CURRENT PROBLEMS IN TUMOUR PATHOLOGY

# ENDOCRINE TUMOURS

## The Pathobiology of Regulatory Peptide-producing Tumours

J. M. Polak & S. R. Bloom

Churchill Livingstone

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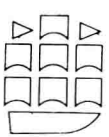

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**Endocrine Tumours**

# CURRENT PROBLEMS IN TUMOUR PATHOLOGY
## THE PATHOBIOLOGY OF MALIGNANT DISEASE

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# Foreword

In the later years of the last century, the signal advances in the clinical appreciation of disease emanated largely from the pathological laboratory with histopathology and morbid anatomy at the forefront. Latterly, however, increases in chemical and physiological knowledge allowed the emergence of the modern biochemical approach to disease and its investigation, which meant the relative eclipse of tissue pathology, from an investigative discipline to a predominantly service-orientated subject.

Recent years have seen a minor revolution in histopathology and our approach to it, and nowhere is this more evident than in the study of maligant disease. The advent of new techniques in cell biology—molecular genetics, in vitro methods, the evolution of monoclonal antibodies to differentiation antigens, light and electron microscopic immunocytochemistry, in situ DNA hybridization and recombinant DNA technology, and in embryology, experimental histochemistry and ultrastructure, have radically changed our views of cell lineage and therefore of tumour classification. The immunocytochemical analysis of tumour markers is profoundly altering both our diagnostic practice and prognostic predictive power. The application of these new, morphologically-based techniques to experimental systems is also allowing pathologists to again make inroads into the more general problems of tumour growth and clonality, invasion and metastasis.

It is the purpose of this current series of volumes to survey critically the impact of this new thinking on what we might call the *pathobiology of maligant disease*. Each book will take a single tumour or group of tumours, and with the relevant experts chosen carefully by the volume editor, will succintly analyse the changes which our new technology has wrought upon our pathological concepts of the particular disease group, will note where these advances impinge upon histopathological diagnosis, and will (hopefully) predict the direction of fruitful new research. It is the fervent hope of the series editors that these ideals will be realized.

We are singularly fortunate in our choice of editors for the first volume of this series: Julia Polak and Stephen Bloom, both of the Royal Postgraduate Medical School, who themselves have built a collaboration

which has been at the forefront of research into endocrine tumours over the decade, have assembled a thoroughbred team of contributors who we think have performed their appointed tasks both critically and attractively. We have no doubt that their efforts will be avidly received by a wide readership.

London, 1985

Nicholas A. Wright
John G. Azzopardi

# Preface

Cancer is a major scourge of mankind. To grow, a neoplasm must, perforce, organize its environment. Thus it is probably true to say that all cancers secrete local regulators of one sort or another. Certain tumours are noteworthy for the fact that they produce large quantities of regulatory peptides and they are the subject of this volume. We are still in the early stages of discovering the nature of the regulatory peptide system and new peptides are being found at, it seems, weekly intervals. Thus it may well be that many more tumour types will be uncovered in the future. The technology necessary to study these neoplasms is, however, mostly in place. Thus four chapters are devoted to understanding peptide biosynthetic pathways. Much of the advance in this area has indeed come from the study of tumours—yet another example of nature being the best research worker. The three chapters on new methods of histological examination form a vital part of the pathologist's repertoire and illustrate the very fast-moving nature of this field. Woe betide anyone who uses yesterday's techniques!

An area of great future emphasis will be the dissecting of tumour biology in culture systems. Although, today, it is not possible to predict clinical behaviour of a tumour by knowledge of its responses in the petri dish, this will surely be the way forward in the future. Pathologists will undoubtedly be advising as to the clinical programme from their knowledge of the nature of the tumour cells.

There are few areas of pathology as exciting as this one, nor, indeed, where knowledge makes such a great difference to the clinical outcome. These growing points have almost immediately become 'essential knowledge'. For this reason, the editors found their task as refreshing as a summer holiday (which they had to do without!).

London, 1985

J.M.P
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# Contents

# Neuroendocrine neoplasms and regulatory peptides. An introduction

## TERMINOLOGY

The idea that the neural and endocrine systems are intimately associated has been reinforced by the discovery that many active peptides are produced and released both by endocrine (APUD) and nerve cells (Polak & Bloom 1983).

A variable terminology has been used for this group of active peptides. Gut endocrinologists are familiar with the term 'gut hormones' as many of these active peptides were first discovered in the gastrointestinal tract, localised in mucosal endocrine cells and released to the bloodstream to act as classical circulating hormones. Neuroscientists, by contrast, are more conversant with the term 'neuropeptides', as many of these active peptides were originally found in the brain.

The apparent barriers between gut endocrinology and neuroscience broke down with the discovery that the brain and the gut contain identical active peptides, thus generating another term, 'gut and brain peptides'.

None of these terms is completely suitable, as it is clear that the peptides are not exclusively localised in the brain and gut, nor do they act simply as circulating hormones or neurotransmitters, since a single peptide may perform either or both of these roles according to its localisation in endocrine cells and/or nerves.

The generic term 'regulatory peptides' was therefore suggested, to cover the whole range of active peptides, whether circulating hormones or neurotransmitters, that control tissue functions.

Neoplasms derived from the neuroendocrine system have been described by a variety of terms, including 'APUDomas', endocrine tumours and, more particularly, pituitary tumours, islet cell tumours and 'carcinoid' tumours. The term apudoma was proposed by Szijj and collaborators in 1969 to describe tumours derived from the APUD system. The term 'carcinoid' is not generally applicable as it is associated with amine-producing tumours giving rise to the classical carcinoid syndrome. Although the term 'islet cell tumours' is appropriate for primary tumours of the endocrine pancreas it is now recognised that identical tumours may

arise from the gut, respiratory tract, pituitary, prostate, urethra, thymus and adrenal gland.

By conventional histological staining neuroendocrine neoplasms are characterised by the presence of tumour cells with the appearance of motor neurons, with a variable amount of eosinphilic cytoplasm and regular nuclei. Tumour cells are arranged in ribbons, irregular masses or glandular structures separated by thin bands of connective tissue. Mitoses are rarely seen but local or vascular invasion can be noted. Although these features are indicative of a neuroendocrine neoplasm, precise characterisation of the tumour and its peptide production can only be achieved by the use of specialised staining techniques (Heitz et al 1982) (see later in this chapter).

## TECHNOLOGY FOR THE INVESTIGATION OF THE DISTRIBUTION OF REGULATORY PEPTIDES

Regulatory peptides are now known to be very widely distributed. That presence in most peripheral tissues has been established mainly by the use of two techniques: radioimmunoassay and immunocytochemistry. Radioimmunoassay provides accurate information on the quantities of peptides in a given tissue and, in combination with chromatography, allows full determination of their chemical nature. The principles and details of the techniques of radioimmunoassay for regulatory peptides are fairly standardised (Bloom & Long 1982). Immunocytochemistry (the principles and applications of this technique are detailed in Ch. 7) provides information about the precise localisation of peptides within endocrine cells or nerves and the technology is still in active flux (Polak & Van Noorden 1983).

## MORPHOLOGY OF PEPTIDE-CONTAINING CELLS AND NERVES AND THEIR DERIVATIVE TUMOURS

The endocrine or APUD cells were first recognised by special histological or histochemical methods, including silver impregnation (argentaffin and argyrophilic methods) (see Ch. 6), masked metachromasia and lead haematoxylin staining (see Ch. 6 and 8) and lately by immunocytochemistry using specific antibodies to peptides, amines or glycolytic enzymes (for the latter see Ch. 7). In luminal organs such as the gut, endocrine cells are classically pear shaped with the widest part towards the basal membrane, and often extended along it (Fig. 1.1). Towards the luminal pole the cells end in a cluster of microvilli. At the electron microscopical level these endocrine cells display characteristic features. The cytoplasm is furnished with numerous electron-dense secretory granules whose shape, density, size and limiting membrane allows distinction between several different cell types (Fig. 1.2A & B) (for details see Ch. 8).

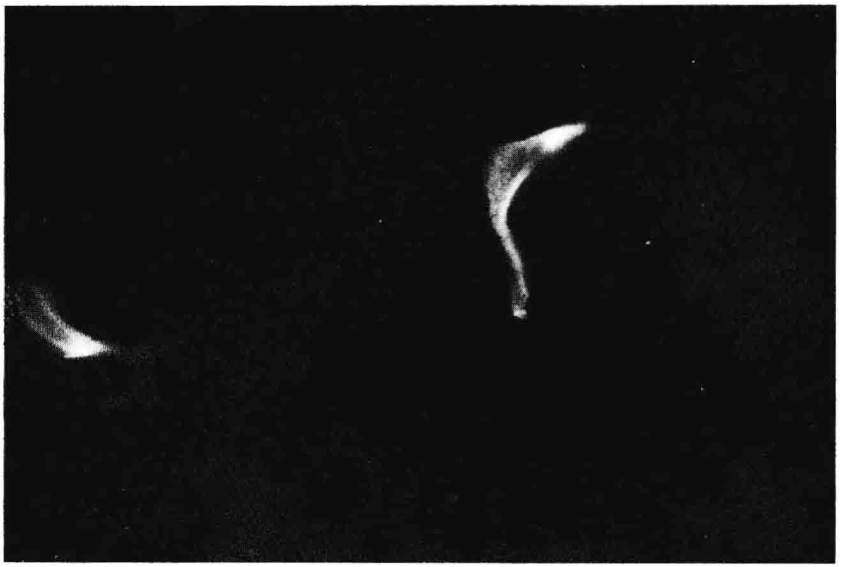

**Fig. 1.1** Enteroglucagon-immunoreactive cells in the mucosal epithelium of human colon. Basal and luminal elongations can be seen. p-Benzoquinone solution fixed, 10 $\mu$m cryostat sections. Magnification = 700 ×

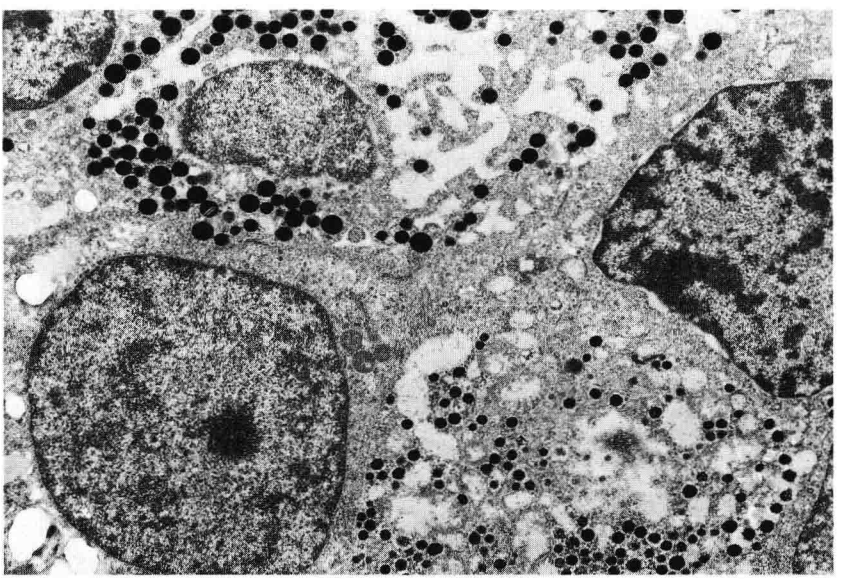

**Fig. 1.2A** Adjacent endocrine cells from human colonic mucosa exhibiting morphologically distinct secretory granule populations. Glutaraldehyde, oxmium tetroxide fixation; uranyl acetate and lead citrate counterstains. Magnification = 7000 ×

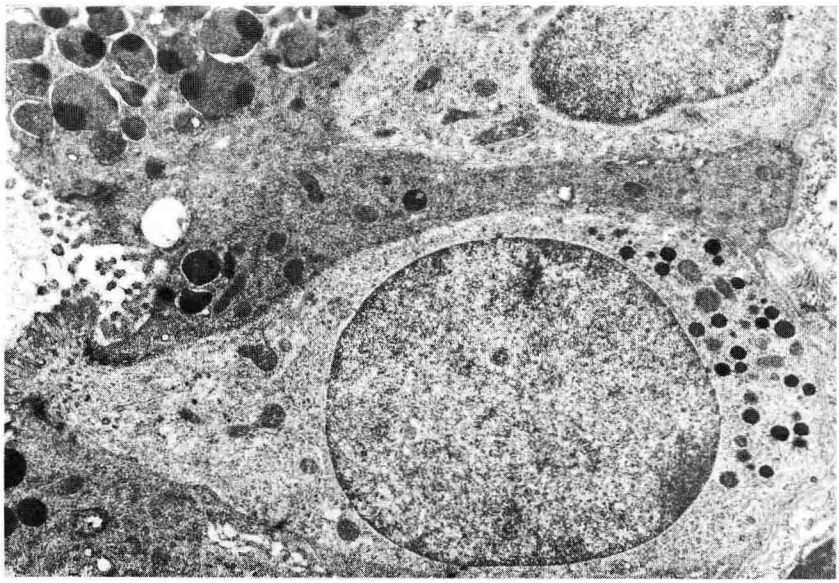

**Fig. 1.2B** Endocrine cell from human duodenum exhibiting basally situated secretory granules (300 nm diameter), large spheroidal nucleus and an array of microvilli at the luminal surface. Cell junctions are clearly visible close to the luminal opening. Glutaraldehyde, oxmium tetroxide fixation; uranyl acetate and lead citrate counterstains. Magnification = 8125 ×

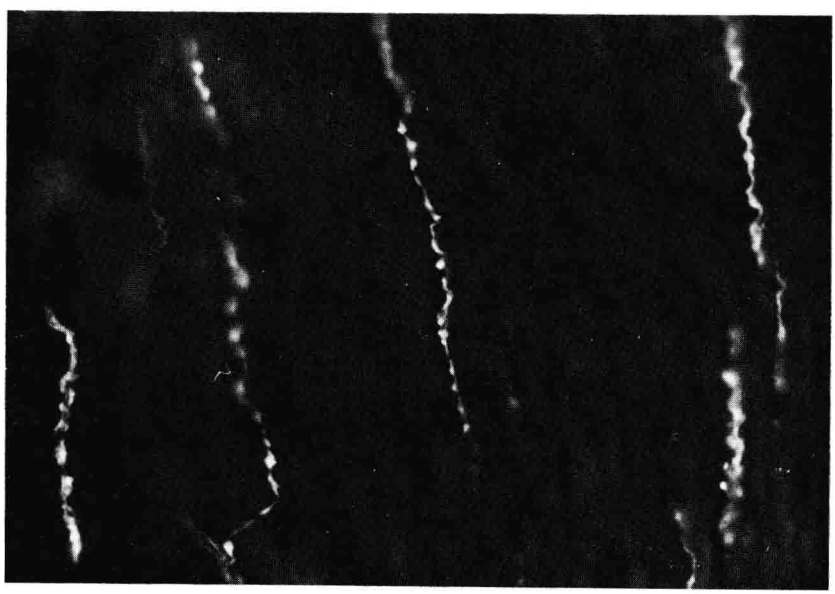

**Fig. 1.3** Circular muscle coat from human colon containing numerous VIP nerves immunostained using the technique of indirect immunofluorescence. p-Benzoquinone solution fixed 10 $\mu$m cryostat sections. Magnification = 520 ×

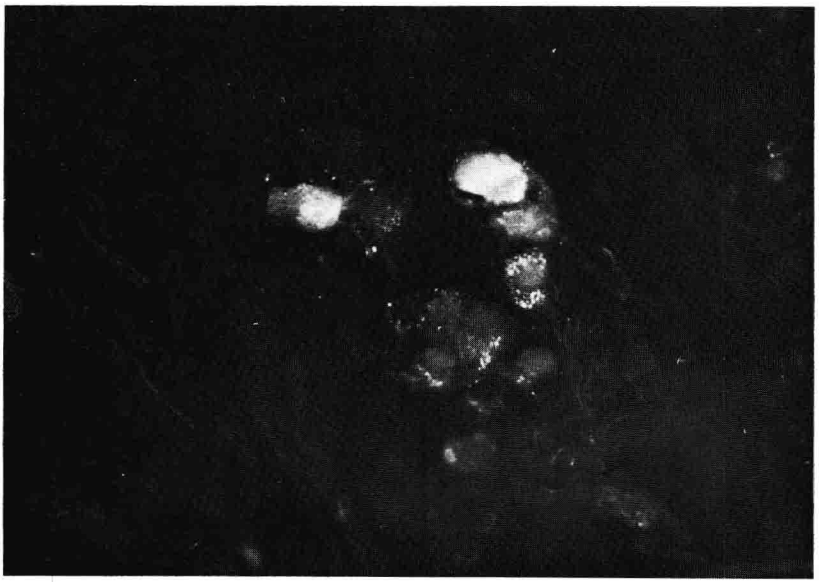

**Fig. 1.4** VIP-immunoreactive and non-immunoreactive ganglion cells in the submucous plexus of porcine small intestine. p-Benzoquinone fixed 10 $\mu$m cryostat sections. Magnification = 375 ×

Peptide-containing nerves show all the features of classical nerves and have a characteristic beaded appearance (Fig. 1.3). These nerve fibres originate from ganglion cells (Fig. 1.4) or primary sensory neurons; the former frequently seen to be present within the innervated tissue.

## DISTRIBUTION OF REGULATORY PEPTIDES

It is beyond the scope of this introductory chapter to analyse in detail the distribution of each regulatory peptide in all peripheral tissues. Many peripheral organs are well provided with the components of the 'diffuse neuroendocrine system' (Polak & Bloom 1979) and thus contain an array of separate endocrine cell types and nerves charcterised by the presence of several dfferent regulatory peptides. Tissues in which peptide-containing endocrine cells and nerves have been well described include the gastrointestinal tract and pancreas (Polak & Bloom 1982), respiratory tract (Polak & Bloom 1983), adrenal medulla, thryroid, pituitary and gall bladder (Cai et al 1983). Tissues like the prostate, urethra and skin (Kazzaz 1974, Gu et al 1981) have been shown to contain both nerves and endocrine cells, but the peptide product of the latter remains to be fully elucidated. In the thymus, endocrine cells containing somatostatin and neurotensin have been described (Sundler et al 1978, Polak & Bloom 1981).

A summary chart of the peptides present in these tissues is shown in the Appendix.