

Contents

Chapter 1 Introduction to Immunocytochemistry and Historical Background	1
1.1 Approaches to Immunocytochemistry	1
1.2 Criteria for an Electron-Microscopic Immunocytochemical Technique	3
1.3 Problems with the Pre-Embedding Techniques	4
References	7
Chapter 2 Fine Structure Preservation	9
2.1 Introduction	9
2.2 Fine-Structure Evaluation in Practice	13
2.2.1 Fine-Structure Stabilization by Fixation	13
2.2.2 Contrasting	13
2.2.3 Analysis	15
2.2.4 Interpretation	17
2.2.5 Selective Destruction	21
2.3 Summary	23
References	24
Chapter 3 Fixation for Fine Structure Preservation and Immunocytochemistry	26
3.1 Fine Structure Preservation	26
3.1.1 Glutaraldehyde	29
3.1.2 Formaldehyde	36
3.1.3 Effects of Aldehydes on Cell Components Other than Proteins	46
3.1.4 Cross-Linking Agents Other than Glutaraldehyde and Formaldehyde	51
3.2 Factors Affecting the Quality of Fixation for Fine Structure Preservation	54
3.2.1 Concentration and Length of Fixation	55
3.2.2 Temperature of Fixation	57
3.2.3 pH and the Buffer Vehicle	57

3.2.4	Osmolarity and Ionic Strength of the Buffer Vehicle ..	60
3.2.5	Purity of the Aldehyde Fixative; Storage and Disposal	63
3.2.6	Tissue Type	64
3.2.7	Methods of Fixation	64
3.2.8	Rate of Fixative Penetration	66
3.3	Fixation Artefacts	67
3.4	Effect of Fixatives on Enzyme Activity	70
3.4.1	Effect of Glutaraldehyde on Enzyme Activity	70
3.4.2	Effect of Formaldehyde on Enzyme Activity	71
3.5	Fixation for Immunocytochemistry	71
3.5.1	Effect of Glutaraldehyde on Antigenicity	73
3.5.2	Effect of Formaldehyde on Antigenicity	75
3.5.3	Effect of Acrolein on Antigenicity	76
3.5.4	Effect of Osmium Tetroxide on Antigenicity	76
3.5.5	Effect of Factors Affecting Cross-Linking on Antigenicity	77
3.5.6	Assessing the Effects of Fixation on Antigenicity	78
3.6	A Concluding Remark	80
	References	80

	Chapter 4 Embedding Media for Section Immunocytochemistry	90
4.1	Resin-Free Sections – Temporary Embedement	91
4.1.1	The PEG Method	92
4.1.2	The Poly-Methylmethacrylate Method	93
4.2	Permanent Embedding Media	94
4.2.1	Water-Miscible Media	94
4.2.2	New Acrylic Resins	96
4.2.3	Epoxy Resins	119
4.3	Freeze Substitution	123
4.3.1	Rapid Freezing	124
4.3.2	Freeze Substitution and Immunolabelling	125
	References	129

	Chapter 5 Cryo and Replica Techniques for Immunolabelling ..	137
5.1	Cryo Sectioning Techniques	137
5.1.1	Historical Perspectives	137
5.1.2	Theory of Freezing	139
5.1.3	The Knife	144
5.1.4	The Microtome	152
5.1.5	Cryo Sectioning Theory	153
5.1.6	The Hydrated Cryo Sectioning Technique	160
5.1.7	Tokuyasu Thawed Cryo-Section Technique	162

5.2	Freeze-Fracture and Replica Labelling Methods	187
5.2.1	Fracture-Label Method	187
5.2.2	Label-Fracture Method	191
5.2.3	Fracture-Flip	192
5.3	Other Replica Techniques for Labelling	192
5.3.1	Surface Replica Methods	192
5.3.2	Replicas of Labelled Sections	197
	References	197

	Chapter 6 Elementary Immunology (BRIAN BURKE) ..	204
6.1	Basic Immunology	204
6.1.1	The Immune Response	204
6.1.2	Cells of the Immune System	205
6.2	The Structure of Antibodies	206
6.3	The Biological Functions of Immunoglobulins	209
6.3.1	IgG	209
6.3.2	IgM	209
6.3.3	IgA	210
6.3.4	IgD and IgE	210
6.3.5	Generation of Antibody Diversity	210
6.4	The Nature of Antigenicity	212
6.4.1	Antigen Size – Carriers and Haptens	213
6.4.2	Antigen Presentation	213
6.4.3	Proteins as Antigens	213
6.4.4	The Interaction of Antibodies with Their Antigens ..	214
6.5	Practical Aspects of Immunology	215
6.5.1	Preparation of Antibodies	215
6.5.2	Production of Polyclonal Antibodies	216
6.5.3	Monoclonal Antibodies	218
6.5.4	Anti-Peptide Antibodies	221
6.5.5	Use of Molecular Biology Approaches to Express Immunoglobulin Molecules in Cells	222
6.5.6	Purification of Antibodies	223
6.6	Affinity Purification	225
6.7	Characterization of Antibodies	227
6.8	Precipitin Techniques	227
6.9	Immunoblotting	228
6.10	Immunoprecipitation	229
6.11	Immunoassay	231
	Appendix	232
	References	234

Chapter 7 Labelling Reactions for Immunocytochemistry	237
7.1 Historical Perspectives	237
7.2 Labelling in Practice	242
7.2.1 Purity of Antibody: Use of Light Microscopy	242
7.2.2 Class of Antibody; Hybrid Antibodies	243
7.2.3 Antibody Concentrations	245
7.2.4 Time, Temperature and Repeated Applications of Antibody	248
7.2.5 Avoiding Non-Specific Labelling	252
7.2.6 Evaluation and Control of Labelling Reactions	254
7.2.7 The Immunocytochemical Controls	254
7.2.8 The Biological Controls	257
7.2.9 Problems in Labelling	258
7.2.10 Accessibility Problems on Sections	259
7.2.11 Sensitivity of Labelling	261
7.3 Approaches for Single Labelling	263
7.4 Approaches for Double Labelling	263
7.4.1 Double Direct Labelling	263
7.4.2 Double Indirect - Simultaneous	265
7.4.3 Double Indirect - Sequential	266
7.4.4 Multiple-Labelling Using Serial-Sections	270
7.4.5 Avidin-Biotin Systems	270
7.4.6 Resolution	271
References	275

**Chapter 8 Particulate Markers
for Immunoelectron Microscopy (JOHN LUCOCQ)** 279

8.1 Colloidal Gold	279
8.1.1 Introduction	279
8.1.2 Properties of Gold Colloids	280
8.1.2.1 Gold Particles in Microscopy	280
8.1.2.2 Electrolyte-Induced Aggregation	282
8.1.3 Preparation of Gold Colloids	283
8.1.3.1 Methods	283
8.1.3.2 Number and Concentration of Gold Particle/Complexes	286
8.1.4 Factors that Influence Protein-Gold Complex Formation	287
8.1.4.1 Protein Concentration	287
8.1.4.2 Surfaces of Protein and Gold	289
8.1.4.3 pH and Ionic Strength	290
8.1.5 Preparation of Protein Gold Complexes	291
8.1.5.1 Selection and Adjustment of Complexing Conditions	291
8.1.5.2 Complex Preparation, Purification and Storage	294

8.1.5.3 Specific Protein-Gold Complexes	297
8.1.6 Ferritin	299
8.1.6.1 Method for Conjugating Protein to Ferritin	300
8.1.7 Imposil	300
Appendix. Silver Enhancement Procedure	301
References	302

**Chapter 9 Non-Immunological High-Affinity Interactions
Used for Labelling** 307

9.1 Lectins	307
9.1.1 Simple Sugar Chemistry	310
9.1.2 Glycoproteins	311
9.1.3 Lecting Labelling	315
9.2 Protein A	320
9.2.1 Properties of Protein A	320
9.2.2 Species Specificity	321
9.2.3 Binding to Fab Regions of Antibodies	322
9.2.4 Kinetics of Reaction and the Effect of pH	323
9.3 Protein G	323
9.3.1 Use of Protein G for Immunocytochemistry	324
9.4 Avidin-Biotin Interactions	325
9.4.1 Properties of Egg-White Avidin	325
9.4.2 Streptavidin	326
9.4.3 Biotin	326
9.4.4 Biotinylation of Proteins	327
9.4.5 The Use of a Linker in the Biotinylation Reaction	328
9.4.6 Avidin-Biotin for Affinity Labelling Studies	328
9.4.7 Biotinylation Studies with Living Cells	331
9.4.8 Streptavidin-Gold	332
9.5 Miscellaneous Labelling Approaches Relying on Non-Immunological High Affinity Interactions	332
9.5.1 The Enzyme Gold Method	332
9.5.2 Receptor/Ligand-Gold Complexes	333
9.5.3 Toxin-Gold Conjugates	335
9.6 In-Situ Hybridization	335
References	337

Chapter 10 Preembedding Immuno-Labelling 345

10.1 Permeabilization	346
10.1.1 Chemistry of Detergents	346
10.1.2 Non-Ionic Detergents - Triton X-100	349
10.1.3 Saponin	350
10.1.4 Digitonin	351

10.1.5	Bacterial Toxins	353
10.1.6	Permeabilization for EM Labelling in Practice	353
10.1.7	The Saponin/Thick Section Approaches	354
10.1.8	The Freeze-Thaw Approach	355
10.2	Markers for Preembedding Labelling	356
10.2.1	Horseradish Peroxidase	356
10.2.2	Particulate Markers for Preembedding	358
10.3	Preembedding Studies of the Nucleus	361
10.4	Preembedding Labelling for Studies of the Nervous System	363
10.5	The Use of Dinitrophenol IgG Conjugates	365
10.6	Agarose Gel Method for Preembedding	366
	References	366

Chapter 11 Quantitative Aspects of Immunocytochemistry ... 371

11.1	General Comments	371
11.2	Basic Stereology	375
11.2.1	General Introduction to Stereology	375
11.3	Estimation of Volume Density and Surface Density in Practice	377
11.3.1	Point and Intersection Counts	377
11.4	Estimation of Volume of Reference Space	398
11.4.1	Organ or Tissue	398
11.4.2	Estimating Mean Cell Volume	398
11.5	Stereological Sampling in Practice	410
11.5.1	Measurement of L_v in Practice	412
11.6	Quantitation in Immunocytochemistry	413
11.6.1	Use of Point and Intersection Counting for Relating the Number of Gold Particles to the Volume, Surface or Length of Structures in Sections	414
11.6.2	Labelling Efficiency	417
11.6.3	Estimation of LE in Practice	421
11.7	Approaches for Quantitation Ignoring Labelling Efficiency	425
11.8	Absolute Quantitation Making Assumptions About Labelling Efficiency	426
11.9	Negation of the Labelling Efficiency Factor: The Matrix Gel Method for Quantitating Souble Antigens Which Are Available in Pure Form	427
11.9.1	Possible Limitations of this Approach	429

11.10	Quantitative Model System Developed for Small Molecular Weight Neurotransmitters	432
11.11	Sensitivity of Labelling: Limits of Detection	436
11.11.1	Soluble Proteins	436
11.11.2	Membrane Proteins	437
11.12	Steric Hindrance	437
11.13	Amplification of Gold Labelling and Quantitation ...	438
11.14	Signal-to-Noise Ratio and the Concentration of Antigen: Those Few Gold Particles!	439
	References	441

Chapter 12 An Overview of Techniques for Labelling at the EM Level 446

Subject Index 451