the protein structure. Moreover, we demonstrated previously that the enzymes of rat, chicken, and bullfrog all have practically the same molecular weight, and that they contain a flavin covalently bound to the apoprotein[9]. Accordingly, it is reasonable to consider that the L-gulonolactone oxidase of species belonging to the 3 classes Mammalia, Aves, and Amphibia, evolved from a common ancestral protein.

1 Correspondence to: K. Yagi, Institute of Biochemistry, Faculty of Medicine, University of Nagoya, Nagoya 466, Japan.
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The effect of calcitonin on calcium uptake in mouse molars in vitro

L.W. Kline

University of Alberta, Department of Oral Biology, Edmonton (Alberta, Canada T6G 2N8), 14 August 1980

Summary. Mouse maxillary second molars were removed at either 24 or 96 h of age and maintained in vitro. Half of the teeth, of each age group, were treated with 50 m-units of synthetic salmon calcitonin. By comparing the initial and final calcium concentrations in the medium, the net uptake or release of calcium was inferred. The treated molars took up significantly more calcium than the untreated groups.

Calcitonin (CT), a hormone secreted by the parafollicular cells of the mammalian thyroid, acts as a hypocalcemic factor[1-3]. This effect is due to the ability of CT to suppress osteoclastic-induced bone resorption[4-6]. CT has also been shown to decrease calcium (Ca) efflux from the bone cells, increase the formation of a type of short-term storage Ca-phosphate material in bone fluid, and increase the uptake of Ca into bone cell mitochondria[7-10]. In this study the in vitro effects of synthetic salmon CT on Ca and phosphate uptake by unerupted maxillary 2nd molars was assessed.

Methods. Maxillary 2nd molars were taken from 24- or 96-h-old white mouse pups. A total of 100 molars were used in each age group. The molars were maintained on a culture medium composed of 90% Weymouth medium 722/1 and 10% fetal calf serum supplemented with 15 mg ascorbic acid/100 ml of medium[11]. Ca was added to the medium to a concentration of 10.5 mg%, and phosphate to levels of 5.8 mg%, both within the normal range. Antibiotic coverage consisting of penicillin (50 U/ml) and streptomycin (50 µg/ml) was supplied. Half the teeth within each age group were cultured on this medium; the other half on the same medium to which 50 m-units of synthetic salmon CT (supplied by Armour Pharmaceutical Co.) dissolved in 0.1% highly purified bovine serum albumin was added. An equal volume of the CT vehicle was added to the medium of the untreated teeth.

2 molars were cultured in each dish containing 2.5 ml medium. The molars were incubated in an atmosphere of 50% O_2, 45% N_2, and 5% CO_2 at 37 °C for 48 h[12]. The media were assayed for Ca using a fluorometric method[13] and phosphate using a colorimetric method[14]. The initial and final media were assayed for both Ca and phosphate as was the medium in 1 dish incubated without teeth. The net release or uptake of Ca or phosphate by the molars was determined by comparing the initial and final concentrations. The mean changes in the Ca and phosphate concentrations of the untreated and treated molars, of the same age, were compared using the Student t-test.

After incubation teeth from both age groups and treatments were decalcified and prepared for histological examination to verify their viability and to detect possible morphological differences.

Results. The results are summarized in the table. Each group of molars started with an equal number of samples, i.e. 100. A total of 22 specimens were discarded due to bacterial contamination.

The CT-treated media, both for 24-h and 96-h age groups, were found to contain significantly ($p < 0.05$) less Ca than the untreated groups. The CT-treated media, from both age groups, contained less phosphate, but not at significant levels.

20 molars, randomly selected from all groups after incubation, were examined histologically. All molars exhibited a normal healthy appearance and no morphological differences were observed between experimental and control molars of the same age.

Discussion. At birth the maxillary 2nd molars lie in their bony cripts and have begun to lay down dentin[15]. As the molars are actively laying down predentin and dentin at this period of development, if CT affects the rate of calcification its addition to the culture media should produce a detectable effect.

The CT-treated molars had a significantly greater uptake of Ca from the media but not phosphate. This uptake presumably involved increased Ca deposition in the dentin, and

Average calcium levels (in mg% with SD) in the culture media

	Initial concentration	Final concentration
Group I (24-h mice)		
Untreated	(n = 100) 10.5 ± 0.10	(n = 96) 11.1 ± 0.12
Treated	(n = 100) 10.6 ± 0.11	(n = 94) 10.0 ± 0.13
		($p < 0.05$)
Group II (96-h mice)		
Untreated	(n = 100) 10.3 ± 0.08	(n = 92) 11.5 ± 0.11
Treated	(n = 100) 10.4 ± 0.10	(n = 96) 9.8 ± 0.13
		($p < 0.05$)

possibly enamel, although this was not measured. These results are similar to Mallek et al.[16]. 1st molar and incisor germs of 1-, 5-, 10-, 15- and 20-day-old rat pups were removed from control and protein-energy malnourished rat pups, minced, and cultured for 4 h in media with and without CT. CT-treated incisors and molars from the 5-day-old protein-deprived rats had increased Ca uptake compared with controls. Ca uptake was also significantly higher in the CT-treated control group than the untreated control group. The present study detected an increased uptake of Ca by intact healthy 1-day-old mouse molars, an effect no observed by Mallek et al. Their failure to detect a change in the 1-day-old rat molar could have been due to the shortness of incubation (4 h vs 2 days) and the difference in the manner in which the tissue was incubated (minced vs intact).

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Open field locomotion by zinc deficient adult male mice

N.E. Erlick, E.D. Engel and R.H. Davis

Department of Physiological Sciences, Pennsylvania College of Podiatric Medicine, 8th at Race Street, Philadelphia (PA 18107, USA), 30 July 1980

Summary. Adult male mice maintained on a zinc-free diet display a statistically significant increase in open field locomotion compared to controls. However, excess zinc (100 times the established recommended daily allowance) does not produce a change compared to controls.

All animals require zinc adequate diets for proper development and maintenance of body functions. When denied zinc for a long time, an animal becomes zinc deficient, manifested by symptoms such as hair loss[1,2], fissuring of the soles[3], disrupted bone metabolism[4], skin lesions[5], and growth retardation[6]. However, little information exists on the behavioral effect from zinc deficiency. Rats denied zinc for 7 weeks displayed decreased exploratory activity[7] and performed poorly on behavioral tests[8–11]. The present study attempts to show the influence of zinc deficiency on the open field behavior of adult male mice.

Adult male mice (Institute of Cancer Research strain; 25–30 g; 18–20 animals per group) were housed in a room with 12 h light cycles over a 6-week experimental period. The pool was divided into 3 groups (control, zinc deficient, and zinc supplemented) each based upon the amounts of zinc animals received. Control mice received a standard zinc adequate diet. Zinc deficient mice received a diet identical to the control diet except the zinc was extracted from it (Teklad Test Diets, Madison, Wisconsin). The zinc deficient mice received zinc-free water. We housed these mice in plastic cages with zinc-free plastic caps on water bottles. Zinc supplemented (excess) mice received the standard zinc adequate diet and dissolved zinc sulfate (Mallindrodt Chemical Co., St. Louis, Missouri) in the drinking water in an amount 100 times the established recommended daily allowance (3 mg/kg, dry weight) for mice[2]. Our 6-week plan for each group allowed the mice to develop body zinc depletion. After weighing each animal, we observed open field locomotion by placing them into a 19×25 cm cage with the bottom divided into 6 cm^2 and tabulated the number of lines crossed in a 5-min period.

In the table, we observe that the zinc deficient mice traversed a mean of 182.9 ± 19.6 squares compared to 112.8 ± 6.1 for the control, a statistically significant increase (p 0.005). On the otherhand, while the zinc supplemented mice traversed more squares than the control, specifically 137.6 ± 15.8, statistically they remained unchanged (p 0.5) as compared to control. Apparently, zinc deficient mice exhibit hyperactivity as demonstrated by their increased exploration. Unlike rats, zinc deficiency in mice produces increased exploratory activity. We also observed over the 6-week period that zinc deficient mice displayed abnormal behavior (compared to control or zinc supplemented) such

Open field locomotion by adult male mice

Mice	Weight	Open field test Squares traversed in 5 min	p-value
Control	41.4 ± 1.0	112.8 ± 6.1	-
Zinc deficient	31.8 ± 0.9	182.9 ± 19.6	0.005
Zinc supplemented	43.1 ± 0.9	137.6 ± 15.8	0.5

Mean ± SE. p-probability value 18–20 mice/group.