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FREE RADICAL PRODUCTION BY HEAVY IONS AT 77° K AND ITS RELATION TO THE THERMAL SPIKE THEORY[1]

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ABSTRACT

Polycrystalline samples of amino acids and proteins were exposed to electrons and beams of helium, carbon, neon, and argon ions from the Berkeley heavy-ion linear accelerator. Both the irradiations and the electron spin resonance observations were carried out at 77° K. The results showed that the same types of radicals were formed independent of the stopping power of the bombarding particle.

The results for the amino acid DL-valine have been interpreted in relation to the "thermal spike model." The irradiation of DL-valine at 77° K results in the formation of a radical which converts upon heat treatment into a secondary radical. On the basis of the thermal spike model and the kinetics for the radical conversion, it is possible to calculate the expected amount of secondary radicals formed at 77° K along the tracks of the heavy ions. The observation that no spectral changes take place with increasing stopping power is clearly in contrast to the predictions from the thermal spike model. Therefore, the model in its present form does not apply to radical formation or secondary radical reactions.

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KEY WORDS: Electron spin resonances, Free radicals, Heavy ions, Thermal spikes.

INTRODUCTION

The efficiency of different types of radiation in producing free radicals in substances of biological interest has been explored previously (1). It was found that at room temperature the types of "secondary radicals" were independent of the linear energy transfer (LET), whereas the yield decreases for radiation with stopping powers above 200 MeV gm^{-1} cm^2. Due to experimental difficulties no attempts were made in these early studies to observe the radicals induced at low temperatures.

In the present work the production of radicals by fast, stripped, heavy ions in biological substances has been studied at 77° K. These studies have a twofold purpose. First, we want to see if the types of radicals produced at 77° K are influenced by the radiation quality, and second, attempts are made to analyze these data in relation to the "thermal spike model."

Several authors (2-6) have suggested that fast heavy ions with a large stopping power will give rise to temporary localized heated regions around the ion tracks. The efficiency of these thermal spikes in causing effects in chemical and biological systems has been questioned, mainly because of their short duration times. In the work reported here some compounds have been selected that are known to give one particular electron spin resonance (ESR) spectrum following irradiation at 77° K and a different one after heat treatments at higher temperatures. With a detailed knowledge of the kinetics for these spectral changes it is possible to test the applicability of the thermal spike model to radical

formation processes.

EXPERIMENTAL PROCEDURE

Sample preparation and irradiation

DL-valine, N-acetyl-DL-valine, L-threonine, L-cysteine (free base), pepsin, lysozyme, and ribonuclease were all obtained commercially and used without further purification.

The samples were irradiated in thin layers (approximately 50 mg/cm^2) in the low-temperature irradiation chamber shown in Fig. 1. The samples were evacuated (approximately 10^{-3} torr) and the entire sample holder with the ESR tube attached was completely submerged in liquid nitrogen. The polycrystalline samples were kept between a thin Mylar window (upstream from the sample) and a brass trap door. After irradiation the trap door was opened and the powdered sample shaken into the ESR tube, which was then sealed off under vacuum without any increase in temperature.

The samples were irradiated with 6.5-MeV electrons from an electron accelerator or with heavy ions from the Berkeley heavy-ion linear accelerator (Hilac). Beams of helium, carbon, neon, and argon ions with an energy of 10.4 MeV per nucleon were used. Doses of the order 2 to 6 Mrads, given at a dose rate of 0.3 Mrads/min, were used. The dosimetry is described elsewhere (1, 7).

In these experiments all the ions except helium were stopped completely in the sample. The average stopping power for these ions was taken to be E/R, where E is the total energy of the particle and R is the range. The values used in the subsequent calculations for the three

heaviest ions are as follows, given in MeV gm^{-1} cm^2: 2.5×10^3 (C^{12} ions), 6.3×10^3 (Ne^{20} ions), and 1.6×10^4 (Ar^{40} ions).

The ESR measurements

The ESR spectra were taken at 77° K with a Varian X-band spectrometer. In these experiments we were mainly interested in the types of radicals formed, i.e., in the form of the ESR spectra. Consequently, second-derivative spectra were recorded to improve sensitivity towards any spectral changes. The magnetic field was measured with a proton resonance field meter and the microwave frequency with an electronic counter.

After the first measurement at 77° K the samples were heat treated for 5 min at different temperatures, brought back to 77° K, and the spectrum observed again.

RESULTS AND DISCUSSION

Types of Radicals at 77° K

In Fig. 2 are presented the ESR spectra obtained when DL-valine is exposed to electrons and different types of heavy ions at 77° K. The spectra are probably due to a radical anion or cation (8). It appears clearly from Fig. 2 that there is no major change in the ESR spectra with LET over this large range. Results similar to those for valine were observed for all the compounds studied. The spectra for threonine, cysteine, and the protein, pepsin, are given in Figs. 3, 4, and 5. The data confirm and extend the results previously obtained at 295° K that the types of radicals formed are independent of the stopping power of the bombarding particle (1).

The substances used in these experiments have been chosen because the ESR spectra, and consequently the types of radicals, induced at 77° K are different from those observed at higher temperatures. Thus, as shown in Figs. 3 and 6, the spectra of threonine and valine change in two steps into the final resonance. The first conversion takes place in the temperature range 110° to 120° K. The cysteine resonance changes into the sulfur resonance of the $R-CH_2-S^{\cdot}$ radical (Fig. 4, bottom curve), and the pepsin resonance into the protein doublet (Fig. 5). The point we want to make from these experiments is that for each substance irradiated at 77° K, the low-temperature spectrum was always observed even for the most densely ionizing particles used (Ar^{40} ions).

The Thermal Spike Model

A large fraction of the energy of an ionizing particle is converted into heat when it is absorbed in a substance. This has prompted the idea that the radiation leads to localized regions of high temperatures (2-6). Along the track of a heavy ion these heated regions overlap and coalesce into a cylindrical thermal spike. Some results in radiation chemistry and biology have been analyzed on the basis of this theory (4-6), but no direct evidence supporting the thermal spike model seems to exist.

Radical formation by heavy ions at low temperatures seems to constitute a system that may be used to test the thermal spike model. This is based on the fact that certain substances exhibit one particular ESR spectrum as long as the temperature is kept below a certain threshold value, but reveal a different resonance pattern after being warmed to higher temperatures. The following discussion is based entirely on our results for DL-valine, but may apply to other substances.

In the thermal spike model the energy given off by a heavy ion initiates a "heat-wave" that spreads out according to the classical laws of heat conduction,

$$\nabla^2 T = \frac{1}{D}\frac{\partial T}{\partial t}, \tag{1}$$

where T is the absolute temperature, t is time, and D is the thermal diffusion coefficient. For a heavy ion the energy is released along the track of the particle and the cylindrical solution of equation 1 can be used:

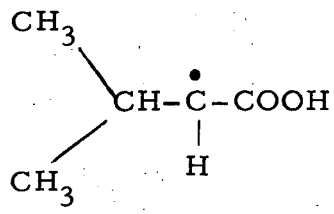
$$T(r,t) = T_0 + \frac{Q}{4\pi c \rho}\frac{1}{Dt}\exp(-r^2/4Dt). \tag{2}$$

Here r is the radial distance from the track core, T_0 is the ambient temperature (77° K), Q is the stopping power of the particle, c is the heat capacity (a value of 0.3 cal/gm °K is used here), and ρ is the density. According to Dienes and Vineyard (2), a value of $D = 10^{-3}\,cm^2/sec$ is appropriate for most substances, as this parameter is most likely proportional to the lattice thermal conductivity only. This value is adopted here, and seems to be a conservative upper value; the choice of a smaller value for D would make the following conclusions even more valid. It is possible to use equation 2 and calculate the maximum temperature reached at different distances from the track core as well as the time elapsed before this value is attained. The results are given in Fig. 7. Equation 2 can also be used to calculate the time elapsed before the temperature at a particular distance decreases to a certain value. In the subsequent discussion we have used the times required to reach 1,000° K and 600° K above ambient.

The kinetics for radical reactions in DL-valine at various temperatures have been studied in detail (9, 10). The sequence of events can be summarized as follows:

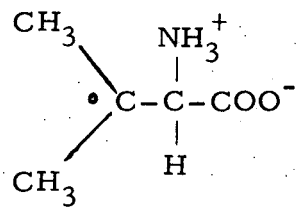
$$\text{Valine} \xrightarrow{\text{ionizing radiation}} R(1) \xrightarrow{K_1} R(2) \underset{K_3}{\overset{K_2}{\rightleftarrows}} R(3) \longrightarrow \text{nonradical products}$$

Figure 6 shows spectra for R(1), R(2), and R(3) in polycrystalline samples. Box et al., (8) have studied single crystals of valine and suggested that R(1) is a cation or an anion, whereas radicals R(2) and R(3) have been identified (8, 11) as



R(2)

and



R(3)

Each step involving spectral changes was found to fit first-order kinetics. The step from R(2) to R(3) seems to involve two first-order processes. Furthermore, the conversion from R(1) to R(2) has an efficiency of unity. The activation energies and frequency factors are given in Table I.

It is evident from the large spectral differences (Fig. 6) that any conversion from R(1) to R(2) or R(3) would easily be detected. If therefore the temperatures in the thermal spikes got high enough and lasted long enough to cause any conversion when valine was bombarded with heavy ions at 77° K a spectrum different from that for R(1) would appear.

The percent conversion F(t) at a particular temperature is given by

$$F(t) = 100 \times [1 - \exp(-Kt)], \qquad (3)$$

where t is the time at temperature T. The reaction constant K is given by

$$K = A_0 \exp(-E_a/RT). \tag{4}$$

Here E_a and A_0 are the activation energy and the frequency factor respectively.

The conversion from R(1) to R(2) as expected from the thermal spike model was calculated in the following way. Equation 2 and Fig. 7 were used to determine the time intervals for which the temperature in the heat-wave was 1,000° K and 600° K above ambient at a given radius. This procedure was repeated for 5-Å increments around the tracks of carbon, neon, and argon ions. These results together with the reaction constants at 1077° K and 677° K as obtained from Table I and equation 4 were then used to calculate the percent conversion expected in the different layers. These results, given in Fig. 8, represent a conservative estimate because the average temperatures were greater than 1077° K and 677° K.

In order to give an estimate of the overall conversion to be expected when valine is bombarded with heavy ions, the distribution of radicals along the track must be known. So far nothing is known about the radical distribution. However, on the basis of the δ-ray spectrum for these heavy ions (12) it is possible to arrive at a rough estimate of the initial energy deposition along the track of the particles. These data are given by the histogram in Fig. 8 (ordinate to the right). Based on these results it is reasonable to assume that the majority of the radicals are formed within a distance of 50 Å from the center of the ion

track. Accordingly, the thermal spike predicts (Fig. 8) that approximately 15% of the valine radicals formed by carbons ions at 77° K would be of type R(2) and that a composite spectrum different from that for electron irradiation should be observed. For neon and argon ions the corresponding values would be about 50% and 80%.

One further calculation is necessary in order to compare the experimental results with the predictions from the thermal spike model. If the radicals in the track core undergo all the reaction steps, including the final decay, the ESR spectrum would be due only to radicals formed by the δ-rays at large distances from the track. These radicals would be away from the thermal effects of the track, and would therefore give a spectrum at 77° K identical to that for electron irradiation. This, however, is not the case. Calculations based on the parameters of Table I for radical decay show that only negligible decay of R(3) would occur, even at the maximum temperatures, for the short times involved. Consequently, the thermal spike model predicts a large conversion of R(1) to R(2), but no final radical decay.

As seen from Fig. 2, all ions gave the same valine spectrum, which was identical to that for electron irradiation. There is no indication of any radical conversion whatsoever. As mentioned above, this failure to observe R(2) or R(3) cannot be explained by thermal decay of radicals in the track core. Therefore, the inescapable conclusion is that the thermal spike model in its present form does not apply to radical formation by heavy ions at 77° K.

ACKNOWLEDGMENTS

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Table I. Activation energies and frequency factors for the radical reactions in irradiated DL-valine.[a]

Reaction step	E_a (kcal/mole)	A_0 (sec^{-1})
Conversion of R(1) to R(2)	7.8 ± 0.2	4.5×10^{11}
Formation of R(3)	6.6 ± 0.3 8.3 ± 0.5	1.1×10^{4} 4.5×10^{4}
Decay of R(3)	22.6 ± 1.0	8.8×10^{7}

[a] These data have been taken from refs. (10, 11).

FIGURE CAPTIONS

Fig. 1. The low-temperature irradiation chamber for heavy ions. The chamber was attached directly to the exit port of the Hilac. The ions penetrated two 0.25-mil Mylar windows before hitting the samples. An enlarged figure of the sample holder is shown below. After irradiation the trap door was opened and the sample shaken down in the ESR tube.

Fig. 2. Second-derivative ESR spectra of DL-valine exposed to different types of radiation at 77° K, measured without any warming. The arrows indicate the positions of the resonance for DPPH (g = 2.0036). Dose, 2 Mrad.

Fig. 3. Second-derivative ESR spectra of L-threonine exposed to electrons and 120-MeV carbon ions at 77° K (the two upper curves). Notations as in Fig. 2. The two lower curves reveal the spectral changes that take place when the samples are heat treated to the temperatures indicated and again measured at 77° K. The intermediate radical is formed at temperatures above 110° K, whereas the final radical is formed at temperatures above 230° K.

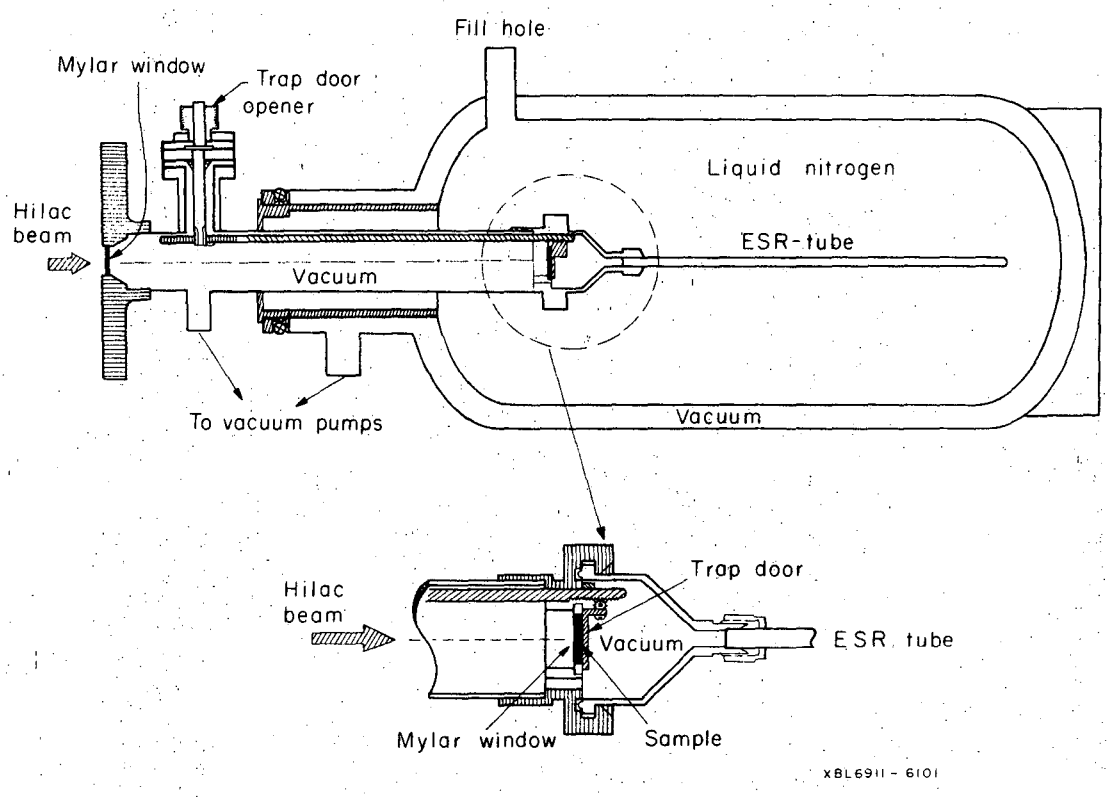
Fig. 4. Second-derivative ESR spectra of the thiol cysteine (free base) exposed to electrons and Ar^{40} ions at 77° K. Notations as for Fig. 2. The lower spectrum, which is entirely due to sulfur radicals of the type $R\text{-}CH_2\text{-}S.$, appears when the samples are heat treated at temperatures above 140° K.

Fig. 5. Second-derivative ESR spectra of pepsin exposed to electrons and Ar^{40} ions at 77° K. Notations as in Fig. 2. The lower spectrum is the usual protein doublet, which in [the case of] pepsin appears at temperatures above 220° K.

Fig. 6. Second-derivative ESR spectra for DL-valine. Valine was irradiated with 6.5-MeV electrons at 77° K and subsequently heat treated at higher temperatures. The spectra after heat treatment to the three temperatures indicated are those for R(1), R(2), and R(3). The observations are made at 77° K.

Fig. 7. Maximum temperatures reached around the tracks of fast carbon, neon, and argon ions from the Berkeley Hilac as calculated on the basis of the thermal spike model (see text). The dashed curve (ordinate to the right) gives the times elapsed from the initial energy deposition until the maximum temperature is reached at different positions outside the track. This time is independent of the LET.

Fig. 8. Percent conversion of R(1) to R(2) for the three different ions as expected from the thermal spike model. The region around the track was divided in layers of thickness 5 Å in these calculations. The curves give the percent of the total number of radicals in the particular layer that should be converted due to the heat wave. The histogram (ordinate to the right) gives a rough estimate of the initial energy deposition around the track. This calculation is based on the assumption that 46% of the total energy is due to δ electrons. The δ-ray spectrum and the stopping power for the secondary electrons as given by Brustad (12) have been used.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Trap door
opener
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Fig. 1

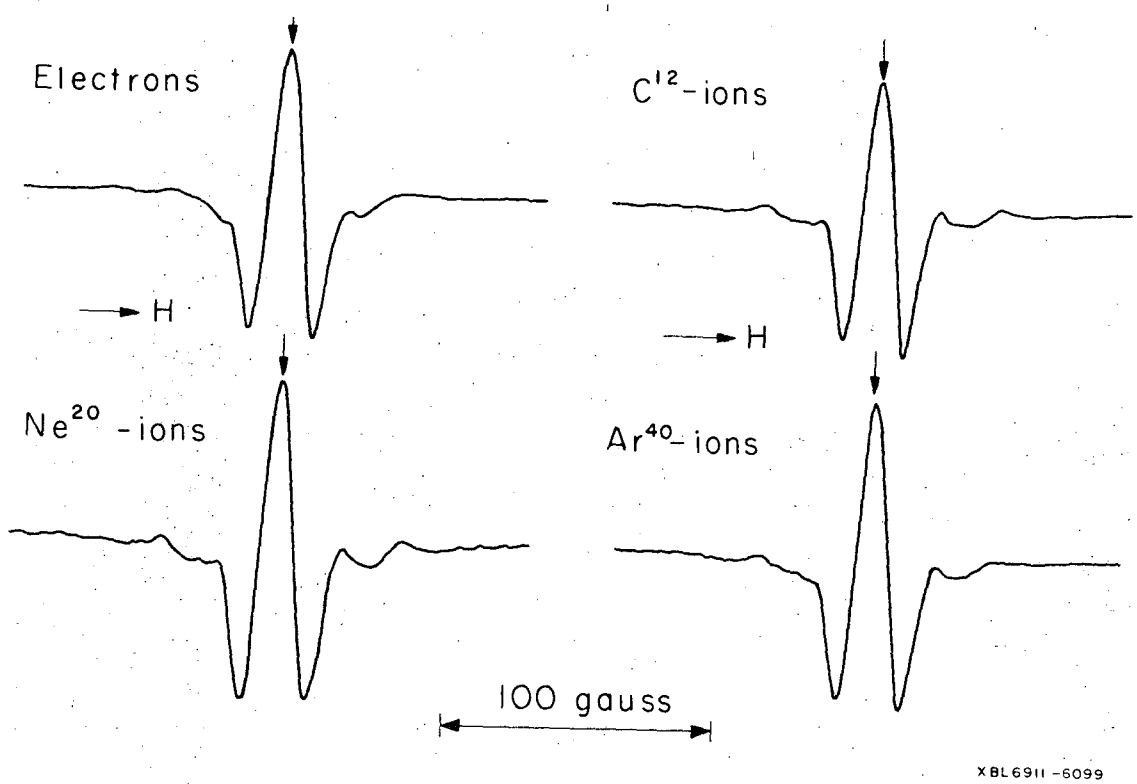
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Fig. 2

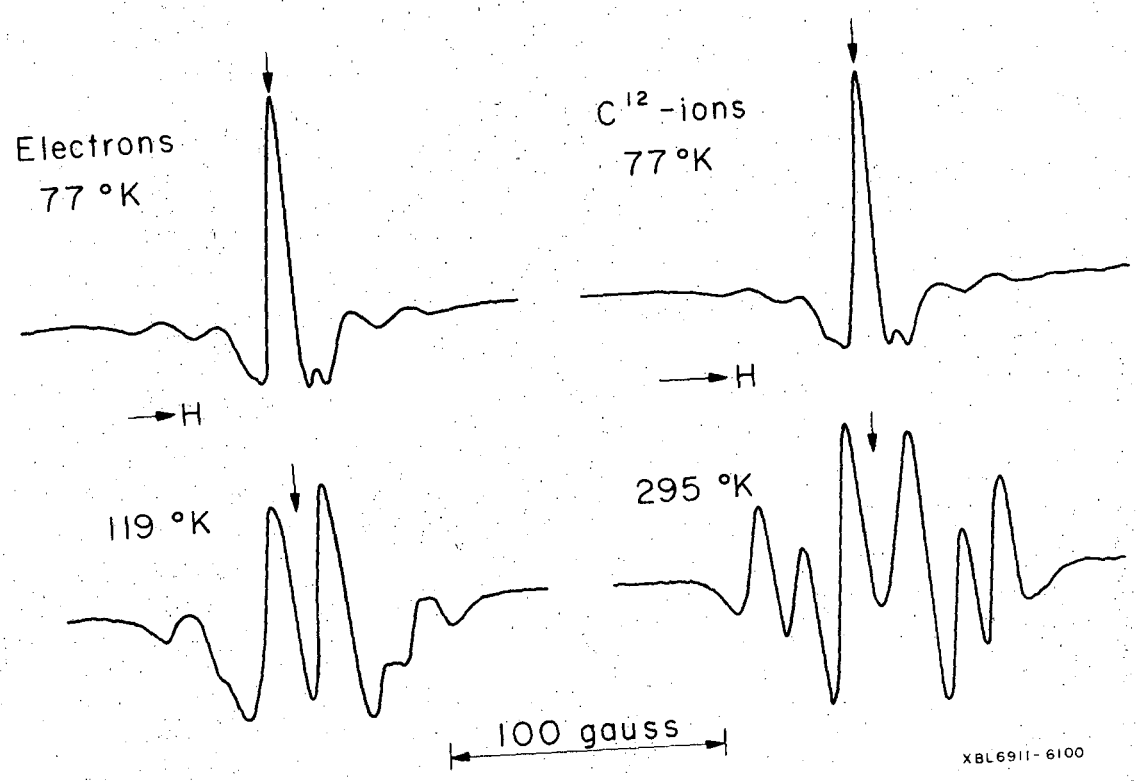
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Fig. 3

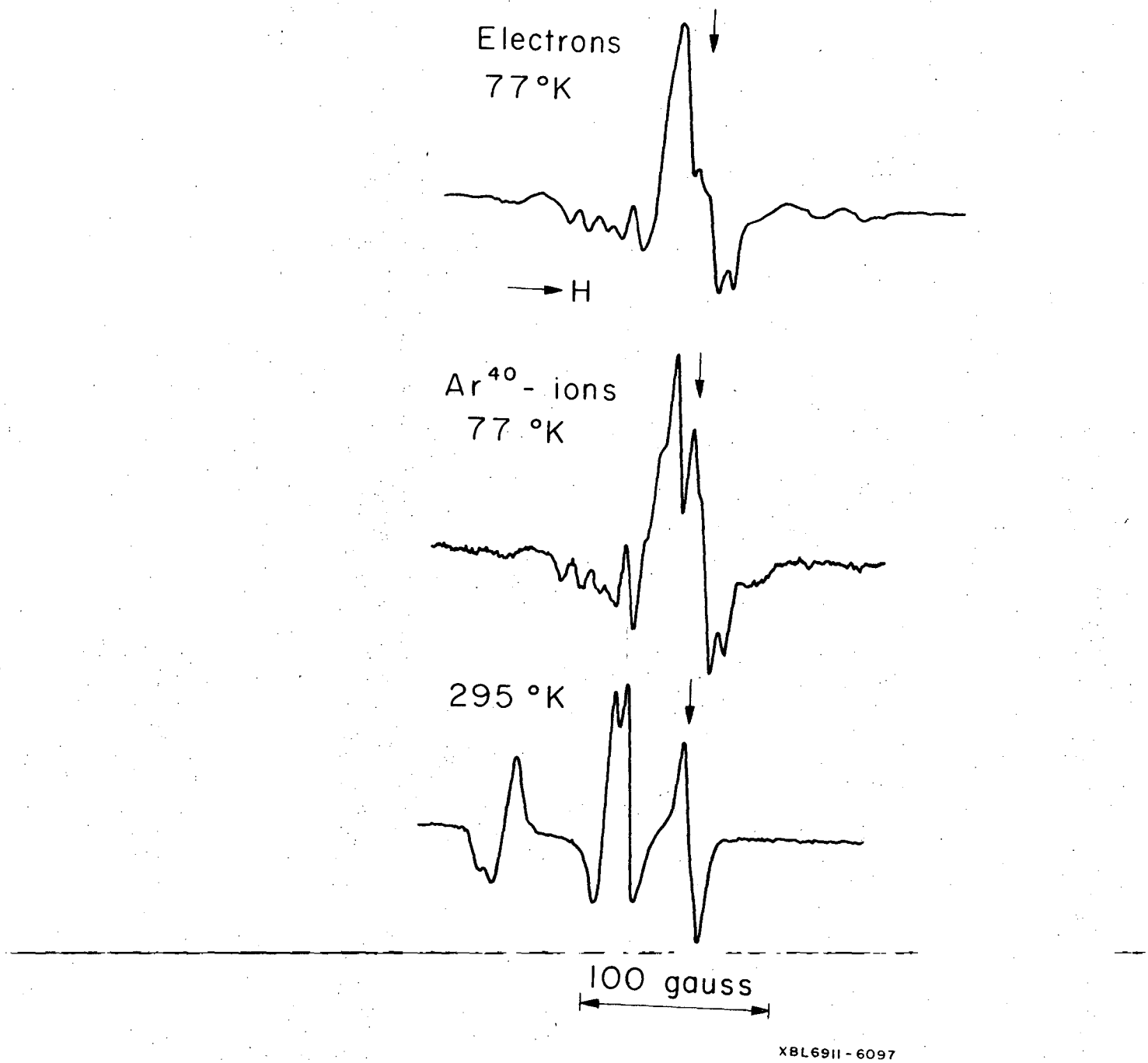

Fig. 4

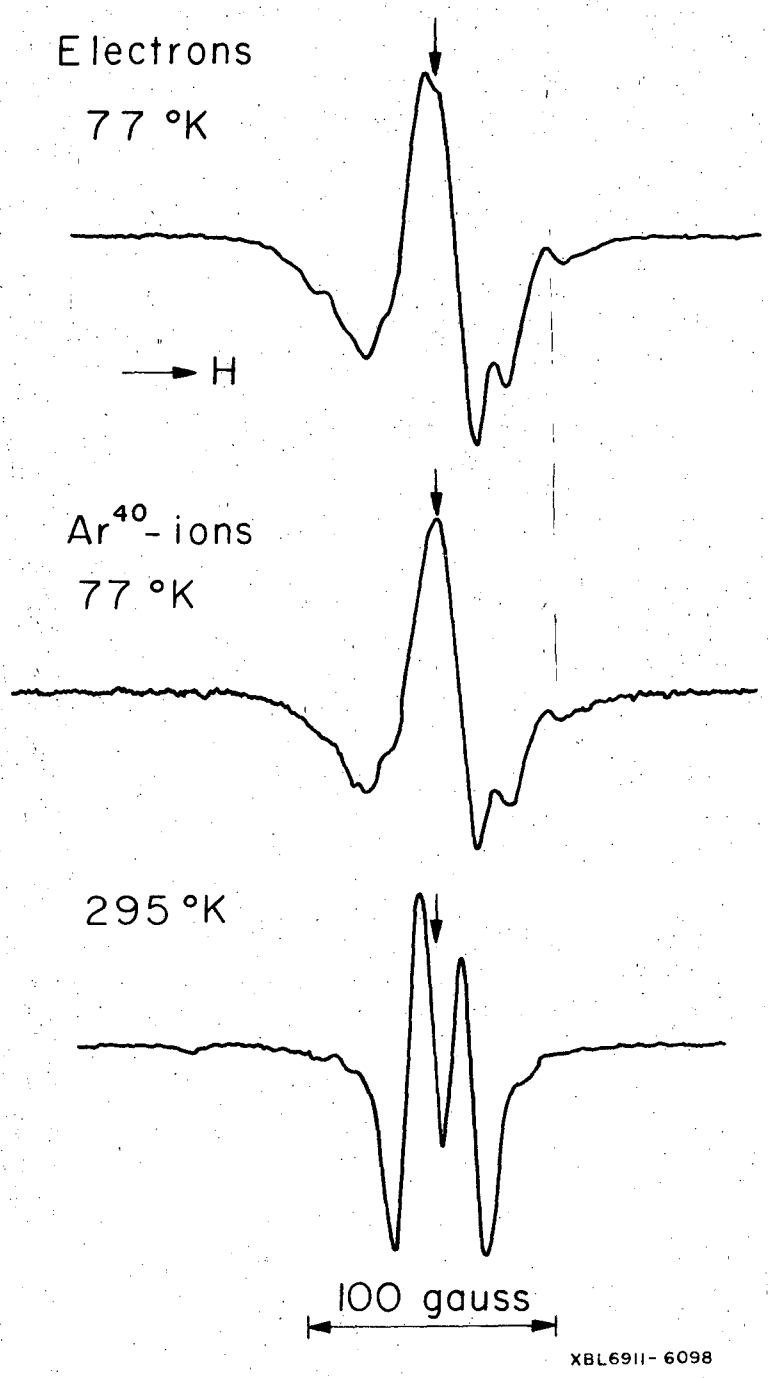

Fig. 5

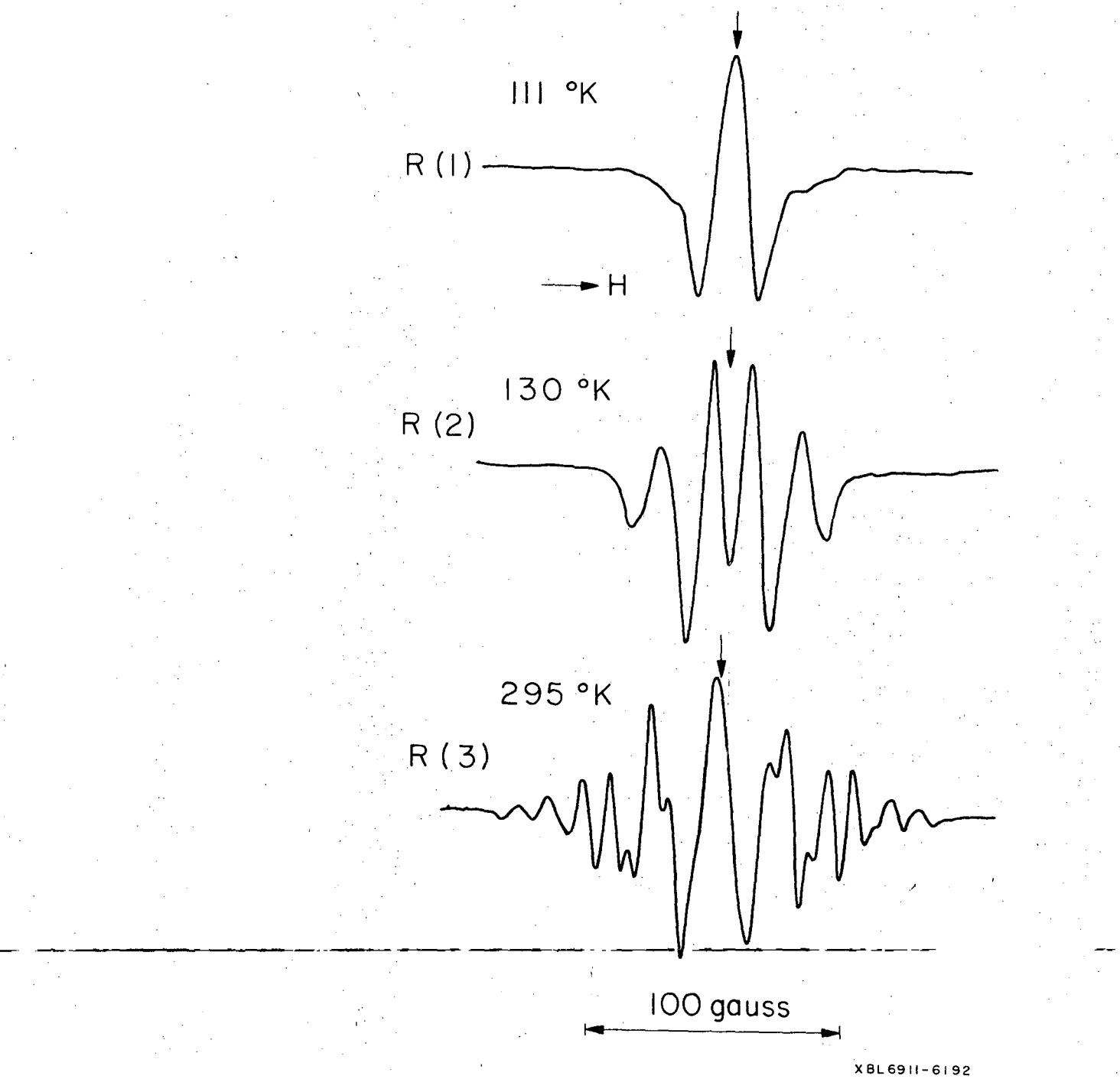

Fig. 6

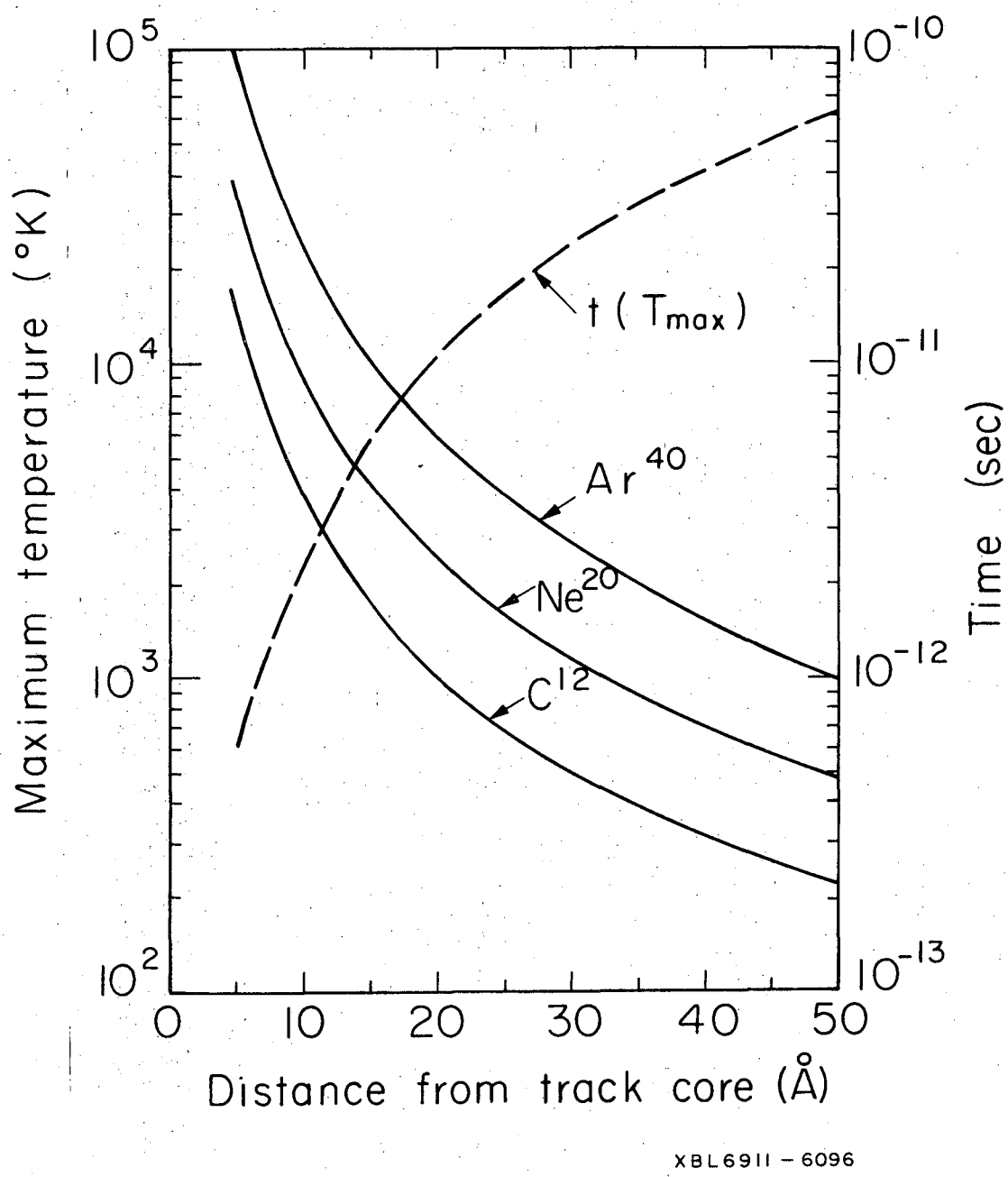

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Fig. 7

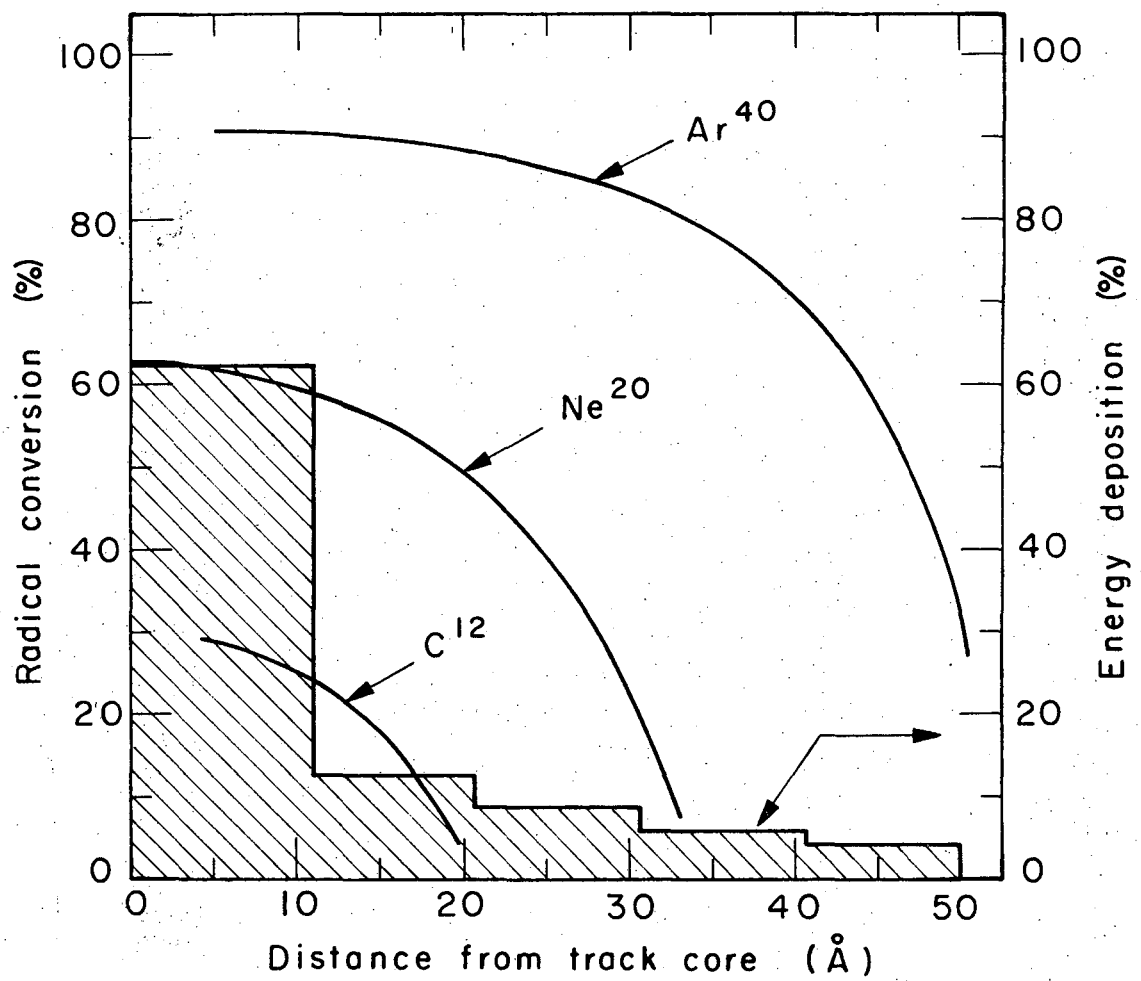

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Fig. 8

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