

# Contents

<b>I.</b>	<b>Introduction .....</b>	<b>1</b>
A.	An Historical Perspective .....	2
1.	The Phenomenon of Viral Interference .....	2
2.	Discovery of Interferon .....	2
3.	Interference Mediated by Other Mechanisms .....	3
B.	An Overview of the Interferon System .....	4
1.	Interferon Inducers .....	5
2.	Induction Mechanisms .....	6
3.	Genetic Information for Interferon Production .....	7
4.	Interferon Messenger RNA .....	7
5.	Interferon Production .....	7
6.	Interferons .....	8
7.	Interferon Binding .....	9
8.	Genetic Information for Interferon Actions .....	9
9.	Cellular Alterations Induced by Interferons .....	9
a)	Antiviral States .....	9
b)	Priming and Blocking .....	10
c)	Enhanced Susceptibility to Toxicity of Double-Stranded RNA .....	10
d)	Effects on Cell Surfaces .....	10
e)	Effects on Cell Multiplication .....	11
f)	Immunomodulations .....	11
C.	The Interferon System <i>in vivo</i> .....	11
1.	Interferons as Prophylactic and Therapeutic Agents in Animals .....	11
2.	Interferons in the Clinic .....	12
<b>II.</b>	<b>Interferon Assays .....</b>	<b>13</b>
A.	General Considerations .....	13
B.	Dose-Response Relationships .....	14
C.	Assay Methods .....	15
1.	Plaque-Reduction Assays .....	15
2.	Yield-Reduction Assays .....	16
a)	Reduction of Infectious Virus Yields .....	16
b)	Reduction of Haemagglutinin Yields .....	16
c)	Reduction of Haemadsorption .....	16
d)	Reduction of Neuraminidase Yields .....	17
3.	Cytopathic Effect (CPE)-Inhibition Assays .....	17
a)	CPE-Reading Method .....	17
b)	Dye-Uptake Method .....	18
4.	Radiochemical Assays .....	19
5.	Other Antiviral Assays .....	19
a)	Reverse Transcriptase Inhibition Assay .....	19
b)	Immunofluorescence Assay .....	20
c)	EB Virus-Expression Assay .....	20
d)	Cytochemical Assays .....	20

e) Agar Diffusion Assays .....	20
f) pH Indicator Assay .....	20
6. Non-Antiviral Assays .....	21
D. Factors Influencing Interferon Assays .....	21
1. Non-Interferon Contaminants .....	21
2. Period of Interferon Exposure .....	22
3. Challenge Multiplicity .....	22
4. Assay Duration .....	22
5. Concentration vs. Volume .....	23
6. Aging Effect .....	23
E. Choice of an Assay System .....	24
1. Assay Method of Choice .....	24
2. Cells of Choice .....	25
3. Virus of Choice .....	25
F. Reference Interferons and Standard Interferons .....	25
<b>III. Interferon Inducers .....</b>	<b>27</b>
A. Animal Viral Inducers .....	34
1. Adenoviruses .....	35
2. Myxo-Paramyxo Inducers .....	36
3. Rhabdoviruses .....	36
4. "Arbovirus" Inducers .....	37
5. Diplorna Viruses .....	37
6. Lymphocytic Choriomeningitis Virus .....	37
B. Non-Viral Inducers of Interferons .....	38
1. "Foreign" Nucleic Acids .....	38
2. Fungal Extracts .....	38
3. Bacteria and Bacterial Products .....	42
4. Other Microorganisms Inducing Interferons .....	43
a) Chlamydia .....	43
b) Rickettsiae .....	43
c) Mycoplasmas .....	43
d) Protozoa .....	43
5. Mitogens and Immune Recognition Induction .....	44
a) Mitogens .....	44
b) Immune Recognition Induction .....	45
c) Induction by Tumor Cells .....	46
6. Synthetic Inducers .....	46
a) Anionic Polymers .....	46
1. Polycarboxylates, Sulfates and Phosphates .....	46
2. Polynucleotides .....	49
b) Low Molecular Weight Inducers .....	53
1. Tilorone .....	53
2. Cationic Dyes .....	53
3. Propanediamine .....	53
4. BL-20803 .....	53
5. MA-56 .....	54
6. AET .....	54
7. U-25, 166 .....	54
C. Interferon (?) Inducers .....	54
D. Uninduced (Spontaneous) Interferons .....	55
<b>IV. Induction Mechanisms .....</b>	<b>58</b>
A. The Interferon Induction-Production Curve .....	58
1. The Induction Phase .....	59
a) True Induction? .....	60
b) Preformed Interferons? .....	61

2. The Interaction of Inducer and Cell .....	62
3. Induction Lag Periods .....	64
a) The <i>in vitro</i> Lag .....	65
b) Induction Lag <i>in vivo</i> .....	67
c) Time of Appearance of Lymphocyte Interferons – Types I and II .....	68
B. Mechanism of Induction by Viruses .....	70
1. Search for Double-Stranded RNA .....	71
2. Viral Events Effecting Induction .....	72
C. Alternative Induction Mechanism Hypotheses .....	74
1. The Double-Stranded RNA Hypothesis .....	74
2. The Repressor-Depletion Hypothesis .....	75
3. The Basal-Level-Interferon Hypothesis .....	75
<b>V. The Genetics of Interferon Production .....</b>	<b>77</b>
A. Hybrid-Cell Analyses of Interferon Production .....	77
1. Production Regulation in Hybrid-Cells .....	77
2. Chromosomal Assignments for Interferon Production in Hybrid-Cell Lines .....	78
B. Chromosomal Assignments for Interferon Production in Aneuploid Cells .....	79
C. Epigenetic Complications of Assessing Genetic Contributions to Interferon Production .....	80
1. Age Effects on Interferon Production .....	80
a) <i>In vivo</i> Age and Interferon Potential .....	81
b) Interferon Production and <i>in vitro</i> Aging .....	81
2. Variations in Interferon Production by Human Diploid Cell Cultures .....	82
D. Genetics of Interferon Production <i>in vivo</i> .....	83
1. Mendelian Analyses of Interferon Production .....	83
2. Non-Specific Factors Influencing <i>in vivo</i> Genetic Evaluations of Interferon Production .....	84
a) Temperature .....	84
b) Hormones .....	85
c) Other Factors Modulating Interferon Production .....	87
<b>VI. Interferon Production .....</b>	<b>90</b>
A. Interferon Production at the Cellular and Molecular Level .....	90
1. Interferon Messenger RNA .....	90
a) Indirect Evidence for Interferon Messenger RNA .....	90
b) Extraction of Interferon Messenger RNA and Its Translation in Heterologous Cells .....	90
c) Translation of Interferon Messenger RNA in <i>Xenopus</i> Oöcytes .....	93
d) Translation of Interferon Messenger RNA in Cell-Free Protein-Synthesizing Systems .....	95
e) Translation of Interferon Messenger RNA in Bacteria? .....	96
2. Superinduction or Superproductions? .....	97
a) Interferon Enhancement by Metabolic Inhibitors .....	97
b) Mechanisms of Superproduction .....	100
3. Regulatory Mechanisms on Interferon Production: The Refractory State <i>in vitro</i> (Hyporesponsiveness and Blocking) .....	103
4. Posttranslational Processing of Interferons .....	106
a) Interferon Precursor? .....	107
b) Glycosylation of Interferon Proteins .....	107
c) Secretion of Interferons .....	108
B. Interferon Production at the Organism Level .....	109
1. Interferon Production <i>in vivo</i> .....	109
a) Production of Interferon in Viral Infections .....	109
b) Non-Viral Interferon Inducers <i>in vivo</i> .....	111
c) Cell-Sources of Interferon .....	115

2. Hyporesponsiveness <i>in vivo</i> : Tolerance to Repeated Inductions .....	124
a) Cross-Tolerance to Different Inducers .....	124
b) Serum Hyporeactive Factor .....	128
C. Mass Production of Interferons .....	129
1. Mouse Interferon .....	129
2. Human Leukocyte Interferon .....	130
3. Human Fibroblast Interferon .....	131
4. Human Lymphoblastoid Cell Interferon .....	132
5. Alternatives to Large-Scale Production .....	133
6. Which Human Interferon Should be Produced? .....	133
<b>VII. Interferons: Their Purification and Characterization .....</b>	<b>134</b>
A. Defining Interferons .....	134
1. Induction of Antiviral Activities .....	135
2. "Species Specificity" of Interferons .....	135
3. Acid-Stability of Interferons .....	145
4. Physicochemical Properties Generally Applicable to All Interferons: The Protein Nature of Interferons .....	149
5. Antigenicities of Interferons .....	150
6. Induction of Non-Antiviral Activities .....	156
7. A Summary Definition of Interferons .....	156
B. Purification of Interferons .....	156
1. Purification of Mouse Interferons .....	158
2. Purification of Human Leukocyte (and Lymphoblastoid) Interferons .....	164
3. Purification of Human Fibroblast Interferon .....	168
C. Characterization of Interferons .....	171
1. Heterogeneities of Interferons .....	171
a) Molecular Weights of Interferons .....	172
b) Stabilities of Interferons .....	174
c) Ligand Affinities of Interferons .....	176
d) Biological Heterogeneities of Interferons .....	176
e) Origins of Heterogeneities of Interferons .....	178
f) Significance of Interferon Heterogeneities .....	182
2. Non-Antiviral Activities of Interferons .....	183
<b>VIII. The Genetics of Interferon Action .....</b>	<b>184</b>
A. Hybrid-Cell Analyses of Interferon Action .....	184
B. Chromosomal Assignments for Interferon Actions in Aneuploid Cells .....	185
1. Antiviral Action of Human Interferon in Aneuploid Cells: Chromosome 21 Dosage Effect .....	185
2. Relationship of Non-Antiviral Actions of Human Interferons to Chromosome 21 .....	186
3. Regulatory Chromosomes for Interferon Actions .....	186
C. Role of Chromosome 21 in Interferon Actions: Interferon Binding .....	187
1. Interactions of Interferons With Cells: Initiation of Interferon Actions .....	187
a) Indirect Studies on Interferon Binding .....	187
b) Direct Studies on Interferon Binding .....	188
2. Chromosome 21 and Interferon Binding .....	189
D. Epigenetic Complications to Assessing Genetic Contributions to Interferon Actions .....	190
1. Age Effects on Interferon Action .....	190
2. Cell Density Effect .....	191
3. Variations in Interferon Sensitivities of Diploid Cells .....	192
4. Other Factors Modulating Interferon Actions .....	192
a) Antagonists .....	193
b) Factors Influencing Early Interferon-Cell Interactions .....	193
c) Miscellaneous Factors .....	193

<b>IX. Mechanisms of Antiviral Actions of Interferons</b>	196
A. General Considerations of Antiviral Actions of Interferons	196
1. Conversion to the Antiviral State	196
a) Kinetics of Development of Antiviral Activity	196
b) Metabolic Requirements for Development of Virus Resistance	198
c) Duration of the Antiviral State	199
2. Character of Interferon-Induced Virus Resistance	200
B. Antiviral Mechanisms: Sites of Interferon Actions Against Viruses	207
1. Inhibition of Virus Attachment, Penetration and Uncoating	208
2. Inhibition of Virus Transcription	208
3. Inhibition of Virus Translation	210
a) Inhibition of Virus Translation in Cells	210
b) Inhibition of Virus Translation in Cell-Free Systems	212
4. Inhibition of Maturation and Release	217
C. An Oversimplified Summation of Interferon-Induced Antiviral Actions	220
<b>X. Non-Antiviral Actions of Interferons</b>	223
A. The Breadth of Interferon Action: The Expanding Realm of Interferonology	224
B. Inhibition of Non-Viral Agents	232
C. Priming	233
D. Blocking	236
E. Cell-Multiplication-Inhibition	238
1. Evidence That Interferon Is the Active Component Inhibiting Cell-Multiplication	238
2. Cellular Systems Inhibited by Interferon	241
3. Metabolic Alterations in Interferon-Treated Cells	242
4. Restraints Imposed by Interferon	243
F. Toxicity Enhancement	244
G. Enhanced Synthetic Activities	248
H. Surface Alterations Induced by Interferons	249
I. Enhanced Immunolysis	250
J. Enhancement of Phagocytosis	251
K. Macrophage "Activation"	252
L. Delayed-Type Hypersensitivity-Inhibition	252
M. Graft-vs-Host Reactions: Effects of Interferon on Transplantation	253
N. Effects of Interferon on Antibody Production	254
1. <i>In vivo</i> Antibody Production	254
2. <i>In vitro</i> Antibody Production	255
<b>XI. Pharmacokinetics of Interferons</b>	257
A. Distribution of Interferon	257
1. Circulating Interferon Levels	257
2. Clearance From the Cerebrospinal Fluid: The Blood-Brain Barrier for Interferon	260
3. Transplacental Passage and Other Barriers to Distribution of Interferon	262
B. Metabolism of Interferons	263
1. Renal Clearance	263
2. Catabolism of Interferon: Role of Carbohydrates	264
C. Effective Interferon Levels	265
<b>XII. Antiviral Actions of Interferons in Animals</b>	266
A. Natural Recovery Processes From Viral Infections	266
1. Progression of Virus Infections	267
a) Localized Infections	267
b) Generalized Infections	269

2. Factors Modifying Pathogenesis: Interactions With Interferon Mechanism	275
a) Restriction of Establishment of Infections	275
b) Elimination of Established Infections	279
B. Antiviral Studies With Interferons and Interferon Inducers in Animals	282
1. Localized Infections	284
a) Effect of Exogenous Interferons	284
b) Effects of Inducers of Endogenous Interferons	285
2. Generalized Infections	286
a) Effects of Exogenous Interferons	286
b) Combined Effects of Interferons and Other Agents	287
c) Effects of Inducers of Endogenous Interferons	287
3. Unifying Speculation on Inducer Toxicities	290
<b>XIII. Antitumor Activities of Interferons in Animals</b>	292
A. Activities Against Virally-Induced Tumors	292
1. Antitumor Effects of Exogenous Interferons	292
2. Antitumor Effects of Inducers of Endogenous Interferons Against Virally-Induced Tumors	296
3. Combined Antitumor Efficacies of Interferon and Other Antitumor Agents	297
B. Activities Against Transplantable Tumors	298
1. Antitumor Effects of Exogenous Interferons	298
2. Antitumor Effects of Inducers of Endogenous Interferons Against Transplantable Tumors	300
C. Activities Against Chemically- and Radiation-Induced Tumors	301
1. Antitumor Effects of Exogenous Interferons	301
2. Antitumor Effect of Inducers of Endogenous Interferons Against Chemically- and Radiation-Induced Tumors	301
D. Mechanisms of Antitumor Activities of Interferons	302
1. Virus-Induced Tumors	302
2. Transplantable Tumors	303
<b>XIV. Interferon in the Clinic</b>	305
A. Preliminary Testing of Interferons: Therapeutic-Index of Interferon?	305
B. Antiviral Activities of Interferons in Man	307
1. Localized Infections	307
a) Prophylaxis With Interferon	307
b) Therapy of Localized Infections	311
2. Systemic Viral Infections	312
a) Disseminated Herpesvirus: Varicella-Zoster	313
b) Cytomegalovirus Infections	313
c) Hepatitis B Virus Infections	315
d) Interferon Therapy in Other Viral Diseases	316
3. Antiviral Effects of Interferon Inducers in Man	316
a) Virus Interference in Man	316
b) Poly rI-Poly rC in the Clinic	317
c) Propanediamine and Its Vehicle	318
C. Cancer Therapy With Interferon	319
1. Antitumor Activity of Interferon Inducers in Man	319
2. Effects of Interferon	320
<b>XV. Prelude to the Interferon System</b>	322
<b>Acknowledgements</b>	323
<b>References</b>	324
<b>Appendix to the References</b>	414
<b>Subject Index</b>	486