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ON THE QUANTITATIVE EVALUATION OF AUTORADIOGRAMS

BY

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Synopsis

Results of an investigation into the possibilities for a quantitative interpretation of autoradiograms on the basis of grain counting or track counting are presented. The relation between track length, number of grains, and initial energy of the beta particle is studied, and the blackening produced by a point source of a beta emitter, e. g. carbon-14, surrounded by nuclear emulsion is described in terms of the distribution of grains in space. It is shown how an analysis of this type can be carried out for any pure beta emitter. However, the present measurements are confined to beta particles of up to c. 400 keV.

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Chapter I

I, 1. Introduction

When ionising particles, such as beta particles emitted by radioactive nuclides, pass through a photographic emulsion, some of their energy is transferred to the emulsion, and a number of silver halide grains is rendered developable. Due to this property, photographic emulsions have been used in order to demonstrate the presence of a source of beta particles in a specimen. In biological terms, this has often involved the recognition or identification of labelled cells in a tissue section or smear.

The autoradiographic techniques that have evolved fall broadly into two categories—those in which a relatively thick emulsion layer (of the order of 100 microns or more) is used and the passage of a beta particle is recognized as a continuous track of silver grains, and those in which a thin layer of emulsion (of the order of 10 microns or less) registers beta particles as a few blackened grains at most.

Clearly, the greater the number of ionising particles traversing a layer of photographic emulsion, the greater the number of tracks or grains one might expect to find. In principle, there is no reason why this method of recording the presence of ionising radiation should not be used in a strictly quantitative manner. In other words, it should be possible to calculate the number of beta particles entering a given volume of emulsion from the number of grains or tracks formed.

I, 2. Relative quantitation

Autoradiography has been used to provide estimates of the degree of labelling of one source relative to that of another by comparing the number of grains or tracks in comparable volumes of emulsion overlying or surrounding the sources studied. Also this type of quantitation, which should be called relative in contradistinction to the absolute measurements referred to in the previous section, requires strict control of technique at four stages, if it is to be reliable.

First, the source to be studied must be presented to the emulsion in such a way that loss or translocation of isotope does not occur, neither before nor during exposure. This prerequisite is difficult to ensure with biological material. The assumption is often made that loss of isotope during histological processing, if it does occur, will be the same in various parts of the tissue, so that a comparison of residual activities in different regions gives reliable information on the relative amounts of radioactive material present *in vivo*. Levi, 1957, working with crystals of S-35 labelled barium sulphate, has drawn attention to the possible movement of even sparingly soluble radioactive material, particularly in ionic form, into and through the emulsion during exposure.

The *second* critical factor in technique is the geometrical relation between source and emulsion. Unless this is shown to be the same for the various structures whose autoradiograms are to be compared, the results cannot be related accurately. If, for example, one cell type is larger than another, or lies deeper in the tissue section (i. e., further away from the emulsion), if the emulsion layer is thicker in one place than in another, or the section varies in thickness, relative quantitation becomes an illusion, unless these factors have been recognised and corrected for (cf. Levi, 1957; Perry, 1961).

The *third* prerequisite for relative quantitation is the standardisation of the photographic processing. Conditions of exposure, viz. temperature, humidity, and the presence of oxidising agents, affect the stability of the latent image. Moreover, the type, the concentration, and the temperature of the developer have considerable influence on the image produced.

Finally, the grains or tracks in the relevant volumes of the emulsion must be counted, and the results compared. This comparison rests on the assumption that the emulsion response is linearly related to the number of incident particles. It is well known that at grain densities higher than those which can be evaluated visually, emulsion saturation begins to play a part, so that proportionality between incident radiation and emulsion response no longer prevails. It is, therefore, important that the exposure time be chosen so that all structures to be compared show blackening which does not exceed the linear part of the characteristic curve (*H*- and *D*-curve; Mees, 1952).

From the above it is clear that relative quantitation of autoradiograms is feasible only under carefully controlled conditions. However, it is worth keeping in mind that the sources of error become increasingly significant as the differences in labelling of different structures to be compared become smaller. Aside from the purely technical problems, it is in the nature of radioactive decay that all observations are subject to statistical fluctuations, which brings an additional uncertainty to the evaluation.

I, 3. Absolute quantitation

The exact determination of the number of beta particles entering a given volume of emulsion, and hence the number of disintegrations taking place in the source per unit time, requires still more stringent control of technique. Obviously, the requirements discussed in the preceding paragraph must be fulfilled and, in addition, the emulsion response, i. e. the number of grains or tracks observed in the emulsion, must be converted into the number of disintegrations taking place in the source.

Grain counting.

Attempts at correlating the number of developed grains observed in the emulsion with the amount of label in the source have been made by several investigators (Howard and Pelc, 1951; Herz, 1957; Lajtha, 1952; Marinelli and Hill, 1948; Lammerton and Harris, 1954; Odeblad, 1950). They defined the term grain yield as the number of grains produced in an emulsion layer by a source which, in the majority of cases, was separated from the emulsion. Grain yield will then depend first of all on the geometrical relation between source and emulsion, and on a great many other variables.

Tables of grain yield are available in the literature (loc. cit.). Each figure represents the grain yield as determined under the conditions of one particular experiment. Some of these determinations have been made by covering a large, uniform source of beta particles with a thin layer of emulsion. The average optical density of the processed emulsion (after exposure) and the average number of incident beta particles were measured independently, and the number of grains produced per incident particle was calculated. Some investigators made use of a point source and determined the "grain yield" as above. However, since grains produced by a point source of beta particles may be found at a considerable distance from their origin, it is misleading to present a figure for grain yield without specifying the volume of emulsion within which the grains were observed.

It is therefore difficult to justify the use of such figures in converting grain counts into terms of disintegrations in the source under different experimental conditions. Autoradiographic grain yield in this sense is comparable to the efficiency of a Geiger counter which is different in each arbitrary counting arrangement.

Track counting.

The determination of the number of disintegrations taking place in a source by means of track autoradiography requires the recognition of every track originating from the source.

A first attempt at correlating the number of beta tracks observed in the emulsion with the amount of label in the source was made by Levi, 1954, who suspended C-14 and S-35 labelled algæ and yeast cells in Ilford G-5 emulsion. In these experiments, absolute quantitation was not even attempted in view of the variation in the degree of labelling of individual cells. When the same author used uniformly labelled barium sulphate crystals as beta emitters (1957), useful information on the possibilities of track autoradiography for absolute quantitation was gained. However, this system proved unsatisfactory mainly because of artefacts due to the solubility of the crystals.

Levinthal, 1957, devised experiments based on absolute quantitation of beta track autoradiography. He suspended virus particles labelled with P-32 in thick layers of G-5 emulsion. The position of a virus was recognised by the star of beta tracks originating from a common centre. Levinthal et al. (loc. cit.) were able to prove that the number of tracks in a star was a direct measure of the number of disintegrations that had occurred in the virus during exposure.

A possible approach.

An approach to absolute quantitation of autoradiograms can be made by establishing the possibility for quantitative *track* autoradiography and from there to proceed to an investigation into the quantitation of *grain* autoradiograms.

The starting point could be the study of a mathematically simple model, for example a point source emitting beta particles with a known energy spectrum, surrounded on all sides by an emulsion layer of infinite thickness as compared to the maximum range of the particles. If the source is small enough for self-absorption to be negligible (a prerequisite fulfilled in the present experiments, but seldom realized when the beta particles originate from biological material), the only adjustments that need be applied to the observed track count are 1) a correction for beta particles that have failed to give rise to a recognisable track and 2) a correction for background tracks due to cosmic radiation.

An adequate description of this simple model must include an analysis of the relationships between particle energy, track length, number of grains per track, and spatial distribution of silver grains relative to the source. It then becomes possible to express the disintegration rate in the source both in terms of track counting and of grain counting.

The question remains, however, whether the description of the simple model will open the way for an analysis of the much more widely used, but mathematically complex systems where the source is covered by emulsion only over part of the space angle, and different media (air, gelatin, glass) surround the source over the remaining space angle. In this latter model, difficulties arise not only from the more complex geometry, but mainly from the fact that media with different densities will make range and yield evaluations extremely difficult. Practically all autoradiographic studies on biological material—by means of stripping film or thin coats with liquified emulsion—fall within the last mentioned category.

The work to be reported in the present paper was undertaken to follow up earlier investigations dealing with the above mentioned simpler case: source embedded in emulsion (Levi and Nielsen, 1959). By studying sources of molecular dimensions in Ilford G-5 emulsion and using beta emitters with a wider range of energies, the relationships between particle energy, number of grains per track, and spatial distribution of grains relative to the source were investigated. Some discrepancies between results reported earlier by Levi and Nielsen (loc. cit.) and by Zajac and Ross (1948) required clarification.

I, 4. Definition of terms

Grain yield.

The factor which relates the number of grains to the number of ionising particles producing them is known as the grain yield. It is defined as the ratio of the number of developed grains in a given volume of the emulsion to the number of ionising particles entering this volume. Several factors affect grain yield. They may be considered in three main groups.

1) The nature of the source: The energy spectrum of the isotope is the first consideration. Beta particles of tritium (E-max = 18.5 keV) will produce fewer grains in emulsion than those of radiophosphorus (E-max = 1.6 MeV).

2) The nature of the emulsion: If one emulsion is more highly sensitised than another with equal grain size, the grain yield will be higher in the former than in the latter. If, in two emulsions of equal sensitivity, the grain size of the first is smaller while the total concentration of silver halide is the same in both, a given loss of energy by an incident particle is likely to produce more grains in the first than in the second emulsion.

3) Conditions of exposure: Conditions of exposure will affect grain yield because, f. ex., oxidising agents or water promote latent image fading. It has also been demonstrated that, to a lesser extent, the time of development, the chemical composition of the developer as well as the temperature will influence the number of grains rendered visible in the emulsion (cf. Demers, 1959; Ahmad and Demers, 1959).

Beta tracks.

Tracks produced by beta particles in nuclear emulsion are characterised by the zigzag path of the particles and the irregular spacing of the silver grains in each track. The higher the energy of the beta particle, the longer its track and the wider its grain spacing in the beginning of the track. With decreasing energy, scattering and grain density (number of grains per unit path) increase.

A track is usually defined as 4 or more silver grains in a row. Therefore, a particle belonging to the low energy part of the continuous beta spectrum of any isotope may not produce sufficient grains to be recognised as a track. In the case of P-32, the proportion of unrecognisable tracks is very small and, at the same time, the majority of the tracks is fairly straight up to a considerable distance from the source (cf. Levinthal's "stars", 1957).

In the case of C-14, the energy of a large fraction of the particles is so low that they will not produce recognisable tracks, because even 3 grains in a group cannot be distinguished from random arrangements of background grains. This problem has been studied in detail by Levi and Nielsen, 1959.

Delta tracks.

Beta particle tracks in nuclear emulsions sometimes branch. Branching occurs when the particle, on its passage through matter, imparts sufficient energy to an orbital electron to knock it out of orbit. Since the ejected electron has the same mass and charge as a beta particle, the characteristics of its track are the same as those of the primary particle. The orbital electron is known as a delta ray. The two branches of the resulting track configuration lie very nearly at right angles to each other.

Range-energy relation.

The term "range" has been applied to beta particles in two different senses. It is frequently used to describe the point-to-point distance travelled by a beta particle of a given energy in a medium of known composition. It is just as often used to denote the furthest distance from the point of origin that a beta particle of given energy will reach in a medium of known composition. Since beta particles undergo scattering on their passage through matter, the "range" by the second definition is always shorter than that given by the first. For low energy betas, f. ex. C-14, the difference between the two distances is appreciable.

To avoid confusion, the grain-to-grain distance travelled by a beta particle in the emulsion will be referred to in the present paper as the *track length*. The distance from the origin of the track to the point furthest away from the origin will be called the *radius* of the track. This is, obviously, the radius of the smallest sphere around the point of origin that contains the entire track.

The mean track lengths of beta particles of known energy can be predicted with great accuracy from theoretical considerations. Demers has constructed a table relating mean track length to energy for beta particles in Ilford G-5 emulsion, and this relationship is confirmed experimentally (cf. Zajac and Ross, loc. cit.).

The maximum probable radii for groups of beta particles have been investigated by interposing absorbers of known density between the source and the detector. This is the familiar "range" determination expressed in terms of mgm/cm^2 (cf. Glendenin, 1948).

The relationship between track length and radius has not been investigated except by Herz, 1949, who studied the depth of penetration of beta particles into a layer of nuclear emulsion.

Chapter II

Experimental

II, 1) Materials

The beta-emitting isotopes used in this work were

1) carbon-14 (E_{max} 155 keV) as labelled glucose in aqueous solution and as labelled yeast cells suspended in water;

2) calcium-45 (E_{max} 250 keV) in ionic form, carrier-free in water;

3) chlorine-36 (E_{max} 714 keV) in ionic form, carrier-free in water.

The emulsion was Ilford G-5 nuclear emulsion in gel form which was used within two weeks of dispatch. Prior to use, the emulsion was stored in a refrigerator at 4° C within a lead shield.

3″×1″ microscope slides were thoroughly cleaned in chromic acid, washed over night, and "subbed", i. e. coated with a thin gelatine layer, as prescribed for use with stripping film.

II, 2) Preparation of slides without cells (sandwich plates).

The stock solution containing the radioactive tracer was diluted with distilled water to a concentration which, by trial and error, was found to produce a reasonable track density in the emulsion in the course of 18–40 h of exposure.

In the dark room, the subbed slides were placed on a levelled glass surface at room temperature (20-25° C). In safelight, the required volume of G-5 emulsion was transferred to a 25 ml measuring cylinder placed in a thermostatically controlled water bath at 42° C. In the course of 10 min, the emulsion had liquified. Subsequently, the molten emulsion was filtered through gauze into another measuring cylinder, and diluted with half its volume of distilled water preheated to 42° C. After 2-4 min of gentle stirring at 42° C, to ensure complete mixing, the diluted emulsion was filtered a second time and kept in a measuring cylinder in the water bath ready for use. Using a Carlsberg pipette with a wide (broken) tip, 1 ml of the molten diluted emulsion was withdrawn and mixed, in a small glass jar, with 100 μl of the aqueous solution containing the tracer.

With another Carlsberg pipette, 1 ml of molten, diluted emulsion was placed on each slide, and spread gently with a fine paint brush to cover the whole surface of the slide. This layer was then left to gel for about 15 min. Next, one drop of the emulsion containing the radioactive isotope was placed in the centre of the slide and spread to cover an area of 2×3 cm^2. Finally, 1 ml of inactive emulsion was pipetted on top and spread to cover the whole slide.

The slides were left on the levelled glass plate in the dark in a gentle current of air for about 3 h, when they appeared dry. They were then placed in plastic slide boxes, and the open boxes were transferred into a desiccator containing dry silica gel. A current of dried CO_2, obtained from evaporating dry-ice in a flask, was passed over the plates for about 20 min and, finally, the slide boxes were stored in a refrigerator at 4° C inside an iron and lead shield.

Blanks were prepared in exactly the same way omitting, however, the central layer of emulsion containing the tracer.

The preparation of, say, 10 plates would require about 80 minutes, until drying began. Since the emulsion does not register tracks while the water content is high, the beginning of exposure cannot be timed exactly. It was estimated that the uncertainty of the exposure time of 20 h. was about 10 %.

Preparation of slides with labelled cells.

A series of plates were prepared containing yeast cells labelled with C-14[1]. The yeast, schizosaccharomyces pombe, was cultured for 24 h in a medium containing C-14 glucose. The cells were carefully washed 3 times in distilled water and resuspended in distilled water. In preparing the slides, a dilute suspension of cells was used to dilute the molten emulsion. 1 ml of emulsion containing the labelled cells was pipetted onto the slides and spread to cover the whole surface. Conditions of drying and exposure were the same as described above.

II, 3) Processing

The developer was made up as follows: 2.2 g of sodium sulphite (7 H_2O) were dissolved in 100 ml of dist. water. 0.46 ml of a sodium hydrogen sulphite solution (spec. gravity 1.34) were added to 210 ml of dist. water. These solutions were mixed and 1 g of Amidol was added. The developer was filtered and used immediately.

Development of the plates followed the temperature cycle method of Dilworth et al. (1948), with a modification suggested by Hauser (1959), who obtained more uniform development of thick plates, if the slides were soaked in full strength developer at 5° C and then immersed in dilute developer during the warm stage. Unless otherwise stated, the following routine was adopted.

The slides were taken from the exposure boxes in the refrigerator and placed in developer at 5°C in the refrigerator for 15 min. They were then transferred to a dish containing one part of developer diluted with two parts of distilled water. The dish was kept in a thermostatically controlled water bath at 20° C and the plates were developed in this warm stage for 25 min. They were then transferred to a stop bath of 1 % acetic acid at room

[1] The authors gratefully acknowledge the help of Dr. C. Chapman Andresen, Carlsberg Laboratory, who prepared the C-14 labelled yeast cells.

temperature for 15 min. During this period, the emulsion surface was gently swabbed a few times with moist cotton wool.

Fixation took place in 3 changes of 30 % hypo at room temperature with gentle mechanical stirring. The volume of each change of hypo was 1 liter and the fixation time 7 h. Finally, the slides were washed in running tap water for 3 h and in distilled water for additional 30 min. They were dried in air in a horizontal position and under a dust cover.

II, 4) Measurement of emulsion thickness

Several slides of both types were taken from the dark room after drying (unprocessed) for measurements of emulsion thickness. The slides were measured mechanically (micrometer), optically (microscope), and by weighing. The optical thickness estimates were obtained by cutting a thin channel through the emulsion layer approximately in the middle of the slide. Under the microscope, the upper surface of the emulsion layer and the upper surface of the glass slide exposed in the channel were brought in focus, and the distance between these surfaces was read on the fine adjustment screw of the microscope. The graduation of the fine adjustment provided a direct measure of emulsion thickness.

All slides were weighed before and after application of the emulsion layer. Since the density of the dried emulsion is given by Ilford to be 3.85, the thickness of the layer could be calculated.

The results obtained by the three methods agreed well, although the thickness determined by weighing was consistently less than that found optically or with the micrometer. The former gives a mean value for the whole surface of the slide, while the two others give the emulsion thickness in the centre of the slide. The results are listed in table I.

TABLE I

Emulsion thickness

Method	Sandwich slides	Single layer
Micrometer	130 μ (125–140)	55 μ (50–60)
Weighing	116 μ	50 μ
Optical	—	57 μ (48–68)
Accepted value	130 μ	55 μ

II, 5) Microscopy

In order to reduce the errors involved in measuring distances in the emulsion in a plane perpendicular to the surface of the slide, re-swelling of the emulsion was found advantageous. After photographic processing, the plates were soaked in 20 % glycerol for 30 min, excess moisture was removed with a fan and, subsequently, the slides were mounted using a drop of Farrant's medium and a cover slip. The resulting preparation was found to be optically satisfactory. The refractive index of the emulsion was determined to be about 1.56.

The thickness of the emulsion after re-swelling was estimated optically. 21 measurements carried out on 7 different sandwich plates ranged from 100-149 microns with a mean of 125 microns, and this was regarded as sufficiently close to the estimated thickness during exposure of 130 microns.

All optical measurements were carried out by one observer (A.W.R.) using a Leitz ortholux microscope with a Ks×45 objective and ×10 Brillenträger oculars. One ocular contained a graduated scale. The length of this scale was calibrated by means of a reference micrometer slide.

Distances parallel to the surface of the slide were measured with this scale, and distances in the optical axis of the microscope were measured using the graduation of the fine adjustment screw of the microscope.

Track lengths were measured from the first grain of the track, which could easily be identified in most cases because beta tracks show a characteristically higher grain density and larger grain size at the termination of the track. For length measurements, each track was divided into a number of relatively straight sections. The projected length of each section in the plane of the slide as well as the "dip", i. e. the length in the optical axis of the microscope, were determined, and the true distance traversed was calculated as the hypotenuse of the right-angled triangle thus constructed. Where delta tracks were encountered, this fact was recorded and the length of the delta track measured.

As mentioned previously, the number of tracks in the emulsion was adjusted so that tracks seldom overlapped or came so close as to cause confusion. At this track density, it was very unlikely for two tracks to lie end-to-end. Wherever doubt arose whether a particular configuration of silver grains represented one or two tracks, the pattern was interpreted as one continuous track.

Chapter III

General outline of the experiments and their interpretation

III, 1) General

The purpose of preparing so-called sandwich slides was to confine the origins of the beta tracks to a narrow layer of emulsion, separated from the supporting glass slide and the air by at least 50 μ of emulsion. This aim was not fully achieved. Although the majority of the tracks started in the centre of the emulsion layer, the origin of some tracks was only within about 30 μ of the glass support and 25 μ of the upper surface. Presumably, the warm emulsion of the active drop melted the inactive emulsion layer immediately beneath it, and a certain amount of mixing occurred also later when the top layer was added. Diffusion of the labelled ions during exposure cannot be excluded, either.

The majority of the carbon-14 tracks was fully contained in the emulsion. When calcium-45 and chlorine-36 were used, increasing proportions (c. 25 $^0/_0$ for Ca and a still higher, but not determined proportion for Cl) of the tracks left the upper and lower surfaces of the emulsion, and a similar increase was noted in the number of tracks re-entering the emulsion from the lower surface due to backscatter from the glass slide.

Most of the sandwich slides showed an artefact: shortly after processing of the emulsion, small brownish grains became visible throughout the emulsion. They were definitely smaller than the blackened grains of the tracks. In the course of a few days, these brownish grains were slowly replaced by large translucent crystals. Beta tracks could often be followed very close to such crystals without noticeable variation in grain size or -density. The nature of this artefact remained obscure. Since neither the visibility nor the grain yield of the tracks was affected, plates showing this artefact were used for counting.

III, 2) Track length and number of grains per track as functions of particle energy

One of the main problems of the present studies was to establish the relationship between beta particle energy and the number of grains produced in the emulsion. However, when using sources emitting a continuous beta spectrum, the initial energy of the particle giving rise to any particular track cannot be specified. Instead, the theoretical "range-energy relation" can be applied (cf. pp. 11 and 32). Zajac and Ross, 1949, found good correlation between calculated and experimentally determined track lengths when using monoenergetic beta particles and a Kodak NT-4 emulsion. Demers, 1958, has calculated the corresponding values for Ilford G-5 emulsion. As might be expected, the two sets of values are practically identical.

An attempt was therefore made towards an indirect determination of the relation between particle energy and number of grains per track by establishing experimentally the ratio between track length and number of grains per track in Ilford G-5 emulsion. On the basis of the theory linking track length and particle energy, it becomes possible to arrive at the desired ratio between the energy of the particle and the number of grains it is likely to produce.

To this end, sandwich slides containing C-14, Ca-45, and Cl-36, respectively, were used and tracks were selected for counting, applying the following criteria (group A, cf. p. 31).

a) The complete track must be contained in the emulsion, i. e. both the beginning and the end of the track must be located at some distance from the surface of the emulsion layer.
b) Tracks were selected which ran predominantly parallel to the plane of the glass slide.
c) Tracks were given preference which did not pass other tracks at very close distance so that confusion could arise.
d) Very tortuous tracks were avoided.

Grain counts were carried out on a total of 101 tracks, namely 30 tracks of C-14, 30 tracks of Ca-45, and 41 tracks of Cl-36. Each track was divided into sections as described on p. 15, the length of each section and the number of grains in it were recorded. The presence of delta tracks was also noted down, and their length and grain count recorded.

The mathematical evaluation of the results is dealt with in a separate chapter (Chapter V.). Summarising the findings, it can be stated that

1) it is justifiable to regard the tracks studied in slides containing C-14, Ca-45, or Cl-36 as one uniform population of tracks;
2) the grain count in, say, the terminal 25 microns of a long track is the same as that of a track whose total length is 25 microns (cf. p. 38);
3) a simple relationship between the mean number of grains per track and the track length has been found, and the same relationship holds for Zajac and Ross' determinations;
4) the standard deviation of the present measurements is of the same order of magnitude as that found by Zajac and Ross.

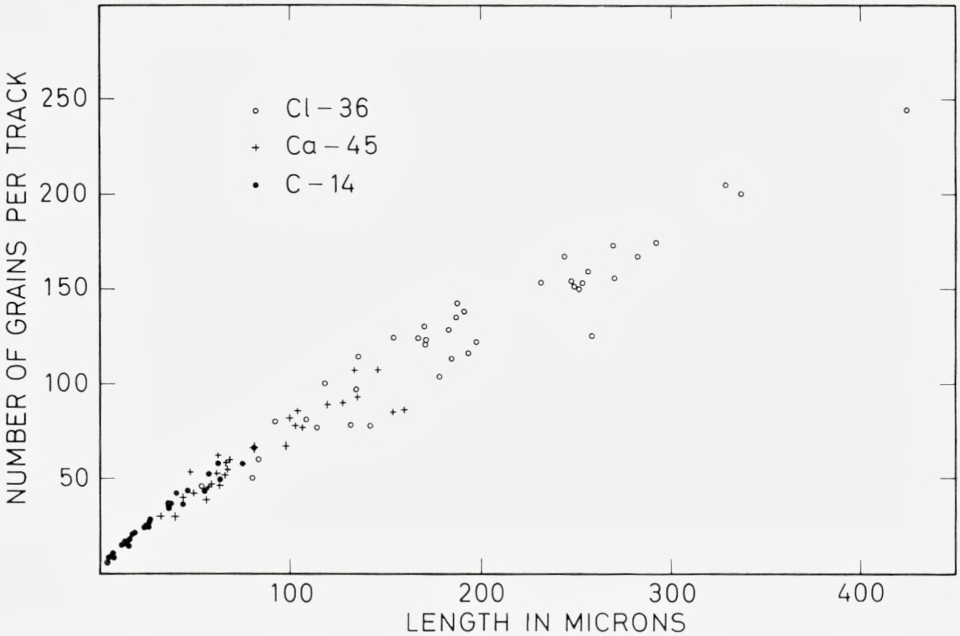

Fig. 1. Number of grains per track vs. track length measured on tracks of C-14, Ca-45, and Cl-36.

These observations are illustrated by Fig. 1 which shows a direct plot of the number of grains per track versus track length.

Using the figures for track length obtained in this series of measurements, the presumed initial energy of each of the beta particles studied was found from the tables given by Demers. The results obtained are plotted in Fig. 2 which shows the number of grains per track versus the calculated particle energy. Both the tracks analysed in the present study and those reported by Zajac and Ross are plotted in the figure. The particles studied had energies between 20 and 390 keV. Where a delta track occurred, it was treated as a separate beta particle, in order to estimate its initial energy. The parent particle was assumed to have lost this energy and to have created the corresponding number of grains at the point of origin of the delta track.

The numerical treatment of these problems is likewise described in chapter V.

III, 3) The energy spectrum of C-14 betas in terms of grains per track

The energy spectrum of C-14 depicted in Fig. 3 rises to a low peak at about 25 keV and then falls to the maximum energy of 155 keV.

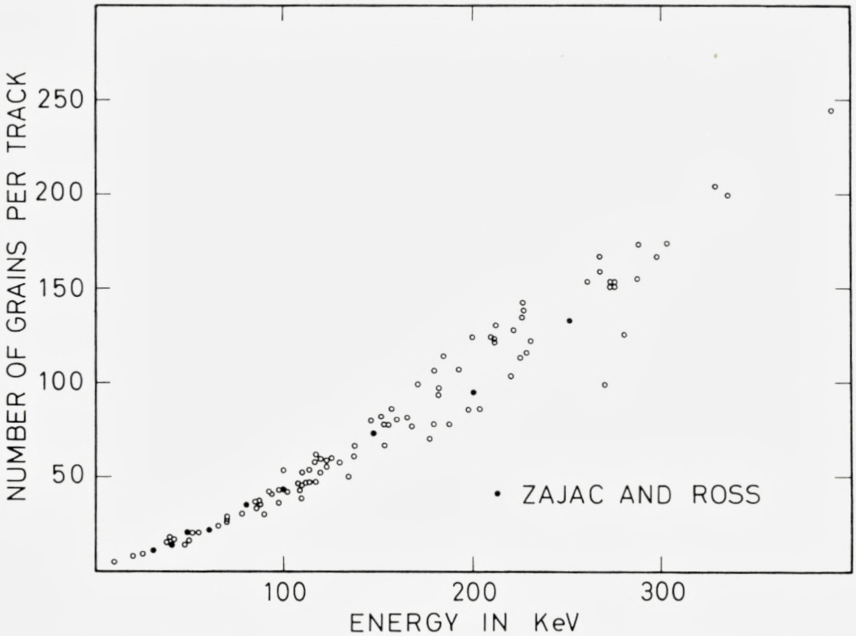

Fig. 2. Number of grains per track vs. presumed initial particle energy.

When calculating the distribution of the number of grains per track on the basis of the energy spectrum, it must be taken into account, however, that we are not dealing with a functional relationship but with a distribution of the number of grains per track for any given energy. On theoretical grounds (Bohr's equation) a considerable straggling in ranges and grain counts can be expected for a group of monoenergetic beta particles. Zajac and Ross (1949) found a standard deviation of about 20 % of the mean in their measurements of track length and their grain counts. If the distribution curve for the number of grains per track is corrected, assuming that each initial energy value will result in a population of grain counts, with a Poisson type distribution about the mean, a curve is obtained which is shown in Fig. 3 together with the energy spectrum. A detailed account of this calculation is given in chapter V.

In order to establish experimentally the distribution of the number of grains per track, a large number of randomly selected tracks was analysed on C-14 sandwich slides and on plates containing C-14 labelled yeast cells. The fields to be scanned under the microscope were chosen by predetermined settings of the mechanical stage (cf. Levi and Nielsen, 1959).

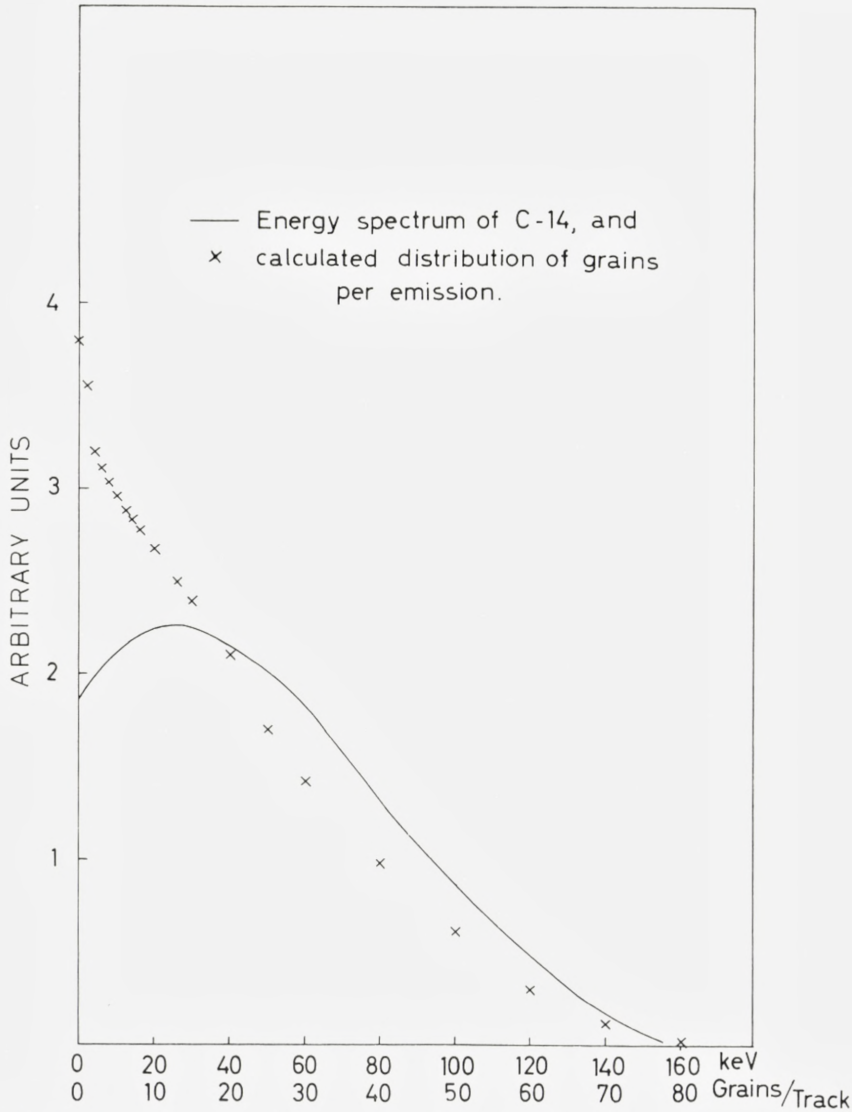

Fig. 3. Energy spectrum of C-14 and calculated distribution of grains per emission.

The following method of choosing tracks to be analysed in these fields of the sandwich plates was adopted (Group B p. 32). (1) All beta tracks lying in the chosen fields, whether complete or leaving the emulsion, were registered and the number of grains was counted. (2) Whenever tracks crossed the boundaries of the chosen field, an attempt was made to decide whether the track origin or its termination lay within the field. Only the tracks whose origin was within the field were included in the counts.

21 fields were scanned, and 191 beta tracks analysed, of which 163 were completely contained within the emulsion while 28 tracks left the emulsion at either top or bottom.

In order to assess the contribution of background electron tracks to this total, and to estimate their energy distribution, 58 fields were scanned on a blank sandwich plate. 90 background tracks were observed, 53 of them completely contained within the emulsion.

The following criteria were adopted for selecting the tracks to be counted on the slides containing labelled yeast cells (Group E, p. 32).

(1) Only single yeast cells, or pairs of cells, in the emulsion were chosen; fragmented cells or cell clumps were omitted.
(2) Yeast cells lying within 20 μ of the top or the bottom surface of the emulsion were omitted.
(3) Yeast cells indicating the presence of a chemographic artefact were omitted[1].
(4) Yeast cells giving rise to 10 or more tracks were excluded because the difficulty of interpreting the track pattern became too great.

The slide was placed in a predetermined position under the microscope, and moved along a straight line from side to side "east-west". Every yeast cell satisfying the mentioned criteria was used for counting. Every beta track originating from the selected yeast cells was included.

A total of 304 beta tracks, originating from 88 yeast cells was analysed. Considering the frequency of background tracks previously determined on blanks, the probability of including a background track that might originate from the area covered by a selected cell was felt to be so slight as to be negligible.

Qualitatively, the results obtained on both types of plates are in good agreement. The observed distribution of the number of grains per track is depicted in Fig. 4.

[1] The slides containing suspended yeast cells showed a chemographic artefact: Where the cells clumped together, a solid mass of silver often surrounded them. However, this mass was completely different in appearance from the discrete grains of the beta tracks. It has been suggested that ruptured cells might be responsible both for the clumping and the chemography. On the same plates, numerous individual cells and pairs of cells were present which were free from silver deposits, and clearly recognisable beta tracks originated from them. These cells were therefore used for counting.

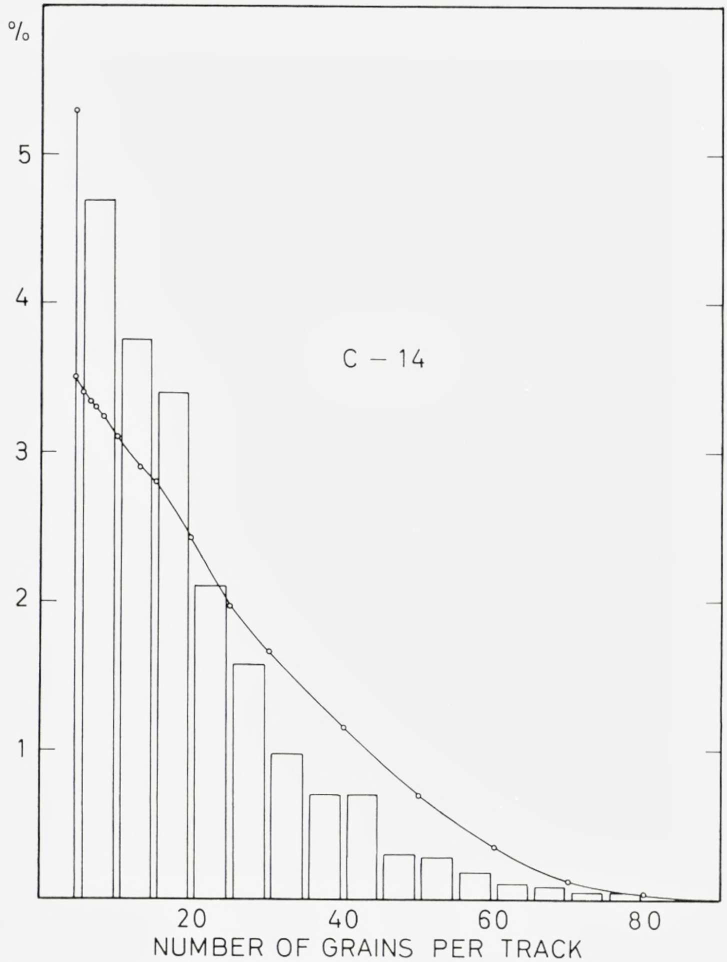

Fig. 4. Observed distribution of the number of grains per track for C-14 (histogram) and calculated distribution (cf. Fig. 3). The first bar of the histogram has been condensed into the line at 4 grains per track.

It appears from Fig. 4 that the shape of the calculated distribution curve agrees fairly well with the observed distribution, although the number of short tracks is somewhat higher than expected.

Since only 4 or more grains in a row form a recognisable track, 14 % of all C-14 beta particles (the sum of the first 4 ordinates) will not be recognised as tracks, and 4 % will not give rise to any grains at all under the conditions of this experiment, as calculated on p. 45.

III, 4) The energy distribution curve of Ca-45 betas in terms of grains per track

An attempt was also made to count the number of grains per track in a Ca-45 sandwich plate, and to compare the values observed with the predicted distribution. The criteria for selecting the tracks and for assessing the contribution of random background tracks to the observed total were the same as described on p. 20.

Thirteen fields were scanned and 195 beta tracks analysed, of which 136 were completely contained in the emulsion and 59 left the emulsion either at the upper or lower surface. As was to be expected, the statistical analysis of these figures was complicated by the higher proportion of tracks leaving the emulsion. Nevertheless, the agreement between the predicted and the observed distribution as illustrated by Figs. 5 and 6 is very close if the straggling effect (cf. above) is taken into consideration. $10\,^0/_0$ of the Ca-45 beta particles must be expected to give rise to less than 4 grains under the conditions of this experiment.

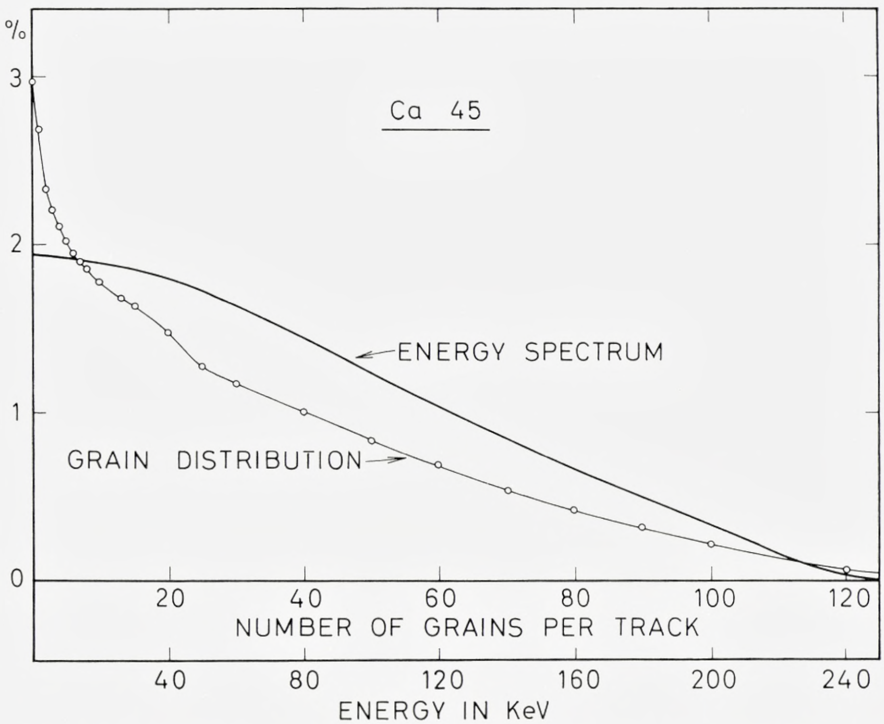

Fig. 5. Energy spectrum of Ca-45 and calculated distribution of grains per emission.

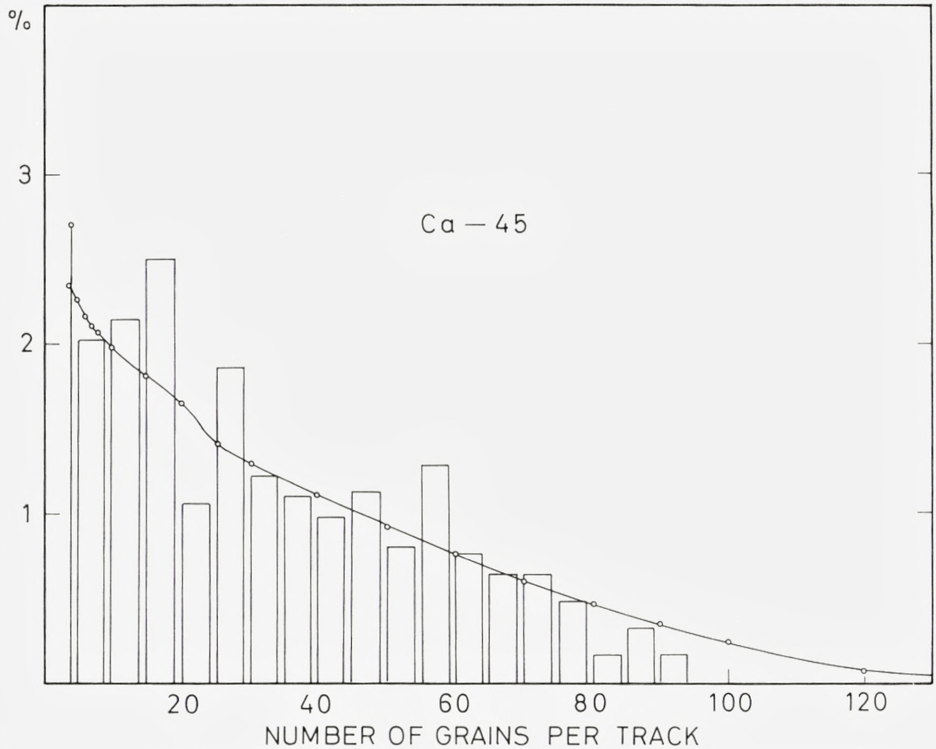

Fig. 6. Observed distribution of the number of grains per track for Ca-45 (histogram) and calculated distribution (cf. Fig. 5). The first bar of the histogram has been condensed into the line at 4 grains per track.

III, 5) The distribution of grains in space relative to the track origin

As discussed in the Introduction (p. 8) the simplest model to be studied with the aim of absolute quantitation must be a point source of a beta emitter surrounded by emulsion on all sides. The questions to be answered are 1) how many grains (or tracks) are being produced in the emulsion by a given number of disintegrations, and 2) what is the radius of the sphere around the source in which all grains (or tracks) will be contained? Therefore, the distance between the origin of a track and the point furthest away is of the greatest interest.

Little information could be found in the literature on the relationship between the track length and the radius of the sphere that will contain the entire track. In order to investigate this problem, two types of measurements were carried out on sandwich slides containing C-14 and Ca-45.

While the counts reported on pp. 20 and 23 were in progress, the distance between the first grain of the track and the grain lying furthest away was measured on every complete beta track encountered. Such measurements were made on 163 tracks of the C-14 slides and on 136 tracks of the Ca-45 slides.

The second type of measurement was performed on the C-14 plates, only (Group D p. 32). Tracks completely contained in the emulsion were divided into sections, each of which could be approximated by a straight line. The positions of the beginning and the end of each section relative to the first grain of the track were measured, and the number of grains in each section recorded. In this way,—and assuming fairly regular spacing of the grains within each section,—a three-dimensional picture of the position of each grain in each track relative to the track origin (the first grain) was obtained. 100 C-14 tracks were measured in this way. From this second type of measurement, the radius of each track can likewise be determined.

The figures obtained for C-14 and Ca-45 were found to constitute a homogeneous population. The mathematical derivation of the function which links the number of grains per track to its radius in microns is given on p. 40 and the distribution of radii is given in Table VI. Using the equations derived in section V, 3 for the interdependence of track length, particle energy, and number of grains per track, an equation is obtained that relates track length with radius, as well as initial particle energy with radius. It follows, for instance (cf. p. 42) that a sphere of G-5 emulsion with a radius of 20 microns around a point source of C-14 will contain 90 % of the beta tracks originating from this source. The corresponding radius of a sphere around a point source of Ca-45 is 45 microns (cf. p. 43).

For C-14, additional information can be obtained from the second type of measurement mentioned above. If the origins of the tracks examined are superimposed, a three-dimensional picture of the distribution in space of silver grains around this model point source can be constructed. Fig. 7 illustrates this distribution graphically. It follows that, for a point source of C-14 completely surrounded by G-5 emulsion, 29 % of the grains produced will lie within 5 microns of the source, 50 % within 9 microns, 75 % within 17 microns and 90 % within 25 microns. The second curve given on Fig. 7 is discussed on p. 45.

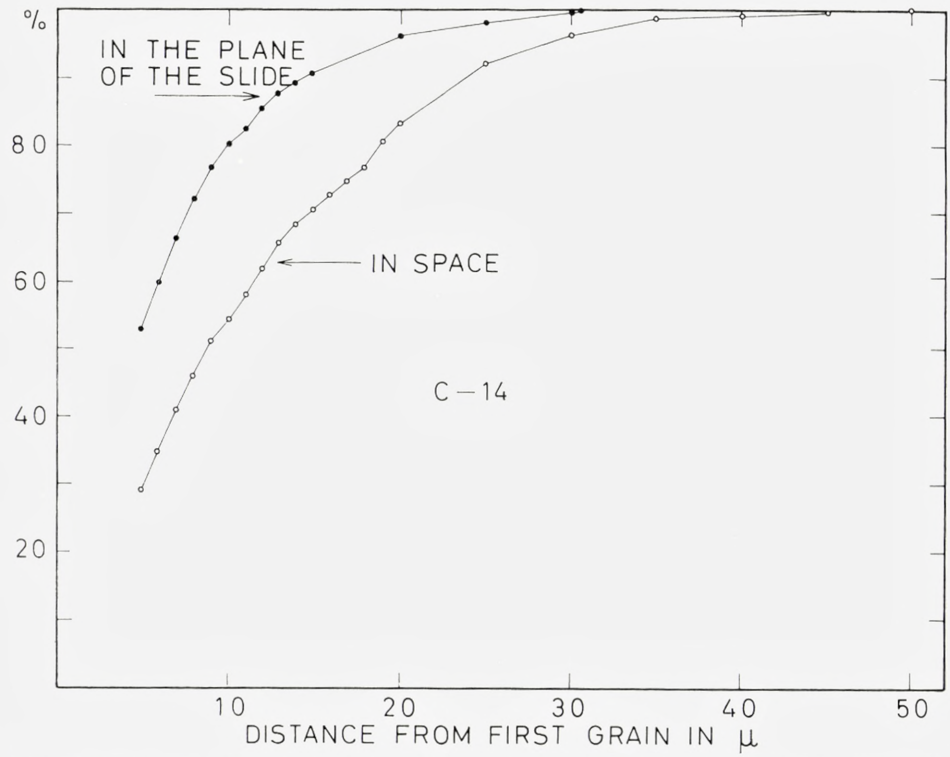

Fig. 7. Distribution in space, and in the plane of the slide, of silver grains around a model point source of C-14.

Chapter IV

Sources of error

The conclusions to be drawn from the experiments described in the preceding chapters rest entirely on the measurements of distances between points in the emulsion, and on grain counts. Some of the sources of error affecting these results are inherent in the processing and re-swelling of the emulsion and the resulting recognisability of detail. Others lie in the optical length and depth measurements, as well as in the criteria used for selecting the tracks to be studied. A critical discussion of these factors is therefore presented in the following sections.

IV. 1) Processing

As discussed earlier, (p. 10) processing conditions have a considerable influence on grain- and track yield. In particular, it was found desirable to investigate the effect of over- or underdevelopment, and to find the optimal development time for the plates prepared in the present study. A separate experiment was therefore carried out using a series of sandwich slides containing Ca-45. The plates were processed as described on p. 13, but the duration of the warm stage of development was varied between 5 min and 40 min. The criteria for the selection of the tracks (Group C p. 32) to be analysed were similar to those mentioned on p. 17. In order to obtain the desired information, only tracks longer than 75 microns were chosen. Using these criteria, the number of grains in the terminal 75 microns of the selected tracks was counted.

After only 5 min development, it proved extremely difficult to find tracks of the desired length. The terminal 20-30 microns could often be identified, but grain spacing was so irregular that the course of the earlier part of the track could only be guessed. This difficulty was not encountered after 10 min development. With increasing development time, the grain size as well as the number of grains increased. After 20 min development, the large grains in the terminal few microns of the tracks tended to fuse into a solid line of silver, which made grain counting difficult in this portion of the tracks. The random grain background was considerably higher after 40 min development as compared with the shorter processing times.

Twelve tracks were analysed at each of the following development times: 5, 10, 15, 20, 25, and 40 min.

The findings are illustrated in Fig. 8 where the grain counts in the terminal 75 microns of track are plotted against the development time. The mean grain counts increased considerably between 5 and 10 min, and more slowly from 10 to 15 and 25 min of developing, remaining practically constant up to 40 min. The grain densities expressed as number of grains per 25 micron sections, measured from the termination of the track, showed a similar pattern for all sections up to 125 microns (cf. p. 38).

From these observations it follows that significant fluctuations in grain yield did not occur as a result of slight variations in development time between 15 and 40 min in the particular conditions of this experiment. Clearly, the length of the plateau shown in Fig. 8 would have been shorter, and its slope steeper, if a more powerful developer had been used, or the temperature of development had been higher. Under different conditions,

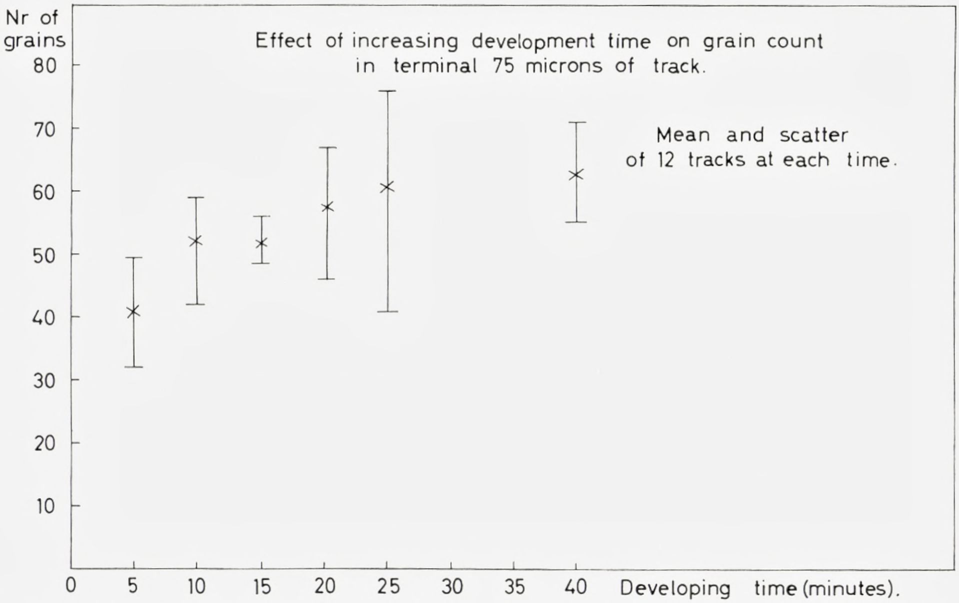

Fig. 8. Effect of increasing development time on grain count in terminal 75 microns of track.

the effect of development time might have been more critical. As mentioned in section II,3, the development time used in the present work was 25 minutes.

IV, 2) Reswelling of the processed emulsion

During fixing, the emulsion layer shrinks considerably, and the grains become superimposed very closely in the optical axis of the microscope. When a steep dip of the track occurs, individual grains cannot be identified. Similarly, delta tracks originating at right angle to the surface of the slide may be missed entirely. In sections of the track that lie perpendicular to the surface of the slide, grain counts are very difficult. Zajac and Ross (1948, 1949) likewise encountered some difficulties in interpreting their track patterns due to the fact that the processed emulsion was viewed without reswelling. Recognition and counting of grains as well as measurements of distances are definitely much easier in the reswollen emulsion. In this connection, it should be emphasized that the discrepancy between the number of grains per track for carbon-14 reported by Levi and Nielsen (1959) and the results obtained in the present study is probably due to the different thicknesses of the processed plates. The material from which the earlier results were derived was re-examined recently. Since the plates prepared by LEVI and NIELSEN had a higher track density, and the emulsion was viewed without reswelling, the track patterns were interpreted differently: whenever portions of a track lay at right angles to the plane of the slide, the heavily superimposed grains gave an appearance which was erroneously interpreted as the dense termination of a track; the continuation of a row of grains at a different focal level was assumed to represent the beginning of a new track. In this manner, long tracks were divided into a number of shorter ones. Undoubtedly, the figures presented in the present paper should be taken in preference to those of LEVI and NIELSEN.

IV, 3) Accuracy of track length measurements

a) When measuring track length, the irregular path of the beta particle was approximated by a series of straight lines. Even in the case of high energy beta particles, producing tracks which appear fairly straight over long distances, multiple scattering occurs between one grain and the next, and hence, the distance travelled by the particle is always greater than the distance measured between the end points of a fairly straight section. ZAJAC and ROSS worked with the oil immersion objective of a Vickers projection microscope, and measured the distance from one grain to the next. This very time consuming method should lead to more accurate results; however, the results obtained by ZAJAC and ROSS agree so closely with those reported in the present paper, that the simpler method of length measurement applied in the present study seems justified.

b) Since beta tracks originate from sources of molecular dimensions, the true point of origin of each track cannot be determined. It was therefore tentatively assumed that the tracks start from the first recognisable grain. In tracks of a total length of about 50 microns, the grains in the first section of the track were approximately 1 micron apart. In tracks 150 microns long, or more, the first grains lay about 2 microns apart. The total track lengths as stated in the present work have therefore been underestimated by not more than 1 or 2 microns, depending on the total length.

c) An estimate of the reproducibility of the measurements in the optical axis of the microscope was obtained in connection with the study of the spatial distribution of grains relative to the track origin. Since it must be assumed that beta particles travel randomly in all directions, the distribution of silver grains around a point source should be spherically symmetrical.

As described on p. 25, the spatial distribution of silver grains recorded for a large number of randomly selected C-14 tracks was evaluated. When

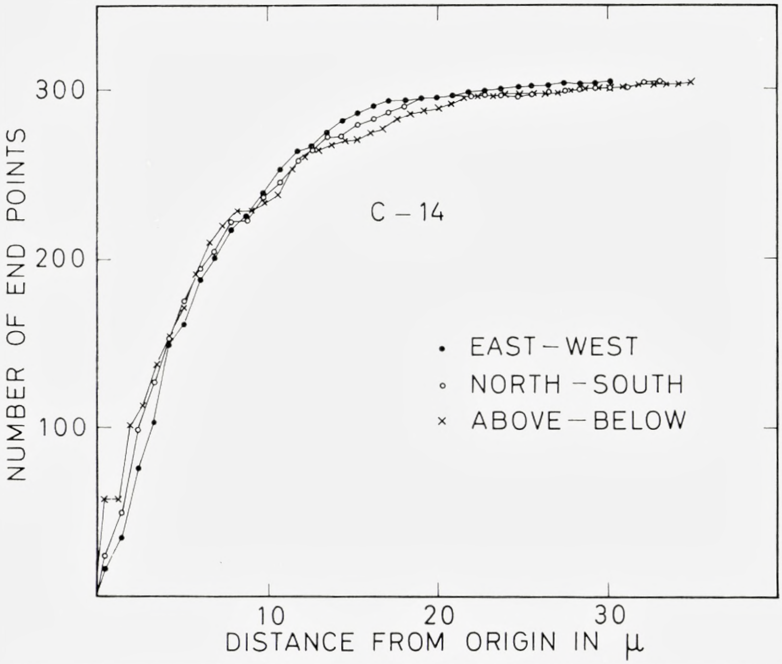

Fig. 9. Distribution of grains about the point of origin of the tracks.

the track origins of all tracks analysed were superimposed, the pattern of grains recorded was found to be symmetrical about the origin with respect to measurements taken in the plane of the slide. The distance measurements in the optical axis of the microscope were more variable, with a greater proportion of zero distances than were observed in the plane of the slide. Fig. 9 may serve to illustrate the distribution of grains about the point of origin of the tracks as being spherically symmetrical, if the very short distances of less than 1 micron which seem to have been recorded as zero, are excluded. This confirms the statement that reswelling restored the processed emulsion very closely to its original thickness.

IV, 4) The accuracy of grain counting

The only appreciable source of error in grain counting lies in the evaluation of the last few microns of the tracks, where the grains can be so large and closely spaced that the silver appears as a rather uneven blob, instead of a group of discrete grains. Since the chances for a short delta track to occur near the termination of the track are likewise high, the number of grains may be underestimated. This error is likely to be consistent for each observer.

IV, 5) The selection of tracks to be analysed

The criteria for selecting tracks to be analysed, out of the practically infinite population of tracks available on each plate, have been stated in the previous chapters. However, some comments are needed if the possibility of a bias is to be evaluated. When the relationship between track length and number of grains per track was studied, very tortuous tracks, and tracks running predominantly in the optical axis of the microscope were avoided. Thereby, length measurements and grain counts were greatly simplified, but other errors may have been introduced.

A beta particle loses most of its energy in multiple scattering whereby a succession of small deviations from its course occur. In addition, abrupt loss of a considerable amount of energy will occur resulting in the formation of a delta track whose length can be measured, provided the delta track lies predominantly in the plane of the slide. If the abrupt loss of energy is due to a nuclear collision, however, the track will show a marked change of direction at the point of collision, but the energy loss cannot be estimated by examining the emulsion. In selecting tracks according to the criteria mentioned above, it is likely that tracks with relatively few abrupt changes in direction were chosen at the expense of those with more delta tracks and more frequent nuclear collisions.

The data thus obtained were used to derive the equation relating initial energy of the particle to the number of grains produced; the resulting equation may describe a situation in which a higher proportion of the particle energy is dissipated by multiple scattering than is normally the case.

Chapter V

The mathematical evaluation of results

V, 1) Groupings

Five series of measurements were carried out in the course of this investigation and, for convenience, they will be referred to in the manner given below.

Group A Selected beta tracks from C-14, Ca-45, and Cl-36 were divided into segments, and the length of each segment as well as the number of grains in it were recorded (cf. p. 17).

Group B Using C-14 plates, Ca-45 plates, and blank plates, randomly selected beta tracks were examined, and both the radius of each track and the number of grains in the track were recorded (p. 20). For each track, a note was made of whether it was completely contained in the emulsion or whether it passed through the top or bottom surface of the emulsion.

Group C A series of Ca-45 plates that had been developed for periods ranging from 5 minutes to 40 minutes was examined (p. 27). Selected beta tracks were divided into segments and the length of each segment, as well as the number of grains in it were recorded.

Group D Randomly selected C-14 tracks were studied (p. 25). Each track was divided into segments and the co-ordinates of the beginning and the end of each segment in three dimensions relative to the track origin (first grain) were recorded, as well as the number of grains in each segment.

Group E The C-14 beta tracks originating from selected yeast cells were examined (p. 21). The number of tracks per cell and the number of grains per track were recorded.

V, 2) The relation between track length, number of grains, and initial energy of the particles

Zajac and Ross (1949) determined the mean track length and the mean grain count for 9 groups of mono-energetic beta particles in Kodak NT 4 emulsion. Tables II and III summarize some of their findings.

Table II

Zajac and Ross' values for mean track length and mean grain count (loc. cit., 1949, table 1).

Particle energy keV	Mean track length (μ)	Mean grain count
E	$\bar{L}$	$\bar{G}$
30	7.0	11.0
40	10.8	13.8
50	15.8	20.4
60	21.4	22.4
80	32.7	35.5
100	46.7	43.3
147	95.4	74.2
200	141	95
250	201	133

TABLE III

Zajac and Ross' values of mean number of grains per micron ($\bar{g}$) at given distance (L) from termination of track (loc. cit., 1949, table 2).

L	$\bar{g}$	L	$\bar{g}$
2.5	1.49	70	0.584
7.5	1.11	90	0.593
12.5	0.93	110	0.545
17.5	0.90	130	0.540
25	0.761	150	0.501
35	0.766	170	0.515
45	0.680	190	0.495
55	0.700	..	..

In Fig. 10 the logarithm of the initial energy of the particles in keV ($\log E$) is plotted against the logarithm of the mean track length in microns ($\log \bar{L}$). The evidently linear relationship between $\log E$ and $\log \bar{L}$ can be described by the equation

$$\log \bar{L} = 1.59 \log E - 1.51 \tag{1}$$

In the same figure, $\log E$ is plotted versa the logarithm of the mean number of grains per track ($\log \bar{G}$) and this relation is described by the equation

$$\log \bar{G} = 1.19 \log E - 0.740 \,. \tag{2}$$

From equations (1) and (2), the functions linking track length and number of grains can be derived, viz.

$$\log \bar{G} = 0.747 \log \bar{L} + 0.385 \tag{3}$$

or

$$\bar{G} = \bar{L}^{0.747}\ 10^{0.385} = 2.43\ \bar{L}^{0.747} \,. \tag{4}$$

The percentage standard deviation reported by ZAJAC and ROSS is fairly constant; the coefficient of variation being approximately 20 $^0/_0$ for the track lengths and slightly lower for the grain counts. However, the coefficients of variation for the grain counts are not incompatible with those predicted by

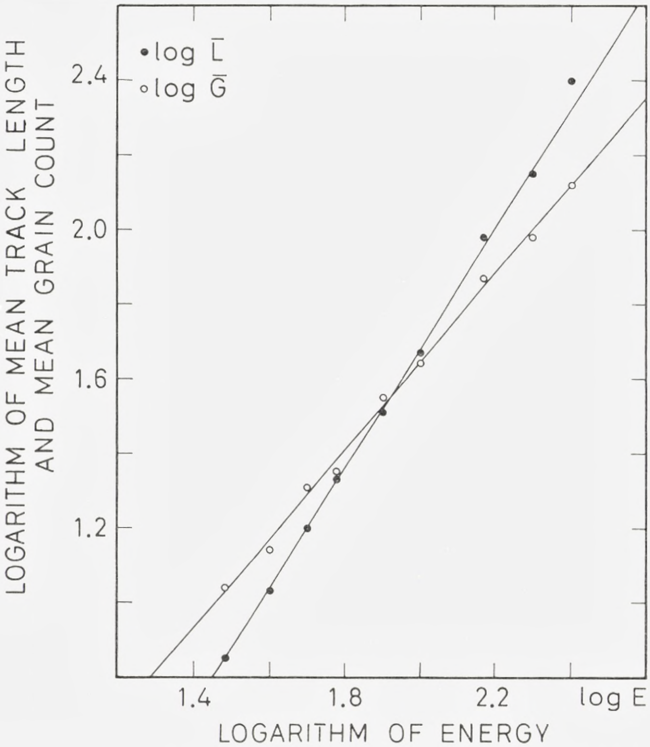

Fig. 10. Zajac and Ross' values for mean track length and mean grain count as a function of initial energy of the particles (log-log scale). These values are listed in Table II.

the Poisson distribution, except for the higher energy groups, where the experimentally determined coefficients are higher than those from the Poisson distribution.

In view of the similarity in composition between Ilford G 5 and Kodak NT 4 emulsion, the relation between particle energy and track length is expected to be the same in the two emulsions. In fact, Zajac and Ross' figures for mean track length agree very well with those predicted on theoretical grounds for both emulsions.

From the group *A* measurements (cf. p. 31), track length and number of grains per track have been determined, but the initial energies of the particles cannot be stated. Fig. 11 shows the number of grains per track plotted against $L^{0.747}$, and, in addition, the energies corresponding to different values of L (equation 1) are plotted on the abscissæ. It can be seen that the

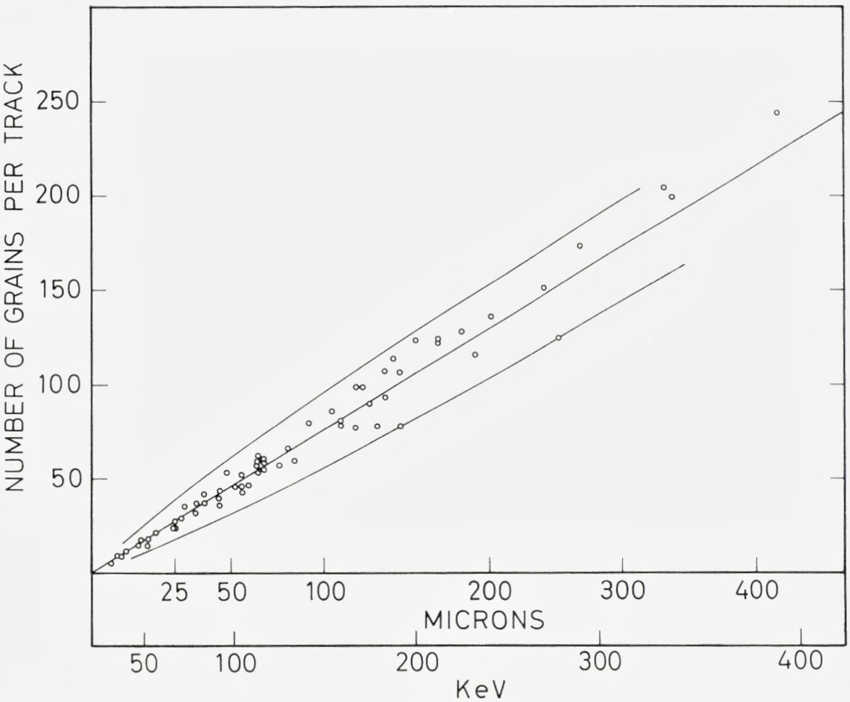

Fig. 11. Number of grains per track versus $L^{0.747}$ and versus initial energy of the particle (cf. equations (2) and (4), section V, 2; Group A).

results obtained for C-14, Ca-45, and Cl-36 tracks form a homogeneous group. The 95 % confidence limits calculated on the assumption of a Poisson distribution with a mean of 2.43 $L^{0.747}$ are shown on the graph. All points observed lie within these limits, which indicates a close agreement with equation (4). Furthermore, the experimental variance of the results is of the same order as that found by Zajac and Ross, or even slightly lower.

In Fig. 12, the number of grains per track versus track length of 100 randomly selected C-14 tracks of group *D* are plotted in a log-log scale. The straight line corresponding to equation (4) is drawn up in full, and it can be seen that this line does not agree too well with the observed points, while the dashed line which has almost the same slope but is placed somewhat below the first mentioned line on the average fits the observed points much better. In addition, it will be noted that the scatter of the points around the straight line is clearly greater for group *D* tracks as compared with tracks of group *A* (cf. Fig. 11).

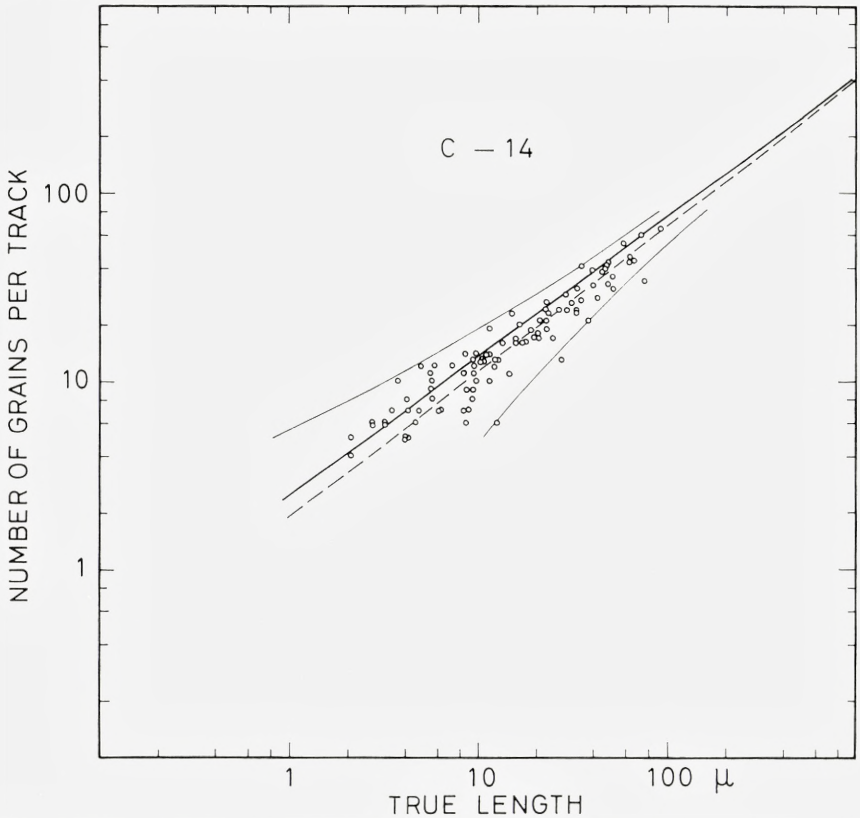

Fig. 12. Number of grains per track versus track length of 100 randomly selected C-14 tracks (Group D; log-log scale).

The difference in the results obtained from these two groups of tracks is undoubtedly due to the fact that the group *A* tracks have been selected so as to be easily recognisable and not very tortuous (cf. p. 17) whereby a lower scatter of the results was obtained. Furthermore, it is reasonable to assume that the difficulties encountered when evaluating the number of grains in sections of track running perpendicular to the plane of the glass slide led to an underestimate of the number of grains per track in group *D*. The scatter of the points of group *D* tracks agrees well with the expected Poisson distribution, while that of group *A* is lower than expected.

Since a beta particle with the initial energy E_0 loses energy on its passage through the emulsion, its energy at a given point in the track will be reduced to, say, E_1. It seems reasonable to assume that the length of the

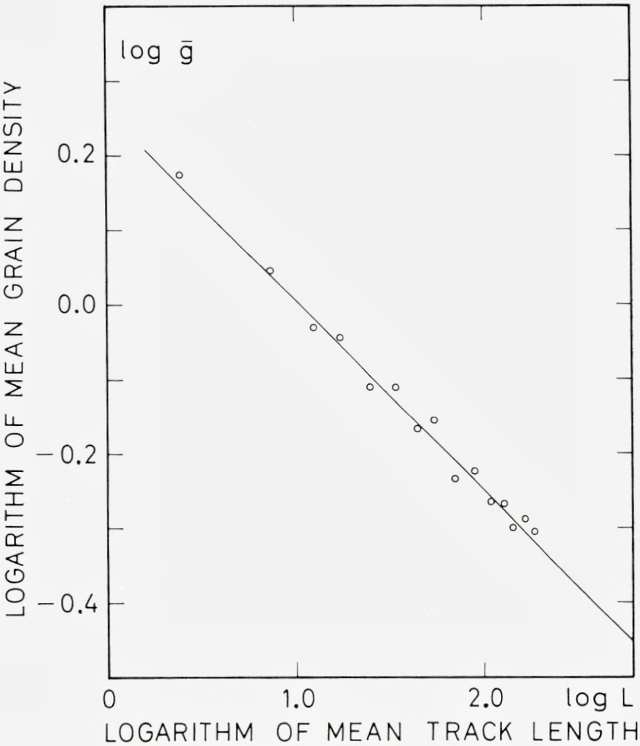

Fig. 13. Zajac and Ross' values of grain density versus track length (log-log scale). These values are listed in Table III.

remaining portion of the track, and the number of grains in that portion, will depend on E_1 in exactly the same manner as for another track produced by a particle with the initial energy E_1. In other words, the terminal section of any track will be independent of the initial energy of the particle and, on the average, independent of whether the complete track is long or short.

On this assumption, the average grain density $\bar{g}$ at a distance L from the termination of the track can be determined by differentiation of equation (4). This leads to

$$\bar{g} = \frac{d\bar{G}}{d\bar{L}} = 2.43 \times 0.747 \ \bar{L}^{-0.253} \quad \text{or} \tag{5a}$$

$$\log \bar{g} = 0.259 - 0.253 \ \log \bar{L} \,. \tag{5b}$$

In Fig. 13, Zajac and Ross' values are plotted with log $\bar{g}$ as the ordinates and log L as abscissæ. There is very good agreement between these observed values and the straight line calculated on the basis of equation (5b).

Table IV

Mean number of grains per 25 microns of track (distance measured from termination).

Distance from end μ	Number of tracks			Mean number of grains.				Theory (Equ. 4)	Variance			
	C-14	Ca-45	Cl-36	C-14	Ca-45	Cl-36	total		C-14	Ca-45	Cl-36	total
0– 25....	17	19	33	26.0	26.2	25.5	25.8	26.9	7.2	11.5	35.8	21.6
25– 50....	6	16	30	17.7	20.1	18.4	18.7	18.3	9.0	10.9	14.5	13.4
50– 75....	2	8	28	16.6	18.9	16.9	17.2	16.0	2.0	4.2	13.3	10.8
75–100....	..	8	24	..	15.4	15.2	15.3	14.7	..	7.1	9.2	8.4
100–125....	..	4	18	..	14.7	14.5	14.5	13.7	..	3.9	7.7	6.8
125–150....	..	..	14	..	..	14.5	14.5	13.1	..	..	8.0	8.0
150–175....	..	..	9	..	..	12.2	12.2	12.5	..	..	7.1	7.1
175–200....	..	..	7	..	..	12.5	12.5	12.1	..	..	4.9	4.9
200–225....	..	..	7	..	..	12.7	12.7	11.7	..	..	2.9	2.9
225–250....	..	..	7	..	..	12.6	12.6	11.4	..	..	4.0	4.0

Tables IV and V give the results obtained from group *A* tracks. The straight segments into which these tracks originally were divided for the purpose of measurements, were of variable length. For convenience of comparison, and in agreement with the considerations outlined above, the tracks listed in tables IV and V have been divided into sections 25 microns long, starting at the termination of the tracks. The number of grains in each such section has been calculated. Table IV records the mean number

Table V

Mean number of grains per 25 microns acc. to total length of track and distance from end.

Total length of track μ	Distance from end of track					
	0–25 μ		25–50 μ		50–75 μ	
	number of tracks	mean no. of grains	number of tracks	mean no. of grains	number of tracks	mean no. of grains
25– 50.............	13	25.8	..	..	..	..
50– 75.............	14	26.4	14	18.3	..	..
75–150.............	19	24.1	18	20.1	18	16.2
150–250.............	14	28.0	11	17.5	11	17.8
250–	9	25.3	9	18.2	9	16.0

of grains found in 25 μ sections of track for beta particles from each of the three isotopes studied. In table V, the mean grain numbers in the three 25 micron sections nearest the termination are listed for tracks of different total length.

It appears from these tables that the grain density, viz. the mean number of grains per 25 micron section of track, is independent of the isotope and of the total track length. Moreover, it can be seen that these values are in close agreement with equation (4) (column 9 of Table IV). The grain density decreases from the termination of the track towards its origin, reaching a reasonably constant figure above 150 microns from the termination. The variance of grain density decreases according to a similar pattern. The high variance found in the terminal 25 microns of track probably reflects in part the great variability in track pattern found in this region, and the increasing difficulty of grain counting when the grains lie very close together.

When delta tracks occur, the relation between total number of grains and total track length cannot be described by equation (4), since both branches represent track terminations. If delta tracks were to be compared with unbranched tracks, one of the branches should be interpreted as a middle section of an unbranched track, and this would involve a different relation between length, number of grains, and energy. A correction could be applied in such cases, but since this correction would be based on an assumption we have set out to prove, it was considered preferable to omit branching tracks from Fig. 11. However, the longest branch of each of these tracks is included in the calculation of grain density listed in tables IV and V.

V, 3) The relation between track length, radius, and distribution of grains in space

Direct measurements of the radius and the number of grains per track are available from group *B* for C-14 and Ca-45, and from group *D* for C-14. These figures are useful, only, if the tracks are fully contained in the emulsion.

For group *D*, it was possible to calculate track length as well as radius. These results are plotted in Fig. 14. In the narrow range from c. 20 keV to 150 keV, the relationship between track length and radius can be expressed as

$$\log R = 0.816 \ \log L + 0.042. \qquad (6)$$

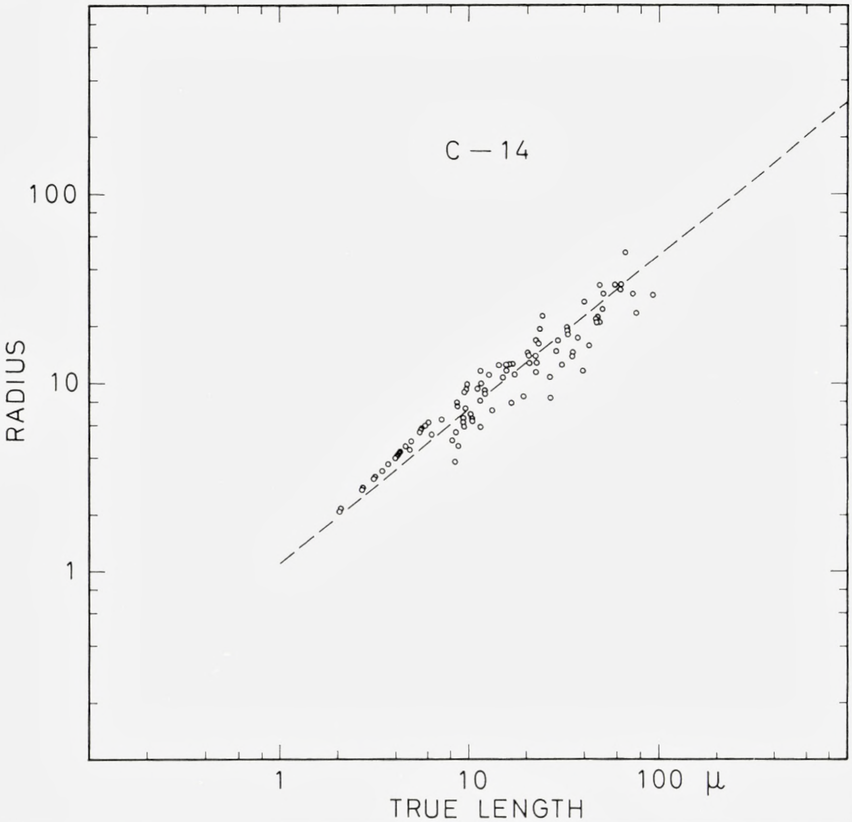

Fig. 14. Track length versus radius for C-14 (Group D; log-log scale).

It can be seen that the points scatter considerably about the mean. Using the relation between log G and log L derived from the dashed line in Fig. 12, viz.

$$\log G = 0.279 + 0.774 \ \log L\,, \tag{7}$$

the following relation between log G and log R is found

$$\log G = 0.229 + 0.949 \ \log R\,. \tag{8}$$

Figs. 15, 16, and 17 illustrate that this relation fits the C-14 tracks of groups B and D and also the Ca-45 tracks of group B. Also here, the scatter of the points about the mean is fairly wide.

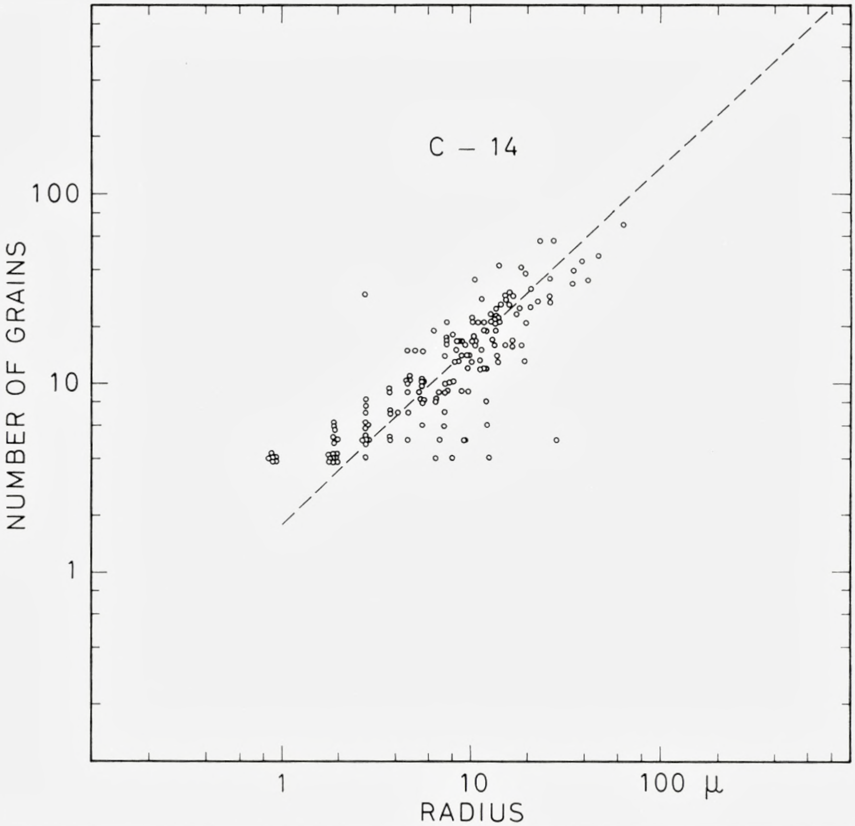

Fig. 15. Number of grains per track versus radius for C-14, (Group *B*, log-log scale).

Since the equations linking the presumed initial energy of a beta particle with its mean track length and mean number of grains have been calculated already, it is possible to apply these equations in order to convert equations (6) and (7) into a function describing the relationship between initial energy and mean radius

$$\log E = 0.784 \log R + 0.967. \tag{9}$$

It must be emphasized, however, that this relationship holds in a very limited range of energies, only (about 20–200 keV). Moreover, the variance of this particular function has not been estimated, and it is likely to be very large. In the absence of any information concerning the shape and width of the distribution curve about the mean radius for a group of mono-

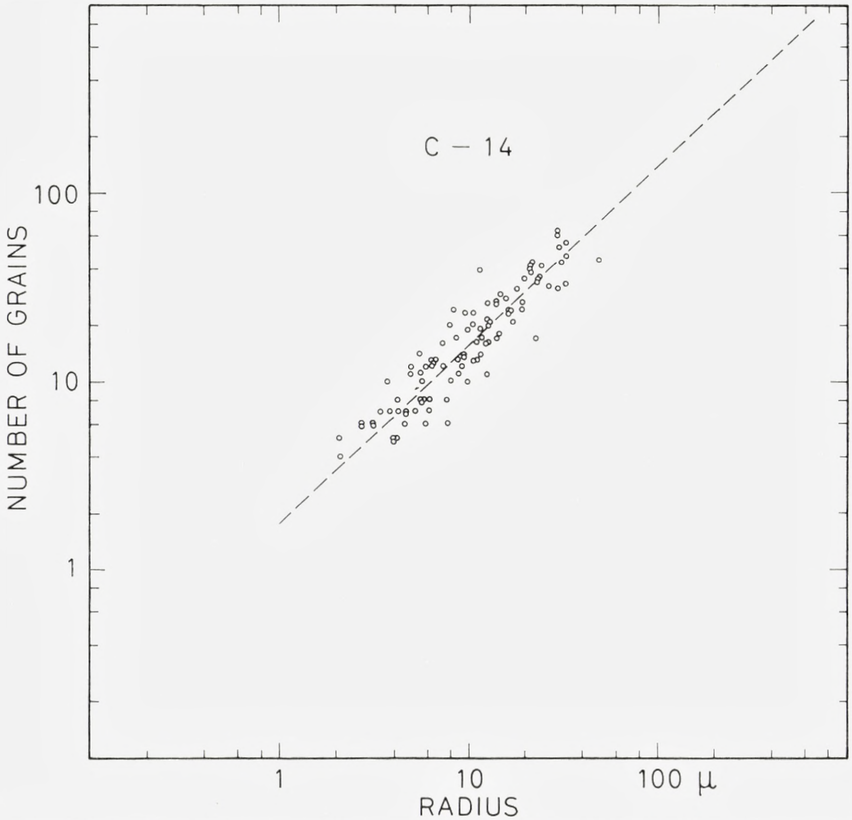

Fig. 16. Number of grains per track versus radius for C-14, (Group *D*, log-log scale).

energetic electrons, it is impossible to predict the probability of finding a C-14 track with a radius greater than a given value.

The data available for C-14 in the measurements of groups *B* and *D*, however, comprise 263 randomly selected tracks. Corrected for particles that have given rise to less than 4 grains (cf. p. 22), this represents 306 disintegrations.

It seems reasonable to assume that all disintegrations giving rise to less than 4 grains have radii shorter than 5 microns. Table VI gives the frequency distribution found for C-14 tracks of different radii. It follows that a sphere of emulsion of a radius of 20 microns around a point source of C-14 will contain about 90 % of the disintegrations.

The data available for Ca-45 are based on fewer tracks and the computation is complicated by the unknown fraction of tracks of group *B* that

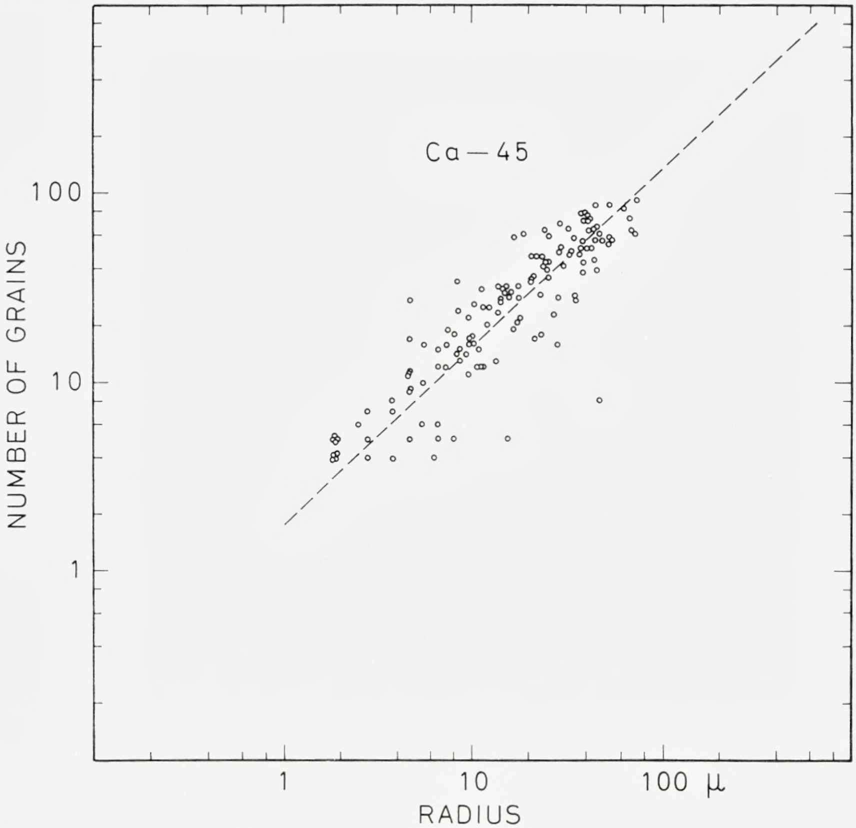

Fig. 17. Number of grains per track versus radius for Ca-45 (Group B; log-log scale).

left the emulsion. As a rough estimate, the sphere of emulsion that should contain 90 % of the disintegrations originating from a point source of Ca-45 would have a radius of 45 microns.

The basic data for calculating the spatial distribution of grains around a point source within the emulsion are available for 100 tracks of C-14 only (group *D*). These measurements were carried out in the following way: The cross in the eyepiece of the microscope was brought to coincide with the first grain of the track to be measured, thus forming the origin of a three-dimensional co-ordinate system. In order to ascertain that no systematic error was made in this system, the symmetry of tracks within the co-ordinate systems was checked using only the end points of all track segments.

TABLE VI

Distribution of radii of randomly selected C-14 tracks.
(Groups B and D, completely contained in the emulsion).

Radius in microns	Nr. of tracks with 4 grains or more	% of all tracks	% of expected nr. of disintegrations
0 – 5.0	71	27.0	37.1
5.1–10.0	76	28.9	24.8
10.1–15.0	55	20.9	18.0
15.1–20.0	25	9.5	8.2
20.1–25.0	14	5.3	4.6
25.1–30.0	10	3.8	3.3
30.1–35.0	6	2.3	2.0
35.1–40.0	2	0.8	0.7
40.1–45.0	1	0.4	0.3
45.1–50.0	2	0.8	0.7
50.1–55.0	0	..	..
55.1–60.0	0	..	..
60.1–65.0	1	0.4	0.3
Total...	263	100.1	100.0

Of 304 end points registered, 104 were situated west of the origin, 173 east of the origin, and 17 just on the north-south axis. Although the difference between east and west seems rather large, it must be assumed that this is a chance occurrence. 148 end points were situated south and 131 north of the origin, while 25 were on the east-west axis. Finally, 138 were above and 108 below in the direction of the optical axis of the microscope, and 58 were in the plane of the origin. The distribution of distances in the three dimensions—however omitting the sign—is depicted in Fig. 9. Apart from the slightly higher uncertainty in the optical axis of the microscope, the differences are insignificant.

From the co-ordinates of the end points of the segments, the co-ordinates of each of the 1968 grains in these tracks have been calculated in approximation. It has been assumed that the distances between the grains in a segment are equal. Moreover, the distance in space of each grain from the origin, i. e. the first grain of the track, as well as the corresponding projected distance in the plane of the glass slide have been calculated.

If the origins of these tracks are superimposed, a model point source of C-14 will result, and the observed distribution of grains around it may be studied.

Since only tracks with 4 or more grains have been registered, a correction must be applied for the tracks with less than 4 grains. According to the calculations described on pp. 22 and 46, 14 % of all C-14 tracks will have 3 grains or less, and the calculated distribution of tracks according to their number of grains has been taken into consideration. On this basis, it can be estimated that 22 grains have been missed in the above total, and these grains are assumed to lie within a distance of 5 microns or less from the first grain.

The distribution of grains in space according to the distance from the first grain is shown in Fig. 7. 50 % of all grains lie less than 9 microns, and 90 % less than 24 microns from the first grain. The figure also illustrates the distribution of grains according to the projected distance from the first grain in the plane of the glass slide. Here, however, only those grains are included which lie less than 5 microns from the first grain in the optical axis of the microscope. When viewed in this manner, 50 % of the grains lie less than 5 microns and 90 % less than 15 microns from the first grain.

The distribution of the distances from the first grain will be very similar to the distribution from the source. For C-14, the mean grain density does not fall below 15 grains per 25 microns at the origin of the longest tracks. It is unlikely, therefore, that the true track origin lay further than 1.5 microns from the first grain, and in many cases it is probably only 1 micron away.

V, 4) The calculation of the distribution of the number of grains per track from the energy spectrum of a beta emitter

In order to deduce a distribution curve for the number of grains per track from the energy spectrum of a pure beta emitter, two assumptions have been made, viz. 1) that the mean number of grains per track for a group of monoenergetic beta particles is given by equation (2) (cf. p. 33)

$$\log \overline{G} = 1.19 \log E - 0.74$$

and 2) that the scatter of the number of grains per beta particle about the mean follows a Poisson distribution.

The energy spectra used in the present study were calculated from the theoretical nomograms of Marshall (1955).

The calculation of the distribution curve proceeded as follows: A large number of equally spaced energy levels was chosen for which the desired distribution of the number of grains per track was calculated (Table VII column 1). These energy levels were 5 keV apart from 2.5 keV and up to 152.5 keV in the case of C-14, and up to 247.5 keV in the case of Ca-45. From the known energy spectrum, the probability of emission of a beta

TA

col. 1 kev	col. 2 $k \cdot p(e)$	col. 3 $m(g)$	col. 4 $p(g\|e)$								
			0	1	2	3	4	5	6	7	8
2.5	3.3	0.5	.6065	.3033	.0758	.0126	.0016	.0002	..	..	.
7.5	3.5	2.0	.1353	.2707	.2707	.1804	.0902	.0361	.0120	.0034	.00
12.5	3.7	3.6	.0273	.0984	.1771	.2125	.1912	.1377	.0826	.0425	.01
17.5	3.8	5.4	.0045	.0244	.0659	.1185	.1600	.1728	.1555	.1200	.08
22.5	3.9	7.5	.0006	.0041	.0156	.0389	.0729	.1094	.1367	.1465	.13
27.5	3.8	9.3	.0001	.0009	.0040	.0123	.0285	.0530	.0822	.1091	.12
32.5	3.8	11.4	..	.0001	.0007	.0028	.0079	.0180	.0341	.0556	.07
37.5	3.7	13.6	..	..	.0001	.0005	.0018	.0048	.0109	.0212	.03
42.5	3.6	16	..	..	..	.0001	.0003	.0010	.0026	.0060	.01
47.5	3.5	18	..	..	..	..	.0001	.0002	.0007	.0019	.00
52.5	3.4	20	..	..	..	..	..	.0001	.0002	.0005	.00
57.5	3.2	22	..	..	..	..	..	..	..	.0001	.00
62.5	3.0	25	..	..	..	..	..	..	..	..	.00
67.5	2.8	28	..	..	..	..	..	..	..	..	.
72.5	2.5	30	..	..	..	..	..	..	..	..	.
77.5	2.3	33	..	..	..	..	..	..	..	..	.
82.5	2.1	35	..	..	..	..	..	..	..	..	.
87.5	1.9	38	..	..	..	..	..	..	..	..	.
92.5	1.7	40	..	..	..	..	..	..	..	..	.
97.5	1.6	43	..	..	..	..	..	..	..	..	.
102.5	1.4	45	..	..	..	..	..	..	..	..	.
107.5	1.2	48	..	..	..	..	..	..	..	..	.
112.5	1.1	51	..	..	..	..	..	..	..	..	.
117.5	0.9	54	..	..	..	..	..	..	..	..	.
122.5	0.8	56	..	..	..	..	..	..	..	..	.
127.5	0.6	59	..	..	..	..	..	..	..	..	.
132.5	0.5	62	..	..	..	..	..	..	..	..	.
137.5	0.3	65	..	..	..	..	..	..	..	..	.
142.5	0.2	68	..	..	..	..	..	..	..	..	.
147.5	0.1	71	..	..	..	..	..	..	..	..	.
152.5	0.0	73	..	..	..	..	..	..	..	..	.
$p(g) \cdot k = \Sigma p(e) \cdot p(g\|e) \cdot k$			2.596	2.425	2.182	2.121	2.067	2.012	1.966	1.931	1.89
$p(g)$ in $^0/_0$			3.81	3.56	3.20	3.11	3.03	2.95	2.88	2.83	2.78
			13.68								
$\frac{p(g)}{0.1368}$ in $^0/_0$			..	..	..	..	3.51	3.42	3.34	3.28	3.22

particle at each of the chosen energy levels ($p\ (e)$) (column 2) was calculated (cf. MARSHALL, loc. cit.). From equation (2), the mean grain number ($m(g)$) corresponding to each energy level was found (column 3). In the case of C-14, these mean grain numbers lay between 0.5 and 73 grains per track, in the case of Ca-45, between 0.5 and 128 grains per track.

0	13	15	20	25	30	40	50	60	70	80	90	100 grain levels
.	..	..	..	..	..	..	..	..	..	..	..	..
.	..	..	..	..	..	..	..	..	..	..	..	..
)28	.0001	..	..	..	..	..	..	..	..	..	..	..
262	.0024	.0003	..	..	..	..	..	..	..	..	..	..
858	.0211	.0057	.0001	..	..	..	..	..	..	..	..	..
219	.0572	.0235	.0009	..	..	..	..	..	..	..	..	..
144	.0987	.0611	.0063	.0002	..	..	..	..	..	..	..	..
740	.1085	.0955	.0239	.0017	.0001	..	..	..	..	..	..	..
341	.0814	.0992	.0559	.0092	.0006	..	..	..	..	..	..	..
150	.0509	.0786	.0798	.0237	.0026	..	..	..	..	..	..	..
058	.0271	.0516	.0888	.0446	.0083	..	..	..	..	..	..	..
020	.0127	.0292	.0809	.0654	.0197	.0002	..	..	..	..	..	..
004	.0033	.0099	.0519	.0795	.0454	.0014	..	..	..	..	..	..
001	.0007	.0027	.0249	.0673	.0677	.0065	.0001	..	..	..	..	..
.	.0002	.0010	.0134	.0511	.0726	.0139	.0002	..	..	..	..	..
.	..	.0002	.0045	.0276	.0631	.0314	.0013	..	..	..	..	..
.	..	.0001	.0020	.0162	.0499	.0447	.0033	..	..	..	..	..
.	..	..	.0005	.0063	.0293	.0598	.0101	.0002	..	..	..	..
.	..	..	.0002	.0031	.0185	.0629	.0177	.0007	..	..	..	..
.	..	..	..	.0009	.0080	.0566	.0328	.0026	..	..	..	..
.	..	..	..	.0004	.0043	.0472	.0431	.0054	.0001	..	..	..
.	..	..	..	.0001	.0015	.0310	.0541	.0128	.0006	..	..	..
.	..	..	..	..	.0005	.0175	.0558	.0243	.0020	..	..	..
.	..	..	..	..	.0001	.0086	.0484	.0373	.0055	.0002	..	..
.	..	..	..	..	.0001	.0050	.0403	.0447	.0094	.0005	..	..
.	..	..	..	..	..	.0020	.0273	.0510	.0181	.0015	..	..
.	..	..	..	..	..	.0007	.0162	.0498	.0290	.0041	.0002	..
.	..	..	..	..	..	.0002	.0086	.0422	.0395	.0089	.0006	..
.	..	..	..	..	..	.0001	.0041	.0315	.0463	.0164	.0017	.0001
.	..	..	..	..	..	..	.0018	.0209	.0473	.0258	.0040	.0002
.	..	..	..	..	..	..	.0010	.0150	.0447	.0322	.0067	.0005
19	1.702	1.640	1.432	1.156	.969	.671	.403	.201	.067	.012	.001	..
7	2.50	2.40	2.10	1.70	1.42	.98	.59	.29	.10	.018	.001	..
9	2.89	2.79	2.43	1.96	1.65	1.14	.68	.34	.11	.020	.002	..

Subsequently, a series of grain levels was chosen (horizontal row 2) and for each value of $(m(g))$, the probabilities of occurrence of a track with the number of grains listed in row 2 were calculated from the standard tables of Poisson distributions. The figures thus obtained were multiplied by the corresponding values of $(p(e))$. The products $p(g|e) \times p(e)$ at each grain level were summed (row 3 from bottom) to give the overall probability of a track containing that number of grains to occur.

This overall probability ($p(g)$) was then normalised to percentage values (row 2 from bottom), and the distribution curve constructed.

Since, according to definition, a track contains 4 grains or more, the percentage values of $p(g)$ for 0,1,2, or 3 grains per track were subtracted from the total, and the remaining values of $p(g)$ were adjusted to give 100 % (last row).

Fig. 4 shows the calculated distribution curve and the observed distribution of the number of grains per track for C-14. The observed distribution was obtained by pooling the results of groups *B*, *D*, and *E*, and it agrees fairly well with the predicted curve. However, the observed distribution shows a preponderance of short tracks, a discrepancy which cannot be explained at present. If assumption (2) mentioned above is not correct, this might account for the difference between observed and calculated values in the beginning of the curve; it is conceivable, for instance, that the scatter of the number of grains per beta particle about the mean has a skewed distribution. It is also possible that, for low energies, the distribution about the mean is wider than that given by a Poisson distribution. Both these factors would tend to increase the probability of occurrence of tracks with few grains.

The predicted and observed distributions for Ca-45 are depicted in Fig. 6. Here, the situation is complicated due to the fact that a larger fraction of the tracks escaped from the emulsion. Still, over a major part of the energy spectrum, agreement between the calculated and the observed grain distribution is good.

Chapter VI

Summary

Beta tracks produced by carbon-14, calcium-45, and chlorine-36 in Ilford G-5 emulsion were studied in order to establish the possibility for accurate evaluation of track- or grain autoradiograms.

From measurements of track length and of the number of grains in the track, equations relating the initial energy of the beta particle to the mean number of grains produced have been derived. On the basis of these relationships, it is possible to predict the pattern of distribution of tracks in terms of the number of grains they contain for any isotope with a known

beta spectrum. In this manner, the percentage of particles that will not produce recognisable tracks can be calculated in each case. Clearly, this fraction will be relatively insignificant for isotopes with a high maximum energy, such as P-32, but it becomes much greater for low energy beta emitters such as C-14 and S-35.

It was found that 14 % of all beta particles emitted by a point source of C-14 will give rise to less than 4 grains, and thus not produce a recognisable track. 4 % will not give rise to any grains at all under the conditions of the present experiments.

Hence, the absolute disintegration rate in a small beta source completely surrounded by Ilford G-5 emulsion can be determined with a considerable degree of accuracy, either by track counting or, if the track density is too high, by grain counting in a well-defined volume of emulsion.

Moreover, the position of silver grains relative to the track origin was measured. From these data, the distribution of grains in space around a point source can be constructed, and the radius of the sphere that will contain a given percentage of the grains can be calculated for any beta-emitter. In the case of C-14, 50 % of all grains lie less than 9 microns, and 90 % less than 25 microns from the source. Viewing the projected distance from the first grain in the plane of the glass slide, and including only those grains which lie less than 5 μ from the first grain in the optical axis of the microscope, 50 % of the grains lie less than 5 microns and 90 % less than 15 microns from the first grain. This resembles the conditions of a stripping film autoradiogram.

Discrepancies in the measurements of track length and grain number as found by Levi and Nielsen versus those found by Zajac and Ross, could be explained on technical grounds, and the values obtained by Zajac and Ross were confirmed in the present study.

The influence of development time on grain yield was investigated and the advantage of re-swelling of the processed emulsion prior to microscopic inspection was demonstrated.

On the basis of the results so far obtained, absolute quantitation in other geometrical arrangements of source and emulsion—such as the familiar tissue section covered by stripping film, or a cell smear coated with a thin layer of nuclear emulsion—is still not directly possible. It is feasible, however, to calibrate small reference sources by track autoradiography, and subsequently to expose them in the conditions of the desired experiment, thus determining the grain yield and, from this, the disintegration rate in the source.

In connection with the calculation of the spatial distribution of silver grains around a point source, the definition of autoradiographic resolution was found to be ambiguous and in need of revision. This problem will be dealt with in a separate communication in the near future.

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