Calling

THE USE OF NUCLEAR TRACK AUTORADIOGRAPHY IN THE STUDY OF THE DISTRIBUTION OF THORIUM AND ITS PRODUCTS IN LIVING ORGANISMS

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ABSTRACT

The thesis describes the use of autoradiographs to study the distribution of thorotrast in the body and to determine the alpha-particle dose to tissues. It reviews the uses of thorotrast (ThO_2) as a radio-opaque medium and its clinical radiation effects. The techniques of autoradiography, particularly the method of coating with Nuclear Research emulsion used here, are described. In the experimental work the alpha-particle activity of thorotrast, its variation with time (age-factor) and the diffusion and adsorption of elements were studied. A formula was derived for the lengths of tracks recorded in autoradiographs and applied to analyse the activity of tissue sections in vitro. By observing the activity over long periods of time the escape of both long-lived and short-lived elements was detected and a formula derived for calculating from the observed activity in vitro and the age-factor of the thorotrast the proportion of elements retained in vivo (retention-factor). The absorption of alpha-particle energy within spherical aggregates of thoro-trast was evaluated by observing their size and thorium content. Tissue dosage was calculated from the effective energy of the alpha-particles which depe ds on the relative activity of the various groups, the total activity per unit volume and the self-absorption. As the thorotrast was distributed non-uniformly this was expressed as the average dose for the whole organ, as the maximum dose in the tissue surrounding the largest aggregates and as the uniform dose from numerous small aggregates. The results are given for the spleen, liver, bone marrow and other tissues from five patients and one rabbit. Variations with the injected dose and the

time since injection are considered. For the correlation of clinical symptoms with radiation dose, a more practicable approach, that of calculating alpha-particle dose from gamma-ray counts in living patients, is illustrated using relationships between alpha- and gamma-activity and the variation of retention-factor and self-absorption with age-factor.

ACKNOWLEDGMENTS

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INTRODUCTION

The experiments described were undertaken in cooperation with the Finsen Institute in Copenhagen, where a study is being made of the radiation effects of thorium deposited in the body. The effects are being studied mainly on patients who were given injections of thorium dioxide sol (thorotrast) for diagnostic X-ray purposes and later developed symptoms of radiation disease. The patients are examined clinically and Geiger-counter measurements are made of the gamma-activity of their organs and of the alpha and beta-activity in breath, excretions, blood and biopsy samples of tissue. In this way it may be possible to correlate the radiation dose received by various tissues with the clinical effects produced and to estimate a tolerance dose for thorium deposited internally. As a supplement to these measurements it was planned here to develop a technique for determining the tissue dosage from alpha-particles using track autoradiographs, and to apply the technique to tissues obtained from some of the Copenhagen patients. This is of value because, for an accurate determination of the tolerance dose, it is important to know not only the total radiation received by the whole body and by separate organs as given by the superficial gamma-counts, but also the distribution of activity within the tissue cells.

The problem of determining how much radiation can be tolerated by the body is complex, as many factors, such as the type of radiation, the manner in which it was administered, the time of exposure and the organs exposed, must be considered. A definition of tolerance dose which applies

to any kind of radiation has been given as "the maximum dose of radiation to which a person of medium weight and stature can be exposed without eventually showing any signs of the known radiation diseases." The dose which we normally receive from natural sources, that is, from the natural radioactivity of the earth, from cosmic radiation and from minute quantities of radioactive materials which are assimilated into the body, amounts to 0.002 reps per week. It is known from human experience and from animal experiments that excess radiation can be dangerous, causing immediate acute illness and fatalities or later chronic illness and genetic changes. On the basis of this data, a maximum permissible level for external radiation has been fixed as 0.3 reps per week. For irradiation from internally deposited material, some data is already available from persons who have been contaminated while working with radioactive materials. In the cobalt mines in Czechoslovakia, an abnormally large number of cases of diseases of the lung in miners was attributed to the inhalation of dust containing particles of radioactive elements of the uranium-radium series. In the dial-painting industry workers ingested orally luminous paints containing radium, and many died years later due to the continuous irradiation of the bone from deposits of radium in the skeleton. Measurements of the amount of radiation received by these patients have made it possible to determine a safe level for radium stored internally. The problem of thorium deposited internally has not been encountered previously and many new problems arise because of the special properties of thorium injected in the form of thorotrast. In this case the colloidal particles of thorium dioxide are deposited in the cells of the reticulo-endothelial system

where they remain indefinitely in an insoluble state and irradiate the surrounding tissues. Some of the decay products are soluble and are free to move to different parts of the body where they are dealt with according to their chemical nature. Because of the long half-life of thorium, the irradiation continues indefinitely, and the evidence suggests that ultimately abnormal effects are produced.

Thorium and its decay products emit alpha, beta and gamma-rays but as only the gamma-rays are sufficiently penetrating to be detectable at the surface of the body, Geiger-counter measurements on living patients are useful only in detecting the presence of radioactivity and in comparing the content of different organs. The alpha-particles carry 90% of the total energy of all types of radiation and as they are absorbed within a very short distance in tissue they contribute most to the tissue dosage. The alpha-particle activity can only be measured using a microscopic technique such as track autoradiography in which thin sections of tissue are used and the alpha-particles recorded in a photographic film. This method has the advantage that as each of the elements present emits a particle of characteristic range in emulsion, the lengths of the tracks may be used to identify the elements and calculate the effective alpha-particle energy. Moreover in the autoradiograph the origin of the particles may be related to the deposits of thorotrast in the cells. These deposits vary in size, some being comparable with, or even greater than, the range of the alpha-particles in tissue, so that part of the energy of the particle is absorbed before reaching the tissue. In the

autoradiographs the size and radioactive content of the aggregates can be determined and the self absorption calculated. As even the most energetic particles which dissipate all their energy in tissue have come to the end of their range in 82 microns, all the energy is absorbed within a small volume of tissue surrounding the aggregates and this results in an intense dose in this region. The measurement of this dose is also possible by means of autoradiographs.

The tissues used in these experiments were obtained from a number of sources, as they became available. The sections from Copenhagen were taken from patients of different medical histories; the autopsy samples included sections from many different organs, the biopsy samples were limited to a few organs, and some samples were obtained from living patients after splenectomy and tonsilectomy. They were mounted some time before it was possible to make the autoradiographs, thus the activity was not necessarily the same as <u>in vivo</u>. Furthermore, no records had been kept of the thorotrast injected into the various patients. This is important, as the activity of the thorotrast varies from batch to batch and with the time since it was prepared. Other material for study was obtained from sources in Britain and in this case it was possible to make exposures almost immediately and to obtain samples of the thorotrast used. Altogether, sufficient material has been examined to give results for five patients at periods of time from less than a day to several years after injection. Animal experiments have also been carried out in Copenhagen and some of these tissues have been examined here, but the work has concentrated mainly on human material as it is important to make use of

this less readily available material whenever the opportunity arises.

As the collecting of results from autoradiographs is very slow it was not practicable here to examine tissues from a sufficient number of patients to allow a statistical analysis of the results and a correlation with clinical symptoms. If it can be shown what factors contribute to the alpha-particle tissue dose in different patients, it might be possible to apply these results to the gamma-ray counts which are more easily obtainable from large numbers of patients. Gamma-ray counts have already been completed on several hundred patients in Copenhagen.

In presenting this subject, the literature dealing with thorotrast has first been summarised briefly, particularly where this gives an indication of the mechanism of the uptake of thorotrast and its properties, or of the details of the clinical effects produced which may be useful in interpreting the experimental results. The Copenhagen investigation has been described separately and in some detail, as it forms the background for the tissue dosage measurements. Before describing the experimental techniques used in the investigation, the field of autoradiography in general has been reviewed as an indication of the techniques available. The remainder of the thesis is concerned with an account of the measurements of the various factors necessary for the calculation of tissue dosage. As an illustration of these measurements and calculations, the results for one patient have been given in detail in the text and then summarised together with those for the other patients in Table XX, which can be turned to at any stage for a comparison of each factor as it is discussed.

CHAPTER 1

SURVEY OF THE USES AND PROPERTIES OF THOROTRAST

1.1. Use of Thorotrast as a Contrast Medium

Thorium dioxide sol was introduced as a contrast medium in X-ray diagnosis by Radt (1929) in Berlin and independently by Oka (1929) in Tokio, being first prepared commercially as "Thorotrast" by the Heyden Company in Germany in 1931. It has many advantages over other contrast media, in particular the high atomic weight of thorium which makes it very radio-opaque, and the absence of immediate unfavourable reactions in patients. The most important use, in cerebral angiography for outlining the vessels of the brain, was developed by Moniz (1933). In this case the thorotrast is injected into the carotid artery and reaches the brain within a few seconds. Alternatively, it is sometimes injected directly into the cavities of the brain (ventriculography) as described by Radovici and Meller (1932). It is also used intravenously for outlining the liver and spleen (hepatosplenography) as shown in the original experiments of Radt and Oka and for the visualization of lymph nodes, vessels and arteries (Ané and Menville, 1933), lung alveoli, placenta, bone marrow and the renal pelvis (general review of Reeves and Stuck, 1938).

Before thorotrast was used for patients it was shown in animal experiments that the doses necessary to outline various organs and body cavities did not produce any toxic effects (Irwin, 1932; Pohle and Ritchie, 1934; Leitner, 1938). For example, doses of the order of 0.5 to 1 ml. per Kgm were found to be sufficient for good visualization

of the liver and spleen while animals could tolerate up to 5 to 10 ml per Kgm. In man, the maximum dose normally given is 75 ml, or approximately 1 ml per Kgm for hepatosplenography. Much smaller doses are given for other purposes, the minimum being of the order of 5 ml in ventriculography.

Although the preliminary results with thorotrast were so favourable, there were some early warnings against the introduction of radioactive substances into the body. It was about this time that Martland (1926 etc.) was publishing reports of persons who had been contaminated with radium and mesothorium while employed as luminous watch dial painters. He found that many years later serious reactions appeared in workers who had ingested even minute quantities of radioactive materials. Those who opposed the use of thorotrast (for example, Council on Pharmacy and Chemistry, 1932; Reeves and Stuck, 1938) pointed to the possibility of similar effects after long periods of time because of the negligible excretion of thorium from the body. On the other hand, many authorities felt that it was such a valuable aid to diagnosis that they were willing to take risks (Yater, Otell and Hussey, 1936) while others urged caution until a sufficiently long time had elapsed to settle the question of late effects, or suggested that its use be restricted to incurable cases (Whitaker, Davie and Murgatroyd, 1933; Pohle and Ritchie, 1934). As a result of these warnings the use of thorotrast has been limited and during the past 25 years many reports concerning the subsequent history of the early patients, as well as further results from animal experiments, have appeared in support of both points of view. Its most common use now is

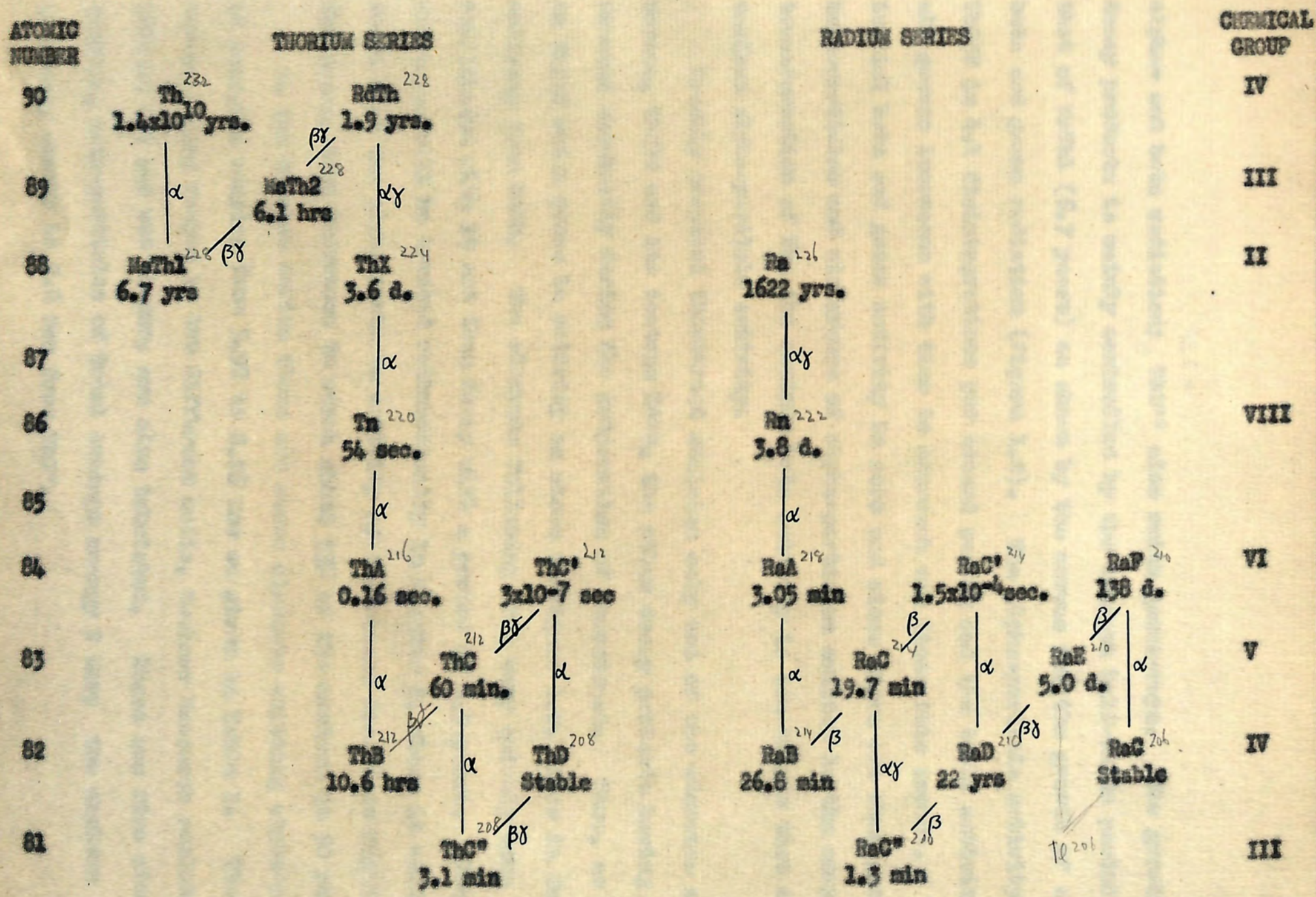
in cerebral angiography, but even then it is usually restricted to terminal cases.

1.2. Physical and Chemical Properties of Thorotrast

According to the specifications thorotrast contains 19 to 20% weight of thorium dioxide as a colloidal suspension in liquid miscible with body fluid. It is stabilised with 16 to 19% by weight of dextrin preparation and contains 0.15% of methyl-p-hydrobenzoate as preservative. By means of electron microscopical examination (Henriques et al, 1950) it was shown that the particles are irregular flat plates 3 to 10 millimicrons in diameter. By measuring the radioactivity it was shown that one millilitre of thorotrast contains approximately 190 mg of thorium. The normal dose injected varies from 5 to 100 ml and therefore contains from 1 to 20 grams of thorium.

Parent thorium (Th232) emits only alpha-particles, but its decay products emit beta and gamma-radiation as well. The relationship between these elements is represented in the decay scheme in Figure 1.1. which contains the atomic number of each element, its half-value period and the radiation emitted. Th232 is followed by mesothorium 1 and 2 (MsTh1 and MsTh2) which emit beta and gamma-rays, MsTh2, of very short half-value period, being transformed almost immediately into radiothorium (RdTh) the next alpha-emitting element. Then follow three more alpha-emitting elements of successively shorter periods. Thorium C (ThC) disintegrates either to ThC' (66%) or to ThC'' (34%) both of which are finally transformed into the stable lead isotope ThD with the emission of further

FIGURE 1.1


ATOMIC NUMBER
THORIUM SERIES
RADIUM SERIES
CHEMICAL GROUP
90
89
88
87
86
85
84
83
82
81
Th 232
1.4x10^10 yrs.
RdTh 228
1.9 yrs.
βγ
MsTh2 228
6.1 hrs
α
αγ
MsTh1 228
6.7 yrs
βγ
ThX 224
3.6 d.
α
Tn 220
54 sec.
α
ThA 216
0.16 sec.
ThC' 212
3x10-7 sec
βγ
ThC 212
60 min.
α
α
ThB 212
10.6 hrs
ThD 208
Stable
α
ThC" 208
3.1 min
βγ
Ra 226
1622 yrs.
αγ
Rn 222
3.8 d.
α
RaA 218
3.05 min
RaC' 214
1.5x10-4 sec.
RaF 210
138 d.
β
β
RaC 214
19.7 min
RaE 210
5.0 d.
α
α
α
RaB 214
26.8 min
β
αγ
RaD 210
22 yrs
βγ
RaG 206
Stable
RaC" 210
1.3 min
β
Tl 201
I
II
III
IV
V
VI
VIII
III
IV

alpha- and beta radiation; ThC'' also emits gamma-rays. The growth of decay products is mainly controlled by the longest half-value period, that of MsTh1 (6.7 years) as shown by the curves for the growth of alpha, beta and gamma radiation (Figure 1.2). The alpha-particle activity of Th232 is 4.1 disintegrations per second per mg and the total activity of all groups increases with time to approach six times this amount. The initial beta and gamma activity is zero and since there are four groups of beta-particles and six groups of alpha-particles emitted in the complete transformation of Th, the maximum beta activity is two-thirds that of the maximum alpha-particle activity.

Freshly prepared thorotrast contains only two of the elements of the series, Th232 and its isotope RdTh, the other decay products having been removed chemically during the preparation of thorotrast. Thus, as well as Th232 which grows in activity as shown in Figure 1.2, there is further activity from RdTh. The elements following RdTh very quickly come to equilibrium with it and then decay with a period of 1.9 years. These processes will be treated mathematically in Chapter 5, where it will be shown that the alpha-particle activity of thorotrast decreases for the first few years, then increases to reach about 95% of the maximum in 30 years.

In the thorium series there are seven elements emitting alpha-particles of energies varying from 3.98 to 8.78 Mev as given in Table I. The corresponding ranges in two different media, Nuclear Research emulsion (Ilford G2) and wet tissue, are also tabulated. There are five elements emitting beta-particles of total average energy 2 Mev; the maximum gamma-ray energy is 2.6 Mev from ThC".

FIGURE 1.2

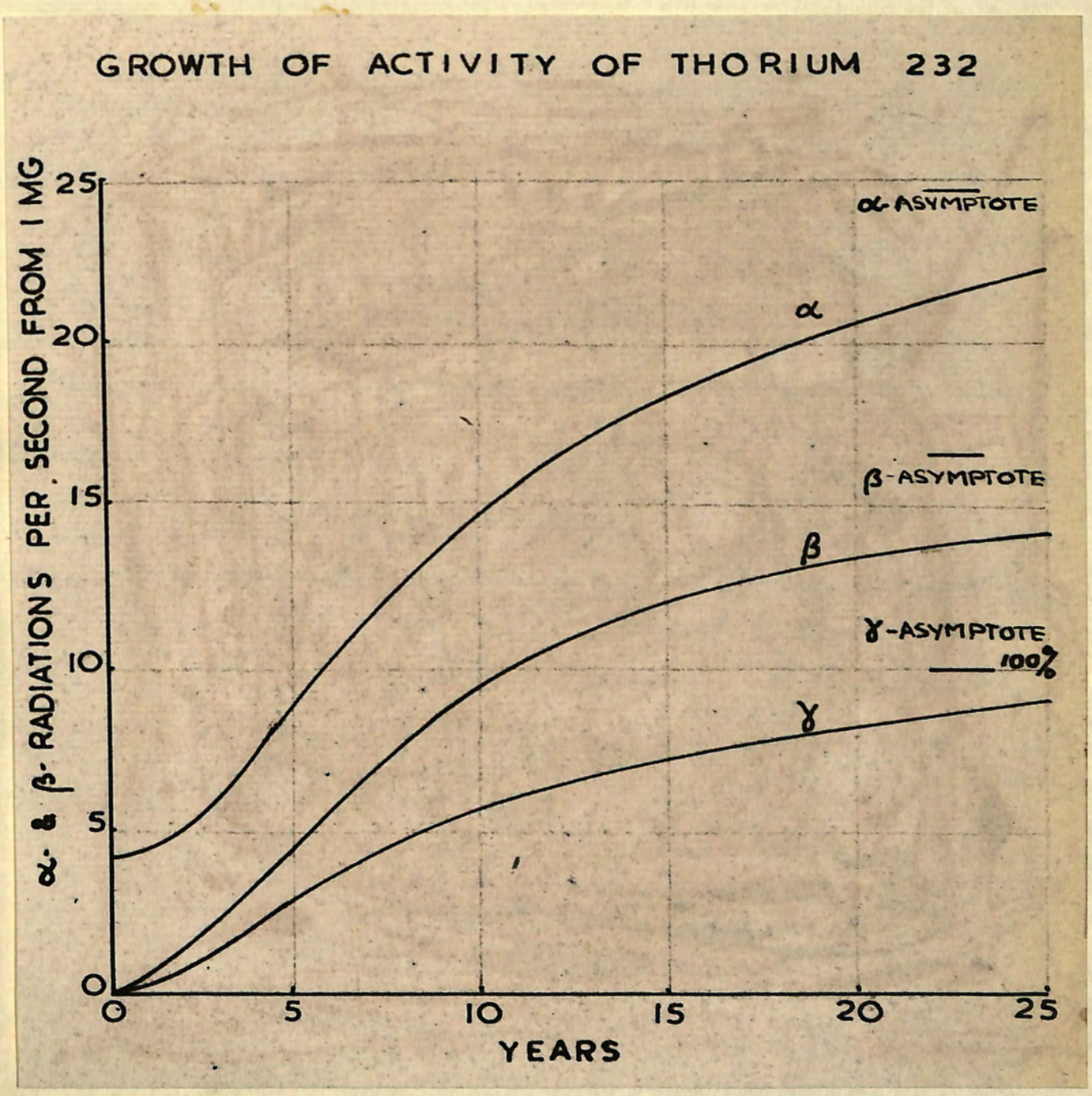

GROWTH OF ACTIVITY OF THORIUM 232
α- & β- RADIATIONS PER SECOND FROM 1 MG
25
20
15
10
5
0
0
5
10
15
20
25
YEARS
α-ASYMPTOTE
α
β-ASYMPTOTE
β
γ-ASYMPTOTE
100%
γ

TABLE I. Energy and Range of Alpha-particles from Thorium and Radium.

Element	Energy of Alpha-particle	Range in C2 Emulsion	Range in Tissue
Th	3.98 MeV	15.7 micron	23.4 micron
RdTh	5.40	24.0	37.5
ThX	5.68	25.8	40.6
Tn	6.28	29.7	47.6
ThA	6.77	33.1	53.6
ThC	6.05	28.2	45.0
ThC'	8.78	48.7	81.6
Ra	4.79	20.2	
Rn	5.49	24.6	
RaA	6.00	27.8	
RaC	5.33 .04%	23.6	
RaC'	7.68 99%	39.8	

As well as the elements of the thorium series, thorotrast may contain traces of radium as an impurity; from the clinical point of view radium is more dangerous than thorium because its activity is 10^7 times as great. The decay scheme of radium is also given in Figure 1.1. In this case the total energy of the four alpha-particle groups is 24.0 Mev, beta-particles 3 Mev, and the maximum gamma-ray energy is 2.2 Mev.

Of the three radiations, the alpha-particles are the most damaging to tissue. They lose all their energy within a short distance (less than 100 microns in tissue) causing intense localized doses of radiation. Also, because of their greater ionising power, they are biologically the most efficient. Gamma-rays react with matter with the production of secondary beta-particles. The gamma-rays are the least damaging but the most penetrating of the radiations, for example the intensity of the gamma-ray from ThC" decreases to one half after passing through 20 cm of tissue. The beta-rays are of intermediate penetrating power, the maximum range in tissue being 1 cm. The alpha-particles are 5 to 20 times as efficient biologically as the beta-particles.

The metabolism of any element when introduced into the body depends on its chemical properties, but in the case of radioactive substances the half-value period also plays a part. As an indication of the chemical properties of thorium and radium and their decay products, the chemical groups to which these elements belong are also tabulated in Figure 1.1. Long-lived Th232 and its isotope RdTh belong to a biologically inactive group of elements, whereas MsThl, ThX and Ra belong to the active group II

containing magnesium, calcium and barium. The behaviour of these elements and their products has been described by Evans (Aub, Evans, Hempelmann and Martland, 1952) with particular reference to their deposition in bone. MsThl, ThX and Ra are metabolized like calcium and are found preferentially in the inorganic part of bone whereas Th and RdTh are found in the organic part. Whether the RdTh formed from MsThl already deposited in the inorganic part is transferred to the organic part, or MsThl and ThX formed from Th and RdTh in the organic part are transferred to the inorganic part, will depend on the half-value periods of the elements and the times of transfer in the body. As MsThl and RdTh have relatively long periods they have more time to be redistributed before decaying. The next elements in the series, thoron (Tn) and radon (Rn) which are gaseous elements like the other elements of group VIII, can diffuse through tissue and escape in the breath but the proportions escaping in this way, 2% and 40% respectively, are different because of their different half-value periods. The elements which have very short periods and have little time to react chemically will remain with the parent element increasing its activity. This will apply to the remaining elements after Tn in the thorium series with the possible exception of ThB, the half-value period of which is probably longer than the time taken for the transfer of elements to different parts of the body. Ra D is also an exception and its long-lived activity is a danger in persons who have inhaled Rn.

An approximate tolerance dose for thorium could be calculated by comparing the activity of thorium with that of radium. The maximum permissible level for radium has been fixed by the International Commission

on Radiological Protection (1954) as a total body content of 0.1 micrograms which is equivalent in alpha-particle activity to 1 gram of thorium or 5 ml of thorotrast. However, for an accurate estimate, the different distributions of radium and thorium in the body, the different amounts of Tn and Rn lost in the breath and the different energies of the radiations emitted must be taken into account.

1.3. Distribution of Thorotrast in the Body after Injection

The distribution of thorotrast in the body depends on whether it was administered intravenously, by intracavitary injection, subcutaneously, or by accident perivascularly, but in all cases at least some of it is taken up in the reticulo-endothelial (R.E.) system. This is easily demonstrated by the appearance of radio-opaque shadows of the liver and spleen some hours after injection. It has been further demonstrated (i) by histological examination of sections of tissue, (ii) by chemical analysis of organs after death and (iii) by physical measurements of radioactivity of living patients and sections of tissue.

(i) The uptake of thorotrast in rabbits was studied by injecting a number of rabbits with various doses, killing them at intervals of time afterwards and examining sections of tissue taken from various organs (Irwin, 1932; Wen and Jung, 1933; Pohle and Ritchie, 1934; Pomeranz, 1934). The distribution always showed large deposits in the liver, spleen, bone marrow and lymphoid tissue, smaller amounts in the adrenals and ovaries and slight traces in the kidney. After injection the thorotrast remained colloidal for five minutes before collecting into

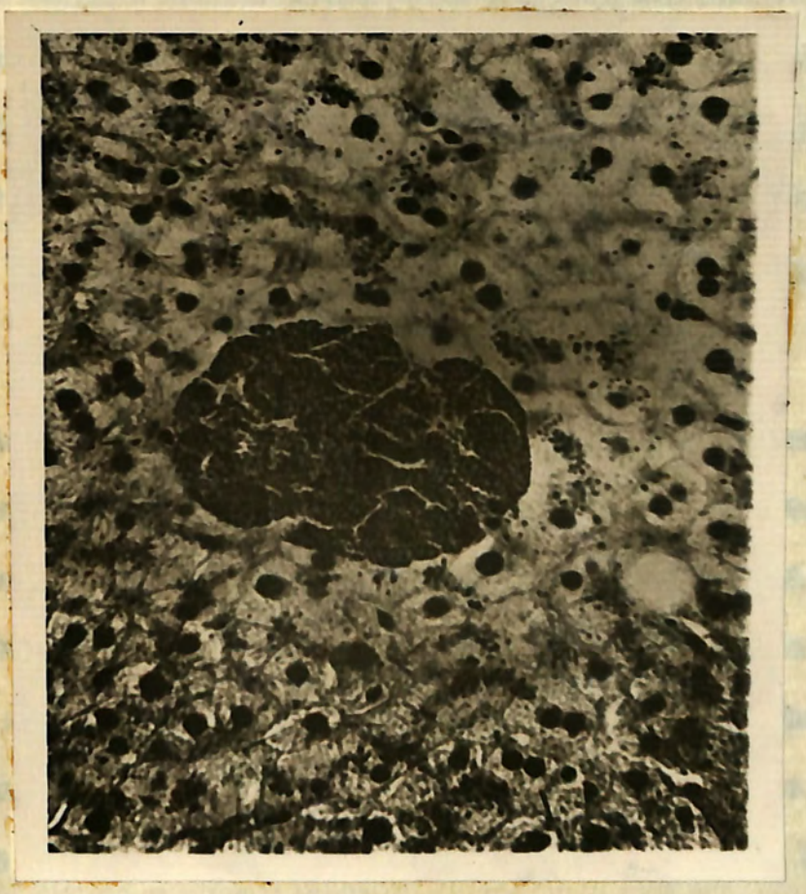
fine granules which were taken up in the cytoplasm of the cells. After 10 minutes, there were already large amounts of thorotrast in the spleen and after 15 minutes fine granules appeared in the liver and bone marrow. At periods from one week to two months after injection the thorotrast-laden cells were grouped in cell masses of 10 to 25 nuclei, similar to that shown in Figure 1.3. A redistribution from the spleen to the liver was also noticed after two months.

In human subjects, Whitaker et al (1933) and Elman and Haworth (1937) found similar deposits of thorotrast in the Kupffer cells of biopsy samples of liver tissue.

Histological studies also revealed some changes in the cells surrounding deposits of thorotrast in the liver, spleen and kidney (Tripoli and Haam, 1932; Shute and Davis, 1933; Pohle and Ritchie, 1934; Pomeranz, 1934; Leitner, 1938) and other reactions (Huguenin, Nemours-Auguste and Albot, 1932). Over a four-year observation period, Orr, Popoff, Rosedale and Stephenson (1938) found necrotic changes and other abnormal effects.

(ii) In chemical estimations, Leipert (1931, 1933) determined the amount of thorotrast present in the liver, spleen, kidney and other organs of both patients and rabbits. Most of the thorotrast was concentrated in the liver and spleen, the amounts in other organs being negligible in comparison. In five patients, the amounts recovered in the liver and spleen, expressed as a percentage of the injection, and the ratio of the amount per gram of tissue in the two organs were as given in Table II.

FIGURE 1.3. Stained section showing a thoriocyte in the liver of a patient several years after injection. (Prepared by Berenbaum and Birch).



It will be noticed that there are variations in the amounts taken up in different patients which do not appear to be related to the dose or to the period of time in the body. Similar variations were noticed in rabbits; for example, in a group of eight rabbits receiving injections of 1.5 and and 3.4 gm, and killed at periods of time from one to 477 days after injection, the fraction of the total injection recovered in the liver and spleen varied between 43% and 76%. In rabbits the ratio of the amount per gram of tissue in the spleen and liver was greater than in man and varied between 8 and 40. The content per gram of tissue in the bone marrow varied from 0.2 to 1.5 times that in the liver and in the lung from 0.1 to 0.6.

(iii) The retention of thorotrast in the R.E. system over long periods of time was demonstrated by means of radioactivity measurements on living patients and autopsy samples of tissue. Taft (1937) detected activity over the liver region of a living patient and compared it with that of a wax phantom of similar dimensions. At autopsy he measured the activity of ashed organs and showed that the liver contained 50% of the amount injected and the spleen 12%. A similar measurement was made by Jacobaeus and Rosenbaum (1938) who found that the liver of a patient five years after injection of 75 ml of thorotrast contained 27% of the original injection.

Berenbaum and Birch (1953) measured the radioactive content of the liver of a living patient 23 years after injection of 10 ml by making autoradiographs of biopsy samples and counting the alpha-particle tracks. From a section of volume 0.0075 mm^3, 100 tracks were recorded in 20 days.

TABLE II. Thorotrast in Liver and Spleen of Patients.

No.	Days between Injection and death	Injection gm.	% of Total Injection in Liver and Spleen	Ratio Specific Activities Spleen/Liver
1	2	15.0	64.3	0.87
2	2	19.5	69.13	1.70
3	4	6.0	56.9	1.02
4	48	29.5	32.54	5.7
5	60	16.5	97.0	1.64

The section was equivalent in volume to 0.0125 mm^3 of unfixed tissue allowing for shrinkage during preparation of the section. Assuming the volume of the liver was 1500 cm^3, and half the total number of alpha-particles emitted were recorded in the autoradiograph, the content of the whole organ was shown to be equivalent to 3.2 ml of thorotrast or 30% of the original injection.

The conclusion that thorotrast remains indefinitely in the body was confirmed by measurements of the amounts excreted. Wichman and Fricke (1932) attempted to estimate by chemical and electroscopic means, the radioactivity of the urine of a patient who had been given thorotrast, but they were unable to trace any thorium or radioactivity. Strenstrom (1941) studied the elimination of radioactive elements from two patients six and seven years after receiving 75 ml and also from animals. The initial alpha-particle activity of the faeces was equivalent to 0.1 ml of thorotrast but this decreased with time. By analysing the decay curve they showed that 95% of the active deposit decayed with the period of ThX and the other 5% with a much longer period. The long-lived activity probably came from the decay products of MsThl which is not itself alpha-active but would be excreted with ThX. They also measured the activity in breath and found that this increased in the first few hours as ThB and C were formed but then decreased, proving that it was Tn which was excreted. They were unable to detect any activity in urine and sputum. These results are important as they indicate that no Th is excreted, but the excretion of the products MsTh, ThX and Tn causes a decrease in the total alpha-particle activity in the body.

The distribution throughout the R.E. system applies to thorotrast entering the blood stream. Various other reactions have been observed after intracavitary injection, some showing that thorotrast remains fixed in the cavity (Radovici, Bazgan and Meller, 1933; Stuck and Reeves, 1938; Beres, 1939), others that some of it is carried to other parts of the body (Alexander, Jung and Lyman, 1934; Freeman, Schoenfield and Moore, 1936). Sometimes during attempts to inject thorotrast intravenously the substance is deposited in the tissue surrounding the vein. In such cases the deposit remains in this position or leaks to neighbouring areas. For example, Kantzman, Gros and Meyer (1950) detected radioactivity at the site of perivascular injection in two patients and there were counts in the region of the liver and spleen also indicating some uptake in the R.E. system.

1.4. Clinical Conditions Produced by Radioactivity

Following the suggestion that thorotrast might have carcinogenic effects, Roussy, Oberling and Guerin (1934, 1936) injected it subcutaneously into rats and mice and found sarcomas at the site of injection from 12 to 18 months later. There was also a correlation between the dose administered and the incidence of tumours after one year, 5 ml producing tumours in 100% of the animals, 2.5 ml in 71% and 0.5 ml in 42%. As the incidence of tumours did not increase proportionately with the dose, it was considered possible that even very small doses might produce tumours. Similar experiments were carried out by Selbie (1936, 1938) who showed a tumour incidence in rats of 33% after 14 months with injections of 0.6 ml and Foulds (1939) who found a tumour incidence of 44% in guinea pigs after

37 months with injections of 0.8 to 1.2 ml. Selbie suggested that the effects were produced by a combination of two factors, namely, the radioactivity of the thorotrast, principally its alpha-particle activity, and the extra susceptibility of inflammatory tissue which is produced by the presence of thorotrast acting as an irritant.

Similar effects did not appear at first in man and several reports reviewed large numbers of cases in which thorotrast had been used without damage. For example, over a period of five years, Yater et al (1936) studied 200 patients, most of whom received injections of approximately 75 ml intravenously. Many of them were seriously ill and died soon afterwards but of 47 still living, some four years later, no new conditions attributable to the radioactivity of thorium were observed. Seven years later Yater and Coe (1943) were still able to report no immediate or remote effects from their experience of 286 cases, 66 of whom had lived from one to ten years after injection. Similar conclusions were drawn by Bigler, Kaucky and Abraham (1935) from their study of 175 patients over a 3½ year period.

On the other hand, isolated cases did appear which were thought to indicate damage produced by radioactivity. Fleming and Chase (1936) cited two cases; in one, deposits in the liver caused abnormal conditions and in the other some thorotrast which had escaped into tissue near a vein caused fibrosis surrounding a granular lump in which the histiocytes were loaded with thorotrast. The latter reaction became quite common, one case being reported by Mora (1940), two by Ziffren (1940), one by Schumann (1943), seven

by Amory and Bunch (1948) and three by Vogtlin and Minder (1952). Struppler (1952) diagnosed a thorostroma 23 years after arteriography and detected radioactivity in the surrounding tissue. Prezyna, Ayres and Mulry (1953), in a similar case, detected the activity using alpha-track autoradiographs and counter measurements and found that after excision the surrounding tissue still contained thorotrast equivalent in gamma-ray activity to 5 to 6 ml and there were also slight counts over the liver and spleen.

More serious reactions were also reported, particularly in recent years: Laborde (1939), aplastic anaemia after 10 years; MacMahon, Murphy and Bates (1947), malignant endothelial cell sarcoma of the liver and aplastic anaemia after 12 years; Jonsell and Lindgren (1944), abnormal liver conditions 10 years after receiving 75 ml; Schmidt, Schulte and Lapp (1950), marrow aplasia 10 years after arteriography; Abrahamson, O'Connor and Abrahamson (1950), lung carcinoma 15 years after 75 ml; Vogtlin and Minder (1952), primary squamous cell carcinoma 18 years after receiving 30 ml; Macaschin, Manti and Germann (1953), fatal panmyelopathy 7 years after 30 ml; Rudolphi (1950), malignant tumour; Ruf and Phillip (1950), Zollinger (1949) and Grebe (1954), blood disorders.

Spier, Cluff and Urry (1947) reported another case of aplastic anaemia nine years after thorotrast visualization of the spleen. Samples of the spleen and bone marrow were analysed and from the activity it was shown that the energy dissipated by alpha-particles per gram of tissue was 123 ergs per hour in the bone marrow and 6.3 ergs per hour in the spleen. The finding that the tissue dosage in the bone marrow is greater than in

the spleen is unusual.

Hughes (1953) diagnosed changes in the central nervous system as due to the presence of thorium in the cranium and spine 13 years after injection of 5 ml into the cerebral ventricles. Alpha-particle counts of the thoron in the breath of the patient indicated that the total content of the body was about 5 ml. They were unable to obtain any gamma-ray counts from the body. The content was also estimated from the area and density of radio-opaque shadows showing that at least 3 ml were present in the brain.

Recently, Looney (1954) has found radiographically that there have been changes in the bones of some of Yater and Coe's patients now 15 to 20 years after injection.

1.5. Discussion

Summarizing the findings of the various observers of the distribution after intravenous injection, it is established that the thorotrast is taken up in the cells of the R.E. system, particularly in the liver, spleen and bone-marrow, where it stays indefinitely, only small amounts of the decay products MsThl, ThX and Tn being removed in the excretions and breath. At first it is present in fine granules in the cells but later accumulates into larger aggregates.

According to different estimates the liver takes up between 30 and 80% of the amount injected and the spleen 3 to 20%. The wide range of values obtained by different observers may show the amount of variation between different patients caused by such factors as differences in the relative

masses or in the functioning of the organs, particularly in patients who are not normal. Or they may indicate fluctuations in the content in the organs in one patient, for example, it has been suggested that the thorotrast is being continually thrown into the blood stream and re-absorbed, or that there is a redistribution of thorotrast among the different organs after some time. On the other hand, the different methods of measurement employed may give different results. For example, results which depend on small biopsy samples are subject to the possible error that these may not be representative of the whole of the organ, and in gamma-counts, in which the count in the organ is compared with that in the thorotrast, it is assumed that there is no loss of soluble products.

From the point of view of tissue dosage, the content per gram of tissue, the specific activity, is important, and allowing for the much greater mass of the liver compared with the spleen, the content per gram of tissue in the spleen is greater than that in the liver by a factor slightly greater than one in man and 10 or more in rabbits. The bone marrow contains almost as much as or even more than the liver per gram and the lymphodes, adrenals, kidneys and lungs considerably less.

Although carcinogenic effects were produced in animals about one year after injection, no changes apart from minor histological ones were observed in patients less than seven years after injection. This suggests that the latent period is longer in man than in animals but it should also be pointed out that the dose producing carcinogenic effects in animals is much greater weight for weight than the maximum dose given to patients, 0.6 ml in a rat weighing about 0.25 Kgm compared with 75 ml in a man

weighing 70 Kgm. Moreover, the subcutaneous dose given to animals remained localized, a condition which only arises in patients when an injection has been badly administered. The concentration at the site of injection in animals probably exceeds that in the various organs in patients by an even greater factor.

Altogether 27 cases which might be regarded as "serious reactions" have been traced in the literature. This number may seem small compared with the total number of persons who must have received thorotrast, but it must be emphasized that thorotrast is given to patients who are seriously ill beforehand and few live more than a few years. Yater and Coe (1943) out of 286 patients could trace only 68 who had lived more than a year and of these only 10 who had lived 10 years up to the time when their report was compiled. The number of patients who have lived longer than the latent period for radioactive effects, which seems to be about 10 years since injection, must be relatively few and the cases which have appeared in the literature must be regarded as significant evidence of late radioactivity effects. This conclusion is further substantiated by the fact that the effects of a carcinogenic nature are mainly concerned with the organs in which the main deposits are found and also by the fact that it is well established that anaemias and other blood disorders are symptoms of radiation damage. An opportunity of studying the question systematically using a large number of cases is therefore of great importance in this field.

CHAPTER 2

ACCOUNT OF THE COPENHAGEN INVESTIGATION

2.1. Scope of the Investigation

In 1948 Finsen in Copenhagen suggested that a number of obscure illnesses occurring in his patients might be due to the toxic effects of thorotrast administered some years earlier and it was decided to investigate this possibility more fully. It was found that during the years 1938 to 1949, 1600 people in Denmark had received thorotrast for various purposes; it therefore seemed an excellent opportunity of carrying out a statistical survey. This was undertaken by Dr. Henriques with a team of pathologists, haematologists and a physicist at the Finsen Institute. Patients who had received thorotrast were examined, and their case histories were brought up to date, while blood tests and measurements of radioactivity were also undertaken. At the same time a series of experiments with animals, involving chemical, histological and haematological analyses, was commenced. In order to avoid alarming the patients, the results have not been published but have been summarised in a number of reports circulated privately from time to time (Henriques et al, 1950; Mitchell, 1951; Henriques, 1952; Henriques, 1953; Johansen, 1953a, 1953b; Hjort and Rundo, 1953). Altogether over 300 patients have been examined in Copenhagen to date. Here it is intended to quote general results for all patients, and to give details of the case histories and measurements of the patients whose tissues have been examined by autoradiography in the present study.

2.2. Radioactivity Measurements

The apparatus was designed by A. Ward (Henriques et al, 1950) and

consisted of the following units.

1. Gamma counters for measuring the activity of living patients.
   (a) A scintillation counter for mapping the activity at a series of points over the surface of the body.
   (b) A gamma ionisation chamber for determining total body radiation.
2. Alpha-ray scintillation counters for determining the radio-activity of blood corpuscles, plasma, excretions and small biopsy samples of tissue. The liquid samples were prepared by spreading thin layers and drying under an infra-red lamp, other samples were prepared by spreading ashed substances, and tissue sections, either paraffin-embedded or freezing microtome, were also used.
3. Beta counters (a) Liquid counters for blood and urine samples.
   (b) End window counters for ash etc.
4. Two arrangements for measuring the alpha-particle activity of thoron in breath.
   (a) Four-litre specimens of exhaled breath were collected in an ionisation chamber.
   (b) The patient breathed continuously into a small ionisation chamber.

With this apparatus it was possible to carry out routine measurements on all patients coming up for examination. The radioactivity was measured over the liver and spleen regions, the legs and neck, also biopsy samples and excretions. The results showed a relationship between counts recorded and the product of the dose injected and the "age factor", the

so-called "effective dose". The "age factor" is a measure of the "age" of the thorotrast, determined by the growth of decay products during the time interval between the injection and measurement, and is defined as the percentage of the maximum activity which is reached when all products are in equilibrium.

The average results for the first 100 patients (quoted by Mitchell, 1951) are given below. In this group of patients the maximum dose was 96 ml, the minimum 7 ml; only four patients received more than 40 ml and the average injection was 19.0 ml. The time interval between injection and measurement varied from 4.5 years to 15.4 years and the average interval was 10.8 years.

Radioactivity of breath		3792 alpha-counts per min. per 4-litre sample
	blood	1.92 alpha-counts per min. per ml
		1.52 beta-counts per min. per ml
	urine	1.09 alpha-counts per min. per ml
	ashed faeces	0.63 alpha-counts per min. per mg
Gamma counts over the surface of the body		
	liver	1033 counts per min.
	spleen	837
	neck	263
	legs	307

The average total content of the liver was 1.5 gm of thorium, of the spleen, 1.4 gm, and of the bone-marrow, 0.4 gm, giving a total content of 3.3 gm. As 19 ml contain 3.6 gm, 92% of the dose injected was

accounted for. The alpha-counts from thoron in breath corresponded to about 10^{-9} curie per litre which is at least 100 times the accepted dose. The radioactivity of the blood was also above tolerance.

For the average figures quoted, the tissue dosage in the spleen was 4 to 30 reps per week, in the liver ½ to 4 reps per week and in the bone marrow 7 to 60 millireps per week. These values were calculated from macroscopic considerations and represent average values. They do not take into account the higher dose in the neighbourhood of the thorotrast.

The results given in later reports (Henriques, 1952), when the number of patients examined was considerably more than 100, confirmed the earlier results for the average patient.

From the radioactivity measurements it was also shown that the activity of the blood corresponded to 10⁻⁵ of the amount of parent thorium in the liver and spleen. Most of the activity was concentrated in the corpuscles (95%) which contained ThB, ThX and possibly MsThl. The same elements were present in the excretions, the total amount of Th excreted being only almost 80 micrograms per day or 300 milligrams in 10 years. Loss of decay products from the organs was also confirmed by the fact that the gamma-activity of the organs removed at splenectomy and autopsy increased with time as the decay products which had been removed while the organ was in the living body accumulated <u>in vitro</u>.

Activity was also detected by means of track autoradiographs in the femur of a rabbit (Rundo and Hjort, 1953). The tracks originated in a granule of thorotrast in a Volkmann Canal; the granule must have been conveyed in the blood stream and lodged in the canal because of its large size.

The facts enumerated above give an indication of the processes involved in the transfer of elements to various parts of the body. The main deposits of thorotrast in the organs of the R.E. system are washed by the circulating blood and some of the soluble elements (ThB, ThX, MsThl) are carried away in the blood stream. Some of the activity is transferred to the lungs where it decays to Tn and is breathed out or gives rise to activity in lung tissue. Some of it is excreted. There is also some transfer of particulate matter by the blood stream.

2.3. Clinical Examination of Patients

The clinical investigation of patients was complicated by the fact that these patients had been given thorotrast for some serious illness and it was difficult to know whether to attribute a clinical symptom to the thorotrast or to the disease for which it was given. A latent period of 5 to 10 years was expected before the appearance of symptoms resulting from radioactivity and it was predicted that a disease caused by thorotrast should increase in severity with time because of the increasing activity. A latent period of 10 to 30 years was expected before the occurrence of malignant tumours.

Most of the Copenhagen patients received thorotrast for cerebral angiography and a few for arthrography of the joints. Radiographic indications corresponded to those described in the previous chapter, that is, distinct shadows in the liver and spleen, smaller ones in the lymph nodes. The most common general symptoms were increasing tiredness and inability to work, accompanied sometimes by a stitch-like pain, loss of

tolerance to alcohol, pain in the joints. The most serious reaction was radiation-induced dyscrasia. In the last report, Henriques (1953) gave data from 234 patients who had been examined and 93 of these had been re-examined after at least one year. A further 150 had been traced who had died from different causes after thorotrast, 30 of them less than two years after contamination with no sign of radiation effects. It was not known whether any of them suffered from severe blood disease. The whole group therefore consisted of almost 400 patients and the 234 who were followed were regarded as a selected group, the most favourable. Henriques gave the following summary.

The Cancer Problem

One patient with hypernephroma after 5 years, studied at autopsy.

One patient still living 9 years after contamination with myeloid leukaemia and larynx tumour.

One patient who was the daughter of a patient contaminated 6 years before, died from retino-blastoma and cystic kidneys at the age of one year.

Two patients died from aplastic anaemia 9 and 13 years after.

So far the cancer incidence in this group of patients was not above normal, but a further 10 years would be necessary before the question could be answered and 20 years before records would be complete.

Accidental Diseases from Paravasal Injections

Three cases of disturbances from paravasal deposits.

Radiation Dyscemia

Two patients died from aplastic anaemia (also mentioned above).

Two patients died from resistant anaemia.

The blood counts of thorotrast patients compared with a group of normal adults showed that more than half suffered slight eurythropenia. A few patients suffered from a more serious anaemia.

One third had cytopenias in the white series.

So far no satisfactory treatment of patients has been devised. A possible approach suggested was to find some substance which would render the aggregates soluble so that they might be removed from the body; various complexing agents have been tried without any success. Five patients have been splenectomised. This reduced the total body content by a large proportion and alleviated some of the symptoms. Anaemia was treated by means of blood transfusions.

2.4. Histological Examination of Tissues

Histological studies of sections from biopsies and autopsies of patients showed deposits of thorotrast in the phagocytic cells of tissue and bone marrow similar to those described in the earlier work. The deposits were usually quite small in recent cases (of the order of one micron) but were up to 50 microns in cases where thorotrast had been present for some years. Often there was evidence of fibrosis in the organs especially in the region of deposits.

In the rabbit series described below, the usual picture of the uptake of fine granules increasing in size and finally aggregating was observed.

The accumulations were found to be in places of the least importance and to be surrounded by extra dense fibrous tissue. Johansen (1953b) concluded that the fibrous tissue, as well as the dense deposits themselves, would absorb all the alpha-particles and a large proportion of the beta and gamma-rays. This would also reduce the rate of diffusion and thus the amount of the soluble isotopes which enters the blood stream. He states: "Everything considered, the investigations undertaken so far suggest that the defensive forces of the organism are able to eliminate no small part of the dangerous alpha-irradiation and to some extent of the beta-rays on the organs that are most sensitive to such rays."

2.5. Chemical Determinations

Chemical determinations of the thorium content of different organs were carried out by Johansen (1953b). A series of rabbits was given injections and killed at various times later. In all organs the preliminary deposition was complete within a few hours. In the liver the amount remained constant after two hours; in the spleen the maximum concentration was reached after two hours, later the organ became enlarged and although the absolute amount continued to rise there was a fall in the concentration; in the lungs there was a slight constant rise with time, and in the bone-marrow there was an evenly increasing deposition in the first 24 hours. The greatest amount in the kidneys was noticed at the beginning while the thorium was still in the circulating blood, after that it decreased. In the adrenal glands, the concentration decreased but the weight of tissue increased so that the absolute quantity remained the same. After the

preliminary deposition which was complete in 24 hours, the amounts in the various organs were as follows -

Liver	27%
Spleen	33%
Lungs	3 to 4%

The remaining 36% was distributed principally in the bone marrow and lymph nodes.

After 15 months, there was a different distribution. The quantity in the liver had increased and there was a decrease in the spleen, lungs and other organs. The amount in the bone-marrow remained constant. After redistribution the liver and spleen contained 70% of the total.

Different distributions were also noticed with different doses. With small doses the liver contained 60% of the total, with higher doses, 50%. The quantity in the spleen increased from 6% with small doses to 20% with larger doses. The bone-marrow was not much affected by different doses. Johansen concluded that the spleen plays the part of a protector of the bone-marrow for large doses.

2.6. Comparison with Radium Study

In the earlier investigation of the contamination of patients with radium, different problems arose because of the different chemical and radioactive properties of radium and thorium. The radium patients were mostly radium dial painters, some were patients who received radium solutions therapeutically, either orally or by injection, and a few were scientists who worked with radium. In all cases the radium was deposited

in the bone substance and many of these patients developed tumours or other changes in different parts of the skeleton, some of which proved fatal. The figures for 30 patients were given by Aub et al (1952). The total body content of the patients was estimated from the Rn alpha-count in the breath and in some cases by the gamma-ray counts from the body also. Some contained MsThl as well as Ra. The patients were grouped according to estimated body burden and the results are summarized in Table III.

The table shows that there is an increase in the number of deaths with increasing body content but that the tumour incidence is approximately constant for all groups with more than 0.7 microgram. The alpha-particle activity of 0.7 microgram of radium, assuming that 50% of the Rn escapes from the patient, is 5.95×10^4 per second. Thus 0.7 microgram of radium is equivalent to 12.8 ml of thorotrast, assuming all products in equilibrium and no escape of Tn. In the thorotrast group of 100 patients considered by Mitchell (1951), 22 had the equivalent of 1.0 microgram of radium, six of these were quite seriously ill and one had died. The maximum content was equivalent to 5.3 microgram. Thus this figure of one third of the patients with more than the equivalent of 1.0 microgram and less than 5.3 microgram who have developed radiation effects, is comparable with the group of radium patients with 0.7 to 7 microgram. In the radium group the latent period for the development of tumours was about 20 years. As this period has not yet been exceeded by any of the thorium patients, it is too early to say whether the effects of radium and thorium are similar.

As well as the total radium content of patients, figures for the tissue dosage at the site of radium deposits in bone are available from

TABLE III. Summary of Data from Radium Patients.

Radium Content micrograms	Number of Cases	Tumour Incidence %	Average Latent Period yrs.	Mortality Rate %	Average Period to Death yrs.
8-23	8	25	13	63	19
2-7	9	33	20	33	26
0.7-2	9	33	19	22	25
0.02-0.5	4	0	-	0	-
All cases	30	27	16	33	22

the alpha-track autoradiograph measurements of Hoecker and Roofe (1951). They noticed that the radium is concentrated within the Haversian systems and calculated the activity of these "hot-spots" from the total number of tracks recorded. The tissue dosage in the femur of two patients varied from 1 to 23 reps per day in different areas.

2.7. Cases - Histories and Measurements of Patients whose Tissues have been examined here.

Patient Z1 (No.4) (Henriques 1950; Mitchell, 1951)

Born 1916. Head injuries 1928. Bilateral arteriography with 22 ml of thorotrast into the carotid arteries on 17/10/38.

Tonsilectomy 1947. Splenectomy 1949; on this occasion biopsy samples of liver and other tissues were also taken.

Radioactivity measurements were carried out in 1949, i.e. after 11 years.

Gamma-counts per min.

Spleen from side	1830	
front	1320	Total side plus front 3150
Liver from side	1450	
front	1730	Total side plus front 3180
Trochantres and legs	956	

Alpha and beta counts

Alpha-particles in breath	8300 counts per min. per 4 litre
in blood cells	5.0 counts per min. per ml
Beta-particles in blood cells	3.5 counts per min. per ml
Alpha-particles in blood plasma	0.3 counts per min. per ml
in ashed faeces	0.71 counts per min. per mg

Alpha-particles in fresh faeces	24	counts per min. per mg
in urine	2.4	counts per min. per ml
Beta-particles in urine	1.6	counts per min. per ml

Present condition - vague symptoms and fatigue, abnormal blood counts. Chemical analysis of the spleen (Rundo, 1955) - thorium content of 0.0272 gm per gm of wet tissue.

Patient Z 361 (No.5) (Rundo, 1952; Johansen, 1953a)

Born 1912. 25 ml thorotrast on 21/6/39. Splenectomy 1950. Radioactivity measurements on 5/2/51, that is after 11.6 years.

Gamma counts from liver plus spleen	2655 counts per min.
Beta activity of blood	2.3 counts per min. per ml
Alpha activity of breath	6100 counts per min. per 4 litre

Died in December, 1952 from aplastic anaemia. Autopsy. Chemical analysis of organs (Rundo, personal communication) - The liver contained 1.34 mg per gram of wet tissue; it was very large and probably weighed about 2 Kgm. The spleen was also large, 335 gm, containing 0.75 gm of thorium.

Patient E 804 (No.3) (Mitchell, 1951; Henriques, 1952)

Born 18/7/1893. Died 23/4/1951 from ruptured aortic aneurysm 14 days after receiving 40 ml of American thorotrast. At autopsy a large number of tissues were obtained and the thorium content estimated. Most tissues were found to contain some activity. Here only the tissues containing the largest amounts which have been used in the autoradiographic determinations will be quoted.

Thorium content of tissue.

Liver hilum	470 microgram per gm
Liver tissue	520
Spleen periphery	3893
Sleen hilum	3745
Bone marrow (2nd lumbar vertebra)	1730

The first two patients (Nos. 4 and 5) are comparable as they received injections of the same order of magnitude and were examined after similar periods of time. Although the product, dose x age factor is similar for the two cases, both the gamma-counts and the clinical symptoms are different. It is therefore evident that further factors are operating, for example, the different susceptibility of patients to radioactivity effects or a different distribution such that more susceptible organs receive a larger dose in the one case than in the other.

CHAPTER 3

GENERAL REVIEW OF AUTORADIOGRAPHY

3.1. Historial Survey

First it is intended to survey briefly the development of autoradiography as a means of locating radioactive substances in biological specimens. Particular aspects of the technique will be discussed in detail in later sections.

This technique depends on the phenomenon of the blackening of photographic emulsions by radioactive substances, discovered by Becquerel in 1896 and first used to study the distribution of radioactive substances in animal tissues by London in 1904. A photographic film was placed in contact with a section of tissue mounted on a slide and held in place for the required exposure time. The film was then separated from the section and developed like an ordinary photograph. The intensity of the blackening in different areas of the film indicated in which part of the organism the radioactive element was located.

London's technique illustrates the essential features of any autoradiograph. At first it was restricted to the study of the distribution of naturally-occurring radioactive substances which were of limited interest biologically. With the introduction of artificially produced radioactive substances, the technique offered new possibilities particularly in the investigation of biological processes by means of radioactive tracers. This type of experiment depends on the fact that the radioactive isotopes are chemically identical with the non-radioactive ones; they

behave in a similar way biologically and they can be located because of their activity. The most useful isotopes in this respect are those of the common constituents of the body. A biologically active compound can be labelled with the isotope of one of its constituents and detected in various tissues by means of autoradiographs. The simple contact type of autoradiograph gives a general indication of where the radioactive substance is present, but for precise localisation of atoms in minute intracellular structures, a higher degree of resolution is necessary. This has been achieved by the use of special photographic emulsions and special techniques for preparing and mounting the autoradiographs.

The types of autoradiographs in general use now are - (a) the contrast autoradiograph in which thin layers of fine grain emulsion record the radioactive substance as a collection of black grains, the density of the grains in different areas being a measure of the activity in that position, and (b) track autoradiographs in which special Nuclear Research emulsions record the tracks of single charged particles which can be traced to points of origin within tissue structures. Both these methods may use a variety of different mounting techniques which ensure good contact between the emulsion and section, a condition which is necessary for good resolution. In any experiment the technique is chosen according to the requirements of the experiment, namely, the type of radiation emitted, the resolution required and the solubility of the labelled compound in the various solutions used for preparing and mounting the tissue. These factors are dealt with in greater detail in the following sections.

3.2. Photographic Emulsions

The manner in which an image is formed in a photographic film is described in many texts (for example, Mees, 1946). The film consists essentially of a layer of silver halide grains from 0.2 to 0.5 microns in diameter, embedded in gelatine. When an ionizing particle passes through one of these grains, it releases electrons from the bromide ions. These free electrons migrate through the crystal until they are trapped at specific points known as sensitivity specks. Here they combine with the silver ions to form a speck of metallic silver known as a latent image. On development, metallic silver is precipitated around the grains which are seen as black dots from 0.5 to 1.0 microns in diameter under a microscope.

The condition for the production of a latent image is fulfilled if a sufficient number of ions are produced in the grain. The probability of a grain being rendered developable therefore depends on the energy lost by ionization by a particle in passing through it. The rate of loss of energy by an ionising particle in passing through any medium is represented by an equation of the form

$$-dE/dx \propto (Ze)^2/v^2$$

where e is the charge on a particle moving with velocity v through a medium consisting of atoms of nuclear charge Ze. For a photographic emulsion, which is a heterogeneous medium consisting of molecules of gelatine and of silver bromide, the energy loss of particles in the gelatine is negligible compared with the loss in the silver bromide grains. The energy loss per unit length of path therefore depends on the size of the

grains and their concentration in the gelatine. The equation shows that the energy loss also depends on the charge and velocity of the particle which is passing through the grain, and hence on the type of particle. These considerations form the basis of methods of preparing emulsions which are sensitive to different types of particles of different energies by varying the size and concentration of grains.

In contrast autoradiography requiring exact localisation, best results are obtained using fine-grained emulsions of high grain content so that the grain size of the image is small compared with the histological structure being examined and each particle has a high probability of being recorded. The loss of sensitivity due to small grain size may be overcome by the addition of sensitizers which increase the intrinsic sensitivity of the grain. The types of emulsion prepared for autoradiography by Kodak are sensitive to 80 to 100 kv electrons (Hers, 1951).

Suppose now that the charged particle passes through a number of grains in succession. Some of these may be rendered developable and if they are sufficiently close together the path of the particle will be recognisable as a series of black dots or a track. The number of grains in the track and its total length depends on the size and concentration of the silver bromide grains and the energy of the incident particle. Emulsions which record particle tracks, known as Nuclear Research emulsions, are prepared with varying sensitivities. Some emulsions record alpha-particles and other heavy particles but the most sensitive types of emulsions now available record all types of particles including high energy beta-particles. The tracks formed are characteristic of the particles, for example, a

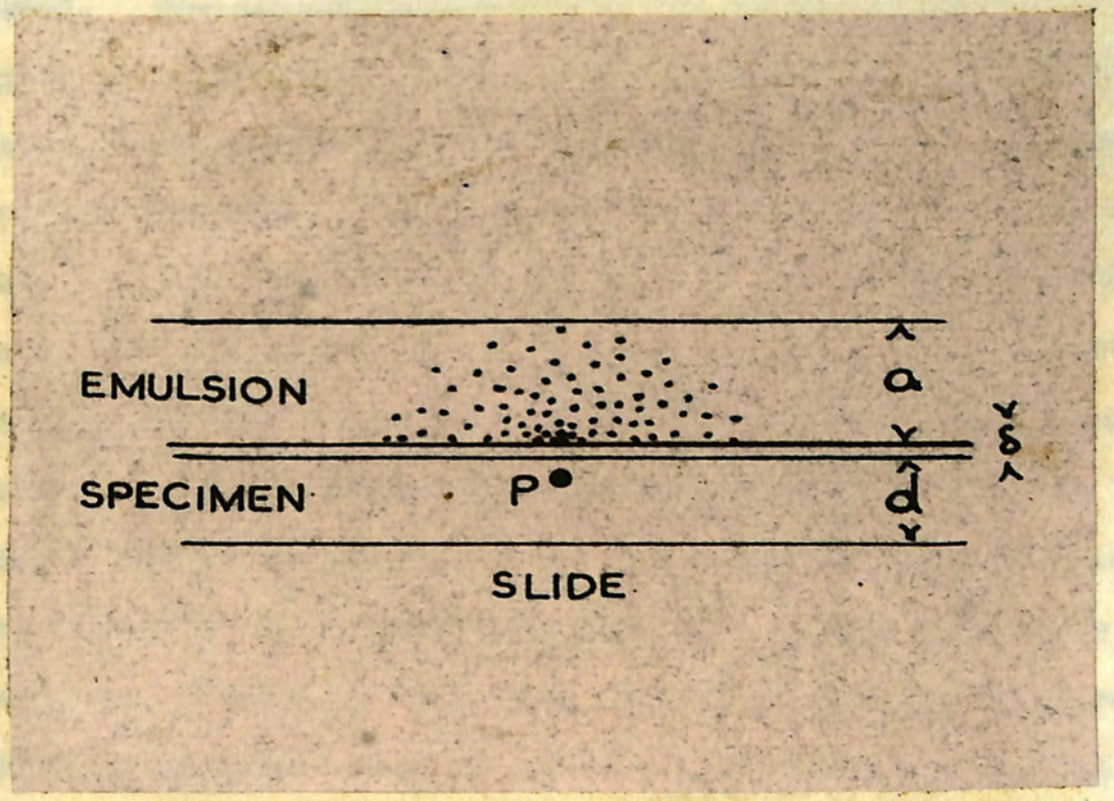
doubly charged alpha-particle produces a straight thick track, whereas a singly charged proton of similar energy produces a lighter track and because of its light mass this particle is more often scattered by the atoms of the emulsion through which it passes; an electron produces a fine straight track at high energies but is highly scattered towards the end of its range. Moreover, since the ionisation per unit length of path increases as the velocity of the particle decreases, the grain density of the track increases towards the end. This applies to all types of particles but in an alpha-particle track the ionisation is so great along the whole track that the effect is not noticeable. The types of emulsion used in track autoradiography are those which are sensitive to alpha-particles (for example Ilford C 2 emulsions) and electrons (Ilford G 5).

3.3. Contrast Autoradiographs

The autoradiograph in general use for locating beta-emitting substances is the contrast autoradiograph. The arrangement is as shown in the Figure 3.1. An emulsion of thickness "a" is placed in contact with a specimen of thickness "d", the separation between the two being "δ". A source of radiation at P emits particles in all directions and is recorded as an area of blackening in the emulsion. If the intensity of the blackening is plotted against the distance from P, the curve obtained has a maximum at P and the width of the curve depends on the spread of the image.

The value of this type of autoradiograph depends on how well one can distinguish between the images of two adjacent sources of activity, that is,

FIGURE 3.1. Diagram of Contrast Autoradiograph



on the resolving power of the method. A mathematical treatment of the geometrical and photographic properties which determine the resolving power was given by Doniach and Pelc (1950). They defined the resolving power for a source of zero diameter as the distance between the centre of the image where the intensity is a maximum and the point at which the intensity is halved. The number of grains in the image at various distances from the point of maximum intensity was given in terms of the photographic response of the emulsion to electrons, the exposure and the geometry of the arrangement defined above. For a source in the form of a rod of zero diameter and thickness d, they obtained an expression for the number of grains at various distances, which, when evaluated for different values of a, d and b, showed that the most important factor in determining the resolution of the autoradiograph is the separation between the emulsion and section. For a section 5 microns thick and emulsion 15 microns, if the gap is reduced from 3 microns to 0.1 microns, the resolving power is improved from 17 microns to 3 microns and if the gap is further reduced to 0.01 microns the resolving power is 2 microns. The thickness of the section and emulsion affect the resolution less. For a section of 2 microns and an emulsion of 2 microns the best resolution is 1.5 microns. An extension of the above calculation of resolving power to sources of finite area was carried out by Gross, Bogoroch, Nadler and Leblond (1951). First they calculated the resolution for a line source and then extended these calculations to plane and cylindrical sources as these more nearly represent the dimensions of sources encountered in practice. Curves for cylindrical sources show that the resolution is never as good as with point

sources and variations in the thickness of the emulsion, section and interspace have a relatively smaller effect on the resolution.

Lamerton and Harriss (1953) introduced a new definition of resolution for sources of finite dimension as "the distance 'd' if the images of two uniformly active cylindrical sources of diameter 'd' can just be resolved when the centres are separated by the distance 2d". They calculated resolving powers for different thicknesses of emulsion and specimen, assuming zero gap as this is the condition approached in practice, by the use of stripping film or liquid emulsion (3.6). For cylindrical sources in a specimen of thickness 2 microns, the resolution is 2.1 microns.

The other important factor in determining the value of the autoradiograph is the intensity of the image produced in a given exposure time. For intense sources a short exposure may be sufficient to give an image which is not distinguishable as separate grains even under high magnification and the blackening in different areas is determined by means of optical density measurements. This type of autoradiograph is used for the investigation of macro-sections using X-ray film where an optical density of 0.5 is suitable. Hers (1951) calculated that only 1.5×10^{-16} gm of P^{32} per mm^2 of tissue are required to produce this density in one day's exposure. On the other hand, in high resolution work, Boniach and Pele considered 10 developed grains above a cell nucleus to be acceptable as an autoradiograph. Assuming one developed grain per beta-particle and an exposure of two half-lives, they concluded that at least 30 radioactive atoms must be present in a cell nucleus to produce an autoradiograph. The grain yield varies with the grain size of the emulsion and the energy of the radiation. In Kodak Stripping

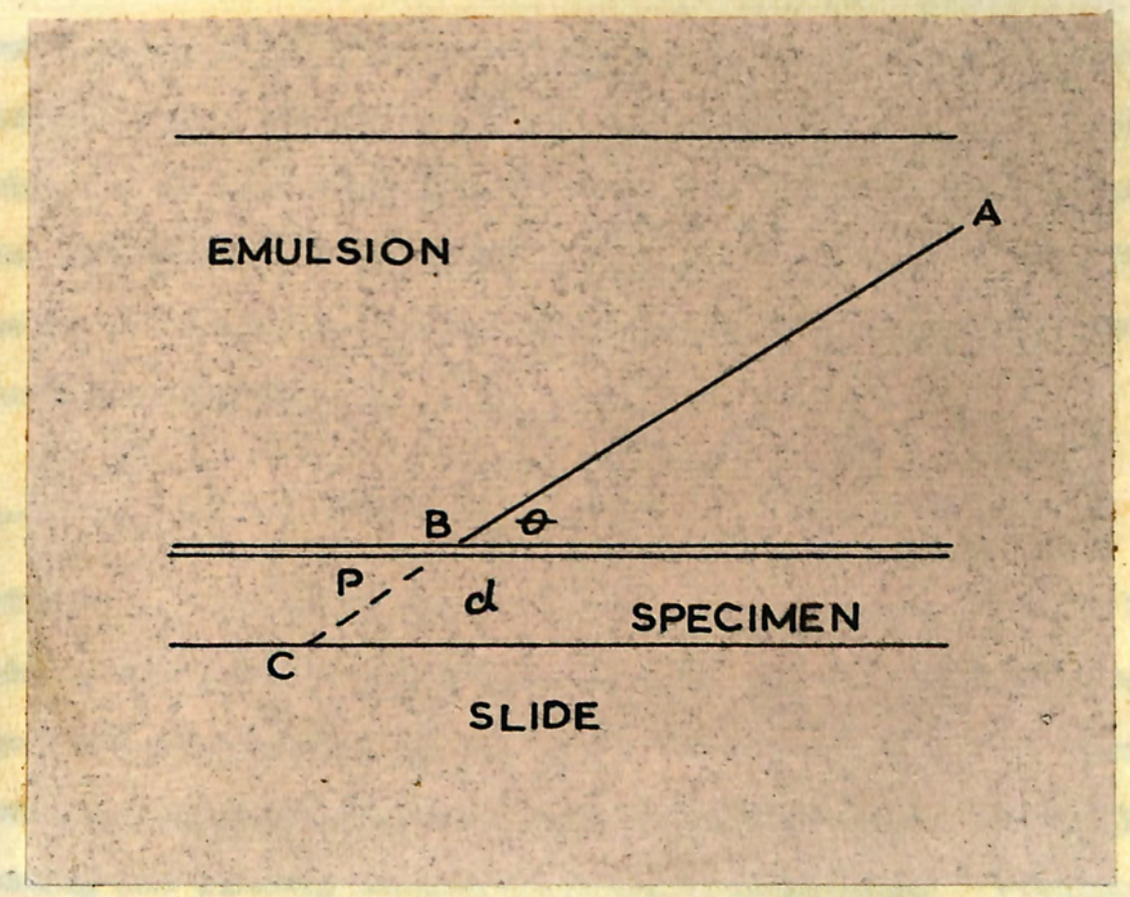
Film (3.6), Lamerton and Harriss found that for normally incident P^{32} electrons, the grain yield was 0.5 grains per electron and for slower S^{35}, 1.8 grains per electron.

In autoradiographs of specimens of low specific activity the limiting factor in obtaining a recognizable result may be the density of the background grains. The background arises from chemical effects and external sources of radiation; with careful handling and pure chemicals, Lamerton and Harriss reduced the background to 1.5 grains per 100 square microns. Even so in one experiment using P^{32} they were still only able to show statistically a doubling of the grain count above background over the nuclei of isolated cells.

3.4. Track Autoradiographs

The arrangement of section and emulsion is similar to that of the contrast autoradiograph but each particle is recorded as a single track. The accuracy in assessing the point of origin of the track depends primarily on the thickness of the section. In the diagram (Figure 3.2), a particle originating at P is recorded as a track AB in the emulsion at an angle θ to the plane of the emulsion. If the position of P is unknown it may be estimated by extending AB to C as P must lie at some point along BC which represents a distance $d/\sin\theta$. The smaller this distance, the greater is the accuracy in placing P. It therefore follows that for greater accuracy the section should be very thin. The accuracy is also greatest for tracks at large angles to the emulsion, being equal to the thickness of the section for tracks perpendicular to the emulsion. This

FIGURE 3.2. Diagram of Track Autoradiograph



relationship holds for alpha-particles and high energy beta-particles which form straight tracks whose direction is easily determined. However, for slow beta-particles which are greatly scattered, the initial direction must be determined from the first two or three grains of the track and a further uncertainty is introduced by the finite size of the grains. Lamerton and Harriss (1953) claim that the resolving power of the contrast autoradiograph is greater than that of the track autoradiograph for beta-particles. However calculations have shown (Blundell, unpublished) that, allowing for the scattering, the error in assessing the point of origin of a beta-particle can be reduced to the order of 0.5 microns for a 1 Mev particle. This is considerably better than the accuracy obtained with the contrast autoradiograph.

3.5. Preparation of Sections

Thin sections of tissue prepared by one of the routine histological procedures are used for autoradiographs. The tissue is first fixed in a chemical which preserves it against deterioration, it is then dehydrated and immersed in some embedding medium which provides a firm block for sectioning and finally sectioned using a microtome. Various chemicals are used for fixing and the choice depends primarily on which special features of the tissue must be preserved. Another consideration which may be important in quantitative estimates of the radioactive content of tissue in autoradiography, is the shrinkage of the tissue. The volume of tissue in the section corresponds to a larger volume of fresh tissue and the shrinkage may vary from 10 to 50% of the volume of fresh tissue depending

on what fixative and embedding medium are chosen. In autoradiography of sections containing radioactive isotopes, the solubility of the isotope in the various solutions will determine which procedure is most satisfactory.

For insoluble isotopes the most common fixatives are formalin and alcohol. Doniach and Pelc (1950) found that alcohol is preferable as there is less interaction between the fixative and the emulsion resulting in artefacts in the autoradiograph. The tissues are usually embedded in paraffin wax and the sections are flattened on warm water before mounting on microscope slides. When preparing the autoradiograph, the wax is removed by dissolving it in xylol.

The method of preparing sections as described above involves a number of operations employing various solvents as well as washing in water and this may not be suitable when the tissue contains soluble isotopes, as a considerable proportion of the isotope may be lost. An alternative is to use sections prepared by freeze-drying as described by Scott-Russell, Sanders and Bishop (1949) and by Holt, Cowing and Warren (1949). The tissue is rapidly frozen to about -190°C and dehydrated at about -40°C, after which it may be embedded in paraffin wax and sectioned in the normal way.

3.6. Mounting Techniques

The Contact Method which was described above has the advantage of simplicity but is limited to rough work and to opaque sections such as bone where other methods are not satisfactory. For most purposes it has now been superseded by one of the following methods which have the common

characteristic that the section and emulsion remain in contact throughout exposure and processing and are viewed simultaneously through the microscope.

In the Mounting Method the section is flattened on water and then mounted directly on a photographic plate. This method was used by Endicott and Yagoda (1947) to prepare alpha-track autoradiographs of sections containing polonium. They used nuclear track plates and found that they could distinguish between tracks which originated in the nucleus or cytoplasm of cells. Evans (1947) used a similar procedure for preparing contrast autoradiographs of I^{131} in thyroid.

The autoradiograph coated with liquid emulsion was first suggested by Belanger and Leblond (1946) who melted emulsion from a photographic slide and painted the liquid over a section of tissue which had been first covered with celloidin. Ilford now prepares special emulsions in gel form for this purpose. These emulsions are sensitive to alpha-particles and electrons (similar to the C2 and C5 series) and may be poured to any desired thickness. Usually the gel is melted and poured over the mounted section as described by Blundell and Rotblat (1951) for the preparation of track autoradiographs of blood smears containing Zn^{65}. In the same way Gros, Guer and Rechenmann(1952) recorded beta-particles from I^{131} and P^{32} and alpha-particles from thorotrast. Levi (1953) recorded alpha and beta-particles from thorotrast simultaneously in a C5 emulsion. Sometimes organisms are mixed with melted emulsion as described by King, Harris and Tkaczyk (1951).

Instead of coating the section with liquid emulsion, it may be covered with a stripping film. Kodak Stripping Film (Berriman, Nora and

Stevens, 1950) consists of a 5 micron layer of sensitive film on a 10 micron gelatine backing. The film and backing are mounted on a glass support from which they are cut and stripped as required. A piece of film sufficient to cover the tissue is floated on water and mounted over the section so that the emulsion is in contact with the section and the gelatine forms a protective covering.

The floating methods of preparing autoradiographs which involve floating either the section or the emulsion on water are not suitable for water-soluble tracers and several modifications have been suggested. Holt, Cowing and Warren (1949) separated the section from the plate by means of a waterproof layer but the resolution is decreased due to the increased separation of the section and emulsion. Wintringham, Harrison and Hammond (1950) took the precaution of excluding water by pressing the stripping film in contact with the section by means of a moist brush. Harris, Sloane and King (1950) used the mounting method but flattened the section on warm mercury and pressed the photographic plate in contact with it so that the section adhered to the emulsion.

Although the above methods of preparing autoradiographs have been found satisfactory for many purposes, there are several disadvantages in keeping the section and emulsion together throughout exposure, processing and staining, such as artefacts due to interaction between the section and emulsion, difficulties in staining the section when the emulsion is present (3.7) and loss of quality in the photographic image. These considerations have led several workers to adopt a method whereby the section and emulsion are in contact during exposure only, are

processed and stained separately, and are brought back to their original positions for measurement. The most difficult part of such a procedure is the realignment of the section and emulsion afterwards, especially when high precision is required. Hoecker, Wilkinson and Kellison (1953) overcame this difficulty by mounting the section on a flexible plastic slide and cementing this at one end to an emulsion-covered plate. During the exposure the two plates were pressed together and for development the plastic slide was held away from the emulsion. When dry it could be replaced to within a micron. This type of autoradiograph was designed for use with bone sections. Blundell (unpublished) solved the problem of realignment using specks of a radioactive substance as fiducial points. These were placed at the end of the slide on which the section was mounted and covered with a small piece of stripping film. During exposure alpha-particles passed through the stripping film into the Nuclear Research emulsion which was also recording the radioactivity of the section. After development the two ends of the alpha-particle tracks which appeared in the stripping film and in the Nuclear Research emulsion were brought into line by moving the slide holding the section and stripping film relative to the emulsion. A special fine adjustment mechanical stage which was fitted to the microscope was designed for realigning the plates. The end of a track could be determined to within a micron using this apparatus.

3.7. Staining Technique

Histological identification of cell structures requires that the tissue should be stained with dyes which are absorbed differentially by

specific parts of the cell. In autoradiographic preparations the section may be stained in the ordinary way before coating it with emulsion but the stain may be impaired when processing the emulsion. In order to protect stained sections, Blundell and Rotblat (1951) covered them with impermeable layers before pouring the emulsion. Other workers (Doniach and Pelc, 1950) preferred to stain the section through the emulsion after processing using diluted solutions of the stain and prolonged soaking. However the loss of detail in the cell structure compared with sections stained without the presence of emulsion reduces the value of the autoradiograph. Belanger (1950) prepared an inverted autoradiograph in which the emulsion-section complex prepared by the coating method was removed from the slide and remounted inverted on another slide for staining. A further modification introduced by Levi (1953) consisted of a repetition of the inversion process after staining so that in the final autoradiograph the emulsion was on top of the section. This was an advantage when dealing with opaque material such as thorotrast as it alleviated the difficulty of counting tracks beneath the thorotrast. The difficulties of obtaining well-stained sections are also overcome by using the realignment techniques (3.6) when the section may be stained quite separately from the emulsion.

3.8. Applications of Autoradiographs

The applications of autoradiographs which have been mentioned above include only cases in which the techniques used have been of special interest. These techniques have been applied to many biological problems which have recently been summarised in several reviews (Gros et al, 1951;

Hers, 1951; Bourne, 1952; Gorbman, 1948; Doniach, Howard and Pele, 1953). Since many of them have little relevance to the present study, only brief descriptions need be given here.

Much work has been done on the location of elements in bone, including calcium, strontium, barium, radium, carbon, phosphorous and fission products. Another important application of which many examples appear in the literature is the uptake of I^{131} in thyroid and the clinical investigation of abnormalities of the thyroid. Applications on the cytological level include the preparation of autoradiographs of single cells and chromosomes demonstrating the incorporation of P^{32} into desoxyribonucleic acid. Autoradiographs have also been prepared of plant tissue, tobacco mosaic virus and rock specimens.

In concluding this survey, some experiments which are more relevant to the present study might be mentioned specifically. The demonstration of alpha-particle tracks associated with thorotrast in the R.E. cells and in bone, and the determination of the thorium content of tissue from particle counts, have been described previously (1.5 and 2.2). Also using particle counts Gros, Cuer and Rechenmann(1952) estimated the density of thorotrast aggregates in tissue. Their results will be discussed later (9.3).

The possibility of determining the position of a ThB atom from the alpha-particle emitted by it was suggested by Rotblat (1949). A track autoradiograph was prepared from a blood smear from a rabbit which had been injected with ThB. Two alpha-particles were shown coming from a single cell and by an analysis of the track lengths it was proposed to

determine the point of origin of the tracks, in particular whether the ThB was attached to the outside of the cell or whether it was within the cell.

The recording of alpha-particle tracks from radium deposits in bone (Hoecker and Roofe, 1951) demonstrates the use of track counts in the determination of tissue dosage. A section of bone was placed in contact with a photographic plate and the total number of tracks recorded, N, from a given area A cm^2 in a given exposure time τ hours was calculated. The thickness of bone in which the particles originated was determined by the range of the particles in bone, R microns, but only $\frac{1}{2}$ of the total number of particles emitted in this layer were recorded. The tissue dosage was calculated from the product of the total number of disintegrations per cm^3 per hour, $^{4N}/A \times R \times 10^{-4} \times \tau$ and the mean energy of the alpha-particles.

CHAPTER 4.

TECHNIQUES USED IN THIS INVESTIGATION

4.1. Requirements of the Autoradiograph

In the determination of tissue dosage from thorotrast, it was necessary to prepare an autoradiograph in which the alpha-particle tracks were visible together with the tissue cells, so that the number in a certain area and their lengths could be determined. The preparation was simplified in the case of thorotrast sections as thorotrast is a very insoluble substance and is not removed to any great extent by the usual histological fixing processes. In staining, minute histological detail was not necessary; it was merely sufficient that the tissue cells and the thorotrast should be correlated with the tracks. These requirements were fulfilled in the coated liquid emulsion autoradiograph. In some experiments it was not necessary to measure the numbers of tracks in a given area of tissue and a simpler contact autoradiograph which could be used for measuring track lengths was adopted.

4.2. Preparation of Sections

Most of the soft tissues used were fixed in formalin and embedded in paraffin wax. In order to determine whether there was any loss of activity in the fixatives, all solutions were preserved from one series of experiments and tested for radioactivity by means of liquid counters. The greatest activity observed in any of the solutions represented only 0.8% of the original injection and it was concluded that the loss in this way was negligible. This result did not agree with the experiments of Hjort (unpublished) in Copenhagen who found some evidence that decay products

were leached out in the solutions but details of his measurements are not available. In our experiments, any loss of short-lived elements did not affect the results as the sections were not examined until some time after they had been prepared and the elements would have had time to accumulate again.

The sections were cut approximately 3 to 10 microns thick and were mounted on 3" x 1" microscope slides. In most cases the slides were cleaned, dried and used without further treatment. Sometimes it was found that the emulsion did not adhere well to the slide when it was placed in the developing solutions and better results were obtained by mounting the sections on prepared slides supplied by Ilford. The slides may also be subbed in a dilute solution of gelatine containing chrome alum (Pelc, 1950). Doniach and.

As well as the 3 micron sections some very thin ones were obtained. Special techniques are necessary for cutting very thin sections; in this case the embedding medium was methyl methacrylate, and the sections were cut from 0.1 to 1 micron thick. The methacrylate was removed using amyl acetate as solvent before pouring the emulsions.

Bone sections presented another problem. Two of the elements of the thorium series, MsThl and ThX are isotopes of radium and might be found in bone substance. Usually bone sections are prepared by removing the calcium but in this case MsThl and ThX would also be removed and the sections would be of no value. Undecalcified bone sections were prepared by embedding the bone in a bioplastic and cutting on a special microtome.

4.3. Preparation of Emulsion

Ilford G2 Nuclear Research emulsion in gel form was used to coat the slides. These emulsions were stored in sealed containers at about 5°C when not in use and were handled in orange safelight for melting and processing.

Before coating wax sections, the wax was removed using xylol and the slides were then taken through descending strengths of alcohol to distilled water. The slides were warmed on a hot plate at about 30°C. The emulsion was melted in a test tube kept at 50°C. The melted emulsion was measured out using a Pasteur pipette and poured over the slide which had been transferred from the hot-plate to a levelled board at room temperature. The emulsion covered the slide to a uniform layer and was left to set. The volume of emulsion used was controlled by the size of the pipette to give a layer of the required thickness. A volume of 0.75 ml of melted emulsion spread on a 3" x 1" slide produces a layer 50 microns thick when dry. This was the thickness used generally in this work so that the longest alpha-particle track from Th C', 49 microns, was fully recorded. When set the emulsion was dried in a stream of warm air, packed away in the dark during the exposure and processed with the section still in contact with the emulsion.

Since the emulsion is recording tracks as soon as it comes in contact with the section, it is important to control the time taken for the emulsion to dry. Initially it contains a large amount of water and since the stopping power of wet emulsion is less than that of dry, tracks formed

during this wet stage are longer than normal. In a normal dry emulsion an alpha-particle track consists of an almost continuous row of grains but when the emulsion is wet the grains are spaced out to such an extent that the particle has to travel a much greater distance to strike the same number of grains. A track formed while the emulsion is wet is thus recognizable because of its wide grain spacing and its greater length. In practice, when a plate is drying, the water content is continually decreasing with the result that the lengths of tracks belonging to one group vary from the range in wet emulsion to that in dry emulsion (5.3). Although some of the tracks formed in the wet emulsion are recognizable and may be avoided in measurement, some are indistinguishable from the normal tracks and it is therefore important to reduce the drying time so that it is short compared with the total exposure. Normally a 50 micron plate takes about 12 hours to dry in the atmosphere at room temperature, a considerable length of time compared with an exposure of 3 or 4 days. Sometimes the exposures were much longer than this, about 40 days, and the drying time is then not so important.

The procedure first adopted for drying plates was to pump out the water in a vacuum system but this was not entirely satisfactory as air bubbles in the emulsion and air locks between the section and emulsion caused wrinkling in the autoradiograph. Drying in a warm current of air was found to be more satisfactory and reduced the drying time to about two hours; an electric hair-dryer placed at a distance of about three feet from the plates was used for this purpose.

For the preparation of contact autoradiographs, the section was prepared and mounted as above. An Ilford 50 micron C2 Nuclear Research plate was placed in contact with the slide and fastened by means of cellulose tape to prevent slipping. The two plates were held together in a press for the required exposure time. The slide bearing the section was then removed and the C2 plate processed for alpha-particles in the usual way. Later the two plates could be matched roughly in order to estimate the activity over certain areas of the section.

4.4. Processing and Staining Techniques

The emulsions were processed following the usual procedure for Nuclear Research emulsions. Since the emulsions are thicker than ordinary photographic films it is necessary to use dilute developers and to prolong the development time so that the developer reaches to all depths of the emulsion. The method used for developing 50 micron C2 emulsions for alpha-particles was to develop for 15 minutes in 1% Azol at 22°C. The development was then arrested by placing the emulsion in 2% acetic acid for 10 minutes. The fixation was carried out in "hypo" at room temperature and speeded up by continual agitation. It takes about one hour to clear a 50 micron plate and the fixing was continued for a further 50% of this time. The plates were then washed in running water for 1 to 2 hours and dried at room temperature. During the fixing the emulsion loses a considerable volume of unexposed silver bromide and its depth shrinks by a factor of about 2.5. The autoradiograph at this stage therefore consists of a layer of tissue covered with a gelatine layer about 20 microns thick.

The procedure described above is suitable for emulsions up to 100 microns thick, but for thicker emulsions precautions must be taken to avoid differences in development in different layers of the emulsion. Although the top layers of a thick emulsion may be developed correctly, the tracks towards the bottom may appear underdeveloped. The temperature development cycle used by Dilworth, Occhialini and Payne (1948) overcomes this difficulty. First the plate is soaked in developer at about 5°C so that the solution penetrates to the bottom of the emulsion but does not develop it appreciably. The temperature is then raised to about 22°C, but in order to avoid further increases in the concentration of the solution in the plate it is first removed from the developer bath. Development is followed by a soaking in cold stop bath, then by a rise in temperature and the same repeated with the fixative. This procedure was followed for poured emulsions 200 microns thick, when the times taken were 30 minutes in 2% Azol developer at 5°C, 15 minutes at 22°C, 30 minutes in cold stop bath, 10 minutes at room temperature. Fixation takes several hours for thick emulsions.

The tissues were stained through the processed and dried emulsion by soaking in 2% haematoxylin for about 12 hours. It was sometimes necessary to first make the emulsion acid by a preliminary soaking in 2% acetic acid; otherwise the emulsion tended to take up the stain to a deep blue and the contrast between section and emulsion was poor. The section was afterwards "blued" in tap water. In the thorotrast sections, the aggregates of thorotrast appeared as yellow granular deposits against the blue-stained cell nuclei. They were more easily distinguishable without using eosin as

a counter stain as is the practice with H. & E. preparations, and gelatine does not withstand prolonged soaking in eosin.

4.5. Observation of Autoradiographs

Sections of tissue from the thorotrast patients were used to prepare autoradiographs by the coating method. Some of the results are reproduced in the Figures 4.2 to 4.8. The autoradiograph is also shown diagrammatically in Figure 4.1 which shows the relative positions of the section, thorotrast and track. In the observation of the autoradiograph using a high magnification, the cells and tracks are not in focus simultaneously as is seen from some of the photographs, for example, Figure 4.3 which was photographed under an objective giving a magnification of 95. Most of the photographs were taken using a 45 times objective and further enlarged in the photographic process. The magnification is indicated by the scales accompanying the photographs.

In the photomicrographs the aggregates of thorotrast are clearly visible as granular material among the cell nuclei. The histological details of the tissue are not well defined as it is difficult to obtain good definition when staining and viewing tissues through 20 microns or more of gelatine. However, it is possible to pick out the nuclei of the cells well enough to distinguish between different types of cells. The tracks can all be correlated with the granular material. In the earlier sections the aggregates are small and are seen to lie within the cell, as in Figure 4.3 showing a Kupffer cell distended with a large deposit of thorotrast. In the later cases, for example, Figure 4.6, the aggregates

FIGURE 4.1. Diagram of Coated Autoradiograph of Thorotrast Section.

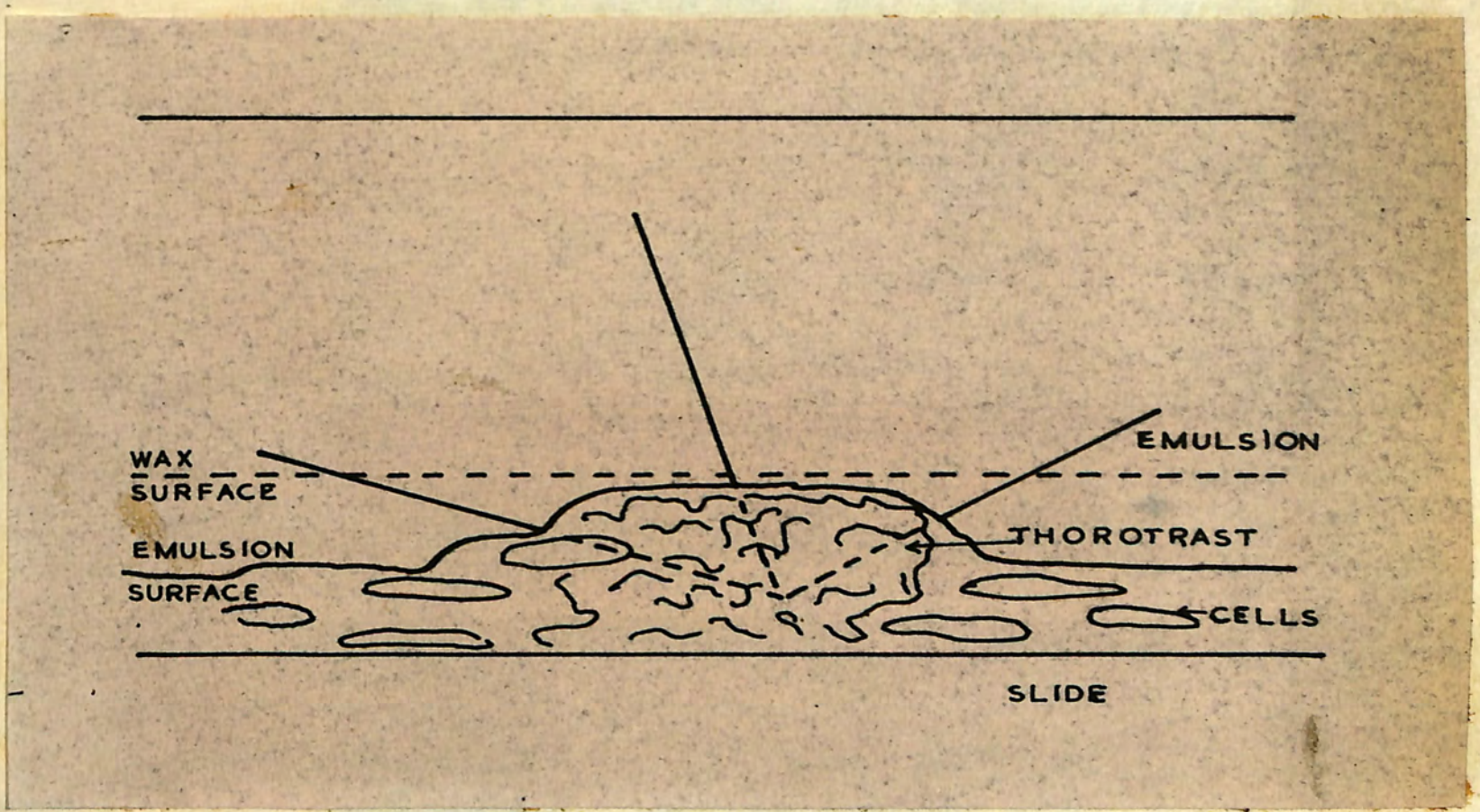


FIGURE 4.2. Autoradiograph of a section of liver from patient No.3. 14 days after injection of 40 ml of thorotrast.

FIGURE 4.3. Enlargement of part of Figure 4.2 showing a Kupffer cell distended with thorotrast.

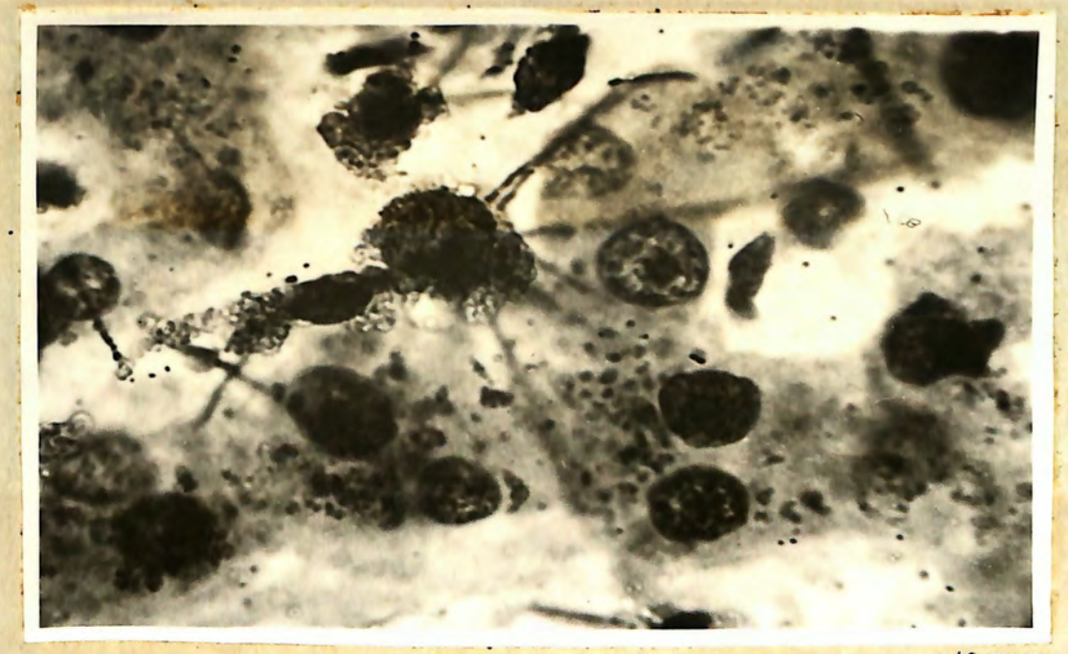


FIGURE 4.4. Autoradiograph of a section of bone marrow from patient No.3, 14 days after injection of 40 ml of thorotrast.

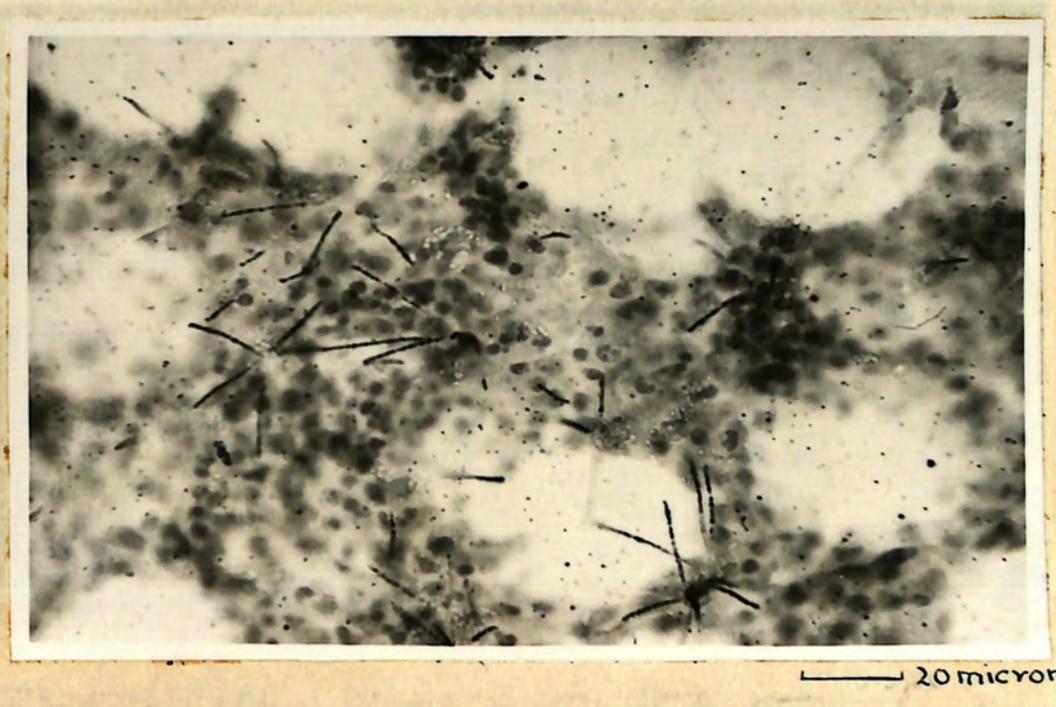


FIGURE 4.5. Enlargement of part of Figure 4.4. showing tracks from thorotrast in a single cell.

FIGURE 4.6. Autoradiograph of a biopsy section of liver from a patient several years after injection (see Figure 1.3)

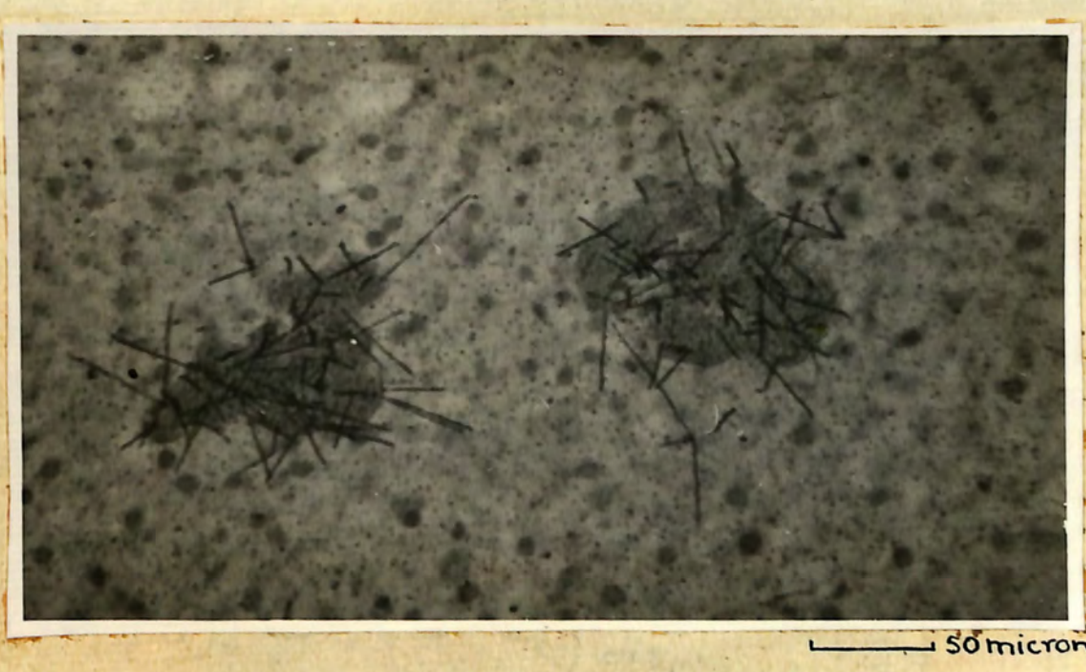


FIGURE 4.7. Aggregates of thorotrast in the spleen of a rabbit 8 months after injection of 2 ml of thorotrast.

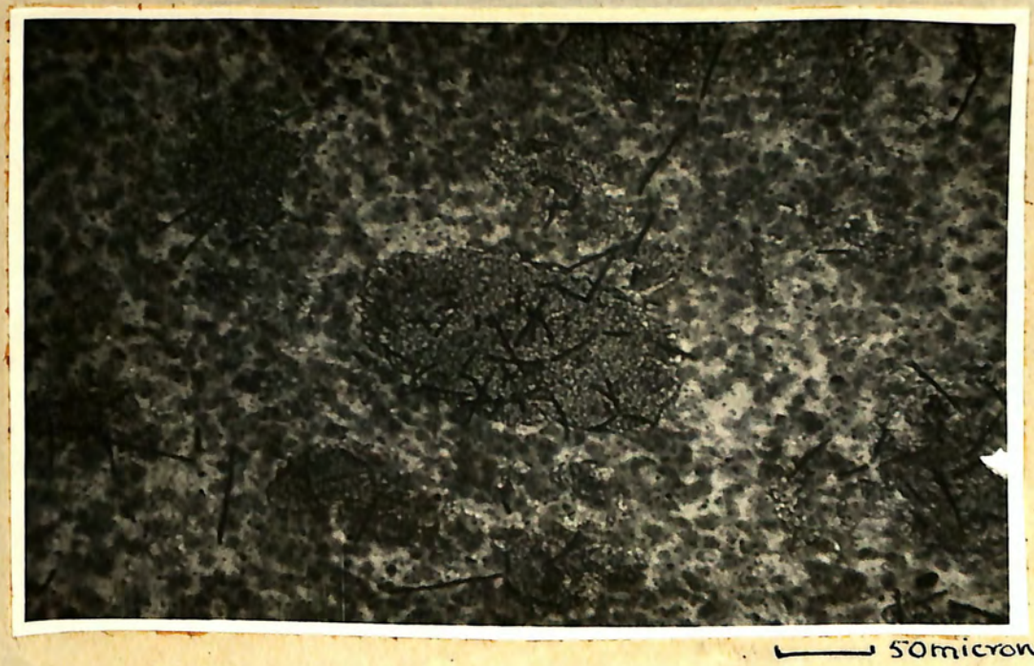


FIGURE 4.8. Radioactive star in the autoradiograph of a section of liver from patient No.2 20 hours after injection of 20 ml of thorotrast.

cover several cell areas and the boundaries of some of the cells can still be seen within the aggregate. The aggregates vary considerably in size and shape. In some cases they form close, almost circular deposits, as is particularly well illustrated in Figure 4.7 showing a section of spleen from a rabbit. In other cases the granules lie in loosely connected shapeless formations.

In Figure 4.8 it is possible to see that several tracks originate at a common point although the actual origin of the tracks is lost within the section. These tracks represent branches of a star formed by the successive disintegration of different elements of the radioactive series. Complete stars are also occasionally recorded. The stars, as well as the track lengths, are of value in identifying the elements present.

4.6. Measurement of Track Lengths

The tracks were measured using a Cooke, Troughton and Simms M4000 microscope at an overall magnification of 1300. The magnification was obtained with a 95X oil immersion objective, 10X eyepieces and a binocular attachment which gave a further magnification of 1.5.

The lengths of tracks were calculated from the horizontal projection and the angle of the track to the plane of the emulsion. The horizontal projection was measured on the graduated eyepiece scale which was calibrated against a stage micrometer; it was measured to the nearest 0.1 micron. The dip was measured from the differences in the reading of the fine-focus scale when the two ends of the track were brought into focus. The fine-focus scale is graduated in microns. In calculating the length of

the track in dry emulsion, the dip must be corrected to allow for the shrinkage factor of the emulsion. This is defined as the ratio of the thickness of the unprocessed emulsion to that of the processed emulsion. The value used here for C2 emulsions was 2.5 (Rotblat and Tai, 1949). Since this factor varies with the processing of the emulsion and is different for large angles of dip, measurements were usually confined to tracks at angles 0 to 30° to the plane of the emulsion in order to minimise errors due to the inaccuracies in the value of the factor used. It was also difficult to measure large dips with accuracy and steep tracks are likely to be distorted. In deciding which tracks to measure when scanning a plate, it was found that with the eyepiece scale used (consisting of 60 scale divisions equivalent to 50 microns) a track was acceptable as lying within 30° to the plane of the emulsion if the horizontal projection in scale divisions was five or more times the dip in microns. It was therefore relatively easy to decide at a glance when a track was acceptable for measurement. In cases where tracks were measured at 0 to 60° the shrinkage was corrected for steep tracks by a 5% decrease in the range.

If "x" is the horizontal projection as measured by the eyepiece scale in microns, "d" is the dip in microns and "s" is the shrinkage factor, then the length of the track is given by $l^2 = x^2 + d^2s^2$. Calculation of the error in l due to errors in x, d and s, shows that $\frac{\delta l}{l} = \frac{x}{l^2}\delta x + \frac{ds^2}{l^2}\delta d + \frac{d^2s}{l^2}\delta s$. If x and d are measured to the nearest micron, then for the maximum angle of 30° and the uncertainty in "s" given by $s = 2.5 \pm 0.5$, the maximum error in l is 13% for the shortest tracks and 6% for the longest tracks. The errors are less for tracks at smaller angles to the plane of the emulsion.

The numbers of tracks per unit area of tissue were measured by counting the number of tracks in a square field of known area. The thickness of the section was determined by reading the depth gauge when focussed on the top and botton layers of the section. The areas of the thorotrast aggregates were measured against a squared eyepiece graticule in terms of the number of squares covering the aggregate.

4.7. Discussion

The coated alpha-track autoradiograph proved to be satisfactory for determining the radioactive content of sections containing thorotrast. Not only did it enable an estimate of the total radioactivity of the section and its location in an organism to be made, but it also allowed an analysis of the kind of radioactive atoms present from the lengths of tracks, an important factor in calculating the tissue dosage.

The main difficulty encountered with some of the coated autoradiographs was the presence of distorted tracks. As the emulsion was liquid when poured over the section it filled in the irregularities in the surface of the section. During processing the emulsion shrank due to the removal of unexposed silver halide grains whereas the tissue did not shrink and the tracks which should have been straight tended to follow the irregularities of the sections. Two possibilities for avoiding distortion have been suggested. (1) It may be possible to prevent shrinkage of the emulsion by soaking it in resin after fixing. The volume occupied by the unexposed silver halide grains is replaced by resin and the original thickness of emulsion is maintained.

(2) The irregularities in the surface of the section may be smoothed out by pouring over it a very thin layer of a solution such as perspex or other transparent substance and allowing this to dry before coating it with emulsion (Blundell and Rotblat, 1951). The thickness of the layer must be controlled so that the gap between the section and emulsion is not increased appreciably with consequent loss of resolution. This procedure also introduces a further difficulty, namely the problem of finding a medium which sticks well to tissue, glass and emulsion. So far these techniques have not been tried because the distorted tracks were not very numerous and could be avoided when making measurements.

The quality of the autoradiograph depended to a great extent on the quality of the material used, best results being obtained with freshly prepared sections. Difficulties were encountered with some of the sections from Copenhagen as the wax was removed some time before the autoradiographs were made. As the tissue was by this time thoroughly dried out and was often already peeling from the slides it did not lie flat when coated with emulsion. Serious errors were therefore introduced when it came to measuring track lengths as the section could not be regarded as a flat layer (6.1). It is also a great disadvantage, from the point of view of staining, to use dried tissues, as the histological details become impaired.

The simpler contact autoradiograph had several advantages over the coated autoradiograph. There was no distortion of the tracks due to differential shrinkage of the emulsion and tissue and the tracks were generally better for measurement. It was also possible to use the same

section to prepare autoradiographs at different times. It was not necessary to remove the wax from the paraffin sections and although the thorotrast is very insoluble, it was desirable to take the sections through as few solutions as possible. Further, there were no tracks formed in wet emulsion.

Another factor which had to be considered when obtaining results from coated autoradiographs, was the leaching of radioactive atoms into the emulsion, described by Levi (1951). This was evident from the number of stars originating in the emulsion instead of in the section. These were mainly four-branch stars and therefore must have consisted of decay products starting from ThX. The number of these stars varied with the time taken to dry the emulsion, as the products more easily diffused through wet emulsion than through dry emulsion. It must also have depended on the proportion of decay products which were present in the thorotrast when the exposure was made.

CHAPTER 5

ANALYSIS OF THE RADIOACTIVITY OF THOROTRAST BEFORE INJECTION

5.1. Theoretical Introduction

It has been mentioned previously (1.2) that the activity of thorotrast varies with time and that it is important to know the age of a sample at the time when it was injected into the patient. Even if the date of preparation of the sample is known, this is not sufficient to define its composition at any time unless the previous history of the thorium dioxide from which it was made is also known.

In the natural state thorium contains all the elements of the radioactive series in equilibrium, that is, the number of disintegrations of each element per unit time is the same for all elements. In the preparation of thorotrast, thorium is treated chemically to remove the short-lived elements, leaving only a mixture of Th and its isotope RdTh. The subsequent activity of the mixture is determined by the relative amounts of these elements present and the half-value periods of Th (10^{10} years), RdTh (1.9 years) and the intermediate product, MsTh 1 (6.7 years). It is the result of two processes which take place simultaneously:

(a) the growth of products from Th

(b) the decay of RdTh originally present.

These processes may be represented mathematically by making use of the theory of successive transformations of radioactive elements. Suppose that P, Q, R etc. represent the numbers of atoms of the successive elements, Th, MsTh 1, MsTh 2, etc. at any time t. The decay constants

of the elements are $\lambda_1, \lambda_2, \lambda_3$, etc. and their activities A, B, C, are given by $A = P\lambda_1$, $B = Q\lambda_2$, $C = R\lambda_3$ etc. The numerical values of the decay constants are given in Table IV. Since MsTh 2 has a very short life compared with the other elements, we may neglect it in these calculations and assume that MsTh1 decays directly to RdTh.

(a) Growth of products from Th. Initially there are P_0 atoms of Th. Then the increase per unit time in the number of atoms of the next element, MsTh1, is the number supplied by the change of Th, minus the number due to the change of MsTh1 into RdTh.

Thus $$dP/dt = -\lambda_1 P \qquad (1)$$

$$dQ/dt = -\lambda_1 P - \lambda_2 Q \qquad (2)$$

$$dS/dt = \lambda_2 Q - \lambda_4 S \qquad (3) \quad \text{etc.}$$

Substituting in (2) the value of P in terms of P_0, that is, $P = P_0 e^{-\lambda_1 t}$,

$$dQ/dt = \lambda_1 P_0 e^{-\lambda_1 t} - \lambda_2 Q$$

The solution of this equation is of the form

$$Q = P_0(C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t})$$

By substitution of initial conditions, namely $Q = 0$ when $t = 0$, we find that

$$C_1 = \frac{\lambda_1}{\lambda_2 - \lambda_1} \text{ and } C_2 = \frac{-\lambda_1}{\lambda_2 - \lambda_1}$$

Thus $$Q = P_0 \frac{\lambda_1}{\lambda_2 - \lambda_1}(e^{-\lambda_1 t} - e^{-\lambda_2 t}) \qquad (4)$$

TABLE IV. Disintegration Constants of the Thorium Series.

Series No.	Element	Radiation	Half-value period	Decay Constant (sec^{-1})
1	Th	α	1.39×10^{10} yr.	1.58×10^{-18}
2	MsTh1	β	6.7 yr.	3.29×10^{-9}
3	MsTh2	β	6.13 hr.	3.15×10^{-5}
4	RdTh	α	1.90 yr.	1.16×10^{-8}
5	ThX	α	3.64 day	2.20×10^{-6}
6	Tn	α	54.5 sec.	1.27×10^{-2}
7	ThA	α	0.158 sec.	4.39
8	ThB	β	10.6 hr.	1.82×10^{-5}
9	ThC	α (34%) β (66%)	60.5 min.	1.91×10^{-4}
10	ThC'	α	3×10^{-7} sec.	2.36×10^{6}
11	ThC"	β	3.1 min.	3.28×10^{-3}
12	ThD		Stable	

Since λ_1 is very small compared with λ_2, this equation can be simplified to

$$Q = P_0 \frac{\lambda 1}{\lambda_2} (1 - e^{-\lambda_2 t})$$

Therefore the activity of MsThl after time t is given by

$$B = A (1 - e^{-\lambda_2 t}) \qquad (5)$$

Substituting the value of Q from equation (4) in (3) it can be shown that for RdTh

$$S = P_0(C_3 e^{-\lambda_1 t} + C_4 e^{-\lambda_2 t} + C_5 e^{-\lambda_4 t}) \qquad (6)$$

where $C_3 = \frac{\lambda_1\lambda_2}{(\lambda_2-\lambda_1)(\lambda_4-\lambda_1)}$, $C_4 = \frac{\lambda_1\lambda_2}{(\lambda_1-\lambda_2)(\lambda_4-\lambda_2)}$,

$$C_5 = \frac{\lambda_1\lambda_2}{(\lambda_1-\lambda_4)(\lambda_2-\lambda_4)}$$

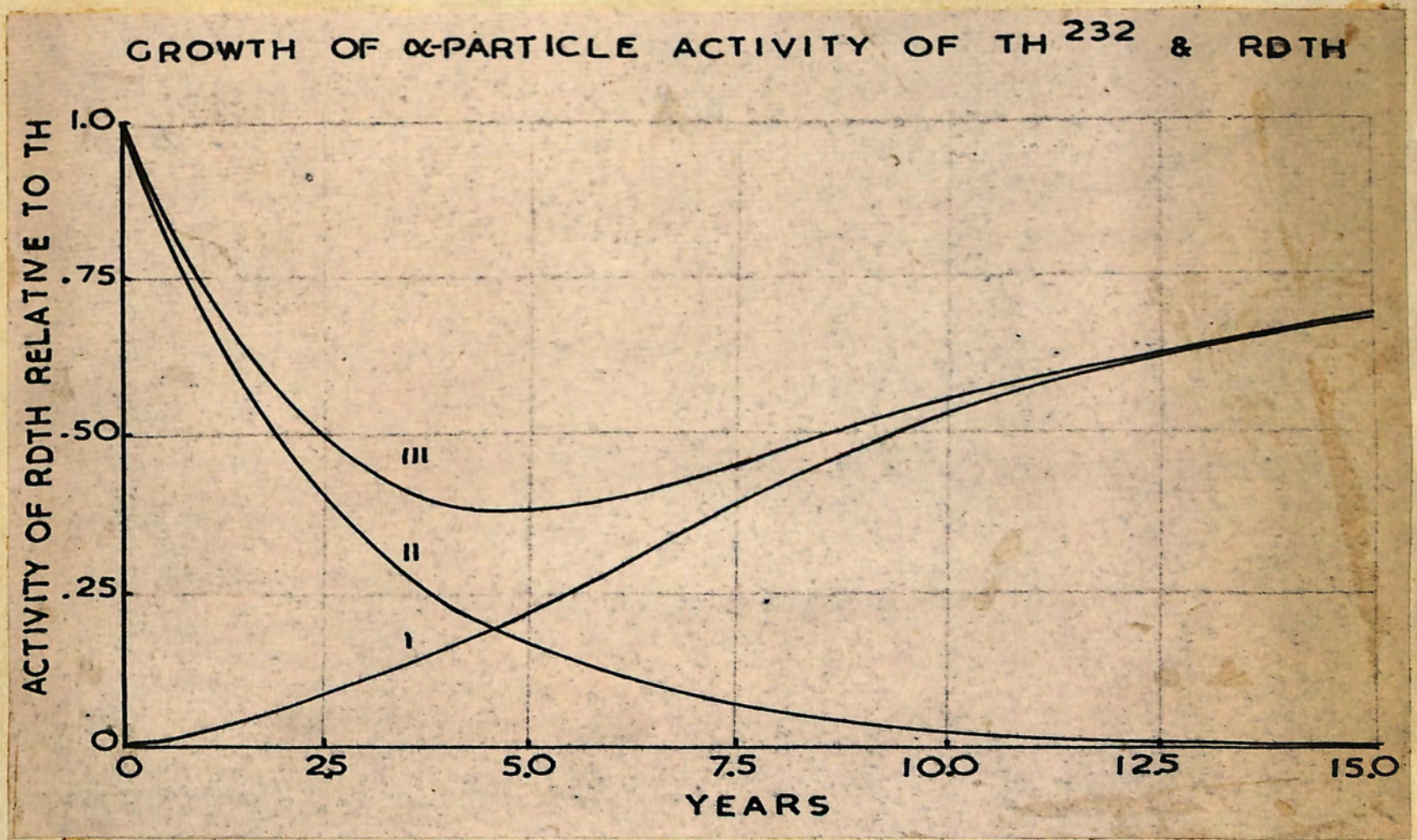
The activity of RdTh after any time t then follows,

$$D = A (1 - \frac{\lambda_4}{\lambda_4-\lambda_2} e^{-\lambda_2 t} - \frac{\lambda_2}{\lambda_2-\lambda_4} e^{-\lambda_4 t}) \qquad (7)$$

Equation(7)represents the growth of alpha-particle activity starting from pure Th. In Curve 1, Figure 5.1, D/A as given by this expression is plotted against t.

(b) Decay of RdTh. If the elements are in equilibrium before the separation of products, the activity of RdTh initially is the same as that of Th, that, is, $P_0\lambda_1 = S_0\lambda_4$

FIGURE 5.1. Curve I Growth of RdTh from Th^{232}
II Decay of RdTh originally present
III Total of I and II



After any time t, $S = S_0 e^{-\lambda_4 t} = P_0 \lambda_1 / \lambda_4 e^{-\lambda_4 t}$

or $D = Ae^{-\lambda_4 t}$ (8)

Equation (8) is plotted in Curve 11, Figure 5.1. The variation of the total activity of RdTh with time is given by the sum of Curves 1 and 11 and is shown in Curve 111.

From these curves, the variation of the total alpha-particle activity of all groups with time may be calculated. Since the products following RdTh have short half-value periods (for example 3.6 days for ThX, the next member of the series) they very quickly reach equilibrium with RdTh, and the total activity of these groups is five times the activity of RdTh throughout. The growth of RdTh continues until all products are in equilibrium again, and the total activity has reached its maximum value of 6A. The total activity at any time t is A + 5D, or expressed as a fraction of the maximum, 1/6 (1 + 5D/A). This ratio corresponds to the age-factor mentioned in Chapter 2 and will be denoted as "β". The value of β calculated from Curve 111, Figure 5.1, and plotted against time, is given in Curve 1, Figure 5.2.

Curve 1, Figure 5.2 represents the case in which the activities of Th and RdTh are initially equal, that is, it applies to the first separation of products from a mixture which has reached equilibrium. Suppose now that after a certain time when the activity of RdTh has fallen as in Figure 5.1, a second separation is carried out. The initial activity of RdTh is then less than the activity of Th, and the variation of total activity is given by a curve such as II or III, depending on what fraction of RdTh remains

FIGURE 5.2.

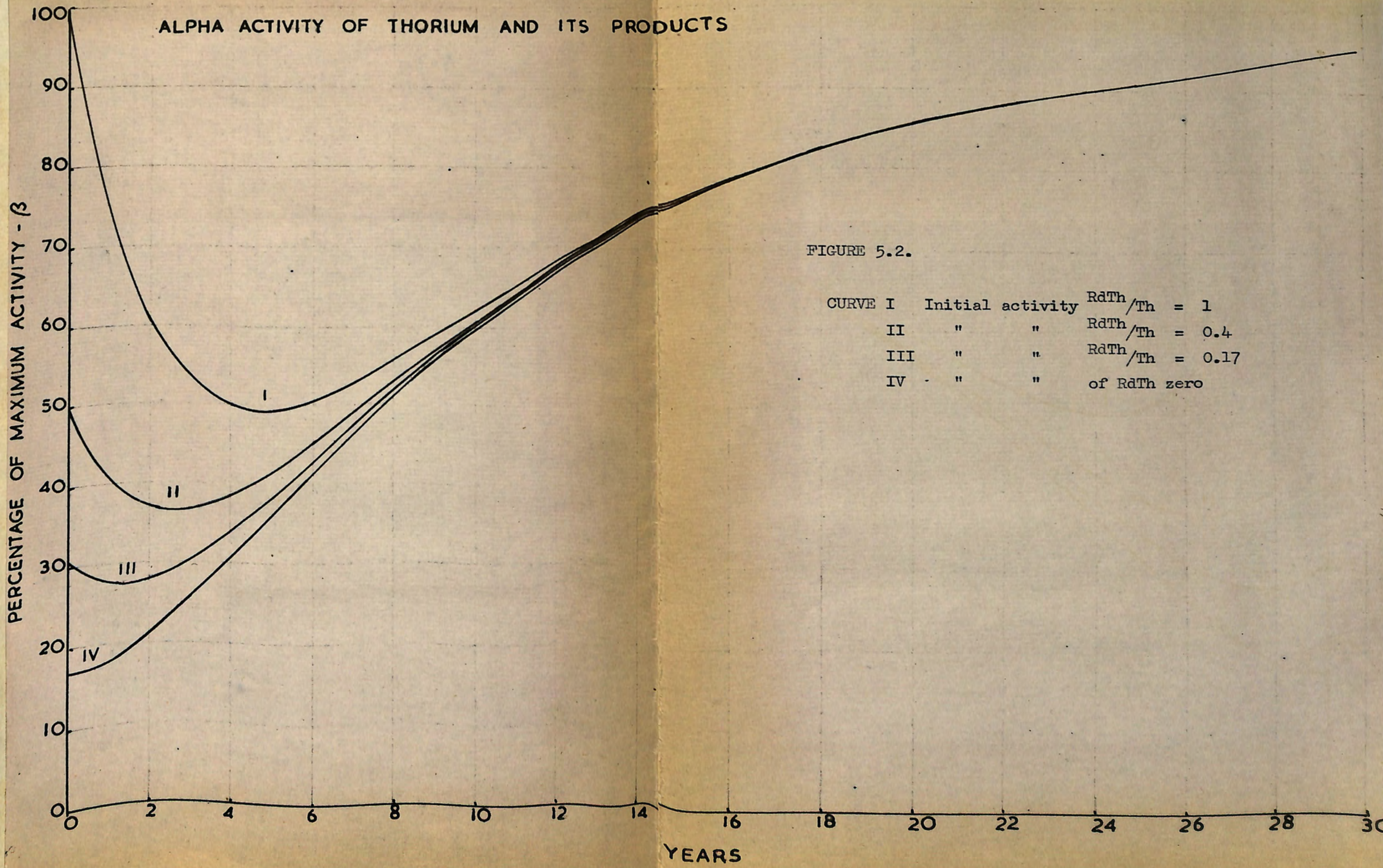


FIGURE 5.2.

CURVE I	Initial activity	$^{RdTh}/_{Th}$	= 1
II	"	$^{RdTh}/_{Th}$	= 0.4
III	"	$^{RdTh}/_{Th}$	= 0.17
IV	"	of RdTh zero	

when the separation takes place. Curve IV represents the limiting case where there is no RdTh present initially. This condition is only approached after a very large number of separations.

For any sample of thorotrast the initial value of β may therefore be anything from 0.17 to 1.0, and even if the date of preparation is known, this is not sufficient unless the relative amounts of Th and RdTh at this time are also known. Alternatively, if a sample is available, the value of β can be determined experimentally at different times and fitted to a curve of the form shown so that the activity at any other time can then be estimated.

5.2. Experimental Method

Nuclear Research emulsions are frequently used to determine the energy spectrum of alpha-particles from a radioactive substance (Powell, 1943) by means of a technique in which the emulsion is soaked in a solution of the substance. In this way the radioactive atoms are incorporated in the emulsion and each alpha-particle emitted is recorded as a track. If the lengths of the tracks are measured the range-number curve shows maxima at ranges corresponding to the alpha-particle groups emitted, the intensity of the maxima being a measure of the relative activities of the different elements present. Further, if the radioactive atoms undergo several disintegrations during the exposure time, several tracks originating at a single point, a star, will be recorded. The number of stars is also a measure of the activity of the different elements.

A similar technique was used to analyse samples of thorotrast using liquid emulsion. A few drops of thorotrast were diluted with distilled water and mixed with melted C2 emulsion. About 1.5 ml of the emulsion were poured on a 3" x 1" microscope slide to produce an emulsion of 100 microns, dried in warm air and left to expose for a suitable length of time. The plates were processed and the tracks measured in the manner described in the previous chapter. The exposure time was adjusted so that a suitable density of tracks for measurement (about 20 in a 50 micron field) was obtained. Between 1000 and 1500 tracks were measured on each plate. The lengths were read to the nearest 0.1 micron and the measurements were confined to tracks at angles 0 to 30° to the plane of the emulsion. The photomicrograph shows the appearance of the tracks and stars. (Figure 5.3)

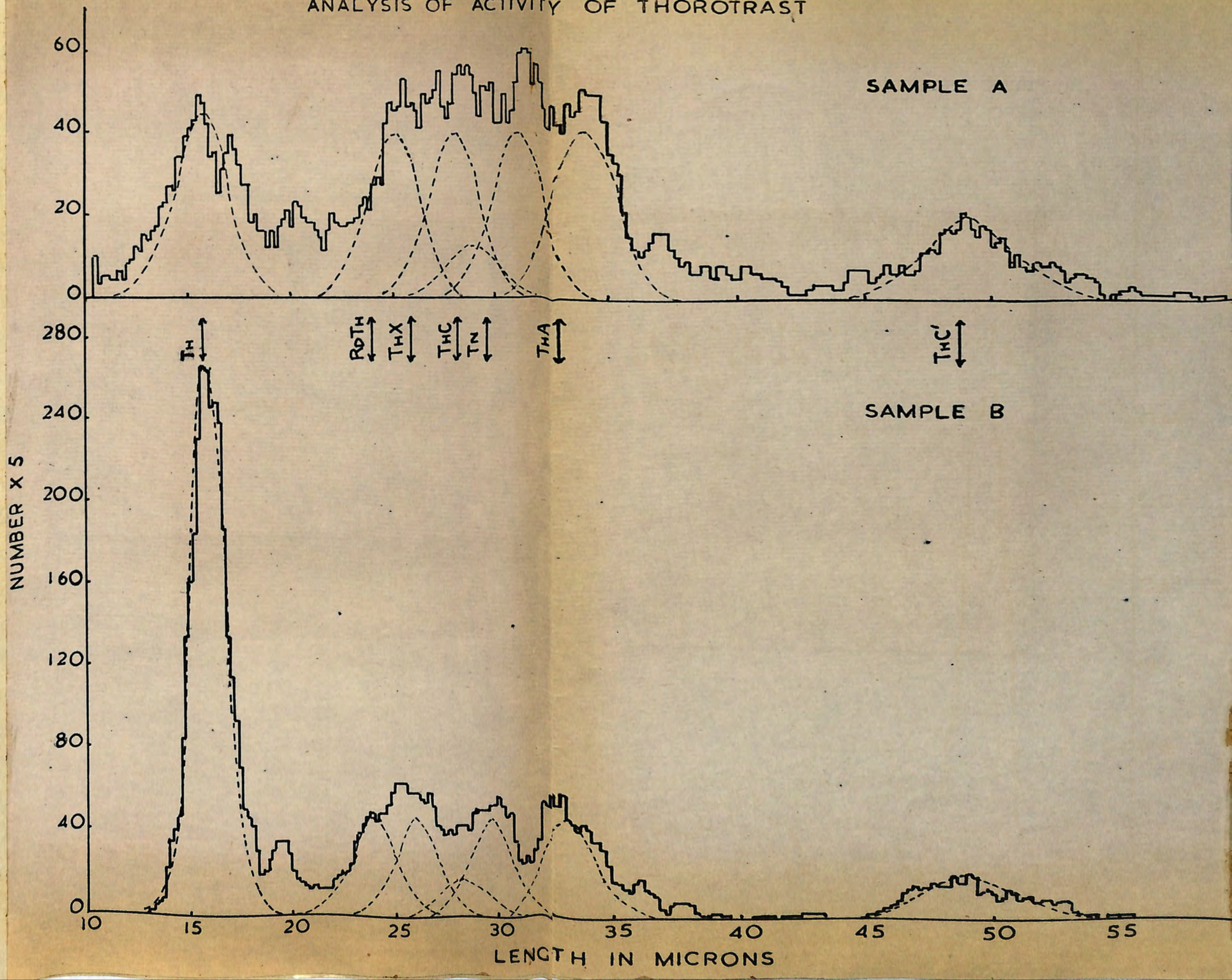
5.3. Analysis of Track Lengths

The distributions of track lengths for two different samples of thorotrast are shown in Figure 5.4 for comparison. Sample A was of German origin and was known to have been prepared at the latest in 1944 when the Hayden factory was bombed. Sample B was of more recent American origin.

It is seen that the distributions consist of a number of peaks corresponding to the different groups of alpha-particles with ranges as given in Table I. The peaks for Th and ThC' alpha-particles are well resolved, but there is some overlapping of the other peaks, particularly in the curve for Sample A. In the curve for sample B, the broad peak at 25 microns corresponds to the superposition of the RdTh and ThX groups;

FIGURE 5.3. Alpha-particle tracks and stars from thorotrast.

ANALYSIS OF ACTIVITY OF THOROTRAST

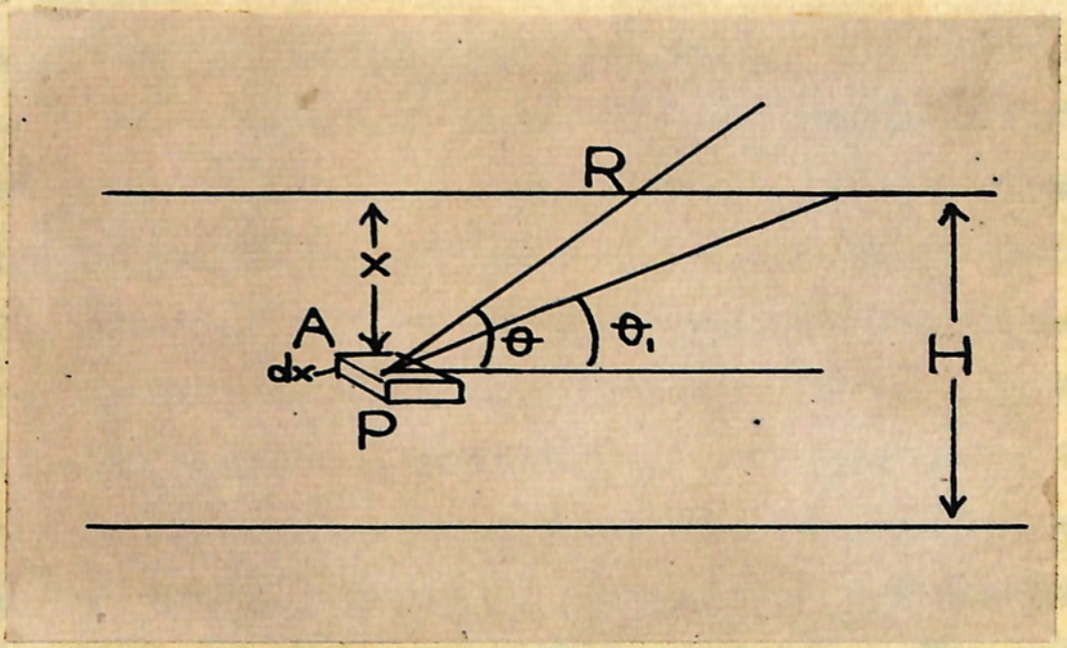


the Tn and ThA peaks can be distinguished at 30 and 33 microns. Because of the straggling of alpha-particles in emulsion, each group is represented by a Gaussian distribution of tracks; the histograms were analysed into curves of this form as shown by the dotted lines on the figure. In both samples the heights of all the peaks except the Th peak are equal, indicating that RdTh was in equilibrium with its products. There are differences in the heights of the Th peaks indicating that in sample A the activity of RdTh was almost as great as that of Th, but in sample B the activity of RdTh was about one fifth that of Th. From the ratio of the number of tracks in the Th group, A, to that in the other groups, 5D, the value of β can be calculated.

Correction for tracks lost. In determining the relative numbers of tracks in each of the groups, a correction must be applied for the tracks which pass out of the top and bottom surfaces of the emulsion. Suppose the thickness of the emulsion is H, tracks are measured in the range of the angles 0 to 30° and the length of the tracks in the emulsion is R (Figure 5.5). The number of tracks originating from an elementary volume of thickness dx and area A at a depth x in the emulsion is NAdx, where N is the number of tracks emitted per unit volume. The tracks which are not fully recorded are contained in the solid angle $(\phi - \theta_1)$ which is given by $2\pi(\sin\theta - \sin\theta_1)$; the number of tracks in this solid angle is $NA(\sin\theta - \sin\theta_1)dx/2$, where $\theta_1 = \arcsin x/R$. Thus the total number of tracks not fully recorded is given by

$$\int_0^{R\sin\theta} \frac{NA}{2}\left(\sin\theta - \frac{x}{R}\right)dx = \frac{NA}{4}R\sin^2\theta$$

FIGURE 5.5.

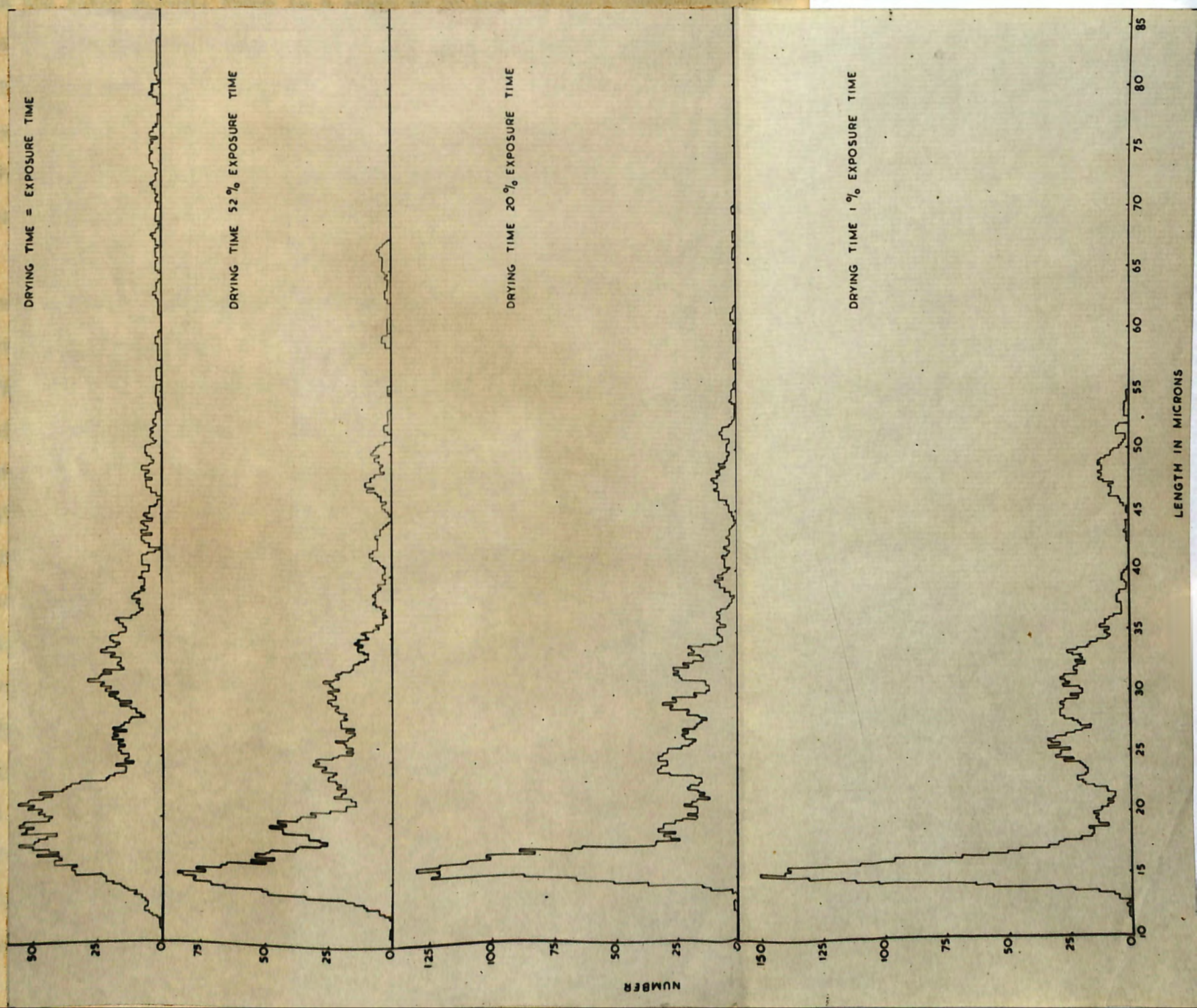


Since the total number of tracks emitted in the solid angle θ is $NA/2H \sin\theta$, the proportion of tracks lost is $R\sin\theta/2H$. If the emulsion is 100 microns in thickness and tracks within the angles 0 to 30° are measured, this fraction has the value 0.125 for R = 50 microns and 0.05 for R = 20 microns.

Correction for tracks recorded in wet emulsion. The curves drawn in Figure 5.4 are wider than expected assuming the straggling of particles in emulsion to be 2 to 3% of the range (Rotblat, 1950); also several of the peaks are shifted to correspond to a range one or two microns greater than normal and there are a number of tracks of range 20 microns and 40 microns which do not correspond to any of the groups from thorium.

Since tracks recorded in wet emulsion would have the effect of increasing the straggling and the mean range of the group, it was thought that it might be possible to improve the resolution of the groups by more careful control of the drying time of the emulsion. The effect was studied in a controlled experiment in which a series of plates was prepared from emulsion containing thorotrast (Sample B). The first plate was developed immediately the emulsion appeared to be dry, that is, after 4 hours, and the other plates at various times up to 14 days later. In this way it was possible to prepare plates in which the drying time represented different fractions of the total exposure time, varying from 100% in the first plate to about 1% in the last plate developed. The lengths of the tracks were measured and the results are plotted in Figure 5.6. In the first plate in which the drying time was equal to the exposure time, the maximum for the Th group of alpha-particles is

FIGURE 5.6. Distribution of track lengths from thorotrast in liquid emulsion for varying times of drying.

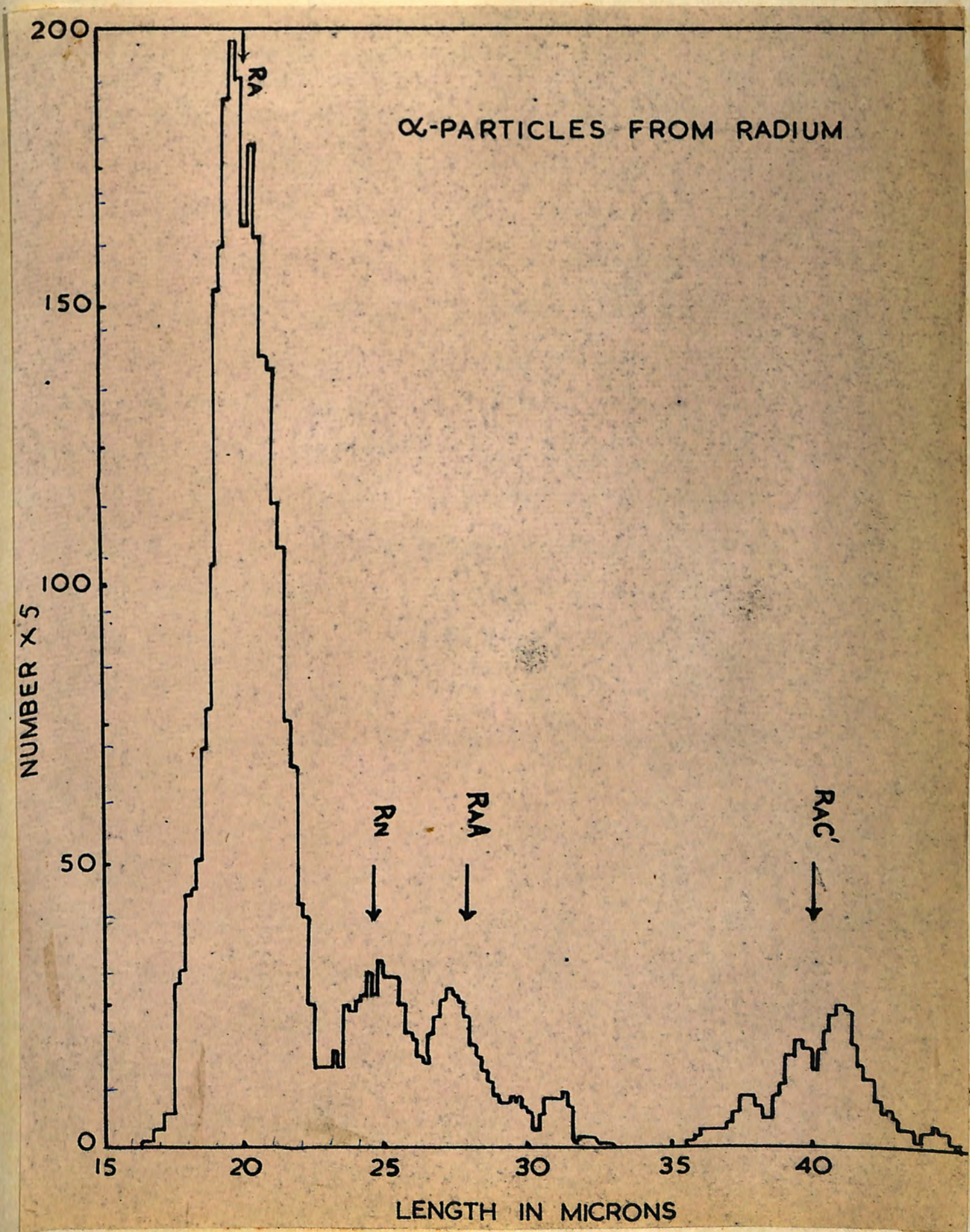


shifted from 16 to 20 microns and the group has a half-width of 3 microns. In the later plates, there is a group at 16 microns and a subsidiary group at 20 microns; the height of the latter decreases to 11% of the tracks in the major group in the plate in which the drying time was 1% of the exposure time. The longest track in the 49 micron group from ThC' is 84 microns, which implies that the stopping power of wet emulsion is slightly greater than half that of dry emulsion.

From the form of the distribution in this controlled experiment, it was possible to analyse the observed distribution (for example Sample A, Figure 5.4), assuming that the tracks greater than 52 microns were tracks from the ThC' group recorded when the emulsion was wet, those in the 35 to 45 micron region were from the central group of five elements and those in the 20 micron region were from the Th group. It was very useful to be able to apply this correction to some of the earliest plates, as these provided valuable points on the age-factor against time graph which could not be repeated. In the preparation of the later plates more care was taken to ensure that the proportion of tracks formed before the emulsion was dry was very small.

Radium Impurity. Although the separation of the various groups was greatly improved by carefully controlling the drying time, there are still a number of tracks of 20 micron range which have not been accounted for. The number of tracks in this group decreases with decreasing time of drying, but not as much as expected. It is possible that the water content of the emulsion was higher than normal for some time after the

FIGURE 5.7.


α-PARTICLES FROM RADIUM
RA
RN
RAA
RAC'
200
150
100
50
0
NUMBER X 5
15
20
25
30
35
40
LENGTH IN MICRONS

5.4. Results of Age-factor Determinations

Four samples of thorotrast were examined at different times. As well as samples A and B mentioned above, two later batches of American thorotrast, C and D, were obtained. The date of preparation of C was unknown; D was said to be less than six months old when purchased in February 1954. The values of β were calculated from the observed distributions of track lengths and corrected as described above. These values together with the dates of analysis are shown in Figure 5.8. Curves of the form shown in Figure 5.2 were fitted to the experimental points, so that the dates of preparation and the initial activities of the samples could be determined. It was intended to compare these figures with the dates of preparation given by the American Heyden Company, but it was found that this company has now discontinued the production of thorotrast and information about earlier batches was not forthcoming. The only check available was the date of purchase of a sample or the earliest recorded date of experiments performed with this batch.

It will be noticed that the values of β obtained for sample B do not correspond accurately with the curve drawn, but it was impossible to fit them to a better curve which did not contradict the known data about this sample, that is, that it was used for experiments in this laboratory in 1951. The reason for the lowered activity observed will be considered later (5.5., 5.6).

In Figure 5.8, the errors represent the statistical error in the number of tracks measured. As mostly 1000 tracks were measured on each

FIGURE 5.8. Determination of Age-factor of Thorotrast.

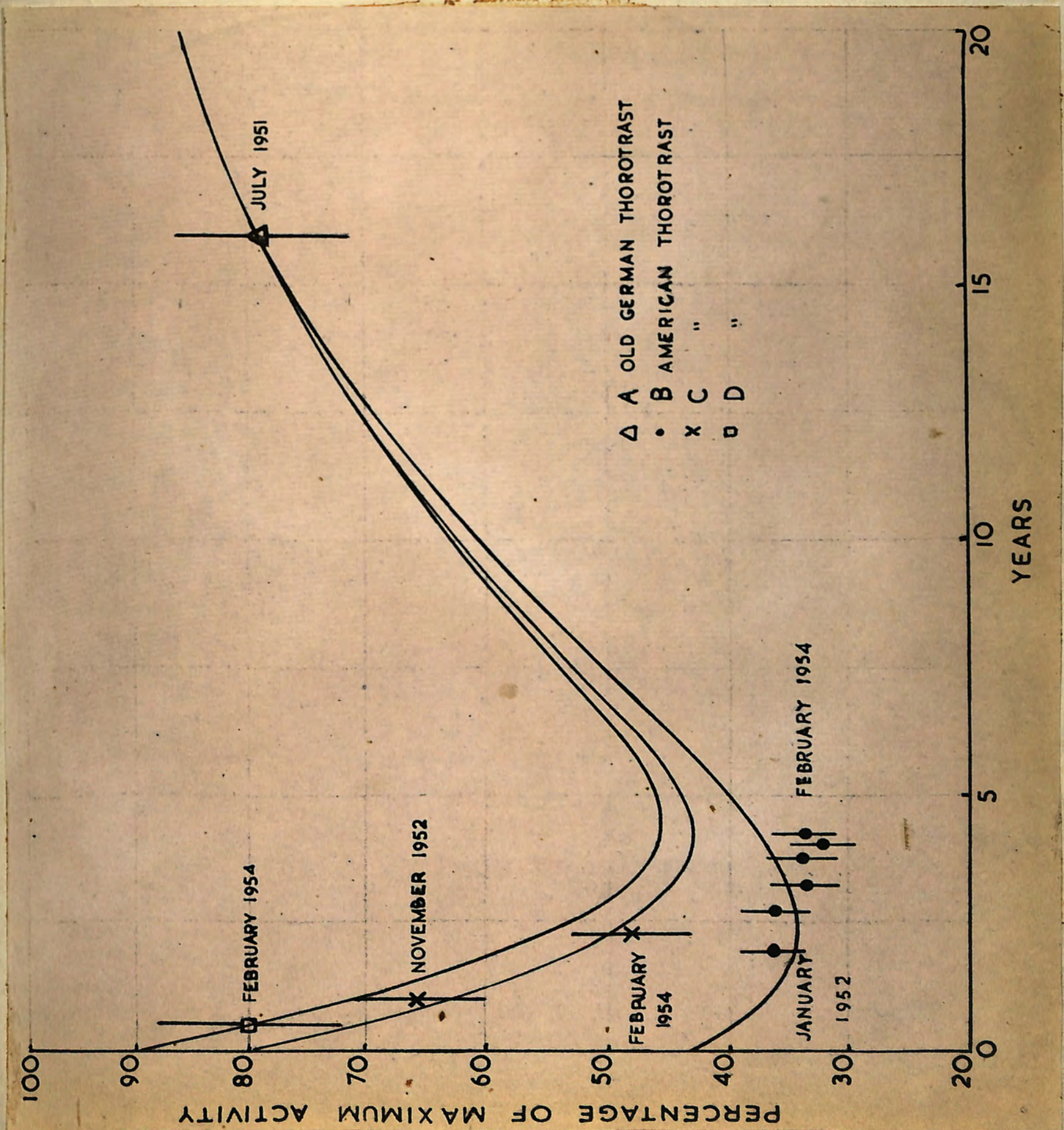


plate and β was calculated by dividing the total into two approximately equal groups, the error was about 10%.

Further errors may have been introduced by inconsistencies in accepting or rejecting a track for measurement, both in deciding whether it lay within an angle of 30° to the plane of the emulsion and also whether it lay within the area being scanned. With regard to the latter some rule must be kept such as measuring only tracks included within the square field or crossing the upper boundary on each traverse of the plate so that neither the long nor the short tracks are favoured. When tracks are orientated in all directions and also at all depths in the emulsion as in this case, measurements often involve moving the square graticule from its fixed traverse and it may not always be replaced accurately. For each observer, personal errors may be considered constant, except perhaps when the observations are made at long intervals of time. The results given were obtained from measurements made by two different observers; it is not known whether consistency between the two observers was sufficiently good to compare results fairly to 10%.

5.5. Diffusion of Elements in Emulsion

In the analysis of the activity of thorotrast using photographic emulsions, it was assumed that no diffusion of elements occurred, that is, that the activity observed in the emulsion was the same as that in the thorotrast itself. This assumption is contradictory to the observed diffusion of Rn from a plate loaded with a radium salt and exposed in exactly similar conditions (5.3). The escape of Rn was easily demonstrated

as a decrease in the activity of Rn relative to Ra (Figure 5.7). As Tn is an isotope of Rn, it was expected that this element would diffuse from a plate loaded with thorotrast, but no decrease in the activity of Tn relative to ThX was observed. A possible explanation is that since Rn has a much longer half-value period than Tn (3.8 days compared with 54 seconds), the proportion of Tn diffusing is probably much less than that of Rn. If there were any diffusion of Tn or any of the other elements, the measured activity would not be a reliable estimate of the age-factor. An alternative method of calculating the age-factor, which is independent of diffusion, was investigated as follows.

Calculation of the Th to RdTh ratio. The age-factor can be calculated either from the activity of Th relative to all the other elements assuming them to be equilibrium, $^{A}/_{5D}$, or from the activity of Th relative to RdTh, $^{A}/_{D}$. Since Th and RdTh are isotopes and both have long half-value periods compared with the exposure time of the emulsion, it can be assumed that the ratio $^{A}/_{D}$ will be unaffected by the diffusion of any element. Thus by observing the Th to RdTh ratio, an alternative estimate of the age-factor, independent of diffusion, may be obtained.

Returning to the distribution of track lengths observed in the thorotrast plates (Figure 5.4), it is seen that the activity of Th can easily be determined, but that for the determination of the activity of RdTh it is necessary to separate the 24 micron group of alpha-particles from RdTh from the 26 micron group from Th X. As a first step in the separation of these groups, the distribution was divided into two separate

groups, one for tracks occurring singly, the other for tracks occurring as branches of stars. The two distributions for one of the thorotrast plates (sample B, exposed in October 1952) are given in Figure 5.9. The distribution for single tracks (Curve I) contains all the Th group, virtually all the RdTh group, some of the ThX, ThC and ThC' groups and a very small proportion of the Tn and ThA groups. The distribution of tracks occurring in stars (Curve II) contains almost all the Tn and ThA groups and the remainder of the ThX, ThC and ThC' groups. The actual numbers of tracks in the various groups are given in Table V. It was not possible to separate the RdTh, ThX and ThC groups in Curve I, but the total number in the combined group is given. The group at 20 microns was attributed to Ra (5.3).

For the accurate separation of the RdTh, ThX and ThC groups occurring as single tracks, a large number of tracks of length 20 to 40 microns was measured very accurately. Measurements were confined to flat tracks to eliminate errors in the measurement of the dip and the value of the shrinkage factor. The distribution was analysed into groups as shown in Figure 5.10, the numbers of tracks in the RdTh, ThX and ThC groups being in the ratio 1:0.34:0.12. The combined group of 223 tracks in Curve I, Figure 5.9, was then divided in the same ratio, as given in Table V. The total numbers of tracks in the various groups are also given; the activities of Tn, ThA and ThC combined with ThC' are equal indicating that these three groups were in equilibrium. There are about 10% less tracks in the ThX group and about 10% more in the RdTh group, but these differences are only slightly greater than the statistical error. Calculation of the age-factor

FIGURE 5.9.

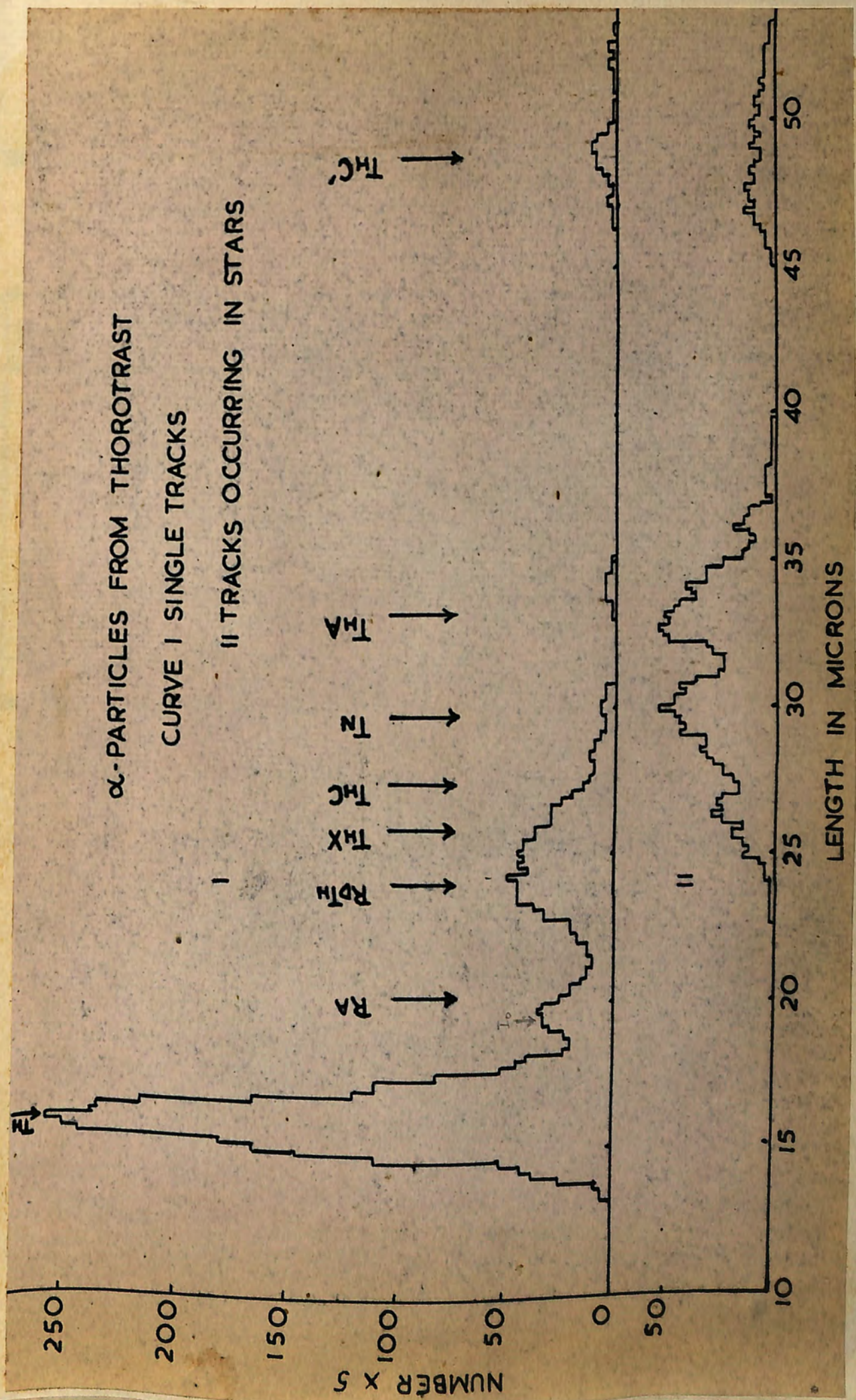
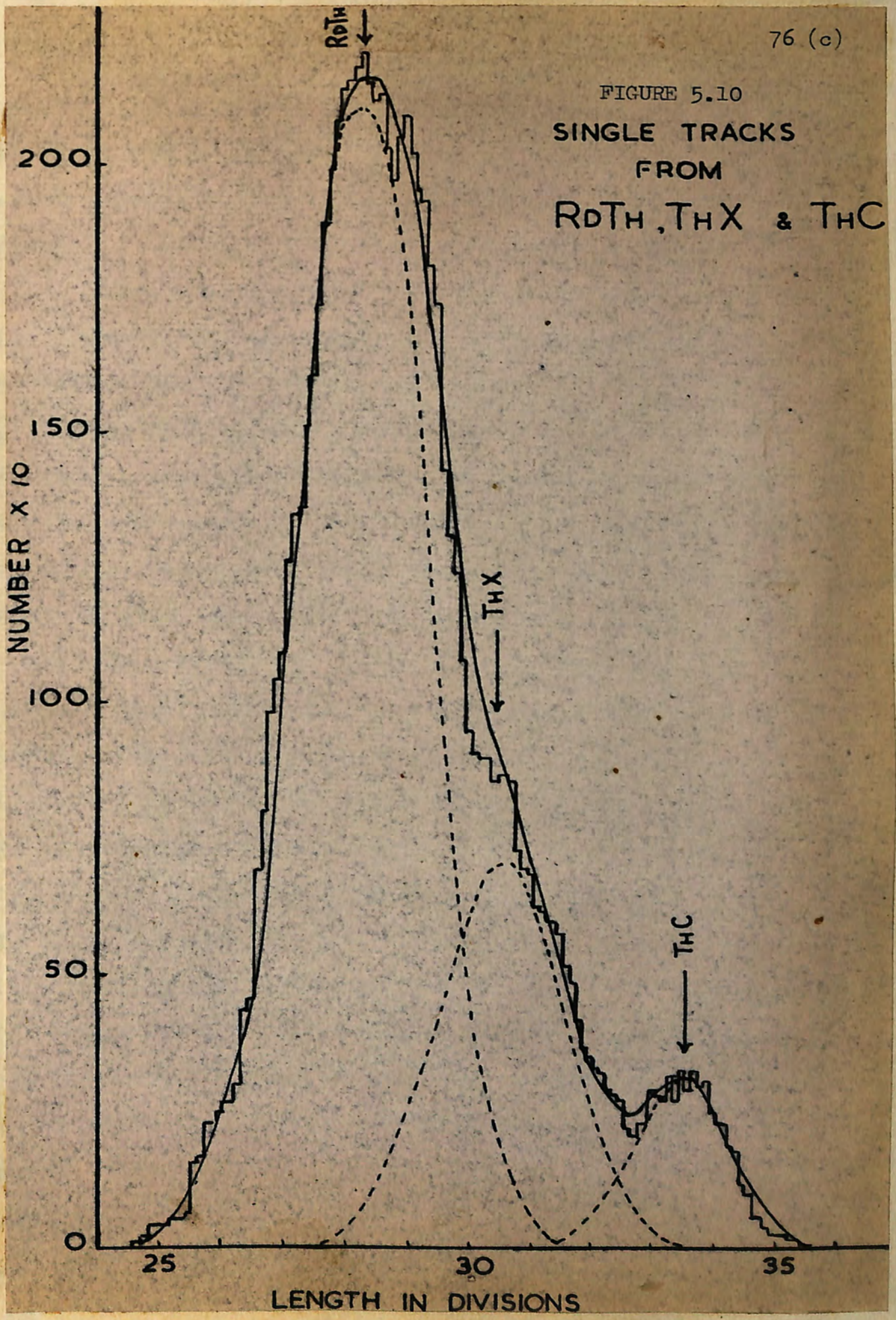

α-PARTICLES FROM THOROTRAST
CURVE I SINGLE TRACKS
II TRACKS OCCURRING IN STARS
ThC'
ThA
Tn
ThC
ThX
RdTh
RA
I
II
LENGTH IN MICRONS
NUMBER x 5
250
200
150
100
50
0
50
10
15
20
25
30
35
40
45
50

TABLE V. Observed Numbers of Tracks in Alpha-particle Groups.
Sample B thorotrast exposed in October 1952
Exposure time = 120 hours. Number of tracks measured = 1350.

Element	Single Tracks		Stars	Totals per Group
	All	20-40 microns		
Th	602		0	602
Ra	54		0	54
RdTh	223	152	2	154
ThX		53	69	122
ThC		18	27	45
Tn	8		132	140
ThA	9		131	140
ThC'	28		65	93

FIGURE 5.10

SINGLE TRACKS FROM RDTH, THX & THC



from the Th to RdTh ratio gave the value 0.39; calculation from the ratio of Th to all other elements, similar to the method used above (5.4), gave the value 0.36. As the two values are within the limits given in Figure 5.8, it was concluded that there was not sufficient evidence of the escape of elements from the emulsion during exposure to account for the lowered value of the age-factor.

Analysis of radioactive stars. While there was little evidence that any elements escaped completely from the emulsion, it is well-known that Tn does diffuse sufficiently to cause a splitting of the radio active stars (Demers, 1947). An example is a 5-branch star formed by the alpha-particles emitted in the transformation of RdTh to the stable element, ThD, which splits into a 2-branch star consisting of alpha-particles from RdTh and ThX, and a 3-branch star consisting of alpha-particles from Tn, ThA and either ThC or ThC'. The centres of the stars may be separated by a few microns indicating that the Tn atom has migrated from the point where it was formed from ThX to the point where it decayed to ThA. Similar migrations of the atoms Rn, ThA, ThB or ThC or ThC' have also been observed.

The types of events which should be recorded in a given exposure time can be predicted by considering the half-value periods of the elements and their sequence in the radioactive series. A few of the transformations in which the parent and daughter elements have comparatively long half-value periods, will lead to the emission of a single alpha-particle only. Other transformations involving a number of short-lived elements will always be recorded as stars. The sequences in which the various types of events arise may be tabulated as follows. (The elements emitting the alpha-

particles are underlined and for brevity ThC is taken to include its alternative daughter products ThC' and ThC'').

Single tracks $\underline{Th} \rightarrow MsThl$

$\underline{RdTh} \rightarrow ThX$

$ThB \rightarrow \underline{ThC} \rightarrow ThD$

3-branch stars $\underline{ThX} \rightarrow \underline{Tn} \rightarrow \underline{ThA} \rightarrow ThB$

4-branch stars $\underline{RdTh} \rightarrow \underline{ThX} \rightarrow \underline{Tn} \rightarrow \underline{ThA} \rightarrow ThB$

$\underline{ThX} \rightarrow \underline{Tn} \rightarrow \underline{ThA} \rightarrow ThB \rightarrow \underline{ThC} \rightarrow ThD$

5-branch stars $\underline{RdTh} \rightarrow \underline{ThX} \rightarrow \underline{Tn} \rightarrow \underline{ThA} \rightarrow ThB \rightarrow \underline{ThC} \rightarrow ThD$

The number of alpha-particles which are recorded as single tracks or as branches of the different types of stars depends on the exposure time of the emulsion and the half-value periods of the elements. When the exposure time is of the order of days it may be assumed that Th always appears as single tracks and the frequency of the stars formed by the later products only need be considered. Suppose there are n disintegrations per second of RdTh and its products and the number of atoms of any one element at any time is N. Then the number of disintegrations of this element is

$$N(1 - e^{-\lambda t}) = \frac{n}{\lambda t}(1 - e^{-\lambda t})$$

where λ is the decay constant of the element. The number associated with the preceding element and contributing to stars is

$$n - \frac{n}{\lambda t}(1 - e^{-\lambda t})$$

and the fraction of tracks in this class is given by

$$1 - \frac{1 - e^{-\lambda t}}{\lambda t}$$

This expression has been evaluated for different elements by Flament (1948) and is reproduced in the curves in Figure 5.11.

Curve I gives the frequency of occurrence of 3-branch stars, ThX → Tn → ThA → ThB.

Curve II is for 4-branch stars, ThX → Tn → ThA → ThB → ThC → ThC' ThD and Curve III for 4-branch stars, ThX → Tn → ThA → ThB → ThC → ThC" → ThD. The ordinates of Curves II and III are in the ratio 2:1 because 66% of ThC disintegrations to ThC' and 34% to ThC". Curves IV and V are for 5-branch stars, RdTh → ThX → Tn → ThA → ThB → ThC → ThC' → ThD and RdTh → ThX → Tn → ThA → ThB → ThC → ThC" → ThD respectively. Curves VII and VIII are for single tracks from RdTh and Th respectively.

From the curves in Figure 5.11, it is seen that if the exposure time is 120 hours, 65% of the RdTh alpha-particles are recorded as single tracks, 6% occur in 4-branch stars (RdTh → ThX → Tn → ThA → ThB) and 29% in 5-branch stars (RdTh → ThX → Tn → ThA → ThB → ThC → ThD), whereas after 500 hours, 24% of the RdTh alpha-particles are recorded as single tracks, 3% in 4-branch stars and 73% in 5-branch stars.

The tracks occurring in stars in the plate studied above (sample B, October 1952) which was exposed for 120 hours, were classified according to whether they occurred in 2-, 3-, 4- or 5-branch stars. Their lengths were plotted on separate distributions which are reproduced in Figure 5.12. From these distributions the numbers of tracks from each element occurring in each class of star were determined. These numbers were calculated as a

FIGURE 5.11

Curve I	3-branch stars	ThX → Tn → ThA → ThB
II	4	ThX → Tn → ThA → ThB → ThC → ThC' → ThD
III	4	ThX → Tn → ThA → ThB → ThC → ThC'' → ThD
IV	5	RdTh → ThX → Tn → ThA → ThB → ThC → ThC' → ThD
V	5	RdTh → ThX → Tn → ThA → ThB → ThC → ThC'' → ThD
VI	4	RdTh → ThX → Tn → ThA → ThB
VII	Single tracks from RdTh	
VIII	Th	

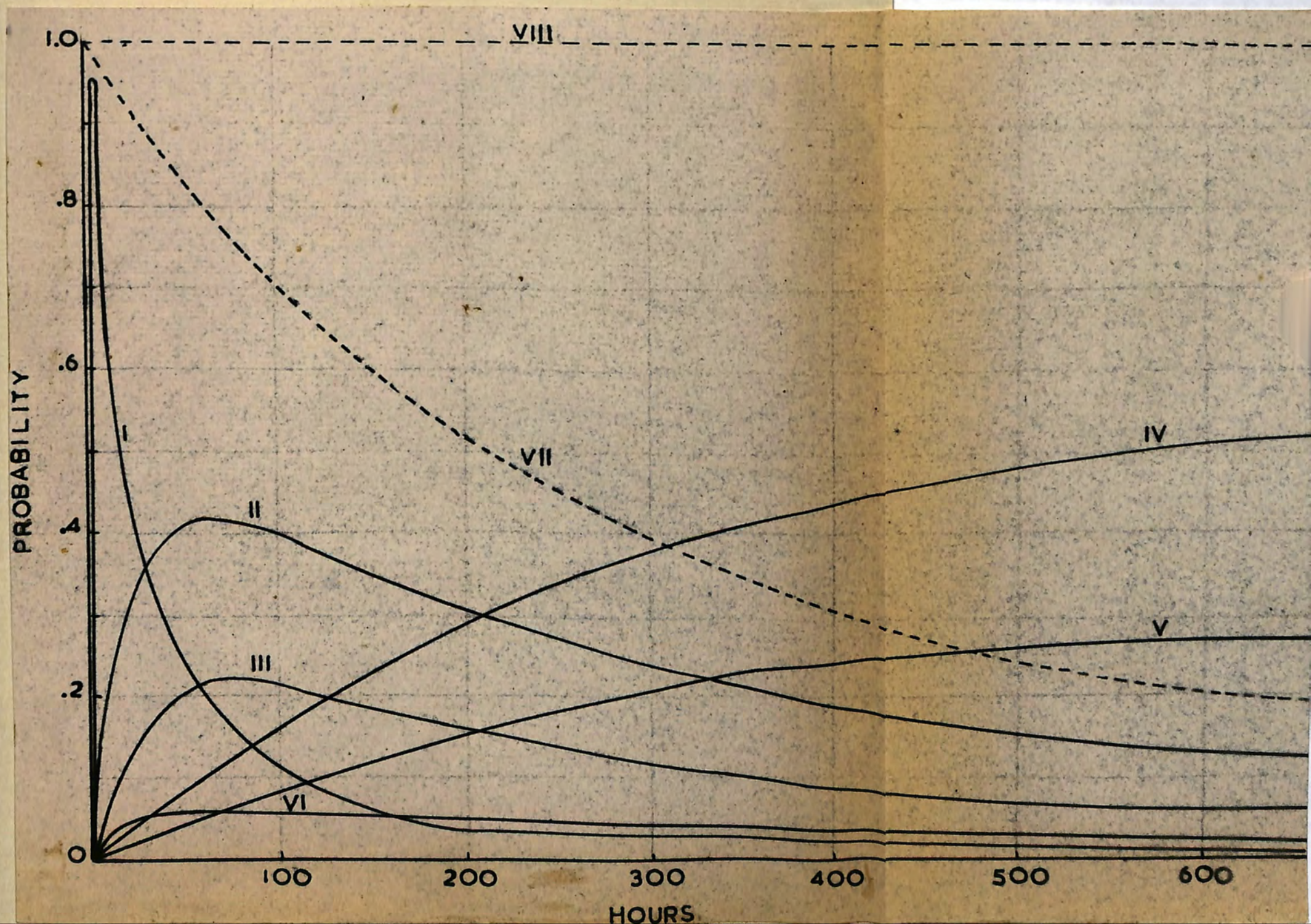
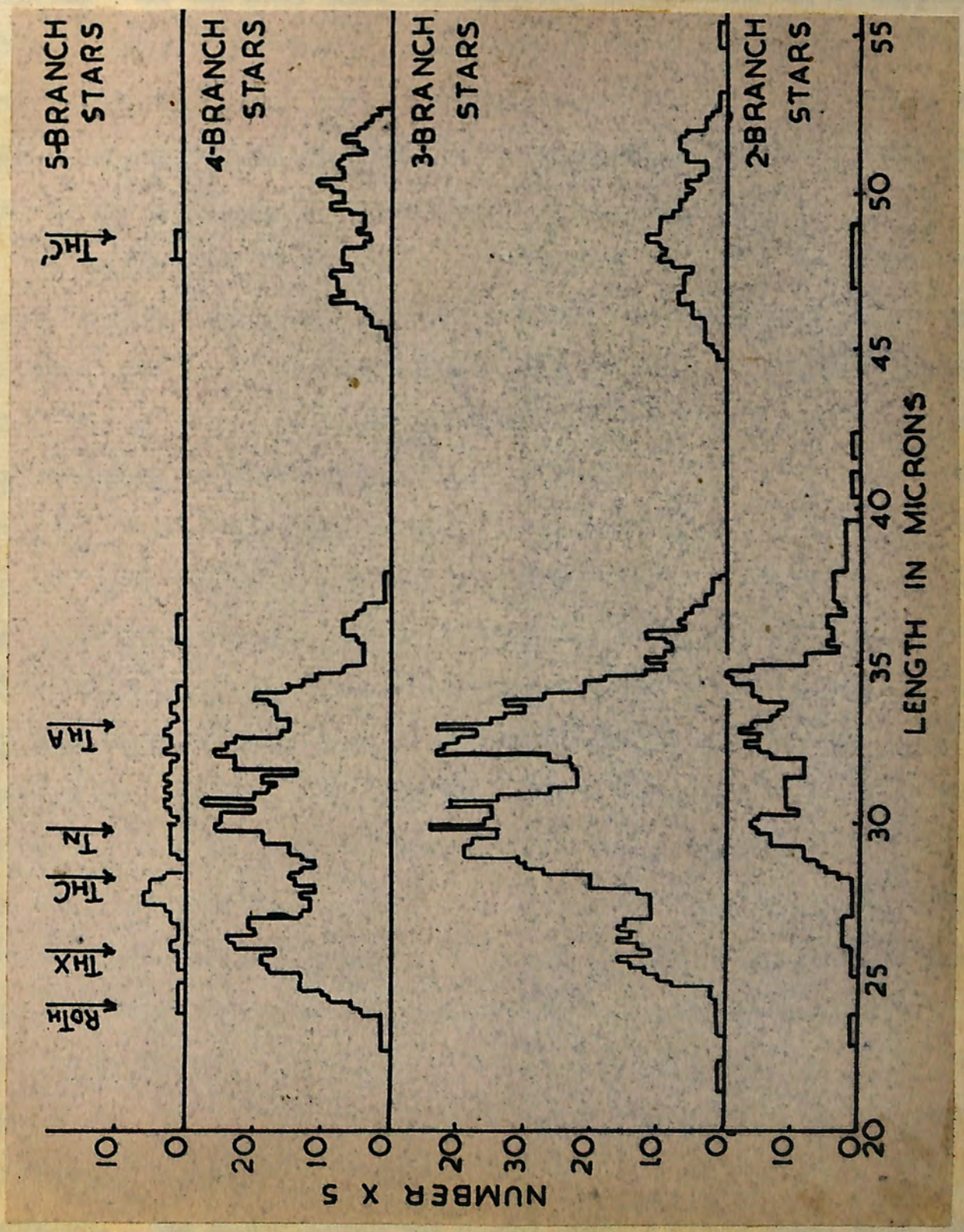


FIGURE 5.12. Alpha-particle tracks from thorotrast occurring in radioactive stars.



percentage of the total number of tracks from the elements as given in Table VI. In the same table the expected distribution for an exposure time of 120 hours is also given. Comparison of the expected and observed distribution shows that there are many more single tracks than expected, particularly from RdTh and ThX; there are a greater number of 3-branch stars, fewer 4- and 5-branch stars and also a number of 2-branch stars.

It is possible to interpret the various events which have been observed by assuming that other elements as well as Tn are capable of diffusing in gelatine. These elements are ThX and one of the elements following ThA, either ThB or ThC. It is not possible by means of alpha-particle studies to distinguish between the two latter elements as ThB does not emit alpha-particles, but as ThB has a longer half-value period than ThC (10.6 hours compared with 60 minutes) it has more time to diffuse before decaying. The single RdTh tracks could arise from the diffusion of ThX in any of the RdTh → ThX sequences. Whereas in the absence of diffusion only 65% of the RdTh alpha-particles should be recorded as single tracks in the given exposure time, 98% were actually recorded. Thus, out of every 33 atoms of ThX formed from the disintegration of RdTh during the exposure, 33 of them have migrated from the position where they were formed before disintegrating, that is 95% of the ThX atoms have diffused. Similarly, the single alpha-particles from ThX could arise from the diffusion of Tn in any of the ThX → Tn sequences. It was estimated from the frequency of such tracks that 43% of the Tn atoms must have diffused. The single ThC and ThC' tracks could arise in the diffusion of ThB or ThC in a number of the sequences. The other unexpected event, 2-track stars, could arise from the

TABLE VI. Distribution of Alpha-particle Tracks in Stars expressed as a Percentage of the total number in each group. Exposure = 120 hours.

Element	Expected Frequency					Observed Frequency				
	Single	2-branch star	3-branch star	4-branch star	5-branch star	Single	2-branch star	3-branch star	4-branch star	5-branch star
Th	100	0	0	0	0	100	0	0	0	0
RdTh	65	0	0	6	29	98	0	0	0	2
ThX	0	0	9	62	29	43	0	22	33	2
Tn	0	0	9	62	29	6	21	46	25	2
ThA	0	0	9	62	29	6	21	46	25	2
ThC	5	0	0	19	10	12	0	12	10	1
ThC'	10	0	0	37	19	23	0	23	19	1

diffusion of Tn in the rare sequence, ThX → Tn → ThA → ThB or from the diffusion of both Tn and ThB or ThC in the sequence ThX → Tn → ThA → ThB → ThC → ThD. The single Tn and ThA tracks may indicate the diffusion of a small proportion of ThA.

A number of other plates containing sample B thorotrast, exposed for various lengths of time, have also been analysed in this way. The proportions of the various elements diffusing are given in Table VII. The plates are arranged in order of increasing exposure time. Those in which the drying time was long compared with the exposure time (5.5) are also included and it is significant that in these plates 100% of the Tn and ThX atoms have diffused because of the higher water content of the emulsion. The mean values in the other five plates are 83% for the diffusion of ThX, 36% for the diffusion of Tn and 15% for the diffusion of ThB or ThC. It was concluded that ThX diffuses more than Tn because of its longer half-value period. Since MsThl is an isotope of ThX it is likely that MsThl may also diffuse but as it is not an alpha-emitter it was not possible to detect this here.

Correlation of single tracks with stars. In the observation of the stars, it was sometimes possible to recognize a single track displaced a short distance from the centre of a star and to measure the displacement of the atom which had diffused. The most common events which could be recognized were those involving the diffusion of Tn, that is, Y-branch stars consisting of tracks from Tn, ThA and ThC or ThC' and single tracks from ThX. Ten of these events were studied by noting the coordinates of the ends of the tracks and calculating the distance of separation of the end of

TABLE VII. Proportions of Elements diffusing in Emulsion.

Plate No.	Exposure Time	Drying Time / Exposure Time	Diffusion of ThX %	Diffusion of Tn %	Diffusion of ThC %
1	4 hours	1.0	100	100	
2	8	0.52	100	100	
3	22	0.20	100	67	
4	3 days		92	45	35
5	5		95	43	21
6	6		80	43	9
7	14		94	13	8
8	28		46	38	3

the single track and the centre of the star. The mean displacement was 9.5 microns; the maximum displacement was 14 microns but it is possible that in some cases the displacement was greater than this and the single track could not be recognized because of confusion with other tracks. Some events were recognized as 3-branch stars with single ThC tracks nearby; the displacement of the ThB or ThC atoms was only of the order of 3 microns in these cases. No events showing the diffusion of ThX could be recognized, probably because the displacement was too great. The relative displacements observed are consistent with the proportions of ThX, Tn and ThC atoms diffusing given in Table VII.

5.6. Loss of Elements by Adsorption

The experiments on the diffusion of elements (5.5) eliminated the possibility that the discrepancy between the predicted age of one of the samples of thorotrast (sample B) and the measured activity could be due to errors introduced into the measurements by the escape of some elements from the emulsion. Another explanation was sought in the adsorption of some of the decay products on the glass container during storage. In this case the activity of the sample removed from the container for use would be lower than that in the liquid remaining in the container.

The presence of adsorbed elements on the inner surface of the container was tested directly by removing the liquid and placing a small piece of stripping film against the glass. The developed film contained a large number of dense black spots consisting of many tracks originating from a common point. These "halos" were similar to the well-known images

produced when drops of a radioactive solution are placed on a photographic plate (Chamie, 1927). They were also occasionally recorded in the plates loaded with thorotrast (5.2); an example is shown in Figure 5.13. The method of formation of haloes was studied by Jedrzejowski (1929) who showed that the atoms tend to group themselves around particulate matter present as an impurity. It is possible that a similar type of phenomenon produced the haloes in thorotrast.

The total number of tracks in the haloes varied considerably. In the smaller ones it was possible to distinguish individual tracks and to measure them when it was found that their lengths corresponded to alpha-particles from RdTh and its products without Th. It was therefore concluded that the intermediate element MsThl adsorbs on the container. A similar conclusion was reached by Rundo (1955) who also found the activity to be less than that predicted from the age of the thorotrast. The reason given was that MsThl does not exist in colloidal form at pH7 and adsorbs strongly on the container where RdTh is formed from it by decay.

It was not possible to determine directly what proportion of MsThl was separated by adsorption, but an attempt was made to determine this indirectly by observing the activity of its isotope, ThX. If ThX were also separated by adsorption, its activity at the time of preparing the emulsion would be reduced, but would increase during the exposure with a half-value period of 3.6 days. If the exposure time of the emulsion were sufficiently short, it might be possible to detect a decrease in the activity of ThX relative to RdTh. If D is the activity of RdTh and a fraction of the RdTh is separated out, the activity of ThX at the beginning

FIGURE 5.13. "Halo" of tracks in thorotrast.

of the exposure time is $(1 - f)D$. Then after time t, the activity of ThX is $(1 - fe^{-\lambda_5 t})D$ where λ_5 is the decay constant of ThX. The activity of ThX relative to RdTh as given by this expression is plotted against t for various values of f in Figure 5.14. From these curves it is seen that if the exposure time is 3 days and one half of the ThX is lost by adsorption, then at the beginning of the exposure time the activity of ThX relative to RdTh is 0.50 and at the end it is 0.75; it should have been possible to detect this easily in the observed distribution of track lengths. If f has the value $^1/_4$ the relative activity varies from 0.75 to 0.86 during the exposure; if f is $^1/_8$, the relative activity varies from 0.88 to 0.94. The latter might not be detected in a distribution in which the number of tracks in each group is known to about 10%. In some cases the exposure time was longer than 3 days, and the effect would be less marked. Since the observed activities of RdTh and ThX were equal to within about 10% in all the plates, the loss of ThX from the thorotrast could not have been more than about 12%. The loss of MsThl might possibly be greater than this because MsThl exists longer than ThX before decaying. The loss cannot be very great in all cases, otherwise an age-factor as high as that observed for sample A would never be reached.

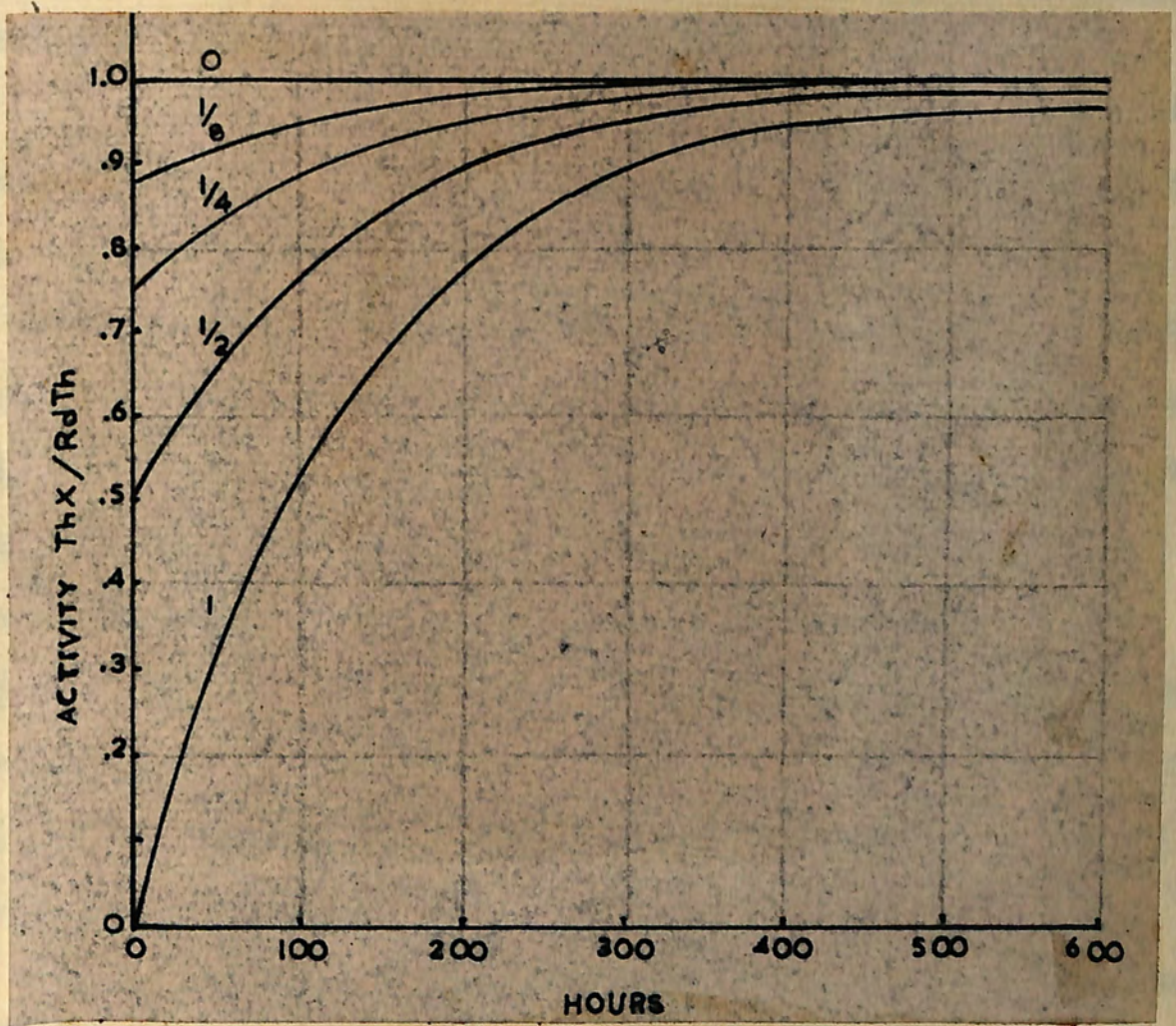
5.7. Discussion

The age-factor defined in Chapter 2 has been expressed in terms of the alpha-particle activity of Th (A) and RdTh (D) by the formula $\beta = {}^1/_6(1 + {}^{5D}/_A)$. Theoretical curves have been given for the variation of the age-factor with time assuming different values of the

FIGURE 5.14. Growth of activity of ThX relative to RdTh

Activity $^{ThX}/_{RdTh} = 1 - fe^{-\lambda_5 t}$

Curves for f = 0, $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, 1



initial Th to RdTh ratio. Several samples have been analysed experimentally and their age determined using the theoretical curves. It has been shown that when the thorotrast is known to be greater than about 10 years old, one measurement of the activity is sufficient to define its age accurately but it is not possible to determine its early history. When the sample has been prepared comparatively recently it is necessary to make at least two or three measurements at various times (for example, at six-monthly intervals); the date of preparation and the activity at any time can then be estimated.

In cases where the time interval between injection and measurement is known from the history of the patient but no sample of the thorotrast is available for analysis, its age-factor can be estimated assuming that the time interval between injection and measurement corresponds to the age of the thorotrast. The accuracy of the estimate increases as the time interval is increased; immediately after preparation the age-factor has limits 0.17 to 1.0, after 2.5 years 0.23 to 0.56, after 5 years 0.35 to 0.50, after 10 years 0.60 to 0.62 and after longer periods of time the limits coincide.

While it is valid to assume that the activity of thorotrast varies in the manner described, in practice some of the elements have properties which may change the activity of samples actually injected into patients. This applies particularly to the element MsThl which tends to be adsorbed on the container during storage, so that the activity is actually less than that predicted from the age. The effect will be quite small in fresh samples of thorotrast in which the MsThl content is low. MsThl atoms also

tend to be deposited on particulate matter forming particles of high activity which may be introduced into the body. This phenomenon is to be distinguished from the aggregation of colloidal particles within tissue cells.

Another way in which elements may escape from thorotrast, altering its activity, is by diffusion. By studying this phenomenon in photographic emulsions, it has been shown that, although the gaseous element, Tn, might be expected to diffuse most, the proportion of ThX diffusing is actually greater, presumably because of its greater half-value period. It was not possible to detect the diffusion of MsThl in the alpha-particle studies, but it is expected that at least a similar amount of this element will diffuse. The amount of diffusion will depend on the medium in which the substance is present. In photographic emulsion it has been shown that the displacement of the diffused atom is only of the order of a few microns. The composition of the emulsion is different from that of tissue containing thorotrast, but it might be assumed that the diffusion of the various elements observed in emulsion would at least give an indication of the diffusion to be expected in tissue. In the case of thorotrast in tissue <u>in vivo</u>, additional factors will operate, on the one hand, the elements are contained within dense aggregates which will tend to prevent the escape of some elements, and on the other hand, the escape of elements will be aided by the blood flow and the solubility of elements in blood. From the observed relative proportions of Tn and ThB diffusing, it might be deduced that a greater proportion of the ThB present in the blood is derived from the decay of Tn escaping from the tissue, than from ThB

escaping directly.

From the alpha-particle studies it was also shown that, in four samples examined, the Ra impurity in the thorotrast did not correspond in activity to more than 10% of the activity of Th.

CHAPTER 6

IDENTIFICATION OF ELEMENTS IN AUTORADIOGRAPHS.

6.1. Derivation of the Distribution of Track Lengths

In the autoradiograph each radioactive element in the tissue emits alpha-particles of characteristic track length which may be used to identify that particular element. If the radioactive atoms were scattered throughout the emulsion the track lengths recorded would be equal to the range R of the particle in emulsion as shown in the previous chapter. In the autoradiograph, however, the alpha-particles originate at all depths throughout a section of finite thickness; some of the energy is absorbed in the section and only the residual range is recorded in the emulsion. Thus, each group of alpha-particles appears as a continuous distribution of track lengths from zero to the full range of the particle.

Suppose the section to be of thickness h, of infinite area and to contain an element emitting a single group of alpha-particles of range R in emulsion. Consider a particle originating at a point P within a distance x of the top of the section and emitted at an angle θ to the plane of the emulsion as shown in Figure 6.1. If l is the length of the track recorded in the emulsion and μ is the ratio of the stopping power of the section and the emulsion, x, l and θ are related by the equation

$$R - l = \frac{x}{\mu \sin\theta} \qquad (1)$$

The probability of the track being of length l to l + dl is given by

$$p(l)dl = p(x)dx\, p(\theta)d\theta$$

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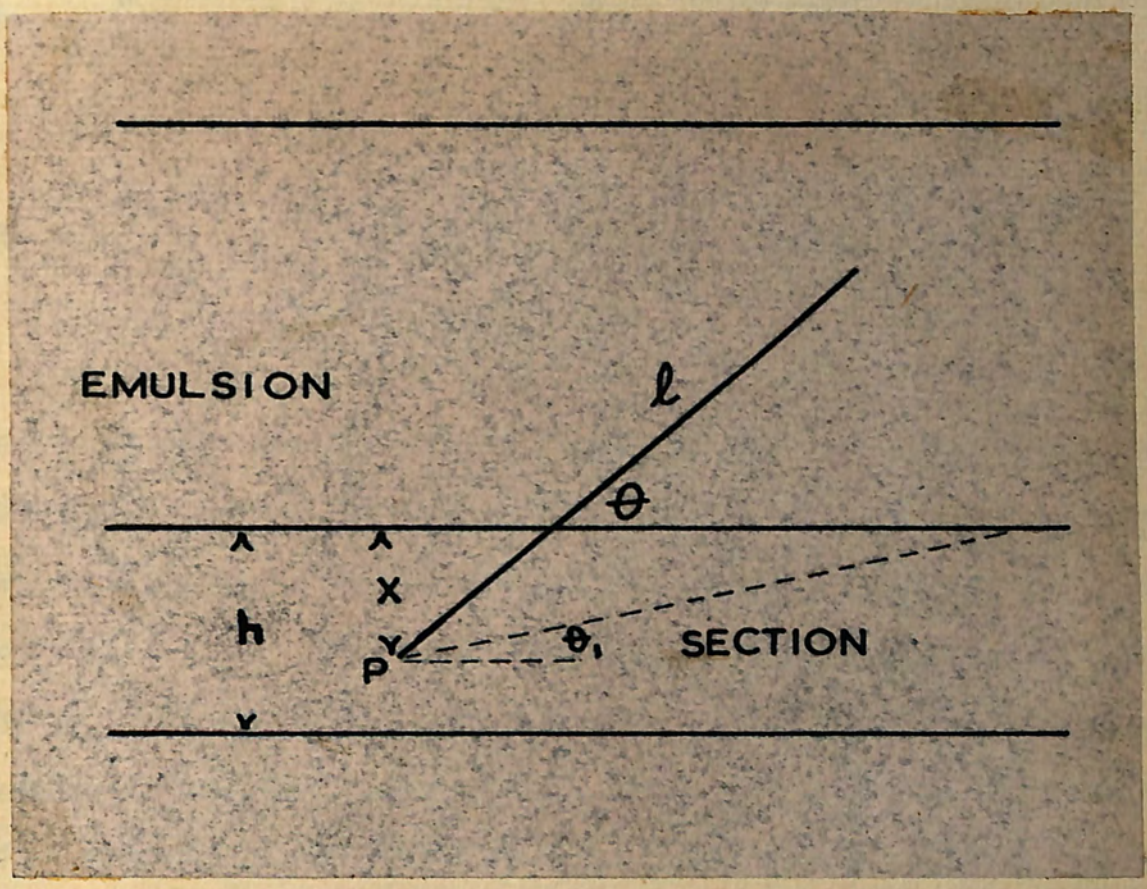
FIGURE 6.1.



where x varies from 0 to h, $p(x) = 1/h$, θ varies from 0 to Θ and $p(\theta)$ is given by the solid angle $d\theta$, that is, $\frac{\cos\theta}{2}$.

Thus $p(l) = \iint \frac{1}{h}\frac{\cos\theta}{2}\, dx d\theta$

The limits of x and θ vary according to l.

For $l < R - \frac{h}{\mu\sin\Theta}$, $0 < \theta < \arcsin\frac{h}{\mu(R-l)}$, $0 < x < h$

$$\text{that is, } p(l) = \frac{h}{2\mu(R-l)} \qquad (2)$$

For $l > R - \frac{h}{\mu\sin\Theta}$, $0 < \theta < \Theta$, $0 < x < \mu(R-l)\sin\theta$

$$\text{that is, } p(l) = \frac{\mu(R-l)\sin^2\theta}{2h} \qquad (3)$$

Here Θ is the maximum angle to which tracks are measured. Plotting p(l) against l a curve is obtained which rises to a maximum at $l = R - \frac{h}{\mu\sin\Theta}$ and decreases linearly to zero at $l = R$. The position of the peak is thus determined by the thickness and stopping power of the section; the intercept on the l-axis is determined by the range of the particle. In Figure 6.2, p(l) is plotted against l for $\frac{h}{\mu\sin\Theta} = 6$ and two different values of R, 16 and 30. It is assumed that the section contains a substance which emits equal numbers of particles of the two ranges. The choice of the value of $\frac{h}{\mu\sin\Theta}$ is arbitrary in this theoretical case. The maximum probability is the same in both cases but the area of the curve for $R = 30$ is greater as the total number of tracks recorded increases with R.

Proportion of total tracks recorded. The fraction of the total number of tracks recorded is obtained from the distribution of track lengths given by equations (2) and (3). Since p(l) is a function of l and θ, the variation of p(l) with l is given by $\partial p(l)/\partial l$ and the total number of tracks

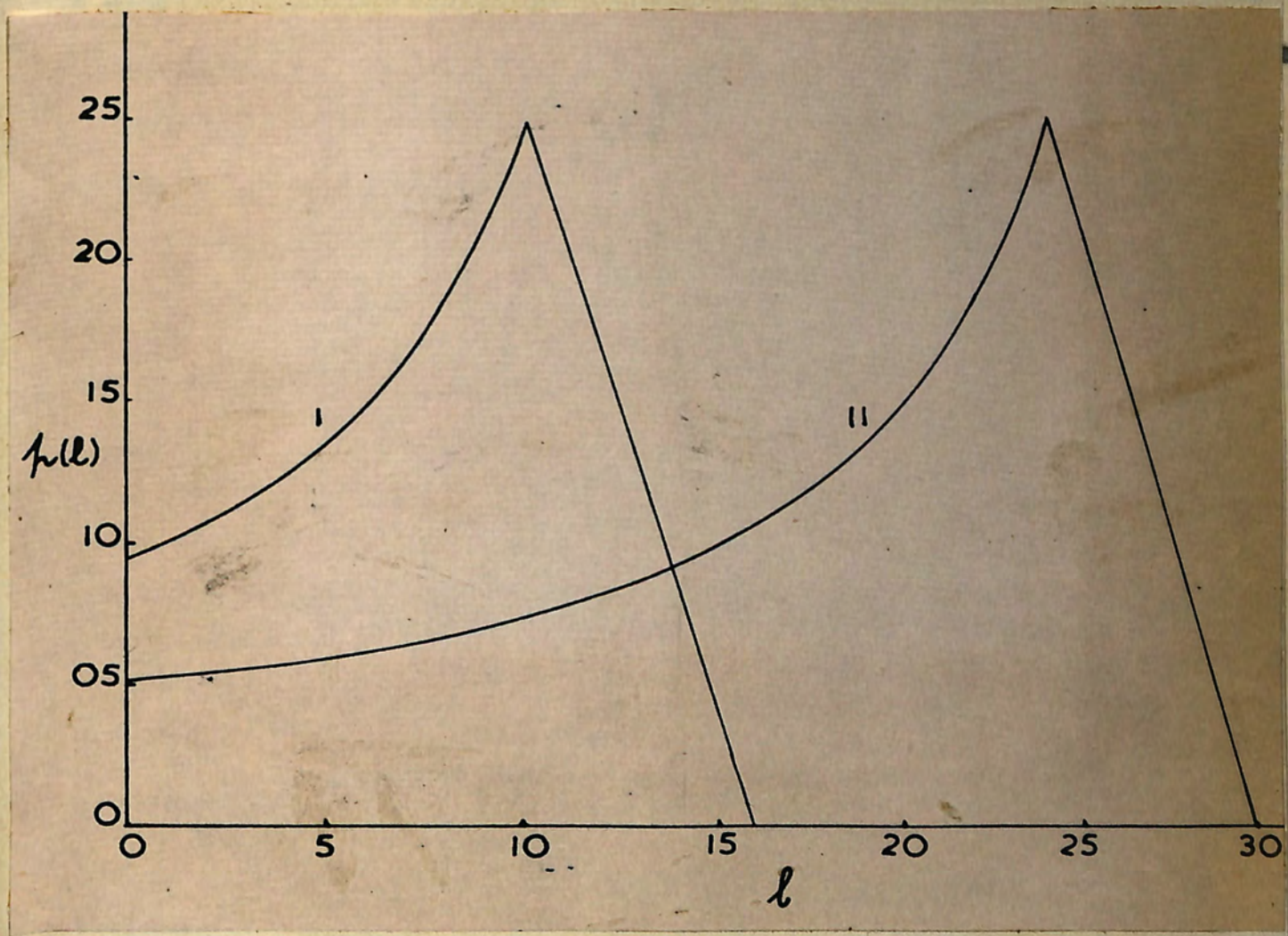
FIGURE 6.2. Plot of equations.

$$p(l) = \frac{h}{2\mu(R-l)} \quad \text{for } l < R = \frac{h}{\mu \sin\theta}$$

$$p(l) = \frac{\mu(R-l)}{2l} \sin^2\theta \quad \text{for } l > R = \frac{h}{\mu \sin\theta}$$

for $\frac{h}{\mu \sin\theta} = 6$ Curve I $R = 16$

II $R = 30$



recorded is

$$\int \frac{\partial n(l)}{\partial (l)}\, dl = \int_0^{R-\frac{h}{\mu \sin\theta}} \frac{h}{2\mu(R-l)^2}\, dl + \int_{R-\frac{h}{\mu\sin\theta}}^{R} \frac{\mu \sin^2\theta}{2h}\, dl$$

$$= \sin\theta - \frac{h}{2\mu R} \qquad (4)$$

A similar result is obtained directly by consideration of Figure 6.1. Of the particles originating at P at a depth x in the emulsion, those which are recorded are included in the solid angle θ_1 to θ where θ_1 is given by $\arcsin {}^{x}/_{\mu R}$, that is, in the solid angle $2\pi(\sin\theta - {}^{x}/_{\mu R})$. The number from the elementary volume at P is given by

$$NA(\sin\theta - {}^{x}/_{\mu R})dx/2$$

and from the whole section assuming $h < R$ by

$$\int_0^h NA(\sin\theta - \frac{x}{\mu R})\frac{dx}{2} = \frac{NAh}{2}(\sin\theta - \frac{h}{2\mu R})$$

The total number of tracks emitted in the direction towards the emulsion is $NA^{h}/2$. Therefore the fraction of tracks recorded in the range of angles 0 to θ is $\sin\theta - \frac{h}{2\mu R}$ as above.

<u>Effect of Straggling.</u> In the derivation of the distribution of track lengths it was assumed that all particles with similar energy have the same range R in emulsion, that is, the straggling of the alpha-particles was neglected. The probability associated with l is more correctly given by

$$p(l)dl = p(x)dx\, p(\theta)d\theta\, p(R)dR$$

where $p(R) = \frac{1}{\sqrt{2\pi}\,\sigma} e^{-(R-R_0)^2/2\sigma^2}$

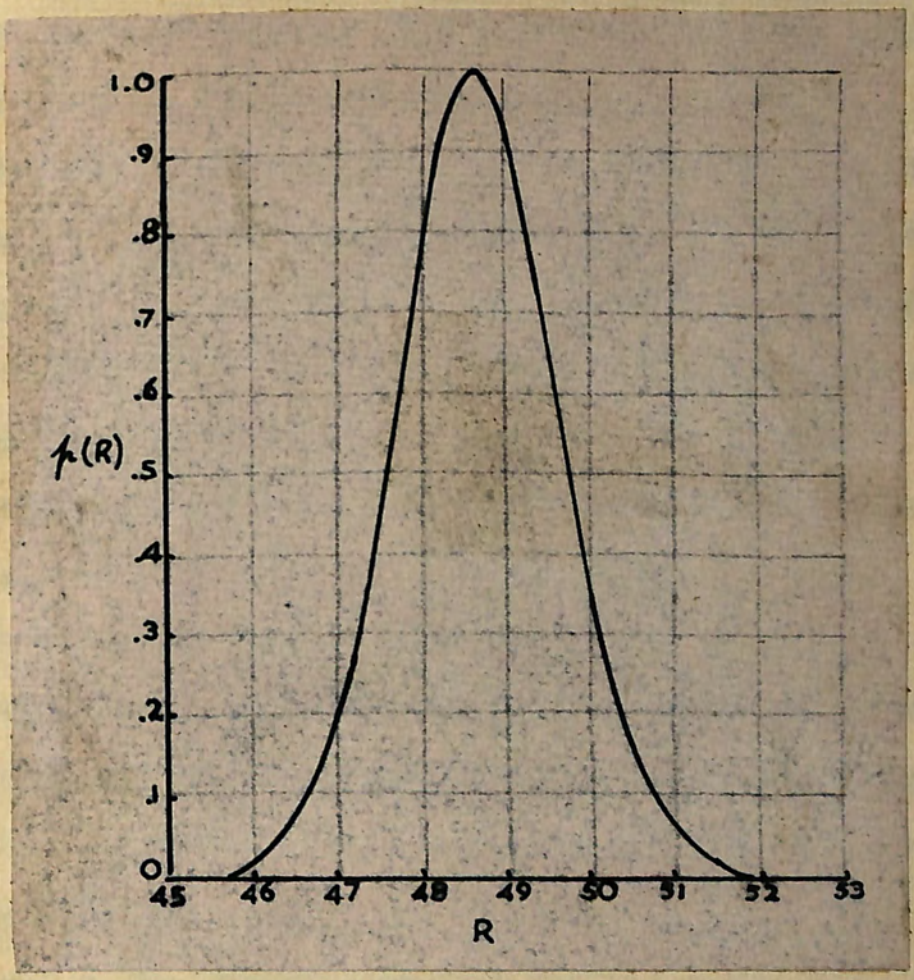
This expression for p(R) is plotted in Figure 6.3 for $R_0 = 48.7$ assuming that for this range the straggling is 2.3% of the range (Rotblat, 1950), that is, that the half-width of the curve at $p(R) = 1/2$ is 1.2. The distribution of track lengths observed in the autoradiographs will be the sum of a number of curves of the form given by equations (2) and (3), one for each different value of R, the height of the curve being proportional to p(R). The effect will be seen as a rounding off at the top of the curve and at the intercept on the l axis, but as the width of the straggling curve is small, the effect of straggling may be neglected as a first approximation.

Irregularities in the thickness of the section. As no section is likely to be of uniform thickness throughout, the observed curve will be the sum of a number of different curves for different values of h, that is, it will be broader than the theoretical curve for a section of uniform thickness. As the amount of variation in h is not known, the best measure of this effect will be the curves actually obtained from autoradiographs.

6.2. Experimental Verification

In order to verify equations (2) and (3) experimentally, the distributions of track lengths from sections containing a known element, namely ThB, were studied. The sections were represented by thin layers of gelatine prepared from a solution containing ThB and poured on microscope slides to varying thicknesses. When the layers were dry, C2 plates were placed against them to simulate contact autoradiographs. The plates were exposed for about one day. It was necessary to adjust the concentration

FIGURE 6.3. Straggling of alpha-particles from ThC' in C2 emulsion.



of the ThB in gelatine so that a suitable number of tracks per unit area was obtained in about two half-value periods of ThB.

ThB emits beta-particles and decays with a half-value period of 10.6 hours to ThC. Two-thirds of the ThC disintegrations to ThC' which emits an alpha-particle of range 48.7 microns in emulsion and the other third emits an alpha-particle of range 28.2 microns. The intensities of the two alpha-particle groups are therefore in the ratio 1:2.

The distributions of track lengths from three different thicknesses were analysed, and are reproduced in Figure 6.4. In the first distribution, section I was very thin and $\frac{h}{\mu \sin\theta} = 3$. Tracks were measured in the range of angles 0 to 30°, hence $\frac{h}{\mu} = 1.5$. The distribution was analysed into two curves giving R values 28 and 49 microns. The heights of the curves are in the ratio 1:2 as expected for tracks from ThB. The shape of the curves also agrees with the theoretical relation.

In pouring section II, 1 ml of the gelatine solution was used and the tracks were measured at angles 0 to 60°. The dependence of the distribution on the angle θ was illustrated by plotting two distributions, one for tracks 0 to 30° and the other for tracks 0 to 60°, that is, for Curve (a) $\theta = 60°$, $\frac{h}{\mu \sin\theta} = 8$, therefore $\frac{h}{\mu} = 6.9$ and for Curve (b) $\theta = 30°$, $\frac{h}{\mu \sin\theta} = 14$, therefore $\frac{h}{\mu} = 7$.
The relative positions of the peaks and the shape of the curves for different values of θ also agree with the formula.

The last two curves are for section III which was poured using 2 ml of gelatine solution. Here again two plots are given, for Curve (a) $\theta = 60°$, $\frac{h}{\mu \sin\theta} = 16$, therefore $\frac{h}{\mu} = 13.8$ and for Curve (b)

FIGURE 6.4.

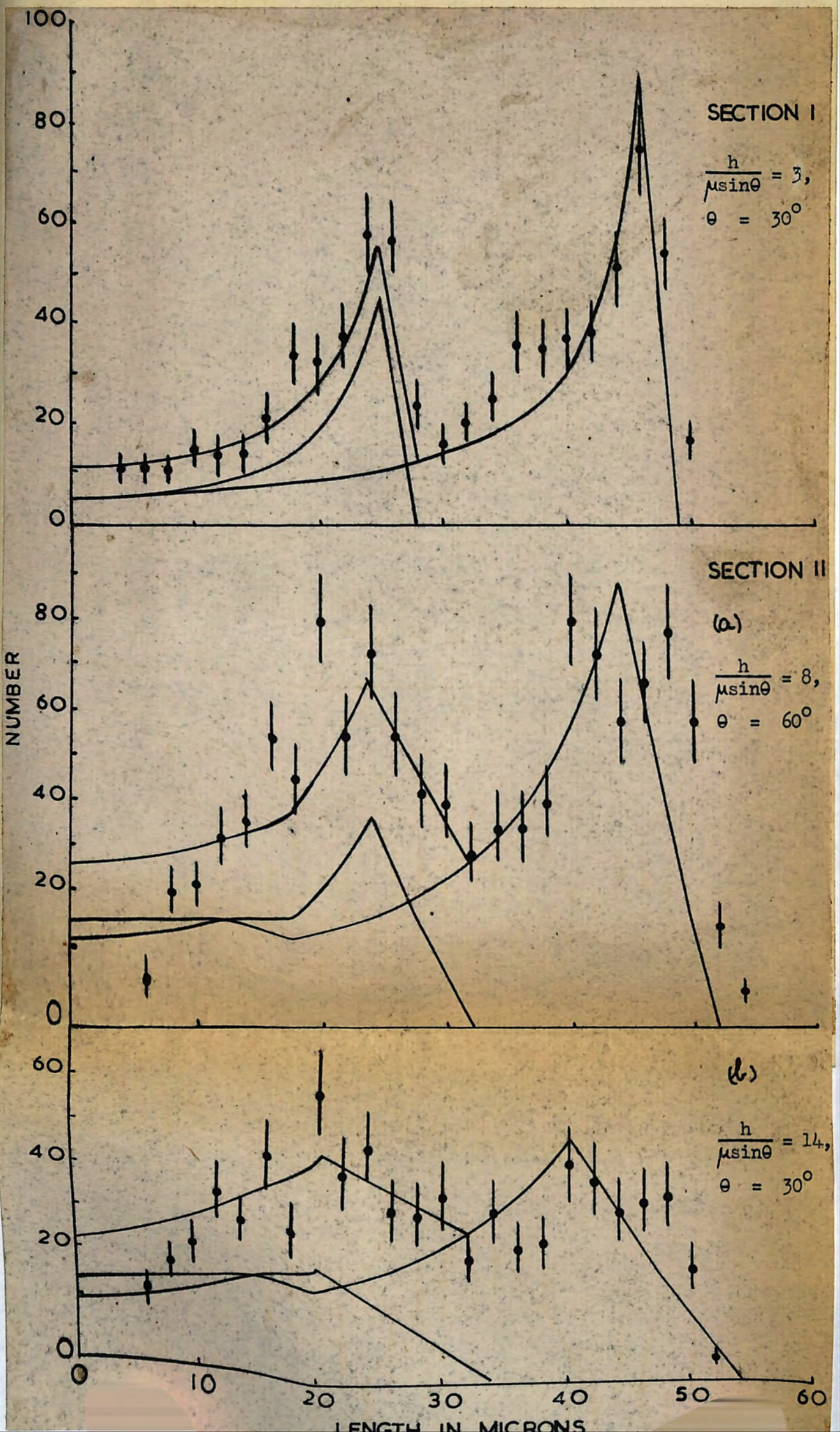
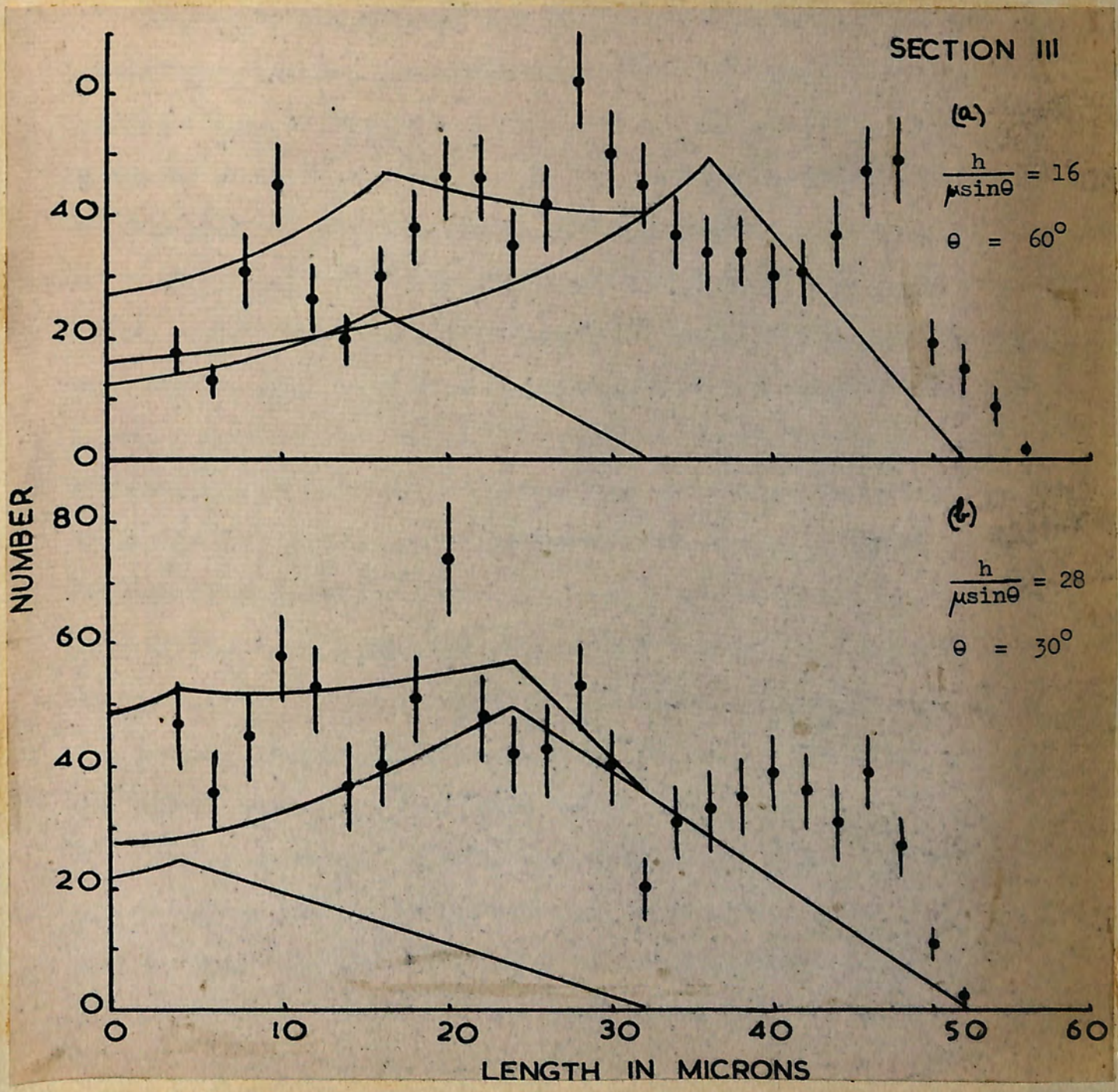

SECTION I
h/μsinθ = 3,
θ = 30°
SECTION II
(a)
h/μsinθ = 8,
θ = 60°
(b)
h/μsinθ = 14,
θ = 30°
NUMBER
LENGTH IN MICRONS
0
10
20
30
40
50
60
80
100

FIGURE 6.4.


SECTION III
(a)
h/μsinθ = 16
θ = 60°
(b)
h/μsinθ = 28
θ = 30°
NUMBER
0
40
20
0
80
60
40
20
0
0
10
20
30
40
50
60
LENGTH IN MICRONS

$\theta = 30^\circ$, $\frac{h}{\mu \sin\theta} = 28$, therefore $\frac{h}{\mu} = 14$.

as expected for a section twice as thick as section II.

In general the shape of the observed distribution agrees with the theoretical form but certain features which are inconsistent with it require consideration, particularly in the case of section III. The maximum length of tracks recorded exceeds that of the range of alpha-particles from ThC' and the best fit was obtained by putting R = 52 or 54 microns, that is, about 10% greater than the true range in emulsion. For steep tracks up to 60° a correction was necessary because of the different shrinkage factor at this angle; this correction has already been applied in the distributions given. Some long tracks will be recorded because of the straggling of the alpha-particles but this correction does not account for all the excess of tracks in the region 45 to 55 microns. In section III there appears to be another distribution for which h has a smaller value, superimposed on the ones given. It is possible that the ThB was not uniformly distributed throughout the layer, and there was a higher concentration near the surface where particles were almost completely recorded. The layer in this case was too thick for good resolution of the two curves.

The width of the peaks is less when steeper tracks are included in the measurements. It would therefore be an advantage to increase θ as much as possible, but difficulties in measuring steep tracks make this impracticable. In practice the width of the peaks is slightly broader than the theoretical distribution because of straggling (6.1).

Another inconsistency is the shortage of tracks less than 10 microns; it is possible that some of these short tracks were missed in the measurements.

FIGURE 6.5. Theoretical distribution of track lengths for $\frac{h}{\mu \sin\theta} = 6$

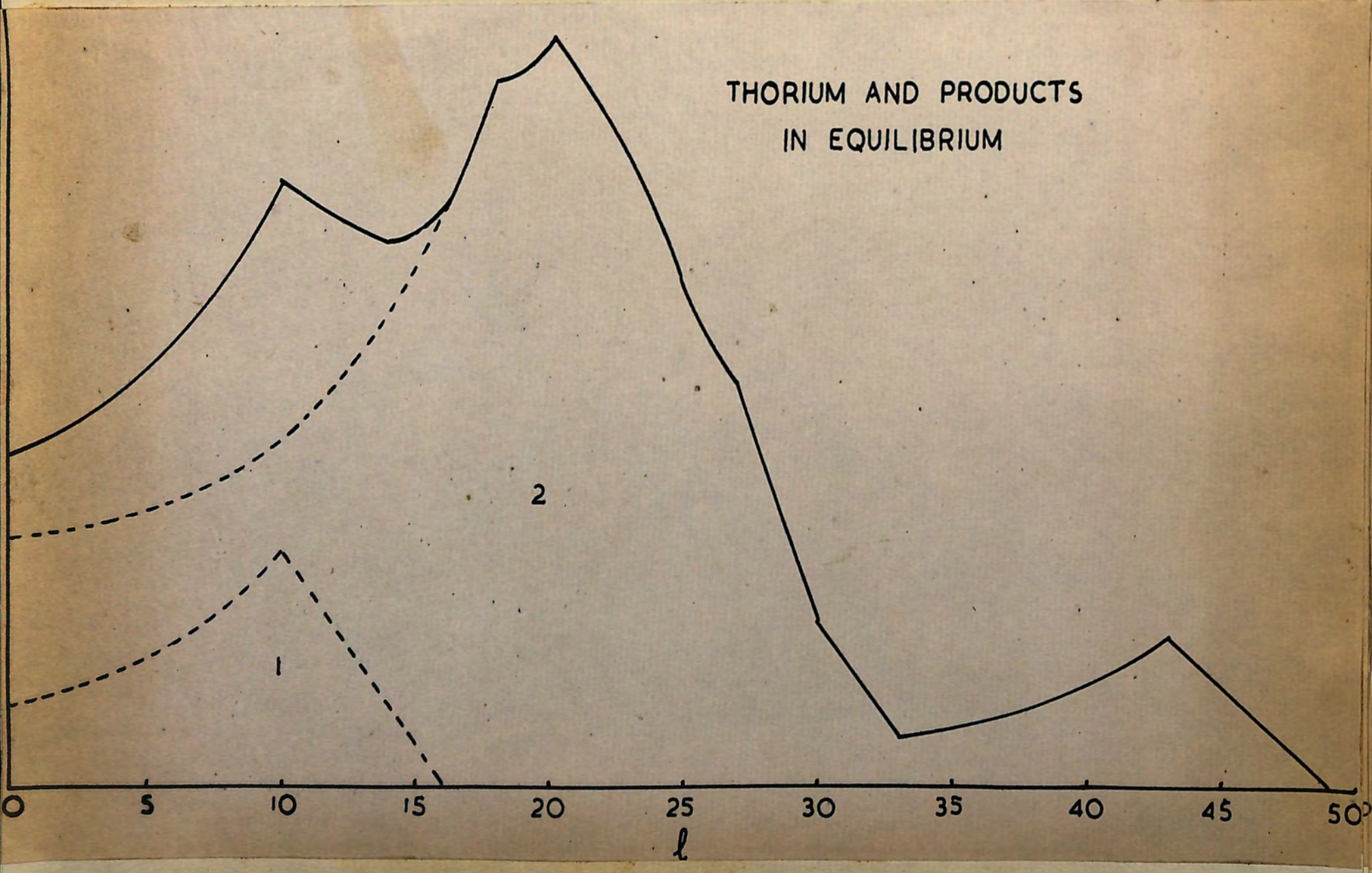
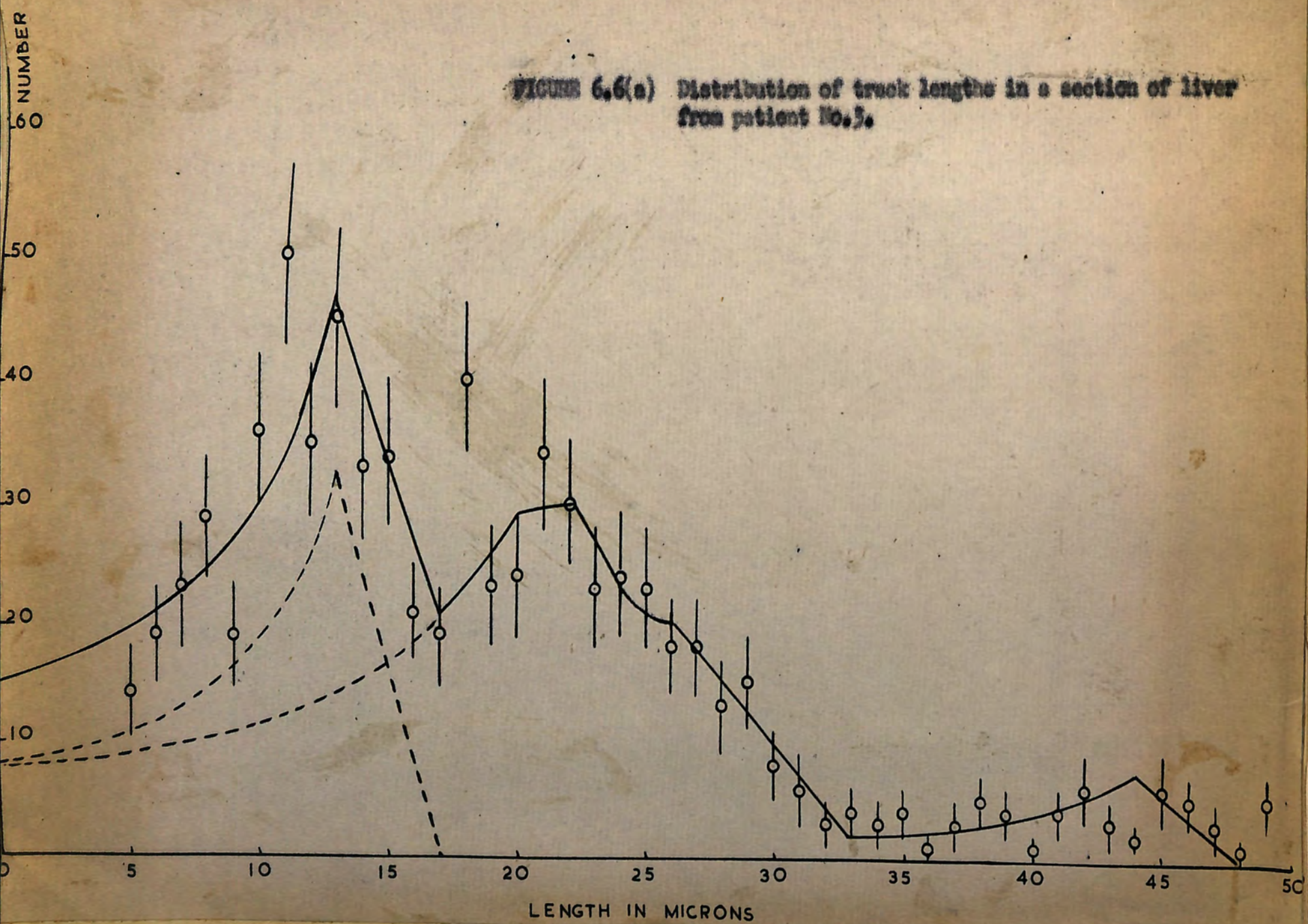


FIGURE 6.6(a) Distribution of track lengths in a section of liver from patient No.3.



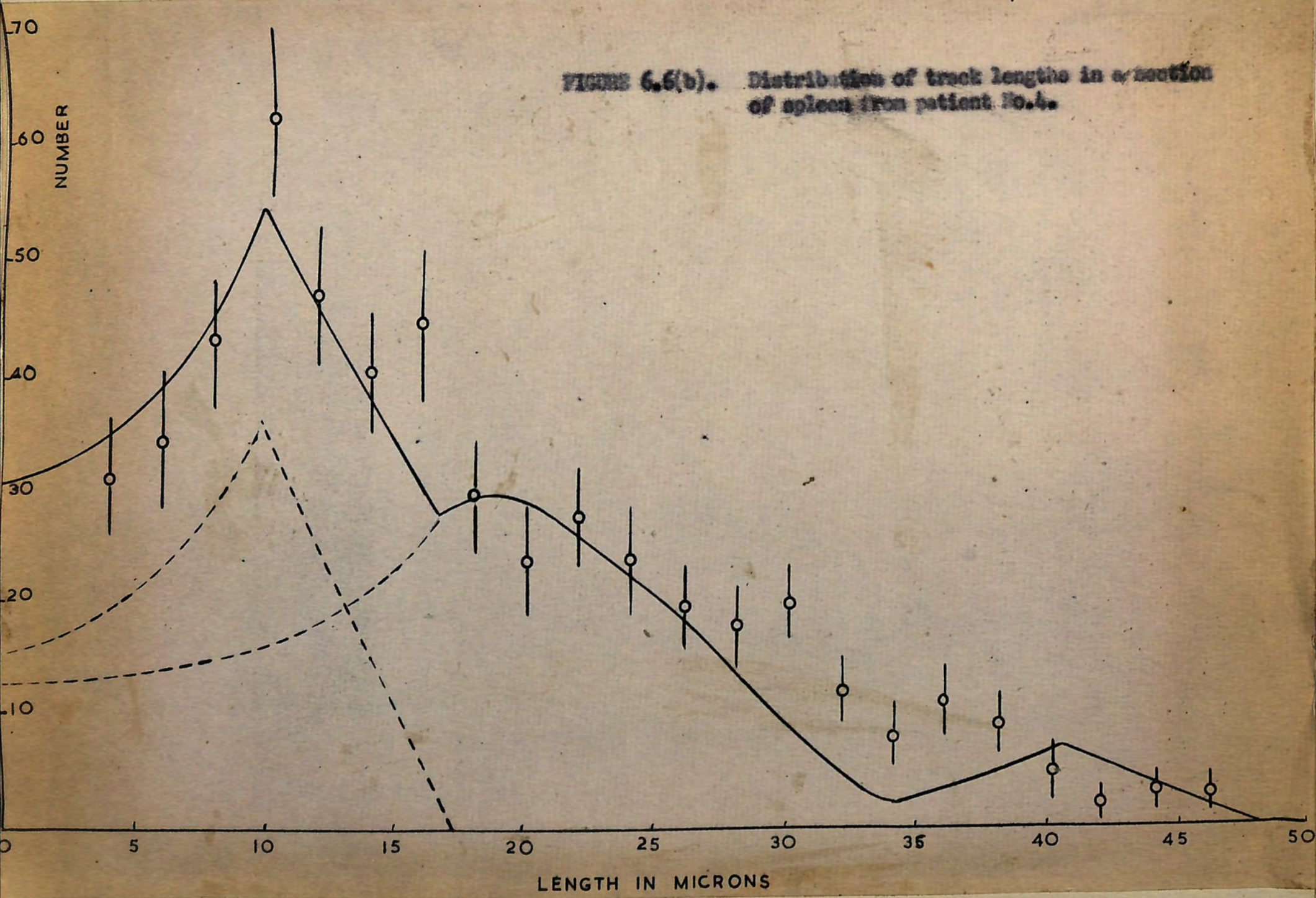


FIGURE 6.6(b). Distribution of track lengths in a section of spleen from patient No.4.

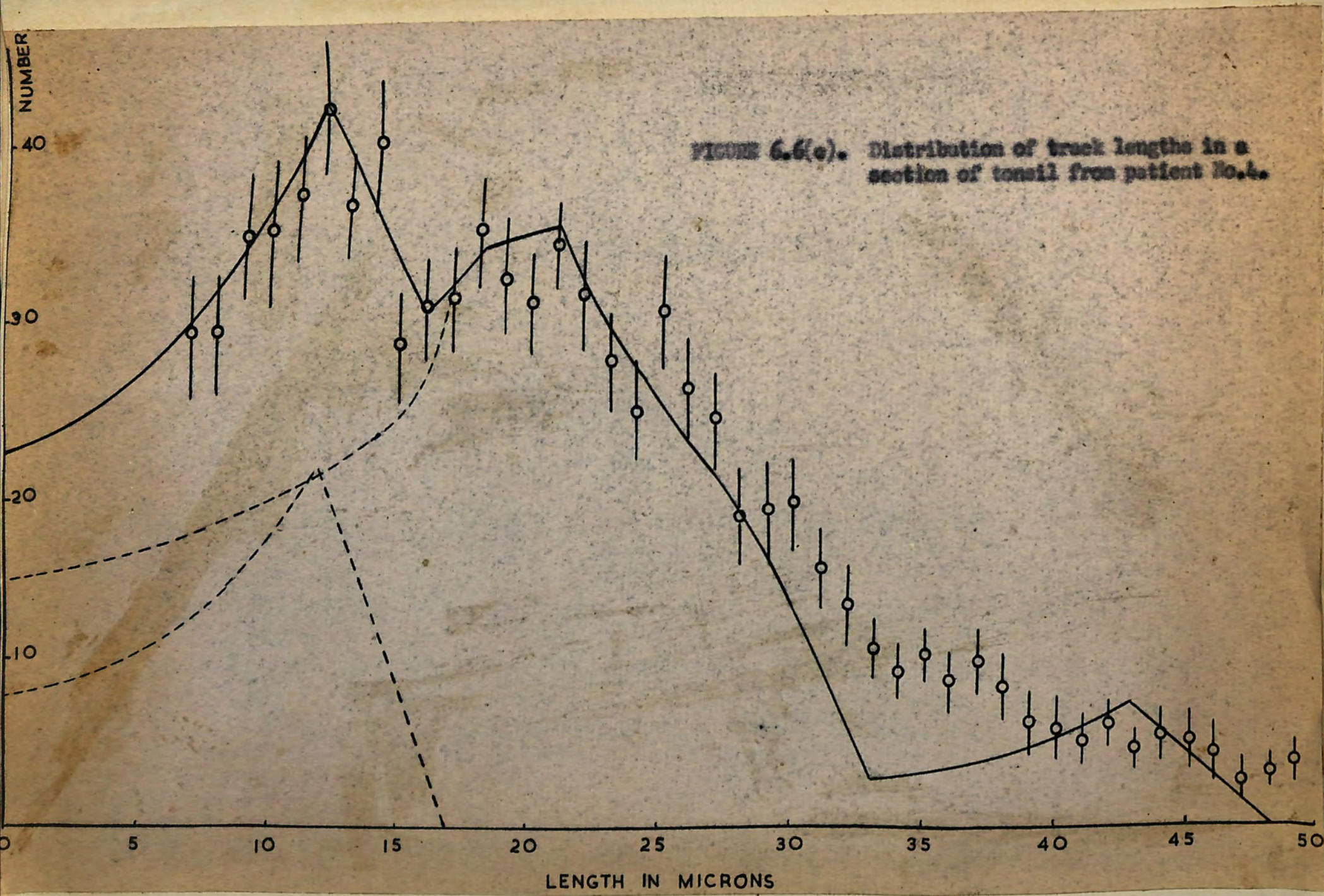


FIGURE 6.6(c). Distribution of track lengths in a section of tonsil from patient No.4.

fitting the curves the position of the maximum of the Th group was noted for the value of $\frac{h}{\mu \sin\theta}$. Using this value the curve for RdTh and its products in equilibrium which gave the best fit to the distribution for l greater than 16 microns was plotted. This was then subtracted from the curve for l less than 16 microns and the curve for Th drawn. In this analysis the tracks less than 5 microns in length were omitted as these were thought to be unreliable. In Figure 6.6. the separate curves are shown by dotted lines and the resultant curve by the full line. It is seen that there are differences in the areas under the curves; in the tonsil section of patient No.4, for example, there are less tracks in the first group in relation to the second than in the spleen section of the same patient.

The values of α calculated from the areas of the two curves must be corrected for the number of tracks absorbed in the section by means of equation (4) (6.1). In the observed distributions, $\theta = 30^{\circ}$, h/μ varies from 2 to 3.5, and R has values 16 microns for the Th group and mean value 30.8 microns for the other groups. The fractions, f, of the total number of alpha-particles emitted from the section which are recorded in the emulsion then have values as follows:

For $h/\mu = 2$, R = 16 microns, f = 0.437
R = 30.8 " f = 0.466
and for $h/\mu = 3.5$, R = 16 microns, f = 0.390
R = 30.8 " f = 0.443

The values of α calculated from the areas of the curves A_1/A_2 must therefore be multiplied by factors 1.06 for $h/\mu = 2$ and 1.14 for $h/\mu = 3.5$.

The values of β calculated from the observed distributions of tracks in the sections from patient No.4 are tabulated in Table VIII, together with several other sections of the same patient which were examined at the same time. The tissues were obtained at biopsies performed at the times given in the date column. The errors quoted allow for the statistical error calculated from the number of tracks measured, but do not allow for errors in the analysis of the observed distributions into groups.

The same analysis has been carried out on some of the sections in other patients, principally those of the greatest activity such as the liver and spleen. These results are given in Table XX. In some of the other cases samples of bone marrow were also examined; these were not available from patient No.4. As well as the three Copenhagen patients, Nos. 3, 4, 5, who were quoted in Chapter 2, tissues from two patients from London Hospitals, Nos. 1 and 2, have been examined and the results are also tabulated. No radioactivity measurements were carried out on these patients while living and all the known data about them is included in the table. These are both recent cases and it is interesting to compare the results with those of the long-standing cases Nos. 4 and 5. The rabbit tissues examined came from one of the experimental rabbits injected in Copenhagen.

6.4. Relative Stopping Power of Section and Emulsion

In the analysis of the distribution of track lengths, a knowledge of the thickness and stopping power of the section is not necessary and, in fact, the observed distribution can be used to calculate the ratio h/μ. In the observed distributions h/μ varied from 2.0 to 3.5; since the

TABLE VIII. Activity in Sections from Patient No.4
Injection 1938. Autoradiographs May 1951, $\beta_o = 0.78$

Section	Date	β	γ_{av}
Spleen medulla	1949	0.54 ± .02	0.66
Spleen	"	0.67 ± .02	0.83
Hilum of spleen	"	0.45 ± .03	0.56
Liver	"	0.74 ± .01	0.91
Lymphode	"	0.54 ± .02	0.66
Tonsil	1947	0.76 ± .01	0.95
Pancreas	1949	No activity	

thickness of the sections was nominally 3 microns, μ has values from 1.5 to 0.85. These values may be compared with the relative stopping power of the section and the emulsion calculated from their density and composition, assuming that the section consists of a mixture of thorotrast and tissue.

The stopping power relative to air of a substance consisting of different kinds of atoms is given by the formula

$$R_{o/R} = \frac{\Delta_o}{\Delta} \frac{N_1S_1 + N_2S_2 + \ldots\ldots + N_iS_i}{N_1A_1 + N_2A_2 + \ldots\ldots + N_iA_i}$$

where R_o is the range of the particle in air, R is the range in the substance, Δ and Δ_o are the densities of the substance and air respectively, N_1, N_2 etc. are the numbers of atoms of each type in the molecule, S_1, S_2 etc. are their atomic stopping powers, A_1, A_2, etc. are the atomic weights.

The composition of dry emulsion, dried tissue and thorium dioxide and the atomic stopping power of the constituents are given in Table IX. In the case of dried tissue, only the main constituents have been given, omitting elements Fe, Mn, Cu etc. which are present in very small quantities. Calculation by the formula shows that

for dry emulsion $R_{o/R} = 0.161 \frac{\Delta_o}{\Delta}$

for dried tissue $R_{o/R} = 0.0935 \frac{\Delta_o}{\Delta}$

and for ThO_2 $R_{o/R} = 0.23 \frac{\Delta_o}{\Delta}$

From these figures, the relative stopping power of emulsion and tissue is 1.72 and of emulsion and ThO_2, 0.70. The values observed in the distributions (1.5 and 0.85) are therefore consistent for a section containing tissue and ThO_2.

TABLE IX. Data for Calculation of Stopping Powers.

Element	Atomic Weight	Atomic Stopping Power	Composition $gmcm^{-3}$		
			Dry Emulsion	Dried Tissue	ThO_2
H	1	0.224	0.049	0.083	
C	12	0.932	0.30	0.711	
N	14	1.02	0.073	0.119	
O	16	1.10	0.20	0.059	1.18
Na	23	1.36		0.006	
Mg	24	1.39		0.002	
P	31	1.62		0.040	
S	32	1.64	0.011	0.010	
Cl	35	1.74		0.006	
K	39	1.84		0.014	
Ca	40	1.86		0.059	
Br	80	2.68	1.465		
Ag	108	3.08	2.025		
I	127	3.30	0.057		
Th	232	4.0			8.69
		Density	4.180	1.109	9.87

6.5. Discussion

For each patient the values of β vary from section to section by a greater amount than the statistical error estimated from the number of tracks measured. When assessing the significance of these differences it is important to consider other errors inherent in the experiment such as irregularities in the thickness of the section and the inhomogeneous nature of the section. In 6.1 the effect of variations in h/μ in different parts of the section was considered and it was shown that the curve may be wider than the theoretical curve assuming a uniform thickness; as the observed distributions were not appreciably wider than the theoretical curves it was assumed that variations in thickness were not very great. Since the tracks originate in aggregates of thorotrast, the assumption of a thin homogeneous section of infinite area may not be valid. A closer approximation may be obtained by considering the distribution of track lengths from aggregates of thorotrast which approximate to thin cylindrical volumes. This effect is emphasized when the wax is removed prior to coating with emulsion; the tissue shrinks more than the dense aggregates and emulsion actually surrounds the aggregates as illustrated in Figure 4.1. There are therefore more of the long tracks recorded depending on the size of the aggregates. It may be significant for example, that in sections in which the aggregates vary greatly in size (No.4) the fit of the experimental points to the calculated curve is not as good as those in which aggregates are of more uniform size (No.3) (9.2). This difference could also be explained by the fact that the former were the earliest sections measured, whereas the latter were some of the latest, when more experience of coating and developing

techniques as well as of track measuring had been gained. On the whole however, it was considered that the shape of the distribution did correspond to the formula calculated (6.1) with sufficient accuracy to justify this analysis.

Another source of error lies in the analysis of the observed distributions into two curves, and this depends on how sensitive the curve is to small differences in the numbers of tracks in each of the groups. Although it is valid to assume that RdTh should be in equilibrium with its products at the time of measurement, small variations may result from the leaching of some elements into the emulsion. The analysis mainly relies upon fixing the heights of two peaks, the first of which is easily distinguishable but the second can be estimated from the general shape of the distribution of the longer tracks. The accuracy of the analysis could be improved by increasing the number of tracks in the longest group so that the height of this peak could be used, but in order to increase the number of tracks to even 100 would mean doubling the total number of tracks measured, in some cases to 1500 on each plate. As this would take rather a long time, it is important to consider first the effect of errors in the measured value of β on the final estimate of tissue dosage.

The variation in the activity among the different organs in each patient indicates that different amounts of the decay products of thorium have been removed from some of the tissues by a biological or chemical process. For example, some of the soluble products may have been carried away in the blood stream and either excreted or deposited in another organ. Material remaining at the site of injection may also have been transferred

via the blood stream to other organs. As the time interval between the preparation of the sections and the autoradiograph exposures was more than a year, any loss of the short-lived elements would no longer be distinguishable; therefore the loss must be of a long-lived product namely MsThl of half-value period 6.7 years. The finding that this element is lost from organs is consistent with the fact that ThX and traces of a long-lived element which was probably MsThl are present in the blood (2.2). However, it is also possible that the diffusion of MsThl may have taken place from the sections in vitro. This point must be settled before it can be concluded that the results indicate a genuine loss from the organ of the living patient. Experiments designed to determine the losses in vitro are described in the following chapter.

CHAPTER 7

ACTIVITY IN LIVING TISSUE RELATED TO MEASUREMENTS FROM AUTORADIOGRAPHS

7.1. Variations in the Activity of Sections in vitro

The activity of tissues *in vivo* may not be the same as that observed in sections *in vitro* for two reasons, first the natural growth of decay products during the time between taking the autopsy or biopsy sample and exposing the autoradiograph and second the possible loss of decay products from the section *in vitro* either during the preparation of sections or afterwards. It has already been shown (4.2) that loss of elements from tissues during the histological processing is negligible. It remains to be determined how the activity varies subsequently *in vitro* and this was done in an independent experiment using animals.

In this experiment mice were given injections of approximately 0.5 ml of thorotrast of known age (sample B) and sacrificed after one day. The sections were prepared within six hours by a special embedding process and exposed immediately to contact autoradiographs; the exposure times varied from one to 140 hours. Other plates were exposed at various times up to almost a year later. Two series of plates were examined, mouse No.1 in December 1951 (spleen and liver) and mouse No.2 in October 1952 (spleen). The activity of the thorotrast at these times was determined as described in Chapter 5. On each of the autoradiographs 750 tracks were measured and the distribution of track lengths analysed as described in Chapter 6. Two of the distributions which refer to mouse No.1 at times 22 hours and 64 days after are shown in Figure 7.1. The activities in all the sections are

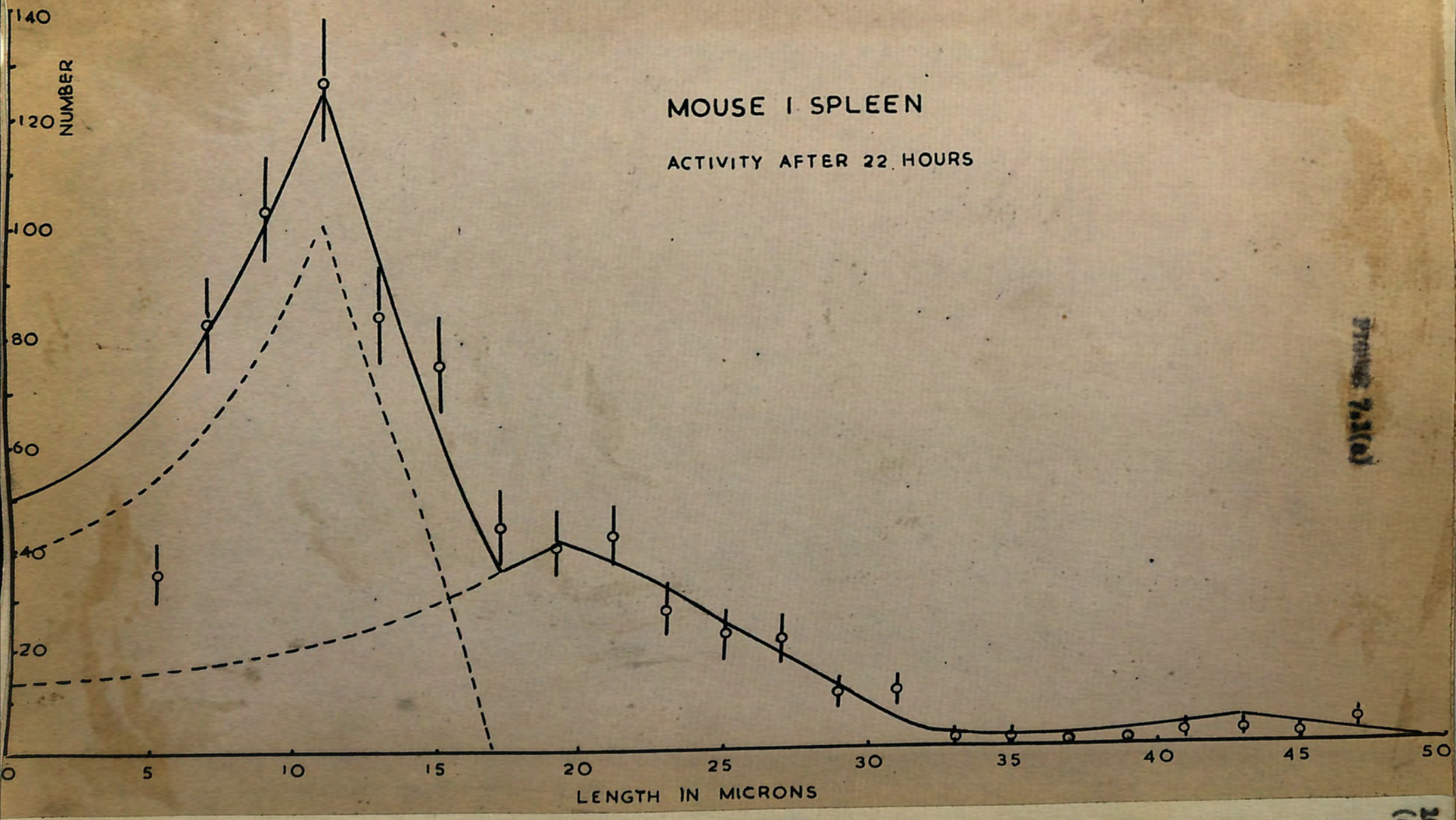
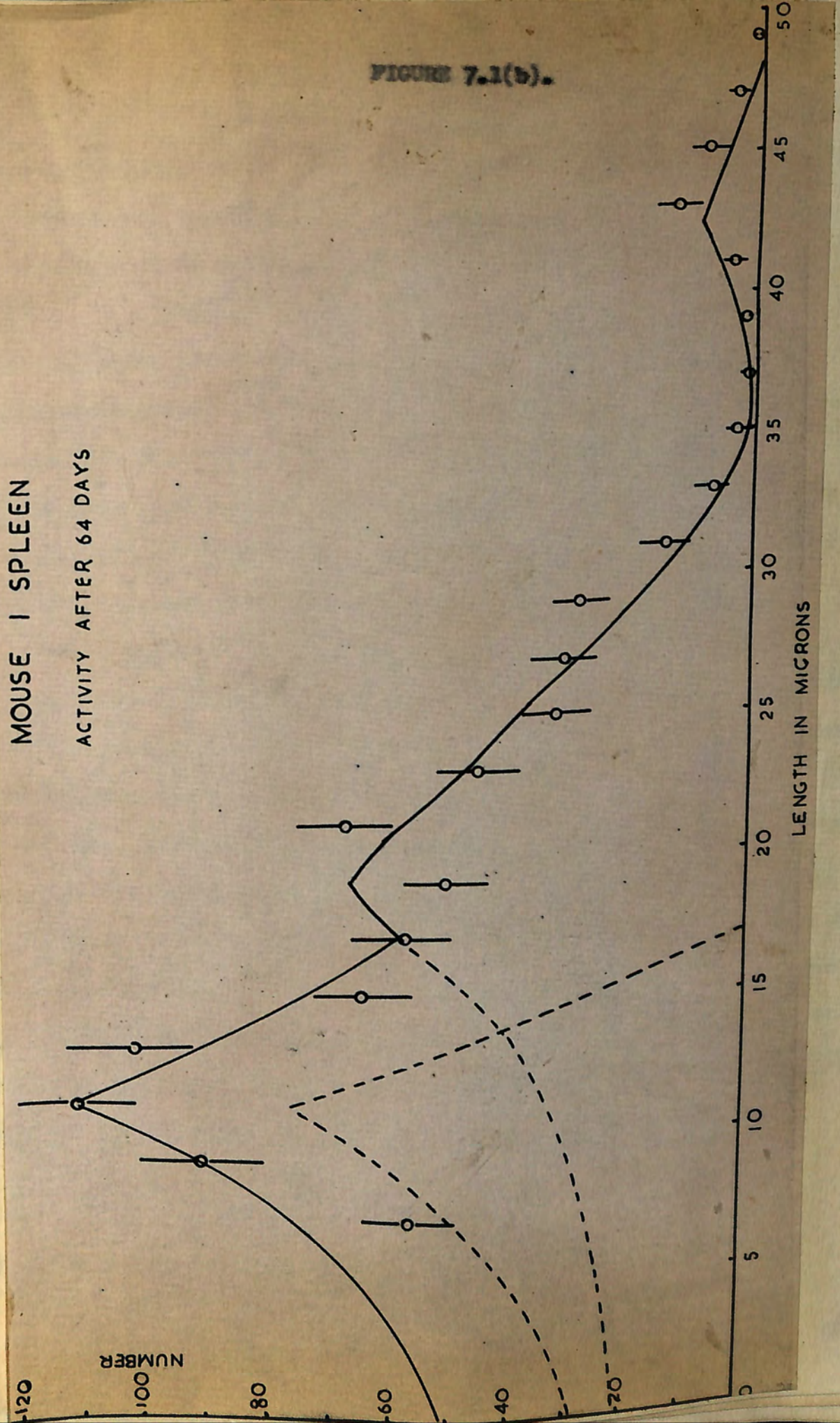

MOUSE I SPLEEN
ACTIVITY AFTER 22 HOURS
NUMBER
140
120
100
80
60
40
20
0
5
10
15
20
25
30
35
40
45
50
LENGTH IN MICRONS

FIGURE 7.1(b).


MOUSE I SPLEEN
ACTIVITY AFTER 64 DAYS
NUMBER
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
50
LENGTH IN MICRONS

given in Table X; t is the time interval between the preparation of the section and the exposure of the autoradiograph and β was calculated from the activity of Th relative to the other groups, $^{A}/_{5D}$ or α, by the relation $\beta = {}^{1}/_{6}(1 + {}^{1}/_{\alpha})$ (6.5). In each series of plates, the value of β, initially lower than the value in the thorotrast at the time of injection, increased rapidly then decreased slowly. The point at 4 hours for the spleen section of mouse No.1 could not be determined very well because with such a short exposure the number of tracks recorded was not sufficient for a reliable estimate. The results can be interpreted by assuming that no decay products escaped from the tissue <u>in vitro</u>.

Let A, B, C etc. be the activities of the various elements and λ_1, λ_2, λ_3 etc. be their decay constants as defined in 5.1. The elements from RdTh onwards were in equilibrium in the thorotrast before it was injected into the animals, so the value of α at that time was $^{A}/_{5D}$. As the time interval between injection and measurement was very short, this also represents the value of α in the thorotrast at the time of sacrificing the animal, assuming no loss of any of the products in the animal. Suppose that the activities at the time of death are given by A_0, B_0, C_0 etc. As ThX will almost immediately come to equilibrium with its products, it may be assumed that ThX but not RdTh are in equilibrium with its products so that

$$\alpha_0 = \frac{A_0}{D_0 + 4R_0} \qquad (1)$$

After time t <u>in vitro</u>, assuming the products grow without loss,

TABLE X. Observed Activity in vitro.

Mouse No.1 December 1951, $\beta_0 = 0.36$

	Spleen	Liver
t	βt	βt
4 hours	0.440 ± .028	
10	0.308 ± .015	
22	0.301 ± .013	
34	0.315 ± .015	0.297 ± .015
46	0.308 ± .015	
70	0.318 ± .015	0.374 ± .020
6½ days	0.356 ± .018	
30	0.487 ± .033	
64	0.437 ± .026	0.445 ± .026
102	0.360 ± .020	0.378 ± .020
259	0.350 ± .018	0.351 ± .018

Mouse No.2 October 1952, $\beta_0 = 0.36$

	Spleen
t	βt
8 hours	0.250 ± .012
23	0.279 ± .012
61	0.282 ± .012
6½ days	0.363 ± .020
28	0.347 ± .018
35½	0.395 ± .023
67	0.409 ± .025
133	0.415 ± .025

$$\alpha_t = \frac{A_t}{D_t + 4E_t} \qquad (2)$$

α_t may be calculated in terms of the activities at death by means of a series of equations for the activity of each element of the series in terms of the initial activity of the parent as given in Chapter 5.

The activity of Th after time t is given by

$$A_t = A_0 ,$$

the activity of MsThl by

$$B_t = A_0(1 - e^{-\lambda_2 t}) + B_0 e^{-\lambda_2 t} ,$$

the activity of RdTh by

$$D_t = A_0\left(1 - \frac{\lambda_4}{\lambda_4-\lambda_2} e^{-\lambda_2 t} - \frac{\lambda_2}{\lambda_2-\lambda_4} e^{-\lambda_4 t}\right) + B_0 \frac{\lambda_4}{\lambda_4-\lambda_2}\left(e^{-\lambda_2 t} - e^{-\lambda_4 t}\right) + D_0 e^{-\lambda_4 t}$$

and the activity of ThX and each of its products by

$$E_t = A_0\left(1 - \frac{\lambda_4}{\lambda_4-\lambda_2} e^{-\lambda_2 t} - \frac{\lambda_2}{\lambda_2-\lambda_4} e^{-\lambda_4 t} + \frac{\lambda_2\lambda_4}{\lambda_5^2} e^{-\lambda_5 t}\right) + B_0\left(\frac{\lambda_4}{\lambda_4-\lambda_2} e^{-\lambda_2 t}\right.$$

$$\left. + \frac{\lambda_4}{\lambda_2-\lambda_4} e^{-\lambda_4 t} + \frac{\lambda_4}{\lambda_5} e^{-\lambda_5 t}\right) + D_0 \frac{\lambda_5}{\lambda_5-\lambda_4}\left(e^{-\lambda_4 t} - e^{-\lambda_5 t}\right) + E_0 e^{-\lambda_5 t}$$

The value of α_t then follows from equation (2) but it is more convenient to calculated $1/\alpha_t$ which is given by $\frac{1}{\alpha_t} = \frac{D_t + 4E_t}{A_t}$

$$= 5 - 5\frac{\lambda_4}{\lambda_4-\lambda_2} e^{-\lambda_2 t} - 5\frac{\lambda_2}{\lambda_2-\lambda_4} e^{-\lambda_4 t} + 4\frac{\lambda_2\lambda_4}{\lambda_5^2} e^{-\lambda_5 t}$$

$$+ \frac{B_0}{A_0}\left[5\frac{\lambda_4}{\lambda_4-\lambda_2}\left(e^{-\lambda_2 t} - e^{-\lambda_4 t}\right) + 4\frac{\lambda_4}{\lambda_5} e^{-\lambda_5 t}\right]$$

$$+ \frac{D_0}{A_0}\left[e^{-\lambda_4 t} + 4\frac{\lambda_5}{\lambda_5-\lambda_4}\left(e^{-\lambda_4 t} - e^{-\lambda_5 t}\right)\right] + 4\frac{E_0}{A_0} e^{-\lambda_5 t} \qquad (3)$$

Equation (3) is simplified by neglecting terms of the order of 10^{-3} when values are given to the constants, thus

$$5 - \frac{1}{\alpha_t} = 5\,\frac{\lambda_4}{\lambda_4-\lambda_2}\left(1 - \frac{B_o}{A_o}\right)e^{-\lambda_2 t} + 5\left(\frac{\lambda_2}{\lambda_2-\lambda_4} + \frac{B_o}{A_o}\,\frac{\lambda_4}{\lambda_4-\lambda_2} - \frac{D_o}{A_o}\right)e^{-\lambda_4 t}$$

$$+ 4\left(\frac{D_o}{A_o} - \frac{E_o}{A_o}\right)e^{-\lambda_5 t} \qquad (4)$$

As a check for the accuracy of equation (4), put $t = o$ and this becomes

$$\frac{1}{\alpha_o} = \frac{D_o}{A_o} + \frac{4E_o}{A_o}$$ as above, equation (1)

For small values of t, say < 20 days, equation (4) becomes

$$5 - \frac{1}{\alpha_t} = 5\left(1 - \frac{D_o}{A_o}\right) + 4\left(\frac{D_o}{A_o} - \frac{E_o}{A_o}\right)e^{-\lambda_5 t} \qquad (5)$$

and for large values of t, that is, > 20 days,

$$5 - \frac{1}{\alpha_t} = 5\,\frac{\lambda_4}{\lambda_4-\lambda_2}\left(1 - \frac{B_o}{A_o}\right)e^{-\lambda_2 t} + 5\left(\frac{\lambda_2}{\lambda_2-\lambda_4} + \frac{B_o}{A_o}\,\frac{\lambda_4}{\lambda_4-\lambda_2} - \frac{D_o}{A_o}\right)e^{-\lambda_4 t} \qquad (6)$$

Equations (5) and (6) were evaluated giving different values to the ratios B_o/A_o, D_o/A_o, E_o/A_o. Consider first equation (6) which is independent of E_o/A_o since by this time the products of RdTh have grown to be in equilibrium. The maximum value of B_o/A_o is given by the value of α in the thorotrast at injection. According to Figure 5.7, this is 0.85 in 1951, two years after preparation. Assuming that there is no MsThl present after preparation of the thorotrast, the activity of MsThl at the time of injection is given by

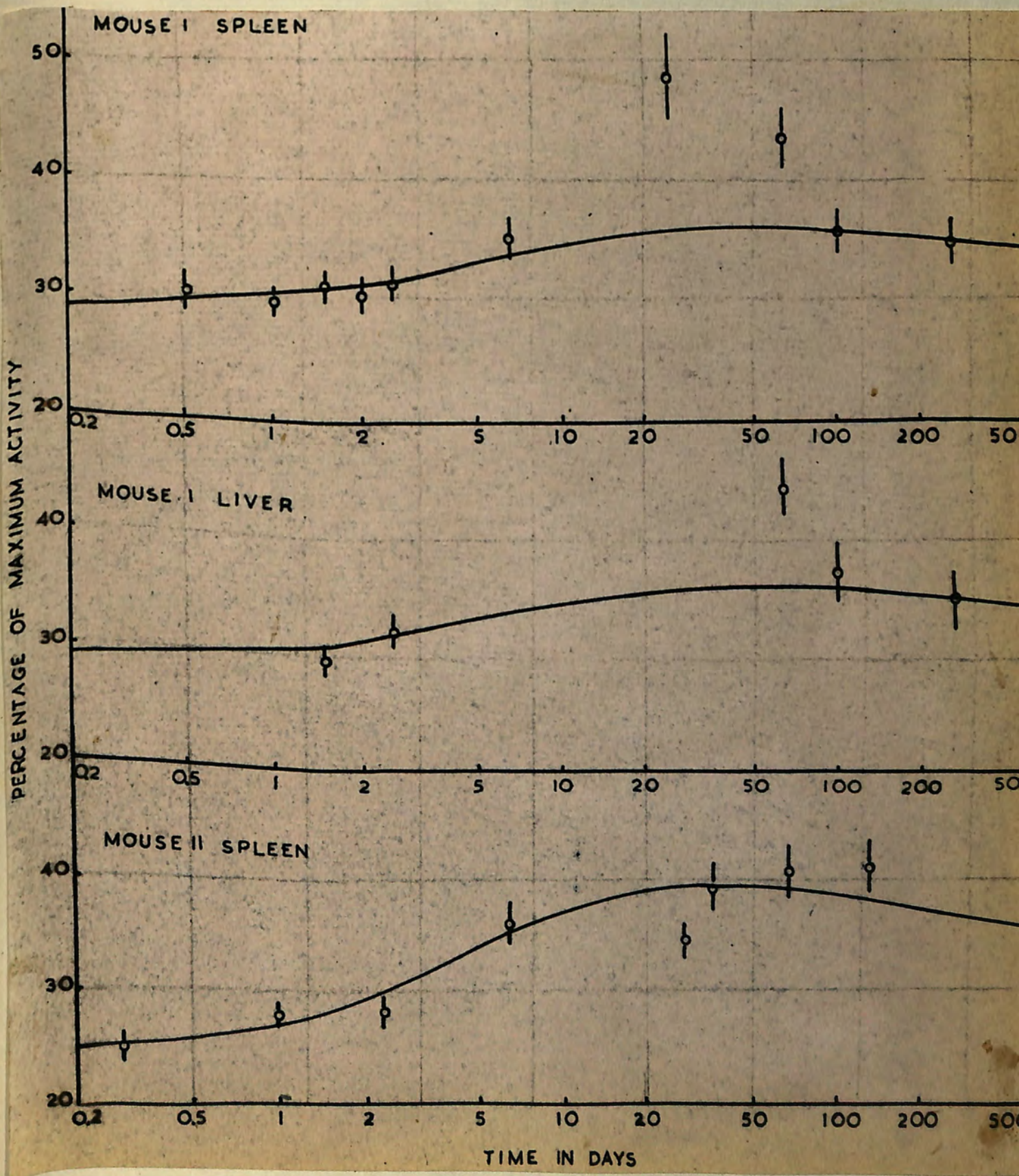
$$B = A(1 - e^{-\lambda_2 t})$$

or $B = 0.2A$ after 2 years

This is the maximum value of B_o in the section but some of the MsThl may have been lost *in vivo*. The possible range of values for B_o is therefore $0 < B_o/A_o < 0.2$. The value of α at injection is 0.85, that is, $A/5D = 0.85$ and the possible values of D_o/A_o are $0 < D_o/A_o < 0.25$, similarly for E_o/A_o. Actually since RdTh is an isotope of Th, there will be no loss of RdTh relative to Th and the ratio D_o/A_o can be assumed to have the constant value of 0.25.

Various values of B_o/A_o and E_o/A_o within the given limits were substituted in (5) and (6) and the corresponding values of β_t, calculated from the relation $\beta_t = 1 - \frac{2 - e^{-\lambda t}}{6}$. The resulting curves were plotted together with the experimental values of β_t using a logarithmic time-scale. In Figure 7.2 the curves for B_o/A_o and E_o/A_o with values in the range 0.05 to 0.10 which gave the best fit to the experimental points are reproduced. With the exception of two high points in the curves for mouse No.1, the experimental points show the same trend as the curves drawn. It was thought that the high points were due to contamination of the plates during exposure and the results for mouse No.2 which applies to the repeat experiment confirm this. In the latter case, the best fit was obtained giving a value of 0.28 for D_o/A_o. This is slightly higher than the value used for the earlier case of mouse No.1, but is still within the limits for the estimate of the age-factor of the thorotrast. It was concluded from these results that there was a loss of MsThl and ThX from the organs *in vivo*, there was no loss of any elements from the section *in vitro* and the activity increased as elements

FIGURE 7.2. Variation of total activity with time.



which had been removed *in vivo* were built up again.

The loss of ThX relative to RdTh could not be detected in the distribution of track lengths because of the difficulty in separating the 24 micron group of alpha-particles from RdTh from the 26 micron group from ThX. As ThX grew again the total number of tracks in the group of products increased in relation to the number in the Th group and the increase was easily detectable.

7.2. Calculation of the Retention Factor in Living Tissues

Since it has been shown that some of the decay products, MsThl and ThX, escape from living organs but that after the tissue is removed from the organ the activity grows without further loss, it is possible to deduce the proportions of the various elements present *in vivo* by comparing the activities in the sections with that in the thorotrast at the same time and allowing for the time interval between taking the sample and measuring the activity. For this purpose it is convenient to define the "retention-factor" as a measure of the proportion of the total activity which is retained in the organ. If β is the activity in the section *in vitro*, β_v *in vivo* and β_0 is the activity in the thorotrast at that time, then the retention-factor $\gamma = \beta/\beta_0$ *in vitro* and $\gamma_v = \beta v/\beta_0$ *in vivo*. The values of β in the thorotrast and in the tissue *in vitro* are related to the corresponding measured values of α thus $\beta_0 = {}^1/6(1+{}^1/\alpha_0)$, $\beta = {}^1/6(1+{}^1/\alpha)$. Since MsThl and ThX are isotopes they behave similarly in the organ and the fraction of ThX escaping will be the same relative to RdTh as that of MsThl relative to Th. Thus by observing the activity of RdTh relative to Th the

loss of ThX from the organ may be estimated. The next two products of ThX, Tn and ThA have very short half-value periods and would not have much time to escape from the organ. We may therefore assume that the ThX left in the organ is in equilibrium with its products. This may not be a valid assumption in the case of ThB which has a half-value period of 10.6 hours and has more time to escape. The activities of the various elements relative to Th are given in the following schemes.

	In Thorotrast	In Vitro	In Vivo
Th	1	1	1
RdTh	$\frac{1}{5\alpha_0}$	$\frac{1}{5\alpha}$	$\frac{1}{5\alpha}$
ThX	$\frac{1}{5\alpha_0}$	$\frac{1}{5\alpha}$	$\frac{1}{5\alpha}\cdot\frac{\alpha_0}{\alpha}$
Other Elements	$\frac{1}{5\alpha_0}$	$\frac{1}{5\alpha}$	$\frac{1}{5\alpha}\cdot\frac{\alpha_0}{\alpha}$

Expressing β in terms of the activities of the various elements, we have

$$\beta = \frac{1}{6}\left(1 + \frac{1}{\alpha}\right)$$

$$\beta_0 = \frac{1}{6}\left(1 + \frac{1}{\alpha_0}\right)$$

$$\beta_v = \frac{1}{6}\left(1 + \frac{1}{5\alpha} + \frac{1}{5\alpha}\cdot\frac{\alpha_0}{\alpha}\right)$$

Combining these equations with the equations

$$\gamma = \beta/\beta_0 \text{ and } \gamma_v = \beta_v/\beta_0,$$

we obtain an expression for the retention-factor in vivo in terms of the retention-factor observed in vitro and the age of the thorotrast.

$$\text{Thus } \gamma_v = \gamma - \frac{1}{5}(1-\gamma)\frac{6\beta_0\gamma - 1}{6\beta_0 - 1}$$

Using this equation the retention-factors in vivo of the various sections were calculated. Those for patient No.4 are tabulated in Table VIII. The thorotrast used for this injection was believed to have

been German thorotrast (sample A) which had an age-factor of 0.78 in 1951 when the autoradiograph was prepared. The highest activity estimated in the autoradiographs was 0.76 in the tonsil section and this corresponds to an age of 15 years in 1951. Since the injection was given in 1938 the thorotrast must have been at least 13 years old in 1951 or at leasttwo years old when the injection was made. An age-factor of 0.78 in 1951 actually corresponds to an age of three years when the injection was made. The value of beta in 1949 when the splenectomy and biopsy were performed was 0.74 and in 1947 when the tonsilectomy was performed it was 0.68. The results for all the other patients are given in Column 12, Table XX. When the age of the thorotrast at injection was unknown, the value of β_o was estimated from the highest value observed in the sections and the time interval between the injection and measurement. For example, in the case of patient No.5, the highest value of β noted in the sections was 0.70 in the spleen; this corresponds to an age of 13 years in 1953 but as the injection was given 14 years before this the age-factor must be at least 0.74 in 1953 and 0.70 *in vivo* in 1952. For patient No.3 the age-factor was calculated from the highest value of β observed, that is, 0.49 in 1953; the age-factor in 1951 *in vivo* would then have a value between 0.39 and 0.59 from Figure 5.2. In the other two cases, Nos.1 and 2, samples of the thorotrast were available for analysis and the content in the section could be compared with the age-factor directly.

The results for all sections show that from 56 to 100% of the products are retained in the organ *in vivo*. In the spleen the retention-factors were found to be 0.83 and 0.96 after 10 and 12 years, 0.68 after 14 days and

1.00 after 12 to 20 hours. As the retention-factor is a measure of the loss of MsThl from the organ it will vary with the amount of MsThl present in the thorotrast. With new samples as in the 12 and 20 hour cases (Nos.1 and 2), the MsThl content of the thorotrast was very low, only 0.02 and 0.04 times the activity of Th, so that loss of MsThl made hardly any difference to the total activity in the organ. With older samples as in the 10 and 12 year cases the MsThl content had grown to about 0.7 times the activity of Th, so loss of this element had a marked effect on the retention factor. In the intermediate case after 14 days the MsThl content could not have been more than 0.4 times that of Th as a maximum. The loss of MsThl will also depend on the size of the aggregates as less diffusion will be possible when the elements are trapped in large aggregates. Since the aggregates grow with time, the loss will be greater soon after injection than after several years (9.2) as indicated by the value of 0.68 after 14 days compared with 0.83 and 0.96 at 10 and 12 years. Comparing the various sections of patient No.5, it is seen that here also the retention factor decreased as the activity and hence the size of the aggregates decreased.

The values in the liver sections are slightly different from those in the spleen, 1.0 after 12 and 20 hours, 0.91 and 0.59 after 10 and 12 years and 1.0 after 14 days. It is difficult to assess the errors in these values when some were calculated without a knowledge of the age-factor of the thorotrast used. In patient No.3 the highest estimated activity in the liver was used, so that the retention factor in this organ was by definition 1.0, whereas it may actually have been lower. Although in the 12 and 20 hour cases, the MsThl content of the thorotrast was so low that no

appreciable loss of activity was apparent, there was probably an appreciable loss of ThX which was no longer detectable when the autoradiographs were prepared some time after autopsy; the retention-factors may actually have been less than 1.0.

Some general confirmation of the order of magnitude of the retention-factors was obtained by Rundo (1955) who, by measuring the activity of an excised liver at various periods of time after excision, showed that 80% of the decay products were retained in this organ. He assumed a lower value, 70% in the spleen mainly based on our measurements for patient No.4. He also assumed that the value would be similar in the bone marrow; this is so in one of our cases (No.3) but not in another (No.5).

7.3. Calculation of the Effective Energy of alpha-particles

From the composition of the activity in the various organs in vivo, the effective energy of the seven alpha-particle groups may be calculated. The effective energy is defined as

$$E = \frac{\Sigma N_i E_i}{\Sigma N_i}$$

where E_i is the energy of the alpha-particles of the ith element and N_i is its relative activity. The values of N_i are obtained from the scheme given in 7.2 and are tabulated for one section, the liver of patient No.4, in Table XI together with the other calculations of the effective energy. Similar calculations were carried out for all the sections and the results are given in Table XI. It is seen that although the retention-factors in the different cases vary from 50 to 100%, the effective energy varies less, only within the range 4.86 to 5.89 MeV.

TABLE XI. Calculation of Effective Energy of Alpha-particles. Patient No.4 - liver.

Element	Relative Activity N_i	Energy of alpha-particle E_i	$N_i E_i$
Th	1	3.98 MeV	3.98 MeV
RdTh	0.69	5.40	3.72
ThX	0.64	5.68	6.63
Tn	0.64	6.28	4.02
ThA	0.64	6.77	4.34
ThC	0.22	6.05	1.33
ThC'	0.42	8.78	3.68
Totals	4.25		24.70

$$\frac{\Sigma N_i E_i}{\Sigma N_i} = 5.81 \text{ MeV}$$

CHAPTER 8

MEASUREMENTS OF THE TOTAL RADIOACTIVE CONTENT OF SECTIONS AND ORGANS

8.1. Total Activity in Tissue Sections

The concentration of thorotrast in the sections was simply measured from the activity, N, or the total number of tracks recorded in the autoradiograph per unit volume of tissue per unit time. The tracks were counted in a measured area of tissue as described in Chapter 4. The sections were nominally 3 microns thick but as there were variations in different parts of the section, the thickness of each section was measured at a number of positions. Because of the different shrinkage of thorotrast and tissue, the thickness of the thorotrast deposits exceeded the thickness of the tissue and as the thorotrast probably did not shrink to any appreciable extent, its thickness should be the more reliable estimate of the thickness of sections when cut. The deposits varied considerably in thickness, some being as much as 10 microns in a 3 micron section. It is thought possible that in sectioning the embedded tissue, the microtome knife does not cut cleanly through the dense material leading to irregularities in thickness. Some of the measurements and the calculated activity of different sections from patient No.4 are given in Table XII. One of the sections from the rabbit is also given for comparison.

Because of the loss of products *in vivo* the total activity *in vivo* is actually lower than that indicated by the number of tracks recorded *in vitro*. The relation between the total activity *in vivo* and *in vitro* was derived by comparing the relative activities of the different elements

TABLE XII. Activity of Tissue and Thorotrast

Subject	Section	Exposure time of ARG. days	R	Area of Tissue mm^2	Tracks counted	Thickness microns	Activity in vitro per mm^2 per min.	Corrected activity in vivo per mm^2 per min.	Area of Thorotrast mm^2	Volume of Thorotrast per mm^3 per min.	Activity of Thorotrast per mm^3 per min.	Activity of Th per mm^3 per min.
Patient No.4	Spleen medulla	2	0.45	0.261	564	5.8	260	203	0.100	0.69	375	116
	Spleen	4	0.33	0.73	289	4.9	28.2	25.4	0.048	0.066	427	106
	Hilum of spleen	5	0.59	1.59	689	9.7	12.3	8.99	0.059	0.037	334	124
	Lymphnode	12	0.45	4.33	322	6.2	1.4	1.13	0.017	0.0041	354	110
	Tonsil	25	0.27	21.2	982	5.3	0.49	0.49	0.021	0.0010	493	106
Rabbit	Spleen	4	0.46	1.00	1800	10.0	62.5	40.1	0.162	0.162	386	122

(7.2). Thus the total activity is <u>in vitro</u> $1 + {}^{1}/_{\alpha}$ and <u>in vivo</u>, $1 + {}^{1}/_{5\alpha} + 4\alpha_0/5\alpha^2$ and the ratio

$$\frac{\text{Total activity in vivo}}{\text{Total activity in vitro}} = \frac{1 + {}^{1}/_{5\alpha} + {}^{4\alpha_0}/_{5\alpha^2}}{1 + {}^{1}/_{\alpha}}$$

From the relations in 7.2 this ratio is the same as the ratio of the retention-factors, that is $\frac{\gamma_v}{\gamma}$ and

$$\frac{\text{Activity in vivo}}{\text{Activity in vitro}} = 1 - {}^{4}/_{5}\,\frac{1-\alpha}{\gamma}\,\frac{6\beta_0 - 1}{6\beta_0 - 1}$$

This ratio was found to have values from 0.70 to 1.0 and the activities <u>in vivo</u> are also given in Table XII.

The results for all the sections from the five different patients and from the rabbit are given in Columns 15 to 17 in Table XI. The sections are arranged in the order spleen, liver, bone marrow, then other tissues - that is, in all cases except one, in order of decreasing activity. Many different sections were examined in the rabbit and the activities of the tissues following bone marrow were found to be very low in comparison. In one case, patient No.5, a section of bone was examined but no activity could be detected. In order to compare the activity in the different organs in each patient, the activity relative to the spleen of each organ has been tabulated (Column 18). It is seen that the liver and bone marrow contain almost as much activity as the spleen in three of the patients Nos.1,2,3) and the spleen contains considerably more in the other two patients, (Nos.4 and 5) and in the rabbit.

In order to compare these results with those of previous investigations and to test the hypothesis that the spleen takes up more material relative

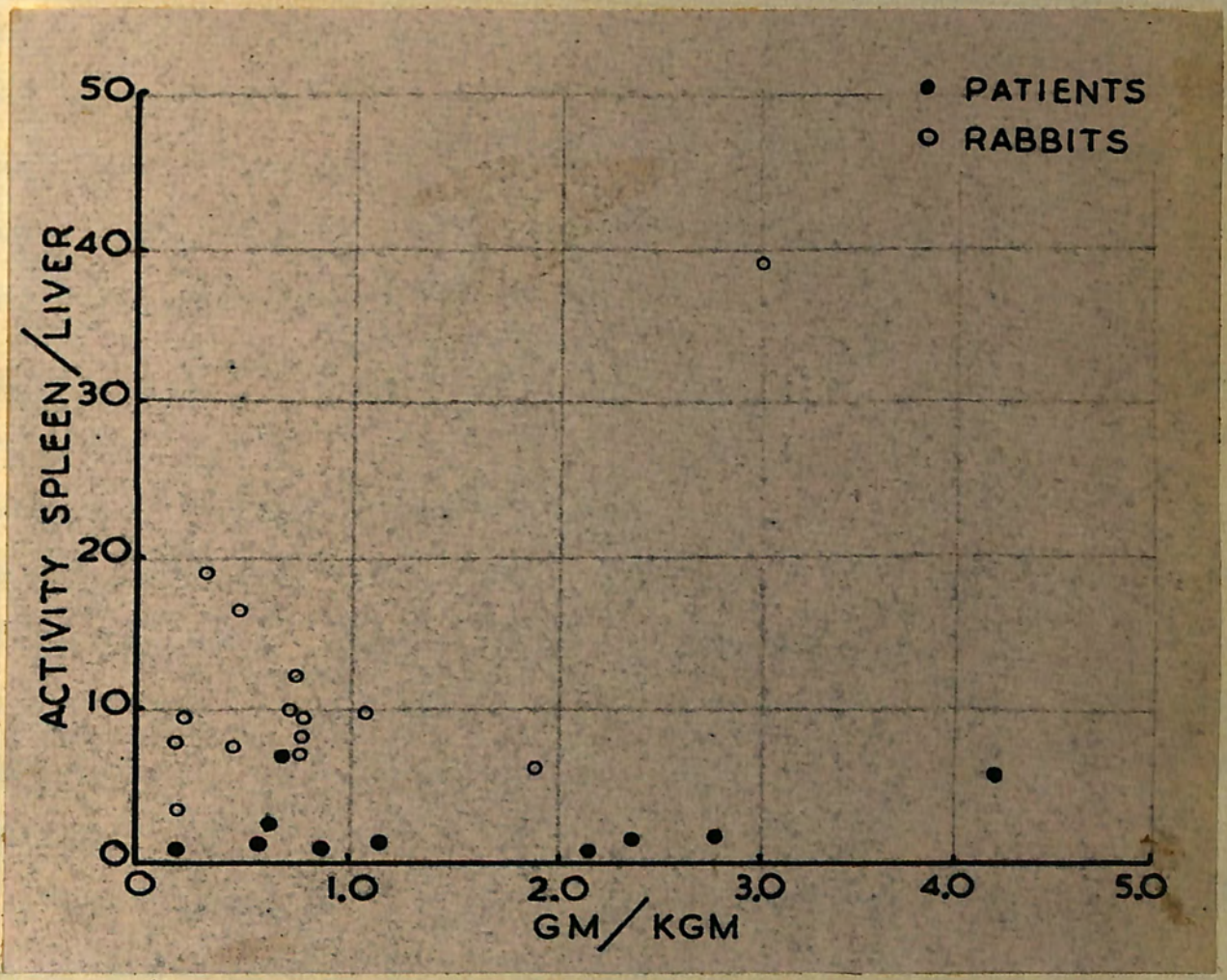
to the liver for large doses, the present results have been plotted together with those of Liepert (Table II) in Figure 8.1. Rabbit results, including those of Liepert and Johansen (2.5) have also been plotted by allowing for the relative masses of rabbits and patients, 2 and 70 Kgm respectively, and expressing the dose in gm per Kgm. For patients there is a tendency for the ratio to increase above unity for the higher doses. For rabbits the ratio, always higher than in patients, may also increase, but variations between individual animals are considerable. As well as variations in the amount taken up in these organs with different doses, these figures will be affected by differences in the relative masses of the two organs. As no figures for the masses of the organs were available in many of the subjects it was not possible to study this effect further.

Another factor causing variations in the ratio of the activities in spleen and liver is the decreasing size of the spleen in thorotrast patients. This would account for the higher ratio in the long-standing cases (Nos.3 and 4) compared with the recent cases (Nos.1, 2 and 5).

8.2. Total Radioactive Content of Organs

The measurements of the activity per unit volume of tissue are based on the activity of minute volumes of tissue compared with the total volume of the organs from which the sections are cut. It is therefore necessary to determine whether the values obtained are representative of the whole organ or whether this varies from section to section in different parts of the organ. A measurement of the total activity of the whole organ would also be of value. These measurements were possible for two of the

FIGURE 8.1. Variation in the ratio of the activity of spleen to liver for different amounts of thorotrast injected.



patients, those from the London Hospitals (Nos.1 and 2). In each case the whole of the organs liver and spleen were obtained at autopsi, these were weighed and several blocks were taken from different parts of the organs for the preparation of the sections. The specific activity, that is the activity per gram of tissue was calculated from the numbers of tracks and the activity of the whole organ was then calculated. An independent measurement of the total activity was obtained using counters and the two values were compared. The results for the two sets of measurements are summarised below and in Tables XIII and XIV.

Patient No.2. This patient received 20 ml of thorotrast (sample C) 20 hours before death. Three blocks were cut from the spleen and four from the liver; from each block 4 micron sections were cut and coated auto-radiographs prepared from them. From track counts on one section from each block the specific activity of the tissue was calculated (Table XIII). In the spleen the mean activity was 4.35 per mm^3 per minute and the maximum variation from this 10%; in the liver, the mean activity was 3.22 per mm^3 per minute and the maximum variation about 5%.

The figures given in Table XIII refer to the activity of sections, that is, of tissue after treatment for sectioning; the equivalent volume of fresh tissue must therefore be found. Unfortunately in this case, it was not possible to make any measurements until after the tissue had been kept in formalin for some time. Blocks were cut from fixed tissue and taken through the various stages to the final embedding in paraffin. The dimensions of the blocks were determined at each stage and it was found that the greatest decrease occurred at the embedding stage. The total

TABLE XIII. Specific Activity in Tissue Sections
(Disintegrations per mm^2 per minute)

	Spleen	Liver
Patient No.1		
Block 1 Section 1	1.67	2.00
2	2.32	1.86
Block 2 Section 1		2.00
2		2.18
Mean	2.00	2.01
Patient No.2		
Block 1	3.85	3.10
2	4.65	3.30
3	4.50	3.40
4		3.10
Mean	4.33	3.22

shrinkage in the linear dimension was 20%, that is, 60% in the volume. This result was compared with that of Berenbaum and Birch (1.5) who found the ratio of unfixed tissue to the equivalent volume in the section to be 1.67 a result which agrees very well. Using their value which would allow for the shrinkage in volume in the fixative, the specific activity of fresh tissue and the activity of the whole of the organs were calculated (Table XIV).

The activity of the thorotrast used in this injection, sample C (5.5) was measured by mixing a known volume with emulsion and counting the number of tracks per unit volume in a given exposure time. In this way it was shown that there were 1.48×10^5 alpha-particle disintegrations per ml per minute. Since 1 ml of thorotrast contains 190 mg of thorium the activity measured corresponds to 780 disintegrations per mg of thorium per minute. Th separated from its decay products has an activity of 240 alpha-disintegrations per mg per minute. This remains constant with time, hence the ratio of tracks from Th to that of all the products is 240/540 = 0.44. This figure was confirmed by comparing it with that measured previously for sample C (5.5) namely 0.38 ± 0.07. The two values agree within the accuracy of the experiment better than expected considering the difficulty in measuring and pouring accurately a volume of melted emulsion. The activity of the liver and spleen were thus equivalent to 16.5 and 0.79 ml of thorotrast.

These results were checked in an independent experiment by digesting small pieces of fixed tissue chemically and determining the radioactivity of the liquid obtained using liquid counters. The results are summarised

in Table XIV. The figures for two different determinations on the same organ are quoted separately to show the amount of variation between different samples of tissue. The liver was re-weighed after fixing in formalin and it was found that its mass was now 1120 gm. Assuming that the mass of the spleen decreases proportionally after fixation, the mass of fixed spleen is 40.7 gm. The counts from the whole organs are then as shown.

The equivalent volume of thorotrast was obtained by diluting a measured volume with water and determining the activity in the liquid counter. The liver contained 13.8 ml of thorotrast and the spleen 0.53 ml, a slightly lower result than was obtained by means of track counts.

Patient No.1. Approximately 7 ml of thorotrast (sample D) were injected 12 hours before death. Two blocks were prepared from the liver and one from the spleen. The sections were 4 microns thick and two sections from each of these blocks were examined. The specific activities of the different sections are given in Table XIII, and the total activities of the whole of the organs in Table XIV. For the sample of thorotrast used, sample D, $\beta = 0.725$ (5.5); the activity of one ml follows from this value. The content in the liver was equivalent to 8.1 ml and in the spleen to 0.70 ml.

In this case the results were checked by taking small samples of fresh tissue, and counting the gamma-activity using a scintillation counter. This was compared with a sample of thorotrast diluted with water, the activity of which was determined using a similar arrangement. The whole liver was found to contain 5.3 ml of thorotrast and the spleen 0.58 ml.

TABLE XIV. Activity of Organs by Track and Counter Measurements.

Thorotrast Injection	Patient No.1		Patient No.2	
	7 ml sample D		20 ml sample C	
	Spleen	Liver	Spleen	Liver
Mass of organs (gm)	118	1361	45	1238
Autoradiograph Measurements				
Mean specific activity of sections (per mm^3 per min.)	2.00	2.01	4.33	3.22
Specific activity in fresh tissue	1.20	1.21	2.59	1.93
Activity of whole organ	1.42×10^5	16.4×10^5	1.17×10^5	24.5×10^5
Activity of thorotrast (per ml per min)	2.02×10^5		1.48×10^5	
Thorotrast Content of organ (ml)	0.70	8.1	0.79	16.5
Counter Measurements				
Mass of tissue sample (gm)	9.71	10.38	1. 1.71 2. 1.60	2.90 4.08
Activity of tissue (cpm per gm)	10.0	7.9	1. 50.3 2. 54.0	48.9 49.4
Mean			52.2	49.1
Activity of thorotrast (cpm per ml)	*2020		*3975	
Thorotrast content of organ (ml)	0.58	5.1	0.53	13.8

*These figures were obtained by using different counters.

Comparing the results from the two patients it is seen that the determination of the activity by means of track counts results in both cases in a higher figure than the determination using counters. There are two possible reasons for this discrepancy. Firstly, the shrinkage of the tissue may have been even greater than the value used, that is, 2.0 and 2.4 in the two cases. Secondly, the counter measurements do not take into account loss of decay products in vivo. If decay products were lost in vivo, the gamma-count from ThC" in the tissue would be reduced compared with that in the thorotrast. The apparent volume of thorotrast in the organ would then be less than the actual volume. The retention factors in the liver and spleen of the two patients were shown to be 1.0 (7.2), that is, the activity in vivo was the same as that observed in vitro, but it was pointed out that as the autoradiographs were exposed some time after the autopsy, the loss of ThX could not be measured. The counter measurements were made much sooner after autopsy, within a day or two, when the loss of ThX should still have been detectable. The results may therefore indicate that there was actually a loss of ThX in vivo.

The results in Table XIII show that there is not much variation between different parts of the organs but the spleen shows slightly more variation than the liver. Measurements made on a single section may therefore be taken to be representative of the organ without serious error. It is possible, however, that the distribution will be less uniform after longer periods of time when the thorotrast has collected in large aggregates. The ratios of the specific activities in the organs (spleen/liver) calculated from both the track and the counter measurements, 1.00 and 1.26

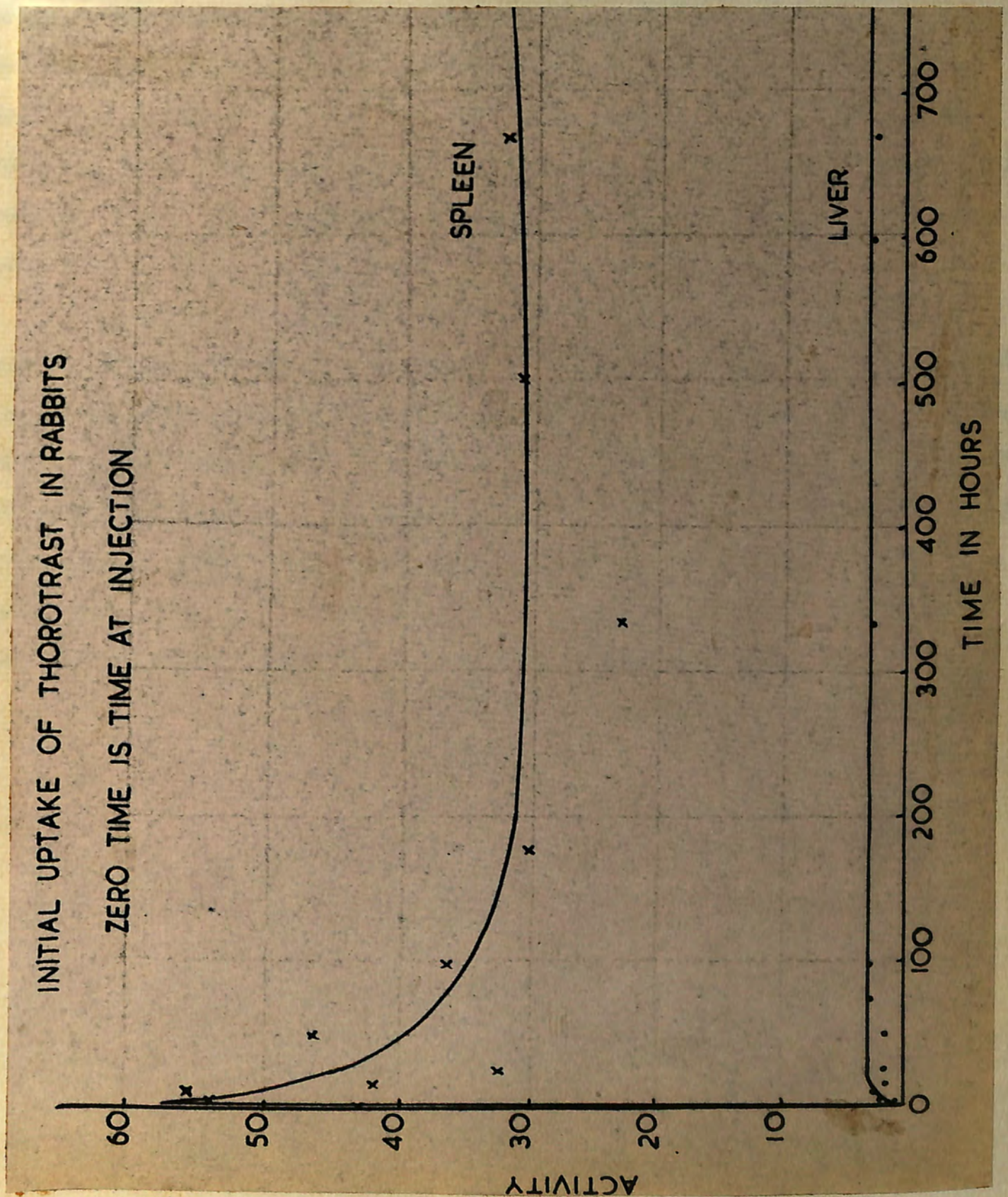
in patient No.1 and 1.34 and 1.06 in patient No.2, are in agreement with the results of Leipert (Table II).

From the figures in Table XIV, the fraction of the total volume of thorotrast injected which was found in the liver and spleen could be calculated. Patient No.1 received approximately 7 ml of thorotrast and a total of 8.8 and 5.9 ml was found in the two organs by track and counter measurements respectively. Allowing that the counter estimate may have been low, it appears that this patient may actually have received a dose of at least 9 ml. It is known that 7 ml was an approximate estimate involving a correction for the dead-space in the 10 ml syringe used to inject the thorotrast. Patient No.2 received 20 ml and the volumes estimated to have been retained in the liver and spleen after 20 hours were 17.3 ml and 14.3 ml by track and counter measurements, representing 89% and 72% of the total injection. These figures are in agreement with the results of Leipert (Table II) although his shortest time was two days and his estimates for different patients varied considerably.

8.3. Rate of Uptake of Thorotrast

In Copenhagen, rabbits were injected with thorotrast and killed at various periods of time afterwards. The organs were analysed for thorium chemically and it was shown that the uptake was complete within a few hours (2.5). Sections from these organs have also been examined by means of autoradiographs and the specific activity estimated from the number of tracks recorded per unit area of section. The results are plotted in Figure 8.2 where the activity is given in average number of tracks per

FIGURE 8.2.


INITIAL UPTAKE OF THOROTRAST IN RABBITS
ZERO TIME IS TIME AT INJECTION
SPLEEN
LIVER
ACTIVITY
TIME IN HOURS
60
50
40
30
20
10
0
100
200
300
400
500
600
700

field of view. The activity in the liver had already reached a steady value after about 24 hours. That in the spleen increased rapidly at first, then decreased to an approximately steady value after about 200 hours. The initial steep rise was interpreted as an initial uptake in the spleen followed by a redistribution to the liver and other organs, principally the bone marrow. The specific activity in the spleen reached a steady value twelve times that in the liver.

The results obtained for patients (8.1, 8.2) show that the rate of uptake may be comparable, for example, in one case 72 to 89% of the amount injected had already been taken up in the liver and spleen after 20 hours. There was no activity in the bone marrow after 12 hours (No.1) but there was activity in the bone marrow comparable with that in the liver after 14 days (No.3). However, this may not be a valid comparison as, in this case, another factor, the different doses (7 ml and 40 ml) will also influence the proportions finally deposited in the different organs.

CHAPTER 9

CALCULATION OF TISSUE DOSAGE.

9.1. Definition of Tissue Dosage

Tissue dosage is a measure of the energy absorbed from any ionising particles by unit mass of tissue in unit time. The unit of absorbed dose is the rad which is defined as 100 ergs per gram (International Commission on Radiological Units, 1954). The rad has replaced the rep as a unit of absorbed dose, the rep being equivalent to 93 ergs per gram.

For alpha-particles from thorotrast the energy absorbed is given by the product of the activity per unit volume of tissue N, and the effective energy of the alpha-particles, $\bar{E}$. Since the thorotrast is concentrated in aggregates, some of the alpha-particle energy is absorbed in the aggregate and only a fraction $\bar{F}$ is dissipated in tissue. The tissue dosage, D, is then given by $N \bar{E} \bar{F}$.

The activity of the tissue, N, was determined from the activity of sections (8.1). The effective energy of the alpha-particles, $\bar{E}$, was calculated from the relative activities of the seven alpha-particle groups (7.3). The fraction of the total energy of the alpha-particles from spherical aggregates of thorotrast which is dissipated in tissue is calculated in the following paragraph.

9.2. Self-absorption in Aggregates

Mathematical derivation. The shape of the aggregates is irregular but may be considered spherical as a first approximation. Suppose the aggregate is of radius r and emits a single group of alpha-particles of range R in

in tissue, N being the number of alpha-particles emitted from unit volume per unit time. The aggregate is shown diagrammatically in Figure 9.1. The number of particles from a point P at a distance x from the centre of the sphere, which are of range L to L + dL inside the aggregate are contained in the solid angle $d\theta$, given by $\frac{1}{2}\sin\theta d\theta$. The number from the elementary volume of the sphere of thickness dx is given by

$$\delta N = \tfrac{1}{2}\sin\theta\, d\theta . 4\pi x^2\, dx\, N$$

Changing the variable from θ to L, by means of the relation $r^2 = L^2 + x^2 + 2Lx\cos\theta$ this becomes

$$\delta N = \frac{\pi N}{L^2}\,(L^2 + r^2 - x^2)\, x\, dx\, dL$$

Therefore, the number of length L to L + dL for the whole sphere is given by

$$N = \int_{r-L}^{r} \frac{\pi N}{L^2}\, dL\, (L^2 + r^2 - x^2)\, x\, dx$$

$$= \pi N\,(r^2 - \tfrac{1}{2}L^2)\, dL$$

As a check for the accuracy of this equation, consider the case of $r < R/2$, when all the particles escape from the aggregate. Integrating with respect to L where L has limits 0-2r

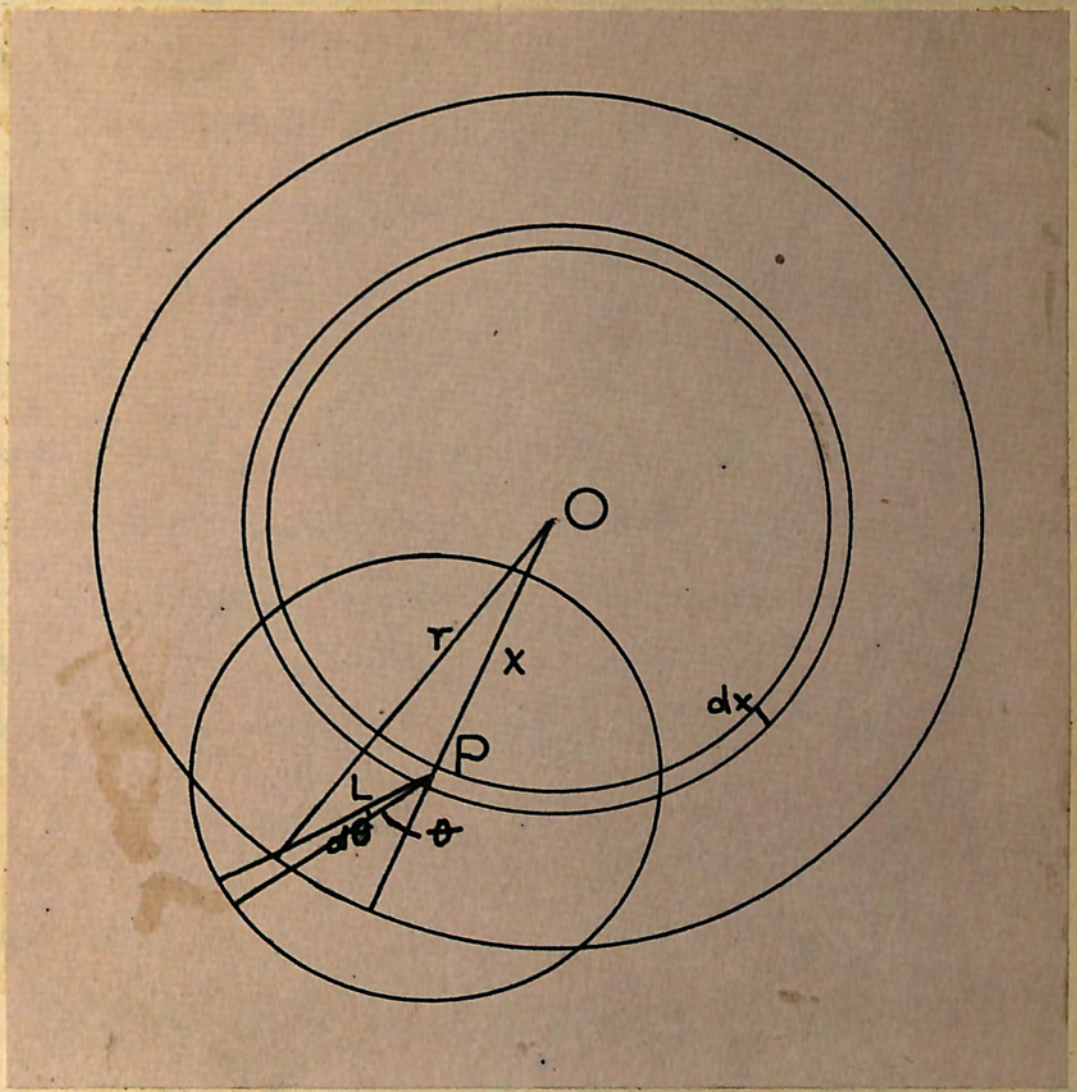
$$N = \int_0^{2r} \pi N\,(r^2 - \tfrac{1}{2}L^2)\, dL$$

$$= 4/3\ \pi\ N\ r^3$$ or the product of N and the volume of the sphere as required. Considering now the number of length l outside the sphere, that is of length l in tissue where $L = \mu(R - l)$ we have

$$N(l) = \pi N\ \mu\left[\, r^2 - \tfrac{1}{4}(R-l)^2 \,\right] dl \qquad (1)$$

Here μ is the ratio of the stopping power of thorotrast to that of tissue.

FIGURE 9.1.



The energy dissipated in tissue by a particle of range l is given by an expression of the form $l = kE^m$ where k and m are constants. The total energy from all particles which is dissipated in tissue, N(E)E, is obtained by substituting this in equation (1) and integrating over all values of E, that is,

$$N(E)E = \pi N \mu k m \int \left[r^2 - \tfrac{1}{4} (R - kE^m)^2 \right] E^m \, dE$$

The limits for E vary according to r, thus

$$\text{for } \mu R > 2r, \quad E_R (1 - 2r/\mu R)^{1/m} < E < E_R$$

and for $\mu R < 2r$, $0 < E < E_R$,

since all the particles from small aggregates dissipate some of their energy in tissue but some of the particles from large aggregates lose all their energy within the aggregate. Expressing the energy dissipated in tissue as a fraction of the total energy of all alpha-particles from the sphere, namely $4/3\ r^3 N E_R$, we have for $\mu R < 2r$,

$$f = 3/4 \frac{m}{m+1} \left[\frac{\mu R}{r} - \tfrac{1}{2} \left(\frac{\mu R}{r}\right)^3 \frac{m^2}{(2m+1)(3m+1)} \right]$$

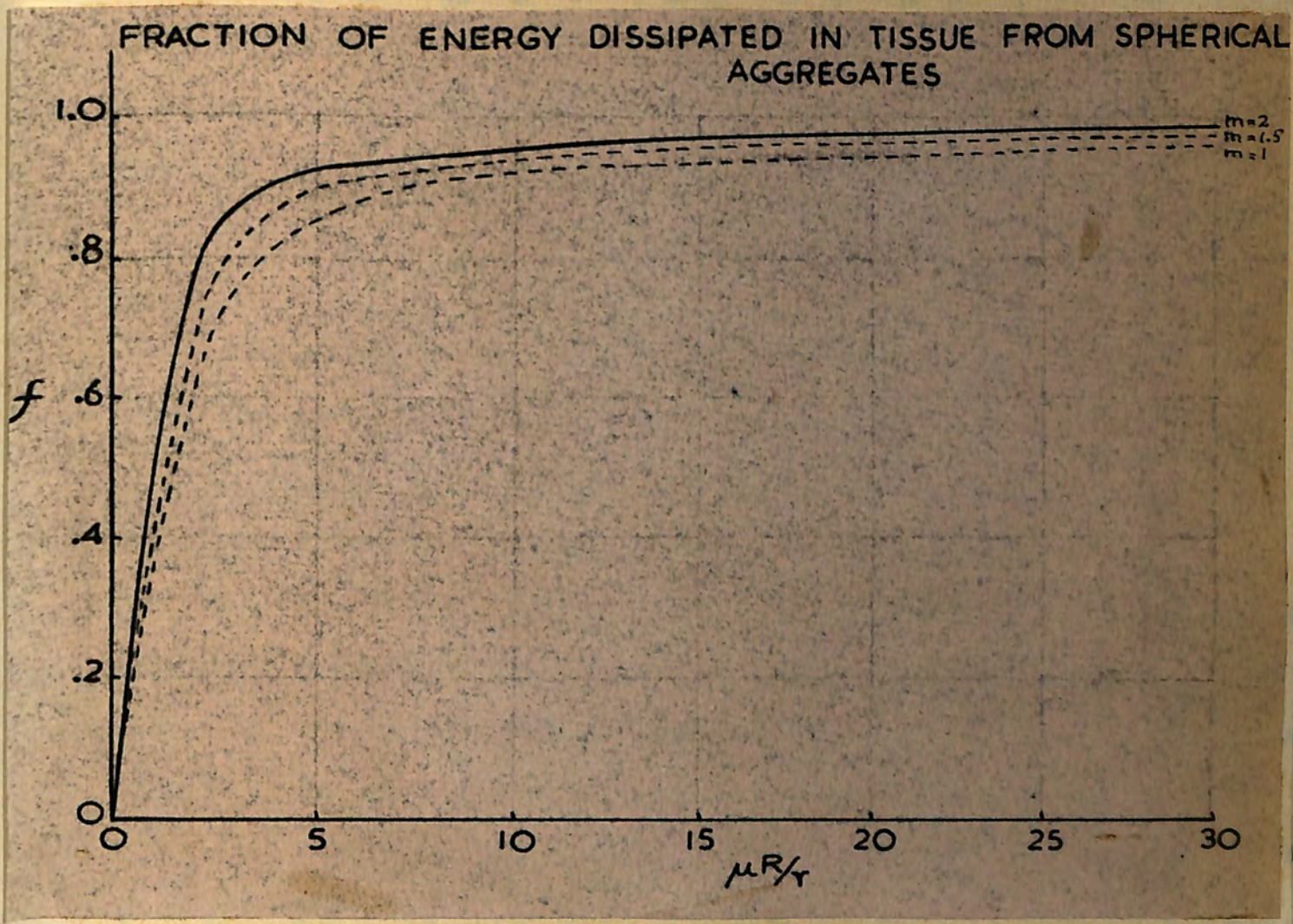
and for $\mu R > 2r$,

$$f = \tfrac{3}{4} \frac{m}{m+1} \left[\frac{\mu R}{r} - \tfrac{1}{2} \left(\frac{\mu R}{r}\right)^3 \frac{m^2}{(2m+1)(3m+1)} \right] -$$

$$\tfrac{3}{4} \frac{m^2}{(m+1)(3m+1)} \left[2 \frac{\mu R}{r} - \left(\frac{\mu R}{r}\right)^2 \frac{m+1}{2m+1} - \tfrac{1}{2} \left(\frac{\mu R}{r}\right)^3 \frac{m}{2m+1} \right] \left(1 - \frac{2r}{\mu R}\right)^{\frac{m+1}{m}}$$

The constant m has a value in the range 1 to 2, and f was calculated for $m = 1, 3/2, 2$. The values of f are plotted against $\mu R/r$ in Figure 9.2. It is seen that the expression is not very sensitive to changes in m in this region, and the value $m = 2$ was chosen so that $R \propto E^2$. The fraction

FIGURE 9.2.


FRACTION OF ENERGY DISSIPATED IN TISSUE FROM SPHERICAL AGGREGATES
1.0
.8
.6
.4
.2
0
f
0
5
10
15
20
25
30
μR/τ
m=2
m=1.5
m=1

f then becomes for $\mu R < 2r$,

$$f = \tfrac{1}{2}\frac{\mu R}{r}\left[1 - \frac{2}{35}\left(\frac{\mu R}{r}\right)^2\right]$$

and for $\mu R > 2r$,

$$f = \tfrac{1}{2}\frac{\mu R}{r}\left[1 - \frac{2}{35}\left(\frac{\mu R}{r}\right)^2\right] - \frac{1}{7}\frac{\mu R}{r}\left[2 - \frac{3}{5}\frac{\mu R}{r} - \frac{1}{5}\left(\frac{\mu R}{r}\right)^2\right]\left(1 - 2\frac{r}{\mu R}\right)^{3/2}$$

This expression is shown as the full line in Figure 9.2.

For tissue containing aggregates of various sizes, the fraction of the total energy of all alpha-particles of range R dissipated in tissue is

$$F = \frac{\Sigma n_i f_i r_i^3}{\Sigma n_i r_i^3}$$

where n_i is the number of aggregates of radius r_i and corresponding energy fraction f_i.

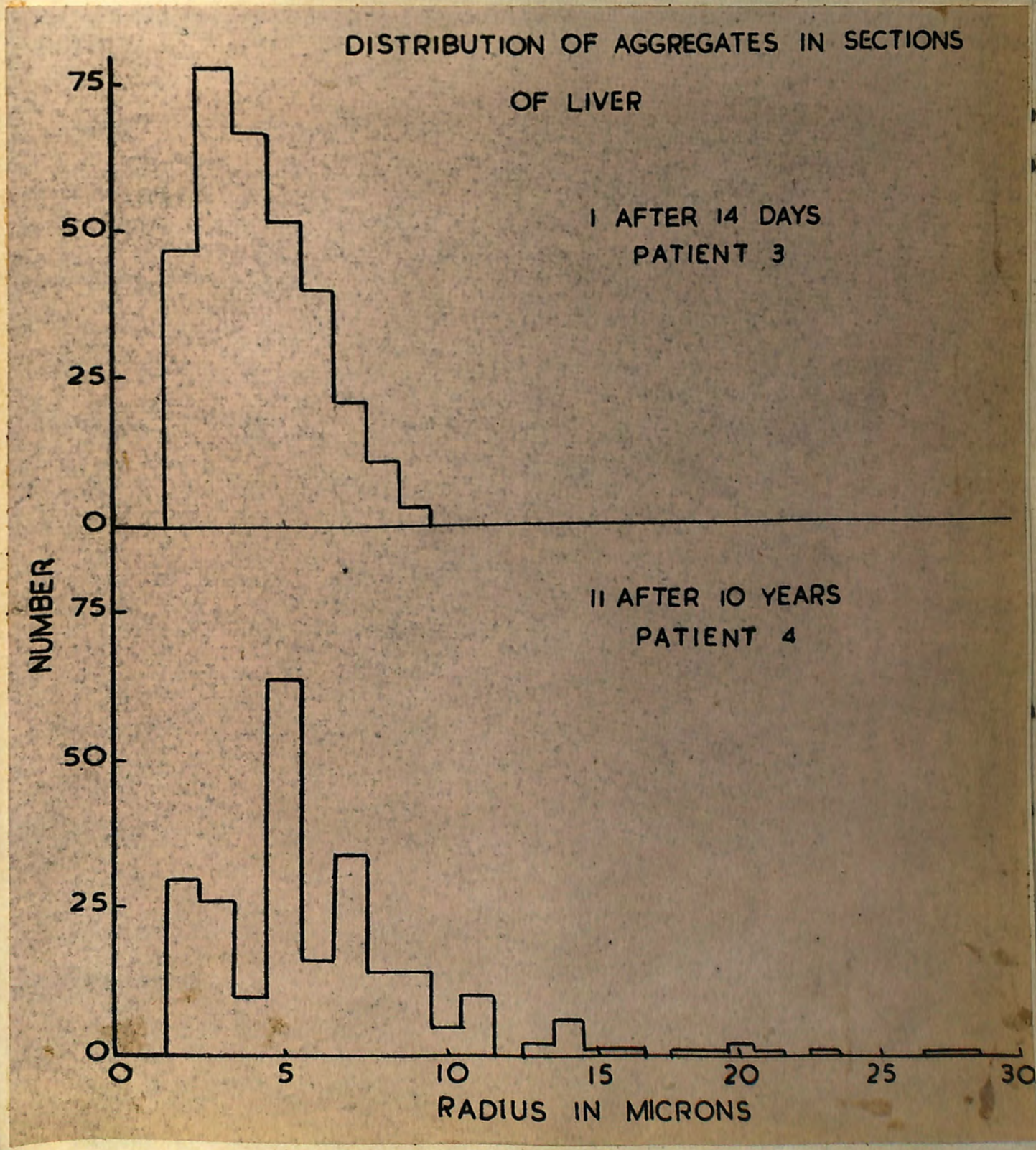
Size of aggregates. The dimensions of the aggregates in the organ are indicated by the areas of the aggregates in the sections, each aggregate of radius r in the organ giving rise to a distribution of cylindrical aggregates of radius ρ in the section. Suppose a spherical aggregate of radius r is cut into a number of parallel sections of thickness dx. The radii of these circular disc sections, ρ, vary with the distance x from the centre of the sphere from which they are cut, the radius of a typical section varying from ρ to $\rho + d\rho$. Since ρ is related to x by the equation $\rho^2 + x^2 = r^2$, $d\rho = x/\rho\, dx$. The number of sections of radius ρ is inversely proportional to $d\rho$, that is proportional to ρ/x. Near the centre of the sphere where x is very small this becomes very large and at the outside of the sphere where $x = r = \rho$, this tends to zero. Hence there are a large number of sections of radii near that of the radius of the sphere and a small number of small radii and it can be assumed as an approximation that the distribution of

radii of the spheres in the organ is the same as the distribution of radii of aggregates in the section.

Results. In the autoradiographs the areas of the aggregates were measured and the radii of circles of equal area were calculated. The results for two sections are shown in Figure 9.3 in which the number of aggregates, n, is plotted against the radius ρ. These distributions are typical of all sections examined in that they show the presence of a large number of small aggregates and a small number of large aggregates. In the liver section from patient No.3 no aggregates of radius greater than 10 microns were found whereas in the same section from patient No.4 the maximum radius was 28 microns.

In making the measurements it was often difficult to decide the extent of the aggregates, for example, whether a small aggregate was a separate aggregate or a fragment of a nearby large one, for it is possible that the aggregates may be broken up in cutting the sections. In order to allow for this uncertainty in the calculations, two limiting cases were considered, one in which the total area of thorotrast in an area 50 microns in diameter (comparable with the range of the alpha-particles) was added together and treated as one large aggregate, the other in which the smallest fragments were treated as separate aggregates. It was found that there was little difference in the calculated values of P in the two cases. As a further check, the distribution of aggregates observed in a thin section was compared with those observed in a thick section approximately 50 microns in thickness. The measurement was not very satisfactory because of the difficulty in seeing through a section of this thickness, but as far as

FIGURE 9.3.


DISTRIBUTION OF AGGREGATES IN SECTIONS OF LIVER
I AFTER 14 DAYS
PATIENT 3
II AFTER 10 YEARS
PATIENT 4
NUMBER
75
50
25
0
0
5
10
15
20
25
30
RADIUS IN MICRONS

could be determined there were no differences in the sizes of the aggregates.

The distribution of the sizes of aggregates may now be used to calculate $\bar{F}$ for the sections quoted. For each value of r the corresponding value of f was obtained from Figure 9.2 by giving a value to μ, the relative stopping power of tissue and thorotrast. The composition of the aggregates is not known, but assuming it consists of ThO_2, the relative stopping powers of ThO_2 and dried tissue calculated from the figures in 6.6 is 0.41. Using this value Table XV was constructed, the values of n and r being obtained from Figure 9.3 and the values of R, the range of alpha-particles in tissue from Table I. Since many of the ranges are rather similar only the groups Th, RdTh, ThA and ThC' were included in the calculation. Results for the other groups were then determined by interpolation. For each value of R the ratios $\mu R/r$ were determined and the corresponding values of f read from the curve in Figure 9.2. Then for each R the values of fnr^3 were calculated and summed to give values of Σfnr^3 as shown in the last row of Table XV. The values of $\bar{F}$ for each group are then as given in Table XVI. Finally, in calculating the average value of F for all groups of particles present in the thorotrast, $\bar{F}$, the relative numbers of particles of each range R given in Table XI were used to determine $N_i F_i$ and $\Sigma N_i F_i$. The average value $\bar{F}$ is given by $\frac{\Sigma N_i F_i}{\Sigma N_i}$. Two examples are given, those illustrated in Figure 9.3. For the liver section of patient No.4, $\bar{F}$ has the value 0.52 and of patient No.3, 0.81. The value in the latter case is higher as there were no large aggregates comparable with those found in the former and these influence the value of $\bar{F}$ most.

TABLE XV. Liver patient No.4

r	n	r^3	nr^3	R = 23.4			R = 81.6		
				$\frac{\mu R}{r}$	f	fnr^3	$\frac{\mu R}{r}$	f	fnr^3
2	30	8	240	4.8	.92	221	16.7	.97	233
3	26	27	702	3.2	.88	618	11.2	.96	674
4	10	64	640	2.4	.82	514	8.4	.95	609
5	64	125	8000	1.92	.76	6090	6.7	.94	7520
6	16	216	3456	1.60	.69	2380	5.6	.93	3220
7	34	343	11662	1.37	.62	7350	4.8	.92	10750
8	14	512	7168	1.20	.56	4020	4.2	.91	6530
9	14	729	10206	1.06	.51	5200	3.7	.90	9180
10	5	1000	5000	.96	.46	2310	3.35	.89	4450
11	10	1331	13310	.87	.43	5730	3.05	.87	11550
13	2	2197	4394	.74	.37	1625	2.57	.84	3680
14	6	2744	16464	.69	.34	5590	2.40	.82	13500
15	1	3375	3375	.64	.33	1115	2.25	.81	2740
16	1	4096	4096	.60	.30	1230	2.10	.79	3240
18	1	5832	5832	.53	.28	1630	1.86	.75	4370
19	1	6859	6859	.50	.26	1785	1.76	.73	5000
20	2	8000	16000	.48	.24	3840	1.67	.71	11350
21	1	9261	9261	.46	.24	2220	1.60	.69	6380
23	2	12167	24334	.42	.22	5350	1.46	.65	15800
27	1	19683	19683	.36	.19	3740	1.24	.58	11400
28	1	21952	21952	.34	.18	3960	1.20	.56	12300
Totals 242		Σnr^3 = 192634		Σfnr^3 = 66518			Σfnr^3 = 144476		

TABLE XV (cont'd). Liver Patient No.3

r	n	r^3	nr^3	$\frac{\mu R}{r}$ R = 23.4	f	fnr^3	$\frac{\mu R}{r}$ R = 81.6	f	fnr^3
2	47	8	376	4.8	.92	346	16.7	.97	365
3	78	27	2106	3.2	.88	1853	11.2	.96	2022
4	67	64	4288	2.4	.82	3516	8.4	.95	4074
5	52	125	6500	1.92	.76	4940	6.7	.94	6110
6	40	216	8640	1.60	.69	5962	5.6	.93	8035
7	21	343	7203	1.37	.62	4466	4.8	.92	6627
8	11	512	5632	1.20	.56	3098	4.2	.91	5125
9	3	729	2187	1.06	.51	1094	3.7	.90	1968
Totals	319	Σnr^3 = 36932		Σfnr^3 = 25275			Σfnr^3 = 34326		

TABLE XVI.

Liver Patient No.4 $\Sigma nr^3 = 193000$

Element	F_i calculated	F_i interpolated	Relative number N_i	$N_i F_i$
Th	0.345		1	.345
RdTh	0.490		.69	.338
ThX		0.53	.64	.340
Tn		0.57	.64	.355
ThA	0.610		.64	.390
ThC		0.55	.22	.121
ThC'	0.750		.42	.315

$\Sigma N_i = 4.25$, $\Sigma N_i F_i = 2.20$

$$\frac{\Sigma N_i F_i}{\Sigma N_i} = 0.52$$

Liver Patient No.3 $\Sigma nr^3 = 36900$

Th	0.687		1	.687
RdTh	0.830		.385	.320
ThX		0.85	.385	.327
Tn		0.88	.385	.338
ThA	0.895		.385	.344
ThC		0.87	.128	.111
ThC'	0.930		.256	.237

$\Sigma N_i = 2.924$, $\Sigma N_i F_i = 2.364$

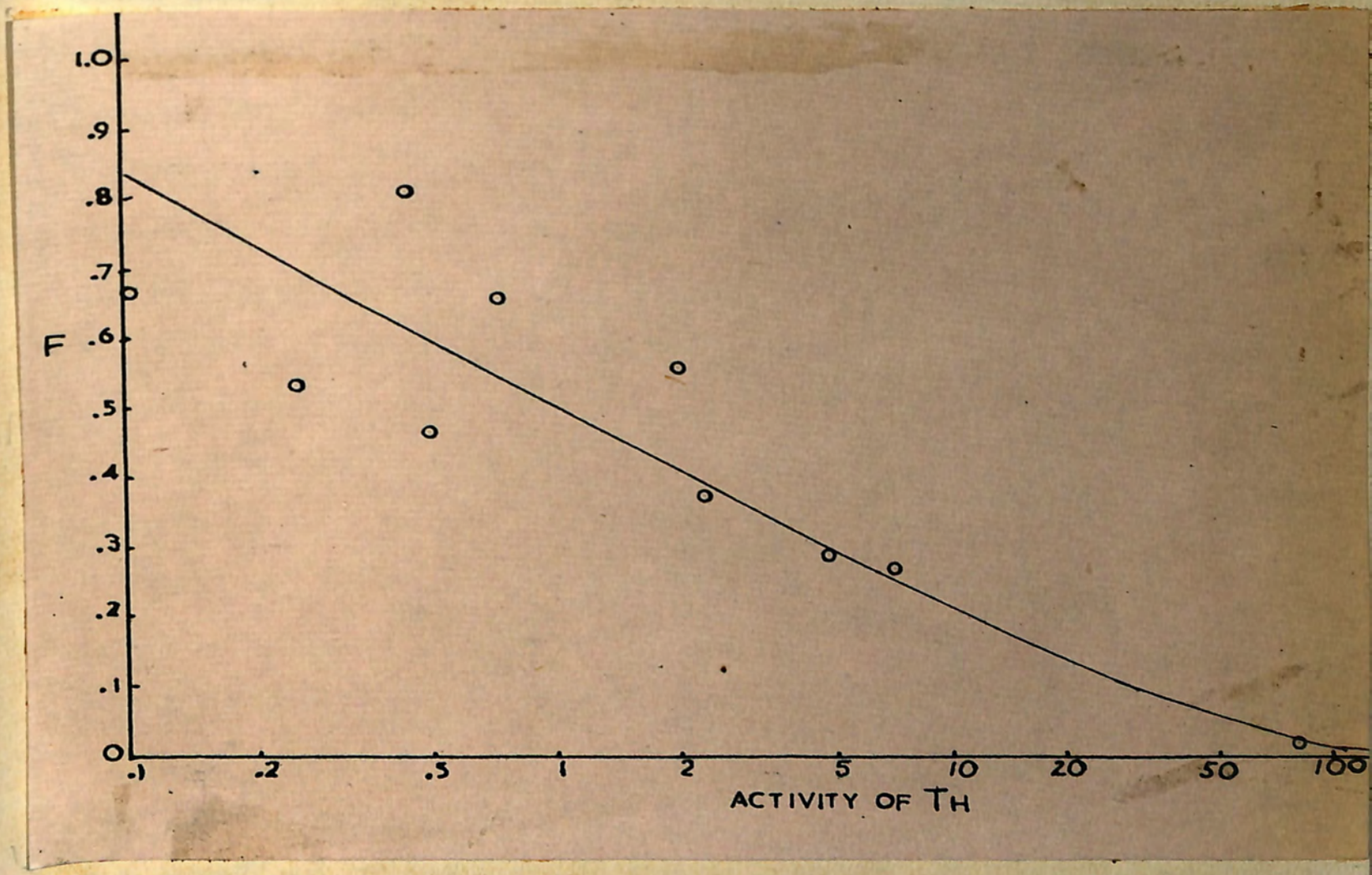
$\Sigma N_i F_i / \Sigma N_i = 0.81$

The values of $\bar{F}$ for all the sections are tabulated in Column 21, Table XI. There is a correlation between $\bar{F}$ and the activity of sections (Column 17) for where there is a large amount of material in the organ it collects in large aggregates which absorb a greater proportion of the energy of the particles. This is particularly well illustrated in the case of the rabbit where a large number of tissues were examined; in those of very low activity the aggregates are so small that almost all the energy is dissipated in tissue whereas in those of highest activity the aggregates are much larger and the fraction is only 30%. Since the amount of material in the organ is more correctly given by the activity of parent thorium, the correlation is best shown by plotting $\bar{F}$ against the total activity multiplied by a factor $\frac{\alpha}{1+\alpha}$, which represents the activity of Th relative to the total activity. This has been done for patients Nos.4 and 5 in Figure 9.4. A logarithmic scale has been used for the activity of Th expressed in disintegrations per mm^3 of tissue per minute. More accurately a separate curve should have been drawn for $\bar{F}$ corresponding to each of the seven groups of particles as $\bar{F}$ is not a simple average value for all groups but takes into account the varying compositions of the activities in the different organs. Figure 9.4 represents the self-absorption after 10 to 12 years. Another curve would apply to earlier times; for patients Nos.1 and 2, the two earliest cases, the values of $\bar{F}$ are in the range 90 to 100% and for patient No.3 at 14 days, 80%.

9.3. Density and Stopping Power of Thorotrast

In the calculation of the fraction of energy absorbed in thorotrast

FIGURE 9.4. Variation of the fraction of energy dissipated in tissue as a function of the amount of thorotrast present.



aggregates (9.2) the stopping power of tissue relative to thorotrast was taken to be the same as the stopping power of tissue relative to ThO_2. An independent estimate of the density of thorotrast is possible from the total number of tracks per unit volume of thorotrast observed in the autoradiographs. The measurements were carried out on some of the sections and the results are tabulated in Table XII. The average total number of disintegrations per mm^3 of the thorotrast aggregate per minute is 395 ± 52 and the number from Th alone calculated from the value of α in the section is 114 ± 7. Since each mg of thorium emits 240 alpha-particles per minute, 1 cm^3 of aggregate contains 480 mg of thorium. The density of liquid thorotrast was measured and found to be 1.39 gm cm^{-3}. Since it contains 190 mg of thorium per ml, the density of thorotrast aggregates must be about 2.5 times this or 3.5 gm cm^{-3}. It is stressed that this estimate is probably not very accurate as the measurements of the volumes of aggregates were subject to large errors and no correction has been made for the effects of the shrinkage of tissue during histological processing. As a comparison, the density has also been calculated from autoradiographs by Gros, Guer and Rechenmann (1952). They counted 436 tracks from $1.4 \times 10^{-7} cm^3$ of aggregate in 15 days. Their value for the number of alpha-particles per unit volume is therefore 288 per mm^3 per minute or of the same order of magnitude as that calculated here.

Another estimate of the relative stopping power of emulsion and section, which is independent of the thickness of sections, follows from the measurement of the fraction of energy absorbed from individual alpha-particles. In 6.1 it was shown that for an alpha-particle of range R

in emulsion the length recorded is given by $R - l = x/\mu\sin\theta$, from which $\mu = x/(R-l)\sin\theta$. For any track, measurements of the length and dip provide values for l and θ but x cannot be determined unless the point of origin of the track is known. This can be determined when the track is recognisable as a branch of a star as shown in Figure 4.1, for if several tracks coming from an aggregate can be traced to a common point, the distance x can also be determined. Doubt may still exist as to the identification of the tracks and the correct value of R to use, except for tracks the recorded lengths of which are greater than 33 microns, as these must be associated with the ThC' 48.7 micron group. The value of μ refers to the relative stopping power of the section and emulsion; if the measurements were confined to tracks which originated in an aggregate, this would be equivalent to the relative stopping power of thorotrast and emulsion, and hence the relative stopping power of tissue and thorotrast could be determined.

For the above condition to be fulfilled a star is measurable if (a) there are at least three recognisable tracks, (b) one of them is greater than 33 microns in length, (c) none of them is too steep for accurate measurement, (preferably all less than 30° to the plane of the emulsion) and (d) they all originate in thorotrast as distinct from tracks which pass partly through tissue and partly through thorotrast. As the number of such events was found to be very small, only a few stars were measured to test the method. One of the difficulties was that with a thin section the lengths of the paths of the particles in the section were of the same order of magnitude as the straggling of the particles in emulsion.

Figure 9.5 is a diagram of one of the stars which originated in an aggregate showing the accuracy with which it is possible to determine the point of origin of the stars. Errors of the order of a micron were introduced by irregularities in the surface of the thorotrast. The coordinates (x, y, z) of the two ends of the tracks were determined using the stage micrometers and the dip was multiplied by the shrinkage factor of the emulsion. Once the identity of the longest track had been established it was possible to identify the others fairly easily. The lengths of the tracks in the star in Figure 9.5 are given in Table XVII. The mean value of μ (0.65) agrees with the value calculated from the composition of ThO_2 and emulsion (0.71) which was used for the calculation of the self-absorption of thorotrast.

Because of the discrepancies between the various estimates of μ, the limits were taken as that calculated assuming that thorotrast has the same density as ThO_2 and as tissue, 0.41 and 1.0 respectively. Recalculation of Tables XV and XVI putting $\mu = 1.0$ gives a value for $\bar{F}$ in the liver of patient No.4 of 0.78 or 50% higher than the previous one; the limits of $\bar{F}$ are therefore 0.52 to 0.78. This range of values will apply approximately to the sections from patient No.5, but in a few cases, for example the spleen of patient No.4, the limits will be greater. In cases where $\bar{F}$ has values 80 to 100% for $\mu = 0.41$ the correction for $\mu = 1.0$ will be quite small.

9.4. Calculation of Dosage

The values for the three factors N, $\bar{E}$ and $\bar{F}$ together with their

FIGURE 9.5. Determination of the point of origin of alpha-particles in a section.

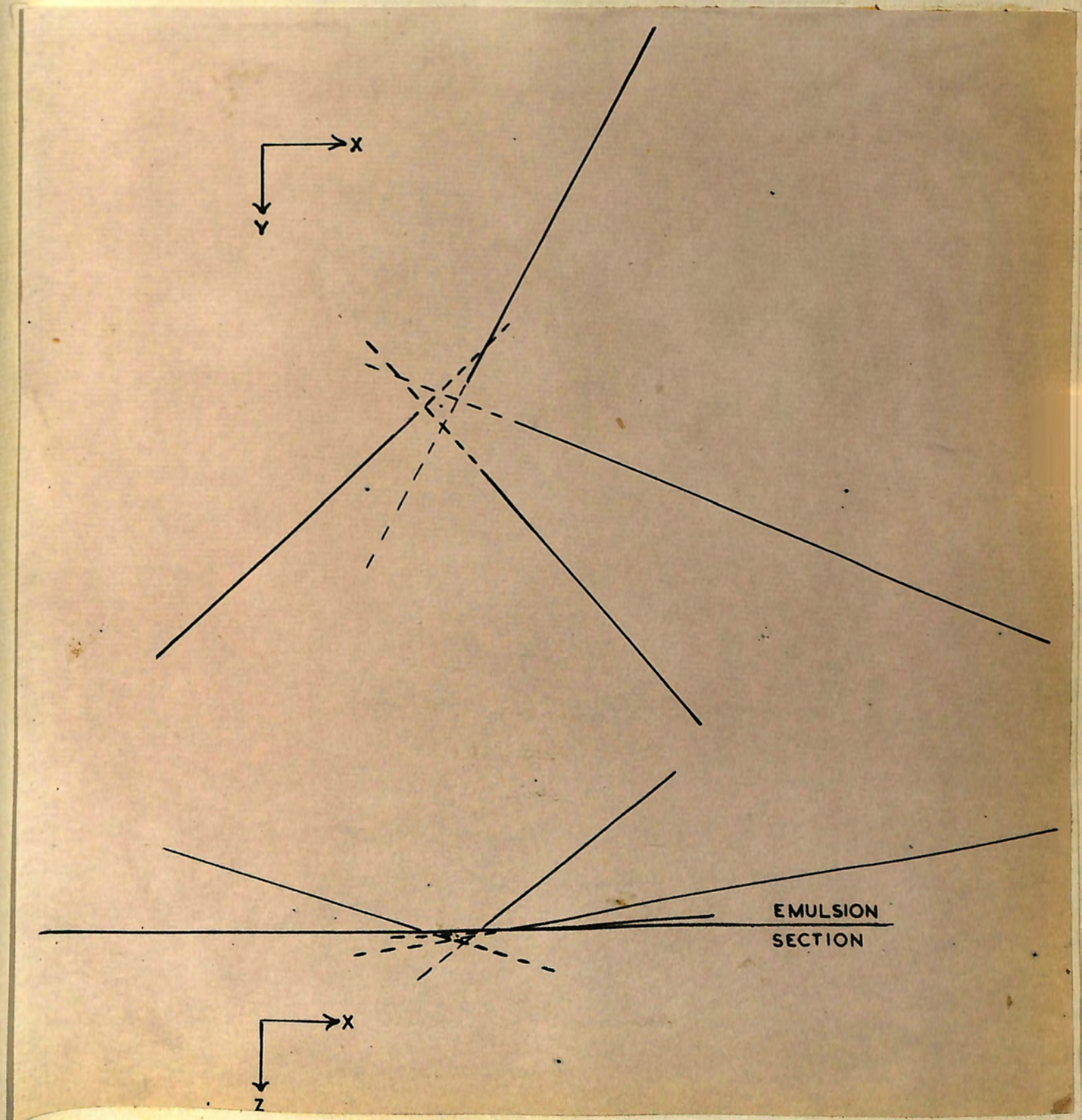


TABLE XVII. Calculation of the Relative Stopping Power of Thorotrast and Emulsion from the Lengths of Tracks in a Radioactive Star.

Element	Track length in emulsion l	Track length in section $x/\sin\theta$	Total range in emulsion R	$\mu = \frac{x}{(R-l)\sin\theta}$
ThC'	40.1 microns	4.2 microns	48.7 microns	0.49
ThA	29.0	2.4	33.1	0.58
Tn	22.8	5.9	29.7	0.85
ThX	21.3	3.3	26.0	0.70
				mean 0.65

product in rads per week are given in Columns 17, 20, 21 and 22 of Table XX. It was assumed that the density of tissue is 1.0 gm cm^{-3}, 1 Mev $=1.602 \times 10^{-6}$ ergs and 1 rad = 100 ergs per gram of tissue.

The figures given in Table XX represent the average doses for the whole organ and do not take into account the fact that the thorotrast is non-uniformly distributed. The large number of small aggregates will give rise to a small uniform dose and the few large aggregates will act as concentrated sources of activity or "hot spots". The values of the uniform and hot spot doses were determined from the following considerations.

Uniform dose. Each group of alpha-particles of range R present in the aggregate irradiates a spherical shell of tissue of thickness R surrounding the aggregate. As there are several groups of alpha-particles of varying values of R, the layers of tissue immediately adjacent to the aggregate are irradiated by all groups of particles but the outer layers are only irradiated by alpha-particles of the maximum range. Although the energy dissipation varies within the irradiated volume, as an approximation this variation will be neglected here and it will be assumed that an aggregate of radius r emits alpha-particles of mean range $\bar{R}$ which irradiate a spherical shell of thickness $\bar{R}$.

The distribution of dose is approximately uniform throughout the tissue if the aggregates are sufficiently close together that their irradiated volumes are adjacent or overlapping. Thus, for uniform irradiation there must be at least one aggregate in a volume approximately equal to that of a cube of side $2\bar{R}$, that is, the number per unit volume, n, must be greater

than $1/(2\bar{R})^3$. Suppose the total volume of all aggregates measured is V and the number of aggregates of radius r observed is n. If the volume of thorotrast per mm^3 of tissue is σ, the number of aggregates of radius r per mm^3 of tissue is $n\sigma/V$. The condition for uniform irradiation is therefore fulfilled if

$$n\sigma/V > \frac{1}{(2\bar{R})^3}$$

The mean range of alpha-particles from the thorium series is 47 microns in tissue. Thus for uniform irradiation the number of aggregates must be greater than $1/94^3$ per $micron^3$ or 1200 per mm^3. Since the distribution of the sizes of aggregates falls off very quickly from a large number of small aggregates to a small number of large aggregates, it is relatively easy to decide which contribute to the uniform dosage and which comprise isolated "hot spots". As an example, the distribution in the liver section of patient No.4 will be considered. From the value of Σnr^3 calculated in Table XV, the total volume of all aggregates observed V is $4/3\pi\Sigma nr^3$. In this section $\sigma = 0.040$ and the number of aggregates of radius r per mm^3 are then as given in Table XVIII, r being grouped in 5 micron intervals for $r > 5$. In Table XVIII the condition for uniform irradiation is fulfilled for $0 < R < 10$. For this group of small aggregates the values of Σnr^3 and Σfnr^3 follow from Table XV and $\bar{P}$ from Table XVI.

Dose at hot spots. The maximum dose from the hot spots was calculated from the following considerations. The volume of the irradiated tissue is $4/3\pi[(\bar{R} + r)^3 - r^3]$. The total energy absorbed per unit time in this volume $= 4/3\pi r^3 N \bar{E}P$ where N is the number of particles emitted from unit volume of thorotrast per unit time and P is the fraction of the total

TABLE XVIII. Number of aggregates of Radius r microns per mm^3 of Tissue - Liver Patient No.4

r	2	3	4	5	10	15	20	25	30
$\frac{n6}{\frac{4}{3}\pi\Delta mr^3}$	1480	1280	494	3160	2620	940	247	148	99

10468

energy of the particles which is dissipated in tissue. The tissue dosage is then given by

$$D = \frac{N \bar{E} P r^3}{R(R^2 + 3Rr + 3r^2)}$$

For example, in the liver section of patient No.4 the maximum radius of the aggregates was 28 microns. The fraction of the energy of a particle of range 47 microns which is dissipated in tissue from an aggregate of radius 28 microns is 0.34 (Figure 9.2). The effective energy of the alpha-particles is 5.80 Mev. Putting N = 395 disintegrations per mm^3 per minute (9.3) the dose-rate to tissue is 6.25 rads per week.

The uniform and maximum dosages were calculated for all the sections of patients Nos. 4 and 5, the two cases in which the aggregates were largest, and are given in Table XIX together with the average doses from Table XX. The maximum dose in the tissue surrounding the aggregates is several times greater than the uniform dose delivered by the small aggregates. In the measurement of the sizes of aggregates, the number of large aggregates observed was very small. It is therefore possible that some larger aggregates may have been missed and the maximum doses may be even greater than those quoted. The maximum aggregate that has been observed was approximately 500 microns in radius giving a dose-rate of 20.9 rads per week.

9.5. Estimation of Errors

The measurements involved in the estimation of the tissue dosage were the determination of the radioactive content of sections from the track

TABLE XIX. Distribution of Dose in Sections of Patients Nos. 4 and 5.

Patient	Section	Maximum Radius of Aggregate microns	Average Dose	Uniform Dose	Maximum Dose
			rads per week		
4	Spleen medulla	500	3.14	-	20.9
	Spleen	61	6.25	0.18	12.0
	Hilum of spleen	55	2.12	0.75	7.56
	Liver	28	5.13	2.50	6.25
	Lymph node	10	0.78	0.23	1.16
	Tonsil	18	0.31	0.06	4.35
5	Spleen	52	3.30	0.41	8.49
	Liver	26	0.55	-	4.98
	Bone marrow 1	32	0.85	0.18	4.00
	2	26	0.27	-	4.75

lengths, the total number of tracks counted in a measured area of tissue, the thickness of sections and the area of the aggregates.

Although the retention-factors were found to vary from section to section from 50 to 100%, the effective energy of the alpha-particles did not vary by more than 16% in the group showing the maximum variation (No.4). The correction factor to convert measured activity in vitro to that in vivo was of the order of 0.7 to 1.0. Errors in analysing the track lengths into groups therefore had a relatively small effect on the final result compared with other errors involved.

There was no difficulty in measuring the number of tracks originating in a measured area of tissue or the area of the aggregates with considerable accuracy. The thickness of the sections was more difficult to estimate and is considered to be the least accurate of the experimental figures used.

The possible limits for the value of the relative stopping power of thorotrast and tissue were considered above, but the greater stopping power of fibrous tissue surrounding the aggregates compared with that of normal tissue should also be taken into account. The dose in the fibrous tissue surrounding aggregates will be greater than the figures given for the hot-spot dose but the alpha-particles will affect a smaller volume of tissue and tissue immediately outside the effective range will be protected. It was also assumed in the calculation of self-absorption of aggregates that they were spherical. This may lead to an underestimate of tissue dosage as less of the total energy will be absorbed within any other aggregates.

Dose-rate in wet tissue. The figures quoted represent the doses in dry tissue and no attempt has so far been made to relate this to the dose in wet tissue. It has already been shown (8.3) that tissue shrinks considerably in preparation for sectioning and the correction has been estimated in two cases by comparing the activity measured in autoradiographs with that estimated in wet tissue using gamma-ray measurements. Applying this correction to the results would decrease the dose-rate to approximately half that quoted for dry tissue. Because the shrinkage probably varies according to the processes used, it cannot be assumed that a similar correction applies generally. In some of the cases from Copenhagen figures are available from chemical estimations of the thorium content of wet tissue (2.7) with which to compare the autoradiograph estimates. The thorium content of tissue from the autoradiograph measurements is given by the total activity per unit volume multiplied by the factor $\frac{\alpha}{1+\alpha}$ (9.2). The Copenhagen estimates apply to two spleens excised at splenectomy and one liver obtained at autopsy. The figures for patient No.3 were not used because it was not known which of the tissues listed corresponded to the tissues studied by means of autoradiographs.

Patient No.4 - Spleen

By chemical analysis the thorium content of this organ was 27.2 mg per gm of wet tissue which agrees with the autoradiograph estimate of 25.6 mg per gram.

Patient No.5 - Liver

By chemical analysis the content was 1.34 mg of thorium per gm of wet tissue compared with the autoradiograph value of 1.71 mg per gm.

Patient No.5 - Spleen

This organ was unusually large (335 gms) containing 0.75 gm of thorium which corresponds to a concentration of 2.24 mg per gm or somewhat less than the autoradiograph estimate of 9.0 mg per gm.

As a general check of the validity of the estimates of tissue dosage it is possible to calculate the maximum dosage in a "standard patient" as defined by Rundo (1955) receiving 20 ml of thorotrast which is distributed in the proportions of 70% in the liver weighing 1700 gm, 25% in the spleen weighing 150 gm, 5% in the bone marrow weighing 1500 gm. The maximum dose will then occur in the spleen when all products are in equilibrium, no decay products are lost from the organ and the thorotrast is distributed uniformly with no self-absorption. Under these conditions the concentration of thorium in the spleen will be 6.33 mg per gm which delivers a dose to tissue of 9.1 rads per week. However, some of the measurements do not show a 3 to 1 ratio for the specific activities in spleen and liver as assumed by Rundo and the content in the bone marrow was found to be comparable with that in the liver. For example, in Patient No.3, the ratios of the specific activities in the spleen, liver and bone marrow were found to be 1:0.76:0.62. Assuming the amount in other parts of the body is negligible and the masses of the organs are as given in the "standard patient" 40 ml distributed in this way would be as 21.7 ml in liver, 2.6 ml in spleen and 15.7 in the bone marrow with corresponding dose-rates on the assumptions given above of 3.5 rads per week in the liver, 4.7 in the spleen and 2.9 in the bone marrow. The age-factor of the thorotrast in this injection was estimated to be approximately 0.5 which makes the

dose-rates 1.8, 2.4 and 1.5 compared with the autoradiograph estimates of 5.4, 6.8 and 4.2 rads per week respectively. The organs in this patient might have been smaller than in "standard man" as in patients Nos.1 and 2 but even so the autoradiograph estimate seems rather high unless a correction factor of approximately 2 is applied in this case also.

It is puzzling that in several of the cases considered the activity per unit volume of dry tissue is greater than that of wet tissue by a factor of about 2 whereas in others the agreement between the two values is good. It may be significant that the shrinkage is greater in cases in which the thorotrast is distributed fairly uniformly in single cells compared with those in which the thorotrast has accumulated into dense deposits which reduce the total shrinkage. It is also possible that in some cases there had been a decrease in the volume of the organ in vivo and the density of the tissue was greater than 1.0. The autoradiograph estimate depends on measurements of volume whereas the chemical estimate depends on measurements of mass.

As the estimates of dose-rate quoted involve several approximations, a range of values for the probable dose-rate in wet tissue has been tabulated (Column 23, Table XX) wherever possible taking as the lower limit that applying a shrinkage factor as deduced above and as the upper limit using the minimum self-absorption. As in some cases these corrections were similar, a result equal to the one quoted for dry tissue is regarded as the most probable, with a 50% possible error. In other cases, Nos.1, 2 and 3 a lower limit only is given as the correction for self-absorption was known to be small when self-absorption was small.

9.6. Calculation of alpha-particle Dose from gamma-ray Counts

As autoradiography does not provide a practicable way of determining the alpha-particle dose in living patients in a routine investigation, it is important to consider whether it is possible to calculate this from gamma-ray counts making use of the data obtained from autoradiographs.

The gamma-ray count is a measure of the amount of ThC" in the organ. Hence the apparent volume of thorotrast in the organ calculated from a direct comparison of this count with the count from a known volume of thorotrast under similar geometrical conditions, will be less than the actual content of the organ unless a correction is made for the loss of decay products *in vivo*. The correction may be calculated by considering the activities of the various elements separately (7.2). If the activity of Th relative to its products is α_o in the thorotrast and α in the organ and a fraction, f, of MsThl is retained in the organ *in vivo*, the activities of the various elements are as given in the following scheme.

	In Thorotrast	In Organ
Th	1	1
RdTh	$\frac{1}{5\alpha_o}$	$\frac{1}{5\alpha_o} f$
ThX	$\frac{1}{5\alpha_o}$	$\frac{1}{5\alpha_o} f^2$
Tn	$\frac{1}{5\alpha_o}$	$\frac{1}{5\alpha_o} f^2$
ThA	$\frac{1}{5\alpha_o}$	$\frac{1}{5\alpha_o} f^2$
ThC	$\frac{2}{3}\ \frac{1}{5\alpha_o}$	$\frac{2}{3}\ \frac{1}{5\alpha_o} f^2$
ThC"	$\frac{1}{3}\ \frac{1}{5\alpha_o}$	$\frac{1}{3}\ \frac{1}{5\alpha_o} f^2$

The apparent volume V_a of thorotrast in the organ as deduced from the gamma-ray count will therefore be different from the actual volume, v, by a fraction f^2, i.e. $v = \frac{V_a}{f^2}$. This fraction may be determined in terms of the retention factor for this organ γ and the age-factor of the thorotrast β_o from the following considerations.

The retention factor γ was defined as β/β_o and has the value

$$\frac{1 + \frac{1}{5\alpha_o} f + \frac{4}{5\alpha_o} f^2}{1 + \frac{1}{\alpha_o}}$$

from which $$f = \frac{1}{8}\left[\left(1 + 80\,\frac{6\beta_o\gamma - 1}{6\beta_o - 1}\right)^{\frac{1}{2}} - 1\right] \qquad (1)$$

so that $$v = \frac{64 V_a}{\left[\left(1 + 80\,\frac{6\beta_o\gamma - 1}{6\beta_o - 1}\right)^{\frac{1}{2}} - 1\right]^2} \qquad (2)$$

Equation (2) gives the actual volume of thorotrast in the organ in terms of the apparent volume, the age-factor of the thorotrast and the retention-factor in this organ. The age-factor of the thorotrast used for any patient is usually not known but in many cases the error involved in assuming that the age of the thorotrast corresponds to the time interval between injection and measurement will not be great. An estimate of the retention-factor is also required. It has been shown that the retention-factor varies with the MsThl content of the thorotrast, and hence its age, and also with the size of the aggregates. The figures obtained (7.2) apply to a few cases with different values of the variables, but are not sufficient to show how to calculate the retention-factor for any given values of the variables. This might be found possible by extending the experiments to

compare (a) the fraction of MsT hl which is retained in animals injected with different samples of thorotrast of different ages and (b) the fraction of MsThl which is retained in animals injected with the same sample of thorotrast at intervals of time as the aggregates increase in size.

For the calculation of tissue dose it is necessary to know the specific activity, or the volume of thorotrast per unit volume or mass of the organ. Having calculated the actual total thorotrast content of the organ from the gamma-count, the specific activity follows if the mass or volume of the organ is known. Direct determination of the mass of organs is not possible in living patients but it might be possible to make an estimate of the volume from the size and density of the radio-opaque areas of the liver and spleen or of the mass from the total mass of the patient. The alpha-particle activity of the organ is then $24.6 \times 190 \frac{V}{v} \gamma\beta_0$ disintegrations per unit volume per second, where V is the volume of the organ, the activity of 1 mg of Th with its products in equilibrium is 24.6 disintegrations per second and 1 ml of thorotrast contains 190 mg.

The next factor in determining the alpha-particle dose is the self-absorption of the aggregates which was shown to be a function of the concentration of thorotrast in tissue (specific activity) and the time interval since injection (9.2). The alpha-particle activity of Th alone is given by $4.1 \times 190 \frac{V}{v}$ disintegrations per unit volume per second from which F can be determined for each value of R, the range of the alpha-particles, from curves of the form shown in Figure 9.4.

Calculation of the alpha-particle dose-rate from the equation $D = \overline{NE}F$ now follows where the factors are given by

$$N = \frac{24.6 \times 190 V_a \gamma \beta_o}{f^2 V} \text{ per unit volume per second.}$$

$$\bar{E} = \frac{\Sigma N_i E_i}{\Sigma N_i} \text{ Mev.}$$

$$\bar{F} = \frac{\Sigma N_i F_i}{\Sigma N_i}$$

where N_i is the relative activity of the i^{th} element in terms of f and α_o. f is given by equation (1) and α_o by $\frac{1}{6\beta_o - 1}$

As an example of the method of procedure, the calculation of the dose-rate from the counts recorded in the "average patient" quoted by Mitchell (2.2) will be given. This patient received 19.0 ml of thorotrast and measurements were made after 10.8 years, when the counts in the liver and spleen were 1033 and 837 cpm respectively. With the counters used, 1 gm of "old" thorium arranged as a 10 x 5 cm^2 area source under 4 cm of water absorber to simulate conditions for liver and spleen counting gave a count rate of 1000 cpm. Assuming "old" thorium refers to Th in equilibrium with decay products, the age-factor will be 1.0 and for the patient after 10.8 years it will be 0.64. Hence the counts in the liver and spleen are equivalent to 1.62 and 1.31 gm of thorium. (Mitchell quoted figures of 1.5 and 1.4 gm in these organs in the "average patient" but it is not stated exactly how these figures were obtained). In Table XX the retention factors measured after comparable lengths of time, 10 and 12 years, had mean values for the two patients (Nos.4 and 5) of 0.75 in both the liver and spleen. The actual amounts of thorium in the liver and spleen by the equation above, putting $\gamma = 0.75$ and $\beta_o = 0.64$, are then 2.57 and 2.08 gm

respectively. (The total amount calculated to be in these two organs is actually greater than the 3.8 gm which were contained in the 19.0 ml injectio but the figures will serve to illustrate the procedure). No figures are available for the average masses of the two organs in this group of patients but assuming these to be the same as in "standard man", namely 1700 and 150 gm respectively, the concentrations of thorium are then 1.51 mg per gm in the liver and 13.9 mg per gm in the spleen. The alpha-particle activities in these organs are then 1.07 and 9.82 disintegrations per mm^3 per minute and the activity of parent thorium alone is 0.37 and 3.42 disintegrations per mm^3 per minute. Comparing these figures with Figure 9.4 the corresponding fractions of energy dissipated in tissue are 0.61 and 0.32 respectively. Since the retention-factors are similar in both organs the effective energy of the alpha-particles is also similar, namely 5.33 meV. The alpha-particle dose-rates are then 0.56 rads per week in the liver and 2.70 rads per week in the spleen. These figures compare very favourably with the results obtained by means of autoradiographs, for patient No.5 who was given 25 ml of thorotrast.

By similar reasoning the alpha-particle dose could be calculated from the gamma-ray counts obtained from one of the patients examined here, No.4, and the values obtained by the two methods could be compared. In this patient the counts were made one year after splenectomy so are not applicable to calculation of the dose-rate in the spleen. The count in the liver 11.4 years after injection was 1225 (mean of side and front counts) which is equivalent to 1.225 gm of "old" thorium or 1.85 gm of thorium of age factor 0.66 corresponding to 11.4 years. The liver of this patient was reported

to be "normal or perhaps small" so assuming it weighs 1000 gm, the apparent content of this organ is 1.85 mg per gm of wet tissue. Allowing a retention factor of 0.75 and an age-factor of 0.66, the actual content is 2.94 mg per gm and the total alpha-particle activity is 2.16 disintegrations per mm^3 per minute. The activity of parent thorium only is 0.725 disintegrations per mm^3 per minute and the fraction of energy dissipated in tissue from Figure 9.4 is 0.52. The effective energy of the alpha-particles is 5.33 MeV. The dose-rate is then 0.96 rads per week, somewhat lower than the autoradiographic estimate, but more in line with the results obtained for patient No.5.

CONCLUSION

Discussion of Results

The findings show that in a patient who has received 20 ml of thorotrast, the dose in the spleen, liver and bone marrow is of the order of 1 to 8 rads per week. This dose rate is smaller than the activity alone would suggest for two reasons, partly the loss of some of the decay products, but more especially the self-absorption in the thorotrast aggregates.

In the measurements of the content of different organs it was found that from 50 to 100% of all the elements are retained in the organs of the R.E. system in which the thorotrast is deposited. The elements lost were shown to be MsThl and ThX, in agreement with the findings of previous workers that these elements are present in the blood stream and are excreted. It was not possible in the experimental conditions existing to detect any transfer from the organs of thoron which is breathed out, or of ThC which is also present in the blood. Some of these elements will be derived from the parent elements present in the blood stream and some may escape from the tissue where it is formed, but as the half-lives of these two elements are smaller than those of MsThl and ThX the fractions of the former transferred directly from the organs are probably much less. As the retention-factor is a measure of the loss of MsThl from the organ, it will depend on the amount of MsThl present and hence on the age of the thorotrast. It was found that in cases where the thorotrast was quite fresh and the MsThl content small the retention-factor was nearly 100% but as the MsThl content increased with time, the retention-factors decreased. Another factor which was shown to influence the proportion of soluble elements transferred

when an organ contains a large amount of material it is protected against radiation damage by the self-absorption of the large aggregates formed. The mechanism of the formation of these aggregates is not clearly understood. When the thorotrast is injected into the body the particles of ThO_2 are of colloidal size (about 7 millimicrons) but when the thorotrast first appears in the cells after about 15 minutes, it has accumulated into granules about 1 micron in diameter. Later these granules accumulate within the cell and eventually there is an aggregation of thorotrast-laden cells with deposits up to 50 microns or more in radius. Histological examination shows that the deposits finally collect in regions where they do least damage to the organ. The formation of some aggregates also clearly shows that they consist of a number of thorotrast-laden cells collected closely in places where groups of cells of the type which take up foreign matter are not normally found. It has been suggested that this is a radioactive effect in which some cells receive a small dose which is sufficient to cause proliferation without killing them and the new cells formed, being of similar type, take up further material which becomes free in the blood stream from time to time, freshly released from cells which have not survived. Experiments are at present in hand to determine whether the phenomenon is connected with the radioactivity. It has been found that when the aggregate size is small (1 to 5 microns in radius) only 10 to 20% of the energy of the alpha-particles is absorbed but later when the aggregates have grown they absorb 60 to 80% of the energy. The factor by which the dose-rate is reduced by self-absorption is shown in Column 24 of Table XX.

In the five cases studied, the time intervals between the injection and the autopsy or biopsy were less than one day in two cases, 14 days in one case and 10 and 12 years in two more cases. While at least two more cases at approximately one and five years would be required to make a satisfactory study of the dose at different times over the whole period, the results obtained so far are sufficient to give an indication of the extent of the variations in the dose with time. For this purpose the values of the dose per 20 ml divided by the age-factor were calculated (Column 25, Table XX), the correction being applied to eliminate variations due to the volume of thorotrast injected and the age of the sample. Any differences therefore indicate the effect of the two factors discussed above, i.e. retention of decay products and self-absorption in thorotrast aggregates, as well as the rate of uptake of thorotrast in the different organs. Data on the rate of uptake are available from measurements in rabbits, in which it was found that the content of the liver and spleen reaches a maximum in a few hours, after which the amount in the liver remains constant, but that in the spleen falls to a steady value at about 100 hours, with a redistribution to the bone marrow and to other parts of the body. There may then be a further redistribution after longer periods of time such as that which occurred in rabbits after 15 months. The measurements on the organs of the 12 and 20 hour patients indicate a similar rate of uptake in human subjects as most of the total volume of thorotrast injected was found in the liver and spleen after these times. There may however be a redistribution to the bone marrow which has already taken place in the 14 day patient and a further redistribution at a later date which has

probably taken place in the 10 to 12 year intervals for the two late cases. The possible range of values for some of the dose-rates calculated are rather large to make a comparison between the various patients satisfactorily. With the early group of three patients, the values are consistent showing a dose-rate in the spleen of about 3 rads per week per 20 ml injected. Results for the other two patients show greater divergence although the doses are still of similar order of magnitude. As the self-absorption varies by a factor of 2 or 3 between the two groups, the other two factors, escape of elements and redistribution of activity must tend to reduce the difference. The results so far therefore indicate that the variation of dose with time is similar to that of the activity of thorotrast, initially of the order of 50 to 100% of the maximum, falling to 30 to 50% within five years, then increasing to the maximum value in about 30 years.

There also appears to be a variation in the relative amounts of thorotrast in the various organs at different times. In the three early cases, there was an approximately equal distribution of material throughout the tissue of the spleen, liver and bone marrow. This result agrees with the earliest experiments involving chemical analyses of tissues at short periods of time after injection. In the long-standing cases there was a greater specific activity in the spleen relative to the liver, similar to the results obtained in Copenhagen. This variation cannot be explained by a redistribution after some time as this is said to occur from the spleen to the liver, but may be explained by a shrinking of the spleen due to the presence of thorotrast.

A dose-rate of 1 to 8 rads per week is much greater than the maximum permissible level for X-rays of 0.3 r per week especially when allowance

is made for the greater biological efficiency of alpha-particles. While the average dose-rate to organs is important in determining radioactivity effects, another important factor may be the dose in localised regions, particularly in determining carcinogenic effects. For this, a comparison may be made between the 'hot-spot' dose-rates from the radium deposits in the Haversian systems in bone and from the thorotrast aggregates in soft tissue. The values of 1 to 3 rads per day for thorotrast patients are comparable with the lower limit of Hoecker and Roofe's estimate of 1 to 23 reps per day in a radium patient who had a total body burden of 50 micrograms of radium, but nothing comparable with their maximum estimate has been observed here. Fifty micrograms of radium actually corresponds to a very large volume of thorotrast, 917 ml, which is about ten times the maximum dose used in patients. Most of the radium patients ingested much less than 50 micrograms and some with only 0.7 microgram suffered ill-effects. In these patients the 'hot-spot' dose-rates will be more comparable with those in thorotrast patients. However, there is a fundamental difference in the nature of the hot spots in the two cases; in the case of thorotrast, the insoluble thorium deposits absorb a considerable fraction of the total energy of the alpha-particles, whereas in the case of radium, the radium atoms are actually incorporated in the bone and the alpha-particles dissipate all their energy in living material. The thorotrast results also show that if the contribution of the hot-spots to the total dose is removed there is still a uniform background dose-rate to tissue which is in some cases several times tolerance.

No correlation of clinical symptoms with the measured radiation dose is possible with these results. The two long-standing cases of 10 and 12 years are the only ones of interest in this respect. Although the amounts of thorotrast injected are similar both their clinical histories and the measured dose-rates differ. The dose-rates in the liver and spleen of patient No.4 are noticeably higher than those in the equivalent organs of patient No.5. In the latter case the rest of the thorotrast is accounted for by a dose-rate in the bone marrow comparable with and slightly higher than that in the liver. There was also a considerable deposit in the side of the neck presumably at the site of injection. No sample of bone marrow was available from patient No.4 who is still living. It would have been interesting to determine whether the dose was higher in the bone marrow of the patient who died from aplastic anaemia, this being the most common fatal reaction so far reported. In one of the cases of aplastic anaemia reported in the literature the bone marrow also contained a very high proportion of thorotrast, even higher than in the spleen. It would seem that where the liver and spleen retain the bulk of the material, the vulnerable bone marrow is protected.

The correlation of dose-rates with clinical symptoms will not be possible until a very large number of cases has been examined. This is not practicable using autoradiographs to study living patients. As the next step, a possible method of calculating the alpha-particle dose-rate from gamma-ray counts using the autoradiograph results has been worked out. From these results it has been shown possible to deduce the self-absorption of alpha-particle energy in the aggregates if the amount of

thorotrast in the organ can be deduced from gamma-counts and the time interval since injection is known. It may also be possible to deduce the proportion of elements retained in any organ given the age of the thorotrast and the time interval since injection, although further measurements will be necessary before this can be established definitely. The method has been illustrated by calculating the dose-rates from some of the results for gamma-ray counting obtained in Copenhagen. The results seem quite promising.

Subjects for Further Research

In the descriptions of experiments and the discussions of results, two further subjects for research were mentioned:-

1. The mechanism of the formation of aggregates.
2. The determination of how the retention of decay products varies with the ThX content of the thorotrast and the aggregate size.

To these might be added several extra experiments which would increase the accuracy of the measurements.

3. The self-absorption of thorotrast aggregates could be measured directly without making any assumptions about their shape and density if they could be actually embedded in emulsion. It might be possible to substitute liquid emulsion for an embedding medium but several new problems would have to be solved such as ensuring an even distribution of silver halide grains throughout the tissue and reducing the distortion caused by the differential shrinkage of tissue and emulsion. The method might not

be useful for very large aggregates of radius greater than the maximum practicable thickness of tissue sections.

4. The loss of soluble products and particularly the amount of the short-lived ones which escapes from the organs could be determined by examining by means of autoradiographs samples of blood taken from patients and animals. The difficulty with this procedure would be that the activity of the blood smears would be so small that the number of tracks recorded might be too few to be useful.

5. As there are uncertainties in the thickness of dried tissue and the equivalent volume of wet tissue, a better measure of the tissue dosage might be to express this as the dose per cell instead of per gram of tissue. This would involve counting the number of cells in the sections rather than measuring the area and thickness.

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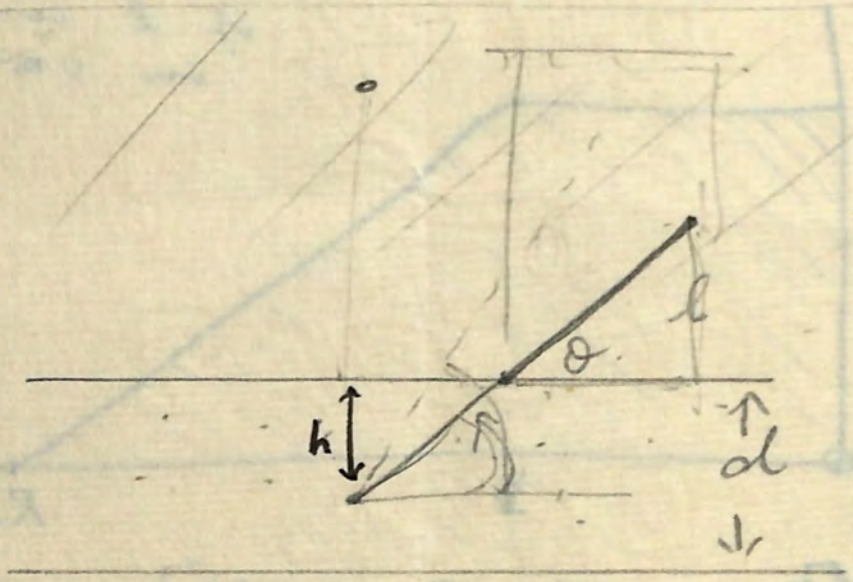
TABLE XX

1 No.	2 Patient	3 History	4 Injection	5 Date	6 Time in the body	7 Age-factor of thorotrast β_o	8 Section	9 Date of ARG	10 α in vitro	11 β in vitro	12 Retention-factor γ	13 Exposure of ARG days	14 Tracks counted	15 Area mm^2	16 Thickness microns	17 Activity per mm^3 per min. in vivo
1	Atkinson Morley Hospital	Autopsy 1954 12 hrs. after injection	7 ml U.S. thorotrast Lot 223 Sample D	May 1954	12 hours	0.73	Spleen Liver Bone marrow	July 1954	0.29 0.30 no activity	0.74 0.72	1.00 1.00	25 25	5811 5747	39.71 40.60	4.0 4.0	2.01 2.01
2	National Hospital	Autopsy 1952 20½ hours after injection	20 ml U.S. thorotrast Lot 211 Sample C	1952 Oct.	20½ hours	0.66	Spleen Liver	Nov. 1952	0.27 0.31	0.78 0.71	1.00 1.00	6.3 6.8	7811 5638	177.5 115.5	4.0 4.0	4.33 3.30
3	Z 804 Copenhagen	Autopsy 1951 14 days after injection	40 ml U.S. thorotrast Batch not known	1951	14 days	0.49	Spleen Liver Bone marrow Hilus hepatis	Dec. 1953	0.76 0.51 0.66	0.39 0.49 0.42	0.68 1.00 0.76	40 40 40 40	2388 9353 3418 269	2.00 11.32 5.95 52.33	3.8 4.1 3.1 3.2	9.95 7.49 6.12 0.054
4	Z 1 Copenhagen	Tonsilectomy 1947, splenectomy and biopsies 1949	22 ml	1939	Tonsils 8 years Others 10 years	0.78	Spleen medulla Spleen Hilum of spleen Liver Lymphnode Tonsil Pancreas	May 1951	0.45 0.33 0.59 0.29 0.45 0.27 no activity	0.54 0.67 0.45 0.74 0.54 0.76	0.66 0.83 0.56 0.91 0.66 0.95	2 4 5 49 12 25	564 289 689 4528 322 902	0.26 0.73 1.59 3.16 4.13 21.2	5.8 4.9 9.7 4.5 6.2 5.3	203 25.4 8.99 9.85 1.13 0.49
5	Z 363 Copenhagen	Splenectomy 1950, autopsy 1952	25 ml	1939	12 years	0.74	Spleen Liver Bone marrow 1 " " 2 Bone	July 1953 Dec. 1953	0.31 0.45 0.58 0.48 no activity	0.70 0.54 0.46 0.51	0.96 0.59 0.47 0.55	33 33 40 40	1323 3016 700 495	12.20 13.6 2.49 5.31	4.7 5.8 4.8 4.2	9.37 1.36 1.55 0.62
	Rabbit 5 Copenhagen	Autopsy 1949 8 months after injection	2 ml German thorotrast	1949	8 months	0.78	Spleen Liver Marrow from femur Femur Lung Hilus of lung Kidney Heart Thymus Lymphnode	Nov. 1951	0.68 0.48 0.55	0.41 0.51 0.47	0.37 0.50 0.45	4 5 6 154 154 154 154 154 154 154	1800 314 171 708 275 213 26 75 118 27	1.00 1.36 0.70 4.13 7.52 6.68 1.43 6.07 13.10 19.55	10.0 5.0 5.0 3 3 3 3 3 3 3	48.1 14.4 8.95 2.38 1.10×10^{-4} 0.96×10^{-4} 0.55×10^{-4} 0.37×10^{-4} 0.27×10^{-4} 0.04×10^{-4}

17 Activity per mm^3 per min. in vivo	18 Activity relative to spleen	19 Activity per 20 ml/ age-factor	20 Effective energy MeV	21 Fraction energy dissipated in tissue	22 Dose to dry tissue rads per week	23 Estimated dose to wet tissue rads per week	24 Reduction in dose due to self-absorption	25 Dose per 20 ml/ age-factor rads per week
2.01	1.0	7.95	5.86	0.89	1.68	0.84	1.1	3.30
2.01	1.0	7.95	5.86	0.89	1.68	0.84	1.1	3.30
4.33	1.0	6.55	5.89	0.95	3.90	1.95	1.1	2.95
3.30	0.76	5.00	5.83	0.95	2.94	1.47	1.1	2.23
9.95	1.0	10.2	5.21	0.81	6.78	3.39	1.2	3.47
7.49	0.76	7.8	5.59	0.81	5.44	2.77	1.2	2.84
6.12	0.62	6.25	5.30	0.81	4.24	2.12	1.2	2.17
0.054	0.0054	0.055	5.33	0.99	0.46	0.23	1.0	0.24
203	8.0	237	5.33	0.018	3.14	1.57 - 4.71	55.5	3.67
25.4	1.0	29.6	5.68	0.27	6.25	3.12 - 9.37	3.7	7.30
8.99	0.35	10.5	5.05	0.29	2.12	1.06 - 3.18	3.4	2.47
9.85	0.39	11.5	5.80	0.56	5.13	2.56 - 7.69	1.8	6.00
1.13	0.045	1.69	5.33	0.81	0.78		1.2	0.91
0.49	0.019	0.58	5.88	0.67	0.31		1.5	0.36
9.37	1.0	9.10	5.76	0.38	3.30	1.65 - 4.95	2.6	3.58
1.36	0.146	1.33	5.31	0.47	0.55	0.27 - 0.82	2.2	0.60
1.53	0.164	1.49	5.21	0.66	0.85		1.5	0.92
0.62	0.066	0.61	5.05	0.53	0.27		1.9	0.29
48.1	1.0	*17.7	4.86	0.31	11.7		3.2	*4.30
14.4	0.30	5.25	5.26	0.65	7.93		1.5	2.92
8.95	0.19	3.27	5.12	0.73	5.40		1.4	1.98
2.38	0.048	0.85		0.72	1.40		1.3	0.51
1.10×10^{-4}				0.77	10^{-5}		1.3	
0.96×10^{-4}				0.88	10^{-5}		1.1	
0.55×10^{-4}				0.72	10^{-5}		1.4	
0.37×10^{-4}				0.93	10^{-5}		1.0	
0.27×10^{-4}				0.93	10^{-6}		1.0	
0.04×10^{-4}				0.97	10^{-6}		1.0	

*Calculated assuming 20 ml in a patient weighing 70 Kgm is equivalent to 0.57 ml in a rabbit weighing 2 Kgm



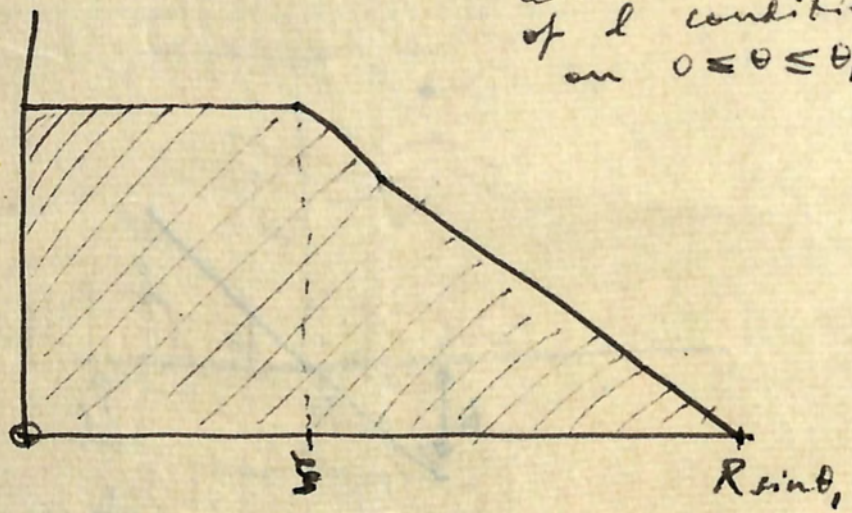
R. Observing. $d \ll R$.
Tracks inclined $\not\perp$ to $\theta - \theta$.

First admit negative as well as positive values of l. With restriction of θ to the range 0 to θ_1, for fixed h the distribution of l is uniform between $-h$ and $-h + R\sin\theta_1$. Integrating with respect to h from 0 to d and discarding negative l, we get a distribution which is ~~triangular from~~ continuous and

{ uniform from 0 to ξ
{ triangular from ξ to a zero ordinate at $R\sin\theta_1$

where $\xi = \max(R\sin\theta_1 - d, \quad 0)$.

distribution of l conditional on $0 \le \theta \le \theta_1$



$[\xi = \max(0, R\sin\theta_1 - d)]$

TISSUE DOSAGE FROM THOROTRAST IN THE BODY

By Prof. J. ROTBLAT and GILLIAN B. WARD

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Tissue Dosage from Thorotrast in the Body

DURING the past twenty-five years a large number of people have been injected with thorotrast for radiographic purposes. Thorotrast is a colloidal solution of thorium oxide which, after injection, is rapidly taken up by the reticulo-endothelial system, particularly the liver and spleen, where the thorium remains practically indefinitely, and only some of its products may be excreted. In the course of time the action of the radiations may give rise to certain clinical symptoms[1], and this fact offers an opportunity for studying the correlation between the clinical effects of radiations and the dosage delivered to tissue. The amount of thorotrast in the given organ is often estimated from a measurement of the γ-ray activity, mainly due to thorium-C″, observed with a Geiger–Müller or scintillation counter[2]. Since, however, more than 90 per cent of the total energy dissipated in tissue is carried by the α-particles, which have a very short range, it is clear that a quantitative study of the radiation dosage requires a technique employing microscopic analysis. Furthermore, it is known[3] that thorotrast in the body forms aggregates of size similar to, or even greater than, the range of the α-particles ; some of the energy of the α-particles is thus absorbed in the thorotrast itself, and in order to determine the energy dissipated in tissue this self-absorption has to be taken into account.

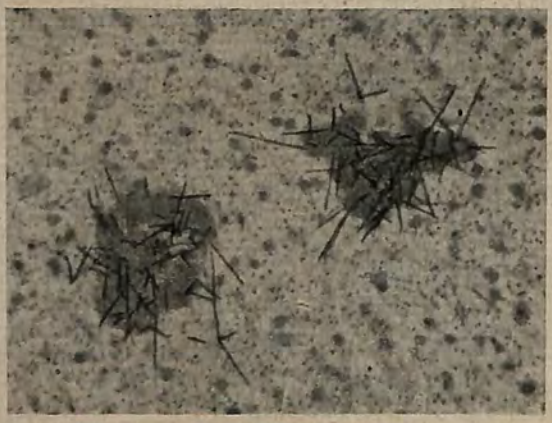
We have studied this problem by using the technique of α-track autoradiography. Histological sections, 3–10 μ thick, from organs containing thorotrast were obtained and used to prepare autoradiographs by means of Ilford *C*.2 nuclear emulsions. Two methods were employed : in one, liquid emulsion was used ; it was poured on to the mounted sections to a thickness of 50–100 μ and then dried quickly. In the second method, *C*.2 plates were placed directly in contact with the sections and exposed under light pressure to ensure intimate contact. After the required period of exposure the sections were processed in the usual way and the α-particles observed through a microscope.

In order to calculate the tissue dosage it is necessary to determine the radioactive content of the section, that is, the activity of thorium and of each of its α-emitting products contained per unit volume of tissue. If the section were infinitely thin the various radioactive substances could be easily identified by

the length of the α-particle tracks in the emulsion. With a section of finite thickness the length of track observed in the emulsion is shorter than the true range ; even a single radioactive isotope in the section gives a continuous distribution of track lengths, from zero to the full range. The shape of this distribution curve can, however, easily be calculated. The measurement of the lengths of the tracks from a given area of the section thus makes it possible to determine the radioactive content of the section. Furthermore, by comparing the relative amounts of radiothorium and thorium in the section with those in the thorotrast itself, it is possible to deduce what the radioactive content was in the organ *in vivo*.

The next quantity necessary for the determination of tissue dosage is the fraction of the total energy of the α-particles dissipated outside the aggregates from which they were emitted. This can be calculated from a measurement of the areas of the aggregates in the sections by means of a square eye-piece graticule. The thorotrast shows up as yellow granular deposits against the blue-stained cell nuclei ; the accompanying photomicrograph shows the appearance of some large aggregates, together with α-particle tracks recorded in the emulsion. From the observed distribution of aggregate sizes it is possible to calculate the fraction of the total energy, F, from all α-emitting substances, dissipated in tissue.

The average tissue dosage is given by the expression $D = NF\overline{E}$, where N is the total activity of the organ, that is, the total number of disintegrations per unit time, per unit volume of tissue, and $\overline{E}$ is the



Photomicrograph of liver section showing large thorotrast aggregates with α-particle tracks

Section	Disintegrations per min. per mm.³ of tissue N	Percentage of energy dissipated in tissue F	Effective energy of α-particles $\overline{E}$ (MeV.)	Energy dissipated in tissue per min. per mm.³ of tissue D (MeV.)	Tissue dosage (rep. per week)
Spleen (interior portion)	260	1·8	5·33	24·9	4·8
Spleen (exterior portion)	28·2	26·5	5·68	42·5	8·3
Hilum of spleen	12·3	29·2	5·05	18·1	3·5
Liver	12·8	56·3	5·80	41·8	8·1
Lymph-node	1·45	81·0	5·33	6·2	1·2
Tonsil	0·49	66·6	5·88	1·9	0·4

'effective energy' of the α-particles, which is related to the radioactive content of the organ.

The accompanying table gives an example of the results of such calculations of tissue dosage. All figures refer to one patient, who was given an injection of 22 ml. of thorotrast in 1938. A splenectomy was performed in 1949 and biopsy samples from several other organs were taken at the same time; the autoradiographs were prepared in 1951.

The figures in the last column of the table give the tissue dosage in rep. per week. In the organs which took up most of the thorotrast, the spleen and liver, the tissue dosage was of the order of 8 rep. per week. Assuming a relative biological efficiency of 20 for α-particles[4], this corresponds to about 160 röntgens of X-rays per week. The accepted value of the tolerance dose for whole-body irradiation is 0·3 r. per week[4].

If all the products of thorium remained in the organ and if the thorotrast were mixed homogeneously with tissue, the tissue dosage to the spleen of the patient referred to in the table would be expected to be about 40 rep. per week. The greatly reduced dosage-rate actually observed is partly due to an escape of some products from the organs but mostly to the formation of the aggregates with the resulting self-absorption of the α-particles. As a consequence of this absorption the tissue dosage is not proportional to the concentration of thorotrast in the organ. This can be seen clearly when comparing the values in columns 2 and 6 of the table. While the total activity of the various organs, which roughly corresponds to the amount of thorotrast in them, varies by a factor of 500, the tissue dosage delivered to these organs varies only by a factor of about 20. The process in

the body which produces the aggregates, probably of a physico-chemical nature, acts thus as a kind of compensating mechanism which results in a reduction of the radiation delivered to tissue.

The tissue dosage shown in the table represents average values for the given organ. Actually, within each organ the dosage is distributed non-uniformly. In most organs the distribution of the thorotrast aggregates shows the presence of a large number of small aggregates, of radii about 5 μ, and a very small number of large aggregates. These few large aggregates contain a considerable proportion of the thorotrast in the organ, and despite the self-absorption they produce a very intense tissue dosage in their immediate neighbourhood. On the other hand, the numerous small aggregates produce a uniform dosage to the whole organ, which is several times lower than the average value. The dosage delivered to tissue consists, therefore, of a uniform radiation of the order of 0·3–0·8 rep. per week, with a few hot spots in which the dosage may be fifty or even a hundred times higher. The significance of these results on the occurrence of clinical symptoms of radiation damage has yet to be established.

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[1] See, for example, Hughes, R., *Proc. Roy. Soc. Med.*, **46**, 191 (1953), where further references are given.
[2] Ward, A. H., and Jensen, P. G., *J. Sci. Instr.*, **29**, 181 (1952).
[3] Bloom, W., "Histopathology of Irradiation from External and Internal Sources", 543 (McGraw-Hill, 1948).
[4] International Recommendation on Radiological Protection, *Brit. J. Radiol.*, **24**, 46 (1951).

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