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Efficiency of radical production by X-rays in substances of biological importance

The efficiency of radical production by X-rays in dry proteins and nucleic acids

A. Müller

KERNREAKTOR
BAU- UND BETRIEBS-GESELLSCHAFT M. B. H.
KARLSRUHE

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EFFICIENCY OF RADICAL PRODUCTION BY X-RAYS IN SUBSTANCES OF BIOLOGICAL IMPORTANCE

Adolf
A. MÜLLER
INSTITUT FÜR STRAHLENBIOLOGIE, KERNFORSCHUNGSZENTRUM KARLSRUHE, FEDERAL REPUBLIC OF GERMANY

Abstract — Résumé — Аннотация — Resumen

EFFICIENCY OF RADICAL PRODUCTION BY X-RAYS IN SUBSTANCES OF BIOLOGICAL IMPORTANCE. Radical concentrations per unit energy of absorbed ionizing radiation in dry amino acids, proteins, and nucleic acids determined in several laboratories are compared and discussed. Measurements in our laboratory were performed using a newly-developed method incorporating a double-sample cavity and a momentum balance. The measured radical yields agree satisfactorily with several other determinations but are at variance with some earlier measurements. Possible explanations are given for these divergencies. An interesting result of our measurements is the small spread and low absolute value of the radical yields, with the exception of amino acids containing aromatic rings. Approximately one radical is produced per 100 eV of absorbed energy (or roughly one radical per primary ionization).

Radical yields in gelatine were measured at various temperatures following irradiation in vacuo at 77°K and 300°K. After warming a sample to 300°K within a few minutes following irradiation in vacuo at 77°K the radical yield found was three times lower than the radical yield observed after irradiating at room temperature. The enhanced yield is not only quenched at low temperature irradiation in vacuo but also at high temperature irradiation in the presence of air. The admission of oxygen or lowering the temperature after irradiation does not diminish the radical yield. The reaction leading to the high yield is much faster than other radical reactions reported previously.

EFFICACITÉ DE LA PRODUCTION DE RADICAUX PAR LES RAYONS X DANS QUELQUES SUBSTANCES BIOLOGIQUES IMPORTANTES. L'auteur compare et étudie le degré de concentration des radicaux produits par unité d'énergie des rayonnements ionisants absorbés dans des acides amines, des proteines et des acides nucléiques, d'après les données obtenues dans plusieurs laboratoires. Dans son laboratoire, il a utilisé une nouvelle méthode combinant l'usage d'une cavité à échantillon double et d'une balance à impulsion; les concentrations mesurées sont très voisines des mesures faites au moyen de plusieurs autres méthodes, mais ne concordent pas avec certains résultats obtenus antérieurement; l'auteur analyse les causes possibles des divergences. Comme caractéristique intéressante ressortant de ses mesures, il signale la dispersion réduite ainsi que la faible valeur absolue des concentrations de radicaux, sauf pour les acides aminés contenant des cycles aromatiques. Il est produit environ un radical par 100 eV d'énergie absorbée (soit approximativement un radical par ionisation primaire).

L'auteur a mesuré la concentration des radicaux produits dans la gélatine à diverses températures, après irradiation in vacuo à 77 et 300°K. Après avoir porté un échantillon à la température de 300°K, dans les quelques minutes suivant l'irradiation in vacuo à 77°K, il a observé que la concentration de radicaux était trois fois moins élevée qu'après l'irradiation effectuée à la température ambiante. L'augmentation de la concentration s'arrête non seulement en cas d'irradiation effectuée in vacuo à basse température, mais aussi en cas d'irradiation effectuée à haute température en présence de l'air. L'admission d'oxygène ou l'abaissement de la température après l'irradiation ne diminue pas la concentration de radicaux. La réaction qui entraîne une concentration élevée est beaucoup plus rapide que d'autres réactions concernant les radicaux et signalées antérieurement.

ПРОДУКТИВНОСТЬ ОБРАЗОВАНИЯ РАДИКАЛОВ ПРИ РЕНТГЕНОВСКОМ ОБЛУЧЕНИИ В БИОЛОГИЧЕСКИ ВАЖНЫХ СУБСТАНЦИЯХ. Сравниваются и обсуждаются полученные в нескольких лабораториях результаты по концентрации радикалов, образующихся на единицу энергии поглощенной ионизирующей радиации в сухих аминокислотах, белках и нуклеиновых кислотах. Измерения производились с помощью нового метода, предусматривающего применение двойного резонатора для

образцов и импульсного равновесия. Выходы радикалов удовлетворительно согласуются с несколькими другими определениями, но отличаются от результатов более ранних измерений. Рассматриваются возможные объяснения этих расхождений. Интересным результатом наших измерений является малое распространение и низкая абсолютная величина выходов радикалов, за исключением выходов аминокислот, содержащих ароматические кольца. Приблизительно один радикал образуется на каждые 100 эв поглощенной энергии (или грубо – один радикал при каждой первичной ионизации).

Выходы радикалов в желатине измерялись при различных температурах после облучения в вакууме при 77 и 300°К. После подогрева образца до 300°К в течение нескольких минут сразу после облучения в вакууме выход радикалов при 77°К был в три раза меньше, чем выход радикалов, наблюдавшийся после облучения при комнатной температуре. Повышенный выход радикалов не уменьшался не толко при облучении в вакууме при низкой температуре, но и при облучении при высокой температуре в присутствии воздуха. Допуск кислорода или понижение температуры после облучения не уменьшает выхода радикалов. Реакция, ведущая к высокому выходу, значительно быстрее, чем другие реакции с радикалами, описанные ранее.

FORMACIÓN DE RADICALES EN SUSTANCIAS DE IMPORTANCIA BIOLÓGICA POR EXPOSICIÓN A LOS RAYOS X. El autor compara y discute los valores de las concentraciones de radicales producidos por unidad de energía de radiación ionizante absorbida en aminoácidos secos, en proteínas y en ácidos nucleicos, obtenidos en varios laboratorios. Para sus propias mediciones el autor recurrió a un nuevo método basado en el empleo de una balanza balística y de una cavidad doble para las muestras. Los rendimientos en radicales medidos concuerdan satisfactoriamente con varias otras determinaciones, pero discrepan con algunas mediciones anteriores. La memoria da posibles explicaciones de estas discrepancias. Un resultado interesante es la dispersión relativamente pequeña de los valores determinados por el autor, y el reducido valor absoluto de los rendimientos, con excepción de los obtenidos con aminoácidos que contienen anillos aromáticos. Por cada 100 eV de energía absorbida, se produce aproximadamente un radical (es decir, alrededor de un radical por ionización primaria).

El autor midió el rendimiento de radicales en gelatina a diversas temperatures tras irradiación en el vacío a 77°K y 300°K. Calentando en pocos minutos hasta 300°K la sustancia irradiada en el vacío a 77°K, obtuvo un rendimiento unas tres veces inferior al observado al irradiar a la temperatura ambiente. El aumento del rendimiento se atenúa no sólo al irradiar a baja temperatura en el vacío, sino también cuando la operación se realiza a temperatura elevada en presencia de aire. La admisión de oxígeno o la reducción de la temperatura después de la irradiación no se traduce en una disminución del rendimiento de radicales. La reacción que da lugar a rendimientos elevados es mucho más rápida que otras reacciones de radicales descritas en la literatura.

INTRODUCTION

Observation of radiation-produced radicals by electron spin resonance (ESR) spectrography in biologically important compounds, such as proteins, nucleic acids, and their constituents was first reported several years ago [1 - 3]. Later, with the same method, radicals were shown to be produced also by irradiating living material [4]. This observation lent strong support to the hypothesis that radical intermediates form important primary steps in radiation damage.

At first only qualitative features of the radical spectra were studied. However, it was soon realized that the radicals observed can be regarded as intermediates in the production of radiation damage only if they are pro-

duced in sufficient numbers. This condition can be tested by measuring the concentration of radicals and the dose of radiation producing them [5]. From these quantities an energy expenditure per radical is derived. This value may be compared to the energy required per biological (or other) event.

For the dosimetry of radiation a variety of standard procedures is known. However, for the measurement of radical concentrations reliable methods had to be developed. Using such methods some values of energy expenditures for radical production in dry amino acids [5 - 12], as well as dry proteins and nucleic acids [6, 13 - 16] have been published in recent years. The earlier results, however, scattered widely. Consequently the authors developed another method for measuring radical yields [12, 17], and using this method we carried out determinations of energy expenditures per radical in a variety of materials.

In the course of our work on different proteins some experiments were performed at liquid nitrogen temperatures. Unexpected results were found with gelatine, leading to the study of this compound in greater detail than others.

MATERIALS AND METHODS

Amino acids, proteins and nucleic acids were obtained commercially* and used without further processing. The amino acids (including glycine) were irradiated and measured in air. The proteins and nucleic acids (and also glycine) were irradiated and measured in vacuo. For this purpose samples of the material under investigation were placed in quartz tubes as used for ESR measurements. These tubes were evacuated on a high vacuum line, in series with a liquid nitrogen trap, and subsequently sealed. Only that region of each tube containing the samples of, generally, 50 - 100 mg was irradiated. The unirradiated part of the tube was used for ESR measurements after shaking the specimen to the other end. Dose-effect curves were usually obtained by using soft X-rays (100 kV, 25 mA, filtered by 0.6 mm of quartz) at a dose-rate of 20 000 r/min. For quantitative determinations of yields (for which absolute values of dose had to be known) irradiations were performed with hard X-rays (150 kV, 20 mA, filtered by 4 mm of borosilicate glass) at 1000 r/min. For irradiations at low temperatures (approximately 77°K) the sealed quartz tubes were flushed by a stream of cooled nitrogen gas. A commercial ESR spectrometer (Varian) was used in conjunction with equipment for temperature control between 77°K and 600°K and a double-sample cavity previously described [18]. Evaluations of the first derivatives of absorption spectra were performed with a momentum balance [12]. Saturation of microwave power was avoided by measuring at various power levels. Dose-effect curves were plotted of all compounds. For yield measurements, doses from the linear part of the dose-effect curves only were delivered.

* Merck, California Foundation.

RESULTS AND DISCUSSION

In Table I are listed results for amino acids and in Table II those for proteins and nucleic acids. A conspicuous trend in the direction of lower values has been noticeable in recent years. This tendency may well be due to the presence of moisture in the samples, or to the underestimation of saturation effects in earlier work. An example in which both microwave *power saturation and dose saturation* occur is given in Fig. 1. In Table I the

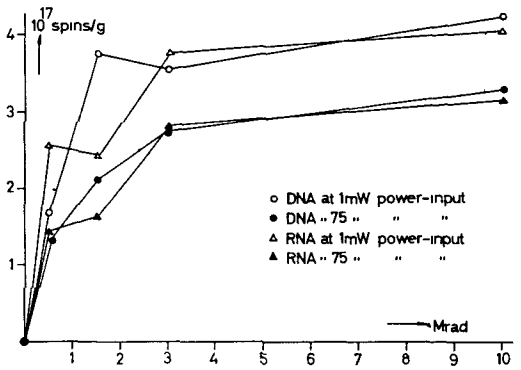

Fig. 1

Radical concentrations in dry DNA and RNA as a function of X-ray dose.

last column lists results from measurements made in air, whereas the values of the two preceding columns were obtained from experiments in vacuo. For any one substance the values of the last three columns vary at most by a factor of three. These differences may be explained partially by the different experimental conditions, though a factor of two contained in these variations may still be due to divergencies in the absolute calibration of the ESR spectrometer. In the first column of Table II figures marked (n) or (d) are inserted. These have been obtained with native (n) or heat denatured (d) preparations. As will be noted, the difference between these values is in contradiction to later measurements, which were performed with native material. A possible explanation of this discrepancy would be a change of power saturation characteristics on denaturation. This effect could cause an apparent difference of the radical concentrations if it is not taken into account. However, variations of moisture content or dose-rate effects [19] may also be responsible.

A general feature of both Tables is the low value of energy expenditures observed in our own experiments. With few exceptions (for substances containing aromatic rings) our values lie below 100 eV for amino acids and below 200 eV for proteins and nucleic acids. The importance of this result is more apparent if we express it in terms of the G value (i. e. the number of radicals per 100 eV of absorbed energy of ionizing radiation); 100 eV is roughly the mean energy transferred to irradiated matter per primary ionization. Hence, the G value indicates directly the number of radicals per

TABLE I

ENERGY EXPENDITURE (eV) PER RADICAL*
Amino acids

Compound	Ehrenberg, Ehrenberg and Zimmer [1957]	Blumenfeld and Kalmanson [1958]	Randolph and Parrish [1958]	Box and Freund [1959]	Kirby-Smith and Randolph [1959]	Henriksen and Pihl [1961]	Prydz and Henriksen [1961]	Köhnlein and Müller [1962]
Glycine	10 - 100	52	160	140	105	17		35
Glycylglycine			100	44	63			23
β-Alanine			1300				25	75
DL-α-Alanine				110	53		13	39
DL-Serine		41					14	37
L-Cysteine						45	34	
L-Phenylalanine							36	
DL-Phenylalanine								172
L-Tyrosine								580
L-Tryptophane							265	
DL-Tryptophane								2500
L-Histidine		82					52	
L-Histidine · HCl								42
L-Valine								42
DL-Valine								47
L-Aspartic acid						45	34	
DL-Leucine			1200	200				
L-Glutamic acid					80	15		

* After different authors.

TABLE I (cont'd)

Compound	Ehrenberg, Ehrenberg and Zimmer [1957]	*Blumenfeld* and Kalmanson [1958]	*Randolph* and Parrish [1958]	Box and Freund [1959]	Kirby-Smith and Randolph [1959]	Henriksen and Pihl [1961]	Prydz and Henriksen [1961]	Köhnlein and Müller [1962]
D-Glutamic acid								23
DL-Glutamic acid · H_2O							15	
DL-Glutamic acid · HCl							5	
L-Proline								51
Creatine								81

TABLE II

ENERGY EXPENDITURE (eV) PER RADICAL*
Proteins and nucleic acids**

Compound	Blumenfeld and Kalmanson [1958]	Kirby-Smith [1961]	Alexander, Lett, and Ormerod [1961]	Libby, Ormerod, Charlesby and Alexander [1961]	Müller [1962]
Egg albumin		5000			
Human serum albumin					70
Bovine serum albumin				100	
Casein	4000(n), 230(d)				160
Pepsin	4000(n)				40
Salmine		5000			
Gelatine		5000			17
Thiogel					14
Ichthyocolla	4000(n), 82(d)				
Haemoglobin					140
Oxyhaemoglobin	4000(n), 190(d)				
Sperm heads			250		
Deoxyribonucleic acid		20000	500		170
Ribonucleic acid					110

* After different authors.
** (n) = native, (d) = denatured

primary ionization. The G values for radical production range from 0.5 to 20. This is of the same order as G values for biological or biochemical reactions produced by ionizing radiation in dry material [20]. In other words, the number of radicals produced in dry matter is generally comparable to the number of damaging events. This conclusion is also valid for nucleic acids. As localized energy deposition in these compounds is generally considered to be the initial event leading to biological radiation damage [21], the participation of radicals in this chain of reaction is further supported.

It has been known for some time that the radical predominantly produced in gelatine by ionizing radiation is typical of a polyglycine radical resembling a doublet [22]. This has been described as being R_1-NH-C·H-CO-R_2 called the glycine-type radical [23]. As is seen from Table II, the efficiency for the production of this radical is unusually high. The same spectrum and the same high yield were observed for irradiated thiogel, which has the same composition as gelatine, but contains 12 SH-groups per molecule of weight 100 000. This is even more striking when the glycine content of gelatine (which is roughly one third) is taken into account. A series of irradiations and measurements were performed at liquid nitrogen temperature in order to determine whether the same radicals observed at room temperature are also found at low temperatures. From Fig. 2 it may be seen that the gelatine spectrum changes qualitatively as the temperature is raised from 77°K (temperature of irradiation) to about 300° K. The transition from the low temperature spectrum to the one observed at room temperature does not occur until the temperature reaches nearly room temperature. The kinetics of this transition have not been studied yet. On lowering the temperature again to 77° K the glycine-type spectrum does not revert to the original one. For other proteins a similar behaviour was found [15, 24].

A comparison of radical yields reveals another interesting feature. The concentration of radicals does not change appreciably going through the aforementioned temperature cycle. In low temperature irradiations the ener-

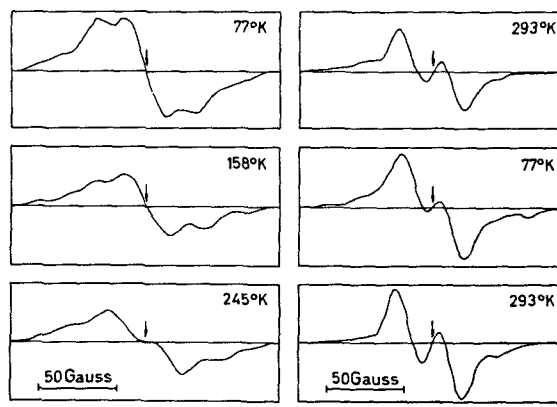

Fig. 2

First derivative of absorption spectrum of dry gelatine at different temperatures following irradiation at 77°K. The temperature was continuously increased from 77° K to 300° K, reversed to 77° K, and returned to 300° K. The arrow indicates a G value of 2 0036.

gy expended per radical is about 50 eV. If, however, the gelatine is irradiated at room temperature, and in vacuo, 17 eV only are needed for the production of one gelatine radical. These figures have been confirmed in measurements on pairs of samples treated identically except that one was irradiated at 77°K and the other at 300°K. The low temperature sample was allowed to warm up immediately after irradiation but still gave a low yield. The same low yield was observed for samples irradiated at room temperature in air. In Fig. 3 radical concentrations and first derivative amplitude of

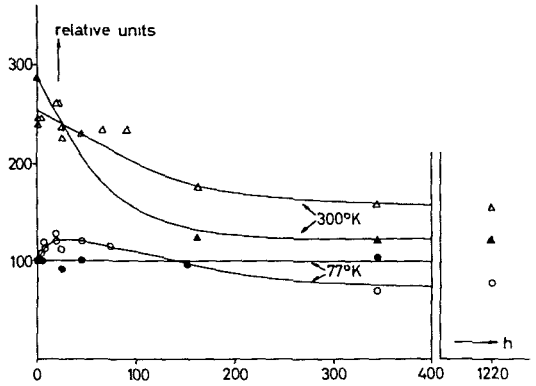

Fig. 3

Decay of radical concentrations and amplitudes of first derivatives of absorption spectra of dry gelatine with time. Concentration (▲) and Amplitude (△) following irradiation at 300°K; concentration (●), amplitude (○) following irradiation at 77°K.

absorption for two identical gelatine samples (irradiated at different temperatures and both measured at room temperature) are plotted in arbitrary units against time after irradiation. Both spectra show a glycine-type doublet. The spectrum observed immediately following room temperature irradiation is somewhat broader than the spectrum of the other sample, as is evident from the ratios of amplitude/radical concentration. This effect is not due to the higher concentration of the sample displaying the broader spectrum, as the same ratio has been observed at different radical concentrations. The amplitude and concentration of the sample irradiated at 300°K decrease in a similar relation with time, which is not the case for the sample irradiated at 77°K. Here, the concentration remains practically constant. The amplitude at first increases slightly, and later diminishes below its initial value.

Tentatively, we conclude from our observations on irradiated gelatine that the same reaction leading to the high radical concentration in the sample irradiated at room temperature is quenched by low temperature and/or oxygen. This conclusion is supported by the finding that, after a sample had been irradiated in vacuo at room temperature, admission of air does not reduce the radical concentration. An upper limit of several minutes for the time constant of the reaction leading to a yield of 1 radical/17 eV is deduced

from the fact that the radical concentration is not enhanced by warming-up the sample immediately after irradiation at low temperature. The speed of this process is at least two orders of magnitude higher than that of the slow reactions observed in other irradiated compounds [25, 26]. An equally slow process in gelatine is the one found by the authors following irradiation at 77° K. Here, one kind of gelatine radical is transformed into another, the total concentration remaining constant. The fraction reacting appears to be small, except if one assumes that both types of radicals display very similar spectra. The spectrum of the end-product is that of a glycine-type radical. Because of the relative slowness, these latter processes appear to be of a chemical nature. The occurrence of the rapid process, however, may lead to further elucidation of two observations in other fields: first, it may constitute a manifestation of an energy transfer mechanism. Such mechanisms have been extensively studied in gelatine, especially by measurement of its conductivity [27]. Second, the similarity of the ratio of three, in gelatine irradiated at high and low temperatures, found for radical concentrations, to the ratio observed in experiments on the radiosensitivity of proteins at the same two temperatures, is very suggestive. The difference in radiation damage observed after irradiation of dry proteins at 77° K and 300° K [28] may be due to the different radical concentrations as observed in our experiment. This is supported by a similar observation on bovine-serum albumin [15].

CONCLUSIONS

1. Radical yields in irradiated dry amino acids, proteins, and nucleic acids are of an equal order of magnitude.
2. In all amino acids, proteins and nucleic acids investigated (except amino acids containing aromatic rings) approximately one radical is produced per 100 eV of absorbed energy (or roughly one radical per primary ionization).
3. In gelatine irradiated in vacuo at 300° K the yield of the glycine-type radical is three times higher than when irradiated in vacuo at 77° K and measured at 300° K a few minutes later.
4. The enhanced yield found after irradiation at 300° K is not only quenched upon irradiating at 77° K in vacuo but also upon irradiating at 300° K in the presence of air.
5. The radical yield is not appreciably decreased by lowering the temperature or admitting oxygen a few minutes after irradiation.

REFERENCES

[1] COMBRISSON, J. et UEBERSFELD, J., Compt. rend. Acad. Sci., Paris 238 (1954) 1397.
[2] GORDY, W., ARD, W. B. and SHIELDS, H., Proc. Natl. Acad. Sci., Washington 41 (1955) 983.
[3] SHIELDS, H. and GORDY, W., Bull. Am. Phys. Soc., Ser. II, 1 (1956) 267.
[4] ZIMMER, K. G., EHRENBERG, L. and EHRENBERG, A., Strahlentherapie 103 (1957) 3.
[5] EHRENBERG, A., EHRENBERG, L. and ZIMMER, K. G., Acta Chem. Scand. 11 (1957) 199.
[6] BLUMENFELD, L. A. and KALMANSON, A. E., Biofizika 3 (1958) 87.
[7] RANDOLPH, M. L. and PARRISH, D. L., Radiation Res. 9 (1958) 170.

[8] BOX, H. C. and FREUND, H. G., Nucleonics 17 (1959) 66.
[9] KIRBY-SMITH, T. S. and RANDOLPH, M. C., Int. J. Rad. Biol., Suppl. 11 (1959).
[10] HENRIKSEN, T. and PIHL, A., Int. J. Rad. Biol. 3 (1961) 351.
[11] PRYDZ, S. and HENRIKSEN, T., Acta Chem. Scand. 15 (1961) 791.
[12] KOHNLEIN, W. and MULLER, A., Phys. Med. Biol. 6 (1962) 599.
[13] KIRBY-SMITH, T., Nuclear Science Series, ed. R. A. Deering, Rep. 31 (1961) 27.
[14] ALEXANDER, P., LETT, J. T. and ORMEROD, M. G., Biochim. biophys. Acta 51 (1961) 207.
[15] LIBBY, D., ORMEROD, M. G., CHARLESBY, A. and ALEXANDER, P., Int. J. Rad. Biol. 4 (1961) 21.
[16] MÜLLER, A., Int. J. Rad. Biol. 5 (1962) 199.
[17] KOHNLEIN, W., Thesis, Univ. Heidelberg (1962).
[18] KOHNLEIN, W. and MULLER, A., Z. Naturforsch. 15b (1960) 138.
[19] DUCHESNE, J., Memoires de l'Académie royale de Belgique, Classe des Sciences 33 3 (1961) 208.
[20] SWALLOW, R. J., Radiation Chemistry of Organic Compounds, Pergamon Press, London (1960) 213.
[21] ZIMMER, K. G., Studies on quantitative radiobiology, Oliver and Boyd, Edinburgh and London (1961).
[22] BOAG, J. W. and MULLER, A., Nature 183 (1959) 831.
[23] DREW, R. and GORDY, W., Bull. Am. Phys. Soc. 6 3 (1961) 258.
[24] PATTEN, F. and GORDY, W., Proc. Natl. Acad. Sci., Washington 46 (1960) 1137.
[25] HENRIKSEN, T. and PIHL, A., Nature 185 (1960) 307.
[26] RAJEWSKY, B. and REDHARDT, A., Nature 193 (1962) 365.
[27] RIEHL, N., Kolloid Z. 151 (1957) 66.
[28] POLLARD, E. C., GUILD, W. R., HUTCHINSON, F. and SETLOW, R. B., Progress in Biophysics 5 (1955) 72.

DISCUSSION

J. LAZURKIN: Did you measure the gas-pressure in your sealed tubes after irradiation?

A. MÜLLER: No.

J. LAZURKIN: Perhaps the deterioration of the vacuum through gas produced from the irradiated samples could result in measured values of G lower than the true values.

A. MÜLLER: I do not think so. The dose-effect curve is linear at low doses, so I do not think that oxygen has an effect in the region from which we computed G values.

J. LAZURKIN: What was the yield at room temperature?

A. MÜLLER: 7 eV.

J. LAZURKIN: That is very interesting. My paper gives a value of 50 for triglycylglycine.

A. MÜLLER: Your value of 50 for triglycylglycine is quite compatible with our value of 7 for gelatine, if the glycine content of gelatine, roughly one third, is taken into account.

J. DUCHESNE: I would like to ask Dr. Müller whether, in the case of DNA's e has found changes in line width on going from low to high doses.

A. MULLER: The commercial samples of nucleic acids with which the yields reported were obtained displayed only singlet lines after irradiation, and these were not found to change. With purified samples of bacteriophage DNA we found absorption and pronounced hyperfine structure. The radical concentration in these samples deviated from the initial linearity at 30 000 rad, but we observed only a small change of the hyperfine structure on the application of higher doses. The general appearance of the absorption was that of a triplet with 40 Gauss separation of the outer peaks of the first derivative curves and about 8 Gauss central-line width.

F. HUTCHINSON: Have you any evidence that small quantities of impurities in your preparations cause changes in the number of spin centres formed? I ask this question particularly in the light of the results given in Dr. Lazurkin's paper and the suggestion that low concentrations of existing radicals can apparently reduce the level of measured spin centres by a process which may involve energy transfer.

A. MÜLLER: The effect of impurities on radiation-produced radical concentration was tested with glycine. Polycrystalline samples of this substance containing various amounts of impurities all yielded the same radical concentration. We also recently compared results from different laboratories on a number of amino acids of different origin, but no differences which could be attributed to impurities were found. However, it may be different for macromolecules, which we have not tested in this respect.

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The efficiency of radical production by X-rays in dry proteins and nucleic acids

ADOLF MÜLLER
Institut für Strahlenbiologie, Kernforschungszentrum Karlsruhe, Germany

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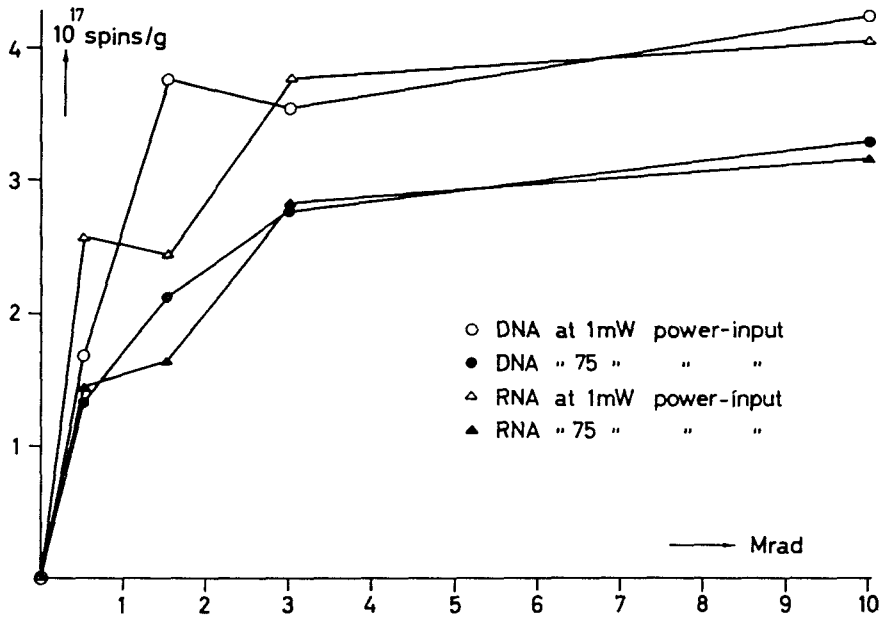
Recently, a reliable and simple method for the measurement of radical concentrations has been described, and yields in several dry amino acids were given (Köhnlein and Müller 1962). In addition yields in a number of dried proteins and nucleic acids have now been measured. The figures reported below should be regarded as preliminary; nevertheless, they demonstrate, along with the data on amino acids published previously, the storage of a substantial part of x-ray energy in the form of radicals. This again emphasizes the importance ascribed to free radicals in primary steps of radiation damage (cf. Zimmer 1961). Gelatine, pepsin, ovalbumin, yeast ribonucleic acid (RNA) and calf-thymus desoxyribonucleic acid (DNA) were obtained from Merck, Darmstadt; Thiogel B was supplied by Schwartz Biochemicals and used without further processing. The human serum-albumin and haemoglobin were a gift of Dr. Braams, Utrecht.

The samples, in powder form, were evacuated on a high vacuum line (10^{-5} mm Hg) for at least 15 hours and sealed off prior to irradiation. These and the dosimetry were executed as reported previously (Köhnlein and Müller 1962). For recording of paramagnetic absorption, the samples were transferred to an unirradiated end of the tubular container. Before spectrographic recordings were taken (appr. 10–30 min after irradiation), radical concentrations had in all cases reached a stable level, changing significantly only after several hours. From the radical concentrations measured and the doses applied, the yields as the number of radicals per 100 ev of absorbed energy (i.e. *G*-values) were computed. Results are given in the table. They are strongly affected by the

Gelatine	6
Thiogel	7
Pepsin	3
Human serum-albumin	1·5
Haemoglobin	0·7
RNS	0·9
DNS	0·6

G-values for radical production in several proteins and nucleic acids.

moisture content of the sample, as has been previously observed (Boag and Müller 1959). Yields are also found to decrease with increasing doses and microwave energy (figure). Consequently we based the results given in the table on measurements done with radiation doses below 0·5 Mrad and input of microwave power to the cavity of 1 mW only. Earlier findings of very low yields (Blumenfeld and Kalmanson 1958, Kirby-Smith 1961) for proteins and nucleic acids may possibly have been caused by neglect of the two saturation effects mentioned, whereas observation of a high yield after efficient drying of nucleic acid was reported previously (Boag and Müller 1959) and recently confirmed (Alexander, Lett and Ormerod 1961).



Radical concentrations in dry DNA and RNA after different doses of x-rays.

Summing up, it may be concluded: 1. Yields in proteins and nucleic acids are of the same order as those in amino acids and not several orders of magnitude lower. 2. The absolute value of yields in biologically-important compounds is big enough to justify continued efforts to elucidate the part played by radicals in primary mechanisms of radiation damage.

References

Alexander, P., Lett, J. T., and Ormerod, M. G., 1961, *Biochim. biophys. acta*, **51**, 207.
Blumenfeld, L. A., and Kalmanson, A. E., 1958, *Biofizika*, **3**, 87.
Boag, J. W., and Müller, A., 1959, *Nature, Lond.*, **183**, 831.
Kirby-Smith, T., 1961, *Nuclear Science Series*, Rep. No. 31, Ed. R. A. Deering, p. 27.
Köhnlein, W., and Müller, A., 1962, *Phys. Med. Biol.*, **6**, 599.
Zimmer, K. G., 1961, *Studies on Quantitative Radiobiology* (Edinburgh and London: Oliver & Boyd).