The effects of 24R,25-dihydroxycholecalciferol and of 1 α ,25-dihydroxycholecalciferol on ornithine decarboxylase activity and on DNA synthesis in the epiphysis and diaphysis of rat bone and in the duodenum

Dalia SÖMJEN,* Itzhak BINDERMAN* and Yosef WEISMAN†

*Hard Tissues Unit and †Vitamin Research Laboratory, Tel-Aviv Medical Center, Sackler School of Medicine, University of Tel-Aviv, Tel-Aviv, Israel

(Received 21 December 1982/Accepted 25 April 1983)

The effect of cholecalciferol metabolites on ornithine decarboxylase activity and on DNA synthesis in developing long bones was investigated in vitamin D-depleted rats. In the epiphysis there was a 6.4-fold increase in ornithine decarboxylase activity 5 h after a single injection of 24R,25-dihydroxycholecalciferol but not of 24S,25-dihydroxycholecalciferol or other vitamin D metabolites. In comparison, in the diaphysis and duodenum, 1 α ,25-dihydroxycholecalciferol, but not other vitamin D metabolites, caused a 3–3.5-fold increase in the enzyme activity. The enzyme activity in the tissues examined attained a maximal value at 5 h after the injection of the metabolites. The activity of ornithine decarboxylase in the epiphyseal region increased dose-dependently as the result of a single injection of 24R,25-dihydroxycholecalciferol and attained a maximal value at a dose between 30 and 3000 ng. In addition, administration of 24R,25-dihydroxycholecalciferol, but not 24S,25-dihydroxycholecalciferol or other metabolites, caused within 24 h a 1.7–2.0-fold increase in [3 H]thymidine incorporation into DNA of the epiphyses of tibial bones. In comparison, 1 α ,25-dihydroxycholecalciferol caused a 1.5-fold increase in [3 H]thymidine incorporation into DNA of the diaphyses and of the duodenum. The present data indicate that 24R,25-dihydroxycholecalciferol is involved in the regulation of epiphyseal growth, whereas 1 α ,25-dihydroxycholecalciferol stimulates the proliferation of cells in the diaphysis of long bones and in the intestinal mucosa.

24,25-Dihydroxycholecalciferol, a metabolite of cholecalciferol, is probably involved in the development of embryonic cartilage and in endochondral bone formation (Ornøy *et al.*, 1978; Endo *et al.*, 1980). The main effect of 1 α ,25-(OH) $_2$ D $_3$, the biologically active metabolite of vitamin D, on bone is stimulation of bone mineral mobilization (Raisz *et al.*, 1972; Brommage & Neuman, 1979). The mechanism of action of 1 α ,25-(OH) $_2$ D $_3$, has been extensively examined in chick and rat intestine (Wasserman *et al.*, 1968; Brumbaugh & Haussler, 1974; Zerwekh *et al.*, 1974; Spencer *et al.*, 1976). Those studies suggest a mode of action of 1 α ,25-

(OH) $_2$ D $_3$ on intestinal mucosa cells similar to that of hormonal steroids on their target organ (O'Malley & Schrader, 1976). Little is known, however, about the mechanism of action of 24,25-(OH) $_2$ D $_3$ in its proposed target tissue. However, we have demonstrated specific receptors for 24,25-(OH) $_2$ D $_3$ in the epiphyses of long bones of vitamin D-depleted rats and in chondrocyte-like cells from chick-embryo limb-bud cell cultures (Sömjén *et al.*, 1982*a,b*). Other studies have shown a specific nuclear uptake of 24,25-(OH) $_2$ D $_3$ and its effects on DNA polymerase activity and proteoglycan synthesis in cultured growth-plate chondrocytes (Corvol *et al.*, 1978, 1980).

Ornithine decarboxylase (EC 4.1.1.17) is the rate-limiting enzyme in the biosynthesis of polyamines (Russell & Snyder, 1968; Pegg & Williams-Ashman, 1968). Ornithine decarboxylase activity is

Abbreviations used: 25-(OH)D $_3$, 25-hydroxycholecalciferol; 1 α ,25-(OH) $_2$ D $_3$, 1 α ,25-dihydroxycholecalciferol; 24,25-(OH) $_2$ D $_3$, 24,25-dihydroxycholecalciferol; 1,24,25-(OH) $_3$ D $_3$, 1,24,25-trihydroxycholecalciferol.

markedly enhanced in growing tissues and is considered to be associated with cell proliferation (Russell & Snyder, 1968; Hölttä & Jänne, 1972). It has been shown that $1\alpha,25\text{-(OH)}_2\text{D}_3$ administered to vitamin D-deficient chicks significantly increases the activity of ornithine decarboxylase in the duodenal mucosa (Shinki *et al.*, 1981). Since the epiphyseal growth plate, the suggested target tissue for $24,25\text{-(OH)}_2\text{D}_3$, is an actively growing tissue, we have examined the effect of cholecalciferol and its metabolites on ornithine decarboxylase activity and on $[^3\text{H}]$ thymidine incorporation into DNA in the epiphyses and diaphyses of long bones and in the duodenum of vitamin D-depleted rats.

Experimental

Animals

Four-week-old female Wistar rats were raised on a vitamin D-deficient diet containing 0.75% calcium, 0.05% phosphorus and soya-bean protein instead of casein (Lawson *et al.*, 1969), and kept in darkness. At 130 days of age the females were mated with normal male rats and maintained during gestation and lactation on the same vitamin D-deficient diet (Sömjen *et al.*, 1982a). The weanling pups were fed with the same diet for 2 (additional) weeks. Vitamin D deficiency was verified by plasma analysis for $25\text{-(OH)}\text{D}_3$ and $1\alpha,25\text{-(OH)}_2\text{D}_3$ (Weisman *et al.*, 1977; Eisman *et al.*, 1976). All animals had no detectable concentrations of $25\text{-(OH)}\text{D}_3$ ($<1.6\text{ ng/ml}$) or $1\alpha,25\text{-(OH)}_2\text{D}_3$ ($<5\text{ pg/ml}$). Then cholecalciferol or its metabolites, in 0.2 ml of 20% (v/v) ethanol in propylene glycol, were injected intraperitoneally.

Effects of cholecalciferol and its metabolites on ornithine decarboxylase activity

At 5 h after the administration of cholecalciferol ($10\mu\text{g}$), $25\text{-(OH)}\text{D}_3$ ($2.5\mu\text{g}$), $24R,25\text{-(OH)}_2\text{D}_3$ (300 ng), $24S,25\text{-(OH)}_2\text{D}_3$ (300 ng) or $1\alpha,25\text{-(OH)}_2\text{D}_3$ (60 ng), the animals were killed. The epiphyses and diaphyses of the tibiae, as well as the duodenal mucosa, were quickly removed, washed thoroughly with cold 0.9% NaCl and homogenized in an Ultra-Turrax homogenizer (homogenizing buffer: 10 mM-Tris/HCl buffer, pH 7.5, containing 250 mM-sucrose, 0.5 mM-dithiothreitol, 0.1 mM-disodium EDTA and 0.1 mM-pyridoxal 5'-phosphate). The homogenate was centrifuged at $38000g$ for 10 min, and the resulting supernatant was used for the enzyme assay.

We have also examined the time course of change in epiphyseal, diaphyseal and duodenal ornithine decarboxylase activity at 2, 5, 14 and 24 h after the administration of $24R,25\text{-(OH)}_2\text{D}_3$ (300 ng) or $1\alpha,25\text{-(OH)}_2\text{D}_3$ (60 ng).

In another experiment we have examined the

dose-dependent stimulation of epiphyseal ornithine decarboxylase activity at 5 h after a single injection of $24R,25\text{-(OH)}_2\text{D}_3$ ($30\text{--}3000\text{ ng}$).

Assay of ornithine decarboxylase activity

Ornithine decarboxylase activity was measured by a modified version of the method described previously (Kaye *et al.*, 1973; Ickson & Kaye, 1976). The reaction mixture (final volume $100\mu\text{l}$) consisted of an enzyme preparation ($0.04\text{--}1.0\text{ mg}$ of protein), 0.5 mM-DL-[1- ^{14}C]ornithine, 0.05 mM-pyridoxal 5'-phosphate and 5 mM-dithiothreitol in 50 mM-Tris/HCl buffer, pH 7.8. Incubation was at 37°C for 60 min in $15\text{ mm} \times 58\text{ mm}$ disposable plastic vials containing a polypropylene centre well suspended from a rubber bottle-stopper, which provided a gas-tight seal. The $^{14}\text{CO}_2$ released was absorbed in 0.25 ml of the organic base mixture Soluene (Packard Instrument Co.), placed in the centre well, which also contained a folded filter paper to increase the absorptive surface. The incubation was ended by injecting 0.5 ml of 3 M- H_2SO_4 into the reaction mixture. Then the tubes were incubated for an additional 25 min at 37°C to complete the absorption of $^{14}\text{CO}_2$.

Effects of $1\alpha,25\text{-(OH)}_2\text{D}_3$ and $24,25\text{-(OH)}_2\text{D}_3$ on $[^3\text{H}]$ thymidine incorporation into DNA

At 24 h after the administration of either $1\alpha,25\text{-(OH)}_2\text{D}_3$ (60 ng) or $24R,25\text{-(OH)}_2\text{D}_3$ (300 ng), the animals were killed and the epiphyses and diaphyses of the tibiae as well as the duodenum were quickly removed. Slices of the tissues were incubated for 2 h in 1 ml of BGJ culture medium (Biological Industries, Beth Haemek, Israel) containing $10\mu\text{Ci}$ of $[^3\text{H}]$ -thymidine. Then the slices were washed and homogenized in distilled de-ionized water in a motor-driven glass/Teflon homogenizer. Incorporation of $[^3\text{H}]$ -thymidine into acid-insoluble material and the content of DNA were measured as previously described (Sömjen *et al.*, 1980; Burton, 1956).

Chemicals

$25\text{-(OH)}\text{D}_3$, $24R,25\text{-(OH)}_2\text{D}_3$ and $1\alpha,25\text{-(OH)}_2\text{D}_3$ were kindly provided by Dr. S. Edelstein, Weizmann Institute of Science, Rehovot, Israel. $24S,25\text{-(OH)}_2\text{D}_3$ was kindly provided by Teva Pharmaceutical Industries, Jerusalem, Israel. Dithiothreitol and pyridoxal 5'-phosphate were purchased from Sigma Chemical Co.

Radioactive materials and measurement of radioactivity

DL-[1- ^{14}C]Ornithine (sp. radioactivity $1\text{--}3\text{ mCi/mmole}$) was obtained from New England Nuclear, and $[^3\text{H}]$ thymidine (sp. radioactivity 5 Ci/mmole) was purchased from The Radiochemical Centre. Radioactivity measurements were made in a Packard Tri-Carb model 3380 automatic liquid-scintillation

Table 1. *Effects of cholecalciferol metabolites on the activity of ornithine decarboxylase in the duodenum and in epiphyses and diaphyses of long bones of vitamin D-depleted rats*

The experimental details are described in the Experimental section. Results are given as means $\pm$ S.E.M. for at least five determinations, except those for the effects of cholecalciferol and 24S,25-(OH)₂D₃, which are means for two determinations. The ratios of experimental to control values are given in parentheses. Statistical significance (by *t* test) of treatment; **P* < 0.001; †*P* < 0.005; ‡ not significant.

Treatment	Ornithine decarboxylase activity (pmol of ¹⁴ CO ₂ /h per mg of protein)		
	Epiphyses	Diaphyses	Duodenum
Untreated (0.2 ml of 20% ethanol in propylene glycol)	540 $\pm$ 50	570 $\pm$ 40	420 $\pm$ 40
Cholecalciferol (10 μ g/animal)	340 (0.55)	860 (1.55)	680 (1.63)
25-(OH)D ₃ (2.5 μ g/animal)	490 $\pm$ 40‡ (0.90)	520 $\pm$ 260‡ (0.91)	420 $\pm$ 140‡ (1.00)
1 α ,25-(OH) ₂ D ₃ (60 ng/animal)	390 $\pm$ 40‡ (0.72)	1810 $\pm$ 410* (3.18)	1430 $\pm$ 160† (3.40)
24R,25-(OH) ₂ D ₃ (300 ng/animal)	3470 $\pm$ 360* (6.43)	730 $\pm$ 100‡ (1.27)	380 $\pm$ 170‡ (0.91)
24S,25-(OH) ₂ D ₃ (300 ng/animal)	540 (1.00)	570 (1.00)	420 (1.00)

Table 2. *[³H]Thymidine incorporation into DNA from tissues of vitamin D-depleted rats after injection of cholecalciferol metabolites*

The experimental details are described in the Experimental section. Results are given as means $\pm$ S.E.M. for at least three determinations, except those for the effect of 24S,25-(OH)₂D₃, which are means for two determinations. The ratios of experimental to control values are given in parentheses. Statistical significance (by *t* test) of treatment: **P* < 0.01; †*P* < 0.005; ‡ not significant.

Treatment	[³ H]Thymidine incorporation into DNA (c.p.m./ μ g of DNA)		
	Epiphyses	Diaphyses	Duodenum
Untreated (0.2 ml of 20% ethanol in propylene glycol)	46 $\pm$ 7	98 $\pm$ 8	34 $\pm$ 5
1 α ,25-(OH) ₂ D ₃ (60 ng/animal)	45 $\pm$ 6‡ (0.97)	147 $\pm$ 20* (1.50)	52 $\pm$ 6* (1.54)
24R,25-(OH) ₂ D ₃ (300 ng/animal)	85 $\pm$ 9† (1.85)	91 $\pm$ 8‡ (0.90)	39 $\pm$ 3‡ (1.15)
24S,25-(OH) ₂ D ₃ (300 ng/animal)	48 (1.01)	98 (1.00)	33 (0.98)

spectrometer. Counting efficiency varied from 47 to 49% for ³H and 58 to 60% for ¹⁴C.

Results

Administration of 24R,25-(OH)₂D₃, but not of 1 α ,25-(OH)₂D₃, 25(OH)D₃ or cholecalciferol, to vitamin D-depleted rats caused within 5 h a 6.4-fold increase in ornithine decarboxylase activity in the epiphyses of tibiae. In comparison, 1 α ,25-(OH)₂D₃, but not 24R,25-(OH)₂D₃, 25-(OH)D₃ or cholecalciferol, caused a 3–3.5-fold increase in the enzyme activity in the diaphyses of the tibiae and in the duodenum (Table 1). Whereas the natural isomer of 24,25-(OH)₂D₃, namely 24R,25-(OH)₂D₃, caused a significant increase in ornithine decarboxylase activity in the epiphyses, the synthetic isomer 24S,25-(OH)₂D₃ had no effect on the enzyme activity. Fig. 1 shows the time course of change in epiphyseal, diaphyseal and intestinal ornithine decarboxylase activity. The enzyme activity in the epiphyses attained a maximal value at 5 h after the injection of 24R,25-(OH)₂D₃. Similarly, ornithine decarboxylase activity in the diaphyses and intestine attained a maximal value at 5 h after the

injection of 1 α ,25-(OH)₂D₃. At 24 h after the injection of 24R,25-(OH)₂D₃ or 1 α ,25-(OH)₂D₃ the enzyme activity declined to the control value in all tissues.

Fig. 2 shows the dose-dependent enhancement of epiphyseal ornithine decarboxylase activity at 5 h after injection of 24R,25-(OH)₂D₃. The enzyme activity attained a maximum value between 30 and 3000 ng of 24,25-(OH)₂D₃.

Administration of 24R,25-(OH)₂D₃, but not of 1 α ,25-(OH)₂D₃ (60 ng), caused within 24 h a 1.7–2-fold increase in [³H]thymidine incorporation into DNA of the epiphyses of tibiae. In comparison, 1 α ,25-(OH)₂D₃, but not 24R,25-(OH)₂D₃, caused a 1.5-fold increase in [³H]thymidine incorporation into DNA of the diaphyses and the duodenum (Table 2).

In contrast with the results obtained with 24R,25-(OH)₂D₃, the administration of the synthetic isomer 24S,25-(OH)₂D₃ had no effect on [³H]thymidine incorporation into DNA of the epiphyses of the tibiae.

Discussion

The question of the biological function of 24,25-(OH)₂D₃ remains controversial. Several studies have

Fig. 1. Time course of change in (a) duodenal, (b) epiphyseal and (c) diaphyseal ornithine decarboxylase activity after intraperitoneal administration of 300 ng of 24R,25-(OH)₂D₃ (O) or 60 ng of 1α,25-(OH)₂D₃ (●). The experimental details are as described in the Experimental section. The activity of ornithine decarboxylase in tissues of untreated rats was: duodenum, 0.42 ± 0.09 nmol of ¹⁴CO₂/h per mg of protein; diaphyses, 0.57 ± 0.04 nmol of ¹⁴CO₂/h per mg of protein; epiphyses, 0.54 ± 0.05 nmol of ¹⁴CO₂/h per mg of protein. Results are means $\pm$ S.E.M. for five determinations.

Fig. 2. Dose-dependence in the stimulation of ornithine decarboxylase activity by 24R,25-(OH)₂D₃ in the epiphyses of vitamin D-depleted rats

The experimental details are described in the Experimental section. 24R,25-(OH)₂D₃ was injected intraperitoneally, and the animals were killed 5 h later. The activity of ornithine decarboxylase in epiphyses of untreated animals was 0.66 nmol of ¹⁴CO₂/h per mg of protein. Results are means $\pm$ S.E.M. for at least four determinations.

suggested that 24,25-(OH)₂D₃ is involved in endochondral bone formation (Ornoy *et al.*, 1978; Endo *et al.*, 1980). Further studies have demonstrated specific receptors for 24,25-(OH)₂D₃ in the epiphyses of long bones of vitamin D-depleted rats and in chondrocyte-like cells from chick-embryo limb bud cell cultures (Sömjen *et al.*, 1982a,b). Other studies have shown a specific nuclear binding of 24,25-(OH)₂D₃ and its effects on DNA polymerase activity and proteoglycan synthesis in cultured growth-plate chondrocytes (Corvol *et al.*, 1978, 1980). Little is known, however, about the mode of

action of $24,25\text{-(OH)}_2\text{D}_3$ in its suggested target tissue. Unlike $1\alpha,25\text{-(OH)}_2\text{D}_3$, which stimulates both specific mRNA and protein synthesis (Haussler & McCain, 1977), no specific $24,25\text{-(OH)}_2\text{D}_3$ -induced protein has been demonstrated yet. The present paper shows that $24\text{R},25\text{-(OH)}_2\text{D}_3$ stimulates both ornithine decarboxylase activity and DNA synthesis in the epiphyseal region of the tibia, whereas $1\alpha,25\text{-(OH)}_2\text{D}_3$ stimulates the enzyme activity in the diaphyses of long bones. Unlike intestinal calcium-binding protein, which is specifically induced by $1\alpha,25\text{-(OH)}_2\text{D}_3$ (Wasserman *et al.*, 1968; Haussler & McCain, 1977), ornithine decarboxylase cannot be considered as a specific marker for vitamin D activity, since many hormones have been shown to stimulate its activity in their respective target tissues (Cohen *et al.*, 1970; Kaye *et al.*, 1971; Takigawa *et al.*, 1979). However, our observation that the enzyme is stimulated in the epiphyseal cartilage by $24\text{R},25\text{-(OH)}_2\text{D}_3$, whereas $1\alpha,25\text{-(OH)}_2\text{D}_3$ stimulates its activity in the diaphyses, suggests that each of these metabolites has its specific site of action in the developing long bone. It is noteworthy that the synthetic isomer, $24\text{S},25\text{-(OH)}_2\text{D}_3$, had no effect on either ornithine decarboxylase activity or [^3H]thymidine incorporation into DNA of the epiphyses. This observation emphasizes the specific role of $24\text{R},25\text{-(OH)}_2\text{D}_3$ in the epiphysis. The enhancement of ornithine decarboxylase activity and DNA synthesis by $24\text{R},25\text{-(OH)}_2\text{D}_3$ is not exclusive to the rat. We have shown that $24\text{R},25\text{-(OH)}_2\text{D}_3$ but not other vitamin D metabolites, stimulates ornithine decarboxylase activity, DNA synthesis and protein synthesis in chondrocytes from cultures of chick-embryo limb-bud cells (I. Binderman & D. Sömjen, unpublished work). The results of the present study are well in accord with previous data (Sömjen *et al.*, 1982a,b) showing the existence of specific receptors for $24\text{R},25\text{-(OH)}_2\text{D}_3$ in the epiphyses of rat long bones and in chondrocytes from cultures of chick-embryo limb-bud cells and of receptors for $1\alpha,25\text{-(OH)}_2\text{D}_3$ in the diaphyses of rat long bones. Accumulating results thus suggest that one of the possible roles of $24,25\text{-(OH)}_2\text{D}_3$ may be stimulation of cell proliferation and differentiation in the epiphyseal growth plate, whereas $1\alpha,25\text{-(OH)}_2\text{D}_3$ may have an effect on the proliferation of differentiating bone cells in the diaphyses. It was shown that both metabolites affect Ca^{2+} transport in primary cultures of bone cells (Eilam *et al.*, 1980). The possibility that changes in the concentration of Ca^{2+} ions may influence DNA synthesis in the epiphyses and diaphyses should be considered (Durham & Walton, 1982). It is known that $24,25\text{-(OH)}_2\text{D}_3$ is converted into $1,24,25\text{-(OH)}_2\text{D}_3$ in vitamin D-deficient animals (Holick *et al.*, 1973), and this could be the final metabolite that acts at the

epiphyseal cartilage. However, it seems that the hydroxy group at C-24 is necessary for the observed biological action on the epiphyseal cartilage, since $1\alpha,25\text{-(OH)}_2\text{D}_3$ has no effect on ornithine decarboxylase activity and DNA synthesis in this tissue. Moreover, the observation that $24\text{R},25\text{-(OH)}_2\text{D}_3$ induces ornithine decarboxylase activity, DNA synthesis and protein synthesis in chick-embryo limb-bud chondrocyte-like cells *in vitro* (I. Binderman & D. Sömjen, unpublished work) suggests that $24\text{R},25\text{-(OH)}_2\text{D}_3$ itself and not a further metabolite is biologically active.

We thank Mrs. E. Berger for excellent technical help. We also thank Mrs. M. Yariv for her help in the enzymic assay and Professor Alvin M. Kaye for his helpful discussions, both being from the Department of Hormone Research, Weizmann Institute of Science, Rehovot, Israel.

References

- Brommage, R. & Neuman, W. F. (1979) *Am. J. Physiol.* **237**, E113–E120
- Brumbaugh, P. F. & Haussler, M. R. (1974) *J. Biol. Chem.* **249**, 1258–1262
- Burton, K. (1956) *Biochem. J.* **62**, 315–322
- Cohen, S., O'Malley, B. W. & Stastny, M. (1970) *Science* **170**, 336–338
- Corvol, M., Dumontier, M. F., Garabedian, M. & Rapport, R. (1978) *Endocrinology* **102**, 1269–1274
- Corvol, M. M., Ulmann, A. & Garabedian, M. (1980) *FEBS Lett.* **116**, 273–276
- Durham, C. H. & Walton, J. M. (1982) *Biosci. Rep.* **2**, 15–30
- Eilam, Y., Szydel, N. & Harell, A. (1980) *Mol. Cell. Endocrinol.* **19**, 263–273
- Eisman, J. A., Hamstra, A. J. & DeLuca, H. F. (1976) *Science* **193**, 1021–1023
- Endo, H., Kiyoki, M., Kawashima, K., Naruchi, T. & Hashimoto, Y. (1980) *Nature (London)* **286**, 262–265
- Haussler, M. R. & McCain, T. A. (1977) *N. Engl. J. Med.* **297**, 974–983
- Holick, M. F., Kleiner-Bossaller, A., Schnoes, H. K., Kasten, P. M., Boyle, I. T. & DeLuca, H. F. (1973) *J. Biol. Chem.* **248**, 6691–6696
- Hölttä, E. & Jänne, J. (1972) *FEBS Lett.* **23**, 117–121
- Ickson, I. & Kaye, A. M. (1976) *FEBS Lett.* **61**, 54–58
- Kaye, A. M., Ickson, I. & Lindner, H. R. (1971) *Biochim. Biophys. Acta* **252**, 150–159
- Kaye, A. M., Ickson, I., Lamprecht, S. A., Gruss, R., Tsafri, A. & Lindner, H. R. (1973) *Biochemistry* **12**, 3072–3076
- Lawson, D. E. M., Wilson, P. W. & Kodicek, E. (1969) *Biochem. J.* **115**, 269–277
- O'Malley, B. W. & Schrader, W. T. (1976) *Sci. Am.* **234**, 32–44
- Ornoy, A., Goodwin, D., Noff, D. & Edelstein, S. (1978) *Nature (London)* **276**, 517–519
- Pegg, A. E. & Williams-Ashman, H. G. (1968) *Biochem. J.* **108**, 533–539
- Raisz, L. G., Trummel, C. L., Holick, M. F. & DeLuca, H. F. (1972) *Science* **176**, 1146–1148

- Russell, D. & Snyder, S. H. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **60**, 1420–1427
- Shinki, T., Takashashi, N., Miyaoura, C., Samejima, K., Nishii, Y. & Suda, T. (1981) *Biochem. J.* **195**, 685–690
- Sömjen, D., Binderman, I., Berger, E. & Harell, A. (1980) *Biochim. Biophys. Acta* **629**, 91–100
- Sömjen, D., Sömjen, G. J., Weisman, Y. & Binderman, I. (1982a) *Biochem. J.* **204**, 31–36
- Sömjen, D., Sömjen, G. J., Harell, H., Mechanic, G. L. & Binderman, I. (1982b) *Biochem. Biophys. Res. Commun.* **106**, 644–651
- Spencer, R., Charman, M., Emtage, J. S. & Lawson, D. E. M. (1976) *Eur. J. Biochem.* **71**, 399–409
- Takigawa, M., Watanabe, R., Ishida, H., Asada, A. & Suzuki, F. (1979) *J. Biochem. (Tokyo)* **85**, 311–314
- Wasserman, R. H., Corradino, R. A. & Taylor, A. N. (1968) *J. Biol. Chem.* **243**, 3978–3986
- Weisman, Y., Reiter, E. & Root, A. (1977) *J. Pediatr.* **91**, 904–907
- Zerwekh, J. E., Haussler, M. R. & Lindell, T. J. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2337–2341