Calcitonin binding and degradation by two cultured human breast cancer cell lines (MCF 7 and T 47D)

David M. FINDLAY, Valdo P. MICHELANGELI, Jane M. MOSELEY and T. John MARTIN*
Department of Medicine, University of Melbourne, Repatriation General Hospital, Heidelberg, Victoria 3081, Australia

(Received 11 November 1980/Accepted 23 January 1981)

Two human breast cancer cell lines (MCF 7 and T 47D) possess calcitonin-responsive adenylate cyclase systems. Suspended cells of both lines specifically bound ^{125}I -labelled salmon calcitonin with mean dissociation constants of 1.7 nM (MCF 7) and 1.4 nM (T 47D); mean receptor numbers were 5300 and 24 400 per cell respectively. Measurement of specific binding to MCF 7 cells was obscured by rapid and substantial degradation of the labelled hormone. Degradation of ^{125}I -labelled salmon calcitonin: (i) was of high capacity; (ii) lacked the specificity displayed by ^{125}I -labelled salmon calcitonin binding to the same cells; and (iii) was not related to binding since cell incubation supernatants retained full degrading activity. The degrading activity was inhibited by corticotropin-(1–24)-tetracosapeptide, insulin and bacitracin. Inclusion of bacitracin in the incubation resulted in apparently fewer numbers of lower affinity receptors on MCF 7 cells, whereas these parameters were identical for T 47D cells incubated in the presence or absence of bacitracin. Eel [2-aminosuberic acid 1,7]-calcitonin was resistant to proteolysis in the presence of either cell line. Analysis of hormone–receptor interactions with calcitonin-responsive cells should take account of potent calcitonin-degrading activities in some cell lines.

Calcitonin has been shown to bind specifically to rat renal and calvarial membranes (Marx *et al.*, 1972a, 1973; Sraer & Ardaillou, 1974; Loreau *et al.*, 1978; Tashjian *et al.*, 1978), lymphoid cells (Moran *et al.*, 1978) and bronchial carcinoma cells (BEN cells) in culture (Hunt *et al.*, 1977; Findlay *et al.*, 1980a). In the course of studies of other cancer cell lines, specific binding of calcitonin to two human breast cancer cell lines has been found and furthermore both cell lines possess a calcitonin-sensitive adenylate cyclase [EC 4.6.1.1; ATP pyrophosphate-lyase (cyclizing)] and a calcitonin-stimulated cyclic AMP-dependent protein kinase (EC 2.7.1.37; ATP:protein phosphotransferase) (Martin *et al.*, 1980; Findlay *et al.*, 1980b). The present paper describes in detail the binding characteristics of calcitonin to the breast-derived MCF 7 and T 47D cells, and their calcitonin-degradation activity.

Abbreviation used: ASu, 2-aminosuberic acid (2-amino-octanedioic acid).

* To whom reprint requests should be sent.

Materials and methods

Hormones and chemicals

Synthetic salmon calcitonin (lot no. K715031; 4700 units/mg) and human [Gly 8]calcitonin were gifts from the Armour Pharmaceutical Co., Kankakee, IL, U.S.A. Synthetic human calcitonin (calcitonin M), and human [Leu 12,16,19]human calcitonin were gifts from Ciba-Geigy, Basle, Switzerland. Eel [ASu 1,7]calcitonin was a gift from Dr. J. Murase, Toyo Jozo Co., Osaka, Japan. Porcine insulin and glucagon were obtained from Eli Lilly and Co., Indianapolis, IN, U.S.A., synthetic corticotropin-(1–24)-tetracosapeptide ('Synacthen') from Ciba-Geigy, and bovine parathyrin was purified in this laboratory by methods previously described (Moseley *et al.*, 1975). Bacitracin (63 units/mg) was obtained from Apothekernes Laboratories, Oslo, Norway. All chemicals were of reagent grade and obtained from standard suppliers. ATP (disodium salt) and cyclic AMP were obtained from Boehringer-Mannheim, and bovine and human serum

albumin from the Commonwealth Serum Laboratories, Parkville, Vic., Australia. [α - 32 P]ATP, cyclic [3 H]AMP and Na 125 I were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

Peptide iodination

Synthetic salmon calcitonin was labelled with 125 I to a specific radioactivity of 160–200 Ci/g as previously described (Hunt *et al.*, 1977) with 2 μ g of chloramine- τ for 10 s. Purified labelled hormone was stored in 20% (v/v) acetone in 1% (v/v) acetic acid in small samples and could be used in binding experiments for at least 4 weeks when stored at -20°C . The same labelling procedure was used for eel [ASu 1,7]calcitonin.

Cell culture

MCF 7 cells were established from a pleural effusion of a scirrhous cancer of the breast by H. D. Soule, Michigan Cancer Foundation, Detroit, MI, U.S.A. (Horwitz *et al.*, 1978), and T 47D cells were originally grown from a pleural effusion from an infiltrating ductal breast cancer (Horwitz *et al.*, 1978). Both cell lines were routinely cultured in RPMI 1640 medium containing 10% foetal calf serum and added insulin (0.1 μM) (Novo crystalline) and hydrocortisone (0.1 μM) in plastic tissue culture flasks (Costar) and maintained at 37°C in an atmosphere of CO_2/air (1:19). The antibiotics minocyclin (1 mg/l) and gentamycin (80 mg/l) were also added. Subcultures were carried out by washing in 0.02% EDTA, followed by treatment with 0.0125% (w/v) trypsin in 0.02% EDTA for 2 min at 37°C , addition of complete medium before centrifugation at 200 g and resuspension in complete medium. Bovine foetal calf serum was obtained from Gibco Biocult and all other culture media from Commonwealth Serum Laboratories.

Adenylate cyclase activity

Cell cultures at or near confluency were used in all assays, unless otherwise stated. Medium was removed from flasks and cells were washed twice with 5 ml of phosphate-buffered saline and once with 25 mM-Tris/HCl, pH 8.2, containing 0.25 M-sucrose, 1 mM-dithiothreitol and 1 mM-EDTA (disodium salt). The cells were scraped from the surface with a plastic 'policeman', homogenized with six strokes of an all-glass hand-held homogenizer in 1 ml of the latter buffer and used directly in the assay. Additives in the adenylate cyclase assay were prepared in buffer A comprising 25 mM-Tris/HCl, pH 8.2, 4.5 mM-Mg $^{2+}$, 30 mM-K $^{+}$ and 0.1% (w/v) bovine serum albumin. The assay method employed has been described in detail previously (Hunt *et al.*, 1976; Findlay *et al.*, 1980a). Protein concentrations were measured by the method of Hartree (1972).

Preparation of cells for binding and calcitonin-degradation experiments

Binding and degradation of labelled calcitonin were studied using intact cells, which were subcultured from confluent flasks by the standard procedure, washed twice in phosphate-buffered saline and resuspended in buffer B of the following composition: 15 mM-Tris, 120 mM-NaCl, 4 mM-KCl, 1.6 mM-MgSO $_4$, 2 mM-NaH $_2$ PO $_4$, 10 mM-glucose, human serum albumin (0.1%, w/v), pH 7.8. In some experiments with MCF 7 cells (see elsewhere in the text) bacitracin (final concentration 1 mg/ml) was added to the cell suspension 10 min before addition of hormone. Incubations with 125 I-labelled salmon or eel calcitonin were carried out with continuous agitation at 20°C in flat-bottomed plastic tubes as detailed for individual experiments in the Figure legends. Non-specific binding was assessed as the binding of 125 I-labelled salmon calcitonin occurring in the presence of at least 2 μ g of unlabelled salmon calcitonin/ml. Cell numbers were counted in a haemocytometer. For assessment of the percentage of 125 I-labelled hormone bound to cells, duplicate 200 μ l samples of incubation mixture were layered on to 100 μ l of chilled buffer B in microfuge tubes, which were centrifuged at 10000 g for 2 min in a Beckman model B microfuge. The tips containing the pellets were excised, and bound and free radioactivity was measured in a Packard automatic scintillation well counter with an efficiency of 70%. For assessment of degradation of 125 I-labelled hormone, 100 μ l or 200 μ l samples of incubation mixture were added to 1 ml of ice-cold 20% (w/v) trichloroacetic acid. The precipitated radioactivity after washing was taken to represent undegraded hormone (deLuise *et al.*, 1970; Marx *et al.*, 1972b).

Results

Activation of adenylate cyclase

Adenylate cyclase activity in broken cell preparations of both cell lines was stimulated by salmon calcitonin (Fig. 1). Half-maximal stimulation of adenylate cyclase in the two cell lines was achieved by a concentration of salmon calcitonin of 10 nM for MCF 7 cells and 0.4 nM for T 47D cells. A range of calcitonin analogues was also effective in causing activation (Fig. 1), the relative potencies of the analogues being in close agreement with those found in other calcitonin-responsive systems, for example hypocalcaemic agents in the rat (Maier *et al.*, 1975).

Degradation of 125 I-labelled salmon calcitonin by MCF 7 and T 47D cells

Calcitonin interaction with MCF 7 and T 47D cells to stimulate adenylate cyclase and protein kinase would reasonably be expected to occur via

Fig. 1. MCF 7 and T 47D cell adenylate cyclase response to calcitonin analogues

Broken cell preparations of (a) MCF 7 cells and (b) T 47D cells were incubated with [α - 32 P]ATP as described in the Materials and methods section, with increasing concentrations of salmon calcitonin (●), human calcitonin (□) and the substituted analogues of human calcitonin human [Gly⁸]calcitonin (■) and human [Leu^{12,16,19}]calcitonin (○). Response is expressed as pmol of cyclic AMP generated per mg of protein during a 10 min incubation. B indicates basal adenylate cyclase activity in the absence of agonist.

cell membrane receptor binding of the hormone. However, experiments to examine the characteristics of binding of 125 I-labelled salmon calcitonin to MCF 7 cells were complicated by rapid and substantial degradation of the labelled hormone. The experiment illustrated in Fig. 2(a) relates degradation of the labelled hormone to the total and non-specific binding of 125 I-labelled salmon calcitonin by MCF 7 cells over 90 min incubation. The increase in both total binding and non-specific binding throughout the incubation implies uptake or binding by the cell of degraded hormone rather than true specific binding. In the case of T 47D cells (Fig. 2b), where non-specific binding increased at a much slower rate than total binding, degradation of 125 I-labelled salmon calcitonin was still considerable, but it occurred much more slowly than with MCF 7 cells.

To determine the capacity and specificity of the calcitonin-degrading activity by suspended MCF 7 cells they were incubated with tracer and increasing concentrations of unlabelled salmon calcitonin, human calcitonin and the biologically inactive human [Gly⁸]calcitonin analogue. After 30 min incubation the percentage degradation was assessed. Fig. 3 shows that even at a concentration of $5 \mu\text{M}$ unlabelled hormone, tracer degradation occurred.

To examine the nature of the proteolytic cleavage, eel [ASu^{1,7}]calcitonin was iodinated and incubated with cells. Fig. 4 shows that 125 I-labelled eel [ASu^{1,7}]calcitonin was more trichloroacetic acid-precipitable initially and was almost totally resistant

Fig. 2. Time course of binding and degradation of 125 I-labelled salmon calcitonin by MCF 7 and T 47D cells. Cells from confluent flasks of (a) MCF 7 cells ($1 \times 10^7/\text{ml}$) or (b) T 47D cells ($6 \times 10^6/\text{ml}$) were incubated as described in the Materials and methods section with 125 I-labelled salmon calcitonin (1.8×10^3 c.p.m./ml; 400 pg/ml) in the presence (●) or absence (○) of $5 \mu\text{g}$ of unlabelled salmon calcitonin/ml. At the indicated times duplicate $200 \mu\text{l}$ portions were taken for assessment of percentage tracer bound to cells (lower panels) or percentage of 125 I-labelled hormone precipitable by 1 ml of ice-cold 20% trichloroacetic acid (upper panels).

Fig. 3. Degradation of ^{125}I -labelled salmon calcitonin by MCF 7 cells in the presence of calcitonin analogues. MCF 7 cells ($4 \times 10^6/\text{ml}$) were incubated for 45 min as described in the Materials and methods section with ^{125}I -labelled salmon calcitonin (3×10^5 c.p.m., 600 pg/ml) in the presence of increasing concentrations of unlabelled salmon calcitonin (Δ), human calcitonin ($\bullet$) or human $[\text{Gly}^8]\text{calcitonin}$ ($\circ$). Duplicate 100 μl portions of incubation mixture were added to 1 ml of ice-cold 20% trichloroacetic acid. The broken line indicates trichloroacetic acid precipitability of ^{125}I -labelled salmon calcitonin incubated in buffer alone.

Fig. 4. Degradation of ^{125}I -labelled salmon calcitonin and eel ^{125}I -labelled $[\text{ASu}^{1,7}]\text{calcitonin}$ in the presence or absence of bacitracin.

MCF 7 cells ($3 \times 10^4/\text{ml}$) were incubated as described in the Materials and methods section with ^{125}I -labelled salmon ($\circ$, $\bullet$) or eel (Δ , $\blacktriangle$) calcitonin (2.5×10^5 c.p.m./ml; 550 pg/ml) in the absence (Δ , $\circ$) or presence ($\blacktriangle$, $\bullet$) of bacitracin (1 mg/ml). At the times indicated duplicate 100 μl portions of incubation mixture were added to 1 ml of ice-cold 20% trichloroacetic acid.

to proteolysis throughout a 45 min incubation, compared with rapid degradation of the labelled salmon hormone over the same period.

Several peptides were tested for their ability to block degradation of ^{125}I -labelled salmon calcitonin by MCF 7 and T 47D cells. Corticotropin-(1-24)-tetracosapeptide at concentrations from 80 to 500 $\mu\text{g}/\text{ml}$ has been used to inhibit the inactivation system of salmon calcitonin present in rat renal membranes without affecting binding kinetics (Sraer & Ardaillou, 1974; Loreau *et al.*, 1978). Insulin (1 μM) blocks degradation of labelled insulin by MCF 7 cells (Osborne *et al.*, 1978). The peptide antibiotic bacitracin has been shown to inhibit the

inactivation of glucagon by microsomal membranes (Desbuquois *et al.*, 1974) and almost to abolish degradation of insulin by MCF 7 cells (Osborne *et al.*, 1978). Table 1 compares the ability of these peptides as inhibitors of ^{125}I -labelled salmon calcitonin degradation as measured by trichloroacetic acid precipitability and indicates that effective control of degradation was achieved by all three agents; bacitracin was routinely used in subsequent binding experiments for MCF 7 cells at a concentration of 1 mg/ml, which was the lowest effective concentration. The effects of bacitracin on binding and degradation of labelled salmon calcitonin and eel $[\text{ASu}^{1,7}]\text{calcitonin}$ are compared in Fig. 4.

Table 1. *Degradation of ^{125}I -labelled salmon calcitonin by MCF 7 and T 47D cells*

MCF 7 and T 47D cells ($6 \times 10^6/\text{ml}$) were incubated as described in the Materials and methods section with ^{125}I -labelled salmon calcitonin ($1 \times 10^6 \text{ c.p.m./ml}$, 2 ng/ml) in buffer alone or buffer containing insulin, bacitracin or corticotropin-(1–24)-tetracosapeptide at the stated concentrations. After 45 min duplicate $200 \mu\text{l}$ portions of incubation mixture were taken for assessment of the percentage of ^{125}I -labelled hormone precipitable by 1 ml of ice-cold 20% trichloroacetic acid. Percentage degradation was calculated by comparing results with those of labelled hormone incubated without cells.

Peptide	Degradation (%)	
	MCF 7 cells	T 47D cells
No addition	70	44
+ Insulin ($1 \mu\text{M}$)	7	9
+ Bacitracin (7 units/ml)	50	7
(70 units/ml)	7	0.7
(140 units/ml)	0	0
+ Corticotropin-(1–24)-tetracosapeptide ($40 \mu\text{M}$)	5	2.2
($80 \mu\text{M}$)	4	2.9

Binding of ^{125}I -labelled salmon calcitonin to MCF 7 and T 47D cells

As we had found with human lung cancer (BEN) cells (Findlay *et al.*, 1980a) the subculturing procedure did not influence the calcitonin binding or stimulation of adenylate cyclase in MCF 7 or T 47D cells. All experiments to demonstrate characteristics of binding of ^{125}I -labelled salmon calcitonin to MCF 7 cells were carried out using cells pre-incubated for 10 min (before hormone addition) with sufficient bacitracin to yield a final concentration of 1 mg/ml in the incubation. Fig. 5 shows the time course of binding of ^{125}I -labelled salmon calcitonin to MCF 7 cells. Although non-specific binding was still a substantial percentage of the total binding (30–50% in most experiments), this increased only slightly over a 90 min incubation, whereas total and specific binding increased rapidly to a plateau after about 45 min.

Fig. 6 provides Scatchard analysis of binding of ^{125}I -labelled salmon calcitonin in the presence of increasing concentrations of unlabelled salmon calcitonin with or without bacitracin in the incubation. Although almost twice as much tracer bound to cells in the absence of bacitracin, unlabelled salmon calcitonin was less effective in competing with label for binding. With bacitracin present a receptor number of 2740 per cell and a dissociation constant of 1.8 nM were indicated; in the absence of bacitracin the Scatchard plot appeared curvilinear and suggested a much higher number of lower-affinity receptors. The high-affinity estimate in the presence of bacitracin is more consistent with a physiologically significant receptor. In kinetic studies carried out over a period of several months receptor numbers [5300 ± 1250 (mean $\pm$ s.e.m.); $n = 7$] and dissociation constants ($1.71 \pm 0.22 \text{ nM}$, $n = 7$) remained fairly constant.

Binding of ^{125}I -labelled salmon calcitonin to T 47D cells could be examined without use of bacitracin.

Fig. 5. *Time course of ^{125}I -labelled salmon calcitonin binding to MCF 7 cells in the presence of bacitracin* MCF 7 cells ($6 \times 10^6/\text{ml}$) were incubated as described in the Materials and methods section with ^{125}I -labelled salmon calcitonin ($1 \times 10^6 \text{ c.p.m./ml}$; 2 ng/ml) in the presence (●) or absence (O) of $1 \mu\text{M}$ -unlabelled salmon calcitonin, in the presence of 1 mg of bacitracin/ml. At the indicated times duplicate $200 \mu\text{l}$ portions of incubation mixture were taken for assessment of percentage of tracer bound to cells. Specific binding (Δ) was calculated as the difference between total binding and binding in the presence of unlabelled salmon calcitonin.

The time course of binding of ^{125}I -labelled salmon calcitonin to T 47D cells is shown in Fig. 2(b). Inclusion of bacitracin resulted in lower non-specific

Fig. 6. Scatchard plots of ^{125}I -labelled salmon calcitonin binding to MCF 7 cells in the presence or absence of bacitracin

MCF 7 cells ($6.3 \times 10^6/\text{ml}$) were incubated as described in the Materials and methods section with ^{125}I -labelled salmon calcitonin (1.7×10^5 c.p.m./ml; 600 pg/ml) in the absence (○) or presence (●) of 1 mg of bacitracin/ml. Scatchard plots were derived from the binding data in each case.

Fig. 7. Scatchard plots of ^{125}I -labelled salmon calcitonin binding to T 47D cells in the presence or absence of bacitracin

T 47D cells ($2 \times 10^6/\text{ml}$) were incubated as described in the Materials and methods section with ^{125}I -labelled salmon calcitonin (1.3×10^5 c.p.m./ml; 260 pg/ml) in the absence (○) or presence (●) of 1 mg of bacitracin/ml. Scatchard transformations of the binding data were carried out in each case.

binding of labelled hormone, but had no detectable effect on binding parameters calculated from Scatchard analysis (Fig. 7). In experiments repeated throughout a period of several months, mean values for the dissociation constant and receptor number for ^{125}I -labelled salmon calcitonin binding to T 47D cells were: $K_D = 1.4 \pm 0.2 \text{ nM}$ (mean $\pm$ s.e.m., $n = 10$); receptor number = $24400 \pm 4300/\text{cell}$ (mean $\pm$ s.e.m., $n = 10$).

Specificity of binding of calcitonin to MCF 7 cells has been shown previously, with a range of unrelated peptide hormones (Martin *et al.*, 1980). The same hormones (human somatotropin, insulin, glucagon, parathyrin, prolactin and corticotropin) at high concentration did not compete with ^{125}I -labelled salmon calcitonin for binding to T 47D cells (results not shown).

Discussion

The proteolytic activity of MCF 7 cells and to a lesser extent T 47D cells is likely to be a function of

their tumour origin. MCF 7 cells can directly resorb bone in culture (Eilon & Mundy, 1978) and therefore probably contain a number of lysosomal enzymes that can be released in certain circumstances. It is possible that suspended cells may release them more readily. Shiu (1980) found that T 47D and MCF 7 cells in culture degraded ^{125}I -labelled prolactin quite slowly and this degradation was related to prolactin binding. In the present experiments suspended cells rapidly degraded ^{125}I -labelled salmon calcitonin and almost full degrading activity was retained in the incubation supernatant. The activity may therefore be due to release of enzymic activity from suspended cells.

Since incubation supernatant retained degrading activity and since salmon and human calcitonin and human [Gly⁸]calcitonin, which display substantially different activities for binding (Martin *et al.*, 1980), gave equal tracer protection, degradation of label

appears to be a separate event from binding. Furthermore, degradation of label appears to be a non-specific phenomenon of high capacity. Salmon calcitonin, human calcitonin and human [Gly⁸]-calcitonin at high concentrations (5 μ M) inhibited tracer degradation. Binding and degradation of insulin by MCF 7 cells has also been described (Osborne *et al.*, 1978), although the insulin-degrading activity at 21°C was not as rapid as for calcitonin, as assessed by either trichloroacetic acid solubility or ability of the tracer to rebind to fresh cells. The proteolytic capacity in MCF 7 cells was high but unlabelled 1 μ M-insulin almost abolished it (cf. Fig. 4). Insulin (1 μ M) and corticotropin-(1–24)-tetracosapeptide (40 μ M) both blocked degradation of ¹²⁵I-labelled salmon calcitonin and presumably act by protecting the labelled salmon calcitonin from non-specific proteolytic enzymes. The mechanism of action of bacitracin is not clear but its peptide nature may allow it to act in a similar manner at high concentration.

Because of the apparent non-specific nature of ¹²⁵I-labelled salmon calcitonin degradation, it is interesting that the synthetic eel calcitonin analogue, eel [ASu^{1,7}]calcitonin, was stable in the presence of MCF 7 cells. This analogue differs from salmon calcitonin by replacement of the *N*-terminal cysteine-1 and cysteine-7 together by 2-amino-suberic acid and by three amino acid replacements at residues 26, 27 and 29 (Noda & Narita, 1976; Yamauchi *et al.*, 1977). Possibly replacement of the disulphide bridge with a carbon-carbon linkage resulted in greater stability of the eel [ASu^{1,7}]calcitonin label.

¹²⁵I-labelled salmon calcitonin binding to MCF 7 cells was decreased in the presence of bacitracin. This result is consistent with the decrease in insulin binding seen by Osborne *et al.* (1978), but contrasts with the significant increase in binding of glucagon to liver cell microsomal preparations in the presence of bacitracin (Desbuquois *et al.*, 1974). This difference may relate to the nature of MCF 7 cells, which are perhaps capable of uptake or non-specific binding of hormone fragments.

It is unlikely that bacitracin had a direct effect on the MCF 7 cell calcitonin receptor; binding parameters (receptor number and dissociation constant) were not different for T 47D cells incubated with or without bacitracin. More complex actions by bacitracin cannot be ruled out, however, since a recent report shows inhibition by the peptide of cluster formation by α_2 -macroglobulin on rat kidney fibroblasts associated with transglutaminase inhibition (Davies *et al.*, 1980).

Binding of calcitonin to MCF 7 and T 47D cells fulfils the requirements for true receptor binding in terms of affinity and specificity and appears to be linked to early calcitonin-activated biochemical

events in these cells. Kinetic and quantitative studies of hormone-receptor interaction should be approached bearing in mind the capacity of cells to degrade the ligand. The significance for either cell line of the calcitonin receptor in terms of growth or function has not been defined. In view of the complex hormonal requirements for normal mammary cell proliferation (Yang *et al.*, 1980) and differentiation (Tonelli & Sorof, 1980) functional calcitonin receptors on these two cell lines would need to be understood in terms of their complement of insulin (Osborne *et al.*, 1978), prolactin (Shiu, 1980), oestrogen, progesterone, glucocorticoid and androgen (Horwitz *et al.*, 1978; Barnes & Sato, 1979), epidermal growth factor (Tonelli & Sorof, 1980; Osborne *et al.*, 1980) and L-3,3',3-triiodothyronine receptors (Burke & McGuire, 1977) and perhaps more importantly our recent finding of receptors for the potent Ca²⁺-mobilizing hormone 1,25-dihydroxyvitamin D (Eisman *et al.*, 1979). These cells provide a system *in vitro* for determining subsequent calcitonin-controlled biochemical events and the role of receptors for Ca²⁺-regulating hormones in breast tumour cells.

This work was supported by grants from the Victorian Anti-Cancer Council and the National Health and Medical Research Council of Australia.

References

- Barnes, P. & Sato, G. (1979) *Nature (London)* **281**, 388–389
- Burke, R. E. & McGuire, W. L. (1977) *Cancer Res.* **37**, 792–799
- Davies, P. J. A., Davies, D. R., Levitzki, A., Maxfield, F. R., Milhaud, P., Willingham, M. C. & Pastan, I. H. (1980) *Nature (London)* **283**, 162–167
- deLuise, M., Martin, T. J. & Melick, R. A. (1970) *J. Endocrinol.* **48**, 181–188
- Desbuquois, B., Krug, F. & Cuatrecasas, P. (1974) *Biochim. Biophys. Acta* **343**, 101–120
- Eilon, G. & Mundy, G. R. (1978) *Nature (London)* **276**, 726–728
- Eisman, J. A., MacIntyre, I., Martin, T. J. & Moseley, J. M. (1979) *Lancet* **ii**, 1335–1336
- Findlay, D. M., deLuise, M., Michelangeli, V. P., Ellison, M. & Martin, T. J. (1980a) *Cancer Res.* **40**, 1311–1317
- Findlay, D. M., Michelangeli, V. P., Eisman, J. A., Frampton, R. J., Moseley, J. M., MacIntyre, I. & Martin, T. J. (1980b) *Cancer Res.* **40**, 4764–4767
- Hartree, E. F. (1972) *Anal. Biochem.* **48**, 422–427
- Horwitz, K. B., Zava, D. T., Thilager, A. K., Jensen, E. M. & McGuire, W. L. (1978) *Cancer Res.* **38**, 2434–2437
- Hunt, N. H., Martin, T. J., Michelangeli, V. P. & Eisman, J. A. (1976) *J. Endocrinol.* **69**, 401–409
- Hunt, N. H., Ellison, M., Underwood, J. C. E. & Martin, T. J. (1977) *Br. J. Cancer* **35**, 777–784

- Loreau, N., Lajotte, C., Wahbe, F. & Ardaillou, R. (1978) *J. Endocrinol.* **76**, 533–545
- Maier, R., Kamber, B., Riniker, B. & Rittel, W. (1975) *Horm. Metab. Res.* **7**, 511–514
- Martin, T. J., Findlay, D. M., MacIntyre, I., Eisman, J. A., Michelangeli, V. P., Moseley, J. M. & Partridge, N. C. (1980) *Biochem. Biophys. Res. Commun.* **90**, 150–156
- Marx, S. J., Woodward, C. J. & Aurbach, G. D. (1972a) *Science* **178**, 999–1001
- Marx, S. J., Fedak, S. A. & Aurbach, G. D. (1972b) *J. Biol. Chem.* **247**, 6913–6918
- Marx, S. J., Woodard, C., Aurbach, G. D., Glassman, H. & Keutmann, H. T. (1973) *J. Biol. Chem.* **248**, 4797–4802
- Moran, J., Hunziker, W. & Fischer, J. A. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3984–3988
- Moseley, J. M., Martin, T. J., Robinson, C. J., Reit, B. W. & Tregear, G. W. (1975) *Clin. Exp. Pharmacol. Physiol* **2**, 547–553
- Noda, T. & Narita, K. (1976) *J. Biochem. (Tokyo)* **79**, 353–359
- Osborne, C. K., Monaco, M. E., Lipmann, M. E. & Kahn, R. C. (1978) *Cancer Res.* **38**, 94–102
- Osborne, C. K., Hamilton, B., Titus, G. & Livingstone, R. B. (1980) *Cancer Res.* **40**, 2361–2366.
- Shiu, R. P. C. (1980) *J. Biol. Chem.* **255**, 4278–4281
- Sraer, J. & Ardaillou, R. (1974) in *Endocrinology 1973* (Taylor, S., ed.), pp. 170–176, Heinemann Medical Books, London
- Tashjian, A. H., Wright, D. R., Ivey, J. L. & Pont, A. (1978) *Recent Prog. Horm. Res.* **34**, 285–334
- Tonelli, Q. J. & Sorof, S. (1980) *Nature (London)* **285**, 250–252
- Yamauchi, H., Shiraki, M., Otani, M., Matsuo, M. & Orimo, H. (1977) *Endocrinol. Jpn.* **24**, 281–285.
- Yang, J., Richards, J., Guzman, R., Imagawa, W. & Nandi, S. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2088–2092