Acta orthop. scand. *54*, 470–478, 1983

# EFFECTS OF SALMON CALCITONIN ON SYNTHESIS AND MINERALIZATION OF COLLAGEN IN RATS

ARNE EKELAND & TRINE UNDERDAL

Institute for Surgical Research, Rikshospitalet, University of Oslo, Norway

The effects of salmon calcitonin (CT) on collagen metabolism and mineral deposition in fractures and intact femora, and on collagen metabolism in healing skin wounds and intact skin have been studied in young male rats. Serum calcium and serum phosphorus were reduced 3 h after the daily subcutaneous CT injection (3 MRC-U/kg body weight), whereas a rebound increase in the serum levels of both minerals was observed at 24 hours after the injection.

CT had an early transient inhibitory influence on the collagen synthesis, and this resulted in a reduced total content of collagen in bones and skin specimens from treated rats compared to controls. The concentration of collagen in bone and skin was, however, increased in treated animals compared to controls after prolonged CT administration.

Following an early transient increase, the incorporation of strontium-85 into the fractured bones was impaired after 30 days of CT treatment. This resulted in a reduced mineral concentration in the fractures of treated rats compared to controls in the last part of the experiment.

The recorded effects of CT treatment, which were most pronounced in healing fractures and intact skin specimens, may be interpreted as an inhibitory influence of CT both on synthesis, mineralization and degradation of collagen.

*Key words:* $^{14}$C-hydroxyproline; calcitonin; callus; fractures; growth; phosphorus; $^{85}$Sr; wound healing

Accepted 11.xi.82

The thyroidal hormone calcitonin (CT) is claimed to stimulate both fracture repair and bone formation (Duriez 1979). It has also been used in the treatment of human fractures (Kaplan et al. 1976, Rasskazov 1977). Several studies have, however, failed to show any significant influence of CT on fracture repair (Bright & Estep 1968, Harris et al. 1975, Schatzker et al. 1979, Lindgren et al. 1981). This discrepancy may be due to differences in the methods used to evaluate the hormonal effects. In order to elucidate this problem, we have studied the effects of CT on fracture repair and bone growth by several methods applied simultaneously in the same animal. Since collagen is the main organic component both of bone and skin, the influence of CT on healing wounds and intact skin was also investigated.

In a recent paper (Ekeland et al. 1983), we reported that salmon CT administration to rats did not influence mechanical properties of healing femoral fractures, intact femora or skin wounds, whereas mechanical properties of intact skin were impaired by the hormone. In the present paper, the bones and the skin specimens used for the mechanical tests have been further investigated to determine the influence of salmon CT on collagen metabolism and on mineralization.

## MATERIALS AND METHODS

*Experimental animals*

A total of 118 male Wistar/Af/Han/Mol SPF rats were used. They were initially about 25 days old, and had a median body weight of 61 g. The rats were divided into two weight-matched groups of 59 animals each. One group was treated with salmon CT (Miacalcic®, Sandoz, Basel, Switzerland), 3 Medical Research Council Units (MRC-U) per kg body weight. The hormone was dissolved in 3 per cent gelatine, and injected subcutaneously once daily (at 10–11 a.m.) from the first postoperative day. The control group received corresponding injections of the vehicle. The rats were fed standard animal pellets containing 0.9 per cent calcium and 0.7 per cent phosphorus (Bl. nr. 3155, Møllesentralen i/s, Oslo, Norway) and water *ad libitum*.

*Experimental fracture and wound*

A standardized, mid-femoral fracture was produced in all the rats, and in 74 of them (37 to be CT treated and 37 controls) a skin incision was made in the midline along the spine, as previously described (Ekeland et al. 1983). Twenty-four hours before the rats with skin wounds were killed, 15 μCi (0.56 MBq) L(U-$^{14}$C) proline (285 mCi (10.5 GBq)/mmol) and 8 μCi (0.30 MBq) carrier-free strontium (Sr)-85 chloride (115 Ci (0.43 TBq)/mmol) (The Radiochemical Centre, Amersham, Buckinghamshire, England) per 100 g body weight were injected intraperitoneally. The final CT injection was given simultaneously, whereas the final CT injection to rats without skin wounds was administered 3 h prior to sacrifice.

At 10, 20, 30 and 40 days after the fracture and the wound had been induced, groups of rats were killed by aortic exsanguination in ether anaesthesia. The collected blood was centrifuged, and the serum stored at –20°C until analyzed for calcium, phosphorus and albumin. Following the mechanical tests (Ekeland et al. 1983) the bones and the skin samples were stored at –20°C until subjected to collagen and mineral analyses.

*Preparation of samples for analyses*

Specimens of fractured and intact femora from rats with skin wounds were prepared by removing epiphyses, cartilage and soft tissues from the bones (Figure 1A). Meticulous care was taken not to remove any parts of the callus.

Samples from wounded skin were prepared from the three specimens used for tensile tests (Ekeland et al. 1983) by excising the scar 3 mm from the incisional site on both sides (Figure 1A). Only the most cranial of the three specimens used for tensile tests of intact skin was employed for collagen analyses (Figure 1B). These specimens were derived from rats which had not received injections of radioactive proline. The collagen synthesis of intact skin was therefore measured in the most cranial of the three skin specimens from the rats with wound, after the skin close to the wound had been excised as described above. The distance from the analyzed specimen to the wound ranged from 3 mm to 15 mm (Figure 1A).

Blood from rats with wounds were collected during the first part of the day, whereas blood from the rats without wounds were collected in the afternoon (Figure 1).

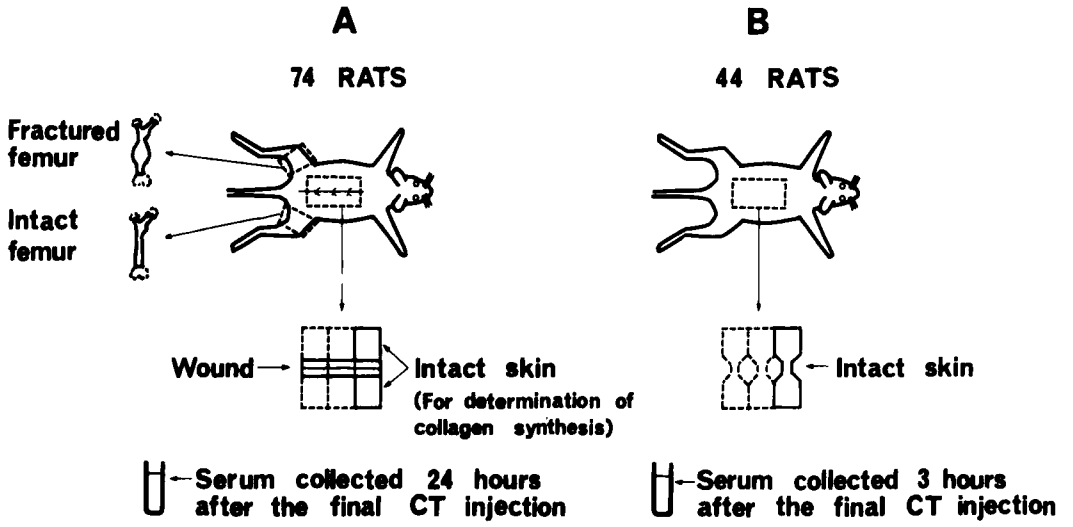


*Figure 1. Schematic drawings of rats with (A) and without (B) skin wounds. The bone, skin and blood samples analyzed are indicated by heavy lines. CT, calcitonin.*

*Collagen analyses*

Bone and skin specimens were defatted in acetone for 7 days with two changes of acetone, and dried in air at 35°C until constant weight (dry weight). The specimens were then hydrolyzed in 4 ml 6 M HCl for 18 h. The collagen content of bone and skin was determined by measuring the amount of hydroxyproline in the hydrolysates colorimetrically (Firschein 1969).

*Mineral analyses*

Calcium and phosphorus concentrations in bone hydrolysates and sera, and serum levels of albumin were measured by a Bichromatic Analyzer (Abbott Laboratories, Diagnostic Division, Pasadena, USA). A-Gent Calcium (Abbott Laboratories), Phosphorus Auto/Stat (Pierce Chemical Corporation, Rockford, Illinois, USA) and A-Gent Albumin (Abbott Laboratories) kits were used for the analytical procedures.

*Radioactive nuclide analyses*

The collagen synthesis of bone and skin and the mineralization of bone were determined by measuring the amount of $^{14}$C-hydroxyproline and of $^{85}$Sr in the previously described bone and skin samples (Firschein 1969).

The bone hydrolysates were first counted in a well counter (Auto-Gamma Scintillation Spectrometer, Packard Instrument Company, Downers Grove, Illinois, USA) to determine selectively the activity of $^{85}$Sr (Firschein 1969). The counting efficiency was 26 per cent.

Chromatography of the hydrolysates on Dowex 50W columns was used subsequently to isolate $^{14}$C-hydroxyproline. Both $^{14}$C-proline, and $^{85}$Sr were retained by the columns, and completely removed from the collected fractions (Firschein 1969, Engesæter et al. 1980). The isolated $^{14}$C-hydroxyproline was counted in a liquid scintillation counter (Mark I, Nuclear – Chicago, Bes Plaines, Illinois, USA). Dilusolve® (Packard Instrument Company) was used as a scintillation solution, and counting efficiency, determined by the two channel ratio method, was 70 per cent.

As the amount of radioactive tracers administered to the rats referred to the specific activity of the tracers at the start of the experiment, all tissue samples were counted simultaneously.

*Statistical analyses*

Median with 0.25- and 0.75-fractiles were used to express the average and the dispersion of the measured values. The statistical significance probability was calculated by Wilcoxon's two-tailed test for two samples (Diem & Lentner 1975). Differences were considered significant when $2P \leq 0.05$.

## RESULTS

### *Growth of the animals*

Compared to controls, the dry weight of fractured femur specimens was transiently reduced after 20 days of CT treatment, whereas the dry weight of standardized, intact skin specimens was reduced both after 20 and 30 days of treatment (Table 1). CT did not significantly influence the dry weight of intact femur and skin wound specimens. Nor

*Table 1. Dry weight of standardized specimens from fractured and intact femora and from wounded and intact skin during 40 days of calcitonin (CT) treatment*

| Days after fracture/ wound | *n* | Fractured femur (mg) | | Intact femur (mg) | | Skin wound (mg) | | Intact skin (mg) | |
|---|---|---|---|---|---|---|---|---|---|
| | | CT treated rats | Controls | CT treated rats | Controls | CT treated rats | Controls | CT treated rats | Controls |
| 10 | 6–10 | 169 (157–175) | 171 (161–190) | 116 (113–118) | 119 (116–119) | 52 (50–54) | 53 (50–57) | 98 (91–103) | 102 (93–107) |
| 20 | 6–10 | 292 * (258–328) | 323 (300–390) | 179 (176–197) | 184 (183–198) | 66 (63–71) | 68 (61–74) | 95 *** (85–101) | 114 (113–128) |
| 30 | 6–10 | 369 (352–394) | 368 (354–379) | 267 (264–288) | 269 (254–279) | 63 (60–69) | 66 (58–69) | 113 *** (112–130) | 152 (146–161) |
| 40 | 5–7 | 445 (405–477) | 384 (350–444) | 340 (327–363) | 328 (325–337) | 77 (66–85) | 87 (75–90) | | |

Significant differences between CT treated and control rats are indicated by asterisks. *** $2P = 0.01$, * $2P < 0.05$. *n* = Number of animals. Median with 0.25- and 0.75-fractiles.

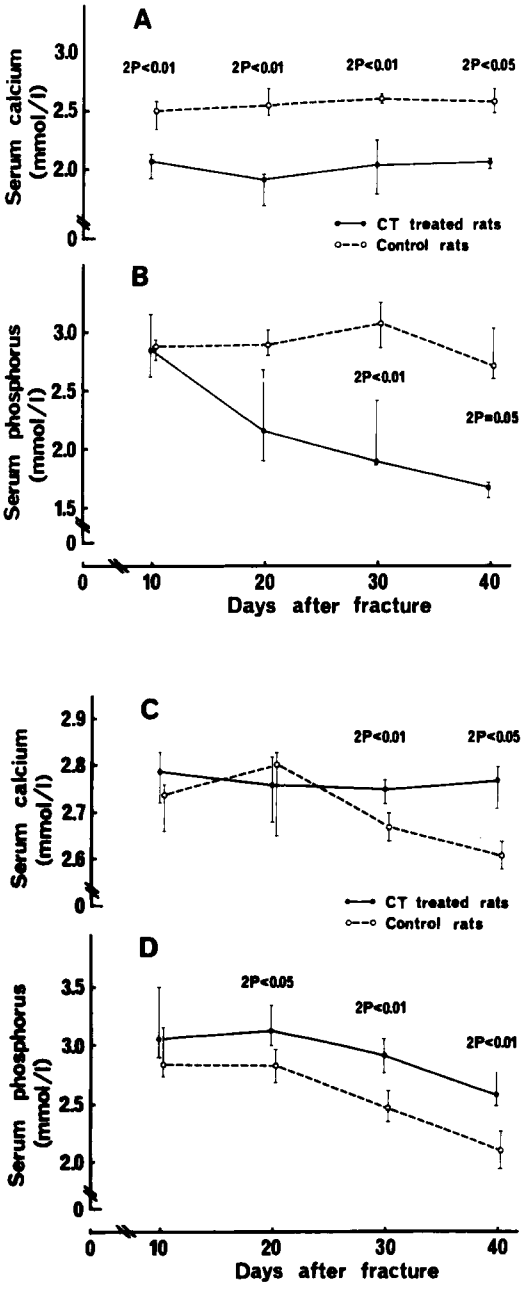


*Figure 2. Serum calcium and serum phosphorus in calcitonin (CT) treated and control rats at 3 h (A and B) and at 24 h (C and D) after injection of the hormone (3 MRC-U/kg body weight). The sera for A and B were sampled from rats without, and those for C and D from rats with skin wounds. There were 4–10 animals in each group. Significant differences between CT treated and control rats are indicated by* P*-values. (Median with 0.25- and 0-75-fractiles).*

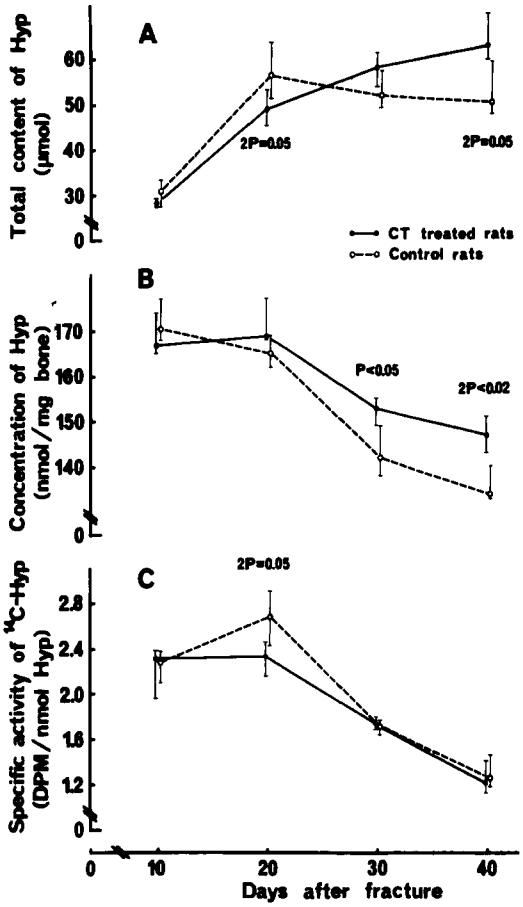


*Figure 3. Total content (A), concentration (B) and synthesis (C) of hydroxyproline (HYP) in standardized specimens of fractured femora during 40 days of calcitonin (CT) treatment. There were 5–10 animals in each group. Significant differences between CT treated and control rats are indicated by* P*-values. (Median with 0.25- and 0.75-fractiles).*

did it change the body weight (Ekeland et al. 1983).

### *Serum analyses*

The serum concentrations of calcium were significantly reduced 3 h after the daily CT injection throughout the study, whereas the serum concentrations of phosphorus were significantly reduced only during the last part of the study (Figure 2A and B). In contrast, the serum levels of both minerals were increased compared to con-

trols 24 h following the CT injection. This occurred, however, only after 20–30 days of treatment (Figure 2C and D).

There were no significant differences in serum albumin between treated rats and controls at either 3 or 24 h after the CT injection. In both groups the serum levels increased from about 45 g/l to about 53 g/l from the 10th to the 40th day of the experimental period.

*Bone analyses*

*Fractured femora.* The total content and the synthesis of hydroxyproline were reduced by about 13 per cent in specimens from CT treated rats compared to controls after 20 days of treatment. Later on, however, the total content and the concentration of hydroxyproline were increased in the fractures of treated rats compared to controls (Figure 3). Following an early transient increase, the incorporation of $^{85}Sr$ into the fractures was significantly impaired by CT treatment, and the concentrations of calcium and phosphorus in the fractured bones were reduced by about 8 per cent compared to controls at the end of the study (Figure 4).

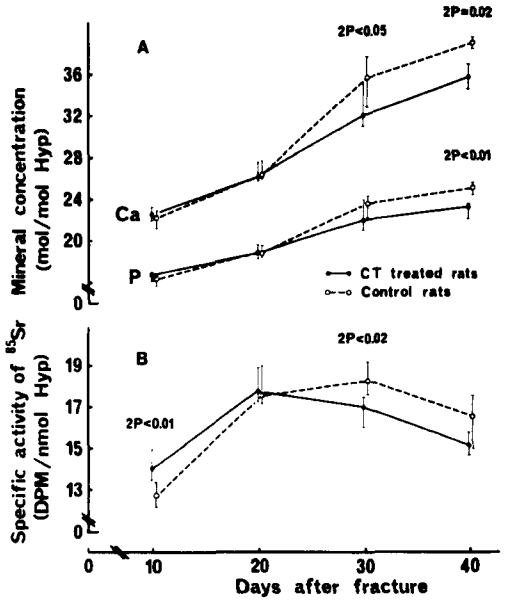


*Figure 4. Concentration (A) of calcium (Ca) and phsophorus (P), as well as incorporation of strontium (Sr) – 85 (B) in standardized specimens of fractured femora during 40 days of calcitonin (CT) treatment. There were 5–10 animals in each group. Significant differences between CT treated and control rats are indicated by P-values. (Median with 0.25- and 0.75-fractiles).*

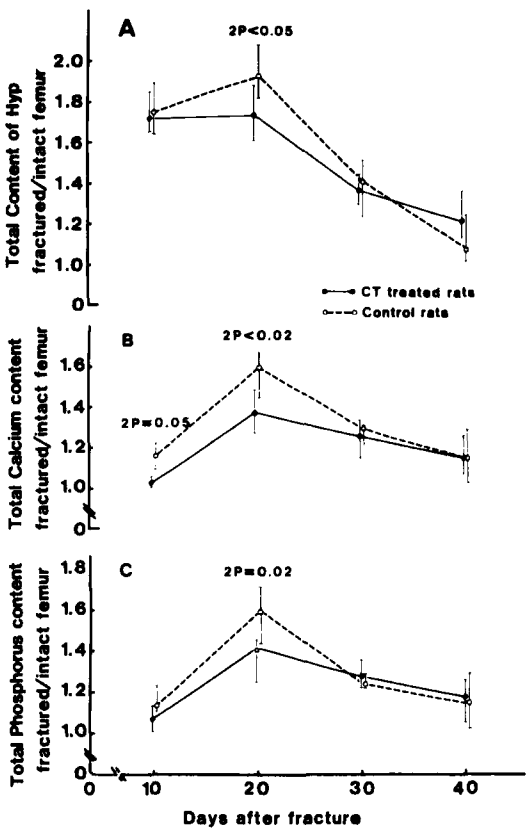


*Figure 5. Ratios of total content of hydroxyproline (Hyp) (A), calcium (B) and phosphorus (C) in fractured to intact femur specimens during 40 days of calcitonin (CT) treatment. There were 5–10 animals in each group. Significant differences between CT treated and control rats are indicated by P-values. (Median with 0.25- and 0.75-fractiles).*

Since the experiment was performed in growing animals, the effect of CT on the fracture callus may be more clearly observed from the ratios of hydroxyproline and minerals in fractured/intact femora (Figure 5).

*Intact femora.* Similar but less consistent effects of CT treatment were observed in specimens from intact femora (Table 2).

*Table 2. Concentration and synthesis of hydroxyproline (Hyp), mineral concentration and mineral incorporation of strontium (Sr) – 85 in intact femur specimens during 40 days of calcitonin (CT) treatment*

| Days after fracture | *n* | Concentration of Hyp (nmol/mg bone) | | Concentration of calcium (mol/mol Hyp) | | Concentration of phosphorus (mol/mol Hyp) | | Specific activity of $^{14}$C-Hyp (DPM/nmol Hyp) | | Specific activity of $^{85}$Sr (DPM/nmol Hyp) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CT treated rats | Controls | CT treated rats | Controls | CT treated rats | Controls | CT treated rats | Controls | CT treated rats | Controls |
| 10 | 9–10 | 143 | 148 | 35 | 34 | 25 | 25 | 1.59 ** | 1.67 | 14.7 * | 13.5 |
| | | (142–148) | (146–149) | (33–35) | (33–34) | (24–26) | (25–27) | (1.45–1.63) | (1.65–1.80) | (14.0–15.8) | (13.0–14.1) |
| 20 | 7–10 | 155 | 153 | 35 | 36 | 25 | 25 | 1.67 | 1.62 | 13.7 | 13.3 |
| | | (150–157) | (151–158) | (34–37) | (35–37) | (23–25) | (24–26) | (1.60–1.75) | (1.52–1.81) | (13.2–14.4) | (12.4–13.8) |
| 30 | 9–10 | 153 *** | 145 | 36 *** | 39 | 24 *** | 25 | 1.39 | 1.32 | 13.7 | 13.7 |
| | | (151–155) | (144–147) | (36–37) | (38–41) | (23–24) | (25–27) | (1.23–1.48) | (1.28–1.39) | (12.8–14.3) | (13.5–14.1) |
| 40 | 7 | 148 | 146 | 38 | 39 | 25 | 26 | 0.86 | 0.87 | 12.9 | 12.7 |
| | | (145–150) | (142–151) | (37–39) | (37–40) | (25–26) | (25–27) | (0.85–0.89) | (0.84–0.93) | (12.5–13.7) | (12.1–13.9) |

Significant differences between CT treated and control rats are indicated by asterisks. *** $2P < 0.01$, ** $2P < 0.02$, * $2P < 0.05$. *n* = Number of animals. Median with 0.25- and 0.75-fractiles.

*Skin analyses*

*Wounds.* Compared to controls, the collagen synthesis was transiently reduced after 10 days of CT treatment, whereas the collagen concentration was increased after 40 days of treatment (Table 3).

*Intact skin.* The collagen synthesis in CT treated rats was reduced by about 12 per cent compared to controls in the beginning of the experiment, and the total collagen content of the skin specimens was significantly reduced after both 20 and 30 days of treatment. In contrast, the collagen concentration of the skin was significantly increased after 30 days of CT treatment (Figure 6).

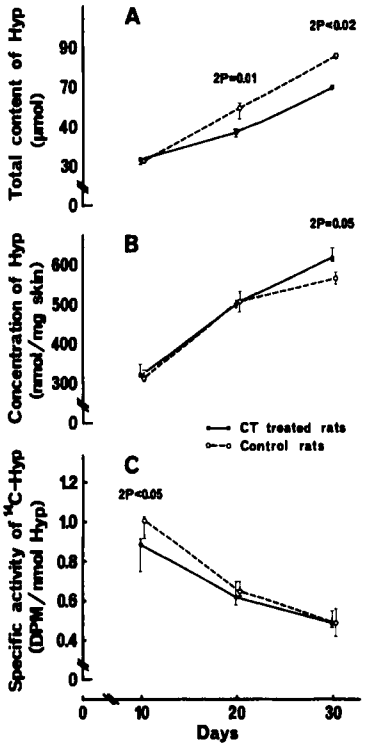


*Figure 6. Total content (A), concentration (B) and synthesis (C) of hydroxyproline (Hyp) in standardized specimens of intact skin during 30 days of calcitonin (CT) treatment. There were 6–10 animals in each group. Significant differences between CT treated and control rats are indicated by* P*-values. (Median with 0.25- and 0.75-fractiles).*

*Table 3. Concentration and synthesis of hydroxyproline (HYP) in standardized specimens from skin wounds during 40 days of calcitonin (CT) treatment*

| Days after wound | *n* | Concentration of Hyp (nmol/mg skin) | | Specific activity of $^{14}$C-Hyp (DPM/nmol Hyp) | |
|---|---|---|---|---|---|
| | | CT treated rats | Controls | CT treated rats | Controls |
| 10 | 8–10 | 319 (308–327) | 314 (307–326) | 0.90 ** (0.79–0.94) | 1.04 (0.95–1.06) |
| 20 | 7–10 | 438 (413–451) | 434 (427–458) | 0.81 (0.76–0.88) | 0.76 (0.75–0.90) |
| 30 | 8–10 | 574 (560–589) | 591 (555–609) | 0.58 (0.50–0.59) | 0.59 (0.49–0.69) |
| 40 | 6 | 498 * (473–539) | 466 (462–470) | 0.45 (0.40–0.48) | 0.42 (0.37–0.47) |

Significant differences between CT treated and control rats are indicated by asterisks. ** $2P < 0.02$, * $2P < 0.05$. *n* = Number of animals. Median with 0.25- and 0.75-fractiles.

## DISCUSSION

The present study seems to indicate a negative influence of salmon CT both on collagen synthesis and on collagen mineralization in rats.

The amount of salmon CT administered to the rats (3 MRC-U/kg body weight/day) is comparable to doses of the drug recommended in human medicine (Hamdy 1977, Pezeshki & Brooker 1977). The reduced serum calcium observed 3 h after each CT injection (Figure 2A), suggests effective CT treatment throughout the experimental period. The increased serum levels of calcium and phosphorus 24 h after the CT injection, observed in the last part of the experiment (Figure 2C and D), appears to be a rebound effect. Similar findings have been reported previously for plasma calcium (Doyle et al. 1968). The hyperphosphatemia observed in the present study makes it less likely that a chronic hypersecretion of parathyroid hormone alone occurs in this situation. Since both hypocalcemia and hypophosphatemia (DeLuca & Holick 1979) and possibly CT (Suda et al. 1981) may stimulate the biosynthesis of 1.25-$(OH)_2D_3$, an increase of this active vitamin D metabolite may explain our findings. The small differences in serum calcium and serum phosphorus of control rats (Figure 2) are probably related to the timing of the blood sampling, and are in accordance with the normal daily fluctuations of circulating calcium and phosphorus in rats (Talmage et al. 1975).

The radioactive nuclides $^{14}$C-proline and $^{85}$Sr have proved to be reliable tools to study and compare the accretion rates of collagen and mineral in bone (Firschein 1969) and of collagen in skin (Laitinen 1967). The amounts of $^{14}$C-hydroxyproline and of $^{85}$Sr in the tissue samples of the present study indicate the net synthesis of collagen and the mineralization (net mineral accretion plus mineral exchange) during a 24-h period (Firschein 1969).

Salmon CT transiently reduced the collagen synthesis by 5–13 per cent both in bone and skin. In two previous experiments in rats where the collagen synthesis was measured in a similar way, either an inhibitory effect (Kalu & Foster 1971) or no effect (Marks 1972) of porcine CT on the formation of collagen was reported. In contrast, an increased collagen synthesis has been reported in morphological and autoradiographic studies both after porcine CT in rats (Zichner 1974) and salmon CT in rabbits (Lupulescu 1974, Lupulescu & Habowsky 1978). Thus, the results seem to be related to the methods used to evaluate the hormonal effects. Healing wounds may increase the collagen metabolism some distance away from the wound (Peacock 1963, Lyall

1974). Our values for collagen synthesis of intact skin may therefore be higher than the real values, since the analyzed specimens were located near skin wounds.

While also other authors have observed an early increased incorporation of mineral tracers into bone after CT treatment (Babický et al. 1976, Bessonova & Petrovich 1976), the specific activity of $^{85}Sr$ and the mineral concentration were reduced in fractured femora of treated rats during the last part of the experiment (Figure 4). This agrees with the study of Doyle et al. (1968), but contrasts with that of Delling & Glueckselig (1971).

We interpret our data as follows: The negative influence of CT on the collagen synthesis in the first part of the study led to less total collagen content both in bone and skin of treated rats. During the last part of the study, however, the inhibitory effect of CT on collagen degradation (Talmage & Cooper 1979) increased both the total content and the concentration of collagen in the fractures, and the concentration of collagen in the bones and skin of treated animals compared to controls (Figures 3 and 6, Tables 2 and 3). Thus, the normal remodeling of the immature, low mineralized callus was probably inhibited by CT. This may explain the lower mineral concentration in the fractures from hormone treated rats compared to controls after 40 days of treatment (Figure 4). The collagen types found in callus and bone, wounds and intact skin are not identical (Prockop 1979), and the degradation of collagen is slower in skin than in bone (Laitinen 1967, Lindner 1973). Therefore, the inhibition of CT on collagen degradation did not prevail to the same extent in intact skin specimans (Figure 6A), even though the collagen concentration increased in specimens from treated rats (Figure 6B). This may explain why the strength of intact skin was reduced by CT treatment, unlike the strength of fractured and intact bones and of wounded skin (Ekeland et al. 1983).

In conclusion, salmon CT (3 MRC-U/kg body weight/day) had a negative influence on fracture repair in rats by a transient reduction of collagen synthesis and an inhibition of collagen degradation. Similar observations were made in intact bones, wounded and intact skin. The effects of CT, were, however, less consistent in intact bones and wounds.

## ACKNOWLEDGEMENTS

The authors thank The Norwegian Research Council for Science and the Humanities for financial support, and Sandoz, Basel, Switzerland for the generous supplies of salmon CT.

## REFERENCES

Babický, A., Pavlík, L., Kolář, J. & Blahoš, J. (1976) Effect of calcitonin on fracture healing in rats. *Endocrinol. Exp. (Bratisl.)* **10**, 73–79.

Bessonova, S. S. & Petrovich, Y. A. (1976) Retabolil and thyrocalcitonin action on the mineral metabolism in the bones of rats following fracture of the mandible. *Farmakol. Toksikol.* **39**, 216–220.

Bright, R. W. & Estep, H. L. (1968) Effects of thyrocalcitonin on calcium deposition and fracture healing. Third International Congress of Endocrinology, Mexico, D.F., June 30 – July 5, 1968. *International Congress Series* No. 157. (Ed. Gual, C.), p. 6. Excerpta Medica Foundation, Amsterdam.

Delling, G. & Glueckselig, W. (1971) The effect of calcitonin on the regeneration of circumscribed tibia defects and on mineral content of bone in the rat. *Isr. J. Med. Sci.* **7**, 367–368.

DeLuca, H. F. & Holick, M. F. (1979) Vitamin D: Biosynthesis, metabolism, and mode of action. *Endocrinology* (Ed. DeGroot, L. J.). Vol. 2, pp. 653–668. Grune & Stratton, New York.

Diem, K. & Lentner, C. (eds.) (1975) Documenta Geigy. *Scientific tables.* 7th ed. p. 192, Ciba-Geigy Ltd., Basle.

Doyle, F. H., Foster, G. V., Bordier, P. & Matrajt, H. (1968) Radiological bone changes produced by calcitonin. In: *Calcitonin* (Ed. Taylor, S.). Proceedings of the Symposium on Thyrocalcitonin and the C Cells, London, England, July 17–20, 1967, pp. 329–337. William Heinemann Medical Books, London.

Duriez, J. (1979) Calcitonine et fracture. *Ann. Chir.* **33**, 3–7.

Ekeland, A., Myhre, L. & Underdal, T. (1983) Effects of salmon calcitonin on mechanical properties of healing and intact bone and skin in rats. *Acta Orthop. Scand.*, in press.

Engesæter, L. B., Underdal, T. & Langeland, N. (1980) Effects of oxytetracycline on mineralization of bone in young rats. *Acta Orthop. Scand.* **51**, 459–465.

Firschein, H. E. (1969) Collagen and mineral dynamics in bone. *Clin. Orthop.* **66**, 212–225.

Hamdy, R. (1977) The signs and treatment of Paget's disease. *Geriatrics* **32**, 89–93.

Harris, J. M., III, Bean, D. A. & Banks, H. H. (1975) Effect of phosphate supplementation, thyrocalcitonin, and growth hormone on strength of fracture healing. *Surg. Forum* **26**, 519–521.

Kalu, D. N. & Foster, G. V. (1971) Effect of calcitonin on ($^3$H)-proline incorporation into bone hydroxyproline in the rat. *J. Endocrinol.* **49**, 233–241.

Kaplan, A. V., Balaba, T. J., Briskin, A. I., Markova, O. N. & Zamyatkina, O. G. (1976) Use of thyrocalcitonin in the therapy of fractures. *Ortop. Travmatol. Protez.* **5**, 1–5.

Laitinen, O. (1967) The metabolism of collagen and its hormonal control in the rat. *Acta Endocrinol. (Copenh.)* **56**, Suppl. 120.

Lindgren, J. U., Narechania, R. G., McBeath, A. A., Lange, T. A. & DeLuca, H. F. (1981) Effects of 1.25 dihydroxyvitamin $D_3$ and calcitonin on fracture healing in adult rats. *Clin. Orthop.* **160**, 304–308.

Lindner, J. (1973) Skin ageing. A review. In: *Connective tissue and ageing* (Ed. Vogel, H. G.). Proceedings of the Workshop Conference Hoechst, Schloss Reitenburg, April 21–22, 1972. *International Congress Series* No. 264, pp. 249–259. Excerpta Medica Foundation, Amsterdam.

Lupulescu, A. (1974) Effect of calcitonin on fibroblasts and collagen formation in rabbits. An ultrastructural and scanning electron microscopic study. *J. Morphol.* **142**, 447–466.

Lupulescu, A. & Habowsky, J. (1978) Effects of calcitonin on wound healing: A morphological study in rabbits. *J. Surg. Res.* **25**, 260–268.

Lyall, M. H. (1974) Increased rate of collagen synthesis in intact skin remote from an experimental wound in the rat. Meeting of the Surgical Research Society, Newcastle, England, July 5–6, 1974. *Br. J. Surg.* **61**, 911.

Marks, S. C. (1972) Lack of effect of thyrocalcitonin on formation of bone matrix in mice and rats. *Horm. Metab. Res.* **4**, 296–300.

Peacock, E. E. (1963) Variations in the amount of saline extractable collagen in skin distant to a healing wound. *J. Surg. Res.* **3**, 250–253.

Pezeshki, C. & Brooker, A. F. (1977) Immobilization hypercalcemia. *J. Bone Joint Surg.* **59**-A, 971–973.

Prockop, D. J., Kivirikko, K. I., Tuderman, L. & Guzman, N. A. (1979) The biosynthesis of collagen and its disorders. *N. Engl. J. Med.* **301**, 13–23, 77–85.

Rasskazov, L. V. (1977) Thyrocalcitonin in the complex therapy of compound fractures. *Ortop. Travmatol. Protez.* **9**, 30–32.

Schatzker, J., Chapman, M., Ha'Eri, G. B., Fornasier, V. L., Summer-Smith, G. & Williams, C. (1979) The effect of calcitonin on fracture healing. *Clin. Orthop.* **141**, 303–306.

Suda, T., Horiuchi, N., Akiba, T., Sunaga, S. & Takahashi, N. (1981) Localization and control mechanism for 25-hydroxyvitamin $D_3$-lα-hydroxylase activity in the mammalian kidney. In: *Hormonal control of calcium metabolism* (Eds. Cohn, D. V., Talmage, R. V. & Matthews, J. L.). Proceedings of the Seventh International Conference on Calcium Regulating Hormones. Estes Park, Colorado, USA, Sept. 5–9, 1980. *International Congress Series* No. 511, pp. 212–221. Excerpta Medica, Amsterdam.

Talmage, R. V. & Cooper, C. W. (1979) Physiology and mode of action of calcitonin. In: *Endocrinology* (Ed. DeGroot, L. J.). Vol. 2, pp. 647–651. Grune & Stratton, New York.

Talmage, R. V., Roycroft, J. H. & Anderson, J. J. B. (1975) Daily fluctuations in plasma calcium, phosphate, and their radionuclide concentrations in the rat. *Calif. Tiss. Res.* **17**, 91–102.

Zichner, L. (1974) Influence of calcitonin on the action of bone cells in rats. *Exp. Pathol. (Jena)* **9**, 27–36.

Correspondence to: Arne Ekeland, Institute for Surgical Research, Rikshospitalet, Oslo 1, Norway.