

VOLUME VIII
SUPPLEMENTS

PART 4

HISTOCHEMISTRY OF THE
ADENOHYPHYSIS

C. GIROD

Lyon

With 37 figures and 41 tables



GUSTAV FISCHER VERLAG · STUTTGART

1976

Contents

Foreword	1
Analytical study	4
Introduction: Discussion of Terminology	4
Some viewpoints	4
Conclusions	5
I. Histochemical Methods	7
A. Methods of Study	7
1. General	7
a) The problem of terminology	7
b) Classification of methods of histochemical analysis	8
2. Procedures	9
a) The PAS and the PAS-orange reaction	9
α) PEARSE's PAS-orange reaction and PURVES and GRIESBACH's variation	9
β) HERLANT's PAS-orange reaction and ELFTMAN's variation	11
γ) WILSON and EZRIN's PAS-orange-methyl blue method	12
δ) LANDING and HALL's diazo blue B-fast green-PAS method	12
ε) DUBOIS and HERLANT's methazol blue-PAS-orange G method	13
ζ) EZRIN's colloidal iron-PAS method	15
b) Alcian blue staining and the Alcian blue-PAS reaction	16
α) HERLANT's Alcian blue-PAS-orange G stain	16
β) Some variations	18
γ) ADAMS and SWETTENHAM's and HEATH's performic acid-Alcian blue-PAS-orange G reaction	20
δ) KLESSEN's lead tetraacetate-sodium bisulphite-Alcian blue-PAS technique	21
c) The paraldehyde fuchsin stain	21
α) HALMI and DAVIES's paraldehyde fuchsin stain	22
β) GABE's paraldehyde fuchsin stain	23
γ) RUSSEFIELD's paraldehyde fuchsin stain	27
δ) ORTMAN's paraldehyde fuchsin stain	27
ε) ELFTMAN's paraldehyde fuchsin-PAS-orange reaction	27
ζ) SWOPE's Alcian blue-paraldehyde fuchsin stain	28
d) The paraldehyde thionine stain	28
α) PAGET and ECCLESTON's paraldehyde thionine-Luxol fast blue-PAS stain	28
β) EZRIN and MURRAY's paraldehyde thionine-PAS-orange G stain	30
e) Other methods	30
α) MCCONAILL's lead haematoxylin stain	30
β) Other methods sometimes used	32
3. Conditions of use	32
a) Fixation	32
α) Variability of fixatives used	33
β) Influence of different fixatives on histochemical results	34

γ) Influence of the manner of fixation	37
δ) Time of fixation	39
ε) Completion of fixation	39
b) Stains and reagents	40
α) Variability of preparations.	40
β) Variability of procedure.	43
B. Results	46
1. General	46
a) Variability of material studied	46
b) Variability of methods of study.	47
2. Results obtained from each group of histochemical methods	47
a) The PAS and PAS-orange reactions and their variations	59
α) Description	59
β) Comments.	59
(1) Validity and significance of the PAS reaction for the cytological study of the anterior pituitary	59
(2) The PAS reaction (or PAS-orange and its variations) and classification of anterior pituitary cells	62
b) Staining with paraldehyde fuchsin	64
α) Description	64
β) Comments.	64
(1) Validity and significance of the paraldehyde fuchsin staining for the cytological study of the anterior pituitary	64
(2) Paraldehyde fuchsin stain (alone or combined with other techniques) and classification of anterior pituitary cells	66
c) Alcian blue staining the Alcian blue-PAS reaction, and their variations	77
α) Description	77
β) Comments.	77
(1) Validity and significance of Alcian blue staining for the cytological study of the anterior pituitary	77
(2) Alcian blue staining (and its variations) in the classification of anterior pituitary cells	87
d) Paraldehyde thionine staining	88
α) Description	88
β) Comments.	88
(1) Validity and significance of staining with paraldehyde thionine for the cytological study of the anterior pituitary	88
(2) Paraldehyde-thionine staining and the classification of anterior pituitary cells	92
e) Lead haematoxylin staining	92
α) Description	93
β) Comments.	93
f) Other methods	95
(1) Presence of biogenic amines in anterior pituitary parenchyma cells	95
(2) Identification of PEARSE'S «APUD-cells» in the anterior pituitary	96
C. Functional significance	99
1. General	106
a) The great heterogeneity of the nomenclature of cell types	106
b) Topographical localisation of cell types	106

c) Cellular renewal and differentiation	108
d) The functional state of anterior pituitary cells	110
2. The functional characterisation of cell types	111
a) Somatotropic cells	112
α) General characteristics	112
β) Histochemical characteristics	112
γ) Evidence of function	114
b) Prolactin cells	119
α) General characteristics	119
β) Histochemical characteristics	119
γ) Evidence of function	121
c) Corticotropic cells.	127
d) Gonadotropic cells	127
α) General characteristics	127
β) Histochemical characteristics	128
γ) Evidence of function	132
e) Thyrotropic cells	138
α) General characteristics	138
β) Histochemical characteristics	139
γ) Evidence of function	144
II. The Contribution of Cytochemical Methods	151
A. Dissociation and Cellular Isolation	151
1. General	151
2. Methods of study	152
a) Cellular dissociation.	152
b) Separation by sedimentation	152
c) Clonal cultures	153
3. Results	153
a) Cellular dissociation	153
b) Separation of cell types	154
c) Clonal cultures	155
B. Cell Fractionation	155
1. General	155
2. Methods	156
a) Differential centrifugation	156
b) «Sub-fractionation» procedures and the isolation of granules	156
3. Results	157
a) Fractionation of anterior pituitary cells	157
α) Description	157
β) Discussion	157
b) Isolation of granules	158
α) Morphology of different fractions	158
β) Biological activity of different fractions	161
4. Discussion	162
C. Autoradiographic Analysis	164
1. Sex steroids and anterior pituitary cells	165
a) Chemical and physiological principles	165
α) Uptake and fixation of ^3H -oestradiol	165
β) Fixation of other sex steroids	166
b) Autoradiographical studies	167

2. Protein synthesis in anterior pituitary cells	168
a) General principles	168
α) General principles of the method	168
β) Stages of intracellular protein synthesis	169
b) Application to the study of the anterior pituitary	169
α) Hormonal biosynthesis	169
β) The metabolism of nucleic acids of the nucleus	170
 III. The Contribution of Immunohistochemical Methods	 172
A. Principle of Immunohistochemistry	172
1. Immunofluorescence	173
a) Description of fluorochromes	173
α) Fluorescein isocyanate	173
β) Fluorescein isothiocyanate	174
γ) Other fluorochromes	174
b) Ways in which fluorochrome is taken up onto protein molecules	176
c) Conditions for use of fluorochromes	176
α) Criteria for use	176
β) Non-specific fluorescence, and counter-stains	179
d) Principles of the immunofluorescence reaction	179
α) The direct reaction	179
β) The indirect reaction	179
γ) The method of inhibition	181
2. Other procedures	181
a) Peroxidase labelling	181
b) The method with unlabelled antibodies shown up by a peroxidase- anti-peroxidase complex	182
B. Application of Immunohistochemical Methods to the Study of Anterior Pituitary Cytology	183
1. Methods of study	183
a) Preparation of antibodies	183
α) Immunisation of animals	183
β) Immunological study of antibodies	184
b) Carrying out the immunofluorescence reaction	186
α) Fixing the pituitary	186
β) Preparation of sections	186
γ) The immunofluorescence reaction proper	186
δ) Histochemical control	187
ϵ) Immunofluorescence reaction with semi-thin sections	188
c) Other immunohistochemical reactions	188
α) Labelling with peroxidase for light microscopy	188
β) Peroxidase labelling for electron microscopy	190
2. Results	191
a) The influence of fixation and embedding	191
b) Specificity of immunohistological reactions	192
c) Identification of anterior pituitary cell categories	192
α) Somatotropic cells	192
β) Prolactin cells	199
γ) Corticotropic, melanotropic and lipotropic cells	205
δ) Gonadotropic cells	224
ϵ) Thyrotropic cells	232
Conclusions	235
References	236
Addendum	296
Author Index	304
Subject Index	316