

CONTENTS

Preface	v
Preface to the First Edition	vii
Acknowledgements	xi
CHAPTER 1	
CLASSICAL NMR SPECTROSCOPY	
1.1 Nuclear Magnetism	2
1.2 The Bloch Equations	7
1.3 The One-Pulse NMR Experiment	16
1.4 Linewidth	18
1.5 Chemical Shift	21
1.6 Scalar Coupling and Limitations of the Bloch Equations	23
References	27

CHAPTER 2**THEORETICAL DESCRIPTION OF NMR SPECTROSCOPY**

2.1	Postulates of Quantum Mechanics	29
2.1.1	THE SCHRÖDINGER EQUATION	30
2.1.2	EIGENVALUE EQUATIONS	31
2.1.3	SIMULTANEOUS EIGENFUNCTIONS	34
2.1.4	EXPECTATION VALUE OF THE MAGNETIC MOMENT	35
2.2	The Density Matrix	37
2.2.1	DIRAC NOTATION	37
2.2.2	QUANTUM STATISTICAL MECHANICS	40
2.2.3	THE LIOUVILLE-VON NEUMANN EQUATION	41
2.2.4	THE ROTATING FRAME TRANSFORMATION	43
2.2.5	MATRIX REPRESENTATIONS OF THE SPIN OPERATORS	46
2.3	Pulses and Rotation Operators	50
2.4	Quantum Mechanical NMR Spectroscopy	54
2.4.1	EQUILIBRIUM AND OBSERVATION OPERATORS	55
2.4.2	THE ONE-PULSE EXPERIMENT	56
2.5	Quantum Mechanics of Multispin Systems	58
2.5.1	DIRECT PRODUCT SPACES	59
2.5.2	SCALAR COUPLING HAMILTONIAN	61
2.5.3	ROTATIONS IN PRODUCT SPACES	65
2.5.4	ONE-PULSE EXPERIMENT FOR A TWO-SPIN SYSTEM	68
2.6	Coherence	70
2.7	Product Operator Formalism	77
2.7.1	OPERATOR SPACES	78
2.7.2	BASIS OPERATORS	80
2.7.3	EVOLUTION IN THE PRODUCT OPERATOR FORMALISM	84
2.7.3.1	FREE PRECESSION	84
2.7.3.2	PULSES	85
2.7.3.3	PRACTICAL POINTS	86
2.7.4	SINGLE-QUANTUM COHERENCE AND OBSERVABLE OPERATORS	88
2.7.5	MULTIPLE-QUANTUM COHERENCE	90
2.7.6	COHERENCE TRANSFER AND GENERATION OF MULTIPLE-QUANTUM COHERENCE	92

2.7.7	EXAMPLES OF PRODUCT OPERATOR CALCULATIONS	93
2.7.7.1	THE SPIN ECHO	93
2.7.7.2	INSENSITIVE NUCLEI ENHANCED BY POLARIZATION TRANSFER	96
2.7.7.3	REFOCUSED INEPT	98
2.7.7.4	SPIN-STATE SELECTIVE POLARIZATION TRANSFER	99
2.8	Averaging of the Spin Hamiltonians and Residual Interactions	102
	References	112

CHAPTER 3

EXPERIMENTAL ASPECTS OF NMR SPECTROSCOPY

3.1	NMR Instrumentation	114
3.2	Data Acquisition	124
3.2.1	SAMPLING	124
3.2.2	OVERSAMPLING AND DIGITAL FILTERS	126
3.2.3	QUADRATURE DETECTION	132
3.3	Data Processing	136
3.3.1	FOURIER TRANSFORMATION	136
3.3.2	DATA MANIPULATIONS	142
3.3.2.1	ZERO-FILLING	142
3.3.2.2	APODIZATION	143
3.3.2.3	PHASING	151
3.3.3	SIGNAL-TO-NOISE RATIO	158
3.3.4	ALTERNATIVES TO FOURIER TRANSFORMATION	159
3.3.4.1	LINEAR PREDICTION	160
3.3.4.2	MAXIMUM ENTROPY RECONSTRUCTION	161
3.4	Pulse Techniques	165
3.4.1	OFF-RESONANCE EFFECTS	165
3.4.2	B_1 INHOMOGENEITY	172
3.4.3	COMPOSITE PULSES	174
3.4.4	SELECTIVE PULSES	179
3.4.5	PHASE-MODULATED PULSES	181
3.4.6	ADIABATIC PULSES	189

3.5	Spin Decoupling	201
3.5.1	SPIN DECOUPLING THEORY	201
3.5.2	COMPOSITE PULSE DECOUPLING	204
3.5.3	ADIABATIC SPIN DECOUPLING	209
3.5.4	CYCLING SIDEBANDS	212
3.5.5	RECOMMENDATIONS FOR SPIN DECOUPLING	216
3.6	B_0 Field Gradients	217
3.7	Water Suppression Techniques	221
3.7.1	PRESATURATION	223
3.7.2	JUMP-RETURN AND BINOMIAL SEQUENCES	224
3.7.3	SPIN LOCK AND FIELD GRADIENT PULSES	227
3.7.4	POSTACQUISITION SIGNAL PROCESSING	232
3.8	One-Dimensional ^1H NMR Spectroscopy	234
3.8.1	SAMPLE PREPARATION	234
3.8.2	INSTRUMENT SETUP	236
3.8.2.1	TEMPERATURE CALIBRATION	236
3.8.2.2	TUNING	237
3.8.2.3	SHIMMING	238
3.8.2.4	PULSE WIDTH CALIBRATION	252
3.8.2.5	RECYCLE DELAY	257
3.8.2.6	LINewidth MEASUREMENT	259
3.8.3	REFERENCING	262
3.8.4	ACQUISITION AND DATA PROCESSING	263
3.8.4.1	ONE-PULSE EXPERIMENT	263
3.8.4.2	HAHN ECHO EXPERIMENT	265
	References	267

CHAPTER 4

MULTIDIMENSIONAL NMR SPECTROSCOPY

4.1	Two-Dimensional NMR Spectroscopy	273
4.2	Coherence Transfer and Mixing	280
4.2.1	THROUGH-BOND COHERENCE TRANSFER	280
4.2.1.1	COSY-TYPE COHERENCE TRANSFER	281
4.2.1.2	TOCSY TRANSFER THROUGH-BONDS	284

4.2.2	THROUGH-SPACE COHERENCE TRANSFER	289
4.2.3	HETERONUCLEAR COHERENCE TRANSFER	290
4.2.4	COHERENCE TRANSFER UNDER RESIDUAL DIPOLAR COUPLING HAMILTONIANS	291
4.3	Coherence Selection, Phase Cycling, and Field Gradients	292
4.3.1	COHERENCE LEVEL DIAGRAMS	293
4.3.2	PHASE CYCLES	295
4.3.2.1	SELECTION OF A COHERENCE TRANSFER PATHWAY	298
4.3.2.2	SAVING TIME	305
4.3.2.3	ARTIFACT SUPPRESSION	307
4.3.2.4	LIMITATIONS OF PHASE CYCLING	310
4.3.3	PULSED FIELD GRADIENTS	311
4.3.3.1	SELECTION OF A COHERENCE TRANSFER PATHWAY	311
4.3.3.2	ARTIFACT SUPPRESSION	313
4.3.3.3	LIMITATIONS OF PULSED FIELD GRADIENTS	314
4.3.4	FREQUENCY DISCRIMINATION	315
4.3.4.1	FREQUENCY DISCRIMINATION BY PHASE CYCLING	320
4.3.4.2	FREQUENCY DISCRIMINATION BY PULSED FIELD GRADIENTS	322
4.3.4.3	ALIASING, FOLDING, AND PHASING IN MULTIDIMENSIONAL NMR SPECTROSCOPY	323
4.4	Resolution and Sensitivity	326
4.5	Three- and Four-Dimensional NMR Spectroscopy	327
	References	331

CHAPTER 5

RELAXATION AND DYNAMIC PROCESSES

5.1	Introduction and Survey of Theoretical Approaches	334
5.1.1	RELAXATION IN THE BLOCH EQUATIONS	337
5.1.2	THE SOLOMON EQUATIONS	338
5.1.3	RANDOM-PHASE MODEL FOR TRANSVERSE RELAXATION	346
5.1.4	BLOCH, WANGSNES, AND REDFIELD THEORY	350

5.2	The Master Equation	351
5.2.1	INTERFERENCE EFFECTS	359
5.2.2	LIKE SPINS, UNLIKE SPINS, AND THE SECULAR APPROXIMATION	360
5.2.3	RELAXATION IN THE ROTATING FRAME	363
5.3	Spectral Density Functions	365
5.4	Relaxation Mechanisms	370
5.4.1	INTRAMOLECULAR DIPOLAR RELAXATION FOR IS SPIN SYSTEM	371
5.4.2	INTRAMOLECULAR DIPOLAR RELAXATION FOR SCALAR-COUPLED IS SPIN SYSTEM	378
5.4.3	INTRAMOLECULAR DIPOLAR RELAXATION FOR IS SPIN SYSTEM IN THE ROTATING FRAME	381
5.4.4	CHEMICAL SHIFT ANISOTROPY AND QUADRUPOLEAR RELAXATION	383
5.4.5	RELAXATION INTERFERENCE	385
5.4.6	SCALAR RELAXATION	387
5.5	Nuclear Overhauser Effect	388
5.6	Chemical Exchange Effects in NMR Spectroscopy	391
5.6.1	CHEMICAL EXCHANGE FOR ISOLATED SPINS	392
5.6.2	QUALITATIVE EFFECTS OF CHEMICAL EXCHANGE IN SCALAR-COUPLED SYSTEMS	401
	References	402

CHAPTER 6

EXPERIMENTAL ^1H NMR METHODS

6.1	Assessment of the 1D ^1H Spectrum	406
6.2	COSY-Type Experiments	409
6.2.1	COSY	410
6.2.1.1	PRODUCT OPERATOR ANALYSIS	410
6.2.1.2	EXPERIMENTAL PROTOCOL	412
6.2.1.3	PROCESSING	415
6.2.1.4	INFORMATION CONTENT	418
6.2.1.5	QUANTITATION OF SCALAR COUPLING CONSTANTS IN COSY SPECTRA	420

6.2.1.6	EXPERIMENTAL VARIANTS	426
6.2.2	RELAYED COSY	429
6.2.2.1	PRODUCT OPERATOR ANALYSIS	430
6.2.2.2	EXPERIMENTAL PROTOCOL	432
6.2.2.3	PROCESSING	432
6.2.2.4	INFORMATION CONTENT	432
6.2.3	DOUBLE-RELAYED COSY	433
6.3	Multiple-Quantum Filtered COSY	437
6.3.1	2QF-COSY	440
6.3.1.1	PRODUCT OPERATOR ANALYSIS	440
6.3.1.2	EXPERIMENTAL PROTOCOL	444
6.3.1.3	PROCESSING	445
6.3.1.4	INFORMATION CONTENT	446
6.3.2	3QF-COSY	449
6.3.2.1	PRODUCT OPERATOR ANALYSIS	449
6.3.2.2	EXPERIMENTAL PROTOCOL AND PROCESSING	451
6.3.2.3	INFORMATION CONTENT	452
6.3.3	E.COSY	455
6.3.3.1	PRODUCT OPERATOR ANALYSIS	457
6.3.3.2	EXPERIMENTAL PROTOCOL	460
6.3.3.3	PROCESSING	461
6.3.3.4	INFORMATION CONTENT	462
6.3.3.5	EXPERIMENTAL VARIANTS	463
6.4	Multiple-Quantum Spectroscopy	463
6.4.1	2Q SPECTROSCOPY	465
6.4.1.1	PRODUCT OPERATOR ANALYSIS	466
6.4.1.2	EXPERIMENTAL PROTOCOL	473
6.4.1.3	PROCESSING	474
6.4.1.4	INFORMATION CONTENT	474
6.4.2	3Q SPECTROSCOPY	481
6.4.2.1	PRODUCT OPERATOR ANALYSIS	481
6.4.2.2	EXPERIMENTAL PROTOCOL AND PROCESSING	483
6.4.2.3	INFORMATION CONTENT	484
6.5	TOCSY	486
6.5.1	PRODUCT OPERATOR ANALYSIS	486
6.5.2	EXPERIMENTAL PROTOCOL	493
6.5.3	PROCESSING	496

6.5.4	INFORMATION CONTENT	498
6.5.5	EXPERIMENTAL VARIANTS	499
6.6	Cross-Relaxation NMR Experiments	502
6.6.1	NOESY	502
6.6.1.1	PRODUCT OPERATOR ANALYSIS	503
6.6.1.2	EXPERIMENTAL PROTOCOL	506
6.6.1.3	PROCESSING	510
6.6.1.4	INFORMATION CONTENT	510
6.6.1.5	EXPERIMENTAL VARIANTS	511
6.6.2	ROESY	517
6.6.2.1	PRODUCT OPERATOR ANALYSIS	517
6.6.2.2	EXPERIMENTAL PROTOCOL AND PROCESSING	520
6.6.2.3	INFORMATION CONTENT	522
6.6.2.4	EXPERIMENTAL VARIANTS	524
6.7	^1H 3D Experiments	525
6.7.1	EXPERIMENTAL PROTOCOL	526
6.7.2	PROCESSING	527
6.7.3	INFORMATION CONTENT	527
6.7.4	EXPERIMENTAL VARIANTS	528
	References	529

CHAPTER 7

HETERONUCLEAR NMR EXPERIMENTS

7.1	Heteronuclear Correlation NMR Spectroscopy	535
7.1.1	BASIC HMQC AND HSQC EXPERIMENTS	536
7.1.1.1	THE HMQC EXPERIMENT	536
7.1.1.2	THE HSQC EXPERIMENT	540
7.1.1.3	THE CONSTANT-TIME HSQC EXPERIMENT	543
7.1.1.4	COMPARISON OF HMQC AND HSQC SPECTRA	544
7.1.2	ADDITIONAL CONSIDERATIONS IN HETERONUCLEAR CORRELATION EXPERIMENTS	546
7.1.2.1	PHASE CYCLING AND ARTIFACT SUPPRESSION	546
7.1.2.2	^{13}C SCALAR COUPLING AND MULTIPLY STRUCTURE	548

7.1.2.3	FOLDING AND ALIASING	549
7.1.2.4	PROCESSING HETERONUCLEAR CORRELATION EXPERIMENTS	552
7.1.3	DECOUPLED HSQC, SENSITIVITY-ENHANCED HSQC, AND TROSY EXPERIMENTS	552
7.1.3.1	THE DECOUPLED HSQC EXPERIMENT	553
7.1.3.2	SENSITIVITY-ENHANCED HSQC	560
7.1.3.3	TROSY EXPERIMENT	566
7.1.3.4	COMPARISON OF DECOUPLED HSQC, PEP-HSQC, AND TROSY EXPERIMENTS	570
7.1.3.5	RELAXATION INTERFERENCE AND TROSY SPECTRA OF LARGER PROTEINS	570
7.1.4	WATER SUPPRESSION AND GRADIENT ENHANCEMENT OF HETERONUCLEAR CORRELATION SPECTRA	573
7.1.4.1	SOLVENT SUPPRESSION	573
7.1.4.2	GRADIENT-ENHANCED HSQC AND TROSY NMR SPECTROSCOPY	574
7.1.5	THE CONSTANT-TIME ^1H - ^{13}C HSQC EXPERIMENT	578
7.2	Heteronuclear-Edited NMR Spectroscopy	581
7.2.1	3D NOESY-HSQC SPECTROSCOPY	582
7.2.1.1	3D ^1H - ^{15}N NOESY-HSQC	585
7.2.1.2	3D ^1H - ^{13}C NOESY-HSQC	588
7.2.2	3D TOCSY-HSQC SPECTROSCOPY	589
7.2.3	3D HSQC-NOESY AND HSQC-TOCSY EXPERIMENTS	591
7.2.4	HMQC-NOESY-HMQC EXPERIMENTS	593
7.2.4.1	3D $^{15}\text{N}/^{15}\text{N}$ HMQC-NOESY-HMQC	594
7.2.4.2	4D $^{13}\text{C}/^{15}\text{N}$ HMQC-NOESY-HMQC	595
7.2.4.3	4D $^{13}\text{C}/^{13}\text{C}$ HMQC-NOESY-HMQC	597
7.2.4.4	PROCESSING 4D HMQC-NOESY-HMQC SPECTRA	599
7.2.5	RELATIVE MERITS OF 3D AND 4D HETERONUCLEAR-EDITED NOESY SPECTROSCOPY	600
7.3	^{13}C - ^{13}C Correlations: The HCCH-COSY and HCCH-TOCSY Experiments	601
7.3.1	HCCH-COSY	603
7.3.2	CONSTANT-TIME HCCH-COSY	607
7.3.3	HCCH-TOCSY	608

7.4	3D Triple-Resonance Experiments	613
7.4.1	A PROTOTYPE TRIPLE-RESONANCE EXPERIMENT: HNCA	614
7.4.1.1	A SIMPLE HNCA EXPERIMENT	618
7.4.1.2	THE CT-HNCA EXPERIMENT	625
7.4.1.3	THE DECOUPLED CT-HNCA EXPERIMENT	626
7.4.1.4	THE GRADIENT-ENHANCED HNCA EXPERIMENT	627
7.4.1.5	THE GRADIENT-ENHANCED TROSY-HNCA EXPERIMENT	628
7.4.2	A COMPLEMENTARY APPROACH: THE HN(CO)CA EXPERIMENT	629
7.4.3	A STRAIGHT-THROUGH TRIPLE-RESONANCE EXPERIMENT: H(CA)NH	632
7.4.4	BACKBONE CORRELATIONS WITH THE ^{13}C O SPINS	637
7.4.4.1	HNCO	637
7.4.4.2	HN(CA)CO	638
7.4.5	CORRELATIONS WITH THE $\text{C}^\beta/\text{H}^\beta$ SPINS	641
7.4.5.1	CBCA(CO)NH	642
7.4.5.2	CBCANH	645
7.4.5.3	HNCACB	650
7.4.6	ADDITIONAL CONSIDERATIONS FOR TRIPLE-RESONANCE EXPERIMENTS	654
7.5	Measurement of Scalar Coupling Constants	656
7.5.1	HNCA-J EXPERIMENT	656
7.5.2	HNHA EXPERIMENT	660
7.6	Measurement of Residual Dipolar Coupling Constants	665
	References	673

CHAPTER 8

EXPERIMENTAL NMR RELAXATION METHODS

8.1	Pulse Sequences and Experimental Methods	680
8.2	Picosecond-Nanosecond Dynamics	685
8.2.1	EXPERIMENTAL METHODS FOR ^{15}N LABORATORY-FRAME RELAXATION	686

8.2.2	EXPERIMENTAL METHODS FOR ^{15}N RELAXATION INTERFERENCE	692
8.2.3	EXPERIMENTAL METHODS FOR $^{13}\text{CH}_2\text{D}$ METHYL LABORATORY-FRAME RELAXATION	693
8.2.4	EXPERIMENTAL METHODS FOR ^{13}CO LABORATORY-FRAME RELAXATION	699
8.3	Microsecond-Second Dynamics	702
8.3.1	LINESHAPE ANALYSIS	704
8.3.2	ZZ-EXCHANGE SPECTROSCOPY	706
8.3.3	$R_{1\rho}$ ROTATING-FRAME RELAXATION METHODS	707
8.3.4	CPMG RELAXATION METHODS	711
8.3.5	CHEMICAL EXCHANGE IN MULTIPLE-QUANTUM SPECTROSCOPY	715
8.3.6	TROSY-BASED APPROACHES	718
	References	721

CHAPTER 9

LARGER PROTEINS AND MOLECULAR INTERACTIONS

9.1	Larger Proteins	725
9.1.1	PROTEIN DEUTERATION	726
9.1.2	RELAXATION IN PERDEUTERATED AND RANDOM FRACTIONALLY DEUTERATED PROTEINS	728
9.1.3	SENSITIVITY FOR PERDEUTERATED PROTEINS	729
9.1.4	^2H ISOTOPE SHIFTS	732
9.1.5	EXPERIMENTS FOR $^1\text{H}^{\text{N}}$, ^{15}N , $^{13}\text{C}^{\alpha}$, $^{13}\text{C}^{\beta}$, AND ^{13}CO ASSIGNMENTS IN DEUTERATED PROTEINS	733
9.1.5.1	CONSTANT-TIME HNCA FOR DEUTERATED PROTEINS	735
9.1.5.2	HN(CA)CB FOR DEUTERATED PROTEINS	737
9.1.5.3	OTHER EXPERIMENTS FOR RESONANCE ASSIGNMENTS	739
9.1.6	SIDE CHAIN ^{13}C ASSIGNMENTS IN DEUTERATED PROTEINS	740
9.1.7	SIDE CHAIN ^1H ASSIGNMENTS	743

9.1.8	NOE RESTRAINTS FROM DEUTERATED PROTEINS	745
9.1.8.1	4D H^N-H^N $^{15}N/^{15}N$ -SEPARATED NOESY EXPERIMENT	745
9.1.8.2	$^{13}C/^{15}N$ -, $^{13}C/^{13}C$ -, AND $^{15}N/^{15}N$ -SEPARATED NOESY EXPERIMENTS ON RANDOM FRACTIONALLY DEUTERATED PROTEINS	747
9.1.9	SELECTIVE PROTONATION	749
9.2	Intermolecular Interactions	753
9.2.1	EXCHANGE REGIMES	753
9.2.2	PROTEIN-LIGAND BINDING INTERFACES	755
9.2.2.1	CHEMICAL SHIFT MAPPING	756
9.2.2.2	CROSS-SATURATION	757
9.2.2.3	TRANSVERSE RELAXATION AND AMIDE PROTON SOLVENT EXCHANGE	759
9.2.3	RESONANCE ASSIGNMENTS AND STRUCTURAL RESTRAINTS FOR PROTEIN COMPLEXES	760
9.2.3.1	ASSIGNMENTS AND STRUCTURES OF PROTEINS IN PROTEIN-LIGAND COMPLEXES	761
9.2.3.2	ISOTOPE-EDITED/FILTERED NOESY TO DEFINE INTERMOLECULAR INTERFACES	762
9.3	Methods for Rapid Data Acquisition	769
9.3.1	NONUNIFORM SAMPLING	770
9.3.2	GFT-NMR SPECTROSCOPY	771
9.3.3	PROJECTION-RECONSTRUCTION	773
	References	775

CHAPTER 10

SEQUENTIAL ASSIGNMENT, STRUCTURE DETERMINATION, AND OTHER APPLICATIONS

10.1	Resonance Assignment Strategies	782
10.1.1	1H RESONANCE ASSIGNMENTS FOR UNLABELED PROTEINS	782
10.1.2	HETERONUCLEAR RESONANCE ASSIGNMENTS FOR ISOTOPICALLY LABELED PROTEINS	792
10.2	Three-Dimensional Solution Structures	796

10.2.1	NMR-DERIVED STRUCTURAL RESTRAINTS	796
10.2.1.1	NOE DISTANCE RESTRAINTS	797
10.2.1.2	DIHEDRAL ANGLE RESTRAINTS FROM SCALAR COUPLING CONSTANTS	798
10.2.1.3	DIHEDRAL ANGLE RESTRAINTS FROM ISOTROPIC CHEMICAL SHIFTS	804
10.2.1.4	RESTRAINTS FROM RESIDUAL DIPOLAR COUPLING CONSTANTS	804
10.2.1.5	HYDROGEN BOND RESTRAINTS FROM AMIDE PROTON-SOLVENT EXCHANGE	805
10.2.1.6	HYDROGEN BOND RESTRAINTS FROM TRANS-HYDROGEN BOND SCALAR COUPLING CONSTANTS	806
10.2.2	STRUCTURE DETERMINATION	806
10.3	Conclusion	813
	References	814
	Table of Symbols	819
	List of Figures	825
	List of Tables	837
	Suggested Reading	839
	Index	841

For the reader's easy reference, the Table of Constants and the Spin-1/2 Product Operator Equations are given on the inside back cover end pages.

growth in the field of nuclear magnetic resonance spectroscopy that continues today originated with the development of Fourier transform NMR spectroscopy by Ernst and his colleagues. The conception of multidimensional NMR spectroscopy by Jeener, Y. D. Currently, NMR spectroscopy and X-ray crystallography are the only techniques capable of determining three-dimensional structures of macromolecules at atomic resolution. In addition, NMR spectroscopy is a powerful technique for investigating the structure and dynamics of biological macromolecules. The former limitation has been alleviated partially by the development of more powerful magnets and more sensitive NMR spectrometers and by advances in techniques for sample preparation (both synthetic and biochemical). The latter limitation has been transcended into a significant advantage by the phenomenal advances in the theoretical and experimental capabilities of NMR spectroscopy (and spectroscopists). The history of these developments has been reviewed by Ernst and by Wüthrich in their 1991 and 2002 Nobel Laureate lectures, respectively (4, 5). In light of subsequent developments, the conclusion of Blah's