Ectopic ACTH syndrome: clinicopathological correlations

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SUMMARY Ten out of 164 cases of bronchogenic carcinoma showed pathological evidence at necropsy of the ectopic ACTH syndrome. All occurred in association with oat-cell carcinoma, constituting 19% of that group. The pathological features consisted of adrenocortical hyperplasia confined to the zona fasciculata and Crooke's hyaline change in the pituitary. Immunoperoxidase stainable ACTH was detected in the pituitary but not in the carcinoma tissue, a surprising finding, which may be due to the different nature of ACTH present in tumour tissue. The ectopic ACTH syndrome was diagnosed ante mortem in only four out of 10 patients on the basis of hypokalaemia and metabolic alkalosis. The lack of clinical pointers in all but terminal cases is discussed as well as possible measures for earlier diagnosis.

Although excessive secretion of ACTH by non-pituitary neoplasms, termed as ectopic ACTH syndrome (Liddle *et al.*, 1965), is now being recognised with increasing frequency (Liddle *et al.*, 1965; 1969; Neville, 1972; Rees and Ratcliffe, 1974; Rees, 1975; Smith, 1975), information is still limited on the clinicopathological correlations as well as on the real incidence of the condition. Attempts to assess the incidence of ectopic ACTH syndrome based on clinical findings failed to provide unequivocal results (Rees and Ratcliffe, 1974; Rees, 1975) chiefly because owing to rapid progress of the neoplastic process, signs of hypercorticism either are not manifest or are overshadowed by the more prominent symptomatology due to the neoplasm. Biochemical studies revealed that ACTH can be extracted from almost every human lung cancer tissue, including squamous-cell carcinoma, adenocarcinoma, and oat-cell carcinoma (Gewirtz and Yalow, 1974) as well as from other APUD cell tumours (Imura *et al.*, 1975; Hirata *et al.*, 1976). It was found, however, that tumour ACTH may lack biological activity while maintaining immunological reactivity (Gewirtz and Yalow, 1974; Rees 1975).

Sustained, excessive secretion of ACTH induces characteristic morphological changes in the adrenal cortex (Symington, 1969; Neville 1972) and sub-

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Received for publication 15 August 1977

sequently, by raising the levels of circulating corticosteroids, it causes easily recognisable changes in the anterior pituitary (Sziij *et al.*, 1969; Neville, 1972). Hence it seemed appropriate to undertake a detailed histological study of the adrenals and the pituitaries obtained at necropsy in patients with various forms of bronchogenic carcinoma in order to detect cases of ectopic ACTH syndrome. The reason for selecting bronchogenic carcinoma for the present work is that ectopic production of ACTH occurs most frequently in association with this type of tumour (O'Riordan *et al.*, 1966; Azzopardi and Williams, 1968). Our study, compared to previous clinical and biochemical investigations, represents an approach from a different viewpoint, it aims to determine the incidence of ectopic ACTH syndrome by using morphological techniques, and to correlate tumour, adrenal, and pituitary histology with the clinical findings.

Material and methods

Necropsies performed at St. Michael's Hospital over the last 20 years provided the material for this study. Cases with bronchogenic carcinoma were traced from the files, and slides of the tumour as well as those of the adrenals and the pituitary were studied. Necropsy had been performed between 3 and 44 hours post mortem.

From 164 bronchogenic carcinoma cases, based on adrenal and pituitary morphology, 10 were regarded as representing ectopic ACTH syndrome.

In 8 out of these 10 cases paraffin blocks and formalin-fixed tissues were also available for further studies. Hence tumour tissue and the remaining half of the horizontally cut pituitary glands were embedded in paraffin and recut. Sections of the tumours were stained with haematoxylin-phloxine-saffron and those of the pituitary glands with haematoxylin-phloxine-saffron, PAS, Goldberg-Chaikoff's trichrome, Mann's, Herlant's tetrachrome erythrosin, Brookes' carmoisine, lead haematoxylin, Gomori's aldehyde fuchsin, and aldehyde thionin techniques.

For immunocytological localisation of ACTH in the tumour and pituitary, the immunoperoxidase technique was used (Mason *et al.*, 1969; Sternberger *et al.*, 1970). Details of the technique have been described elsewhere (Kovacs *et al.*, 1976). The ACTH antiserum was raised in rabbits against synthetic 1-24 ACTH (Organon, Oss, Holland; kindly donated by Dr S. Hane and Dr P. H. Forsham, Metabolic Research Unit, University of California Medical Center, San Francisco, California, USA). Instead of applying horseradish peroxidase and antihorseradish peroxidase individually, a horseradish peroxidase-antihorseradish peroxidase complex was used (Cappel Laboratories Inc, Downingtown, Pennsylvania, USA). Binding sites of immunological reaction were demonstrated by 3,3'-diaminobenzidine. The specificity of immunostaining was verified by absorption of the anti 1-24 ACTH with excess antigen and by replacing the specific antibody with normal rabbit serum or phosphate buffered saline. No cross reaction was found toward other pituitary hormones. We did not attempt to extract ACTH from tumour or pituitary tissue to determine its tissue concentration.

Results

Based on the histology of the adrenal cortex and pituitary, 10 cases were regarded as representing ectopic ACTH syndrome. The morphological changes were distinctive and characteristic, and there were no difficulties in separating these 10 cases from those showing no adrenocortical and adeno-hypophysial changes or from those which exhibited other abnormalities.

INCIDENCE

The number of bronchogenic carcinoma cases studied in the present work is shown in Table 1. It is evident from the Table that the occurrence of the ectopic ACTH syndrome in cases of bronchogenic carcinoma is not rare (6%). All 10 cases were associated with oat-cell carcinomas (19%). No morphological changes indicating ectopic

Table 1 Incidence of ectopic ACTH syndrome in bronchogenic carcinoma

Tumour type	No. of cases	Cases with ectopic ACTH syndrome	%
Squamous-cell carcinoma	56	0	0
Adenocarcinoma	31	0	0
Oat-cell carcinoma	53	10	19
Anaplastic large-cell carcinoma	24	0	0
Total	164	10	6

ACTH syndrome were associated with squamous-cell carcinoma, adenocarcinoma, or anaplastic large-cell carcinoma. The present material reflects neither the exact number of bronchogenic carcinoma necropsies nor the incidence of the different histological types that had occurred at St. Michael's Hospital.

ADRENAL CHANGES

The adrenal glands in the 10 cases were considerably enlarged (Fig. 1), both weighing between 20 and 38 g. The cortex was brown on the cut surface, indicating a marked decrease in lipid content. By histology, the most conspicuous findings were the widening of the cortex and the loss of lipids (Fig. 2). Compact fasciculata cells predominated, and congestion was frequent. Zona glomerulosa cells were seen only in focal areas as isolated small islands, and in many places large fasciculata cells extended up to the

Fig. 1 (A) Control adrenal; (B) adrenal with ectopic ACTH syndrome. Enlargement and lipid depletion are apparent in the cortex. Arrow shows metastatic carcinoma. The primary tumour is a bronchogenic oat-cell carcinoma.

Fig. 2 Compact cell hyperplasia is noted in the adrenal cortex. Ectopic ACTH syndrome associated with bronchogenic oat-cell carcinoma. Haematoxylin-phloxine-saffron $\times 250$

fibrous capsule. In six cases foci of metastatic carcinoma were also found. The medulla showed no consistent abnormalities. The adrenocortical changes were characteristic and well defined. Based on the extent of involvement, the adrenal changes were divided into three classes showing slight (+), moderate (++), or severe (+++) abnormalities. The assessments were undertaken by two independent observers without knowing the clinical history or other pathological findings. The results are shown in Table 2.

PITUITARY CHANGES

The anterior pituitary showed various degrees of Crooke's hyalinisation in all 10 cases. These changes consisted of a deposition of glassy hyaline material

in the cytoplasm of the corticotroph cells (Fig. 3). The intensity of hyalinisation was assessed by a similar method to that in the adrenals, and the results are seen in Table 2. In cases of extensive changes, the hyaline material filled almost the entire cytoplasm, and only a few secretory granules remained visible in the perinuclear region and close to the plasma membranes. The hyaline material showed no staining with PAS or with lead haematoxylin, whereas the secretory granules showed intense coloration with these two staining procedures. The corticotroph cells, including the Crooke's cells, exhibited strong immunostaining for ACTH. Concordant with the results of Phifer *et al.* (1970), no immunoreactive ACTH was demonstrated in the hyaline material (Fig. 4).

Table 2 Pathological findings

Patient	Liver weight (g)	Metastasis in liver	Adrenal weight (g)	Metastasis in adrenals	Intensity of adrenocortical changes	Pituitary weight (g)	Extent of Crooke's hyalinisation
JG	5310	Yes	24.7	No	+++ - +++	0.6	+ - +
DL	1780	Yes	29.5	Yes	+++ - +++	0.4	+++ - +++
AL	4250	Yes	21.0	No	+++ - +++	0.5	+ - +
RH	1100	Yes	33.0	Yes	+++ - +++	0.4	+++ - +++
NS	2050	No	—	Yes	+++ - +++	0.3	+++ - +++
FW	2995	Yes	38.0	Yes	+++ - +++	0.5	+++ - +++
JN	3200	Yes	27.0	No	+++ - +++	0.5	+++ - +++
PB	2800	Yes	27.5	Yes	+++ - +++	0.7	+++ - +++
RE	3150	Yes	30.0	No	+++ - +++	0.5	+++ - +++
HN	1000	One small metastatic focus	20.0	Yes	+++ - +++	0.5	+ - +

ACTH immunostaining in tumour negative in all cases

Fig. 3 Extensive Crooke's hyalinisation (arrowheads) is evident in the anterior pituitary of a man with ectopic ACTH syndrome due to bronchogenic oat-cell carcinoma. PAS $\times$ 400

Fig. 4 Immunoperoxidase technique reveals the presence of immunoreactive ACTH in the cytoplasm of corticotrophs. The Crooke's hyaline material is negative. Immunoperoxidase technique $\times$ 400.

HISTOLOGY OF TUMOUR

In all 10 cases the tumours were typical examples of bronchogenic oat-cell carcinoma. Pleomorphism was, in general, pronounced and necrotic foci were common. The histology of the tumours

associated with adrenocortical compact cell hyperplasia and adeno-hypophysial Crooke's hyalinisation was indistinguishable from the histology of those which failed to show adrenocortical and pituitary changes. By using the immunoperoxidase technique,

no immunoreactive ACTH was demonstrated in any of the tumours. The cytoplasm of the tumour cells was also negative with the PAS technique and with the lead haematoxylin stain.

CLINICAL FINDINGS

The data are presented in Tables 3 and 4.

Five out of 10 patients had radiological evidence of an intrathoracic mass, one had hilar adenopathy, and one a pleural effusion with malignant cells detected in the sputum. The diagnosis of probable or definite bronchogenic carcinoma was, therefore, made in seven out of 10 patients.

Only two patients had Cushingoid features, consisting of facial plethora and increased pigmentation. Three patients had definite proximal myopathy. However, the most striking clinical feature, affecting eight out of 10 patients, was cachexia. More than half the patients were hypertensive and hyperglycaemic. The other striking finding was the presence of oedema, affecting six out of 10 patients and probably largely related to the sodium retaining effect of glucocorticoids, although hypoalbuminaemia may have played a part but was not documented. A presumptive diagnosis of ectopic ACTH syndrome was made only in the four patients

who had hypokalaemia and metabolic alkalosis; in two of these, plasma cortisol levels were measured and found to be grossly raised. Markedly raised cortisol levels are, however, often found in sick patients and by themselves are not diagnostic. The abnormal transaminase levels in one-third of the patients reflect carcinomatous liver involvement.

The mean survival time was only 12 days, half the patients surviving for less than one week after admission. This illustrates the diagnostic problem which exists in many of these cases, as the biochemical markers may be present only in the terminal phase of the disease or not even then, as shown in this study.

Discussion

The validity of the present study depends on how reliable this approach is to detect ectopic ACTH syndrome cases by morphological investigation of the adrenals and the pituitaries. Symington (1969) found that the adrenal changes were so characteristic that they could be recognised easily and the diagnosis of ectopic ACTH syndrome made convincingly by histologically examining slides

Table 3 Clinical features

Patient	Sex	Age	Cushingoid features	Cachexia	Myopathy	Hypertension	Tachycardia	Oedema	Icterus
JG	M	48		+	+	+	+	+	+
DL	M	58		+				+	
AL	M	60		+		+	+	+	+
RH	F	80			+				
NS	M	50	+		±		+		
FW	M	66				+	+	+	
JN	M	47		+				+	
PB	M	51		+			+		
RE	M	52		+		±		+	
HN	M	68	+	+	+	+	+		
Total positive			2/10	8/10	3-4/10	5-6/10	6/10	6/10	2/10

Table 4 Laboratory findings

Patient	Chest x-ray	K ⁺	HCO ₃ ⁻	Blood sugar	Liver enzymes	Clinical diagnosis	Survival (days)
JG	Hilar adenopathy	N	N	N	↑	Alcoholic liver disease	3
DL	Mediastinal mass	?	?	↑	?	Oat-cell ca. (sputum)	12
AL	?N	N	N	↑	↑	Ca. peritoneal tap	5
RH	Hilar mass	N	N	N	↑	? Bronchial ca.	41
NS	?N	↓	↑	↑	N	Bronchial ca.	18
FW	Pleural effusion	↓	↑	↑	N	Bronchial ca. (sputum)	5 h
JN	N	↓	↑	↑	?	Nephrotic syndrome Alcoholic liver disease	4
PB	LUL mass	?	?	↑	?	Bronchial ca. (sputum)	13
RE	L hilar mass	↓	↑	?	↑	Bronchial ca.	14
HN	Mediastinal mass	N	N	N	?	Oat-cell ca. (sputum)	12
Total positive	7/10	4/8		5-6/9	3-4/7		

ACTH secreting tumour considered *ante mortem* in 4/10. N = normal

taken from the adrenal cortices. Concordant with Symington's (1969) view, the present study also showed that in cases of ectopic ACTH syndrome, distinctive histological changes can be demonstrated in the adrenal glands very similar to those seen in patients after protracted administration of pharmacological doses of ACTH (Studzinski *et al.*, 1963; Symington, 1969; Neville and Mackay, 1972). It is known that, in association with prolonged illnesses or severe stress reactions, the adrenals are enlarged and the cortices depleted of their lipid content (Symington, 1969). In these cases, however, compared to ectopic ACTH syndrome, adrenocortical hyperplasia is not so extensive and Crooke's hyalinisation in the anterior pituitary is absent. One condition that may be accompanied by similar adrenocortical changes indistinguishable in some cases from ectopic ACTH syndrome is Cushing's disease (Symington, 1969; Neville and Mackay, 1972). This is not unexpected, since in both diseases the adrenal cortices are under the stimulatory effect of ACTH. Although in many cases of Cushing's disease the adrenal enlargement is nodular and not as extensive as that of ectopic ACTH syndrome and, in addition, diffuse or focal hyperplasia of clear cells is also frequently apparent (Symington, 1969), the adrenal changes may be identical in the two conditions, and Crooke's hyalinisation in the anterior pituitary may also be present.

Recently, corticotropin releasing (CRF) activity was detected in extracts of several cases of bronchogenic carcinoma (Upton and Amatruda, 1971; Imura *et al.*, 1975). Whether tumours secreting only CRF cause adrenocortical and pituitary changes similar to those seen in cases of ectopic ACTH syndrome cannot be stated at present.

The formation of Crooke's hyaline material in the cytoplasm of corticotroph cells can be considered as a specific indication of glucocorticoid excess, occurring in no other condition (Halmi *et al.*, 1971; Uei and Suzuki, 1974). Thus the presence of Crooke's hyalinisation in our cases provides strong evidence that blood corticoid levels were indeed increased.

Hence, based on these considerations, it is reasonable to conclude that the 10 cases represent true examples of the ectopic ACTH syndrome, including even those which clinically showed no apparent symptoms. It may be that the incidence is more common than that shown in our figures, since some cases might have been overlooked because the histological changes in the adrenals and the pituitaries were not sufficiently prominent. Symington (1969) noted that Crooke's hyalinisation may be an inconsistent finding in cases of ectopic ACTH syndrome. This may well be so in cases associated

with a slight or short lasting rise of blood corticoid levels.

According to a recent review (Rees, 1975), the incidence of ectopic ACTH syndrome in cases of bronchogenic carcinoma, assessed on the basis of clinical symptoms and laboratory findings, varies between 0 and 22%. Our study provides new information from another angle, showing that secretion of ACTH by bronchogenic carcinoma is not infrequent. All 10 cases which exhibited adrenocortical compact cell hyperplasia and adeno-hypophyseal Crooke's hyalinisation in our material represented oat-cell carcinomas, indicating that oat-cell carcinoma possesses the appropriate genetic code to produce ACTH.

Biochemical studies convincingly proved that ACTH can be extracted from the tumour tissue in cases of ectopic ACTH syndrome (Liddle *et al.*, 1969; Orth *et al.*, 1973; Rees, 1975). The presence of immunoreactive ACTH in tumours associated with ectopic ACTH syndrome was also demonstrated by immunofluorescent techniques (Jarrett *et al.*, 1964; O'Neal *et al.*, 1968). Dense bodies of various sizes found in the cytoplasm of tumour cells by electron microscopy in cases of ectopic ACTH syndrome were interpreted as representing secretory granules and were assumed to contain ACTH (O'Neal *et al.*, 1968; Corrin and McMillan, 1970; Kay and Willson, 1970; Pimstone *et al.*, 1972; Rawlinson, 1973). However, the majority of the ACTH appears to be present as big ACTH (Gewirtz and Yalow, 1974), which is immunoreactive but not significantly bioreactive, and a variable but often large amount of the hormone is present as C-terminal fragments which do not cross-react with the anti N-terminal 1-24 serum and are biologically inactive (Ratcliffe *et al.*, 1972; Orth and Nicholson, 1973). Tissue ACTH concentrations have been studied (Gewirtz and Yalow, 1974; Orth and Nicholson, 1977) and showed a wide range. Correlation between tissue and plasma levels correlated poorly, which may explain the relatively infrequent occurrence of the clinical syndrome despite the virtually invariable finding of ACTH in these tumours.

The fact that most ACTH appears to be present as a C-terminal fragment may be the reason why no immunoreactive ACTH was demonstrated in tumour tissue in our study using an N-terminal directed antiserum which was shown to stain with pituitary tissue in the same patients. Other possibilities are that the hormone may not have been stored in large enough amounts to allow for detection using this technique or that the antigenic determinants were not free, being bound to a protein molecule, or were changed, dissolved, or inactivated during the fixation and embedding procedure. We have

recently detected ACTH in fresh necropsy material of an oat-cell carcinoma, using the same technique. This may point to a problem of degradation or fixation of the hormone as the cause of non-staining in the previous specimens which we are studying further by means of antisera directed against other parts of the ACTH molecule. Obviously, more work is required to establish the value of the immunoperoxidase technique in detecting ACTH in tumour tissue in cases of ectopic ACTH syndrome.

The clinicopathological correlations were only satisfactory in those patients showing hypokalaemic alkalosis, who constituted 50% of those in whom electrolytes were determined. Only two patients had a plethoric appearance suggestive of Cushing's syndrome. Cachexia was the most frequent feature, with hypertension, tachycardia, and oedema also commonly seen.

Clearly, the biochemical disturbance of hypokalaemia with metabolic alkalosis and hyperglycemia are dependent on the degree of hypercorticism and will thus be missed in many instances. Striking electrolyte changes are rarely seen in Cushing's Syndrome precisely because of the usually lower levels of cortisol secreted. Hence, these are crude markers for the ectopic ACTH syndrome. High plasma cortisol levels showing a lack of diurnal pattern and non-suppressed with 8 mg. of Dexamethasone might be better markers, although sick patients from whatever cause would be expected to show similar values in some instances. However, as an initial marker, plasma cortisol suppressability might be useful when bronchogenic or other APUD cell malignancy is suspected, both as a diagnostic tool and because adrenal suppression by medical or surgical means has been shown to prolong survival in many of these patients (Liddle *et al.*, 1969; Gewirtz and Yalow, 1974; Ayvazian *et al.*, 1975). More specific markers may be the detection of big ACTH or its C-terminal fragments in the circulation. In view of the low survival statistics in those patients in whom the diagnosis is first picked up radiologically, the importance of finding an earlier marker cannot be overstressed.

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