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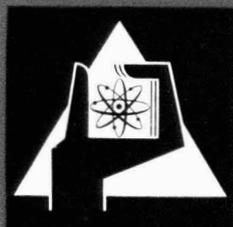
INSTITUT FÜR STRAHLENBIOLOGIE

ABSOLUTE YIELD MEASUREMENTS OF RADIATION - PRODUCED
RADICALS BY ELECTRON SPIN RESONANCE

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Bau- und Betriebs-Gesellschaft m. b. H.
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**Absolute Yield Measurements of Radiation-Produced
Radicals by Electron Spin Resonance**

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§ 1. INTRODUCTION

IN the course of our investigations into the possibilities of using electron spin resonance (E.S.R.) spectrography for quantitative determinations of the yield of free radicals produced by ionizing radiation, we have recently achieved some improvement in the technique of measurement. A report of the main features of our methods is thought to be useful at the present stage, as the difficulties inherent in such measurements do not appear to be generally recognized (cf. Müller and Zimmer 1959).

In addition, we have redetermined the yields for several amino acids in the way described below and compared the results with those of other authors.

§ 2. INSTRUMENTS AND METHODS

2.1. *Dosimetry and Irradiations*

Obviously, an exact determination of dose is an essential part of the measurements of radical yields. This is somewhat difficult to attain with soft x-rays from a tube with 1.5 mm beryllium window operated at 100 kv and 25 mA, and used without additional filter for short irradiation times. The yields obtained in these irradiations were, therefore, checked by using hard x-rays from another tube operated at 150 kv and 20 mA and emitting radiation of a H.V.L. of 6 mm Al. The radiation dose from this tube was measured by three independent methods: (i) the Fricke-actinometer (ferrous sulphate oxidation), (ii) a Baldwin-Farmer ionization chamber calibrated by the British NPL, (iii) an ionization chamber (PTW, Freiburg) calibrated by the German PTB. The mean deviation of all determinations, including those with soft x-rays, was 5%.

2.2. *Recording of the Spectra*

The measurements of the radical concentrations were performed in several steps.

A commercial E.S.R. spectrograph (Varian Associates) operating at 9500 Mc and recording first derivatives of microwave absorption, was used. The ordinary cavity was replaced by an improved double sample cavity shown in fig. 1, the principle of which has already been described (Köhnlein

and Müller 1960). A new feature is the possibility of high frequency modulation (100 kc) which is fed into the cavity by four straight conductors connected outside the cavity so that they form two loops at

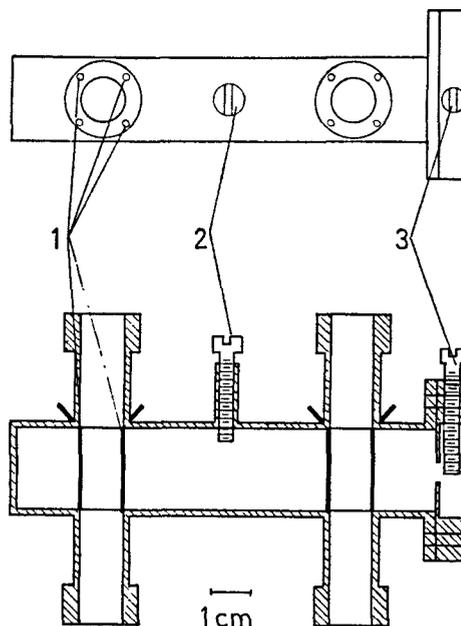


Fig. 1. Double cavity for 100 kc modulation. 1. Modulation loops. 2. Frequency tuning screw. 3. Iris coupling screw.

each sample†. This arrangement leaves sufficient space for a low temperature insert or liquid sample cells, and for spatial adjustment of sample tubes. Between the sample holes a tuning screw for the resonant frequency is provided, in order to avoid tuning the klystron which tends to reduce its life.

The modulation loops are energized separately at each sample from the secondary coil of an output transformer, via a low impedance flexible conductor made of copper strips separated by a thin insulating foil.

With the double cavity it is possible to record for each measurement a control spectrum of a secondary standard under identical microwave field strength immediately after registration of the sample spectrum under investigation.

2.3. Evaluation of the Spectra

From the recorded first derivatives one arrives by double integration at a figure F proportional to the spin concentration (Andrew 1953). Alternatively, the first moment M of the first derivative curve can be

† This arrangement was suggested to us by Dr. K. H. Hausser, Heidelberg.

determined. This can be shown in the following way: if $f(H)$ denotes the absorption as a function of the magnetic field strength H , it follows by partial integration:

$$\int_{-\infty}^{+\infty} f(H) dH = Hf(H) \Big|_{-\infty}^{+\infty} - \int_{-\infty}^{+\infty} Hf'(H) dH \dots (1)$$

In any actual spectrum the first term on the right hand side of eqn. (1) vanishes, as can be verified by inserting Gaussian or Lorentzian functions. Now

$$F = \int_{-\infty}^{+\infty} dH \int_{-\infty}^H f'(H) dH = \int_{-\infty}^{+\infty} f(H) dH \dots (2)$$

and

$$M = \int_{-\infty}^{+\infty} Hf'(H) dH. \dots (3)$$

Hence, the values of F and M are equal. However, a calculation of the errors involved reveals that these can be substantially reduced by evaluating the first moment instead of integrating twice (Collins 1959). Taking advantage of this fact, a simple but accurate determination of the first moment was achieved by weighing with a modified sensitive balance, the principle of which was suggested by Burgess (1961). One of the scales of the balance, schematically drawn in fig. 2, was replaced by a light aluminium table mounted rigidly onto one arm of the balance. The spectra were cut out from the recording paper and placed upon the table as shown in fig. 2. The homogeneity of the paper (weight per unit area) was checked and found to affect the results by less than 2%. As the spectra are not recorded to infinity, the error introduced by finite lengths of the cut out spectra was also determined. For this purpose we calculated the corresponding integrals of the Gauss and Lorentz functions. The results are given in table 1. Taking successively Gaussian and Lorentzian single-line spectra, the width ($2a$) of the integration interval,

Table 1. Limits of integration for Gaussian and Lorentzian distributions in multiples of the width between points of maximum slope for fractions of the infinite integral which are given in the first column

$\frac{\int_{-a}^{+a} f'(H) H dH}{\int_{-\infty}^{+\infty} f'(H) H dH}$	$2a/\Delta H$	Gauss	Lorentz
0.95		2.8	41.6
0.90		2.5	22.6
0.85		2.3	14.8
0.80		2.2	11.0

which will include any given fraction of the area under the infinite curve, is presented in table 1 as a multiple of the interval (ΔH) between points of maximum slope on the curve.

It can be seen that a sufficient accuracy can easily be obtained with a Gaussian distribution whereas it is rather difficult to reach with a Lorentzian distribution. In practice, $2a/\Delta H = 15$ was chosen, which according to table 1 corresponds to an error of 15% with the Lorentzian and to a negligible error with the Gaussian distribution.

2.4. Calibration of Standard Samples

Secondary standards of carbon diluted with calcium carbonate were calibrated with a 10^{-4} molar solution of diphenylpicrylhydrazyl (DPPH) in carbon tetrachloride. The radical content of the DPPH was determined by titration with quinone (Goldschmidt and Renn 1922). The resulting value was also checked by other methods (Köhnlein, to be published).

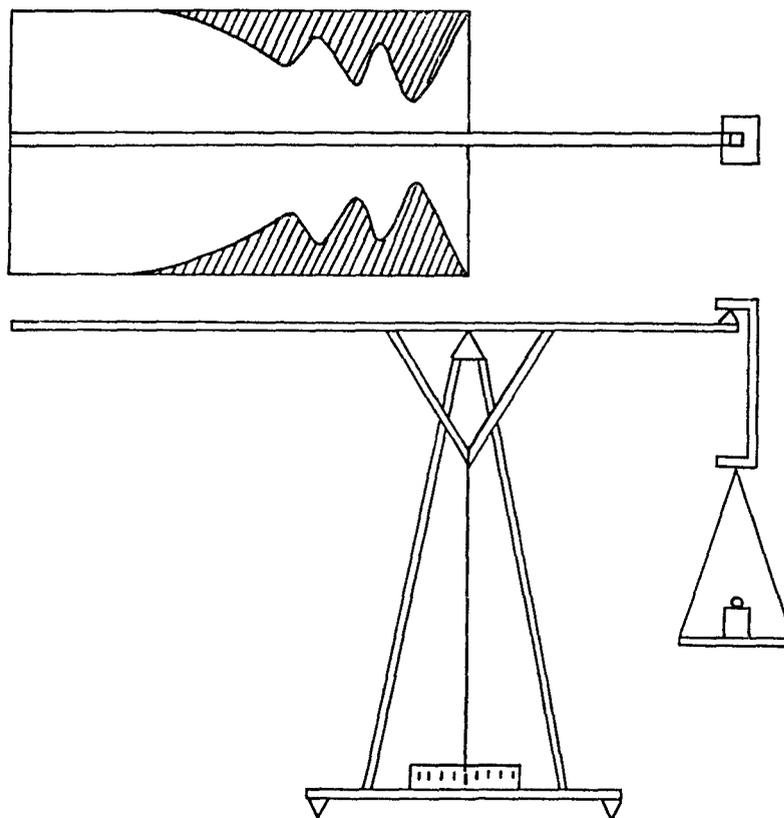


Fig. 2. Schematic top and front view of first moment balance.

§ 3. RESULTS

Numerical values of the radical yields of some amino acids are given in table 2. The chemicals used by us were obtained from the California Biochemical Research Foundation and from Merck, Darmstadt. Irradiations and E.S.R. measurements were done in air at room temperature. The main sources of error in determinations of the yields of radicals produced are the following: (i) radical decay, (ii) saturation of radical concentration, and (iii) saturation of microwave power. The last phenomenon in particular may be the cause of some of the larger discrepancies shown in table 2, as we noticed that it occurred at very different power levels for different compounds. The first source of error was avoided by evaluating only samples which did not show any decay over a period of 10 min and which had been irradiated 10 min before the measurement. Saturation of the radical concentration usually occurs between 5 and 10 megarads. Yields given by us were obtained from compounds irradiated with different doses in the linear dose-effect region. Finally, the microwave power was reduced sufficiently to prevent saturation.

Table 2. Energy expenditure per radical (electron-volts)

Compound	Zimmer, Ehrenberg and Ehrenberg 1957	Blumenfeld and Kalmanson 1958	Randolph and Parrish 1958	Box and Freund 1959	Kirby-Smith and Randolph 1959	Henriksen and Pihl 1961	Present work
Glycine	10-100	60	160	140	105	17	35
Glycylglycine			100	44	63		23
DL- α -Alanine				110	53		39
β -Alanine			1300				74
DL-Phenylalanine							172
Creatine							81
DL-Leucine			1200	200			
Histidine		92					42
L-Proline					300		51
DL-Serine		46					37
L-Valine							42
DL-Valine							47
DL-Tryptophane							2500
L-Tyrosine							580
Glutamic Acid					80	15	23
Cysteine						45	

It is seen (last column) that the values for amino acids containing aromatic rings, i.e. phenylalanine, tryptophane and tyrosine are higher than for the other substances, which vary between 20 and 80 eV. This is in accord with the results obtained by other authors, except some early figures given by Randolph and Parrish (1958).

SUMMARY

The method of measuring the concentration of paramagnetic centres is considered systematically. Some new points of view, and some instrumental accessories are discussed

in detail, especially a double sample cavity for comparisons at different temperatures which employs 100 kc modulation, and also a first moment balance which allows the determination of relative concentrations in a very simple way.

In addition the yields of radicals produced by x-radiation in amino acid compounds were re-measured and the results compared with those of other authors. The values are between 20 and 80 ev per radical, except for aromatic amino acids which need higher energies.

RÉSUMÉ

On a considéré d'une manière systématique la méthode de mesure de la concentration de centres paramagnétiques. On discute en détail quelques nouveaux points de vue, ainsi que quelques instruments accessoires, en particulier une cavité double à échantillons pour effectuer des comparaisons aux températures différentes, en employant une modulation de 100 kc/s ainsi qu'une balance de moments qui permet de déterminer les concentrations relatives d'une manière très simple par pesage des sections individuelles du spectre.

D'ailleurs on a déterminé de nouveau la dépense d'énergie pendant la génération de radicaux dans les composés des amino-acides, et les résultats ont été comparés avec ceux d'autres auteurs. Ces valeurs se trouvent entre 20 et 80 ev par radical, excepté les amino-acides aromatiques, pour lesquels on a obtenu des valeurs plus grandes.

ZUSAMMENFASSUNG

Die Methode der Messung von Konzentrationen paramagnetischer Zentren wird zusammenhängend betrachtet. Einige neue Gesichtspunkte und instrumentelle Hilfsmittel werden ausführlicher behandelt, insbesondere ein Doppelhohlraum für Vergleichsmessungen bei beliebiger Temperatur mit 80 kHz Modulation und eine Momentenwaage, auf der relative Konzentrationen durch Wägung ausgeschnittener Spektren auf sehr einfache Weise bestimmt werden können.

Ausserdem wurden der Energieaufwand für die Erzeugung von Radikalen in Aminosäureverbindungen durch Röntgenstrahlen neu bestimmt und die Ergebnisse mit den anderer Autoren verglichen. Sie liegen zwischen 20 und 80 ev pro Radikal, abgesehen von aromatischen Aminosäuren, für die sich höhere Werte ergeben.

Резюме

Метод измерения концентрации парамагнитных центров подвергался систематическому обзору. Некоторые новые точки зрения и некоторые вспомогательные приборы подвергались подробному обсуждению, в особенности двойная полость для сравнений при разных температурах, с модулированием в 100 кГц, а также весы первого момента, позволяющие определять очень простым методом относительные концентрации.

Кроме того вновь определялись выходы радикалов, производимых рентгеновскими лучами в соединениях аминокислот, причем полученные результаты сравнивались с результатами других исследователей. Полученные значения колеблются между 20 и 80 эв на радикал, за исключением ароматических аминокислот, для которых требуются более высокие значения энергии.

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