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LOCAL ENERGY DISTRIBUTIONS FOR α-PARTICLES OF DIFFERENT ENERGIES IN RELATION TO THE EVALUATION OF CRITICAL SIZES AND ENERGY REQUIREMENTS INVOLVED IN THE INDUCTION OF DAMAGE IN MAMMALIAN CELLS

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ABSTRACT

Event size spectra have been measured with a tissue-equivalent proportional counter in which energy was deposited by α-particles from ^{210}Po. The energy of the particles entering the sensitive volume was varied by introducing absorbers between the source and the counter. Energy deposition patterns for α-particles having a mean energy loss per unit path length (LET_∞) in unit density tissue of 110, 126, 148, 200 and 215 keV/μm respectively, were investigated in cylindrical volumes of various simulated tissue dimensions. These dimensions were simulated by variation of the gas pressure. The simulated volumes have dimensions corresponding to 5.6, 1.2, 0.63, 0.3, 0.15 and 0.075 μm in unit density tissue respectively.

From a comparison of the measured physical data with cross-sections for impairment of the reproductive capacity derived from survival curves for cultured cells of human kidney origin (T-1 cells), it can tentatively be deduced that the critical structure in the cell, in which a given minimum damage must be produced in order to cause impairment of the capacity for unlimited proliferation, has dimensions smaller than 0.07 μm.

INTRODUCTION

For a quantitative analysis of the relation between the biological effectiveness of different ionizing radiations and their energy deposition patterns, the best approach is provided by investigations of the most simple experimental systems and conditions for which sufficiently accurate data can be obtained.

With respect to the physical aspects, many complicating factors are reduced or eliminated if mono-energetic directly ionizing particles are used in conditions whereby the biological objects are traversed by selected short portions of the particle tracks, because in these conditions the distributions of dose in LET are relatively narrow. Energy dissipations from X-rays, γ-rays and fast neutrons are always characterized by wide distributions of dose in LET and the track lengths of the secondary ionizing particles vary from very short to long in comparison to cell diameters. This renders the interpretation of biological effects in relation to microdosimetric data very complex.

With regard to the biological system, it is important to employ the most simple systems for which an unambiguous dependence of the relative biological effectiveness (RBE) on the radiation quality has been observed. For the inactivation of enzymes or phages, the RBE depends very little on the energy deposition pattern and consequently more complex cellular systems must be employed. With multicellular systems however, interaction between damaged cells and unaffected cells may occur, causing very complex responses.

In a series of experiments performed during the past ten years, the track segment method has been employed for the measurement of responses of cultured cells of human kidney origin to irradiations with deuterons, α-particles and other heavy ions up to neon as well as with X-rays, fast neutrons and stopping negative pions (1-8).

Attempts have been made previously to analyze the data obtained, in terms of a hypothesis, in which it is assumed that for the initiation of the sequence of events which causes impairment of the capacity for clone formation by these mammalian cells, an interaction of a minimum number of primary molecular lesions is required and that the distance within which this interaction can occur is limited (4, 9). Because of a lack of adequate physical

data concerning frequency distributions of local energy densities, for the various types of radiations used, a number of unverified assumptions had to be made for the analysis of the radiobiological data, and the results could only provide a first approximation for estimates of local energy densities required and sizes of critical structures involved in the interaction (4). The tentative conclusions indicated that energies in excess of 300 eV must be deposited within volumes with dimensions of 5 to 20 nm, in order to initiate the chain of events leading to cell reproductive death.

In the present contribution results will be described of measurements of frequency distributions of local energy densities for α-particles from ^{210}Po, at energies and in conditions similar to those for which survival curves have been obtained with cultured cells of human kidney origin. Comparison of these measured frequency distributions with the Vavilov theory showed good agreement. Therefore frequency distributions for other α-particle energies and for still smaller volumes have been derived from the Vavilov theory. From these distributions, cross-sections versus LET curves have been calculated, assuming various dimensions of critical structures in cells, involved in the induction of mammalian cell lethality. Comparison of these cross-section versus LET curves with the curve derived from radiobiological studies of the induction of cell reproductive death, indicates that the biological results are consistent with the assumption that the critical structure has dimensions of less than 50 nm and that the minimum energy which must be deposited in this structure from ionizing radiation is of the order of 500 eV.

TECHNIQUES

Event size distributions for α-particles have been measured with a tissue-equivalent proportional counter. The construction of the cylindrical counter has been described previously (10). The inner diameter of the counter is 5 mm and the length of the central wire is 50 mm. The counter was flushed at different gas pressures by a tissue-equivalent gas, existing of a mixture of C_2H_4, C_2H_6, N_2 and Ne (11). The sensitive volume corresponds at the different gas pressures employed to cylinders with diameters of 5.6, 1.2, 0.63, 0.3, 0.15

and 0.075 μm of unit density tissue respectively.

The counter pulses are pre-amplified by a Tennelec TC 133, shaped and amplified by a Tennelec TC 200 and subsequently sorted by a multichannel analyzer.

Gas multiplication factors employed were in the order of 10^3. ^{210}Po α-particles, after passage through a number of Mylar absorbers of 0.54 mg/cm^2, enter the sensitive volume through a circular aperture of 0.2 mm diameter in the tissue-equivalent plastic wall of the counter.

The axis of the aperture is perpendicular to the central wire, and the particles pass the anode at a distance of about 0.5 mm. The length of the trajectory of the α-particles in the sensitive volume is equal to a chord length of 4.9 mm.

The energies of the α-particles which entered the sensitive volume, was varied by changing the number of Mylar absorbers inserted between the ^{210}Po source and the counter. Since at least two Mylar windows are required to enclose the source and the proportional counter respectively, the number of extra-absorbers was varied between 0 and 4.

RESULTS

Measured distributions of energy loss (ε) for α-particles, which have passed through two Mylar absorbers are presented in figures 1 and 2 for different simulated diameters (d_s) of tissue cylinder of density 1. On the horizontal scale the values of ε/d_s have been plotted. The contribution at high LET values beyond the main peak, especially manifest at large diameters, might be due to e.g. large angle scattering of the α-particles in the counter gas and wall, interaction of particles with the wall of the aperture and straggling effects in the source and counter windows.

In figures 3 and 4 energy loss distributions of α-particles, which have passed through different numbers of absorbers are presented for equivalent diameters of 0.15 and 0.075 μm respectively. The figures demonstrate the increase in contribution of small energy deposition events at decreasing diameters.

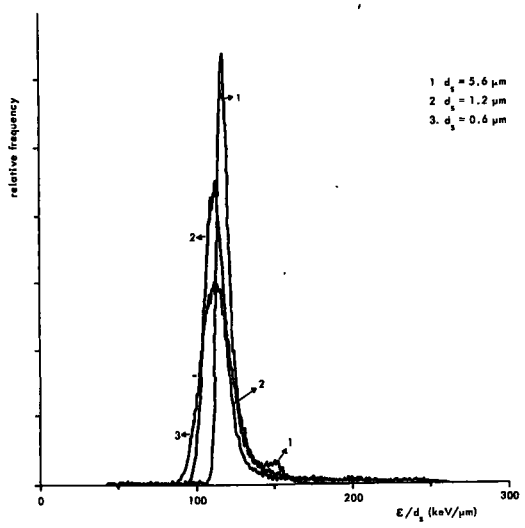

Figure 1. Energy loss (ε) distributions of ^{210}Po α-particles which have passed through two Mylar absorbers for simulated diameters (d_s).

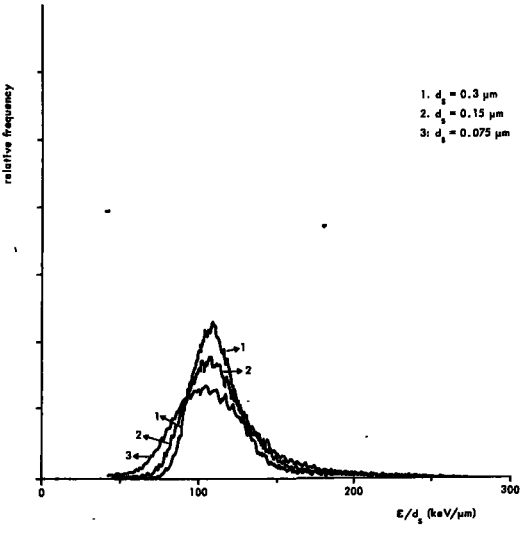

Figure 2. Energy loss (ε) distributions of ^{210}Po α-particles which have passed through two Mylar absorbers for simulated diameters (d_s).

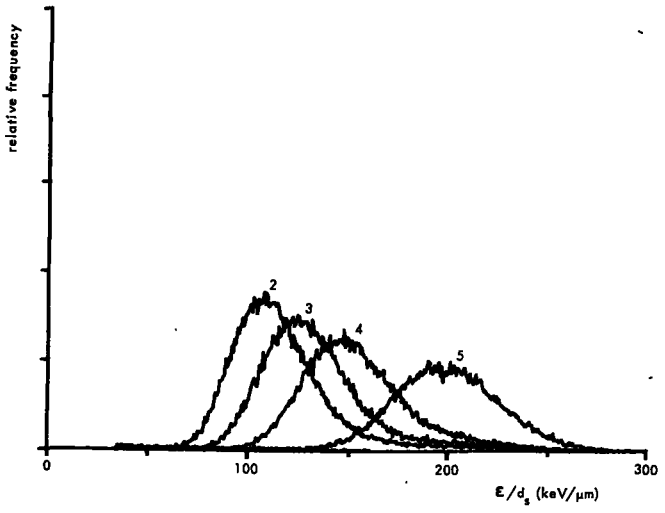

Figure 3. Energy loss (ε) distributions of ^{210}Po α-particles at simulated diameter(d_s) of 0.15 μm, having passed different numbers of absorbers. Numbers correspond to number of absorbers.

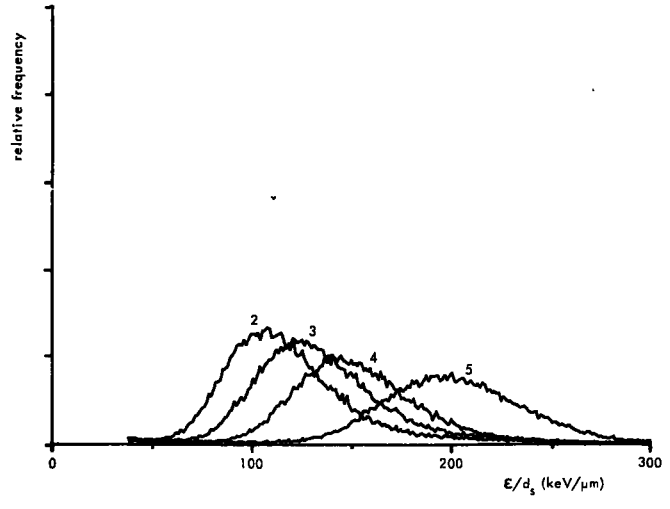

Figure 4. Energy loss (ε) distributions of ^{210}Po α-particles at simulated diameter (d_s) of 0.075 μm, having passed different numbers of absorbers. Numbers correspond to number of absorbers.

DISCUSSION

1. LET values of the α-particles of different energies

The mean values of the measured energy loss distributions in simulated diameters of 1.2 μm and 0.075 μm for α-particles which have passed through different numbers of absorbers, have been fitted to the LET_∞ versus range curve for α-particles derived from data published by Walsh (12). The results of this analysis are presented in figure 5.

The data points for the simulated diameter (d_s) of 1.2 μm are shifted over 0.6 μm to the left with respect to the corresponding points for d_s of 0.075 μm, reflecting the fact that for the large path length of 1.2 μm the track average LET is determined, instead of the LET of the particle at entrance of the sensitive volume.

From the curve of figure 5 the LET_∞ of the α-particles, which have passed 2, 3, 4, 5 and 6 absorbers is determined to be 110, 126, 148, 200 and 215 keV/μm respectively.

For volumes with decreasing diameter the number of energetic δ-rays depositing their energy outside the volume will increase. As a consequence the mean of the distribution is no longer a measure of the LET_∞. From the fact that the respective values for a d_s of 1.2 μm and 0.075 μm are very close together, it can be concluded that within the limits of error, in the case of a simulated diameter of 0.075 μm of unit density tissue equilibrium conditions still apply.

2. Experimental distributions compared to Vavilov distributions

The distributions of local energy deposition measured in our experiments could be carried out only for α-particles with LET_∞ of 110 keV/μm and larger. For a comparison of these distributions with the relation between the biological effectiveness of α-particles and their LET_∞, as derived from experiments with cultured cells of human kidney origin by Barendsen, it is of particular interest to obtain similar data for LET_∞ values between 10 and 100 keV/μm (4). As a first approximation, distributions have been derived based on the Vavilov theory (13).

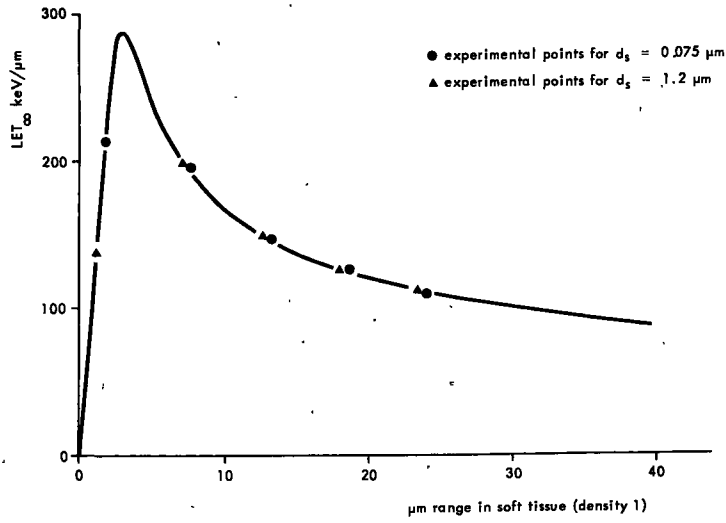

Figure 5. LET_∞ versus range in soft tissue for α-particles. The curve is obtained from data published by Walsh (12).

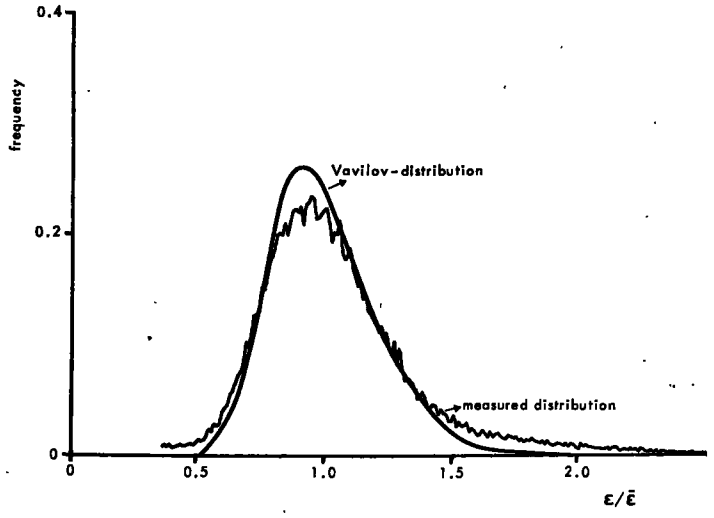

Figure 6. Measured and theorectical energy loss (ε) distributions from α-particles of 110 keV/µm at a simulated diameter (d_s) of 0.075 µm.

In order to show that for the measured distributions a fair agreement is obtained with calculated distributions, a comparison is presented in figure 6 for α-particles with an LET_∞ of 110 keV/µm and a simulated diameter of 0.075 µm. The Vavilov distributions were obtained from tabulated data from Seltzer and Berger (14).

The differences between the experimental and theoretical curve might be due not only to the effects of small collision numbers and of resonance collisions and δ-ray escape as mentioned by many authors (i.e. 15), but are mainly produced by loss in resolution of the actual energy deposition distribution by gain variation of the counter, the noise induced by the pre-amplifier and geometrical resolution loss. It can be concluded that as a first approximation the Vavilov theory can be used to obtain distributions of local energy deposition for α-particles of lower LET_∞ values than those for which measured data are available.

3. Comparison of biological effectiveness and energy deposition spectra

From dose-survival curves for cultured cells irradiated with α-particles of different LET_∞ values, Barendsen had earlier derived a curve relating to relative effectiveness per particle, expressed as a cross-section to the LET_∞ (4). This curve and the experimental points on which it is based are shown in figure 7. The maximum cross-section at LET_∞ values in excess of 160 keV/µm is equal to 35 μm^2, indicating that a sensitive structure exists in the cell which presents a cross-section of 35 μm^2 to the parallel beam of incident particles. A factor of two reduction from the maximum cross-section is observed at about 80 keV/µm. This implies that at 80 keV/µm, one-half of the particles passing through the critical structure is ineffective for producing impairment of the capacity for unlimited proliferation. On the basis of the hypothesis mentioned in the introduction, this would be due to the fact that in 50 per cent of the passages insufficient damage is produced, within a critical distance along the particle track, to initiate the chain of events causing the final effect. It is possible to deduce further from the solid curve of figure 7 that at an LET_∞ of 110 keV/µm about 15 per cent of the passages of α-particles are still ineffective for producing the biological effect.

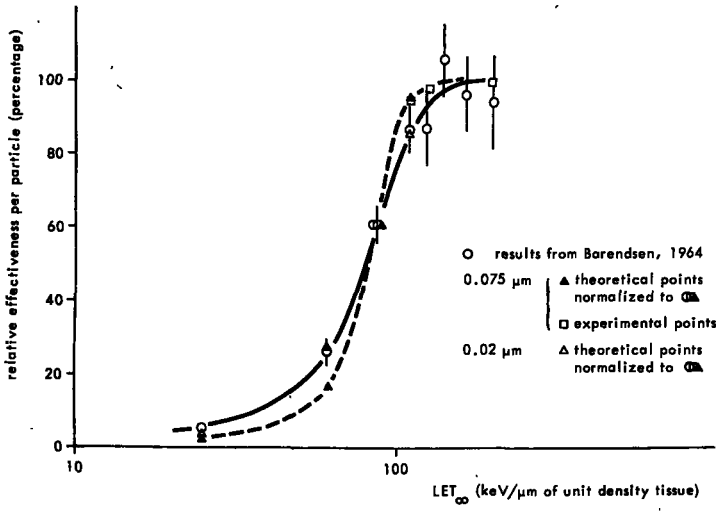

Figure 7. Relative effectiveness per particle versus LET_{∞}.

From figures 1 and 2 it can be deduced that this 15 per cent cannot be accounted for if the critical track length would be either 5.6, 1.2 or 0.6 μm because for all these distributions in more than 99 per cent of the α-particle traversals ε/d_s is larger than 80 keV/μm. Even for the still smaller values of d_s of 0.3, 0.15 and 0.075 μm the distributions shown in figure 2 are not wide enough to account for an efficiency of only 0.85 from the maximum value. At 0.075 μm, for 110 keV/μm, a decrease in effectiveness of only about 5 per cent would be expected as indicated by the square at 110 keV/μm in figure 7. This indicates that the critical distance must be smaller than 0.075 μm. It is important to note that the conditions employed in the measurements of the energy deposition distributions and for the measurements of cell survival conform very closely. Furthermore as discussed earlier, even for the smallest d_s, δ-ray equilibrium is still prevailing and consequently the LET_∞ value is the valid parameter.

Using the Vavilov theory, calculations for $d_s = 0.075$ μm have been carried out for those LET values, lower than 110 keV/μm, at which biological data were available. The results are shown by the closed triangles in figure 7. The dotted curve through these points is clearly much steeper than the measured cross-section versus LET_∞ curve derived from the biological data. It can further be noted that the squares derived from measured distributions are in good agreement with the theoretical curve.

Further calculations have been carried out, based on the Vavilov theory, for a critical distance of 0.02 μm. The results are represented by the open triangles, showing that these data are very close to the curve for the biological effectiveness for the α-particles used for cell irradiation experiments. It can be concluded that the critical distance in which a given minimum energy deposition is required, is of the range of 0.01 to 0.04 μm. Further experimental data of distributions of energy deposition are required however to obtain more precise estimates.

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D I S C U S S I O N

Mr KELLERER

You refer to a critical structure. Do you assume that you have one of these critical structures in the cell or several, and if so, how many?

Mr HOGEWEG

It is impossible from this type of calculation to state whether there are one or more critical structures in the cell.

Mr KELLERER

The question is important because you derive probabilities for cellular inactivation, and these probabilities are clearly functions of the number of sites.

Mr HOGEWEG

On the basis of the hypothesis that we are dealing in the critical structure with a two-state system and a minimum energy level, the calculations can only result in a critical distance in which this energy has to be deposited.

Mr KELLERER

Are you not assuming in your calculations that an alpha particle traverses exactly one site? You derive inactivation cross-sections for a site and then you compare them with the experimentally observed cross-sections for inactivation of the cell.

Mr BARENDSEN

In the case of an alpha-particle passing through the sensitive structure, we think that one passage is enough to cause that particular effect and it does so by deposition of an amount of energy in excess of a certain threshold, relevant for that particular structure. However, the structure extends over a much wider area than a distance of 200 Å, as you can deduce from the maximum cross-section, which is about 40 μ^2. Of course, we have never stated that only one critical site exists with a diameter of 200 Å.

Mr KELLERER

I am afraid I did not quite understand. Is it your conclusion that in traversing the nucleus the alpha-particle must produce a specified local energy concentration of within 200 Å at one place exactly in order to inactivate the cell?

Mr BARENDSEN

My interpretation involves the assumption that a large structure or set of unique molecules exists, quite probably located in the cell nucleus and presumably identical with the DNA histone complex or with part of it. The physical measurements cannot tell us anything about the correctness of this latter part of the hypothesis, but a number of biological arguments support it. Damage finally causing cell reproductive death is now in my interpretation due to changes in a small portion of the DNA histone complex which are initiated by deposition of energy in a high concentration locally. This damage can occur, however, in any part of this complex and cause that particular part of the chromosomal material to reproduce incorrectly. As I pointed out earlier, interaction distances might be larger with X-rays, however. I think it is important to keep in mind that the total amount of DNA in a mammalian cell is of the order of 10-20 picograms.

Mr KELLERER

So it is indeed an essential postulate that the alpha-particle passes through exactly one critical structure.

Mr BARENDSEN

May I clarify my ideas a little further? In the case of other radiations, e.g., X-rays, it is quite possible that one electron will pass through a particular part of the nucleohistone complex at one point and that at a distance of, say, 1000 Å from that point another particle passes through, these two events interacting to cause the same effect as observed through a single passage of a high LET ion. I don't think that the other electron has to pass through within the same critical distance of 200 Å. From our experiments we can not deduce what the distance is along which these two lesions can interact.

Mr BICHSEL

I wish to refer to the figure which compares the VAVILOV theory with the experimental spectra. First of all I would like to say that you are comparing here the energy loss of an alpha particle with the energy taken up by the gas counter. One of the possible differences, for example, if you have the excitation of a K-electron of a gas atom is that the X-ray which is emitted upon de-excitation of that atom can escape from the counter without producing any ionization. Therefore the alpha-particle will lose an energy which may be greater by 500 eV or whatever the K-excitation energy is. Now, this was the first point. The explanation for the excess of low energy depositions in the counter is probably to be found in the escape of delta-rays from the counter. We have made some calculations of this effect. We found that pulses are reduced in magnitude and therefore appear shifted to the left. On the other hand, delta-rays which are knocked out of the wall by the alpha-particle would increase the pulse heights which you observed and would shift them to the right. We have not calculated the total effect for alpha-particles, but I would estimate that the total number of alpha-particles involved in these losses and gains would be of the order of several %, which approximates to what you have observed. Finally, I

would like to say that the width of the curve which you have here should not really agree exactly with the VAVILOV calculation because this calculation does not take into account the quantum mechanical bound state corrections to the second moment and this width is, of course, related to the second moment. Now it could be that you are already in that part of the correction for the second moment which goes down again towards or below one and if so, this would be quite interesting to know (Ref: Proceedings of the Second Symposium on Microdosimetry, 1969, p. 521).

Mr HOGEWEG

First of all, as to your criticism about the distribution fitting these VAVILOV distributions, I must mention that it is very difficult to obtain exact agreement for there is a larger discrepancy between these measured distributions and the VAVILOV distribution. We show the measured distribution without correction for the loss in resolution from the noise of the preamplifier. If you apply this correction for the energy loss distribution, then the fit becomes better, especially in the lower region. I agree that the measured distribution in the high energy region is much broader than the VAVILOV distribution. This is in our opinion due to delta-rays originating from the wall of the aperture. We don't claim, however, that this VAVILOV distribution will be the exact description of the measured distribution, but the discrepancies are at a first approximation of minor importance.

Mr ROSSI

If you measured these distributions with a solid state detector, they would, of course, be much narrower. If you measured them with, say, a photgraphic emulsion, they would be much broader and your attempt to get the size of the critical structure in terms of the width of these distributions is, it seems to me, equivalent to the assumption that each of the ionizations that occurs to the structure is vital and none of them is wasted.