascites, though they may have arrested the growth in the liver.

The patient left the district, and routine follow-up by letter in April, 1954, yielded the information that the patient was alive, and better in health, though still troubled by ascites. In October 1954, however, her doctor wrote that she had oedema, ascites, and dyspnoea, and that her condition was deteriorating.
Figure 3h. Case 9. Biopsy of fungating growth. Section stained H & E. x 500.
Autoradiography.

Stripping film autoradiographs were made with sections cut at 5μ and at 10μ from each of the specimens. Over parts of the 10μ sections of the lymph node an autoradiograph was demonstrated by counting grains. This amounted to $8 \pm 4$ grains per 100 $μ^2$ above the background level of 4 grains per 100 $μ^2$; only the most densely cellular areas were above the background level. A count of 8 grains per 100 $μ^2$ in 2 half-lives with a 10μ section would correspond, on the calculation of Chapter II, to an activity of 0.3 μc/gram. The mean activity, by Geiger counter, of the other half of the lymph node, was 0.17 μc/gram, which is quite consistent with a value of 0.3 μc/gm for the more active parts. Any close agreement must be regarded as fortuitous in view of the uncertainty in counting and the lack of precise information on the sensitivity of the emulsion.

With a marginal autoradiograph such as this, the resolution is extremely poor, and it is not possible to localize the activity to particular cells, but only to groups of cells.

In the cutaneous nodule, the endotheliomatous tissue also gave an autoradiograph of the same order of intensity at least in some areas. One could say with confidence that the grain density was probably as great, and was not much greater, than that in the autoradiograph of the lymph node.
Case 9. Carcinoma of the ovary; secondary carcinomatosis of peritoneum and skin.

Clinical Notes.

The patient was a married woman aged 57. She was first seen at the Radiotherapeutic Centre in May 1952. Three weeks previously a carcinoma of the left ovary had been removed at operation, but blood-stained ascites had been found and many secondary nodules in the pelvic peritoneum had been left. The pathological report (Dr. J.H. Dean) was: "Tubular and papillar columnar celled carcinoma.....".

A radical course of X-ray treatment was administered to the pelvic region, and the patient remained well until January, 1953. At this time a lump appeared at the umbilicus, which was poulticed at home, and ulcerated. When seen, she had a large fungating growth, and the abdomen was distended with ascitic fluid.

P³², 7.8 mc. was given by mouth on 9.2.53 and 24 hours later a biopsy of the umbilical tumour was taken for uptake measurement and autoradiography. Microscopic examination showed infiltration of the skin by acinar and solid spheroidal-celled carcinoma (Fig. 34). The differential absorption for radiophosphorus was approximately 2. In the meantime,
conventional radiotherapy was given to the tumour, which responded well.

Two weeks later other masses were palpable in the abdomen, and a lymph node in the axilla. The patient died in May, 1953.

**Autoradiography.**

The specimen (weight 0.03 gm.) was fixed in acetic acid-alcohol mixture, dehydrated and embedded in paraffin wax. Sections of thickness 5μ and 10μ were set up for stripping film autoradiography, and exposed for 2 half-lives. No autoradiographs were obtained.

The original activity was too low to expect anything but a marginal autoradiograph, on the basis of uniform distribution, and in this case over half the activity was extracted by the fixative.
Figure 35  Case 10.  Squamous cell carcinomatosis at site of old gun-shot wound.

Figure 36  Case 10.  Section stained Feulgen.  x 175.
Case 10. Squamous cell carcinoma of chest.

Clinical Notes.

The patient was a man aged 70, a machine minder, and was first seen at the Radiotherapeutic Centre on 23.5.52. He was complaining of a growth on the chest of 2 months' duration. The growth was at the site of an old gunshot wound (1915). He had had dyspnoea on exertion for 2 months and swelling of the left arm for one month.

The wound on the chest had been a glancing one, and had healed within a month. Eight years later there had been a discharge of pus from the wound, but again it had healed in a month, and had given no further trouble until the present.

On examination there was an extensive papilliferous lesion of the skin of the left chest, a large mass in the left axilla, and an enlarged lymph node at the root of the neck on each side. X-ray of the chest showed no evidence of pulmonary metastases.

A biopsy, carried out before he was referred, had shown a poorly differentiated squamous carcinoma, with abundant mitoses and microscopical evidence of lymphatic spread.

It was decided to investigate the possibility of treatment by radio-phosphorus, conventional
radiotherapy to the skin lesions and lymph nodes being started in the meantime. Accordingly, 5 mc. of \( \text{P}^{32} \) was given by mouth on 28.5.52, and biopsy specimens were taken from the chest lesion at 24 hours and at 48 hours after. Geiger counter measurements showed that there was not sufficient uptake to give hope of successful treatment by \( \text{P}^{32} \). Palliative radiotherapy was therefore continued by conventional methods, with satisfactory temporary response, and the patient was better for three months. Then new skin lesions appeared at the angle of the mouth and near the anus, and mediastinal obstruction developed. He died in December, 1952.

**Autoradiography.**

The two biopsy specimens were fixed in acetic acid/alcohol mixture, dehydrated, and embedded. Sections were cut at 8\( \mu \), and set up for stripping film autoradiographs, and for electron tracks by the "coated autograph" technique. In the coated autographs, the density of tracks was definitely raised over the epidermis of normal skin, but not over the dermis. Over the carcinoma, the autoradiograph was more marked than over normal skin, being highest over some
superficial groups of close-packed cells with abundant mitoses (Fig. 36). It was also marked over tissue showing an inflammatory response to invading carcinoma. The dermis underlying carcinoma was slightly active. Keratin and extravasated blood showed little or no activity. These features could be made out in both the 24-hour and the 48-hour specimens, and no difference was demonstrated between the two. The activity was too low to give clear autoradiographs by the stripping film technique.

Neither the original activity of the specimens nor the activity extracted by the fixative was measured in this case.
Case 11. Mycosis fungoides (from Dr. R. Braams).

Clinical Notes.

The patient was a railway guard aged 55 when first seen, in 1948, at the Dermatological Department at Utrecht. The disease was at a fairly early stage, with widespread isolated lesions in the skin, which were entirely superficial. Treatment by X-rays was followed by satisfactory remission.

In 1951 he was seen again with a recurrence of the disease and had very widespread lesions with infiltration of the skin and subcutaneous tissue. No lymph node involvement was detected. A course of X-ray treatment was not fully successful, and a second course was followed by severe leucopenia and ulceration of some of the lesions. The patient became very ill, but responded slowly to blood transfusions and antibiotics.

In February, 1953, it was decided to try radioactive phosphorus, and a test dose of 1 mc. of $P^{32}$ showed that the uptake was greater in the affected skin than in nearby healthy skin, by a factor which ranged from 3 to 7.5. The lesions most active clinically were the ones which took up the greatest amount of radioactive phosphorus, but they also lost it more quickly, and the calculated ratio of radiation dose between the lesions and healthy skin was nowhere better than 5:1.
Figure 37 Case 11.

Section stained H & E. X 230.
A slight clinical improvement was noted. Two further doses were administered, as follows:

13.3.53 ... 1.6 mc.
23.3.53 ... 2.2 mc.

A biopsy of a cutaneous nodule was taken 24 hours after the last administration.

The total radiation to the lesions due to the radio-phosphorus was estimated at only 50 rep, but nevertheless it was considered that this did cause a temporary arrest of the progress of the disease. The patient's general condition improved gradually, and a further course of X-ray treatment with soft X-rays of carefully calculated penetration, and small fields, caused a marked remission, and he was able to return to work.

He remained well until April 1954, when neurological signs appeared in the legs. His condition deteriorated rapidly, and he died from extensive visceral involvement only 6 weeks after giving up work. The skin remained in good condition.
Specimen.

A small piece of tissue from the cutaneous nodule was received from Dr. Braams on 26.3.53. $^{32}$P had been administered by mouth as follows:

- 13.3.53 ... 1.6 mc.
- 23.3.53 ... 2.2 mc.

He stated that about 30% of the activity was lost by excretion after each dose, and that the activity as measured in vivo by a Geiger counter was 5 times as high over this nodule as over normal skin. This is the only available estimate of the differential absorption ratio. Accepting these figures, and making allowance for the residual activity from the first dose of $^{32}$P, and for decay, an original activity in the region of 0.15 $\mu$C/gm. would have been expected in the specimen.

Some of the activity was found in the fixing fluid, but well below half of that originally present in the specimen.

In view of the low activity, the electron track technique was used for autoradiography, but no definite autoradiograph was obtained.
Figure 38 Case 12. Photomicrographs showing the two main types of structure seen in the tumour. Sections stained H & E.
Case 12. Carcinoma of the bronchus, treated surgically.

Clinical Notes.

The patient was a retired police officer, aged 56. He was first seen at the Nelson-Langemann Hospital, Papworth, in October, 1953, complaining of intermittent loss of voice during the past 9 months, shortness of breath on exertion for 6 months, and increasing tiredness. He had a chronic "smoker's" cough, and had gained 1 stone in weight during the past year. X-ray of the chest showed a shadow coming out from the hilum of the left lung. A first bronchoscopy yielded little information; at a second, a tumour was seen.

On 2/12/53, left pneumonectomy was carried out by Mr. C.F.A. Cummins, a tumour approximately 12 x 10 x 8 cm. being found in the lung. Pathologically it was a moderately well differentiated carcinoma. In the surrounding lung, and in a bronchial lymph node there were many foci of epithelioid cells, but no infiltration with carcinoma.

The post-operative course was uneventful, and the patient was discharged home. When last seen in April, 1954, his condition was satisfactory and he could walk over a mile without breathlessness.
Figure 39 Case 12. Autoradiograph of fresh specimen (natural size).

Figure 40 Case 12. A. The tumour embedded in wax and faced up. B. The corresponding autoradiograph (natural size).
Investigation with Au$^{198}$.

As soon as the operation was complete, the surgeon used the resected lung to make a test of the technique of implanting radio-active colloidal gold by injection. The radioactive suspension was injected into the tumour and surrounding tissue, as would be done therapeutically in a case which was found at thoracotomy to be inoperable. When this had been done, the tumour was cut in half, one part being examined at Papworth, and the other brought to the Radiotherapeutic Centre, where Mr. Haybittle laid it on X-ray film, with a protective layer, and obtained autoradiographs of which one is reproduced in Figure 39. The darkest area (right-hand side) corresponded to uninvolved lung.

The apparently uninvolved lung tissue was then cut away, and the tumour fixed and embedded in paraffin. Further autoradiographs were taken from the block after trimming and facing (Fig. 40). There are considerable areas of fairly uniform activity, but also quite large inactive areas. The tumour was not cystic, and the large clear areas seen in both of the figures corresponded to solid tumour into which the injected colloid did not penetrate. Elsewhere in the tumour the distribution, though not uniform, is no less so than, for example, radio-iodine in thyroid tumours.
If some improvement in the technique is possible, to avoid large untreated regions, considerable palliative effect might well be achieved, because of the very large mean dose which is attainable. Alternatively, it might be better to use an isotope of which the radiation is more penetrating.
THE CHOICE OF RADIOACTIVE ISOTOPES FOR CLINICAL USE.

Reference to a table of isotopes such as that of Hollander, Perlman & Seaborg (1953) shows that for nearly every element of the periodic table there is known not one but a number of radioactive isotopes. The properties of these isotopes, even of one element, vary over a wide range as regards the half-life and the nature and energy of the radiations emitted. Comparatively few of them are listed in the Atomic Energy Research Establishment Catalogue (1954), and not many more in the American Tracerlab Catalogue. Others, no doubt, could be made available if there were demand for them. Others again, the majority of known isotopes, cannot be made by neutron irradiation in the "pile", and have been prepared, usually in minute amounts only, by means of cyclotrons and high voltage machines.

Up to the present, not more than two or three radioactive isotopes of any one element have been used in clinical work or biological research, the choice being limited by ready availability, which itself often depends upon the half-life as well as the method of preparation. As the mechanism of their action, particularly with regard to the treatment of malignant disease, becomes
known in more detail, it may well be that more precise requirements as to the most desirable radioactive properties for each different application will become clear.

The work described in this thesis points to the conclusion that this situation has arisen in the case of radioactive iodine for treatment of carcinoma of the thyroid. In the cases described there is evidence that radio-isotope treatment would have been more effective if an isotope which produced its effects at a somewhat greater distance could have been used. Either a more penetrating $\beta$-ray, or a higher proportion of $\gamma$-rays, or better still a very soft $X$-ray with half-value layer of a few millimetres of tissue, would have given a more uniform radiation dose to the tumours in question, without giving an excessive dose to surrounding tissues.

Table X is a complete list of the known isotopes of iodine. Some of them are excluded from practical consideration for radiotherapy because of short half-life, and one ($^{129}_I$) because of excessively low activity and long life. For each of the others, the dose rates due to the different radiations emitted have been calculated. In some cases the physical information is incomplete, but the figures quoted are not likely to be in error enough to alter the conclusions. Some of the lower isotopes transmute by electron capture, leaving excited
### TABLE X.

**ISOTOPES OF IODINE**

Theoretical X- and γ-ray doses, compared with β-ray doses for uniform distribution.

<table>
<thead>
<tr>
<th>Mass No.</th>
<th>Half-life</th>
<th>Type of decay</th>
<th>Mean β-ray energy (MeV)</th>
<th>Mean total γ-ray energy per disintegration</th>
<th>Number of quanta of soft &amp; K-radiation</th>
<th>Radiation dose per μc destroyed per grm</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>30 m</td>
<td>β+</td>
<td>1.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>121</td>
<td>1.5 h</td>
<td>β+</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>122</td>
<td>3.6 m</td>
<td>β+</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>123</td>
<td>13 h</td>
<td>K</td>
<td>-</td>
<td>0.16, 1</td>
<td>0, 65</td>
<td>0.5, 3</td>
</tr>
<tr>
<td>124</td>
<td>4.5 d</td>
<td>(K (70%))</td>
<td>-</td>
<td>2.5, 0.7</td>
<td>65, (50)</td>
<td>64, (49), (3)</td>
</tr>
<tr>
<td>125</td>
<td>60 d</td>
<td>K</td>
<td>-</td>
<td>0, ~1.5</td>
<td>0, 0</td>
<td>0, ~80</td>
</tr>
<tr>
<td>126</td>
<td>13 d</td>
<td>(K (58%))</td>
<td>-</td>
<td>~0.4, 0.58</td>
<td>140, (87)</td>
<td>~30, (18), (4)</td>
</tr>
<tr>
<td>127</td>
<td>STABLE</td>
<td>β-</td>
<td>0.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>128</td>
<td>25 m</td>
<td>β-</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>129</td>
<td>2.107γy</td>
<td>β-</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>130</td>
<td>12.6 h</td>
<td>β-</td>
<td>0.29</td>
<td>2.1, 0.02</td>
<td>12, 127</td>
<td>6.3, 0.01, 0.5</td>
</tr>
<tr>
<td>131</td>
<td>8.0 d</td>
<td>β</td>
<td>0.20</td>
<td>0.39, 0.03</td>
<td>127, (92)</td>
<td>18, (13), 0.2</td>
</tr>
<tr>
<td>132</td>
<td>2.3 h</td>
<td>β-</td>
<td>0.45</td>
<td>2.1, 0</td>
<td>3.6, 0</td>
<td>1.2, 0</td>
</tr>
<tr>
<td>133</td>
<td>21 h</td>
<td>β-</td>
<td>0.41</td>
<td>? 0.6</td>
<td>3.6, 28</td>
<td>? 3, 0</td>
</tr>
<tr>
<td>134</td>
<td>52 m</td>
<td>β-</td>
<td>0.7</td>
<td></td>
<td>6.9, ?</td>
<td></td>
</tr>
<tr>
<td>135</td>
<td>6.7 h</td>
<td>β-</td>
<td>0.31</td>
<td>? 0</td>
<td>6.9, ?</td>
<td></td>
</tr>
<tr>
<td>136</td>
<td>86 s</td>
<td>β-</td>
<td>2.1</td>
<td></td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>131+</td>
<td>3.5 d</td>
<td>β-</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
NOTES ON TABLE X.

Column (1) gives the mass numbers of all the known isotopes of iodine.

Column (2) gives the half-lives, in seconds, minutes, hours, days, or years. Only those isotopes with half-lives of hours or days need be considered for therapeutic applications.

Column (3) gives either the sign of the $\beta$-particle emitted (positron or electron), or the symbol "K" for decay by electron capture, which is nearly always an electron from the K-shell. One quantum of tellurium K-radiation is then emitted, of energy 30 keV.

Column (4) gives the mean energy of the $\beta$-particles emitted.

Column (5) gives the total $\gamma$-ray energy emitted per disintegration, obtained by multiplying the energy of each $\gamma$-ray line in the spectrum by the proportion of disintegrations at which it is emitted, and summing over the whole spectrum. It includes two quanta of 0.51 MeV. annihilation radiation for positron emitters, and the $\gamma$-rays from any short-lived metastable states of daughter nuclei.

Column (6) gives the proportion of disintegrations in which Te$\kappa$ radiation, or similar soft X-rays, are emitted.

Column (7) gives the calculated $\beta$-ray dose per microcurie destroyed per gram of tissue, on the basis of complete absorption. The first figures are for complete physical decay of the isotope, and where figures in brackets are given these are recalculated allowing for excretion with a biological half-life of 20 days.

Columns (8) & (9) give the dose due to $\gamma$- and to X-radiation, calculated for the centre of a sphere of diameter 5 cm., and uniform distribution. These figures would be only slightly affected by non-uniform distribution, in contrast to the $\beta$-ray dose.

Column (10) gives the ratio of $\gamma$-ray plus X-ray dose to $\beta$-ray dose.

Information on the decay schemes is from Hollander, Perlman & Seaborg (1953), and on $^{132}$I dosimetry from Emery & Veall (1954). For $^{124}$I the source is Mitchell, Mei, Maienschein & Peacock (1949); the figures for K-capture and for $\gamma$-radiation have not, unfortunately, been accurately determined.
atoms of tellurium which therefore emit the characteristic X-radiation of this element. The K-radiation has energy 30 KeV., and absorption coefficient 0.18 per cm. in tissue (Marinelli, Quimby & Hine, 1948a). The half-value layer is therefore 4 cm., which would ensure very uniform dose (falling off, of course, from centre to periphery) in a large tumour, though the dose to neighbouring structures would be rather large. I\textsuperscript{125} is worth consideration as a possible isotope for therapy.

No mention appears in Hollander, Perlman, and Seaborg's table of any iodine isotope emitting a softer X-ray than the Te K-radiation (except for traces of L-radiation emitted along with the K-radiation). The most desirable radiation for this particular purpose would be X-rays of as low an energy as 15 KeV (H.V.L. 5 mm.); and it is not impossible that undiscovered metastable states of nuclei, of reasonable half-life, may exist, and may emit such soft radiation. Such metastable states might have been overlooked in the presence of isotopes emitting radiations of higher energy; there is, however, little chance that their preparation in large amounts would be a simple matter.

Among the β-emitters, I\textsuperscript{124} has outstanding advantages. Its half-life is not inconvenient. The mean β-ray energy is three times that of I\textsuperscript{131}, and the range is therefore approximately four times as great.
This fact would contribute usefully towards achieving a satisfactory dose level at the inactive regions. The ratio of $\gamma$- and $X$-rays to $\beta$-rays is also much more favourable from this point of view, and although information on $K$-capture is incomplete in this case, the final ratio of $\gamma$ plus $K$ radiation to $\beta$-radiation is probably seven times that for $I^{131}$. Some of the $\gamma$-radiation is annihilation radiation of 0.5 MeV, which is emitted at the end of the positron tracks, making a further slight improvement in the uniformity of dosage where the distribution of activity is irregular.

$I^{124}$ can be made in the cyclotron by the reactions

$Sb^{121} (a, n) I^{124}$

and

$Sb^{123} (a, 3n) I^{124}$.

The yield unfortunately is likely to be low, and the product will tend to be contaminated with the 13-day isotope $I^{126}$ (Professor W.E. Burcham, personal communication). It is doubtful whether existing cyclotrons can make millicurie amounts of $I^{124}$, but experiments to determine the yield, as a preliminary step, are being started.

The isotopes of astatine may also be considered in this connection, because they are highly concentrated in thyroid tissue. Several of them have half-lives of some hours, but none is sufficiently long-lived to be suitable for therapy. $At^{211}$ (half-life 8.5 hours) emits $\alpha$-particles, and may prove to be of value in
research, particularly with regard to intracellular localization.

In the case of carcinoma of the thyroid, the slow and variable natural course of the disease makes it very difficult to assess the value of any new form of treatment. A large number of cases would be required, and these would have to be followed for as long as ten years, before any but the most dramatic improvement in therapy could be established. It does not therefore provide the most favourable circumstances to urge an attempt to make large amounts of a special isotope at very great expense. But in the development of large scale nuclear processes, particularly the bombs, just as difficult technical problems have been solved many times; and, given sufficient reason, as much could surely be done for medical purposes. No proposed therapeutic use of a radio-active element should be permanently abandoned solely because the most suitable isotope cannot easily be prepared.

Another isotope of iodine $^{132}$I, is now being made available, both in America (Stang, Tucker, Banks, Doering & Mills, 1954), and in Britain (Emery & Veall, 1954). The short half-life (2.3 hours) more than counterbalances the favourable characteristics of the $\beta$-radiation for purposes of therapy, since the activity would largely have decayed by the time the thyroid tissue had reached
its maximum concentration of the administered iodine; but there may prove to be significant advantages for some diagnostic applications. For example, for estimation of the thyroid clearance rate for iodide the short half-life would not seriously complicate the physical measurements, but the total radiation dose to the thyroid would be reduced by at least a factor of 20. This increased margin of safety would be of particular advantage in studies of thyroid function in children. Another application, on which preliminary work is being done, is the use of this isotope to follow the decline of function of thyroid carcinoma during the course of therapy with $\text{I}^{131}$. $\text{I}^{132}$ may also prove of value in the use of radio-iodine to detect distant metastases of a functional carcinoma of the thyroid because one can safely give a larger amount. An incidental advantage is the very transitory activity of waste containing the isotope, which reduces the minor problems, in clinical work, of disposal of excreta and avoidance of contamination. An ingenious system for supplying this isotope has been devised, by which the parent isotope Te$^{132}$ (half-life 3.2 days) is supplied in a form from which known amounts of $\text{I}^{132}$ can be extracted at will by a simple chemical process.

For interstitial use by the "injection implant" technique, the isotope requirements are rather similar
to those for radio-iodine therapy of carcinoma of the thyroid. The autoradiographs from Case 12 illustrate the non-uniformity of distribution, which is probably inevitable, and radiation with a range of the order of millimetres is likely to give the best results. The optimum range will depend upon the dimensions of hard nodules into which it is not possible to implant any activity, and experience may show that the same isotope is not the best for all types of tumour to which this method of treatment is applied. The range chosen should be the shortest which is consistent with the need to irradiate every part of the tumour adequately. The field of choice is a wide one, since almost any element can be made into an insoluble compound and used in colloidal form or as a fine suspension. Either a metastable state emitting soft nuclear \( \gamma \)-rays, or a decay by electron capture leaving the product atom ionized in the K- or L-shell, should provide a better therapeutic agent than Au\(^{198} \), or any \( \beta \)-emitter. Much higher activities would be required to give the same average dose, but the associated risks of handling need not be greater; in fact, if hard \( \gamma \)-rays were absent the protection problems would be simplified. The choice of an isotope depends upon suitable radiation, convenient half-life, and availability. It has then to be prepared in an insoluble form which must be chemically
# Isotopes of Phosphorus

<table>
<thead>
<tr>
<th>Mass No.</th>
<th>Half-life</th>
<th>Type of Radiation</th>
<th>Max. ( \beta )-ray energy (MeV)</th>
<th>Mean ( \beta )-ray energy (MeV)</th>
<th>Max. ( \beta )-ray range (cm)</th>
<th>( \beta )-ray absorption coefficient (cm(^{-1}))</th>
<th>Half-value layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>4.6 s</td>
<td>( \beta^+ , \gamma )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>2.5 m</td>
<td>( \beta^+ )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>STABLE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>14.3 d</td>
<td>( \beta^- )-only</td>
<td>1.70</td>
<td>0.70</td>
<td>0.8</td>
<td>3.9</td>
<td>1800( \mu )</td>
</tr>
<tr>
<td>33</td>
<td>25 d</td>
<td>( \beta^- )-only</td>
<td>0.27</td>
<td>0.09</td>
<td>0.07</td>
<td>65</td>
<td>120( \mu )</td>
</tr>
<tr>
<td>34</td>
<td>12 s</td>
<td>( \beta^- )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**References**

Isotopes: Hollander, Perlman & Seaborg (1953).


Absorption coefficient: formula of Rossi & Ellis (1952).
inert in the tissues of the body.

A number of elements are known to be concentrated in various malignant tissues with differential absorption ratios greater than unity, although far lower than the ratios found to be necessary according to the simple type of calculation discussed in Chapter V. In such cases, there may be the chance of effective therapeutic use if the element is concentrated in or near to vital structures within the cell, for example chromatin or mitochondria, and if an isotope of this element emits rays of exceedingly short range - of the order of the dimensions of a cell, or even of the dimensions of an intracellular structure.

Most α-particles from radioactive isotopes have ranges between 20μ and 40μ; but few elements of biological interest emit α-particles. For β-rays the half-value layer in water is a better guide, and values calculated from the formula of Rossi & Ellis (1952) for low energy β-ray spectra are:

\[ E_{\beta_{\text{max}}} = 0.2 \text{ MeV.} \quad HVL = 67\mu \]
\[ E_{\beta_{\text{max}}} = 0.1 \text{ MeV.} \quad HVL = 23\mu \]

Apart from iodine, phosphorus is the only element of established value for radio-isotope therapy by internal administration. There are only two known isotopes of phosphorus which have half-lives long enough to be useful (Table XI). The readily available one, P\(^{32}\), emits a hard β-ray of half-value layer 1 - 2 mm.
(authorities differ). It is made by neutron bombardment of natural phosphorus, $^{31}\text{P}$, in the "pile". The other, $^{33}\text{P}$, emits a much softer $\beta$-ray, of which the half-value layer is approximately 120$\mu$. This is still large compared with the dimensions of malignant cells, but the difference would greatly affect the relative contributions of radiation dose which a cell would receive from radio-phosphorus within itself and that from neighbouring cells. $^{32}\text{P}$ irradiates compact tissues which pick up the element, such as lymph nodes, testis, and solid tumours, relatively more than isolated cells, or cells in very small groups and thin cords. With $^{33}\text{P}$ there would be less difference. Provided there are no viable malignant cells which fail to take up the element, $^{33}\text{P}$ can never be worse than $^{32}\text{P}$ in this respect, and may be much more useful where delicate strands of infiltrating cells or minute tumour emboli are present, and probably also in polycythaemia rubra vera. Among the cases studied in the present series, Case 6 in particular showed a form of tumour growth where the shorter range radiation would have been advantageous.

$^{33}\text{P}$ can be prepared from relatively rare natural isotopes of sulphur by the reactions:

$$^{16}\text{S}^{33} (n,p) ^{15}\text{P}^{33}$$
$$^{16}\text{S}^{34} (Y,p) ^{15}\text{P}^{33}$$

or from the common isotopes of chlorine:

$$^{17}\text{Cl}^{35} (Y,2p) ^{15}\text{P}^{33}$$
$$^{17}\text{Cl}^{37} (Y,a) ^{15}\text{P}^{33}$$
$^{33}P$ is not at present available from Harwell or Tracerlab, but it could probably be prepared if there was serious demand for it. Any clinical trials should be combined with autoradiography, from which more information could be expected with this isotope than with $^{32}P$, both because of the more favourable ratio of photographic action to tissue dose, and because of better resolution with the $\beta$-rays of shorter range.

The radioactive isotope of hydrogen, tritium, is one which may prove of value if it can be incorporated, in a stable situation, into an organic compound which is concentrated at vital points in growing cells. The mean energy of the $\beta$-rays is only a few thousand volts, and radiation damage to tissues in general would be proportionately slight; yet the ionization within a few microns of the point of emission of a $\beta$-ray is actually greater than that due to the emission of a $\beta$-ray of higher energy. An intracellular structure, to which the active atom had become attached, would therefore be disrupted, with minimal damage to nearby structures. Autoradiographic studies with the highest possible resolution would be necessary to find the exact situation within the cell at which $\beta$-emission occurred. The long half-life of tritium (12 years) is a little inconvenient for therapeutic applications, but the theoretical
advantage of radiation of such short range is so great that this isotope should be given serious consideration.

Finally, in Table XII, a list is presented of all the known isotopes up to atomic number 78, which emit either α-particles of any energy or β-rays of maximum energy below 0.2 MeV., and of which the half-life is sufficiently long to allow time for processing and transport. The use, on a large scale, of isotopes with half-lives longer than a few weeks, would involve some difficulties with regard to disposal of excreta, and when patients died or discharged themselves from hospital. From the therapeutic point of view, it might be possible to remove some longer-lived isotopes from the body when desired, by an agent such as di-mercapto-propanol, in order to reduce the risk of carcinogenesis or excessive general irradiation. In general, however, difficulties in the use of isotopes are likely to increase when the half-life in the body lies outside the range 1 - 30 days.

Above atomic number 78 (platinum), almost every element has at least one isotope which disintegrates by α-emission. These, and the isotopes listed in Table XII, have the definite advantage of short-range radiation, over all other isotopes. They are the ones most worth considering for incorporation into compounds which
## TABLE XII.

### Isotopes which emit $\gamma$-rays of low energy, or $\alpha$-particles.

<table>
<thead>
<tr>
<th>Element</th>
<th>A.N.</th>
<th>A.W.</th>
<th>$E_{\text{max.}}$ (or $E_0$)</th>
<th>Half-life</th>
<th>Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen</td>
<td>1</td>
<td>3</td>
<td>0.018</td>
<td>12.5 y</td>
<td>A</td>
</tr>
<tr>
<td>Carbon</td>
<td>6</td>
<td>14</td>
<td>0.155</td>
<td>5600 y</td>
<td>A</td>
</tr>
<tr>
<td>Sulphur</td>
<td>16</td>
<td>35</td>
<td>0.17</td>
<td>87 d</td>
<td>A</td>
</tr>
<tr>
<td>Nickel</td>
<td>28</td>
<td>63</td>
<td>0.07</td>
<td>85 y</td>
<td>A</td>
</tr>
<tr>
<td>Niobium</td>
<td>41</td>
<td>95</td>
<td>0.16</td>
<td>35 d</td>
<td>F</td>
</tr>
<tr>
<td>Technetium</td>
<td>43</td>
<td>97</td>
<td>conv. 0.1</td>
<td>90 d</td>
<td>A</td>
</tr>
<tr>
<td>Ruthenium</td>
<td>44</td>
<td>103</td>
<td>0.22</td>
<td>40 d</td>
<td>A ) ) F</td>
</tr>
<tr>
<td>Ruthenium</td>
<td>44</td>
<td>106</td>
<td>0.04 plus (Rh106 3.5 (30 sec))</td>
<td>1.0 y</td>
<td></td>
</tr>
<tr>
<td>Palladium</td>
<td>46</td>
<td>112</td>
<td>0.2</td>
<td>21 h</td>
<td></td>
</tr>
<tr>
<td>Silver</td>
<td>47</td>
<td>110</td>
<td>{0.09 (60%), 0.53 (35%)}</td>
<td>270 d</td>
<td>A</td>
</tr>
<tr>
<td>Tellurium</td>
<td>52</td>
<td>127</td>
<td>conv. 0.09 (conv. 0.7)</td>
<td>115 d</td>
<td>A</td>
</tr>
<tr>
<td>Samarium</td>
<td>62</td>
<td>151</td>
<td>0.08</td>
<td>73 y</td>
<td></td>
</tr>
<tr>
<td>Europium</td>
<td>63</td>
<td>145</td>
<td>conv. 0.2</td>
<td>5 d</td>
<td>(see Tb$^{149}$)</td>
</tr>
<tr>
<td>Element</td>
<td>Z</td>
<td>A</td>
<td>Activity</td>
<td>Half-life</td>
<td>Source</td>
</tr>
<tr>
<td>-----------</td>
<td>----</td>
<td>-----</td>
<td>----------</td>
<td>------------</td>
<td>--------</td>
</tr>
<tr>
<td>Europium</td>
<td>63</td>
<td>147</td>
<td>conv. 0.2</td>
<td>24 d</td>
<td>A &amp; F</td>
</tr>
<tr>
<td>Europium</td>
<td>63</td>
<td>155</td>
<td>{0.15 (80%)</td>
<td>2 y</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>{0.24 (20%)}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gadolinium</td>
<td>64</td>
<td>148</td>
<td>α 3.2</td>
<td>&gt; 35 y</td>
<td></td>
</tr>
<tr>
<td>Gadolinium</td>
<td>64</td>
<td>150</td>
<td>α 2.7</td>
<td>long</td>
<td></td>
</tr>
<tr>
<td>Terbium</td>
<td>65</td>
<td>149</td>
<td>α 4.0</td>
<td>4.1 h</td>
<td></td>
</tr>
<tr>
<td>Terbium</td>
<td>65</td>
<td>151</td>
<td>α 3.4</td>
<td>19 h</td>
<td></td>
</tr>
<tr>
<td>Holmium</td>
<td>67</td>
<td>162</td>
<td>{0.8 (15%)}</td>
<td>65 d</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>{conv. 0.1 (9%)}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thulium</td>
<td>69</td>
<td>168</td>
<td>0.10</td>
<td>85 d</td>
<td></td>
</tr>
<tr>
<td>Tantalum</td>
<td>73</td>
<td>177</td>
<td>conv. 0.1</td>
<td>53 h</td>
<td></td>
</tr>
<tr>
<td>Tantalum</td>
<td>73</td>
<td>179</td>
<td>conv. 0.1</td>
<td>600 h</td>
<td></td>
</tr>
<tr>
<td>Rhenium</td>
<td>75</td>
<td>189</td>
<td>0.2</td>
<td>150 d</td>
<td>A</td>
</tr>
<tr>
<td>Osmium</td>
<td>76</td>
<td>191</td>
<td>0.14</td>
<td>16 d</td>
<td>A</td>
</tr>
<tr>
<td>Iridium</td>
<td>77</td>
<td>196</td>
<td>0.08</td>
<td>9 d</td>
<td>A</td>
</tr>
<tr>
<td>Mercury</td>
<td>80</td>
<td>203</td>
<td>0.21</td>
<td>48 d</td>
<td></td>
</tr>
</tbody>
</table>

Note: "conv." = internal conversion electrons.

"A" = available by neutron irradiation in the Harwell pile.

"F" = available separated from fission products at Harwell.

Isotopes with half-lives shorter than 12 hours are not included.

The isotopes Se^79, Zr^93, Pd^107, I^129, Cs^135, Sm^147, are omitted because of very long half-life and therefore low specific activity. (C^14 is included because it is readily available).

NEARLY EVERY ELEMENT OF HIGHER ATOMIC NUMBER THAN 78 (Pt) HAS AT LEAST ONE ISOTOPE WHICH EMITS α-PARTICLES.
concentrate in malignant cells; and an isotope should not necessarily be rejected because it cannot at present be obtained in sufficient amounts. The range of their chemical properties is wide, and a suitable isotope should not be lacking for incorporation into any tumour-seeking compound which may be discovered.
APPENDIX 1.

REDUCTION OF AUTORADIOGRAPHIC SENSITIVITY FOR ISOLATED OBJECTS.

Let us assume that, in order to add usefully to the autoradiographic image, a $\beta$-particle must render one silver grain developable in the emulsion, within a certain horizontal distance $a$ from the vertical line through the radioactive focus from which it originates. On the average, it will do this if its track (assumed straight) is still within the horizontal distance $a$ when it has penetrated half the thickness of the emulsion. If, for example, the point of origin is half way through the section, the only $\beta$-particles which are effective are those emitted within a cone whose half angle is $\alpha$, where

$$\tan \alpha = \frac{a}{b + \frac{1}{2} t}.$$

The ratio of effective $\beta$-particles to the total number...
emitted is the ratio of the solid angles, namely

\[
\frac{2\pi (1 - \cos \alpha)}{4 \pi} = \frac{1}{2} (1 - \cos \alpha) = \frac{1}{2} \left[ 1 - \frac{x}{\sqrt{(a^2 + x^2)}} \right].
\]

Whereas the proportion effective, if every upward-travelling β-particle contributes to the autoradiograph, is \(\frac{1}{2}\).

If we take the half emulsion thickness, \(b\), equal to \(2\mu\), the section thickness equal to \(5\mu\), and the distance \(a\) equal to the radius of a circle whose area is \(100\ \mu^2\),

then \(a = 5.65\mu\)
and \(x = 4.5\ \mu\)

and the fraction of upward-travelling β-particles which are effective is

\[
1 - \frac{4.5}{\sqrt{(31.8 + 20.3)}} = 1 - 0.62 = 0.38
\]

To take the average for all possible positions of the supposed radioactive speck, or, what is equivalent, the value for a thin rod of radioactivity running through the section, it is necessary to integrate over the whole thickness from \(x = b\) to \(x = b + t\). The fraction of upward-travelling β-particles which are effective
is then

\[ \frac{1}{2\pi t} \int_{b}^{b+t} x \int_{b}^{b+t} 2\pi (1 - \cos a) \, dx \]

\[ \begin{align*}
&= \frac{1}{t} \int \left[ 1 - \frac{x}{\sqrt{t(x^2 - a^2)}} \right] \\
&= 1 - \frac{1}{t} \sqrt{a^2 + (b+t)^2} + \frac{1}{t} \sqrt{a^2 + b^2}.
\end{align*} \]

For the case taken above, \( a = 5.65 \mu \), \( b = 2 \mu \), \( t = 5 \mu \), the ratio becomes

\[ 1 - \frac{1}{5} \sqrt{(31.8 + 49)} + \frac{1}{5} \sqrt{(31.8 + 4)} \]

\[ = 1 - 0.60 \]

\[ = 0.40 \]

which is almost identical with the previous example.

The fraction of upward-travelling \( \beta \)-particles which are effective, is lowest for radioactivity near the bottom of the section, and rises to unity for radioactivity at the upper surface.

Under these circumstances, doubling the section thickness does not double the average autoradiograph density. The effective fraction, for \( t = 10 \mu \), has fallen to 0.28, and the total number of useful grains is therefore only increased in the ratio 40:56 by doubling the section thickness.

Any distance separating section from emulsion has to be added, in effect, to the half-thickness \( b \) for
the purpose of this calculation. Separation therefore still further reduces the effective fraction.

The area of the radioactive focus makes little difference to this calculation if it is not greater than 100 μ². For larger areas of activity, the oblique β-particles from one point add to the autoradiograph over neighbouring points, and the sensitivity is therefore increased towards the value which it would have if every upward-travelling β-particle were effective.
REFERENCES


Crookes, W., (1914) Phil. Trans. (A) 214, 433. On acquired radioactivity.


Evans, T.C., (1947) Radiology 49, 206. Preparation of autoradiographs of thyroid tumours for study at high magnification.


Fink, R.M., (1951), Science 114, 143. A new approach to high resolution autoradiography.


Jolles, B., "X-ray Sieve Therapy in Cancer" (London: Lewis, 1953).


p.485: Localisations histologiques spéciales du polonium à l'intérieur de certains organes.

p.487: Localisations ...... à l'intérieur des organes hématopoïétiques.


Leblond, C.P., (1943) J. Anat., Lond. 77, 149. Localization of newly administered iodine in thyroid gland as indicated by radio-iodine.


Marinelli, L.D., Quimby, E.H., & Hine, G.J., (1948a) Nucleonics, 2, no.4, p.56; no.5, p.44. Dosage determination with radioactive isotopes, I.


Müller, J.H., & Rossier, P.H., (1947) Experientia 3/2, 75. De l'emploi d'isotopes radioactifs artificiels dans le but d'exercer un effet radio-biologique localisé.III.


Philipp, K., (1950) Strahlentherapie 82, 51. Anwendung radioaktiver Isotope in der Medizin.


Taylor, S., (1952) Lancet 1, 175. The size of follicles in non-toxic goitre.

Teorell, T., (1937) Arch. int. Pharmacody. 57, 205 Kinetics of the distribution of substances administered to the body.


