

Endocrine cells in tumour-bearing lungs

J D H Sheard, J R Gosney

Abstract

Background – When hormones are detected in the serum of patients with bronchial carcinoma they are generally considered to originate from the tumour, but this may be not the only explanation. Pulmonary endocrine cells proliferate in lungs affected by non-neoplastic disease and their products are often demonstrable in the serum. The aim of this study was to examine the pulmonary endocrine systems of a series of tumour-bearing lungs to see whether any changes in them could possibly account for raised levels of pulmonary peptides in the blood.

Methods – The morphology, number, distribution, and content of pulmonary endocrine cells in 30 pairs of tumour-bearing lungs from patients coming to necropsy with bronchial carcinoma were examined. These features were related to the pathology of the tumour and to other pathological changes present in the lungs, and compared with pulmonary endocrine cells in 10 pairs of control lungs from patients without pulmonary disease.

Results – Increased numbers of endocrine cells, often in the form of large abnormal aggregates, were present in 17 pairs of tumour-bearing lungs where they were associated not with the tumour but with non-tumoral pathology, especially inflammation and changes associated with cardiac failure. Appropriate and inappropriate peptides were identified within them.

Conclusion – The possibility is raised that, in some subjects with bronchial carcinoma who have raised serum hormone levels, the source of these substances might be the endocrine cells in the diseased lung around the tumour.

(Thorax 1996;51:721-726)

Keywords: pulmonary endocrine cells, ectopic secretion, bronchial carcinoma.

When increased levels of amine, peptide, or protein hormones are found in the blood of patients with bronchial tumours, they are generally assumed to be products of the neoplasm. However, it has often proved difficult or impossible to localise the hormone in question to the tumour allegedly producing it, irrespective of how it is sought and even when the whole of the tumour has been available for study.^{1,2} This is usually attributed to the kinetics of synthesis and secretion or inadequacies of the available technology,³ but an alternative explanation has been proposed^{1,4} – namely, that

the substance or substances in question may arise not from the tumour itself, but from proliferating endocrine cells in the diseased lung around it. With this hypothesis in mind, we have investigated the nature and extent of any changes that might have developed in the pulmonary endocrine system of lungs containing primary bronchial malignancies, and have considered whether they could possibly explain the increased hormone levels occasionally found in the serum of such patients which are usually attributed to the tumour itself.

Methods

Thirty pairs of lungs from patients coming to necropsy with primary bronchial carcinoma were studied, together with 10 pairs of control lungs from age matched subjects with no clinical or necroscopic evidence of pulmonary disease. Cases were included only if the necropsy had been performed within 48 hours of the death of the patient. Necropsy material was used because it allowed extensive sampling, not only of the tumour and the lung adjacent to it, but also of the remaining ipsilateral and the contralateral lung. This was essential for studying the entire endocrine cell population irrespective of its topographical relationship to the neoplasm. Lungs were distended with 10% neutral buffered formalin, fixed for 24–48 hours and extensively sampled, 10 blocks of tissue being taken from the primary tumour, 5–10 from the pulmonary tissue immediately adjacent to it (that is, from the lobe into which it had grown or within which it had arisen), and 5–10 blocks from each of the remaining four pulmonary lobes.

Tissue was embedded in paraffin wax. Sections from all blocks were stained with haematoxylin and eosin and examined by light microscopy to allow classification of the tumour⁵ and histopathological scrutiny of both lungs. Adjacent sections were immunolabelled by the avidin-biotin complex technique⁶ for neuron specific enolase (NSE) and protein gene product 9.5 (PGP 9.5), general markers of cells of the diffuse endocrine system,¹ as well as for a range of secretory products of human pulmonary endocrine cells including gastrin releasing peptide (GRP), calcitonin, calcitonin gene-related peptide (CGRP), and 5-hydroxytryptamine (5-HT; serotonin), all of which are found in the endocrine cells of normal lungs, and certain substances which have been described in pulmonary endocrine cells in diseased lungs^{1,7,8} – namely, adrenocorticotrophin (ACTH), arginine vasopressin (AVP), leucine (leu-) enkephalin, somatostatin, human growth

Department of Pathology, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK
J D H Sheard
J R Gosney

Correspondence to:
Dr J R Gosney,
University Department of Pathology,
Duncan Building,
Royal Liverpool University Hospital,
Daulby Street,
Liverpool, L7 8XW, UK.

Received 23 June 1995
Returned to authors
6 September 1995
Revised version received
17 January 1996
Accepted for publication
25 January 1996

Antisera used for immunohistochemistry

<i>Antisera*</i>	<i>Source</i>	<i>Dilution</i>	<i>Incubation</i>	<i>Tissue controls</i>
PGP 9.5	Biogenesis (Bournemouth, UK)	1:2000	18 hours	Human pancreas
NSE	DAKO Ltd (High Wycombe, UK)	1:200	45 minutes	Human pancreas
Calcitonin	DAKO Ltd (High Wycombe, UK)	1:2000	45 minutes	Medullary carcinoma of human thyroid gland
GRP	DAKO Ltd (High Wycombe, UK)	1:2000	18 hours	Human fetal lung
CGRP	Peninsula Labs (St Helens, UK)	1:4800	45 minutes	Medullary carcinoma of human thyroid gland
5-HT	Peninsula Labs (St Helens, UK)	1:1000	45 minutes	Human ileum
ACTH	DAKO Ltd (High Wycombe, UK)	1:250	45 minutes	Human pituitary
HGH	DAKO Ltd (High Wycombe, UK)	1:800	45 minutes	Human pituitary
AVP	ICN Biomedicals Ltd (High Wycombe, UK)	1:800	45 minutes	Human pituitary
Somatostatin	Seralab (Crawley Down, Sussex, UK)	1:400	45 minutes	Human pituitary
Leu	Peninsula Labs (St Helens, UK)	1:800	45 minutes	Human adrenal medulla
VIP	Various	Various	Various	Various

PGP=protein gene product; NSE=neuron-specific enolase; GRP=gastrin-releasing peptide; CGRP=calcitonin gene-related peptide; 5-HT=5-hydroxytryptamine (serotonin); ACTH=adrenocorticotrophin; HGH=human growth hormone; AVP=arginine-vasopressin; Leu=leucine-enkephalin; VIP=vasoactive intestinal polypeptide.

hormone (HGH), and vasoactive intestinal polypeptide (VIP).

Following treatment with hydrogen peroxide in methanol to inactivate endogenous peroxidase and with normal swine serum to prevent non-specific binding of antibody, sections were incubated with the primary anti-serum for a variable period of time depending on its nature and concentration. This was then linked to the avidin-biotin complex by biotin conjugated donkey anti-rabbit immunoglobulin (for polyclonal antisera) or donkey anti-mouse immunoglobulin (for monoclonal antisera). The chromogen used was 3',3'-diaminobenzidine tetrahydrochloride dihydrate. Appropriate positive and negative tissue controls were employed in all cases. The source, working dilution, incubation period, and the nature of the control tissue used for each of the primary antisera are shown in the table. Immunolabelled sections were examined by light microscopy and the presence or otherwise of the above substances was sought in the tumour as well as in the endocrine cells of the lung, their morphology, distribution, and content all being noted and topographically interrelated.

There are various ways in which pulmonary endocrine cells can be quantitated.¹ The most accurate is expression of their number in terms of either the total epithelial population (endocrine cells/10³ epithelial cells) or of the unit length of epithelium (endocrine cells/cm epithelium). However, in this study detailed quantitation proved impossible to apply with any meaning because the changes in endocrine cells observed were not only present in parenchyma as well as in the airways, but were frequently gross and focal and of an irregular distribution. For the same reasons, defining endocrine cell subpopulations by their content of secretory products in this way also proved unworkable. Changes in endocrine cells were

therefore expressed semiquantitatively according to the sequence of morphological changes they have been shown to pass through when they increase in number and become gradually disorganised. The first of these is an increase in their number so that, instead of being evenly scattered throughout the bronchial and bronchiolar mucosa as is normally the case, they line up to form interrupted rows (IRs) along the basement membrane. The next stage involves the formation of nodular aggregates (NAs) of more than 10 cells arranged in a disorganised fashion basally and suprabasally, sometimes ensheathing the entire lumen of the small airways or arising in the parenchyma.

The degree of endocrine cell proliferation was expressed according to the number of IRs and/or NAs in the tissue examined as follows: 0–5 IRs per five sections = +; 5–10 IRs per five sections = 2+; >10 IRs per five sections = 3+; 0–5 NAs per five sections = 4+; > 5 NAs per five sections = 5+.

Results

All 10 pairs of control lungs (mean age 75 years, range 58–89) contained endocrine cells with a morphology, distribution, and content typical of the endocrine system of healthy human lungs (fig 1).⁹ Thus, the majority were solitary with a regular distribution in intrapulmonary bronchi and bronchioles and always basally located in the epithelium. The only three clusters of endocrine cells identified in these control lungs were small (comprising 2–3 cells), basal, compact and regular, the typical appearance of neuroepithelial bodies¹⁰ which are normally present in adult human lungs but in very small numbers.⁹ About two thirds of the pulmonary endocrine cells revealed with antisera to NSE and PGP 9.5 contained GRP and most of the remainder contained calcitonin

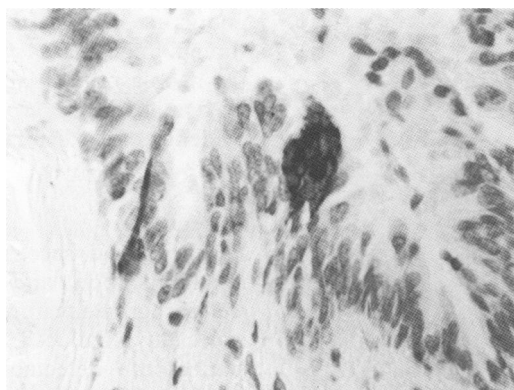


Figure 1 One solitary endocrine cell and one cluster, probably a neuroepithelial body, in a terminal bronchiole in one of the control lungs. This normal arrangement of endocrine cells was seen also in 13 of the 30 pairs of tumour-bearing lungs in which there was no non-tumoral pathology. ABC method for PGP 9-5; original magnification $\times 600$.

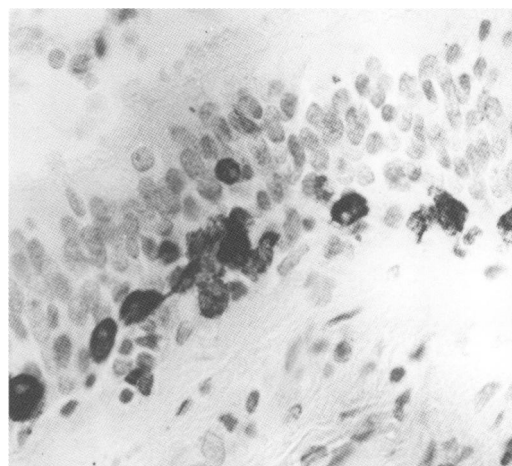


Figure 2 An interrupted row of endocrine cells in a tumour-bearing lung affected by bronchopneumonic consolidation. ABC method for PGP 9-5; original magnification $\times 600$.

or CGRP. Occasional cells were weakly immunoreactive for 5-HT, but none of the other secretory products sought was identified.

Of the 30 bronchial neoplasms, 12 were small cell and 18 were non-small cell tumours. Nine of the latter were squamous, five adenocarcinomas, two mixed adenosquamous, and two morphologically undifferentiated. Eight of the 12 small cell tumours were immunoreactive for NSE and/or PGP and there was focal immunopositivity for 5-HT in three. Two contained ACTH or a substance crossreacting with it. None of the other substances sought was identified in the group of small cell lesions, although five of the non-small tumours showed immunoreactivity for NSE and/or PGP.

In 13 pairs of tumour-bearing lungs the pulmonary endocrine cells appeared entirely normal in number, distribution and content, with characteristics identical to the equivalent populations in the 10 pairs of control lungs. In none of these lungs was there any significant non-tumoral pathology apart from focal collapse in the region of the tumour. In particular, there was no evidence of consolidation, cardiac failure (intra-alveolar haemorrhage or oedema), or fibrosis.

In the remaining 17 pairs of tumour-bearing lungs, however, proliferation of pulmonary endocrine cells had occurred to varying degrees in the form of IRs (fig 2), NAs (fig 3), or both, and in these lungs there was significant non-tumoral pathology, the most common of which was infective consolidation. This was a feature of 10 of the peritumoral and 11 of the ipsilateral/contralateral specimens. It was usually associated with the most marked form of endocrine cell proliferation, namely NAs (fig 3). These structures were seen in five of the 10 specimens of peritumoral lung (proliferation 4+) and eight of the 11 specimens of ipsilateral and contralateral lung (proliferation 4+) affected by consolidation. In the nine remaining specimens of pulmonary tissue showing infective consolidation, pulmonary endocrine cells had proliferated to form IRs in six (proliferation + in two, 2+ in one, and 3+ in three; fig 2), being normal in just three. The

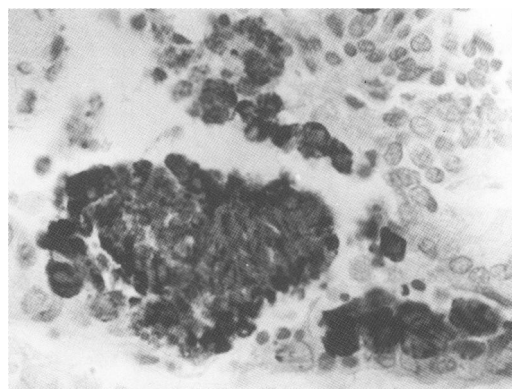


Figure 3 A nodular aggregate of endocrine cells in a tumour-bearing lung affected by bronchopneumonic consolidation. ABC method for PGP 9-5; original magnification $\times 600$.

most florid proliferation of all was seen in a subject with candidal infection in whom NAs were numerous and contained very large numbers of cells (proliferation 5+; fig 4). Of the remaining five specimens in which NAs were found but in which there was no consolidation, evidence of cardiac failure was present. In all of these proliferation was 4+.

There was no suggestion of any association between endocrine cell proliferation and tumoral pathology. Of the 12 lungs with NAs of pulmonary endocrine cells, six contained small cell and six non-small cell tumours (NS, Fisher's exact test). There was no suggestion either that NAs were more prevalent closer to the tumour and, in fact, most were found in ipsilateral lung distant from the tumour or in contralateral lung rather than in a peritumoral location. In two samples of lung which appeared entirely normal, the endocrine cells were also normal. The endocrine cells were also normal in five samples of lung where there were pathological changes – two in which there was consolidation alone, one in which there was consolidation as well as evidence of cardiac failure, and two in which evidence of cardiac failure was accompanied by changes of chronic bronchitis or bone marrow embolism.

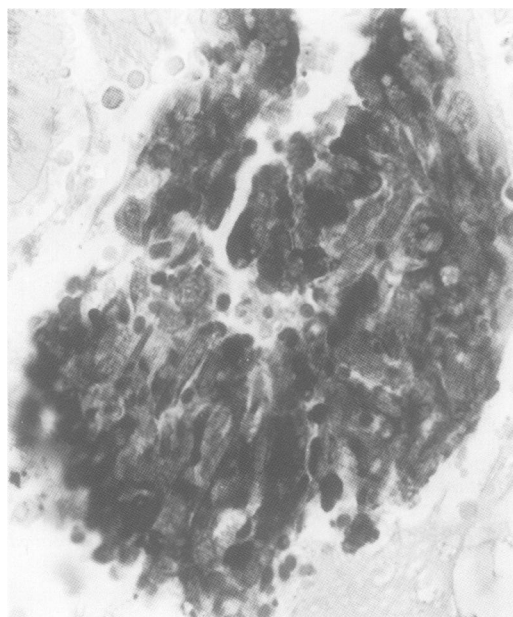


Figure 4 A very large nodular aggregate of endocrine cells in a lung affected by a severe candidal pneumonia. ABC method for PGP 9-5; original magnification $\times 600$.

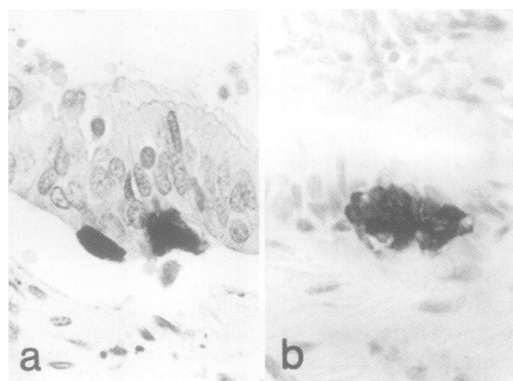


Figure 5 Immunoreactivity of proliferating endocrine cells for (a) ACTH and (b) HGH in candidal pneumonia. ABC method for ACTH and HGH; original magnification $\times 600$.

In areas of tumour-bearing lung where endocrine cells were normal, their content was predominantly of GRP and calcitonin, as in the control lungs. In IRs calcitonin often predominated but, apart from one case in which there was candidal infection, aberrant substances – that is, products not found in normal human pulmonary endocrine cells – were not generally seen. In this particular case, however, in which proliferation of endocrine cells was particularly florid (5+), ACTH and HGH were both seen (fig 5). In three further cases ACTH was demonstrable but HGH was not. In one further case HGH was found but immunoreactivity for ACTH was absent. AVP, somatostatin, and leu-enkephalin were not seen in any cells. Labelling for VIP was abandoned because of inadequate results with control tissue and despite using a variety of antisera and labelling conditions. In a number of IRs and NAs none of the secretory products sought – normal or aberrant – could be demonstrated.

There was no obvious relationship between the substances synthesised by any of the tumours and those found in the proliferating

endocrine cells in the surrounding lung, although in one case ACTH was present in both the neoplasm (a small cell carcinoma) and the NAs in the lung around it where there was evidence of cardiac failure.

Discussion

Synthesis and secretion of a wide range of amine, peptide, and protein hormones is a well recognised feature of bronchial carcinoma, especially small cell carcinoma.¹ These substances are detectable in a significant proportion of patients with the disease and occasionally cause clinical effects, Cushing's syndrome due to release of ACTH-like substances being the classical example. So accepted has this association become that increased levels of such hormones in patients with pulmonary tumours are automatically assumed to be due to secretion by the neoplasm, despite the fact that the substance in question is often undetectable in tumour tissue. Our findings suggest that an alternative mechanism, namely leakage of these substances from proliferating endocrine cells in diseased lung out with the tumour, might be responsible.

Although the functions of pulmonary endocrine cells remain unclear, there is considerable evidence now to suggest that they are almost certainly involved in the pathogenesis of or response to a variety of pulmonary diseases, including such conditions as bronchial asthma, respiratory infections, chronic bronchitis and emphysema, cystic fibrosis, hypertensive pulmonary vascular disease, eosinophilic granuloma, and several respiratory disorders of early life.^{1,7,8,11-18} In all of these conditions endocrine cells increase in number in what appears to be a fairly stereotyped way, their normal evenly spaced sparse distribution being first replaced by interrupted rows of cells (IRs) and then, probably only if the insult persists, by nodular aggregates of cells (NAs) which pile up to protrude into airways or alveoli.¹ In tandem with these changes, calcitonin often replaces GRP as their predominant secretory product and, occasionally, aberrant products appear.¹ The observation of this pattern of endocrine cell proliferation has been made repeatedly in lungs affected by the wide range of non-neoplastic conditions outlined above,^{1,7,8,11-18} and we consider this response to pulmonary injury to be well established in the literature.

There is evidence also to suggest that the products of proliferating endocrine cells sometimes leak into the circulation. Most attention has focused on calcitonin, with hypercalcaemia and hypercalcaionuria well documented accompaniments of a range of inflammatory pulmonary diseases,^{19,20} but ACTH is also sometimes increased in patients with non-neoplastic pulmonary disease^{21,22} in whom it is predominantly in the "big" form and is non-suppressible, features which suggest that it is not of pituitary origin. The results of investigations such as these, and of the present study, suggest that increased serum levels of a variety of hormones should be frequently detectable in the blood of patients with non-

neoplastic pulmonary disease. Surveying the available literature reveals that this is, in fact, the case.¹ Indeed, one of the problems which has confounded the use of numerous initially promising hormonal serum markers of bronchial carcinoma over the years is the prevalence of increased levels in the blood in patients with inflammatory disorders of the lungs.¹ It is this lack of specificity, rather than sensitivity, which has led to eventual disappointment in their practical use as markers of pulmonary malignancy.

The characteristics of the endocrine cell proliferation described in this study are typical of that described in previous work from this^{12 13 17} and other^{7 8 11 14–16 18} laboratories and raise currently unanswered questions about their possible role in the development of or response to a range of pulmonary disorders. The clear association between proliferation of these cells and non-tumoral pathology gives support for such a role, whereas the absence of any link with the tumour itself suggests that there is no direct association between them. A gradually increasing body of evidence from studies of normal pulmonary development as well as diseased lungs points strongly towards a role for peptides such as GRP in the growth, development, and repair of pulmonary tissues,^{1 23} an hypothesis supported by the findings of the present study. Why the most marked proliferation (NAs) should be associated with consolidation of the parenchyma or its filling with oedema fluid or haemosiderin-laden macrophages due to cardiac failure is unclear, but it is interesting to note that pulmonary infarction due to thromboembolism, in which the alveoli are flooded with blood, has also been associated with proliferation of pulmonary endocrine cells¹⁷ (and, indeed, with "ectopic" secretion of ACTH²²). Perhaps the severity of the parenchymal damage is an important factor.

The lungs we studied were taken at necropsy, at the end point of the natural history of bronchial carcinoma when the associated non-tumoral pathology described is common. However, earlier in the course of the disease, such as at initial presentation, these changes are less often present, so that the endocrine cell proliferation we describe might be less marked and less likely to account for the increased serum levels of hormones sometimes seen in such patients at this earlier stage. We have no way of knowing whether significant endocrine cell proliferation is present at this time, a question which could be adequately answered only by studying surgically resected lungs. Evidence from studies of the endocrinology of acute inflammatory pulmonary disease, however, suggests that the proliferation of endocrine cells in such circumstances is a rapid event and often extremely florid^{12 20} so that a causal relationship with increased serum hormone levels early in the course of bronchial carcinoma seems by no means implausible.

Why aberrant substances such as ACTH and HGH should appear at a time of marked proliferation of endocrine cells is uncertain, but it is interesting to note how both substances have been demonstrated in tumourlets,²⁴ the

locally invasive aggregates of pulmonary endocrine cells first described by Whitwell²⁵ which probably represent a further stage in their proliferation beyond that of NAs and which are seen only when the pulmonary damage stimulating it is particularly severe or prolonged.¹ The appearance of these substances is probably just a functional accompaniment of increasing morphological disorder.

The undisclosed products that might be present in the IRs and NAs which immunolabelled only for NSE and PGP 9.5 is a matter for speculation. A range of substances beyond those we were able to seek has been described in human pulmonary endocrine cells in occasional studies,²⁶ but whether they were present in some of the proliferating cells in the present investigation we cannot say.

Our hypothesis is not unprecedented. Recent studies of hepatobiliary disease describe neuroendocrine differentiation in and elaboration of parathyroid hormone-related protein by biliary epithelium, suggesting that the paraneoplastic hypercalcaemia sometimes seen in patients with hepatocellular carcinoma may be a consequence of changes in the bile ducts rather than inappropriate secretion by the tumour.^{27 28} Because we were confined to necropsy material, a consequence of needing to sample both lungs extensively, we were unable to measure serum levels of the substances we localised by immunolabelling so the mechanism we suggest remains unproven and based on only circumstantial evidence. A logical next step would be to extend the study to surgically resected pulmonary tumours and the endocrine cells of the pulmonary tissue removed along with them, and to relate the findings to serum levels of hormones measured in blood taken before surgery.

- Gosney JR. *Pulmonary endocrine pathology: endocrine cells and endocrine tumours of the lung*. Oxford: Butterworth-Heinemann, 1992.
- Coates PJ, Doniach I, Howlett TA, Rees LH, Besser GM. Immunocytochemical study of 18 tumours causing ectopic Cushing's syndrome. *J Clin Pathol* 1986;39:955–60.
- Blackman MR, Rosen SW, Weintraub BD. Ectopic hormones. *Adv Intern Med* 1978;23:85–113.
- Gosney MA, Gosney JR, Lye MDW. Serum adrenocorticotrophin and calcitonin in non-small cell carcinoma of the bronchus. *Life Sci Adv* 1991;10:53–7.
- World Health Organization. The World Health Organization histological typing of lung tumours. 2nd edition. *Am J Clin Pathol* 1982;77:123–36.
- Hsu SM, Raine L, Fanger H. Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. *J Histochem Cytochem* 1981;29:577–80.
- Gould VE, Linnoila RI, Memoli VA, Warren WH. Neuroendocrine components of the bronchopulmonary tract: hyperplasias, dysplasias and neoplasms. *Lab Invest* 1983;49:519–37.
- Tsutsumi Y, Osamura RY, Watanabe K, Yanaihara N. Immunohistochemical studies on gastrin-releasing peptide and adrenocorticotrophic hormone-containing cells in the human lung. *Lab Invest* 1983;43:623–32.
- Gosney JR. Neuroendocrine cell populations in postnatal human lungs: minimal variation from childhood to old age. *Anat Rec* 1993;236:177–80.
- Lauweryns JM, Peuskens JC. Neuroepithelial bodies (neuroreceptor or secretory organs?) in human infant bronchial epithelium. *Anat Rec* 1972;172:471–82.
- Stanislowski EC, Hernandez-Garcia J, Mora-Torres MA, Abajian-Polanco E. Lung neuroendocrine structures. Topography, morphology, composition and relation with intrinsic asthma (non-immune). *Arch Invest Med* 1981;12:559–77.
- Allibone RO, Gosney JR. Rapid changes in pulmonary endocrine cells in acute bronchopneumonic consolidation. *J Pathol* 1990;161:347.
- Gosney JR, Sissons MCJ, Allibone RO, Blakey AF. Pulmonary endocrine cells in chronic bronchitis and emphysema. *J Pathol* 1989;157:127–33.

- 14 Fukayama M, Hayashi Y, Shiozawa Y, Furukawa E, Funata N, Koike M. Human chorionic gonadotropin alpha-subunit in endocrine cells of fibrotic and neoplastic lung. *Lab Invest* 1990;**62**:444-51.
- 15 Johnson DE, Wobken JD, Landrum BG. Changes in bombesin, calcitonin and serotonin immunoreactive pulmonary neuroendocrine cells in cystic fibrosis and after prolonged mechanical ventilation. *Am Rev Respir Dis* 1988;**137**:123-31.
- 16 Aguayo SM, King TE, Waldron JA, Sherritt KM, Kane MA, Miller YE. Increased pulmonary neuroendocrine cells with bombesin-like immunoreactivity in adult patients with eosinophilic granuloma. *J Clin Invest* 1990;**86**:383-44.
- 17 Heath D, Yacoub M, Gosney JR, Madden B, Caslin AW, Smith P. Pulmonary endocrine cells in hypertensive pulmonary vascular disease. *Histopathology* 1990;**16**:21-8.
- 18 Johnson DE, Georgieff MK. Pulmonary neuroendocrine cells. Their secretory products and their potential role in health and chronic lung disease in infancy. *Am Rev Respir Dis* 1989;**140**:1807-12.
- 19 Becker KL, Nash D, Silva OL, Snider RH, Moore CF. Increased serum and urinary calcitonin levels in patients with pulmonary disease. *Chest* 1981;**79**:211-6.
- 20 Becker KL, Silva OL, Snider RH, Moore CF, Geelhoed GW, Nash D, *et al.* The pathophysiology of pulmonary calcitonin. In: Becker KL, Gazdar AF, eds. *The endocrine lung in health and disease*. Philadelphia: WB Saunders, 1984:277-99.
- 21 Ayvazian LF, Schneider B, Gerwitz G, Yalow RS. Ectopic production of big ACTH in carcinoma of the lung: its clinical usefulness as a biologic marker. *Am Rev Respir Dis* 1975;**111**:279-87.
- 22 Dupont AG, Somers G, VanSteirteghem AC, Watson F, Vanhaelst L. Ectopic adrenocorticotropin production: disappearance after removal of inflammatory tissue. *J Clin Endocrinol Metab* 1984;**58**:654-8.
- 23 Miller YE. Pulmonary neuroendocrine cells and lung development: dim outlines emerge. *J Clin Invest* 1994;**91**:1861.
- 24 Gosney JR, Green ART, Taylor W. Appropriate and inappropriate neuroendocrine products in pulmonary tumourlets. *Thorax* 1990;**45**:679-83.
- 25 Whitwell F. Tumourlets of the lung. *J Pathol Bacteriol* 1955;**70**:529-41.
- 26 Polak J, Becker KL, Becker E, Cutz E, Gail DB, Goniakowska-Witalinska L, *et al.* Lung endocrine cell markers, peptides and amines. *Anat Rec* 1993;**236**:169-71.
- 27 Roskams T, Willems M, Campos RV, Drucker DJ, Yap SH, Desmet VJ. Parathyroid hormone-related peptide expression in primary and metastatic liver tumours. *Histopathology* 1993;**23**:519-25.
- 28 Burt AD, MacSween RNM. Bile duct proliferation - its true significance? *Histopathology* 1993;**23**:599-602.