Effect of Tetracycline on Retention of Calcium and Strontium in Rodents.* (29132)

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Antibiotics of the tetracycline family have an affinity for bony tissues. Thus in addition to their primary value as clinical therapeutic agents, they have been used as fluorescent markers for *in vivo* labeling of calcified zones in bone(1). Several recent studies have been concerned with the effects of tetracycline on the deposition and retention of radiostrontium in mammals. The results of these latter efforts are conflicting. Ogawa *et al*(2) and Richards *et al*(3) indicate that the agent has some value in reducing a body burden of radiostrontium if treatment is begun either before or shortly after a dose of the radioactive material. Nakatsuka *et al* on the other hand observed no significant effect(4). A report by Catch, which appeared while the present study was underway, concluded that tetracycline was effective but only if given prior to the radionuclide(5).

The purpose of the work reported here was to assess the value of tetracycline for minimizing the retention of potentially hazardous radiostrontium in the body, and to learn whether this bone-seeking antibiotic exerts gross effect on normal calcium metabolism.

Methods. Groups of mice or rats were each divided into 2 subgroups, one of which received tetracycline treatments, the other serving as control. Equal doses of Ca^{47} and/or Sr^{85} were administered intraperitoneally to both treated and control subgroups. The retention-time curve for the radioisotope was determined for each animal by repeated measurements of its total body gamma activity, using a 3″ × 3″ NaI scintillation detector in a large iron shield and pulse height analysis to distinguish between the two radionuclides where necessary.

Experiment 1: Twenty, male Sprague-Dawley rats, 25 days old were divided into 2 groups of 10. The experimental group received 6 intramuscular injections, each containing 10 mg of oxytetracycline (OTC) in 0.9% saline during a period of 24 days. The total dose was approximately 1200 mg/kg body weight. The OTC was shown to be pure by a chromatographic technique(6). The control group was given injections of 0.9% saline only. Body weights were recorded at time of each injection. On the 27th day, 5 experimentals and 9 controls survived. Two from the experimental and 4 from the control group were then each given 5 ml of a non-radioactive 3.2% strontium lactate solution intraperitoneally. Urines and feces were collected for 24 hours, after which the animals were sacrificed and blood samples obtained. The retention of strontium in "total plasma units" was calculated by the method described by Harrison and Fraser(7). Analyses for stable Sr were performed with a flame photometric technique(8). Each of the remaining 3 OTC treated rats and 5 controls was given an intraperitoneal injection of carrier free Sr^{85} chloride (Abbott Laboratories) and subjected to total body counting for 20 days. The femurs were then removed, thermally ashed and assayed for radioactive Sr^{85} content.

Experiment 2: Twenty-five female mice, 24-30 g, were selected. Thirteen each received 167 mg of tetracycline (Tetrasyn-Pfizer) per kg body weight in 0.25 ml water, given intraperitoneally (i.p.) 4 hours prior to a single dose of 0.5 μc Sr^{85} i.p. (day 0) and thereafter on days 1, 2, 3, 4, 16, 17, 18, 19 and 20 for a total of 1250 mg/kg. Twelve controls received only a single injection of 0.5 μc Sr^{85}.

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TABLE I. Affinity of Sr^{++} and Ca^{++} for Oxytetracycline.

Ion	1:1 Complex			2:1 (Metal:Ligand) Complex		
	No. determinations	Avg assoc constant	± S.D.	No. determinations	Avg assoc constant	± S.D.
Ca^{++}	15	950	22	8	78	26
Sr^{++}	21	313	89	22	15	6

The pH was held at 7.36 with Tris buffer $\mu = 0.16$, t = 21°C. Detailed experimental procedure has been previously published(9).

Experiment 3: Fifteen of 30 female mice, 24-32 g each received 83 mg of tetracycline (TC) per kg in 0.25 ml water, given i.p. 4 hours before a single i.p. dose of 1.0 μc Ca^{47} (Oak Ridge Laboratory) and daily thereafter for 13 days, except for omissions on days eight and nine. Total dose was 996 mg/kg. Fifteen controls received an equal dose of Ca^{47}, and, in addition 250 mg ascorbic acid per kg in 0.25 ml water, i.p., on the same days as the TC treatments. Ascorbic acid was used to simulate the effects, if any, of the small amounts of ascorbic acid incorporated in the Tetrasyn preparation.

Experiment 4: Thirty mice, 26-34 g each received a mixture of 0.44 μc Sr^{85} and 0.53 μc Ca^{47}. The treated group of 15 received 75 mg of TC per kg in 0.1 ml water given i.p. daily for 4 days *prior* to the i.p. dose of Ca^{45} and Sr^{85}, and on days 0, 1, 2, 3, 4, 7 and 10 thereafter. Total dose 825 mg/kg. Controls received 75 mg ascorbic acid per kg in 0.1 ml water on the same days, in addition to the radioisotopes.

Experiment 5: The intramuscular (i.m.) route for treatment was evaluated by administering 8 mg per kg of TC in 0.1 ml water i.m. to each of 12 male rats daily for 5 days prior to a single i.p. injection of a mixture containing 0.3 μc Sr^{85} together with 3.1 μc Ca^{47}, and on days 0, 1, 3, 5, 7, 11, 12 and 13 thereafter, totaling 500 mg/kg. Twelve controls received $Sr^{85} - Ca^{47}$ and i.m. ascorbic acid equivalent to that in the TC doses on the same days. Aseptic techniques were used for all injections. The rats ranged from 170-230 g, but were all from 106-108 days old.

Results. In Experiment 1 the intent was to attain a high level of oxytetracycline in the skeletons of rats prior to administration of strontium and to observe possible pathological effects in histological sections of bone tissue.

For the rats receiving stable Sr lactate the calculated mean retention was 401 total plasma units for the OTC treated group and 308 for the controls. The mean Sr^{85} whole body retentions were 63% for the treated and 59% for the control group. The large quantity of OTC definitely inhibited the growth of these immature rats. The stunting resembled that observed in rats reared on a calcium deficient diet. Unstained thin sections of tibial cortex under ultraviolet light showed the fluorescence characteristic of the tetracyclines—primarily in the endosteal and periosteal surfaces. Histological sections of bone stained with hematoxylin-eosin, showed structures suggesting a decreased rate of calcification, attenuated metaphyseal bone and an increase in thickness of the epiphyseal plate. These findings suggested that OTC in large doses may sequester calcium and thereby produce effects similar to those observed in hyperparathyroidism. The OTC did not appear to diminish strontium body burdens, but since so few rats were used, this particular observation was not taken as conclusive, pending the outcome of the later experiments.

The affinity of oxytetracycline for Ca in aqueous solutions was demonstrated by utilizing a bathochromic absorption shift which occurs upon chelation(9). The affinity for calcium was considerably greater than for strontium (Table I) which suggests that OTC in the blood or deposited at bone sites is much more likely to be complexed with Ca ions than with Sr ions.

In the remainder of the experiments male albino rats or mice received repeated injections of a tetracycline (TC) preparation (Tetrasyn-Pfizer). In all these experiments

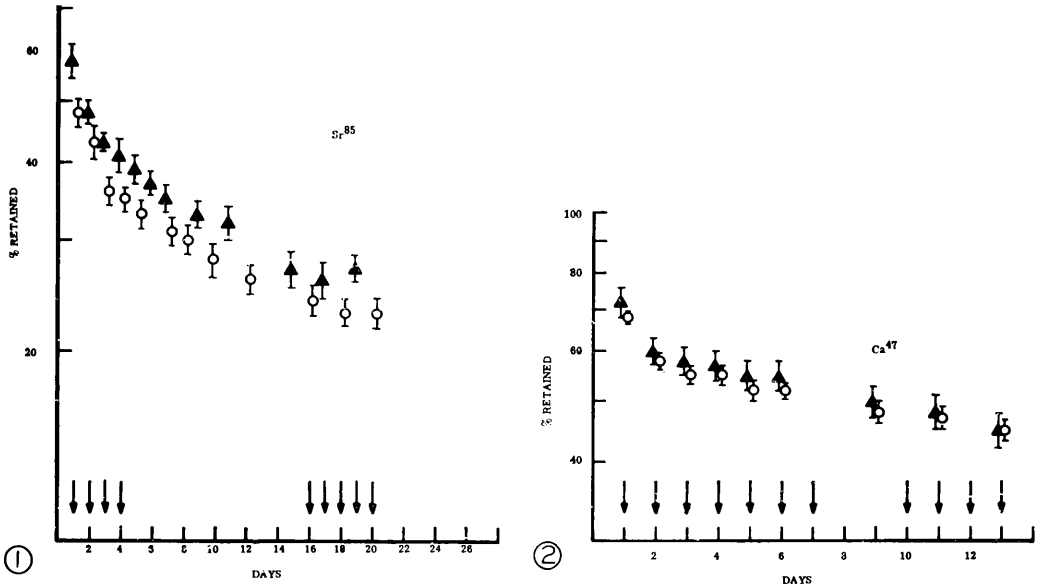

FIG. 1 and 2. Percent of radioisotope dose retained in whole body, as a function of time post-injection. Open circles represent mean value of controls ± 2 times standard deviation of the mean. Triangles represent mean value for treated group ± 2 standard deviations of the mean, arrows indicate days of tetracycline treatment after injection of radioisotope. Tetracycline also given on day 0, four hours prior to radioisotope. Fig. 1 presents data for exp 2; Fig. 2 for exp 3.

save No. 2, the control animals received injections of ascorbic acid in amounts approximating that incorporated in the Tetrasyn preparation by the manufacturer as a buffer.

Fig. 1 and 2 present the total body retention data for Exp. 2 and 3, respectively. For the sake of brevity, curves from the other experiments are not presented. No significant effect of TC treatment was observed. The daily values for control mean % retention ± 2 standard deviations of the mean overlapped those for the TC treated groups, both for Ca^{47} and Sr^{85}. In all cases where both isotopes were given to the same animal, Sr^{85} daily retention was always less than Ca^{47} retention. Table II presents comparisons of radioactivities in the bones.

The total body retention of Sr^{85} was not significantly reduced by TC treatment in mice or rats, even when administered *before* the radioisotope. Indeed, in Exp. 2 total body retentions were very slightly lower in the mice *not* treated with tetracycline. The skeletal contamination with Sr^{85} was also slightly lower in these control mice (Table II). In the same manner, in Exp. 5 the bone burdens of the control rats were somewhat lower than the treated group, but this difference had a low probability of significance.

The skeletal burdens of Ca^{47} were unaffected by the TC treatments except in Exp. 5 where the mean radioactivity in femora and tibiae in untreated rats was about 84% of that in the treated animals. The total body retentions of Ca^{47} were almost identical for treated and control animals except in Exp. 4 where the mean values for the tetracycline group were reduced below those of the control group. The differences were not great and had a low value for probability of statistical significance. These control mice all became ill from bacterial infections probably contracted from the multiple injections which were given without observing aseptic precautions. The TC treated mice all remained healthy in this experiment, presumably because of the antibiotic nature of the agent.

Neither the ascorbic acid nor the saline which accompanied the TC injections affected radioisotope retention. This was proven by injecting 30 rats (102-104 days old) with a Ca^{47}–Sr^{85} mixture. One group of 10 re-

TABLE II. Radioactivity in Total Body and in Bones. Symbols T and C represent treated and control groups, respectively. Radioactivity in bones is given in counts per minute per whole femur, except in Exp 5 where both femora and both tibiae were pooled.

Exp No.		Total body % retention on day 11 Mean	Range	Radioactivity in bones (c/m/femur) Mean ± S.E.	n	P	Days
2. Mice (Sr^{85})	T	30	29-34	338 ± 8	12		
	C	27	23-31	283 ± 11	22	<.01	24
3. Mice (Ca^{47})	T	48	37-54	34.6 ± 1.2	26		
	C	47	38-53	33.3 ± .8	30	>.3	16
4. Mice Ca^{47}	T	32	22-36	29.3 ± 1.8	12		
	C	36	28-43	31.9 ± 1.6	13	.3	11
Sr^{85}	T	21	15-27	985 ± 69	12		
	C	22	17-29	951 ± 57	13	>.3	11
5. Rats Ca^{47}	T	66	59-72	27,964 ±1757	11		
	C	66	59-80	23,461 ±1723	10	.1	15
Sr^{85}	T	52	40-64	6759 ± 567	11		
	C	53	47-66	6296 ± 469	10	>.3	15

S.E. is standard error of mean value $= \sigma \div \sqrt{n}$, where n = number of bones assayed except in Exp 5 where n = number of animals assayed. P = probability that the difference between the mean values is due to chance, by the t test. The Days column gives interval between radioisotope injection and sacrifice.

ceived no other treatment. A second group of 10 received daily intraperitoneal injections of aqueous ascorbic acid (250 mg/kg body weight) beginning 4 days prior to administration of the radioisotopes. The third group received injections of 0.85% sodium chloride on the same schedule. Sterile techniques for injection were utilized to minimize complications which might arise from bacterial infections. Daily total body counting and radioisotope assays of the femurs after the twelfth day following Ca^{47}–Sr^{85} administration disclosed no significant differences between the 3 groups of rats.

Summary. Tetracycline (TC) administered repeatedly in doses totaling as much as 1200 mg/kg body weight, by either the intramuscular or intraperitoneal route, had no significant effect on the body retention of either Ca^{47} or Sr^{85} injected into mice or rats. Nor were the amounts of these radionuclides found in the bones reduced by such treatment. Oxytetracycline (OTC) was shown to have a greater chemical affinity for calcium ions than for strontium ions *in vitro*. Repeated large doses of OTC in the toxic range stunted the growth of young rats and caused pathological changes in bone tissue resembling those observed in hyperparathyroidism. Although TC and OTC in large amounts can alter the normal metabolic interrelationships between calcium ions and bone tissue, they have no significant value as agents for reducing a potentially hazardous body or skeletal burden of radiostrontium.

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