Some aspects of neuroendocrine pathology

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SUMMARY The advent of modern microscopical investigative methods for the determination of neuroendocrine differentiation has increasingly given credence to the original concept of a “diffuse endocrine system”. These methods include a variety of silver impregnation techniques, technologically advanced light and electron microscopical immunocytochemistry, and, lately, the ability to localise specific binding sites by in vitro autoradiography and mRNA species by in situ hybridisation. Further insight has been gained into the possible role of regulatory peptides contained in the so called “diffuse endocrine system” and into the nature of disease processes by investigating the role of the system in benign and malignant disease.

The idea that control of bodily functions was regulated almost exclusively by “endocrine” glands and “classical” nerves began to be challenged at the turn of this century, when Bayliss and Starling discovered a “hormonal” principle originating from a supposedly non-endocrine organ, the gastrointestinal tract. It was then shown that this “hormonal” principle was, indeed, capable of mimicking effects which had been attributed until then to the organ’s innervation (Pavlovian concept of nervism). In spite of the revolutionary start in the 1900’s and of the early recognition by Feyrer of the existence of a set of specialised “clear” or “endocrine” cells, diffusely distributed throughout the body (“diffuse endocrine system”), progress in the recognition of this other facet of neuroendocrinology was hampered by the lack of appropriate tools for its investigation.

The 1960’s and 70’s were, however, exciting times, as methods were found for the extraction and purification of peptides present in tissues in exceedingly low concentrations, and advanced detection systems were applied. Immunocytochemistry was used for the accurate localisation of active principles in endocrine cells or nerves, or both; radioimmunoassay for the accurate measurement of concentrations of material extractable from tissues; and chromatography for its chemical characterisation.

The concept of the existence of a “diffuse endocrine, or neuroendocrine” or “amine precursor uptake and decarboxylation system” (APUD) is now well established and the production of active peptides by the components (endocrine cells or nerves) of this system well recognised.

Morphological methods for the investigation of the diffuse neuroendocrine system

SPECIAL HISTOLOGICAL METHODS AND IMMUNOCYTOCHEMISTRY

Numerous special histological methods have been shown to be helpful for showing the presence of endocrine cells and nerves, the preferred methods being those using silver impregnation—in particular, the Grimelius method, which is capable of showing most peptide producing endocrine cells. Immunocytochemistry, however, has now been established as the method of choice for the study of this novel endocrinology.

General neuroendocrine markers

Several antibodies to specific cytoplasmic components have now been proposed as capable of staining endocrine cells, nerves, or both components of the “diffuse neuroendocrine system” simultaneously. Neuron specific enolase (NSE) is an isozyme of the glycolytic enzyme enolase, originally extracted from the brain. Antibodies to neuron specific enolase immunostain all components of the “diffuse neuroendocrine system”. The intensity of immunostaining for neuron specific enolase is unrelated to the presence of secretory granules. The pattern of staining is quite unique as it stains both endocrine cells and their innervation. Because neuron specific enolase is related chemically to an enzyme with glycolytic properties, its presence may be related to the metabolic state of the cell, and because of its cytoplasmic non-granular localisation, immunostaining for neuron specific
enolase is particularly useful for the visualisation of poorly granulated neuroendocrine tissue, such as neuroendocrine tumours of the lung. It is important to be selective when choosing antibodies to neuron specific enolase as this enzyme is a large protein and thus antibodies are likely to react to different epitopes of the entire molecule. It is prohibitive in terms of cost to produce the enzyme synthetically and thus extracted natural material is used for immunisation. It is therefore likely that the antigen will be contaminated with material other than pure NSE and unwanted staining to non-neuroendocrine elements is therefore to be expected. A cocktail of high quality monoclonal antibodies recognising several epitopes of the molecule will be an ideal tool for morphologists.

Chromogranins are a family of proteins first extracted from the adrenal medulla by Blashko in 1967. It has been shown that chromogranins coexist with catecholamines in the storage granules of adrenal medullary cells and in sympathetic nerves. The nucleotide sequence of human prochromogranin has recently been disclosed and shown to contain within it an identical sequence of another recently described secretory protein found in the parathyroid. It has recently been reported that the chromogranins can be released together with catecholamines from normal tissue and from neuroendocrine tumours and that circulating chromogranin immunoreactivity, as measured by radioimmunoassay, may be useful for monitoring sympathoadrenal secretion and for the diagnosis of neuroendocrine tumours. Although the functions of the chromogranins have yet to be fully established, it is thought to establish the intragranular matrix. Both polyclonal antiseraum and monoclonal antibodies are available, the former reputed to stain endocrine cells and neural elements; the latter, raised by Ricardo Lloyd's group, consistently picks up peptide producing endocrine cells (fig 1). The association of chromogranin immunoreactivity and dense cored secretory granules of endocrine cells has recently been shown by an immunolabelling technique (fig 2).

7B2 or anterior pituitary pig (APPG) is a large protein originally extracted from porcine and human pituitary glands. The name 7B2 derives from the initial chromatographic peak obtained during analysis of this material. 7B2 was later found to have a molecular weight of 21000 and to contain about 180 amino acids. The porcine protein and its human counterpart are highly homologous, differing by only one amino acid (position 12) in the first 77 of the N-terminal sequence. Sequence homologies have been reported with Rous sarcoma virus and with insulin growth factor. 7B2 is broadly distributed in central and peripheral nerves and in nearly all endocrine cells. β cells and their derivative tumours, however, seem to contain larger concentrations of this protein than other endocrine cell types and tumours (figs 3a and b).

PGP (protein gene product) 9.5 is a recently extracted soluble protein of 27000 molecular weight. PGP is broadly distributed and its presence in neuroendocrine tumours has recently been reported. Antibodies to PGP stain all classes of nerves consistently and with great clarity, and PGP is thus a use-

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**Fig 1** Chromogranin immunoreactive endocrine cells in human ileum. (Freeze dried, formaldehyde, vapour fixed, semithin resin section. Immunoperoxidase (PAP)).
ful marker for analysis of neural abnormalities, in particular those of peripheral tissues. The validity of PGP antibodies as a universal stain for endocrine cells remains doubtful. Some endocrine cells of the gut are said to be stained by some monoclonal antibodies currently available but only on acidic material fixed in alcohol (Wilson POG, Barber PC, Hamid QA, et al, unpublished observations).

Neurofilament triplet proteins, glial fibrillary acidic proteins (GFAP), and S-100 The variety of nerves containing not only classical neurotransmitters such as acetylcholine, noradrenaline, 5-hydroxytryptamine and gamma-amino-butyric acid, but also putative peptide neurotransmitters, can now be fully
and extensively visualised by the use of polyclonal and monoclonal antibodies to neurofilaments. Neurofilaments belong to the group of cytoskeletal proteins known as intermediate filaments. These antibodies have been extensively used in immunocytochemistry to visualise the entire innervation as they stain both neuronal cell bodies and nerve fibres. Antibodies to supporting elements, both glial and Schwann cells, are also available—glial fibrillary acidic protein (GFAP) and S-100. GFAP is known to stain astrocytes, in particular those of the central nervous system, whereas S-100 is a good marker for Schwann and glial cells of peripheral tissues. Thus antibodies to neurofilaments, to GFAP, and to S-100 are recommended for visualising the pattern of innervation.

Neurofilaments are of three types with different molecular weights of 68,000, 150,000, and 200,000, the predominant type depending on the degree of maturation of the cell. Monoclonal antibodies are likely to pick up a specific epitope of the polypeptide triplet and therefore may not pick up the entire innervation. Polyclonal antisera of excellent quality are available, and again a good mixture of high quality monoclonal antibodies, recognising the various components of the polypeptide triplet of neurofilaments, may be the ideal route for staining.

Specific peptide markers
Antibodies to regulatory peptides are easily available and work well on conventionally fixed and paraffin embedded tissue. They are particularly applicable to the study of endocrine component of the diffuse neuroendocrine system. Furthermore, antibodies are now raised to the various regions of pre-propeptides, thus giving a wider range of localisation possibilities in the study of regulatory peptides.

Electron microscopy
Electron microscopy has been instrumental in showing the existence of dense core secretory granules with specific morphological features, such as size, limiting membrane, electron density and halo. Morphology alone permits a prediction of the type of peptide in a particular secretory granule, but immunocytochemistry, increasingly applied at the electron microscopical level, provides more certainty. Electron immunocytochemical techniques can be broadly divided into pre-embedding and post-embedding methods. The pre-embedding method using peroxidase-antiperoxidase complexes is useful when synaptic contacts or membrane visualisation is required, as the end product of the peroxidase reaction can be made electron dense by osication of the material following the immunoreaction. For post-embedding procedures, a variety of gold labelling methods are now preferred. Gold particles of various sizes can be attached by covalent forces to immunoglobulins. Different antibodies can be labelled with different gold particles, thus enabling multiple immunostaining procedures to be carried out. The advantage of this technique over the peroxidase method is that the delicate gold particles, when deposited, do not mask the electron-dense neurosecretory granules.

Functional markers
Efforts have recently been centred on the possibility of developing a "functional" morphology and thus expanding the confines of neuroendocrine morphology, which has so far dealt only with the analysis of stored molecules; thus attention has been paid to microscopical imaging tools that could serve to indicate localisation of a specific mRNA species, or peptide binding site, and to visualise intracellular events leading to peptide formation and release. These aims are beginning to be realised by the development of novel techniques, including those of in situ hybridisation, in vitro autoradiography and monitoring of intracellular pH as an indicator of peptide synthesis in progress.

In situ hybridisation
This novel technique shows specific mRNA molecules directing the synthesis of a given peptide. It is based on the property of complementary sequences of nucleic acids to hybridise within the cell (fig 4a). Thus a specific probe can be constructed and suitably labelled that will specifically hybridise with defined mRNA molecules. Numerous techniques have been proposed and are broadly divided into radioactive and non-radioactive methods (fig 4b). The preferred radioactive labels include 32p, 35s, and 3h. Most non-radioactive techniques make use of biotin-avidin systems, but the sensitivity of this method has recently been questioned, although its use for in situ hybridisation at the electron microscopical level remains unchallenged.

Localisation of cellular binding sites (receptors)
Numerous techniques for the localisation of cellular binding sites have recently been proposed and can be broadly divided into three main groups: (i) In vitro autoradiography, a technique originally proposed by Young and Kuhar based on the incubation of tissue sections with radioactive ligands which are subsequently autoradiographically developed, by exposure either to a special film or to emulsion coated coverslips (figs 5a and b); (ii) The production of specific antibodies to purified receptors; this has been used in particular for localisation of oestrogen receptors.
Fig 4  (a) In situ hybridisation of prolactin mRNA in rat pituitary using $^{32}$P-labelled probe. (Paraformaldehyde fixed tissue.) (b) Schematic representation of various label types used with probes for in situ hybridisation.
Fig 5  (a) Dark field photomicrograph of LKB Ultrafilm autoradiogram produced after incubating unfixed cryostat section of guinea pig kidney with 200 pM human $^{125}$I-$\alpha$-atrial natriuretic peptide (ANP). $^{125}$I-ANP binding is concentrated over the glomeruli, renal arteries, and collecting tubules in outer medulla; (b) Negative image of a) to show details.

Fig 6  (a) Diagrammatic representation of binding of divalent bombesin ligand (DBN) to cell surface receptor (R) and its visualisation using anti-bombesin (Anti-BN) and antibodies labelled with gold. (b) Electron micrograph of cell surface (N = nucleus) with receptor sites (arrows) visualised by immunogold labelling as described in (a). (Taken from Lackie et al. Histochemistry 1984;83:57–9.)
(iii) In certain instances, however, and in particular when specific antibodies to receptors are not available, it may be necessary to use highly sensitive methods to detect low numbers of receptors.

A technique has recently been proposed that is based on the availability of a specific monoclonal antibody to a given peptide; of the knowledge of the region specificity of this antibody; and of the active site (recognised by the receptor) of the peptide. Fig 6a illustrates the basic principle. Using this principle we have recently been able to show and further confirm biochemical findings of the existence of receptors to a growth promoting factor called bombesin or gastrin releasing peptide (GRP) on the surface of rapidly growing tumour cells of a small cell carcinoma (fig 6b).

**Diseases of the “regulatory peptide”-containing system**

It is beyond the scope of this paper to provide an extensive review of all reports indicating a potential pathogenetic role for regulatory peptides. Thus only the most established and salient features of the pathology of the diffuse neuroendocrine system will be given.

**BENIGN DISEASE**

**Genitourinary system**

The genitourinary systems of man and animals has been shown to contain a variety of regulatory peptides localised in particular in the organ's innervation. Endocrine cells have also been described, but no particular peptide has been consistently associated with
them. Nerves containing vasoactive intestinal polypeptide (VIP) are especially prominent in the genitourinary system and have been shown to be associated with smooth muscle, secretory glands, blood vessels and surface epithelium. A role in modulating muscle tone, glandular secretion, and blood vessel diameter has been attributed to VIP. It was therefore of interest to see pronounced abnormalities of nerves containing VIP in the smooth muscle of the bladder in patients with "unstable" bladder\(^\text{25}\) and around the erectile tissue in the penis of impotent men (figs 7a, b, c and figs 8a and b).\(^\text{26}\) Neuronal and endocrine abnormalities have recently been observed in patients with chronic cystitis (Hamid QA, Rode J, Flanagan AM, \textit{et al.}, unpublished observations). And the possibility of a neuronal factor trophic to the endocrine cells has been mooted.

**Respiratory tract**

Endocrine cells and nerves containing peptide in the respiratory tract of man and mammals are abundant.\(^\text{27}\) Peptides found in endocrine cells of the respiratory tract include calcitonin, possibly the enkephalins, and in particular, bombesin. The presence of bombesin immunoreactivity in numerous mucosal endocrine cells in the growing fetal and neonatal lung\(^\text{28}\) is of interest: strong evidence supports the current contention that bombesin or gastrin releasing peptide may be one of the key growth promoting factors. Bombesin cells are particularly few in number in hypoplastic lungs that are associated with the respiratory distress syndrome (figs 9a, b, and c).\(^\text{29}\)

Bombesin immunoreactive cells are, however, numerous in hyperplastic lungs (bronchopulmonary dysplasia). Abnormalities of endocrine cells around lung tumours and in emphysematous or bronchiectasic lung have also been reported\(^\text{30}\). VIP immunoreactivity is particularly abundant in main airways and nerves containing VIP are closely associated with smooth muscle and secretory glands. Thus the abnormalities of such nerves of the lung seen in asthma and cystic fibrosis fit well with the poor relaxation of smooth muscle and abnormalities of bronchial secretion that are the main clinical features of these diseases (Shipperbottom A \textit{et al.} Abstract presented at 154th meeting of the Pathological Society of Great Britain and Ireland, 1987). Such nerves have also been shown to be very hyperplastic in certain cases of vasomotor rhinitis.\(^\text{31}\)

**Diseases of the central nervous system**

The central nervous system contains a large number of neuropeptides whose projections and pathways have been extensively investigated.\(^\text{32}\) Neuropeptides have been shown to be abnormal in several neuro-pathological states, including Alzheimer's disease or senile dementia (abnormal somatostatin and neuropeptide tyrosine in the cortex) (figs 10a and b) Huntington's chorea (reduction of substance P and cholecystokinin in the striatum), schizophrenia (decrease of somatostatin and cholecystokinin in the hippocampus), and motorneurone disease (pan-neuropeptide changes).
Fig 9  (a) Neonatal human lung immunostained with anti-bombesin serum. Many epithelial endocrine cells displaying bombesin-like immunoreactivity are seen in specimen from infant dying of non-respiratory disease.  
(b) Only one cell (arrow) in equivalent area of lung from case of respiratory distress syndrome. (Wax sections (5 μm) of formalin fixed tissue, PAP method, haematoxylin counterstain.)  
(c) Chart showing results of radioimmunoassay measurement of bombesin in neonatal human lung. (Taken from Ghatei et al. J Clin Endocrinol Metab 1983;57:1226–32.)
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Fig 10  (a) NPY-immunoreactive neurons in cortex of normal human brain. Cell shows varicose dendritic processes.  
(b) NPY-immunoreactive neurons in cortex of brain from patient with Alzheimer's disease. Cell damage is shown by presence of short, stumpy dendrites.
Gastrointestinal tract

The gastrointestinal tract is probably one of the best investigated organs in terms of regulatory peptides and endocrine cells and enteric nerves, perhaps because it has attracted attention since the onset of investigations into regulatory peptides.

Hyperplasia of endocrine cells in the stomach G cell hyperplasia was first described using immunocytochemistry. Functional studies were subsequently carried out and the entity has now been recognised as G cell hyperplasia/hyperfunction. G cell hyperplasia can be found in severe cases of duodenal ulceration with symptoms closely resembling those associated with gastrin producing tumours (gastrinomas) encountered in the Zollinger–Ellison syndrome. No tumour, however, is present. G cell hyperplasia is also often found in cases of atrophic gastritis with or without pernicious anaemia.

Since 1969 a characteristic endocrine cell type has been reported to be present in the fundic mucosa. This cell, which has an elongated shape and is not in contact with the lumen, was named enterochromaffin-like (ECL) cell. The peptide product of these cells, which contain characteristic secretory granules, is as yet unknown. ECL cells are stained by silver impregnation methods. These cells have been shown to be sensitive to increased gastrin concentrations, as in either hyperfunction of G cells or a gastrinoma. In addition, an excessive release of gastrin from G cells for lack of gastric inhibition, can lead to ECL cell hyperplasia and subsequent formation of micronodules and carcinoids (figs 11a and b). These findings are becoming increasingly common as drugs capable of producing maximum gastric acid suppression (new generation of H2 receptor blockers, proton pump inhibitors etc) are now available for the treatment of duodenal ulcer and other hypersecretory conditions. These drugs, taken long term, produce a complete blockade of gastric acid secretion and subsequent oversecretion of G cells leading to chronically increased circulating gastrin concentration.

Endocrine cells are often observed in areas of intestinal metaplasia. No correlation is found between endocrine cell population and the type of intestinal metaplasia, thus sulphomucin producing foci of intestinal metaplasia (the so called "colonic type") and the sialomucin producing foci ("small intestinal type") do not differ greatly from each other as far as the endocrine cell population is concerned. Interestingly, secretin and cholecystokinin cells, which are not normally seen in the colon, are found in both types of intestinal metaplasia. Abundant gastrin cells are commonly seen in intestinal metaplasia of antral mucosa with atrophic gastritis.

Barrett's oesophagus The presence of endocrine cells in Barrett's oesophagus has recently been reported. In Barrett's oesophagus the normal stratified squamous epithelium is replaced by specialised columnar epithelium, similar to that of the gastric fundus, or a combination of the two. Recently, endocrine cells have been detected immunocytochemically in the abnormal epithelium. Three cell types were
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observed: enterochromaffin (EC), somatostatin (D), and gastrin (G) cells.

Coeliac disease The presence of numerous immunoreactive cells in coeliac disease has often been reported.\textsuperscript{41, 42} The findings of so-called secretin and cholecystokinin cell hyperplasia are particularly relevant in coeliac disease, as it has been recognised for some time that anatomical abnormalities of the duodenal mucosa lead to an impairment of hormone release by intraluminal stimuli. Consequently, pancreatic bicarbonate and enzyme secretion are defective. By contrast, if secretin and cholecystokinin are given intravenously the pancreatic response is normal. These findings suggest that there is an impairment of hormone release from the gut in patients with coeliac disease. This explains the intensely immunostained cells previously interpreted as hyperplastic. These findings fit in well with dynamic studies which indicate an impairment of hormone release after normal intraduodenal acid stimulus.\textsuperscript{43} Changes in other endocrine cell types have also been reported in coeliac disease.

Sparing of endocrine cells in graft versus host disease\textsuperscript{44} A remarkable preservation (sparing) of endocrine cells in the gut of patients with graft versus host disease has recently been reported. These findings are

Fig 12  (a) Submucosa of normal human colon, taken at resection for carcinoma, immunostained for VIP using indirect immunofluorescence technique. Small, scattered bundles of varicose VIP nerves can be seen.
(b) Colonic submucosa from patient with Crohn's disease, immunostained as for (a). VIP nerves are much more numerous and their fine, varicose appearance is replaced by coarse, thickened, densely immunostained profile.
(c) Normal ileal mucosa, removed at resection for caecal carcinoma, immunostained for chromogranin. Immunoreactive endocrine cells are scattered in epithelium.
(d) Ileal mucosa with chronic inflammatory changes associated with long standing Crohn's disease, immunostained as for (c). Mucosal epithelium is packed with large numbers of endocrine cells.
Fig 13  (a) Haematoxylin and eosin stain of tumour in transgenic mouse. Tumour cells are arranged in acini and trabeculae.
(b) Tumour in transgenic mouse immunostained for insulin showing immunoreactivity in most tumour cells.
(Bouin’s fixed tissue, 5 μm wax sections. PAP stain with weak haematoxylin counterstain).
(c) Section of B cell tumour from transgenic mouse immunostained with antibodies to large T antigen. Many nuclei of the tumour cells show dense immunoreactivity whilst the exocrine pancreas is unreactive.
(d) Electron micrograph showing secretory granules in tumour cell from transgenic mouse pancreas. Presence of proinsulin in granules was visualised using immunogold labelled antibodies.
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seen in a setting of mucosal epithelial cell and crypt destruction with focal polymorphic infiltrates and are in keeping with the reported sparing of other endocrine tissue in this disease, with the selective damage to certain cell types, and with the finding that this disease affects especially rapidly proliferating tissue (such as lymphoid tissue and gut epithelium) rather than nervous tissue or muscle.

Inflammatory bowel disease (Crohn's disease) Neuronal hyperplastic changes have been reported to occur in the bowel of patients with Crohn's disease. These changes have now been shown to be due to abnormalities of the VIPergic and also of the peptide histidine isoleucine (PHIergic) components of the enteric nervous system. Nerves containing VIP seem to be severely hyperplastic, distorted, and intensely immunostained. Changes are particularly evident in the mucosa and lamina propria. VIP immunostaining could thus be a useful tool for diagnosing inflammatory bowel disease in endoscopic biopsy specimens. Endocrine changes in the mucosa of patients with inflammatory bowel disease have also been noted (figs 12a and b, c and d).

MALIGNANT DISEASE

Experimental endocrine tumours

Until recently the experimental production of endocrine tumours has not been easy. Some reports, however, have shown the successful production of insulinomas in rodents after treating animals with streptozotocin and nicotine and endocrine tumours have been commonly observed in old animals. The problem has recently been circumvented by the application of novel and exciting techniques that rely on the production of hybrid genes.

A hybrid gene containing the simian virus 40 (SV40) gene early (transforming) region and the rat insulin II gene (regulatory) region was recently established in a mouse germ cell line. It was found that tissue specific, pancreatic insulin producing B cell tumours were heritably produced in the transgenic offspring (Power RF, Holm R, Bishop AE, unpublished observations). The transformed B cells could be identified by their proliferation and by expression of large T antigen (the oncogene encoded in simian virus 40) (figs 13a, b, c and d).

The production of transgenic mice entails the microinjection of a solution of DNA into one of the pronuclei of the fertilised mouse oocyte, after which the injected embryos are reimplanted into the oviducts of pseudopregnant female mice and allowed to develop. About 20% of the newborn mice carry the injected DNA as a heritable genetic element, which they then transmit to their offspring, thus generating a line of transgenic mice which generally manifest the phenotype encoded for by the new gene. Transgenic mice have proved useful for studying oncogenesis, in particular through the use of oncogenes that are manipulated to be expressed specifically in a certain cell type, thereby eliciting a particular type of tumour.

Human endocrine tumours

Pituitary tumours The anterior lobe of the pituitary gland (adenohypophysis) produces at least six hormones, and numerous cell types have been described by special staining, immunocytochemistry, and electron microscopy. The production of novel peptides by the anterior pituitary has recently been recognised. These peptides include neurotensin, vasopressin, dynorphin A, and in particular, B255 and neuromedin B. Neuromedin B is a decapeptide originally isolated from the pig spinal cord and designated "B" because of the similarity of its amino acid sequence to that of bombesin and its ability to stimulate peptide release in a manner similar to that of bombesin. Neuromedin B has been shown to be present in thyrotrphs of the anterior pituitary, and B2 has been shown in gonadotrophs and gonadotrophinomas (figs 14a and b).

Pancreas The pathology of the endocrine tumours of the pancreas has been extensively described, and readers are referred to several publications. The table summarises the main features. Neuroendocrine tumours of the lung have also been extensively reviewed, and we will focus only on some interesting recent findings.

Active peptide hormones have been shown to be produced and released from neuroendocrine tumours of the lung; the most commonly found include calcitonin, ACTH, vasopressin, neurotensin, and especially bombesin. Immunoreactivity to bombesin has been shown to be closely associated with small cell carcinoma of the lung. Bombesin may exert a local action on tumour cell surface receptors (autocrine effect). The structure of human pro-bombesin has recently been disclosed by molecular biological methods and shown to consist of a signal peptide (bombesin) and a C terminal extended segment. Three mRNAs have been found to code for bombesin, and thus the length of the C terminal extended fragment varies according to the splicing sites of the transcribed message. Specific antibodies to an N-terminal portion (21 amino acids) of the C terminal fragment show stronger immunoreactivity than antibodies to bombesin itself in pulmonary and non-pulmonary small cell carcinomas and large concentrations of extractable chemically characterised, C terminal flanking peptide were detected by specific radioimmunoassay. Furthermore, it has recently been shown that immunoreactivity to bombesin has a more or less similar distribution in carcinoids, atypical carcinoids, and small cell carcinomas. By contrast, the
Fig 14  Serial sections (2 μm) of rat anterior pituitary immunostained for a) 7B2 and b) luteinising hormone; c) Neuromedin B and d) thyroid stimulating hormone. Most immunoreactive gonadotrophs also show 7B2 immunoreactivity. Both antigens are present in same cells (arrowed).
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Fig 15  (a) Haematoxylin and eosin stained section of small cell carcinoma.
(b) Small cell carcinoma lung, immunostained using antibodies to bombesin. Scattered cells are positive for PAP immunostaining.
(c) Diffuse strong immunostaining for C terminal peptide of human pro-bombesin in small cell carcinoma. (Formalin fixation PAP immunostaining.) (Taken from Hamio et al. Virchows Arch (Pathol Anat) 1987;411:185–92.)
C terminal peptide of human pro-bombesin is detectable in a greater proportion of cases of more malignant tumours (small cell carcinomas and atypical carcinoids), raising the question not only as to whether this C terminal fragment is a good pointer to the biological behaviour of the tumour but also whether this may, indeed, be an active part of pro-bombesin molecule with perhaps additional trophic actions. The high expression of the C terminal peptide of pro-bombesin in a large number of small cell carcinomas, which are known generally to be poorly granulated, may indicate an alternative non-granular pathway from peptide synthesis to release (figs 15a–c). It would thus be justifiable to speculate that peptides could be produced and voided by a cell, particularly a transformed or otherwise compromised cell, without packaging into storage granules.

**Conclusions**

We have advanced a great deal from the early descriptions of the diffuse neuroendocrine system to the recognition of the production by components of this system (endocrine cells and nerves) of numerous active peptides sustaining specific sets of functions. The role of this “regulatory peptide system” in experimental and human diseases further advances our understanding of the nature of those diseases and provides evidence that these peptides do, indeed, exert powerful actions that control numerous bodily functions.

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