

Biological and Biochemical
Applications
of
Electron Spin Resonance

D. J. E. INGRAM

M.A., D.Phil. D.Sc., Hon.D.Sc., F.Inst.P.

Professor of Physics, University of Keele

ADAM HILGER LTD

LONDON

CONTENTS

I INTRODUCTION

- 1.1 The potentialities of electron resonance in biological investigations 1
- 1.2 The basic principles of electron resonance 3
- 1.3 Main features of E.S.R. spectra
—*Integrated intensity—Line width—g values* 8
- 1.4 Electronic splitting
—*Atoms or molecules with more than one unpaired electron—Electronic splitting in organic molecules—Electronic splitting in transition group atoms* 14
- 1.5 Hyperfine splittings and their origins
—*Interaction with nuclear magnetic moments—Hyperfine interaction with only one nucleus—Angular variation of hyperfine pattern—Hyperfine pattern from more than one nucleus—Superhyperfine patterns* 23

2 EXPERIMENTAL TECHNIQUES

- 2.1 Simple spectrometer systems 39
- 2.2 Sensitivity considerations 41
- 2.3 Detector noise and its reduction
—*Factors affecting spectrometer sensitivity—Noise variation with crystal current—Variation of crystal noise with frequency of detection* 56

3 SPECTROMETER SYSTEMS

- 3.1 High-sensitivity spectrometers
—*Elimination of low frequency crystal noise—High frequency field modulation spectrometers—Superheterodyne spectrometer systems—The use of microwave circulators* 65
- 3.2 Averaging and integrating methods 75
- 3.3 The integration of rapidly changing spectra 79
- 3.4 The study of transient reactions
—*Methods of studying non-repetitive transient reactions—Continuous flow systems—Sudden freezing techniques* 84
- 3.5 Zero field spectrometers 92
- 3.6 Millimetre wave spectrometers
—*Magnetic field requirements—4 mm spectrometer system—2 mm wavelength spectrometer* 96
- 3.7 Saturation effects 103
- 3.8 Measurement of relaxation times 108
- 3.9 Double resonance—ENDOR
—*Basic principles—Experimental techniques in ENDOR* 112

4 FREE-RADICAL AND IRRADIATION STUDIES

- 4.1 General features of a free radical spectrum 123
- 4.2 The analysis of hyperfine patterns 126

4.3	Radicals formed by high energy irradiation — <i>Early experimental observations—More recent studies— The mechanism of formation of secondary radicals—Correlation of secondary radicals and biological damage</i>	131
4.4	Radicals produced by other forms of radiation — <i>Production by thermal hydrogen atoms—Study of transient radicals formed by high-energy electrons</i>	147
4.5	Studies on photo-synthesis and triplet state excitation — <i>E.S.R. studies on photo-synthesis—Triplet state excitation in proteins</i>	151
4.6	Radicals formed by pyrolysis	158
4.7	E.S.R. and carcinogenic activity	166
5	ENZYME STUDIES	
5.1	The application of E.S.R. to the study of enzymes	177
5.2	E.S.R. spectra from metal ions — <i>g values of metal ions—Hyperfine structure in metal ions</i>	178
5.3	General features of enzyme reaction	187
5.4	Studies on xanthine oxidase	190
5.5	Studies on aldehyde oxidase	197
5.6	Studies on the dehydrogenases	198
5.7	The $g = 1.94$ signal associated with iron atom	200
5.8	Studies on catalase and peroxidase	202
5.9	Studies on enzymes containing copper	207
6	THE INVESTIGATION OF METALLO-ORGANIC COMPOUNDS	
6.1	Transition group atoms and their role in biochemistry	212
6.2	Atomic orbitals and energy levels associated with transition group atoms	213
6.3	g -values and molecular field splittings	221
6.4	Proteins containing copper	226
6.5	Biochemical molecules containing cobalt	229
6.6	Studies on haemoglobin and related derivatives — <i>The haem plane and its surroundings—High spin and low spin states of the iron atom—Energy levels and anisotropy of the high spin state—Haem plane orientation as determined from high spin g values—Structural information from low spin g values— The study of line widths and hyperfine splittings—Determina- tion of the zero-field splitting parameters</i>	231
6.7	Electron resonance studies on non-haem iron in proteins	265
7	RECENT DEVELOPMENTS AND FUTURE PROSPECTS	
7.1	The application of saturation and double resonance studies to biochemical compounds	270
7.2	Relaxation studies on biochemical molecules	272
7.3	The application of double resonance techniques	277
7.4	Spin labelling techniques	285
7.5	Future prospects	290
	AUTHOR INDEX	301
	SUBJECT INDEX	305