Summary

1. Amounts of prostaglandin E and prostaglandin F have been measured by radioimmunoassay in extracts of renal cortical carcinoma and benign and malignant breast tumours after solvent extraction and column chromatography.

2. Substantial amounts of prostaglandin E were found in extracts of benign and malignant breast tumours and in renal tumours. Much lower amounts of prostaglandin F were present in all tumour types.

3. Co-cultivation of tumour explants with mouse calvaria led to significant bone resorption in 10 of 13 renal carcinomas, three of eight malignant breast tumours, and two of nine benign breast tumours. Tumours associated with bone resorption had higher concentrations of prostaglandin E in culture media at the end of incubation than did non-resorbers.

4. Indomethacin (14 μmol/l) greatly reduced bone resorption in the presence of the tumour, but this was not always complete, particularly with breast tumours. Indomethacin had no effect on prostaglandin-induced bone resorption. Theophylline (2-2 mmol/l) significantly increased prostaglandin E production and resorption by an effect on the tumour.

5. It is concluded that prostaglandins may be important in mediating the effects of renal cortical carcinoma and possibly breast cancer on bone destruction. A non-prostaglandin mechanism may also contribute to bone destruction by breast carcinoma.

Key words: bone resorption, breast cancer, cyclic AMP, hypercalcaemia, kidney cancer, prostaglandins.

Introduction

The demonstration by Klein & Raisz (1970) that prostaglandins are potent stimulators of bone resorption has been amply confirmed. Although the bone-resorbing activities of prostacyclin and thromboxane A₂ have not been reported, prostaglandin E₂ is more potent in this respect than any of its metabolites (Seyberth, Hubbard, Oelz, Sweetman, Watson & Oates, 1977; Tashjian, Tice & Sides, 1977). Cells of most tissues can produce prostaglandins, particularly inflammatory and neoplastic cells (Jaffe, Parker & Philpott, 1971; Ferraris, DeRubertis, Hudson & Wolfe, 1974; Bray, Gordon, Morley & Myatt, 1975; Tolone, Bonasera, Brai & Tolone, 1977). Thus prostaglandins may play a role in the excessive bone destruction which accompanies certain inflammatory and neoplastic diseases (Tashjian, Voelkel, Levine & Goldhaber, 1972; Goodson, Dewhirst & Brunetti, 1974; Robinson, Tashjian & Levine, 1975; Voelkel, Tashjian, Franklin, Wasserman & Levine, 1975).

Although prostaglandin E₂ may be the cause of hypercalcaemia in two animal cancers (Tashjian et al., 1972; Voelkel et al., 1975), the data in human
cancer are less compelling. Seyberth, Segre, Morgan, Sweetman, Potts & Oates (1975) found elevated urinary concentrations of the major prostaglandin E metabolite in patients with hypercalcaemia associated with various solid tumours. Inhibitors of prostaglandin synthesis reduced both the hypercalcaemia and the concentrations of metabolite in urine.

Bone resorption can be stimulated by breast carcinoma tissue in culture (Powles, Clark, Easty & Neville, 1973; Dowsett, Easty, Powles, Easty & Neville, 1976a; Jenkins, Polanska & Wills, 1976; Powles, Dowsett, Easty & Neville, 1976). Furthermore, the ability to stimulate bone resorption is not confined to malignant breast tumours, but can occur also with benign lesions (Dowsett, Gazet, Powles, Easty & Neville, 1976b; Jenkins et al., 1976). The nature of the agents responsible for this resorption has not been conclusively determined, since although prostaglandins are produced by the cultures, resorption cannot always be fully inhibited by prostaglandin synthesis inhibitors.

Atkins, Ibbotson, Hillier, Hunt, Hammonds & Martin (1977) stimulated resorption of mouse calvaria during culture with nine of 13 renal cortical carcinomas. The activity was associated with elevated concentrations of prostaglandin E, and could be inhibited by indomethacin. Carcinomas of the kidney and breast are commonly associated with the development of skeletal metastases and hypercalcaemia. In the present work we have investigated the ability of these tumours to produce bone reabsorption, and have measured prostaglandins extracted or released from tumours to assess their role in skeletal effects.

Materials and methods

Materials

BGJ culture medium (Biggers, Gwatkin & Heynor, 1961) was prepared from powder. The final solution was supplemented with antifungal agent, ascorbic acid (150 µg/ml) and 15% heated horse serum as described previously (Webster, Atkins & Peacock, 1974). The parathyroid hormone used was partially purified bovine hormone with a potency of 1000 units/mg (Moseley, Martin, Robinson, Reit & Tregear, 1975). Prostaglandins and their metabolites were gifts from Dr J. E. Pike, Upjohn Co., Kalamazoo, U.S.A. Antibodies, raised in rabbits, directed against prostaglandin E2 and prostaglandin F2α, were gifts from Dr Lawrence Levine, Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts, U.S.A. 3H-labelled prostaglandin E2 and prostaglandin F2α (specific radioactivities 120–170 Ci/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks, U.K.

Calvaria for use in bone culture were obtained from 5- to 7-day-old Swiss albino mice bred in our own colony. Tumour tissue was obtained from patients undergoing routine surgery or breast lump or carcinoma of kidney; histological examination confirmed the diagnosis in all cases.

Methods

Extraction of prostaglandins from tumours

Tumour samples were collected on ice immediately on excision and either processed within 45 min or frozen at −20°C until used within 24 h. Tissue was blotted dry, weighed and washed rapidly in Tris [0.1 mol/l of sodium chloride (saline: 50 mmol/l)] solution, pH 7.4, at 4°C, then cut with scissors and homogenized for 2 min in a Polytron homogenizer in 4 ml of buffer/g of tissue together with 3 vol. of an extraction mixture consisting of ethyl acetate/propan-2-ol/hydrochloric acid (0.1 mol/l) (3:3:1, by vol.). Care was taken to ensure uniformity of the procedure for all samples. Extraction and chromatography were then carried out as described below.

Prostaglandin radioimmunoassay

Radioimmunoassay of prostaglandins E and F were performed on culture medium from bone resorption experiments after 3 days of culture and on extracts of tumour homogenates. To increase assay specificity and minimize interference all samples for radioimmunoassay were extracted into acidified ethyl acetate (Jaffe & Behrmann, 1973). Further separation of prostaglandins was achieved by chromatography of extracts on microcolumns of silicic acid (60–200 mesh) with a benzene/ethyl acetate/saline: 50 mmol/l (1:3:1, by vol.). Care was taken to ensure uniformity of the procedure for all samples. Extraction and chromatography were then carried out as described below.
Prostaglandins and cancer

1 h with 0.1 ml of [3H]prostaglandin E₂ (4000 c.p.m.) and 0.1 ml of antibody (at a final dilution of 1 in 500 for prostaglandin E and 1 in 1000 for prostaglandin F). Separation of free from bound labelled prostaglandin was achieved with dextran-coated charcoal (Herbert, Lau, Gottlieb & Bleicher, 1965). A standard curve for the appropriate prostaglandin was included in each assay.

Bone-resorption bioassay

The method used for the detection of bone resorption in vitro has been described previously (Webster et al., 1974; Atkins et al., 1977). Calvaria were explanted and equilibrated in bulk in 40 ml of BGJ culture medium for 24 h at 37°C in an atmosphere of 5% CO₂ in air. Each calvarium was then placed on a stainless-steel grid in a small dish containing 2 ml of fresh culture medium. Tumour tissue was obtained fresh and transported from the operating theatre in cold sterile sodium chloride solution (150 mmol/l) for use in culture within 2 h of excision. Tissue explants (4 mm × 1 mm³) were placed around the bone but not in contact with it. The viability of tumour tissue was assessed by histological examination at the beginning and end of the culture period. For each tumour, experiments were performed to assess calcium release from calvaria alone, or in the presence of parathyroid hormone (1 unit/ml), tumour explants with or without indomethacin (14 μmol/l) or theophylline (2.2 mmol/l). Between six and 12 replicate bone cultures were carried out for each treatment. At the end of 3 days of culture, the calcium concentration in each medium was measured by automatic titration with a Corning model 503 calcium analyser. Differences from controls were assessed by means of Student's t-test for unpaired data; values of P < 0.05 were considered to be statistically significant.

Results

Preliminary results

Prostaglandin E₂ was more effective in promoting bone resorption than any other prostaglandin, metabolite or analogue (Table 1). The threshold responsiveness of bone resorption to prostaglandin E₂ in this system is 5–10 ng/ml (Atkins et al., 1977). Concentrations of theophylline used in later experiments had no effect on the bone resorption produced by maximally or submaximally effective concentrations of prostaglandin E₂ or parathyroid hormone. Indomethacin (14 μmol/l) did not inhibit the bone resorption induced by either agent.

The radioimmunoassays for prostaglandin E and F were sensitive to 0.1 ng of prostaglandin E and 0.2 ng of prostaglandin F/tube, where sensitivity was determined as the mass of prostaglandin required to exhibit binding of tracer to antibody by 10%. When a known mass of prostaglandin was added to culture medium and the medium treated by extraction and chromatography as described above, the curve obtained with the appropriate

<table>
<thead>
<tr>
<th>Table 1. Potency of various prostaglandins and metabolites in stimulating bone resorption in cultured mouse calvaria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone resorption was estimated by measuring calcium release in 48 h cultures in response to each substance (at least three concentrations), with prostaglandin E₂ as standard. Potencies were related to prostaglandins E₂ (=100) by noting the amount required to achieve 50% of maximal stimulation of resorption.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prostaglandin</th>
<th>Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostaglandin E₁</td>
<td>100</td>
</tr>
<tr>
<td>Prostaglandin E₂</td>
<td>50</td>
</tr>
<tr>
<td>Prostaglandin F₂α</td>
<td>10</td>
</tr>
<tr>
<td>Prostaglandin A₂</td>
<td>5*</td>
</tr>
<tr>
<td>Prostaglandin D₂</td>
<td>0</td>
</tr>
<tr>
<td>Prostaglandin B₂</td>
<td>0</td>
</tr>
<tr>
<td>Thromboxane B₂</td>
<td>0</td>
</tr>
<tr>
<td>(15S)-9α,11α-(epoxymethano)-prosta-(5Z,13E)-dienoic acid</td>
<td>5*</td>
</tr>
<tr>
<td>(15S)-11α,9α-(epoxymethano)-prosta-(5Z,13E)-dienoic acid</td>
<td>5*</td>
</tr>
<tr>
<td>13,14-Dihydro-prostaglandin E₂</td>
<td>10–20</td>
</tr>
<tr>
<td>13,14-Dihydro-prostaglandin E₁</td>
<td>10–20</td>
</tr>
<tr>
<td>15-Keto-13,14-dihydro-prostaglandin E₂</td>
<td>1–2*</td>
</tr>
<tr>
<td>15-Keto-prostaglandin E₂</td>
<td>1–2*</td>
</tr>
</tbody>
</table>

* Approximate estimates only, since partial and non-parallel responses were obtained.
TABLE 2. Prostaglandins E and F in extracts of breast tumours

Each value (ng/g wet weight of tumour) represents the mean of three to five estimations obtained from separate portions of individual tumours, with SD in parenthesis. Significance of results (Student's t-test): prostaglandin E, malignant vs benign \( P < 0.1 \); prostaglandin E, malignant vs normal \( P < 0.0025 \); prostaglandin E, benign vs normal \( P < 0.1 \).

<table>
<thead>
<tr>
<th>Malignant histology</th>
<th>E</th>
<th>F</th>
<th>Benign histology</th>
<th>E</th>
<th>F</th>
<th>Normal breast</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Scirrhous adenocarcinoma</td>
<td>32.0 (4-2)</td>
<td>4.3 (1.1)</td>
<td>(a) Fibroadenoma</td>
<td>50.0 (5-5)</td>
<td>6.6 (0-8)</td>
<td>E</td>
</tr>
<tr>
<td>2 Scirrhous adenocarcinoma</td>
<td>20.4 (6-1)</td>
<td>4.8 (0-4)</td>
<td>(b) Fibroadenoma</td>
<td>6.2 (0-7)</td>
<td>2.7 (0-3)</td>
<td>F &lt; 2.0</td>
</tr>
<tr>
<td>3 Scirrhous adenocarcinoma</td>
<td>78.2 (8-3)</td>
<td>3.2 (0-3)</td>
<td>(c) Fibroadenoma</td>
<td>8.8 (1-0)</td>
<td>&lt; 2.0</td>
<td>(i) 2.2 (0-3)</td>
</tr>
<tr>
<td>4 Scirrhous adenocarcinoma</td>
<td>17.0 (3-7)</td>
<td>Not tested</td>
<td>(d) Fibroadenoma</td>
<td>12.3 (2-2)</td>
<td>&lt; 2.0</td>
<td>(ii) 12.4 (0-6)</td>
</tr>
<tr>
<td>5 Scirrhous adenocarcinoma</td>
<td>29.0 (2-7)</td>
<td>3.4 (0-4)</td>
<td>(e) Fibroadenoma</td>
<td>26.0 (2-9)</td>
<td>3.6 (0-4)</td>
<td>(iii) 2.0 (0-3)</td>
</tr>
<tr>
<td>6 Scirrhous adenocarcinoma</td>
<td>11.0 (1-8)</td>
<td>2.8 (0-3)</td>
<td>(f) Fibroadenoma</td>
<td>28.3 (1-2)</td>
<td>2.0 (0-4)</td>
<td>(iv) 2.0 (0-3)</td>
</tr>
<tr>
<td>7 Scirrhous adenocarcinoma</td>
<td>64.6 (16-0)</td>
<td>3.2 (0-8)</td>
<td>(g) Mammary dysplasia</td>
<td>26.8 (1-0)</td>
<td>3.8 (0-5)</td>
<td>(v) &lt; 2.0</td>
</tr>
<tr>
<td>8 Cellular adenocarcinoma</td>
<td>38.2 (4-3)</td>
<td>&lt; 2.0</td>
<td>(h) Mammary dysplasia</td>
<td>11.8 (1-6)</td>
<td>2.8 (0-3)</td>
<td>(vi) 2.4 (0-6)</td>
</tr>
<tr>
<td>9 Medullary carcinoma</td>
<td>18.2 (4-3)</td>
<td>&lt; 2.0</td>
<td>(i) Mammary dysplasia</td>
<td>13.9 (1-6)</td>
<td>2.7 (0-3)</td>
<td>(vii) 2.4 (0-6)</td>
</tr>
<tr>
<td>10 Medullary carcinoma</td>
<td>24.6 (4-9)</td>
<td>6.8 (0-3)</td>
<td>(j) Mammary dysplasia</td>
<td>6.0 (0-4)</td>
<td>&lt; 2.0</td>
<td></td>
</tr>
<tr>
<td>11 Medullary carcinoma</td>
<td>64.0 (7-6)</td>
<td>2.1 (0-4)</td>
<td>(k) Mammary dysplasia</td>
<td>82.0 (15-0)</td>
<td>2.2 (0-6)</td>
<td></td>
</tr>
<tr>
<td>12 Medullary carcinoma</td>
<td>28.4 (4-3)</td>
<td>Not tested</td>
<td>(l) Duct ectasia</td>
<td>28.0 (1-6)</td>
<td>&lt; 2.0</td>
<td></td>
</tr>
<tr>
<td>13 Intraduct carcinoma</td>
<td>62.2 (9-8)</td>
<td>2.2 (0-4)</td>
<td>(m) Duct ectasia</td>
<td>6.2 (0-9)</td>
<td>&lt; 2.0</td>
<td></td>
</tr>
<tr>
<td>14 Intraduct carcinoma</td>
<td>18.5 (1-9)</td>
<td>2.0 (0-6)</td>
<td>(n) Cytosarcoma</td>
<td>&lt; 2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 Intraduct carcinoma</td>
<td>41.3 (6-4)</td>
<td>2.8 (0-6)</td>
<td>(i) Cytosarcoma</td>
<td>&lt; 2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 Intraduct carcinoma</td>
<td>18.0 (1-9)</td>
<td>Not tested</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 Cystosarcoma phylloides</td>
<td>38.2 (4-4)</td>
<td>4.1 (0-4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 3. Prostaglandin E in extracts of renal tissue
Mean of three determinations (ng/g wet weight of tissue) with SD in parenthesis.

<table>
<thead>
<tr>
<th></th>
<th>Carcinoma</th>
<th>Normal kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>73.0 (10.0)</td>
<td>8.4 (0.8)</td>
</tr>
<tr>
<td>B</td>
<td>225.0 (9.6)</td>
<td>2.4 (0.2)</td>
</tr>
<tr>
<td>C</td>
<td>22.3 (1.4)</td>
<td>3.0 (0.3)</td>
</tr>
<tr>
<td>D</td>
<td>10.0 (0.9)</td>
<td>7.0 (0.7)</td>
</tr>
<tr>
<td>E</td>
<td>6.2 (1.4)</td>
<td>Not tested</td>
</tr>
<tr>
<td>F</td>
<td>110.0 (11.0)</td>
<td>Not tested</td>
</tr>
</tbody>
</table>

A chromatographic fraction was parallel to the curve with prostaglandin in buffer. The recoveries of [3H]prostaglandin E2 and [3H]prostaglandin F2α added to culture medium before extraction and chromatography were 71.4 ± 2.2% (n = 106) and 72.2 ± 2.6% (n = 102) respectively. Addition of 2 ng and 10 ng of unlabelled prostaglandin E2 to culture medium yielded recoveries of 97.5 ± 3.0% (n = 5) and 95.5 ± 0.6% (n = 4) respectively after correction for recovery of added label. The chromatography step avoided problems related to cross-reactivity of the prostaglandin E antibody with prostaglandins B2 and A2, which elute in a different chromatographic fraction (Jaffe & Behrman, 1973). No distinction could be made between prostaglandins E1 and E2, nor between prostaglandin F1α and F2α. No other prostaglandin or metabolite cross-reacted in either assay to any significant extent.

In view of the previously demonstrated (Samuelsson, 1973) ability of some tissues to synthesize prostaglandins rapidly during handling, preliminary experiments were performed on 10 tumours in which amounts of prostaglandin were measured in extracts of tumour and treated as in the Materials and methods section, and of tumour treated in the same way but with the inclusion of indomethacin (14 μmol/l) in the buffer used for cutting and homogenizing the tissue. Identical results were obtained in the presence and absence of indomethacin.

Prostaglandins E and F in extracts of breast and renal tumours

Table 2 shows the amounts of prostaglandins found in extracts of 17 malignant breast tumours, 14 benign breast tumours and six samples of histologically normal breast tissue obtained at a site remote from the malignant process from patients with breast carcinoma. The amounts of prostaglandins E and F tended to be low in normal breast tissue, whereas the amounts of prostaglandin E in malignant tumours were significantly higher than in controls. The concentration of prostaglandin F in tumour extracts was invariably lower than that of prostaglandin E. Table 3 lists the prostaglandin E concentrations in extracts of six renal cortical carcinomas and of four controls consisting of normal renal tissue (i.e. uninvolved by tumour) from the same specimens. Four of the six tumour extracts were compared with normal tissue, and in each case the amount of tumour prostaglandin E was greater than normal.

Bone resorption induced in vitro by renal and breast tumours

Bone-resorption data are shown in Figs. 1 and 2, and culture medium concentrations of prostaglandins E and F in Table 4, from a series of eight malignant breast tumours, nine benign breast tumours and 13 renal carcinomas cultured together with mouse calvaria in vitro. Of the renal cortical carcinomas, four (G, H, J and N of Table 4) were
reported before (Atkins et al., 1977). They are included here because they were the first of a series in which prostaglandins were measured. The histology of individual breast tumours is listed in Table 2. Only three of the malignant breast tumours caused significant bone resorption and in one this occurred in the presence of theophylline (Fig. 1). Bone resorption occurred with two of nine benign breast tumours (Fig. 1) and 10 of 13 renal carcinomas (Fig. 2).

Cultures of renal carcinomas which resorbed bone contained prostaglandin E in the media in amounts which are known to cause bone resorption. Less prostaglandin E was present in incubates of non-resorbing tumours (Table 4). The concentrations of prostaglandin F detected were invariably lower than those of prostaglandin E, and were far below those needed to induce bone resorption (Table 1). With benign breast tumours bone resorption was associated with elevated concentrations of prostaglandin E but not of prostaglandin F (Table 4). Two malignant breast tumours (nos. 7 and 8) induced calcium release from calvaria despite the presence of moderate or very low concentrations of prostaglandin E in the medium at the termination of culture.

Another tumour (no. 1) induced resorption only in the presence of theophylline, associated with an elevation of medium prostaglandin E concentration.

Indomethacin (14 μmol/l) reduced but often did not prevent the calcium release induced by resorbing tumours (Figs. 1 and 2). Indomethacin reduced the calcium release by all the renal carcinomas and benign breast tumours, but only by seven out of nine malignant breast tumours (Figs. 1 and 2). Theophylline (2-2 mmol/l) significantly increased the medium prostaglandin E concentrations at the end of incubation of renal cortical carcinomas and in seven out of 10 instances significantly increased the bone resorption (data not shown). Theophylline did not significantly alter prostaglandin concentrations in incubates of either benign or malignant breast tumours with mouse calvaria (Table 4).

Discussion

Carcinomas of the breast and renal cortex frequently metastasize to bone and are commonly associated with hypercalcaemia (Warwick, Yendt & Olin, 1961). Increased osteoclastic resorption near the site of metastatic tumour deposits in bone may be a major mechanism of malignant hypercalcaemia (Faccini, 1974; Galasko, 1976), but the factors responsible for stimulating osteoclasts are not yet clearly understood.

There is little radioimmunoassay information on prostaglandins extracted from human tumours, although extracts of human breast tumours have been bioassayed (Bennett, Simpson, McDonald & Stamford, 1975; Bennett, Charlier, McDonald, Simpson & Stamford, 1976; Bennett, Charlier, McDonald, Simpson, Stamford & Zebro, 1977). Our results suggest that high amounts of prostaglandin E may be extracted from some renal carcinomas, and benign and malignant breast tumours. The finding of large amounts of prostaglandin in extracts of some benign breast tumours may seem to be at variance with the results of Bennett et al. (1975, 1976, 1977) but might be related to the different methods used. Fibroadenomas homogenized in Krebs' solution yield low amounts of prostaglandin, but incubated slices yield high amounts (A. Bennett, M. Harris, D. J. F.
TABLE 4. Prostaglandin concentrations in pooled media after cultivation of tumour and calvaria

At the end of period of cultivation of tumour with bone, 0.25 ml of medium was taken from each incubation and pooled with its replicates before extraction, chromatography and assay for prostaglandins. Results are expressed in ng/ml. The degree of bone resorption induced by each tumour can be seen in Figs. 1 and 2. NT, Not tested.

Paired t-tests for prostaglandin E values: renal carcinoma, control vs theophylline, \( t = 3.057, P < 0.01 \); control vs indomethacin, \( t = 3.91, P < 0.005 \); malignant breast, control vs theophylline, \( t = 1.039, \) not significant (NS); control vs indomethacin, \( t = 1.640, \) NS; benign breast, control vs theophylline, \( t = 0.838, \) NS; control vs indomethacin, \( t = 1.5440, \) NS.

Histology of breast tumours not given in Table 2: no. 18 = scirrhous carcinoma; o = fibroadenosis; p, q = fibroadenoma.

<table>
<thead>
<tr>
<th>Prostaglandin E</th>
<th>Prostaglandin F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+theophylline</td>
</tr>
<tr>
<td>Renal carcinoma</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>24.8</td>
</tr>
<tr>
<td>B</td>
<td>32.5</td>
</tr>
<tr>
<td>G</td>
<td>3.4</td>
</tr>
<tr>
<td>H</td>
<td>5.5</td>
</tr>
<tr>
<td>C</td>
<td>34.9</td>
</tr>
<tr>
<td>I</td>
<td>18.5</td>
</tr>
<tr>
<td>J</td>
<td>6.1</td>
</tr>
<tr>
<td>K</td>
<td>12.9</td>
</tr>
<tr>
<td>L</td>
<td>0.3</td>
</tr>
<tr>
<td>M</td>
<td>24.2</td>
</tr>
<tr>
<td>E</td>
<td>0.3</td>
</tr>
<tr>
<td>N</td>
<td>4.3</td>
</tr>
<tr>
<td>Malignant breast</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4.4</td>
</tr>
<tr>
<td>8</td>
<td>0.3</td>
</tr>
<tr>
<td>9</td>
<td>0.1</td>
</tr>
<tr>
<td>18</td>
<td>0.3</td>
</tr>
<tr>
<td>1</td>
<td>2.4</td>
</tr>
<tr>
<td>2</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>0.0</td>
</tr>
<tr>
<td>14</td>
<td>0.4</td>
</tr>
<tr>
<td>Benign breast</td>
<td></td>
</tr>
<tr>
<td>k</td>
<td>13.0</td>
</tr>
<tr>
<td>g</td>
<td>0.0</td>
</tr>
<tr>
<td>o</td>
<td>14.3</td>
</tr>
<tr>
<td>i</td>
<td>0.0</td>
</tr>
<tr>
<td>f</td>
<td>0.5</td>
</tr>
<tr>
<td>b</td>
<td>0.2</td>
</tr>
<tr>
<td>e</td>
<td>0.2</td>
</tr>
<tr>
<td>p</td>
<td>0.2</td>
</tr>
<tr>
<td>q</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Rowe, I. F. Stamford & J. E. Wright, personal communication). Although we have prevented generation of prostaglandins during tissue cutting, we cannot exclude the possibility that much of the prostaglandin in our extracts of breast tumours was formed during surgical excision. Jenkins et al. (1976), Dowsett et al. (1976b) and ourselves have obtained bone resorption in some experiments with slices of benign breast tumour, and we have shown that prostaglandin E is released.

Using chromatography and radioimmunoassay, we have obtained evidence that extracts of breast tumours contain prostaglandin E rather than prostaglandin F. Other studies using bioassay indicate that various prostaglandins may be present, and that 'prostaglandin F' may be predominant in extracts of some tumours (Bennett et al., 1975, 1977). It is of interest in this context that some animal tumours and tumour cell lines produce predominantly, and sometimes exclusively, prostaglandin E2 (Tashjian et al., 1972; Hammarstrom, Samuelsson & Bjursell, 1973; Hamprecht, Jaffe & Philpott, 1973; Voelkel et al., 1975; Burstein, Gagnon, Hunter & Maudsley, 1976, 1977). Prostaglandins and cancer
taglndin F$_{2a}$ seems to be much less potent than prostaglandin E$_2$ in stimulating bone resorption, although Dowsett et al. (1976a) disagree. It is possible that prostaglandin F$_{2a}$ has a role as a local mediator of bone resorption when tumours become localized in bone.

Bone resorption was induced in vitro by most of the renal carcinomas studied, extending the observations of an earlier study (Atkins et al., 1977). The prostaglandin assay results strongly suggest that the bone resorption was due to released prostaglandin E, but other prostanoids or their metabolites might participate. The actual medium concentrations of prostaglandin E at the end of incubations are less important in relation to the amount of bone resorption produced than is the fact that prostaglandin E production continued throughout the culture period. The reduction of bone resorption by indomethacin, at a concentration thought to be selective for inhibition of prostaglandin synthesis, is further evidence that the resorption was due to prostaglandins. However, the frequent inability of indomethacin to inhibit completely the calcium release in the presence of tumours, despite suppression of prostaglandin E production, suggests the involvement of non-prostaglandin material which resorbs bone. Dowsett et al. (1976a) and Galasko (1976), using different approaches, have also produced evidence of non-prostaglandin-induced bone resorption. The nature of this additional mechanism is obscure, but our data (Atkins et al., 1977) suggest that parathyroid hormone is not involved. The role of osteoclast-activating factor (Horton, Raisz, Simmons, Oppenheim & Mengenhagen, 1972) in bone resorption by solid tumours has not been defined, but a similar molecule occurs in fractions from malignant ascitic fluids (Nimberg, Humphries, Lloyd, Badger, Cooperband, Wells & Schmid, 1978). Some breast carcinomas can produce considerable quantities of proteolytic enzymes in vitro (Easty, Dowsett, Powles, Easty, Gazet & Neville, 1977), and bone resorption in vitro by the supernate from an established breast cancer cell line which was independent of osteoclastic activity has also been described (Mundy, Eilon, Altman & Dominguez, 1978).

Co-incubation with theophylline increased prostaglandin E concentrations in culture media (Table 4) and enhanced tumour-induced bone resorption. Although cyclic AMP is involved in the action of parathyroid hormone in bone (Chase & Aurbach, 1970), addition of large amounts of cyclic AMP to bone cultures does not cause bone resorption in the system used here. However, cyclic AMP enhances prostaglandin production in some situations (Hamprecht et al., 1973; Thomas, Philpott & Jaffe, 1974). The effect of theophylline in these cultures may have been to reduce cyclic AMP hydrolysis, thus stimulating prostaglandin synthesis by tumour tissue and increasing bone resorption. This conclusion derives from the present data, which extends the observations made in a few renal cortical carcinomas (Atkins et al., 1977). Adenlate cyclase activity in renal cortical carcinoma is consistently elevated (Hunt, Shortland, Michelangeli, Hammonds, Atkins & Martin, 1978), and the generated cyclic AMP may increase prostaglandin production.

Since bone resorption and prostaglandin production occur with benign breast tumours, the phenomena are not peculiar to malignant tissue. Perhaps inflammatory cells, which occur to some extent in all tumours, contribute to prostaglandin synthesis. Various types of cell found in inflammatory reactions can elaborate prostaglandins (Ferraris et al., 1974; Brat et al., 1975; Tolone et al., 1977). The recent study of a case of hypercalcaemia related to benign breast hypertrophy may also be relevant (Marx, Zusman & Umiker, 1977).

In view of the rapid metabolism of circulating prostaglandin E, it seems likely that the bone-resorbing effects of this prostaglandin become important only after the tumour has spread to bone, although prostaglandin synthesis by metastatic tumour cells may facilitate such spread to bone. Infusion of prostaglandin E can cause hypercalcaemia in experimental animals (Franklin & Tashjian, 1975; Robertson & Baylink, 1977) but large amounts are required. The mechanisms of hypercalcaemia in human cancers which have not overtly metastasized to bone remain largely unknown. In a few instances this may be due to a prostaglandin. The ectopic production of parathyroid hormone has been documented (Greenberg, Martin & Sutcliffe, 1973), but this does not explain all cases of non-metastatic hypercalcaemia in cancer (Powell, Singer, Murray, Minkin & Potts, 1973), and may indeed be invoked only very rarely (Martin & Atkins, 1979).

Although our data do not exclude an important role for prostaglandins in the bone effects of breast cancer, it is less apparent than with renal cortical carcinoma, and furthermore there are indications from this and other work that non-prostaglandin mechanisms may be involved. These need to be explored with breast cancer, in addition to the possibility that generation of labile prostanoids
(prostacyclin, thromboxanes) by tumour deposits in bone might contribute towards localized bone destruction.

Acknowledgments

This work was supported by grants from the Yorkshire Council of the Cancer Research Campaign and the Victorian Anti-Cancer Council. The authors are grateful to the following surgeons, who provided tumour material: Professor H. Duthie, Mr W. Morris Jones, Mr D. Randall and Mr C. Talbot of the Sheffield Royal Infirmary, and Mr J. Williams of the Sheffield Royal Hospital.

References


POWLES, T.J., CLARK, S.A., EASY, G.C. & NEVILLE, D.M. (1973) The inhibition by aspirin and indomethacin of osteolytic tumour deposits and hypercal-


