Recent Advances in HISTOPATHOLOGY

EDITED BY

P. P. ANTHONY

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Preface

In contrast with the last edition of *Recent Advances in Pathology* only a comparatively brief interval separates this issue from its immediate predecessor. Some of the topics covered in that volume appear again in this one, notably reviews of certain aspects of hepatic, renal and lymph node pathology. Since the disorders affecting these tissues constitute such an important part of pathological practice the editors feel that no apology need be made for what might seem slavish treading of well-worn paths.

Much of the book consists of material which should be of direct use to the clinical histopathologist. However, precise histological evaluation as distinct from 'pattern recognition' demands understanding of the cell and tissue processes involved. For this reason we have included some chapters which relate to basic pathological mechanisms rather than to morphology in the hope that our colleagues will find them interesting and useful despite their apparent lack of relevance to the art of histological diagnosis.

1978

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Contents

1. The APUD cell system

R. P. Gould

The story of the APUD cell system can be thought to have started when Nonidez (1932) showed that thyroid parafollicular cells (C cells) contained argyrophil granules and suggested they were the antecedent of an endocrine secretion poured directly into blood vessels. Later, Copp et al (1962) showed the existence of a calcium lowering hormone (eventually called calcitonin) but thought it originated in the parathyroid glands. Subsequently Foster et al (1964a) demonstrated it originated from the thyroid and Foster et al (1964b) and Bussolati and Pearse (1967) showed unequivocally by immunofluorescent localisation that the cells of origin of calcitonin were Nonidez's 'parafollicular' thyroid cells or C cells.

Pearse (1966a,b, 1968, 1969) thoroughly investigated the cytochemistry of the C cell and found it had a number of cytochemical and ultrastructural characteristics in common with other, apparently unrelated, polypeptide hormone-producing cells. This suggested to him that all the cells in the Vertebrata which possess these characteristics formed a distinct class of cells which he called the APUD system (Pearse, 1968).

THE SCIENTIFIC BASIS OF THE APUD CONCEPT

Over the years more cells have been added to the original system and a fairly complete list can be found in Table 1.1. Clearly there is considerable overlap between the APUD cell series and the clear cells (Helle Zellen) of the diffuse endocrine (paracrine) system described by Feyrter (1969, 1972) which, as they are mainly gut located, he considers to be endodermal in origin. There is also an apparent identity between the APUD concept and the unifying concept proposed by Weichert (1970) in his review of the multiple endocrine adenomatoses. But as Pearse and Polak (1974) tartly comment, 'any identity of opinions is confined to the title. Weichert's concept is based not on experiment or observation but on a series of misconceptions derived from the literature.' For a discussion of Weichert's views the reader is referred to Pearse and Polak's (1974) article.

In Pearse's original formulation of the APUD concept (1968) three sets of characteristics were used for categorising an APUD cell, namely: morphological, cytochemical, and embryological. Some comment on the current status of these characteristics would seem appropriate at this stage in the development of the concept.

The morphological characteristics

These are:

1. Low levels of rough endoplasmic reticulum.
2. High levels of smooth endoplasmic reticulum.
3. High content of free ribosomes.
4. Electron-dense, fixation-labile mitochondria.

Table 1.1 APUD cell system

Origin	Cells	Known polypeptide	Possible polypeptide	Other product	Apudoma	Syndrome
Anterior pituitary	Corticotroph*	ACTH	—	(T)	Basophil chromophobe or acidophil adenoma or carcinoma	Cushing's syndrome
	Melanotroph*	MSH	—	(T)		Pigmentation
	Somatroph	ST	—	—		Acromegaly
	Mammotroph	PL	—	—		
Thyroid and ultimobranchial body	C cell*	Calcitonin	—	(5-HT)	Medullary carcinoma	—
Islets of Langerhans	B*	Insulin	—	DA or 5-HT	Islet cell adenoma or carcinoma	Hypoglycaemia
	A	Glucagon	—	DA or 5-HT		Diabetes, etc
	D	Somatostatin	—	—		Zollinger–Ellison (Z-E) syndrome
Stomach	G	Gastrin	—	DA or 5-HT	Carcinoma	Z-E syndrome
	A	Enteroglucagon	—	—	?	?
	D	Somatostatin	—	—	—	—
	EC	—	—	5-HT	—	—
	EC-like	—	—	—	—	—
Duodenum and small intestine	S*	Secretin	—	—	?	?
	D	Gastrin inhibitory polypeptide (GIP)	—	—	?	?
	EG (L)	Enteroglucagon	—	—	?	?
	EC	—	Motilin	5-HT	—	—
	H	Vasoactive intestinal (VIP) peptide	—	—	—	Verner–Morrison syndrome
	D	Somatostatin	—	—	—	—
GI tract, lung teratomas	'Kultschitsky' cell	ACTH, MSH, calcitonin, insulin	—	5HT, 5HTP, histamine, kinins, prostaglandin	Carcinoid	Carcinoid syndrome
Carotid body	Type I cell	—	—	NA or DA	Chemodectoma	—
Skin	Melanoblast	—	—	—	Melanoblastoma	—
Adrenal medulla	A*	—	—	Adrenaline	Phaeochromocytoma	
	NA*	—	—	Noradrenaline		
Lung	F (Feyrter)	—	Vasoactive lung peptide (VLP)	— Biogenic amine	—	
Urogenital tract	U	—	Urogastrone	5-HT	—	—

PL = prolactin; T = tryptophan; 5-HT = 5-hydroxytryptamine; DA = dopamine; NA = noradrenaline
* Pearse's original list of APUD cells (he also included mast cells)

5. Prominent microtubules and centrosomes.
6. Tendency to produce fine protein microfilaments (especially when neoplastic).
7. Membrane-bound secretory vesicles with osmiophilic contents, best preserved in glutaraldehyde, varying in density and with an average size of 100 to 200 nm.

Since their original formulation (Pearse 1968, 1969) it has become apparent that these ultrastructural characteristics are not really very specific since most protein-secreting cells exhibit these features to a greater or lesser degree. For example, the amount of rough endoplasmic reticulum present in a protein-secreting cell will depend on whether it is actively synthesising and secreting its protein product or storing it and Pearse (1971) accounts for its low level in APUD cells by suggesting that their usual state is in the storage phase. However, a survey of published electronmicrographs of APUD cells, e.g. mammotrophs, somatotrophs, argentaffin cells and C cells, etc. (Farquhar, 1971; Gould and Hodges, 1971; Solcia et al, 1975) shows that there is a wide variation in their content of rough endoplasmic reticulum which is dependent on the stage in the secretory cycle the cell is in and the species studied.

These comments also apply to the second feature of high levels of smooth endoplasmic reticulum, usually present in APUD cells in the form of vesicles. In the literature the term smooth endoplasmic reticulum has become restricted to describing the smooth membrane profiles that are associated, for example, with steroidogenic cells (Christensen and Gillin, 1969) or are found associated with glycogen deposition in hepatocytes (Cardell, 1976). However, if the term is used so as to include the membranes surrounding storage granules, then exocrine cells such as the pancreatic acinar cells which are full of such granules, would also have to be considered as having high levels of smooth endoplasmic reticulum, a somewhat misleading view.

As to the mitochondrial characteristics of 'electron density' and 'fixation lability', these features are so variable among all cell types, depending upon the fixative used, its pH, osmolality, temperature and method of application (by perfusion or immersion) that very little reliance can be placed on them as being particularly distinctive features of APUD cells.

A similar criticism can be made with respect to the APUD characteristics of prominent microtubules and centrisomes and 'numerous microfilaments'. The variations of these structures in all cell types is so great, depending very largely on the fixative employed, as to make it difficult to regard them as clear-cut features of APUD cells.

Characteristic 7, the presence of numerous membrane-bound secretory vesicles, although not unique to them, is an undoubted characteristic of APUD cells. However, their variation in shape (Farquhar, 1971; Solcia et al, 1975) is greater than that suggested by Pearse (1971) (Figs. 1.1, 1.2).

In summary, it can be said that the ultrastructural characteristics of APUD cells are fairly non-specific and, not surprisingly, merely indicate that the cells in this system have the general features exhibited by most peptide secreting cells.

The cytochemical characteristics

On the other hand, the cytochemical characteristics of APUD cells are the principal criteria used for including a cell in the series and, as Pearse and Welbourn (1973) state, the first three of these characteristics are of 'paramount importance, significance and constancy'.

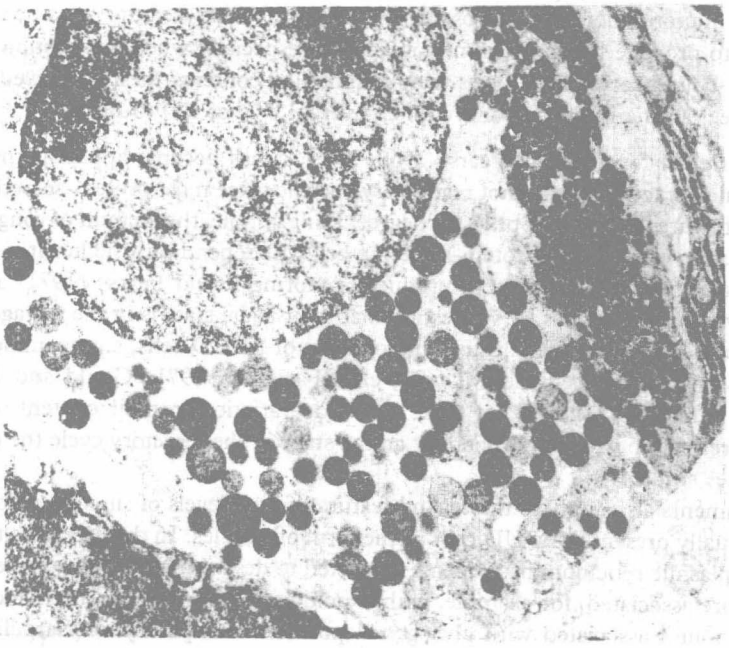

Fig. 1.1 Electron micrograph of human pyloric gland showing a thin extension of an EC cell with small elongated, strongly osmiophilic granules, as well as a D cell with large, round weakly osmiophilic granules, × 10 000 (by permission of Dr G. Vassallo)

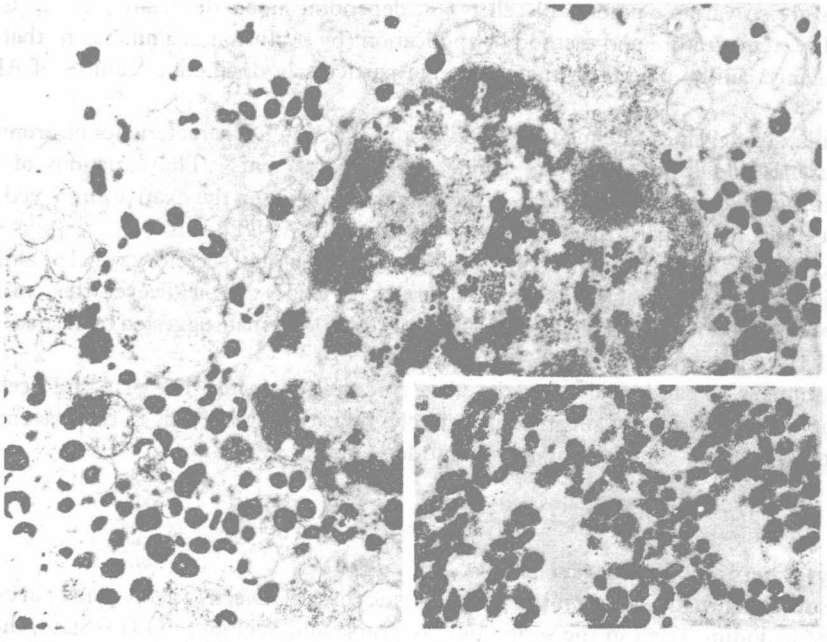

Fig. 1.2 Ultrastructure of EC granules in a carcinoid tumour of the ileum. Inset: granules of the same tumour stained by the argentaffin reaction, × 11 000 (from Solcia et al, 1975)

APUD cells are so named because they

1. (A) Contain a fluorogenic amine (catecholamine, 5-HT, etc.) or can secondarily take it up.
2. (PU) Can take up amine precursors (5-HTP, DOPA).
3. (D) Contain an amino acid decarboxylase.

The next three characteristics show greater variability and less constancy between species and are therefore of less importance.

4. Possess sufficient concentration of side chain carboxyl groups to enable their demonstration as masked metachromasia.
5. Contain non-specific esterases and/or cholinesterases.
6. Contain α-glycerophosphate dehydrogenase.

The final characteristic is not considered by Pearse and Welbourn (1973) to be a true characteristic but is an essential stage in identifying an APUD cell, namely demonstrating its polypeptide hormone product by,

7. Specific immunofluorescence.

As Pearse (1969, 1971) has discussed in detail the importance of each of the above characteristics only a brief review of them will be attempted here.

The amine-handling capacity of APUD cells (characteristics 1–3) is their most important cytochemical characteristic. They either contain a biogenic amine or have the capacity to take it up. Further, they can take up an amino acid precursor (e.g. 5-hydroxytryptophan, 5-HTP; or dihydroxyphenylalanine, DOPA) and decarboxylate it to the respective amine (Fig. 1.3).

To demonstrate the amines they are first immobilised in the tissue by immersing it in liquid nitrogen and then freeze-drying it. The amines are subsequently converted to a

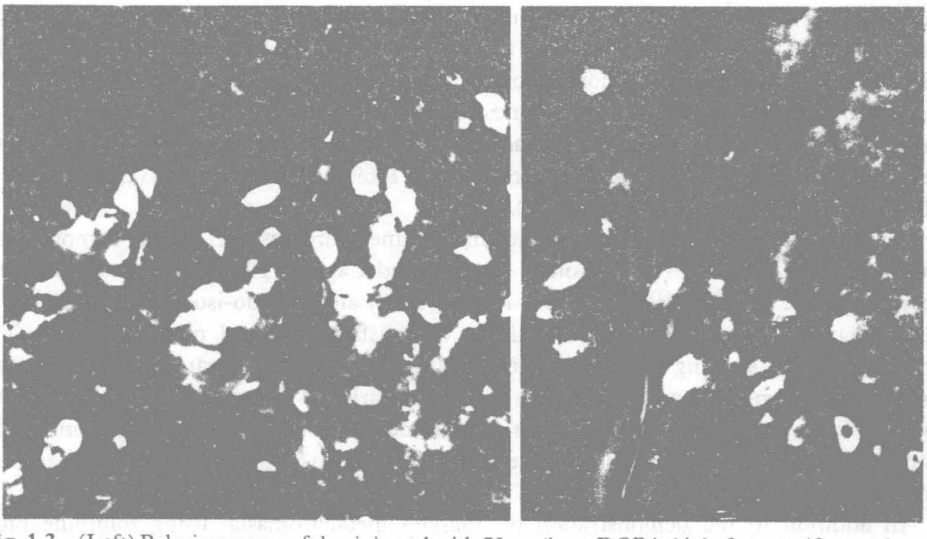

Fig. 1.3 (Left) Pyloric mucosa of dog injected with 50 mg/kg L-DOPA 4 h before sacrifice, and viewed under ultraviolet light. (Right) Gastrin immunofluorescent cells in crypts of human duodenal mucosa, × 280 (from Solcia et al, 1975)

fluorescent compound and localised using an ultraviolet microscope. The method, that of Falk et al (1962), is based on the capacity of formaldehyde gas to convert certain biogenic monoamines such as catecholamines and 5-hydroxytryptamine into highly fluorescent compounds. The reaction is favoured by the presence of proteins. Initially formaldehyde reacts with the arylethylamine (phenylethylamine or indolethylamine) to form tetrahydroisoquinoline or tetrahydro-β-carboline derivatives respectively. In the second step, these weakly fluorescent compounds are dehydrogenated to the intensely fluorescent dihydro derivatives. The method is extremely sensitive and as little as 5×10^{-6} pmol of noradrenaline can be detected within one nerve terminal (Fuxe and Jonsson, 1973). The fluorophores formed from DOPA and the catecholamines emit light with a maximum about 480 nm whereas the indolethylamines, such as 5-hydroxtryptophan and 5-HT, emit light with a maximum of 535 nm. The demonstration of histamine by fluorescence microscopy involves its condensation with o-pthaldehyde (OPT) (Håkanson and Owman, 1967); however, the nature of the fluorophore formed is unknown and the specificity of the OPT method is low (Larsson, Sundler and Håkanson, 1975).

Some care must be taken in interpreting the results of these formalin-induced fluorescence (FIF) methods. For example, non-specific uptake of either amine (dopamine and 5-HT) occurs in lysosomes of cells not in the APUD series; and extra- or intracellular decarboxylation of high levels of injected amine precursors may provide an excess of circulating amine which is then taken up non-specifically by non-APUD cells, thus giving a false positive fluorescence.

An alternative procedure to the above fluorescence methods is to identify the labelled amine or its precursor autoradiographically (Ericson, 1970; Nunez and Gershon, 1972). This has an additional advantage, if carried out for electron microscope study, in that it enables a fairly precise intracellular location to be ascribed to the amine. Results from these studies suggest that the newly synthesised peptide is packaged together with the amine in the secretory granules and would appear to be released together when granule exocytosis occurs. One possible reason for this close relationship between amine and polypeptide hormone is that the amine may increase local vascular permeability and so allow easier access of the hormone to the bloodstream but at the moment the functional meaning of the so-called APUD mechanism remains unclear. It provides a convenient means for the fluorescence microscope detection of polypeptide hormone cells that do not contain histochemically demonstrable arylethyl amines. It is now clear that the APUD mechanism is also shared by other non-hormone, actively protein-synthesising cells, e.g. pancreatic exocrine cells (Alm, 1969; Alm, Ehinger and Falk, 1969), gastric chief cells (Håkanson et al, 1970) and Paneth cells (Ahonen, 1973).

The fourth cytochemical characteristic, 'masked metachromasia', is usually demonstrated with toluidine blue at pH 5·0 (Solcia, Vassallo and Capella, 1968). The fluorescent dye coriphosphine O (Bussolati, Rost and Pearse, 1969) and pseudo-isocyanin (Solcia et al, 1968) have also been used successfully to demonstrate 'masked metachromasia'. The rationale of this staining method has been discussed in detail by Pearse (1969, 1971) and appears to depend primarily on enough side-chain carboxyl groups (glutamic and aspartic acid residues) or carboxyamido groups (glutamine and asparagine) being present in the storage form of the hormone to be converted by hot acid to carboxyl groups which give the staining reaction.

In addition to the demonstration of 'masked metachromasia' using toluidine blue, several other very useful techniques have been employed at both the light and electron

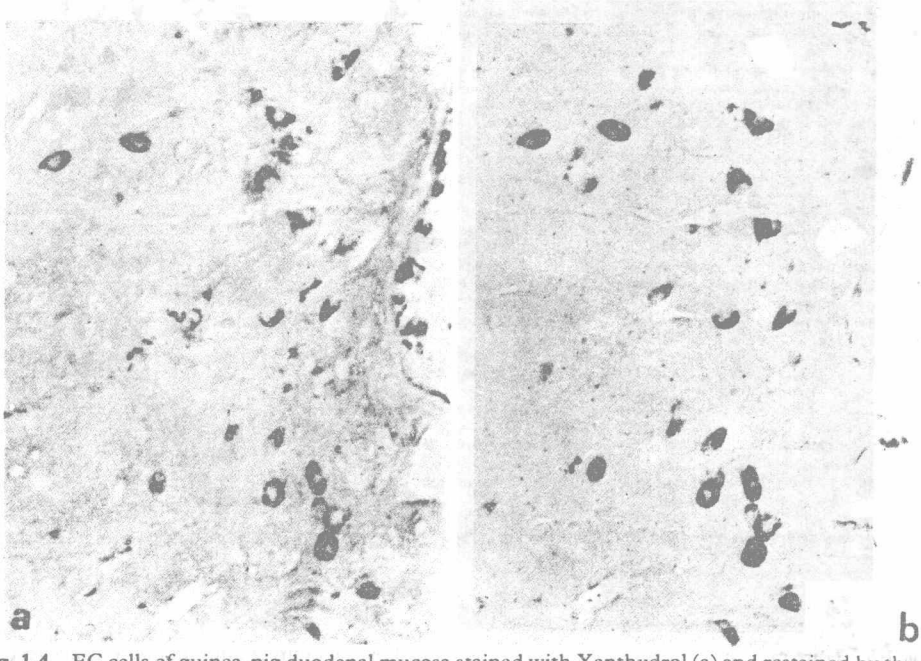

Fig. 1.4 EC cells of guinea-pig duodenal mucosa stained with Xanthydrol (a) and restained by the diazonium reaction (b), × 250 (from Solcia et al, 1975)

microscope levels to demonstrate endocrine cells of both the APUD system and Apudomas (Figs. 1.2, 1.4).

They are listed as follows:

a. Lead haematoxylin method (Solcia, Capella and Vassallo, 1969).
b. Standard argyrophil method (Grimelius, 1968; Vassallo, Capella and Solcia, 1971).
c. Diazo coupling method (Pearse, 1972).
d. Masson Fontana method (Pearse, 1972).

The high levels of non-specific esterase and/or cholinesterase are regarded by Pearse and Welbourn (1973) as being relatively non-specific features of these cells because there is both considerable species variation and a high activity is often found in other non-APUD cells (Hoyt, Hamilton and Tashjian, 1973).

The final cytochemical characteristic of APUD cells is the demonstration in them, by specific immunofluorescence, of the peptide hormone they synthesise and secrete (Figs. 1.3, 1.5). This has been achieved with respect to many of the APUD cells (see Table 1.1, column 3). Strictly speaking until this feature of a putative APUD cell has been clearly demonstrated it is not a full member of the club, it is only on probation. However, until its peptide hormone product has been fully established it remains a stimulus to further investigation as to its function.

The APUD hypothesis

To explain the ultrastructural and cytochemical similarities of the widely varying cell types that make up the APUD series, Pearse (1969) advanced three theories:

1. Although of diverse origin the cells have evolved similar biochemical mechanisms for the production of similar products (polypeptides);

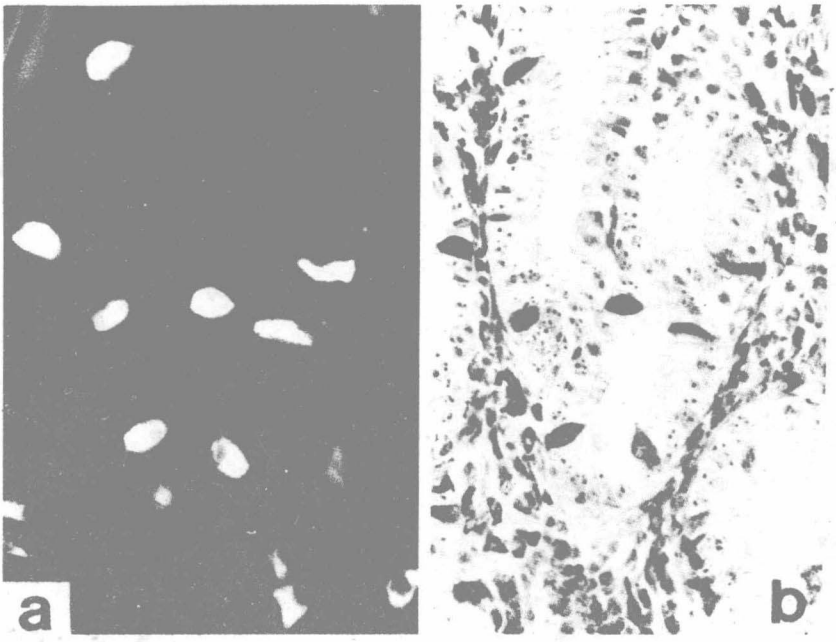

Fig. 1.5 Human pyloric mucosa showing G cells stained by the indirect immunofluorescence test using antibodies against synthetic gastrin I (a), and restained by Grimelius' silver technique (b), × 400 (from Solcia et al, 1975)

2. Although of diverse origin, the cells have evolved a similar set of biochemical mechanisms in response to similar specific secretory stimuli (aminergic or cholinergic);
3. The cells differentiate from a common embryological precursor cell.

Of these three possibilities, the first two are strictly evolutionary (i.e. historical) postulates and in principle would be difficult to test scientifically. However the third hypothesis is eminently suitable for scientific investigation and in his earliest formulation of the hypothesis, Pearse (1966) proposed the neuroendocrine cell of the neural crest as the only possible candidate for the embryological precursor. As new data accumulated, Pearse and Takor Takor (1976) have modified the original suggestion that the neural crest was the sole origin of the APUD cells to the broader concept of neuroectoderm, which includes the neural crest, neural ridges, neural tube and the specialised ectoderm of the placode. In his most recent formulation of the hypothesis, Pearse (1976) states that 'all peptide-hormone-producing cells' are derivatives of 'specialised ectoderm' and therefore, effectively, of cell lines derived from the epiblast and programmed for ultimate neuroendocrine function.

The rest of this section will be devoted to an examination of the embryological and histological evidence for this concept with respect to the major APUD cell types.

Adrenal medullary chromaffin tissue and melanoblasts

The evidence for the neural crest origin of adrenal chromaffin tissue is substantial and derives from the study of its development by classical embryological methods (Hamilton, Boyd and Mossman, 1972), by the labelling experiments of Weston (1970) using ^{3}H

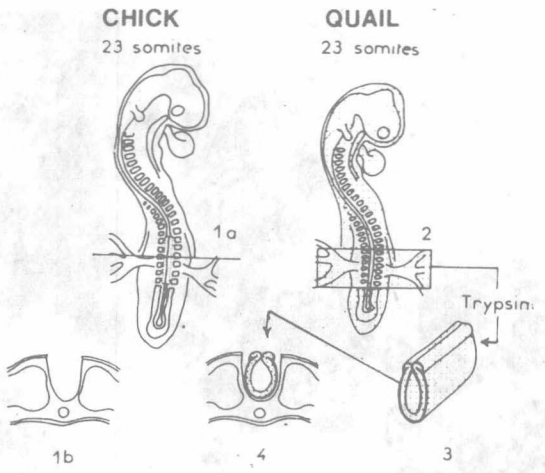

Fig. 1.6 Experimental procedure of isotopic and isochronic graft of a fragment of a quail neural primordium into a chick embryo. (1a) Dorsal view of the chick embryo showing the level of the surgical removal of the folds. (1b) Transverse section at the level of the excision. (2) Quail embryo at the same developmental stage in which the corresponding transverse area is taken. (3) Quail neural tube isolated by trypsinisation. (4) Graft of the quail neural primordium into the chick host (from Le Douarin, 1974)

thymidine, by quail allografts of neural crest (Le Douarin and Teillet, 1971) and FIF cytochemical amine studies (Polak, Rost and Pearse, 1971).

As yet no polypeptide hormone has been isolated from chromaffin tissue, although recently Pearse and Takor Takor (1976) have shown, using an indirect immunofluorescence technique, a vasoactive intestinal peptide-like activity in the adrenalin and noradrenalin cells of the mouse.

The neural crest origin of melanoblasts, long believed, has received strong proof from the quail/chick chimaera experiments of Teillet and Le Douarin (1970) (see below).

Thyroid and ultimobranchial body C cells and carotid body Type I cells

The most powerful evidence for a neural crest origin for the C cell and the carotid body Type I cell comes from the allograft experiments of Le Douarin (Le Douarin and Le Lievre, 1971; Le Douarin, Le Lievre and Fontaine, 1972), in which isotopic, isochronic grafts of quail neural anlage are inserted into the appropriate level of the host chick embryo neural axis (Figs. 1.6, 1.8, 1.9). The subsequent fate of the quail graft cells can be determined because of the clear-cut difference in the structure of their interphase nuclei compared with their chick counterparts. In the chick, the interphase chromatin is evenly dispersed in the nucleoplasm; while in the quail a large amount of DNA is condensed into one or more strongly Feulgen positive heterochromatic masses (Le Douarin, 1974) (Fig. 1.7). The species used are related intimately enough to allow a perfect integration of the tissues grafted from one to the other in the resulting chick/quail chimaera.

Thus, in these experiments quail neural crest cells grafted into the chick were eventually found in the chick ultimobranchial body as C cells and as Type I cells in the carotid body. The cytochemical marker studies of Pearse and Polak (1971a) and Pearse et al (1973b), in which FIF amine positive cells were traced from a neural origin ventrally to their final organ destination, provided confirmatory evidence for the aforementioned biological

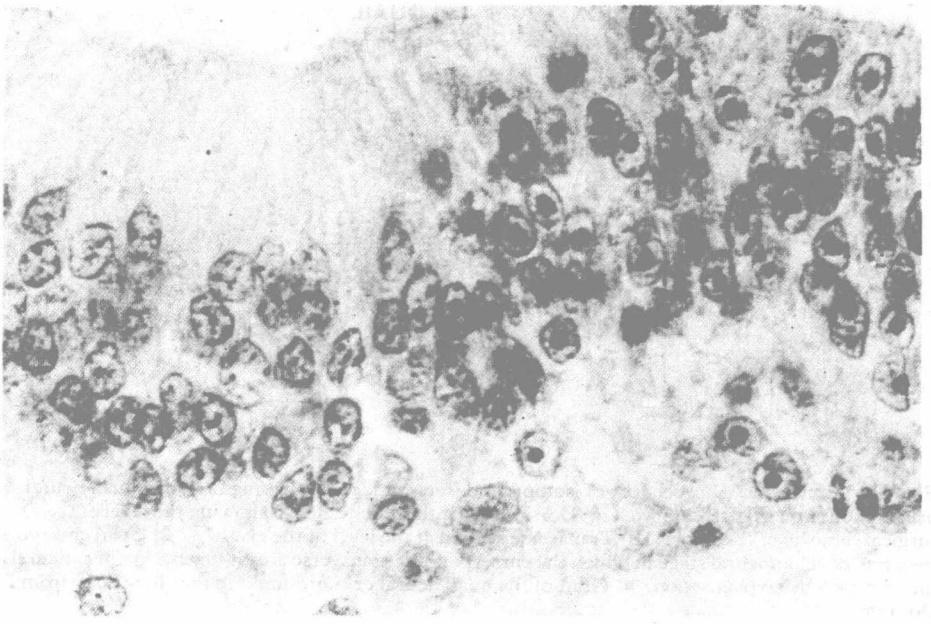

Fig. 1.7 In vivo association of embryonic mesencephalic neuroblasts of quail (on the right), and chick (on the left) at seven days of incubation after the isotopic and isochronic graft of the right mesencephalic quail neural primordium in a 10-somite chick embryo host: Feulgen–Rossenbeck staining, × 400 (from Le Douarin, 1974)

marker experiments. So far, the avian ultimobranchial gland is the only example in which 'total proof' (Pearse and Takor Takor, 1976) has been provided for the production of a peripheral polypeptide hormone, calcitonin, by cells of undoubted neural crest origin (Polak et al, 1974b); although there is little reason to doubt that the calcitonin-secreting C cells, also embryologically ultimobranchial in origin, in the mammalian thyroid, are also of neural crest origin.

Pancreatic and gastrointestinal APUD cells

The embryological evidence supporting a neural crest origin for pancreatic islet endocrine cells and the enterochromaffin/argentaffin/argyrophil cells of the gastrointestinal tract is much less clear cut. Hitherto, most studies on the development of the endocrine pancreas (see Pictet and Rutter, 1972, for a review of the literature), have agreed that the islets of Langerhans are derived from duct epithelium, i.e. are endodermal in origin, as is generally believed of the rest of the gastrointestinal epithelial lining. But Pearse and Polak (1971b) and Pearse, Polak and Heath (1973a), using FIF amine labelling, studied the development of the pancreas and concluded that there was strong evidence to make tenable a neural crest origin for all islet endocrine cells.

Against this conclusion a considerable body of histological and embryological evidence has accrued during recent years. If the exocrine and endocrine cells of the pancreas have different embryological origins, it is perhaps surprising that mixed endocrine/exocrine cells have been found so frequently and in widely differing species (Orci et al, 1970; Pictet and Rutter, 1972; Melmed, Benitez and Holt, 1972). However, such intermediate or mixed cells could be produced in a number of different ways which would not exclude their having different ontogenetic origins. For example, as they are usually found at the