

VOLUME VIII

# SUPPLEMENTS

## PART 1

HISTOCHEMISTRY OF THE ISLETS  
OF LANGERHANS

R. H. LANGE

Giessen

ENZYMOLOGY OF THE THYMUS  
LUCIE ARVY

Paris

With 86 Illustrations and 130 Tables



GUSTAV FISCHER VERLAG STUTTGART  
1973

# Contents

R. H. LANGE

## Histochemistry of the Islets of Langerhans

Preface . . . . .	1
I. Introduction . . . . .	2
A. General Remarks about Islet Morphology, Methodology and Documentation of Data . . . . .	2
B. Explanation of Symbols . . . . .	5
II. The Cell Types of the Pancreatic Islets . . . . .	8
A. So-called Specific Islet Staining Methods . . . . .	8
1. So-called specific B-cell staining methods . . . . .	8
a) Aldehyde-fuchsin . . . . .	9
α) Chemical specificity . . . . .	9
β) Specificity for islet B-cells . . . . .	9
γ) Specificity for a defined subcellular component of B-cells . . . . .	11
b) Pseudoisocyanine . . . . .	12
2. So-called specific non-B-cell staining methods . . . . .	12
B. Definition of the Islet Cell Types and Examples of their Distribution in Systematic Groups . . . . .	14
1. Cyclostomes . . . . .	14
2. Cartilaginous fishes . . . . .	15
3. Bony fishes . . . . .	15
4. Amphibians . . . . .	16
5. Reptiles . . . . .	18
6. Birds . . . . .	19
7. Mammals . . . . .	20
III. Histochemistry of the Islet Cell Types . . . . .	22
A. Chemistry of the Hormonal Secretory Products . . . . .	22
1. Chemistry of B-cell secretory products . . . . .	22
Primary structure p. 22; Tertiary structure p. 23; Immunology p. 23; Measurement p. 23; Solubility p. 27; Crystallization p. 27; Insulin and metals p. 29.	
2. Chemistry of A <sub>2</sub> -cell secretory products . . . . .	29
Primary structure p. 29; Immunology p. 31; Crystallography p. 31; GLA, GLI p. 32.	
3. Chemistry of secretory products of A <sub>1</sub> - or related cells . . . . .	32
Gastrin p. 33.	
B. The Secretory Granules of Islet Cells . . . . .	34
Consideration of osmolarity of the granule content p. 34; "Light scattering" p. 34.	
1. B-cell secretory granules . . . . .	35
a) B-granules <i>in situ</i> (following routine electron microscopy preparation) . . . . .	35
Periodic fine structure p. 35; Shape of the granules p. 36; Significance of crystalline B-granules p. 40.	
b) Isolated B-granules . . . . .	40
Density p. 41; Chemical properties p. 41; Stabilizing and solubilizing conditions p. 41; Morphology p. 41.	
2. The secretory granules of A <sub>2</sub> -cells . . . . .	44
Observation of "light scattering" p. 44; Shape p. 46.	

3. Secretory granules of A <sub>1</sub> -cells and others . . . . .	46
4. Chemistry and morphology of secretory granules of chromophobe cells . . . . .	46
C. Inorganic Material in Islet Cells . . . . .	47
Microincineration p. 47; Light inorganic ions p. 47; Exogenous cobalt p. 47; Zinc, heavy metals p. 47; Experimental effects on heavy metal content p. 54; Hypotheses for the role of zinc in islet cells, especially in B-cells p. 55.	
D. Histochemistry of Proteins in Islet Cells. . . . .	55
1. Proteins in B-cells . . . . .	55
Immunohistochemistry p. 55; SS and SH groups p. 57; Gomori-type methods and pseudoisocyanine p. 57; Quantitative determinations of insulin in islet tissue p. 58; Glutathione p. 58.	
2. Proteins in A <sub>2</sub> -cells . . . . .	59
Immunohistochemistry p. 59; Tryptophan p. 61.	
3. Proteins in A <sub>1</sub> -cells or related cells . . . . .	62
Immunohistochemistry p. 62; Masked basophilia p. 62.	
E. Ribonucleic Acids in Islet Cells . . . . .	63
F. Carbohydrates in Islet Cells . . . . .	65
Glucose p. 65; Glycogen p. 65; Conditions that modify glycogen content p. 66.	
G. Lipids in Islet Cells. . . . .	67
H. Other Organic Material in Islet Cells (Except Enzymes) . . . . .	68
Vitamin C p. 68; Biogenic amines p. 69; Alloxan p. 72; Various compounds p. 72.	
I. Enzyme Histochemistry of Islet Cells . . . . .	72
1. Activities of Oxidoreductases . . . . .	74
a) (I.I.I.VIII, I.I.IC.V) Glycerol-3-phosphate Dehydrogenase Activity . . . . .	74
b) (I.I.I.XXVII) Lactate Dehydrogenase Activity . . . . .	75
c) (I.I.I.XXX) 3-Hydroxybutyrate Dehydrogenase Activity . . . . .	77
d) (I.I.I.XXXV) 3-Hydroxyacyl-CoA Dehydrogenase Activity . . . . .	77
e) (I.I.I.XXXVII etc.) Malate Dehydrogenase Activity . . . . .	77
f) (I.I.I.XLI) Isocitrate Dehydrogenase Activity . . . . .	78
g) (I.I.I.XLIII) Phosphogluconate Dehydrogenase Activity . . . . .	80
h) (I.I.I.II) Glucose-6-phosphate Dehydrogenase Activity . . . . .	80
i) (I.II.I.XII) Glyceraldehydepsphosphate Dehydrogenase Activity . . . . .	82
j) (I.III.IC.I) Succinate Dehydrogenase Activity . . . . .	83
k) (I.IV.I.II etc.) Glutamate Dehydrogenase Activity . . . . .	83
l) (I.IV.III.IV) Monoamine Oxidase Activity . . . . .	84
m) (I.VI.IV.II) Glutathione Reductase Activity . . . . .	84
n) (I.VI.IV.III) Lipoamide Dehydrogenase Activity . . . . .	84
o) (I.VI.IC.I) Reduced NADP Dehydrogenase Activity . . . . .	85
p) (I.VI.IC.II) Reduced NAD Dehydrogenase Activity . . . . .	85
q) (I.IX.III.I) Cytochrome Oxidase Activity . . . . .	86
r) (I.XII.I.VII) Peroxidase Activity . . . . .	86
2. Activities of Transferases . . . . .	86
a) (II.I.III.III) Ornithine Carbamoyltransferase Activity . . . . .	86
b) (II.II.I.I) Transketolase Activity . . . . .	86
c) (II.II.I.II) Transaldolase Activity . . . . .	87
d) (II.III.II.I) Glutamyltransferase Activity . . . . .	87
e) (II.IV.I.I) $\alpha$ -Glucan Phosphorylase Activity . . . . .	87
f) (II.IV.I.XI) UDPGlucose-Glycogen Glycosyltransferase Activity . . . . .	88
g) (II.VI.I.I) Aspartate Aminotransferase Activity . . . . .	88
h) (II.VI.I.II) Alanine Aminotransferase Activity . . . . .	88
i) (II.VII.I.I etc.) Hexokinase Activities . . . . .	88
j) (II.VII.I.XI) Phosphofructokinase Activity . . . . .	88

## Contents

VII

k) (II.VII.I.XL) Pyruvate Kinase Activity . . . . .	88
l) (II.VII.II.III) Phosphoglycerate Kinase Activity . . . . .	89
m) (II.VII.V.I) Phosphoglucomutase Activity . . . . .	89
n) (II.VII.V.III) Phosphoglyceromutase Activity . . . . .	89
o) (II.VII.VII.IX) UDPGlucose Pyrophosphorylase Activity . . . . .	89
<b>3. Activities of Hydrolases . . . . .</b>	<b>90</b>
a) (III.I.I.II etc.) Arylesterase Activity . . . . .	90
b) (III.I.I.III) Lipase Activity . . . . .	90
c) (III.I.I.VII etc.) (Acetyl)Cholinesterase Activity . . . . .	90
d) (III.I.III.I) Alkaline Phosphatase Activity . . . . .	91
e) (III.I.III.II) Acid Phosphatase Activity . . . . .	92
f) (III.I.III.V) 5'-Nucleotidase Activity . . . . .	95
g) (III.I.III.IX) Glucose-6-phosphatase Activity . . . . .	96
h) (III.I.III.XI) Hexose-diphosphatase Activity . . . . .	97
i) (III.I.IV.c etc.) 3',5'-Cyclic Nucleotide Phosphodiesterase Activity . . . . .	97
j) (III.I.VI.I) (Aryl)Sulphatase Activity . . . . .	98
k) (III.II.I.XXI) $\beta$ -Glucosidase Activity . . . . .	98
l) (III.II.I.XXIII) $\beta$ -Galactosidase Activity . . . . .	98
m) (III.II.I.XXXI) $\beta$ -Glucuronidase Activity . . . . .	98
n) (III.IV.I.I etc.) Aminopeptidase Activity . . . . .	98
o) (III.IV. . .) Proinsulin Converting Enzyme Activity . . . . .	99
p) (III.VI.I.III) Adenosinetriphosphatase Activity . . . . .	99
q) (III.VI.I.VI) Nucleoside Diphosphatase Activity . . . . .	101
r) (III.IX. . .) Phosphoamidase Activity . . . . .	101
<b>4. Activities of Lyases, Isomerases and Ligases . . . . .</b>	<b>101</b>
a) (IV.I.I.XXVI) Dopa Decarboxylase Activity . . . . .	101
b) (IV.I.II.VII) Ketose-1-phosphate Aldolase Activity . . . . .	101
c) (IV.II.I.III) cis-Aconitase Activity . . . . .	101
d) (IV.II.I.XI) Phosphopyruvate Hydratase Activity . . . . .	102
e) (V.I.III. . .) Ribulose-5-phosphate Epimerase Activity . . . . .	102
f) (V.III.I.I) Triosephosphate Isomerase Activity . . . . .	102
g) (V.III.I.VI) Ribulose-5-phosphate Isomerase Activity . . . . .	102
h) (V.III.I.IX) Glucosephosphate Isomerase Activity . . . . .	102
i) (VI.IV.I.I) Pyruvate Carboxylase Activity . . . . .	102
<b>IV. Remarks on the Secretory Cycle of B-Cells . . . . .</b>	<b>103</b>
<b>A. Synthesis of B-Cell Secretory Product . . . . .</b>	<b>103</b>
Morphological maturation p. 103; Chemical maturation p. 104.	
<b>B. Insulin Release . . . . .</b>	<b>104</b>
Release of newly synthesized insulin p. 105; Release mechanism p. 105;	
Coupling of stimulus and release p. 106.	
<b>C. Storage Phase . . . . .</b>	<b>106</b>
<b>V. Concluding Remarks on Methodology . . . . .</b>	<b>107</b>
1. Sequential Staining . . . . .	107
2. Correlative Light and Electron Microscopy . . . . .	108
3. Fixation . . . . .	109
<b>References . . . . .</b>	<b>110</b>
<b>Author Index . . . . .</b>	<b>259</b>
<b>Systematic Species Index . . . . .</b>	<b>266</b>
<b>Subject Index . . . . .</b>	<b>269</b>

LUCIE ARVY

**Enzymology of the Thymus**

Introduction . . . . .	143
I. Enzymes of the Thymus . . . . .	147
A. Phosphatase Activity . . . . .	147
1. Chemically detectable activity . . . . .	147
a) Alkaline phosphatase . . . . .	147
Mouse . . . . .	147
Rat . . . . .	147
Calf . . . . .	148
α) Experimentally induced variation . . . . .	148
β) Pathological variations . . . . .	149
Leukaemic mice . . . . .	149
Mice stricken with thymome . . . . .	150
b) Acid phosphatase . . . . .	151
α) Inter-strain variation . . . . .	151
β) Experimentally induced variation . . . . .	151
Corticosteroids . . . . .	151
Trypan blue and anti-typoid vaccine . . . . .	152
X-ray irradiation . . . . .	153
Mercaptourine . . . . .	153
c) Adenosine-triphosphatase . . . . .	154
α) Experimentally induced variations . . . . .	155
Irradiation . . . . .	155
Nitrogen mustards . . . . .	156
Radio-protectors . . . . .	157
d) 5'-nucleotidase . . . . .	157
e) Other phosphatases . . . . .	158
α) Dialkyl-fluoro-phosphatase . . . . .	158
β) Phosphodiesterases I and II of RAZZELL (1961) . . . . .	158
2. Histologically detectable activity . . . . .	158
a) Alkaline phosphatases . . . . .	158
α) Pre-natal . . . . .	159
Chicken embryo . . . . .	159
Rat foetus . . . . .	159
Human foetus . . . . .	159
β) Post-natal . . . . .	159
Mouse . . . . .	159
Rat . . . . .	160
Dog and Rabbit . . . . .	160
Man . . . . .	160
γ) Experimentally induced variations . . . . .	160
Lysozyme . . . . .	160
Corticosteroids . . . . .	161
Stress . . . . .	162
Leukaemogenic virus . . . . .	162
Other factors . . . . .	162
b) Acid phosphatases . . . . .	162
α) Experimentally induced variation . . . . .	163
Irradiation . . . . .	163
Corticosteroids . . . . .	163
c) Adenosine-triphosphatase . . . . .	163
d) 5'-nucleotidase . . . . .	164

B. Proteolytic Activity . . . . .	164
1. Chemically detectable activity . . . . .	164
a) Calf . . . . .	164
b) Rat . . . . .	165
c) Mouse . . . . .	166
d) Man . . . . .	166
e) Variation in proteolytic activity . . . . .	166
Age . . . . .	166
Adrenalectomy . . . . .	167
Prednisolone . . . . .	168
Cortisol . . . . .	168
Irradiation . . . . .	168
Leukaemia . . . . .	169
Lymphosarcoma. . . . .	170
2. Histologically detectable activity . . . . .	170
C. $\beta$ -Glucuronidase . . . . .	171
Man . . . . .	171
Calf . . . . .	172
Rat . . . . .	172
$\alpha$ ) Experimentally induced variation . . . . .	172
Starvation . . . . .	172
Steroids . . . . .	172
Irradiation . . . . .	174
$\beta$ ) Pathological variation . . . . .	175
D. Amylase . . . . .	175
E. Histone-deacetylase . . . . .	176
F. Histaminase . . . . .	176
G. Histamine-decarboxylase . . . . .	176
H. Orotidine 5'-phosphate decarboxylase. . . . .	176
I. Sulphatase . . . . .	177
J. RNA- and DNA-depolymerases . . . . .	178
1. Ribonucleases . . . . .	178
a) New RN-ase . . . . .	181
b) Experimentally induced variation . . . . .	182
Irradiation . . . . .	182
Fluoroprednisolone . . . . .	183
2. Deoxyribonucleases . . . . .	184
a) Experimentally induced variation . . . . .	188
Adrenal and 11-oxisteroids . . . . .	188
Radiation . . . . .	190
Triethylenemelamine. . . . .	193
K. Glutaminase. . . . .	193
L. Deaminases . . . . .	193
M. Hyaluronidase. . . . .	193
N. Aldolase . . . . .	193
O. Acetylglucosaminidase . . . . .	194
P. Phosphoglucoisomerase . . . . .	194
Q. Nucleoside-phosphorylase . . . . .	195
R. Phosphoribosyl-transferase . . . . .	195

S. Carboxylic Esterases . . . . .	195
1. Chemically detectable activity . . . . .	195
` a) Cholinesterase . . . . .	196
b) Simple esterases . . . . .	196
c) Phospholipases . . . . .	196
2. Histologically detectable activity . . . . .	197
Rat . . . . .	197
Man . . . . .	197
T. Oxido-reductases . . . . .	198
a) Cytochromeoxidase . . . . .	200
Irradiation . . . . .	202
b) Succinoxidase . . . . .	202
c) Mono-aminoxidase . . . . .	203
U. Dehydrogenases . . . . .	203
1. Succino-dehydrogenase . . . . .	204
a) Experimentally induced variation . . . . .	205
Poisoning . . . . .	205
Irradiation . . . . .	206
Corticosteroids . . . . .	206
2. Glutathion-reductase . . . . .	206
3. Dihydrofolate-reductase . . . . .	207
4. Tetrahydrofolate-reductase . . . . .	207
5. 11- $\beta$ -hydroxysteroido-dehydrogenase . . . . .	208
6. 17- $\beta$ -hydroxysteroido-dehydrogenase . . . . .	209
7. Lactic and malic-dehydrogenases . . . . .	209
8. Glucose-6-phosphate and -6-phosphogluconate-dehydrogenases . . . . .	211
9. Other reductases . . . . .	212
V. Peroxidase . . . . .	213
W. Catalase . . . . .	213
X. Synthesizing Enzymes . . . . .	213
1. Transaminases . . . . .	214
a) Man . . . . .	215
b) Rat: Experimentally induced variation . . . . .	215
Hydrocortisone . . . . .	215
Cortisol . . . . .	215
Cortisone and progesterone . . . . .	216
Adrenocorticotropic hormone . . . . .	216
Adrenalectomy . . . . .	216
Castration . . . . .	216
Irradiation . . . . .	217
Ultrasonic vibrations . . . . .	217
c) Calf . . . . .	217
d) Mouse . . . . .	218
Leukaemia . . . . .	218
2. Aspartate-carbamoyltransferase . . . . .	219
3. Methylases . . . . .	219
a) Protein-methylases . . . . .	220
b) RNA-methylases . . . . .	220
Hamster . . . . .	220
Mouse . . . . .	221
Rat and other mammals . . . . .	221
4. Kinases . . . . .	221
a) Thymidine-kinase . . . . .	222
Changes occurring in the course of infections . . . . .	223

b) Deoxycytidine-kinase . . . . .	224
c) Phosphofructo-kinase and hexokinase . . . . .	224
d) Synthesis of ATP . . . . .	225
5. Thymidylate-synthetase . . . . .	225
6. DNA- and RNA-polymerases . . . . .	226
a) DNA-polymerase . . . . .	226
$\alpha$ ) Experimentally induced variation . . . . .	229
$\beta$ ) Pathological variation . . . . .	230
b) RNA-polymerase . . . . .	230
$\alpha$ ) Experimentally induced variation . . . . .	231
Corticosteroids . . . . .	231
Antibiotics . . . . .	233
II. Coenzymes of the Thymus . . . . .	234
a) Coenzyme I (dinucleotide of nicotinamide-adenine = NAD) . . . . .	234
Irradiation . . . . .	235
Trenimon . . . . .	236
b) Coenzyme A . . . . .	236
III. Inhibitors of the Enzymes of the Thymus . . . . .	236
a) Histones . . . . .	237
b) Inhibitors of deoxyribonuclease . . . . .	237
c) Inhibitor of oxytocinase . . . . .	237
Conclusions . . . . .	237
References . . . . .	239
Author Index · Histochemistry of the Islets of Langerhans . . . . .	259
Systematic Species Index · Histochemistry of the Islets of Langerhans . . . . .	266
Subject Index · Histochemistry of the Islets of Langerhans . . . . .	269
Author Index · Enzymology of the Thymus . . . . .	284
Subject Index · Enzymology of the Thymus . . . . .	288