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Acid phosphatase activity in carcinoma of the bronchus

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Nicholls, D. P. and Davies, J. S. (1977). *Thorax*, **32**, 472–477. **Acid phosphatase activity in carcinoma of the bronchus.** Two cases are presented in which an undifferentiated small-cell carcinoma of the bronchus was associated with a considerably raised serum tartrate-labile acid phosphatase concentration. In neither case was there any evidence of a carcinoma of the prostate which, with a few rare exceptions, is the only condition associated with such increased enzyme activity. A survey of acid phosphatase concentrations in 30 other male patients with carcinoma of the bronchus showed that five patients had a raised total acid phosphatase concentration, but only one patient had a raised tartrate-labile acid phosphatase concentration. When the histological type was taken into account, elevated total acid phosphatase concentrations were associated with squamous-cell and adenocarcinoma but not with undifferentiated, small-cell carcinoma. Raised tartrate-labile acid phosphatase concentrations were associated only with squamous-cell carcinoma, in contradistinction to the two individual cases reported. In no group was there any correlation between acid and alkaline phosphatase concentrations.

Histological studies in one of our patients showed little tissue acid phosphatase staining in the primary tumours but intense staining in the cytoplasm of pancreatic cells surrounding a metastasis. Sections of pancreas from six normal subjects showed only slight tissue acid phosphatase activity. The possible sources of the increased serum acid phosphatase are discussed, and it is suggested that the pancreas could be the source of such increased activity in our second case.

Acid phosphatase (AcP) activity in the serum is derived from several sources. The fraction arising from the prostate gland is usually isolated by tartrate-inhibition (Abul-Fadl and King, 1949), but the specificity of this has been questioned (Dow and Whitaker, 1970; Yam, 1974). Both the total and the tartrate-labile AcP in the serum may be increased in diseases other than carcinoma of the prostate (Sullivan *et al.*, 1942; Herbert, 1946; Cooke *et al.*, 1962) or by artefact (Bonner *et al.*, 1954; Ozar *et al.*, 1955). Marked elevation of either fraction is rarely found except in carcinoma of the prostate (Bensley *et al.*, 1948; Cline *et al.*, 1955) and has not previously been described in association with carcinoma of the bronchus.

Case reports

CASE 1

A 63-year-old man, a non-smoker, presented with a deep vein thrombosis in the left calf. There were no other significant findings and rectal examination was normal. Investigations included: bilirubin 23·8 mmol/l (1·4 mg/100 ml); serum aspartate aminotransferase 124 IU/l (normal 13–42); serum alanine aminotransferase 38 IU/l (normal 11–55); alkaline phosphatase 320 IU/l (normal 25–92); total AcP 16·0 IU/l (normal 0·5–6·0); tartrate-labile AcP 14·5 IU/l (normal 0–2). A chest radiograph showed two ill-defined opacities in the right upper zone, and radiographs of the lumbar spine and pelvis were normal. During the course of investigation his clinical state rapidly deteriorated and he died three weeks after admission.

Necropsy showed a carcinoma arising from the right upper lobe bronchus, about 2 cm in diameter, and distal bronchopneumonic consolidation. There were multiple secondary deposits in the liver, left adrenal, and para-aortic lymph nodes. The prostate showed moderate hypertrophy of the median

lobe, but serial sections of the gland showed no abnormality. Histology showed the primary tumour to be an undifferentiated small-cell carcinoma.

CASE 2

A 50-year-old man, a heavy smoker, presented with pain in the arms. The pain was relieved by analgesics but recurred some four months later along with dyspnoea on exertion, weight loss, and slight haemoptysis. On examination there was diminished air entry at the right lung base. Investigations included: alkaline phosphatase 73 IU/l; total AcP 11·0 IU/l; tartrate-labile AcP 10·0 IU/l; calcium 2·15 mmol/l (8·6 mg/100 ml); phosphate 0·90 mmol/l (2·8 mg/100 ml). A chest radiograph showed a mass at the right hilum with heavy shadowing in the right lower zone. In addition the left hilum was prominent with faint shadowing in the left mid-zone.

After bronchoscopy, which confirmed bilateral tumours, his condition rapidly deteriorated. He died some nine months after the onset of illness, following several episodes of profuse haemoptysis. Necropsy showed a large tumour mass arising from the right main bronchus with consolidation of the middle and lower lobes and an abscess in the lower lobe. There was also a tumour mass arising from the left lower lobe bronchus. Several metastases were present in the pancreas, but not elsewhere, and the prostate appeared normal.

Histological examination of both tumours and the metastases showed undifferentiated small-cell carcinoma. The pattern of growth suggested two separate primary sources. The prostate showed focal chronic prostatitis only, and the pancreas itself appeared normal.

Methods

The measurement of serum total and tartrate-labile AcP was carried out on a Vitatron digital concentration photometer at pH 4·9, using phenyl disodium orthophosphate as substrate (Gutman and Gutman, 1938) and tartrate inhibition (Abul-Fadl and King, 1949). Phenyl phosphate is more sensitive, but less specific, than other substrates for measuring prostatic enzyme activity (Walker *et al.*, 1954; Woodard, 1959). Freshly separated unbuffered serum was used. No patient was constipated, catheterised, or had been examined rectally in the preceding 24 hours. Because of the reduced sensitivity of the photometer at low values, readings below 0·5 IU/l cannot be accurately quantified. Such values are regarded as 0·5 IU/l in the data analysis, which would tend to reduce the difference between the sample groups.

For a control series, samples were taken from 30 male hospital inpatients with unrelated disorders, age range 41–69 (mean 54) years. Samples were then taken from 30 male patients with carcinoma of the bronchus proven by histology (21) or cytology (9). The two cases described above were not included. The age range was 40–76 (mean 58·3) and none had symptoms of prostatic disease. Analysis of the results was carried out using Student's two-sample t test.

From case 2 sections of both the primary tumours and a pancreatic metastasis were stained using sodium α-naphthyl-phosphate as substrate (Grogg and Pearse, 1952). The sections were obtained at routine necropsy and were fixed in formalin. There was no evidence of autolysis. Figure 1 shows the section of pancreas containing a metastasis, at low and high power magnification.

To establish the normal staining of pancreas for AcP activity, sections of pancreas were removed at necropsy from six cases, three male and three female, age range 6–90 (mean 69·7) years. All had died from unrelated disorders. Each section was divided into two, one portion being fixed in formalin, and the other transported in saline before being frozen within 24 hours. Routine histological preparations, stained with haematoxylin and eosin, were taken from the formalin-fixed specimens.

Results

From the control series, the normal range for our laboratory of total AcP, expressed as mean ± 2 standard deviations, to the nearest 0·5 IU fraction was 0·5–6·0 IU/l, and for tartrate-labile AcP 0–2·0 IU/l. Table 1 compares the total AcP levels in the various groups with the control series, and Table 2 compares the tartrate-labile AcP levels.

There was no significant correlation in any group between acid and alkaline phosphatase levels.

On histochemical staining of the tumours and metastasis from case 2, the pancreatic tissue surrounding the metastasis showed intense AcP activity in the cytoplasm, whereas the other tissues showed very little activity. In the control series, no histological abnormalities were seen. Very light lysosomal AcP staining was noted, especially in the islet cells. Early autolytic changes caused some increase in this staining, and more severe autolytic changes caused disruption of the normal histology and focal heavy AcP staining. There was no apparent difference between the formalin-fixed and the frozen sections. In no specimen was the tissue AcP staining comparable to that seen

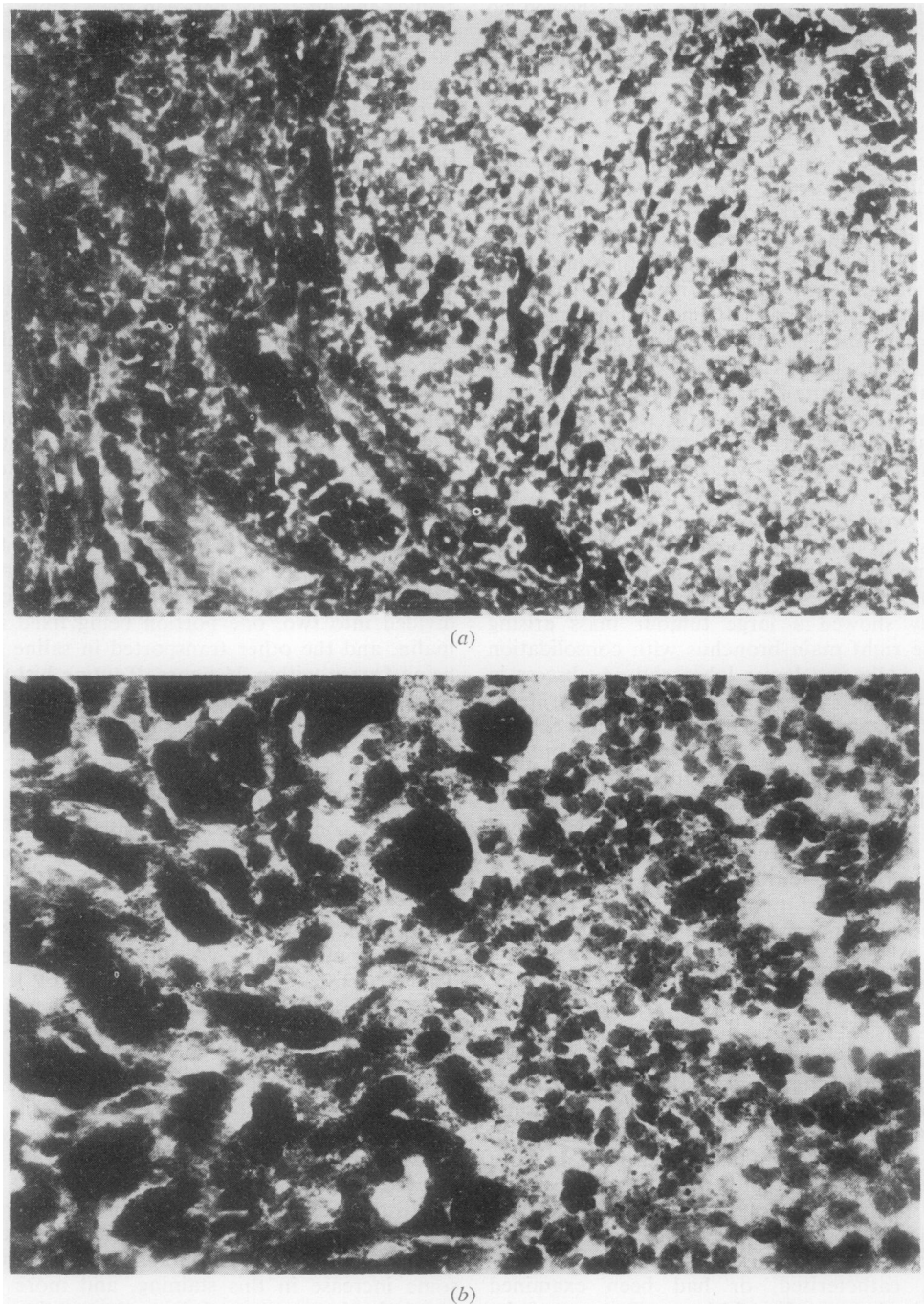

Fig. 1 *Case 2. (a) Low-power photomicrograph of pancreas, stained for acid phosphatase, showing a metastasis on the right. (b) High-power view of the same section showing intense staining of the pancreatic cells for acid phosphatase but poor staining of the tumour cells.*

Table 1 *Total AcP levels*

Group	*No.*	*Range*	*Mean*	*SD*	*No.* > 6	P
Control	30	1·0–6·0	3·17	1·38	0	—
Undifferentiated small-cell	17	1·0–7·0	3·59	1·56	1	> 0·05
Squamous-cell	9	2·0–9·5	5·22	2·24	3	< 0·01
Adenocarcinoma	4	3·0–7·0	5·00	1·83	1	< 0·05
Overall carcinoma bronchus	30	1·0–9·5	4·82	2·00	5	< 0·05

Table 2 *Tartrate-labile AcP levels*

Group	*No.*	*Range*	*Mean*	*SD*	*No.* > 2	P
Control	30	0·5–2·0	0·75	0·50	0	—
Undifferentiated small-cell	17	0·5–2·0	0·91	0·51	0	> 0·05
Squamous-cell	9	0·5–3·0	1·39	0·78	1	< 0·01
Adenocarcinoma	4	0·5–2·0	1·13	0·63	0	> 0·05
Overall	30	0·5–3·0	1·08	0·53	1	< 0·05

in the pancreas from case 2.

Discussion

A raised serum total AcP has previously been associated with carcinoma of the bronchus (Sullivan *et al.*, 1942; Mathes *et al.*, 1956), but the elevation was moderate, being less than twice the upper limit of normal. A previous survey of serum AcP in pulmonary disease, including carcinoma of the bronchus, showed no marked elevations (Pelocchino and Concina, 1956). Increased AcP activity has also been noted in association with carcinoma of the stomach (Simon and Nygaard, 1959), carcinoid tumour of the rectum (Davidson and McDougal, 1976), Gaucher's disease (Tuchman *et al.*, 1959), multiple myeloma (Frenkel and Tourtellotte, 1962), leukaemias (Bases, 1966), thromboembolic disorders (Schoenfeld *et al.*, 1962), and multiple endocrine adenomatosis (Mick, 1972).

Recent isoenzyme studies on AcP have suggested that abnormal patterns may be more frequent than is usually recognised, as the total AcP level may be normal (Yam, 1974). This technique may also help to distinguish the source of increased serum AcP activity, as izoenzyme 2 is produced by the pancreas and isoenzyme 4 by the lung (Lam *et al.*, 1973). However, both are produced by the prostate, and tartrate-inhibition cannot distinguish between them. These variations in the source of AcP activity may account for the fact that tartrate-labile AcP activity is the same in normal men and women (Ozar *et al.*, 1955). In the case of carcinoma of the stomach mentioned above (Simon and Nygaard, 1959), most of the increased serum AcP activity was tartrate-labile, whereas in the case of carcinoid tumour of the rectum (Davidson and McDougal, 1976) most of the activity was not inhibited by tartrate but was inhibited by ethanol. Unfortunately, isoenzyme electrophoresis was not available to us.

The nuclei of almost all cells show AcP activity on histochemical staining, but only neurones and prostatic epithelium show significant cytoplasmic staining (Woodard, 1942). Nuclear staining is increased in human neoplasms (Lemon and Wisseman, 1949). Cytoplasmic staining is sometimes increased, especially in carcinoma of the prostate (Gomori, 1941). Normal bronchial mucosa shows little activity (Gomori, 1941), and bronchial neoplasms do not usually stain heavily (Biressi and Concina, 1957). Normal pancreas shows little tissue AcP activity (Gomori, 1941), and our findings seem to confirm this. In the case of carcinoid tumour of the rectum mentioned above (Davidson and McDougal, 1976), heavy tissue AcP staining was noted in the primary tumour and its metastases, whereas in our case 2, increased cytoplasmic activity was found in the pancreas surrounding a metastasis but not in the primary tumours or metastases. It is interesting to note that the case of multiple endocrine adenomatosis previously reported (Mick, 1972) included a non-β islet-cell carcinoma of the pancreas with liver metastases.

It seems therefore that increased serum AcP activity may arise in different ways. Possible sources in our cases include thromboembolism, liver, circulating red cells, bone, pancreas, prostate, or the tumour itself.

Although case 1 had an undoubted deep vein thrombosis at the time his serum AcP level was noted to be high, there was no evidence of venous thrombosis in case 2. The liver has been

suggested as the source of increased AcP activity in carcinoma patients (Schoenfeld, 1972). Liver AcP is tartrate-labile (Abul-Fadl and King, 1949). There was no evidence of liver metastases in case 2, but as increased tissue AcP activity has been noted in a histologically normal liver in a case of myeloma (Frenkel and Tourtellotte, 1962), it is not possible to exclude the liver as the source of increased AcP activity in our cases. Red cell AcP is increased in carcinoma patients (Schoonover and Ely, 1935), but this is not inhibited by tartrate (Woodard, 1959). Moderate elevations of AcP may be present in metastatic bone disease (Sullivan *et al.*, 1942), but in our cases there was no clinical evidence of bone metastases, and none was demonstrated in the limited radiological and necropsy studies.

It is more likely that the increased serum AcP activity arose from the tumour itself, the prostate, or (in case 2) the pancreas. Many cases of carcinoma of the prostate have a reduced tissue AcP activity (Woodard, 1952), and the raised serum AcP is presumed to be due to the breakdown of some natural barrier around the gland, permitting leakage of AcP into the circulation, and an increase in tissue bulk (Yam, 1974). The same mechanisms may act in carcinoma of the bronchus, but bronchial mucosa is not a natural reservoir of AcP activity. It is possible that the primary tumour or its metastases produces an unidentified 'releasing factor' acting on the prostate gland, but this would not seem to be the explanation in other cases which have a raised total AcP but normal tartrate-labile AcP.

In view of the intense pancreatic tissue AcP activity found in case 2, it seems probable that the pancreas could be the source of increased serum AcP in that case at least. This may be simply a cellular reaction to the presence of neighbouring neoplastic tissue. This concept of a non-specific response seems to fit the observations already noted that increased serum AcP activity of different types may arise in a wide variety of seemingly unrelated disorders and that AcP itself is found in almost all cells. Our observations confirm that raised serum tartrate-labile AcP concentrations may on occasion be associated with conditions other than carcinoma of the prostate.

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