

KFK-255

KERNFORSCHUNGSZENTRUM
KARLSRUHE

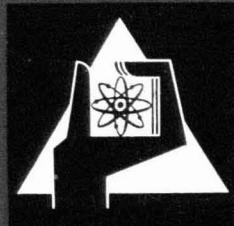
August 1964

KFK 255

Institut für Strahlenbiologie

Comparative E.S.R.-measurements of radical production in
amino acids by ^{210}Po alpha- and ^{60}Co gamma-radiation

Adolf Müller, Philip E. Schambra and Eleonore Pietsch



GESSELLSCHAFT FÜR KERNFORSCHUNG M.B.H.

KARLSRUHE

Comparative E.S.R.-measurements of radical production in amino acids by ^{210}Po alpha- and ^{60}Co gamma-radiation

ADOLF MÜLLER, PHILIP E. SCHAMBRA and ELEONORE PIETSCH

Institut für Strahlenbiologie, Kernforschungszentrum, Karlsruhe,
Germany

(Received 22 January 1964)

The spectra and radical yields for eight different amino acids after irradiation with ^{210}Po α -particles were investigated by E.S.R.-spectroscopy, and compared to those for ^{60}Co γ -rays and x-rays. Radical-concentration as a function of dose of α -particles was determined, as well as the effect of storage time on the radical-concentration and amplitude of the first derivative signal.

The hyperfine spectra obtained from the aromatic amino acids after α -irradiations were identical with those observed after γ -irradiations, in contrast to our findings for aliphatic amino acids. After α -irradiation, the radical yields of the aliphatic amino acids were about a factor of six less than those obtained with γ -rays, while for the aromatic amino acids the reduction in yield was much smaller. With post-irradiation storage time, a stronger reduction in radical-concentration was observed after α -irradiation than that reported after x-irradiation. The dependence of radical-concentration on radiation dose was compared for α - and x-rays. The typical saturation of radical-concentration was found to occur independently of the actual concentration reached and at nearly equal doses of different radiations.

1. INTRODUCTION

Dry amino acids have frequently been used as model substances in the electron-spin-resonance (E.S.R.) investigation of radiation damage to biological material. The first quantitative E.S.R.-measurements of radical yields were carried out on glycine after irradiation with x-rays (Ehrenberg, Ehrenberg and Zimmer 1957). These authors found a linear increase of radical yield with radiation-dose up to 350 kr and a G -value between 1 and 10.

While most of the subsequent investigations of this type have been concerned with the effects of x- and γ -rays, only a few experimenters have made use of densely-ionizing radiations in such work. The radical yield in glycine after α -irradiation was reported by Kirby-Smith and Randolph (1957) as amounting to only 10 per cent of the yield obtained with ^{60}Co γ -radiation. In a subsequent publication (Kirby-Smith and Randolph 1960) 30 per cent was given for this ratio, and the yields of several other substances after irradiation with ^{60}Co γ -rays, x-rays, fast neutrons (14 Mev), and ^{210}Po α -particles were listed. No change of the glycine spectrum with radiation dose was reported, although such changes had been observed after irradiation with high doses of 1.5 Mev electrons at very high dose-rates of about 10^{10} rad/sec (Boag and Müller 1958). Quantitative measurements on irradiated glycine and other substances were found to show

saturation of radical-concentration at high doses (Randolph 1961, Prydz and Henriksen 1961) following the discovery of the same effect at lower doses in irradiated seeds (Zimmer, Ehrenberg and Ehrenberg 1957). Recently, a decrease of radical-concentration in a glycine single crystal at doses of more than 6 Mrad of x-rays has been reported by Schirmer and Sommermeyer (1962), and in polycrystalline glycine above 40 Mrad of γ -rays by Rotblat and Simmons (1963).

Schirmer and Sommermeyer interpret their dose-effect curve of radical-concentration as a function of the second order :

$$C + kC^2 = aD, \quad (1)$$

where C is the concentration and D is the dose, while a and k are constants. Rotblat and Simmons relate their results to a simple exponential function :

$$C = C_\infty [1 - \exp(-D/D_{37})], \quad (2)$$

where C_∞ is the saturation value of radical-concentration and D_{37} is the dose at which 63 per cent ($= 1 - 1/e$) of the saturation value has been reached. The same function has also been reported as fitting the results on irradiated nucleic acid (Müller 1963). Rotblat and Simmons also confirmed the change of the glycine spectrum with very high doses of fast electrons delivered at a very high dose-rate. These authors interpreted the observed spectral changes as being due to an increased diffusion of oxygen into the irradiated material, whereas Boag and Müller (1958) attributed these changes to variations in the dose saturation of different radicals. Schirmer and Sommermeyer also found dose saturation of radical-concentration on irradiation of glycine with α -particles and fitted the resulting dose-dependence to a rather complicated function different from equation (1). However, the expression they derive for the yields after α -irradiation is not much different from the simple exponential expression of equation (2). As in the work of Kirby-Smith and Randolph, no change of spectrum at high doses was reported by Schirmer and Sommermeyer, while the radical yield of α -irradiated glycine and other amino acids was found to be lower compared with γ -irradiated substances by a factor similar to that reported by Kirby-Smith and Randolph (1960).

The present work was started before Schirmer and Sommermeyer had published their results, as the available data on E.S.R.-measurements of radical yields with densely-ionizing radiations appeared to need extension. Such a view still holds because our results and interpretations differ qualitatively and quantitatively from the results which have since been published.

2. EXPERIMENTAL PROCEDURE

2.1. Materials

Amino acids were obtained as crystalline powders of the highest purity available from three different firms and used without further purification. The following substances are included in the present investigation : glycine, glycylglycine, DL- α -alanine, DL-phenylalanine, DL-tryptophane (California Corp. for Biochem. Res.), glycine, β -alanine, DL-valine, L-tyrosine (Merck), glycylglycine (Nutritional Biochemicals). No systematic variations in the measurements reported here were observed between 'identical' compounds obtained from different sources.

Comparative E.S.R.-measurements

2.2. Radiation sources and dosimetry

For irradiations with α -particles, a ^{210}Po -source of 1.8 Curie activity was obtained from the Monsanto Research Corporation. The ^{210}Po had been deposited on a metal disc as an active area of 0.6 cm diameter and was covered with a stainless-steel foil of about $3.8\ \mu$ thickness, transmitting α -particles with a nominal energy of 3.4 mev. The disc was mounted in a plexiglass vessel, which also contained the samples for irradiations. The absorbed dose was measured by replacing the samples by Fricke dosimeter solution (containing 1 mM FeSO_4). Samples and dosimeter were irradiated while the container was continuously flushed with helium gas at atmospheric pressure, thus obtaining maximum particle range at atmospheric pressure and avoiding the presence of oxygen in the irradiation vessel. A G -value of 3.5 was used for the anoxic oxidation of Fe^{++} ions. The concentration of Fe^{+++} was measured by the optical extinction at $304\text{ m}\mu$ wavelength. The absorbed dose was measured at three different source-sample distances (11 mm, 15 mm, and 21 mm). The results were checked in two ways : (i) calculations of the dose-rate from the formula of Hart and Terandy (1958); (ii) measurement of the particle flux and energy distribution by solid-state detectors and conversion into dose-rate. The results of the three methods agreed within 10 per cent.

Later, another Po-source of 2.2 Curie and otherwise identical specifications was obtained. The dose delivered was again measured with the Fricke dosimeter under a helium atmosphere. The results were checked with a parallel-plate ionization chamber by determining the saturation current induced by the α -particles in various gases. Again, the differences were smaller than 10 per cent. For comparison with the effects of α -irradiation, sparsely-ionizing radiation was used from a ^{60}Co γ -source at a dose-rate of $1.03 \times 10^4\text{ rad min}^{-1}$. The samples were placed in glass vessels through which a slow stream of helium gas was passed.

3. IRRADIATIONS

Samples of 50–200 mg of the dry powdered material were weighed into plexiglass cups of 17 mm internal diameter and 10 mm depth. For α -irradiations these were subsequently placed on top of a vertical rod extending from a magnetic vibrator into the irradiation vessel. The thickness of the amino-acid layer was sufficient to ensure that all α -particles arriving within the cup diameter were absorbed in sample material and would not reach the supporting plexiglass through holes accidentally formed in the layer of irradiated powder. To improve on the resulting very inhomogeneous dose-distribution due to the extremely short range of the α -particles (about 2 mg/cm^2 or $20\ \mu$ in organic matter of unit density), the samples were vibrated during irradiations so that the exposed surface layer was continually replaced.

4. E.S.R.-SPECTROMETRY

First derivations of E.S.R.-spectra were recorded with a commercial spectrometer (Hilger & Watts) working at 3 cm wavelength. Microwave saturation curves were recorded for all irradiated samples and the results corrected, if necessary (see below). The cavity supplied with the spectrometer was replaced by a double cavity (Köhnlein and Müller 1960) with external modulation of

100 kc/sec frequency. The 100 kc/sec output of the spectrometer was transformed to match the impedance of the cavity coils, which were modulated one after another. Qualitative evaluations of the spectra were performed with a moment balance (Köhnlein and Müller 1962) or a commercial moment planimeter (Amsler).

5. RESULTS

To study the influence of microwave saturation on the E.S.R.-spectra, the amplitudes of the first derivative spectra were measured at various attenuations of microwave power. This was done with all substances included in the present work. The results on glycylglycine and DL-tryptophane, which are representative also for the other compounds, are plotted in figure 1 along with measurements

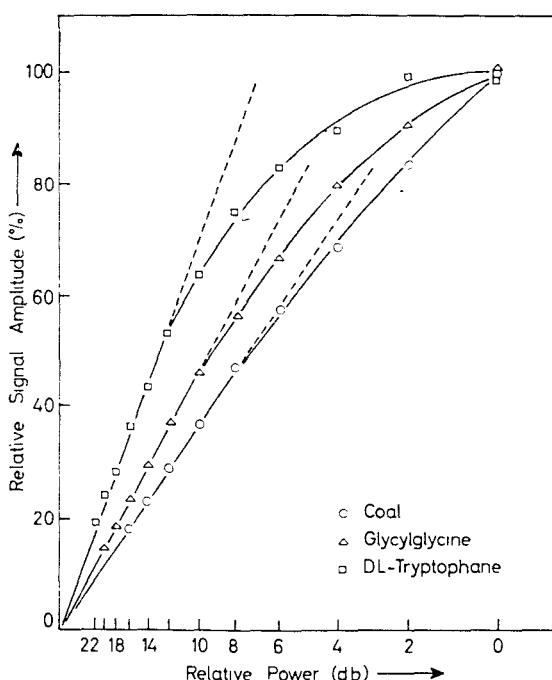


Figure 1. Amplitudes of the first derivative spectra of carbon and of α -irradiated glycylglycine and DL-tryptophane in relative units versus relative microwave power.

on a carbon standard sample. Corresponding to the theoretical dependence in absence of saturation, a linear rise is found only at reduced power-levels. Spectra were recorded at either 0 dB or 10 dB attenuation, and a correction for microwave saturation was applied, if necessary. The dependence of the amplitude of the first derivative spectra for the aliphatic amino acids studied was linear for microwave attenuations to at least 10 dB, while for DL-tryptophane and L-tyrosine some deviation from linearity was observed even at 10 dB attenuation. As the Q -values of the loaded sample cavity were close to each other, a given attenuation corresponds to a definite microwave field-strength or power-level within the cavity. Therefore the attenuation at which the signal amplitudes deviate from linearity in figure 1 is a measure of the relaxation time of the observed radicals.

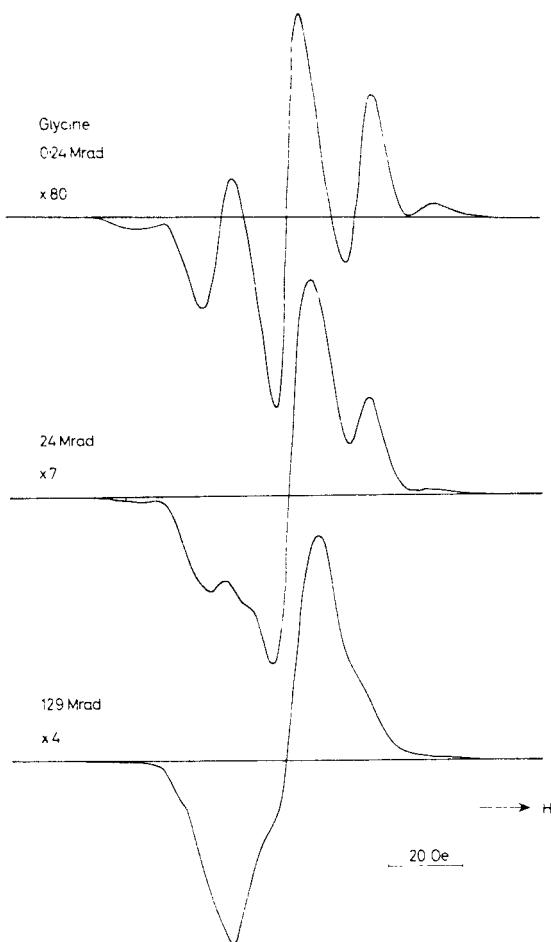


Figure 2. First derivative spectra of glycine after various doses of α -particles. The amplifier gain used for recording the spectrum is also indicated.

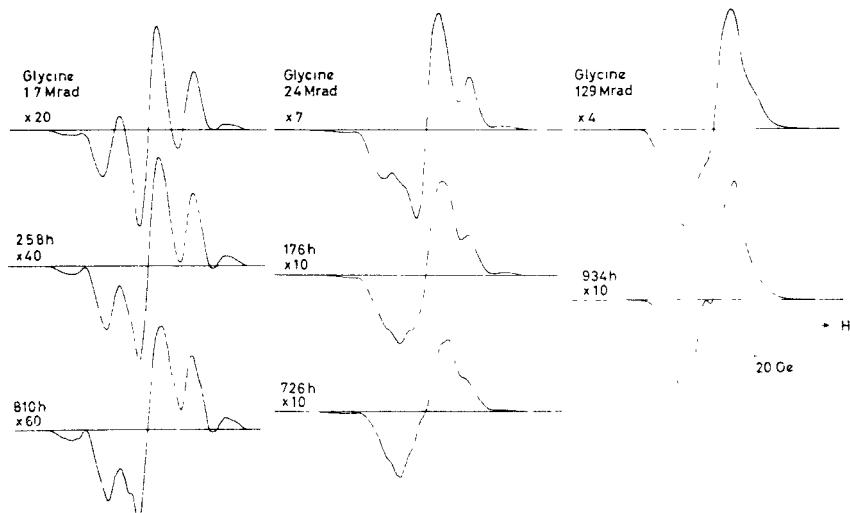


Figure 3. First derivative spectra of glycine as a function of the dose of α -particles and of storage time in air after irradiation. The amplifier gain used is indicated.

The first derivation spectra of glycine recorded immediately after α -irradiation are shown in figure 2. After a dose of 1.7 Mrad the usual triplet spectrum is found; with increasing dose the structure is gradually lost until at 129 Mrad a broad single line with small indications of structure is seen. These changes of the glycine spectrum are very similar to those observed after high doses of fast electron pulses (Boag and Müller 1958). On storage in air all glycine spectra recorded are subject to further modifications which are represented in figure 3. The changes at low doses are similar to those reported previously

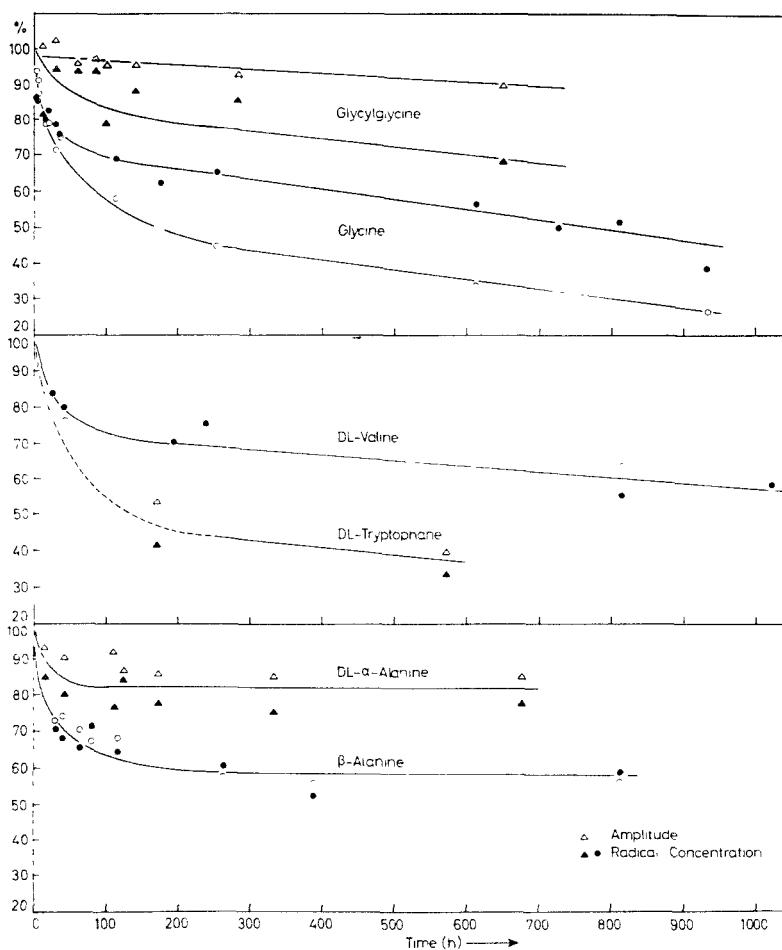


Figure 4. Radical concentration and first derivative amplitudes versus storage time in air after irradiation with α -particles.

following irradiation of glycine with x-rays (Ehrenberg *et al.* 1957). In contrast to earlier measurements of x-irradiated glycine, where the radical concentration changed only very little during storage in air (Zimmer, Köhnlein, Hotz and Müller 1963), the radical-concentration after α -irradiation decreased considerably and in addition to the described changes in spectrum. In figure 4, radical-concentration and amplitude of the first derivative signal at various times after irradiation are plotted for several substances. For all doses the spectrum of

glycylglycine was a doublet (shown in figure 5), as first reported after γ -irradiation by Gordy, Ard and Shields (1955). It will be noted from figure 4 that for glycylglycine a minor reduction of the first derivative amplitude is accompanied by a substantial reduction of the radical-concentration. This indicates a progressive narrowing of the absorption spectrum with time for glycylglycine, whereas an opposite effect was observed in the glycine spectrum. This result differs from that reported after x-irradiation of glycylglycine by Zimmer *et al.* (1963) who found a decrease in amplitude but no spectral changes. A similar narrowing was observed with the doublet spectrum of gelatine after x-irradiation (Müller 1962).

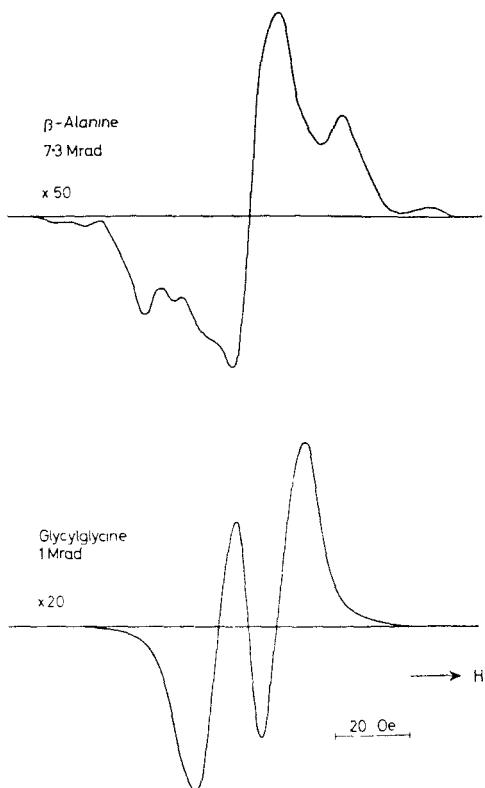


Figure 5. First derivative spectra of β -alanine and glycylglycine after irradiation with α -particles. The gain used is indicated.

The spectrum of α -irradiated β -alanine, also shown in figure 5, displays a less-hyperfine structure than the spectrum observed after x-irradiation (Zimmer *et al.* 1963) and γ -irradiation. A similar reduction of structural resolution following α -irradiation is found in the spectra of the two other aliphatic amino acids investigated : DL- α -alanine and DL-valine (figure 6) in comparison to the spectra of x-irradiated samples published by Zimmer *et al.* (1963) and as observed with γ -rays. As is evident from figure 4, the radical concentrations in α -irradiated β -alanine, DL- α -alanine, and DL-valine decrease in close proportion to the signal amplitudes, indicating that no major spectral changes occur during storage. In contrast to the aliphatic amino acids the aromatic amino acids

investigated: DL-tryptophane, L-tyrosine and DL-phenylalanine showed identical hyperfine structure after α - and γ -irradiation. The small satellite absorption lines which appear in all three spectra shown in figure 7 and especially in the spectrum of L-tyrosine, have not been reported in previous work, though they were observed after irradiation with both α - and γ -rays in our experiments. Again, radical concentration and signal amplitude for the aromatic acids show the same dependence upon post-irradiation storage time (figure 4).

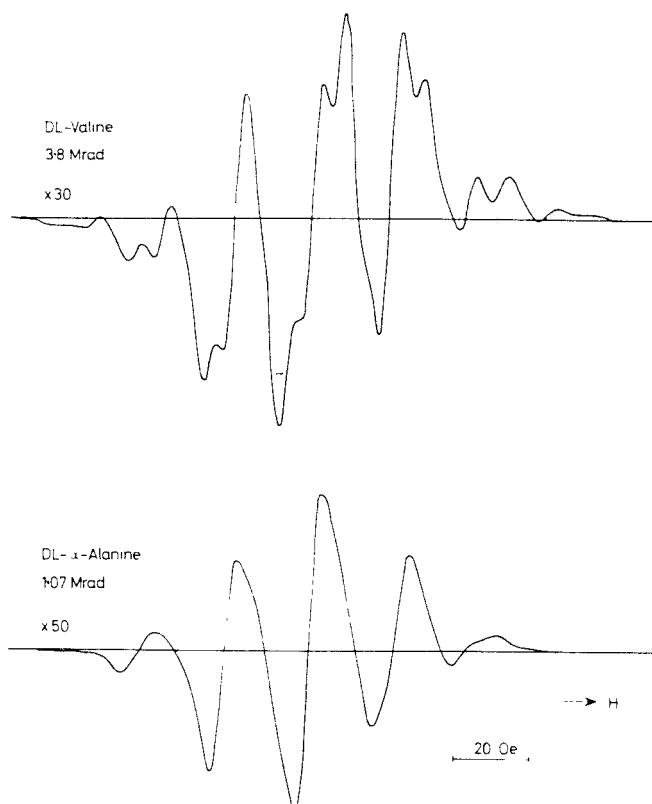


Figure 6. First derivative spectra of DL-valine and DL- α -alanine after irradiation with α -particles. The gain used is indicated.

In figure 8 the measured radical concentrations are plotted versus dose on double-logarithmic scale. The solid lines represent equation (2) as given on p. 588. The D_{37} doses are indicated by arrows, and the initial linear parts of the curves are extrapolated by dashed lines. G -values for radical production are derived from the straight portions of the dose-effect curves and tabulated in the table together with the D_{37} values. The results of measurements after γ -irradiation and after x-irradiation are also listed in the table. The data for x-rays were taken from the work of Zimmer *et al.* (1963) in which the samples were irradiated in air, while the γ -irradiations were performed under the same conditions as the α -irradiations, namely in a helium atmosphere. Whether the differences between the experiments using x- and γ -rays are due to the absence of oxygen

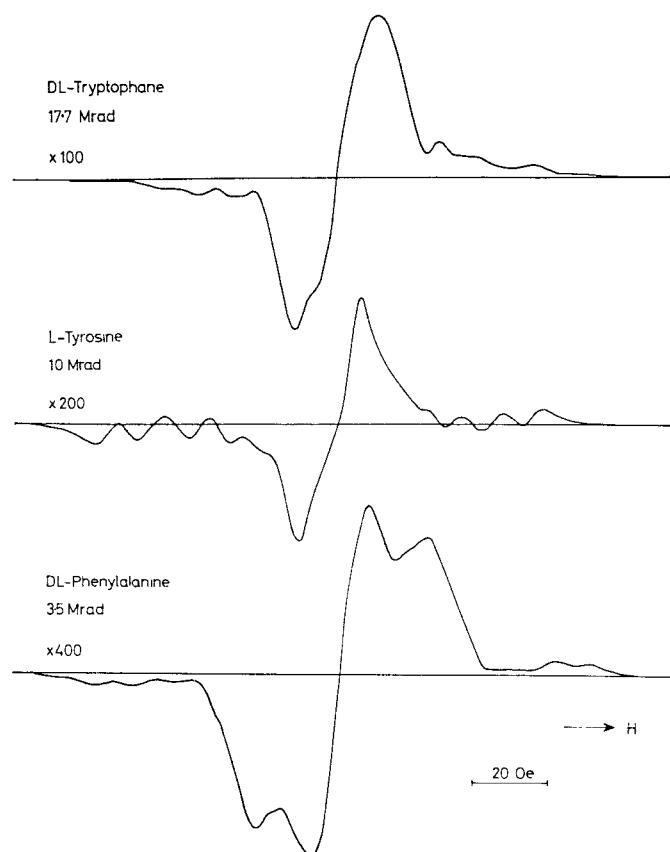


Figure 7. First derivative spectra of DL-tryptophane, L-tyrosine, and DL-phenylalanine after irradiation with α -particles. The gain used is indicated.

in the latter case, or to an LET effect, or to other causes is not known. The data for radical-concentrations as a function of x-ray dose from the work of Zimmer *et al.* (1963) are plotted for the purpose of comparison in figures 9 and 10 together

Compound	G-values			Ratios of G-values		D_{37} (Mrad)	
	α	γ	x	α/γ	α/x	α	x
Glycine	0.7	5.3	4.1	0.13	0.17	7.0	7.7
Glycyglycine	2.0	—	5.7	—	0.35	50	25
DL- α -alanine	0.8	6.0	3.5	0.13	0.23	23	18
β -alanine	0.2	1.3	2.0	0.15	0.10	19	46
DL-phenylalanine	0.6	1.9	0.9	0.32	0.67	—	—
L-tyrosine	0.13	0.3	0.26	0.43	0.50	—	—
DL-tryptophane	0.09	0.06	0.06	1.5	1.5	55	—
DL-valine	0.9	5.4	3.3	0.17	0.27	40	—

Quantitative results of radical production in amino acids.

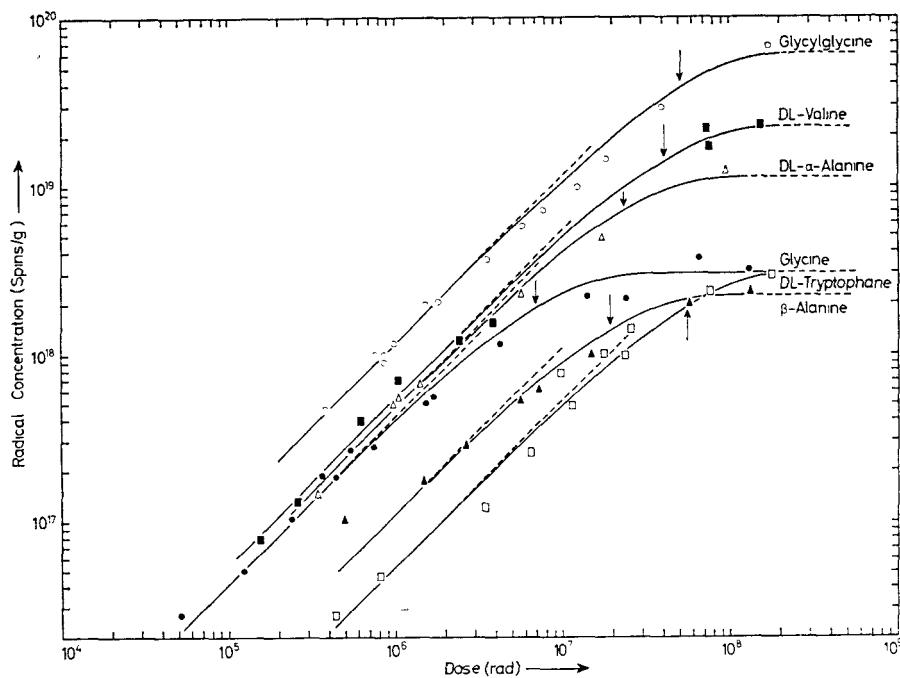


Figure 8. Radical concentrations versus dose of α -particles. The solid lines represent equation (2). The dashed lines are extrapolations of the linear portions, i.e. of the initial slope of the dose-effect curves. The D_{37} -doses are indicated by arrows.

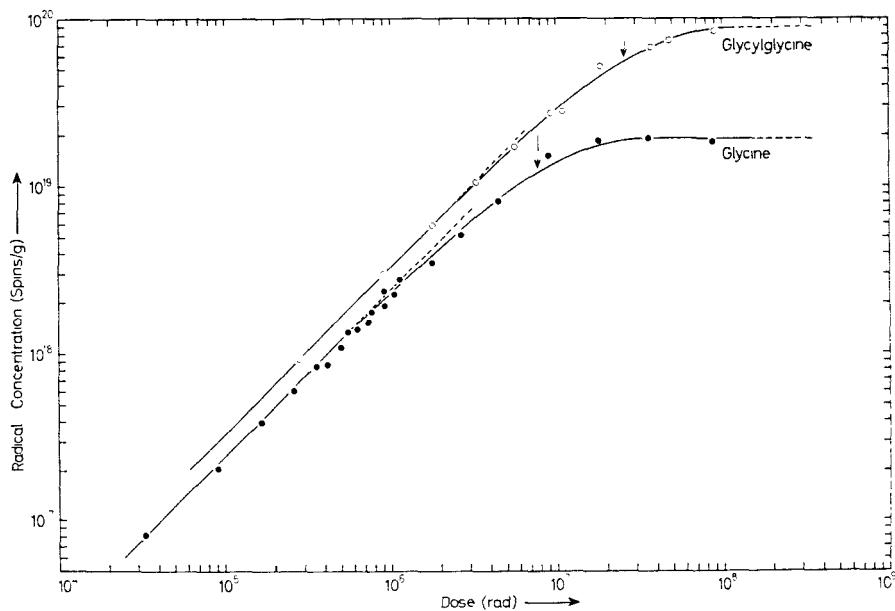


Figure 9. Radical concentrations versus dose of x-rays according to Zimmer *et al.* (1963). The solid lines represent equation (2). The dashed lines are extrapolations as described for figure 8. The D_{37} -doses are indicated by arrows.

with the solid lines giving the best fit to equation (2). In addition to the other data, the ratios of G -values obtained with α - and γ -irradiation, and also with α - and x-irradiation, are included in the table.

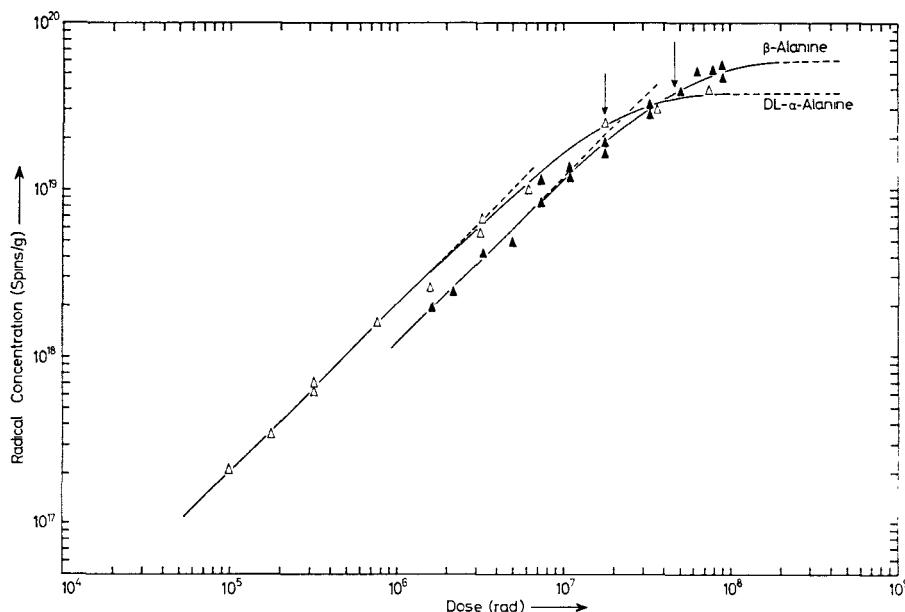


Figure 10. Radical concentrations versus dose of x-rays according to Zimmer *et al.* (1963). The solid lines represent equation (2). The dashed lines are extrapolations as described for figure 8. The D_{37} -doses are indicated by arrows.

6. DISCUSSION

The spectra obtained with α -irradiated glycine at very high doses show qualitative changes which previously have been observed with similar doses of fast electrons at very high dose-rates (Boag and Müller 1958, Rotblat and Simmons 1963). Rotblat and Simmons suggest that the observed spectral changes, which they found to be accompanied by a decrease in radical-concentration, were due to an increased diffusion of oxygen into the samples. Since in our experiments, however, the samples were irradiated in a helium atmosphere, this assumption appears unlikely. The explanation given by Boag and Müller, who attributed the spectral changes to variations in dose saturation of different radicals, also seems unlikely. In view of the high doses needed for this effect, another possibility should be considered : the occurrence of secondary reactions in which radicals absorb radiation energy for a second time resulting in the destruction or transformation of the affected radicals. The high concentration of energy released by α -particles, or short electron pulses of very high intensity, seems to be necessary for this secondary reaction as so far it has not been observed with x- or γ -rays. In view of these findings it is not surprising that a considerable reduction of radical concentration with time is observed after α -irradiation (figure 4), but not after x-irradiation. Similarly, the exceptionally small value for the D_{37} of glycine which is also given in the table is probably correlated to the other unusual phenomena observed with glycine.

As has already been mentioned, the spectra of the other aliphatic amino acids display less-hyperfine structure after α -irradiation. The radical yields are about six times smaller than after γ -irradiation and between four and ten times smaller compared with x-irradiation. On the other hand, the yields with the aromatic amino acids are much less reduced on α -irradiation, and with tryptophane would appear to be even higher than after γ -irradiation. Since the radical yield of tryptophane after γ - or x-irradiation is the lowest of all the compounds studied here, it would seem that the lower the radical yield the larger the ratio of G -values between α - and γ -irradiations. In other words, the smaller the radical yield the more efficient is radical production by densely-ionizing radiation. The D_{37} -values presented in the table fall between 20 and 55 Mrad, with the exception of glycine which has already been discussed. In contrast to the wide range of G -values obtained, the D_{37} -values for x- and α -irradiation are fairly similar. That is, by using α -radiation the saturation concentrations C_∞ are lowered in the average by the same ratio as the G -values. This result cannot be ascribed to an inhomogeneous dose distribution produced by the α -rays since samples with different mean sizes were used in the irradiations : no difference in the dose-saturation level corresponding to the surface-to-volume ratio of these samples was observed. The variations in saturation doses between different compounds correspond rather to the observed differences in G -values.

It therefore must be concluded that the phenomenon of dose saturation does not depend on the final concentration of radicals induced, but on the applied dose of various radiations. This fact has to be taken into account in all attempts to find a model for the dose-effect curves observed.

Les spectres et les rendements en radicaux de huit acides aminés irradiés par les particules α du ^{210}Po ont été étudiés par spectroscopie de résonance paramagnétique électronique (R.P.E.) et comparés à ceux obtenus après irradiation par les radiations γ du ^{60}Co et par les rayons x. La concentration en radicaux en fonction de la dose appliquée des particules α a été déterminée, ainsi l'évolution de cette concentration et de l'amplitude du signal de la dérivée première en fonction du temps de stockage.

Les spectres hyperfines obtenus pour les acides aminés aromatiques irradiés par les particules α sont identiques à ceux observés après irradiation par les radiations γ , contrairement au cas des acides aminés aliphatiques. Après irradiation par les particules α les rendements en radicaux pour les acides aminés aliphatiques sont d'un facteur de six plus faibles qu'après irradiation par les radiations γ , tandis que pour les acides aminés aromatiques la réduction du rendement est beaucoup moins importante. Pendant le temps de stockage après irradiation, on observe une réduction de la concentration des radicaux plus importante après irradiation par les particules α que celle rapportée pour le cas de l'irradiation par les rayons x. On compare l'évolution de la concentration en radicaux en fonction de la dose pour les radiations α et x. La saturation typique de la concentration des radicaux s'établit, indépendamment de la concentration atteinte, à des doses voisines pour les différents types de radiations.

Spektren und Radikalausbeuten von acht verschiedenen Aminosäuren wurden nach Bestrahlung mit ^{210}Po - α -Teilchen mit Hilfe der E.S.R.-Spektrographie untersucht und mit den nach ^{60}Co - γ - und Röntgenbestrahlung erhaltenen verglichen. Die Radikal-Konzentration wurde als Funktion der α -Strahlendosis bestimmt sowie der Effekt einer Lagerungszeit auf die Radikal-Konzentration und auf die Amplitude der ersten Ableitung des Absorptionssignals gemessen.

Die von den aromatischen Aminosäuren nach α -Bestrahlung erhaltenen Hyperfeinspektren waren identisch mit den nach γ -Bestrahlung beobachteten, im Gegensatz zu den bei aliphatischen Aminosäuren gefundenen Ergebnissen. Nach α -Bestrahlung waren die

Radikalausbeuten der aliphatischen Aminosäuren etwa um einen Faktor sechs kleiner als die mit γ -Strahlung erhaltenen, während die Reduktion der Ausbeute bei den aromatischen Aminosäuren viel kleiner war. Mit zunehmender Lagerzeit nach der Bestrahlung wurde nach α -Bestrahlung eine stärkere Abnahme der Radikalkonzentration beobachtet als diejenige, welche nach Röntgenbestrahlung von anderer Seite berichtet wurde. Die Abhängigkeit der Radikalkonzentration von der Strahlendosis wurde für α - und Röntgenstrahlen verglichen. Es wurde gefunden, daß die typische Sättigung der Radikalkonzentration unabhängig von der tatsächlich erreichten Konzentration bei vergleichbaren Dosen mit verschiedenen Strahlenarten eintritt.

REFERENCES

- BOAG, J. W., and MÜLLER, A., 1958, *Nature, Lond.*, **183**, 831.
EHRENBURG, A., EHRENBURG, L., and ZIMMER, K. G., 1957, *Acta chem. scand.*, **11**, 199.
GORDY, W., ARD, W. B., and SHIELDS, H., 1955, *Proc. nat. Acad. Sci., Wash.*, **41**, 983.
HART, E. J., and TERANDY, J., 1958, *Rev. sci. Instrum.*, **29**, 962.
KIRBY-SMITH, J. S., and RANDOLPH, M. L., 1957, *Bull. Amer. phys. Soc.*, **2**, 278 ; 1960,
Int. J. Rad. Biol., Suppl., 11.
KÖHNLEIN, W., and MÜLLER, A., 1960, *Z. Naturforsch.*, **15b**, 138 ; 1962, *Phys. Med. Biol.*,
6, 599.
MÜLLER, A., 1962, *The Effects of Ionizing Radiations at the Molecular Level* (Vienna :
International Atomic Energy Agency), p. 61 ; 1963, *Int. J. Rad. Biol.*, **6**, 137.
PRYZDZ, S., and HENRIKSEN, T., 1961, *Acta chem. scand.*, **15**, 791.
RANDOLPH, M. L., 1961, *Free Radicals in Biological Systems*, ed. M. S. Blois et al. (New
York : Academic Press), p. 249.
ROTBLAT, J., and SIMMONS, J. A., 1963, *Phys. Med. Biol.*, **7**, 489 ; **7**, 499.
SCHIRMER, O., and SOMMERMEYER, K., 1962, *Atompraxis*, **8**, 1.
ZIMMER, K. G., EHRENBURG, L., and EHRENBURG, A., 1957, *Strahlentherapie*, **103**, 3.
ZIMMER, K. G., KÖHNLEIN, W., HOTZ, G., and MÜLLER, A., 1963, *Strahlentherapie*, **120**, 161.