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On the Attempt to Establish a Model on Steroid-Induced Osteoporosis in Bones of Rats.

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A diet low in calcium caused a consistent and significant demineralization and rarefaction in the tibia, femur, and mandible of rats. There was no effect on incisor or molar teeth, nor on height nor form of alveolar bone. Administration of various adrenal corticosteroids (including cortisone, hydrocortisone, and prednisolone) caused less changes in bones than did the low calcium diet. There was a trend towards increased density and calcium immobilization in bones due to steroids. No effects were seen in teeth or alveolar bone.

It was suggested that these results are in accord with the antianabolic rather than the catabolic concept of steroid action, and these short term experiments indicate that, if steroids were administered to rats over a long period of time, an osteoporosis would occur.

Chronic administration of adrenocortical hormones to humans is generally believed to lead to skelatal osteoporosis. Adams and Jewsey (1), pointed out, that osteoporosis is commonly found in patients suffering from Cushing's Disease and in those treated with large doses of adrenal corticosteroids. Krane (2) defined osteoporosis as a "disorder in which there is reduction in bone mass without significant reduction in ash content." He further pointed out that the condition may not be so simple and there may be structural variations more profound than a simple decrease in normal bone mass per unit volume. Rose (3), felt that there exists chemical differences between osteoporosis and normal bone. According to Nordin (4), osteoporosis and osteomalacia (in which ash content of the bone is reduced) can exist simultaneously. Thus, without clearly defining the conditions in which it has occurred, the use of the term "osteoporosis" carries limited meaning.

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Cortisone therapy is used in the treatment of diseases where osteoporosis may be a manifestation of the disease itself. For example, Copeman (5) listed several cases of generalized osteoporosis as one of the features of severe rheumatoid arthritis, and Talbott and Ferrandis (6) included it as a feature of dermatomyositis and systematic scleroderma. Studies of steroid effects in man have been mainly qualitative, employing radiologic and histologic techniques.

The early studies of cortisone-induced skeletal effects in rats done by Follis (7), Storey (8), and Sissons and Hadfield (9), showed:

(a) ratardation of skeletal growth (diminished osteogenesis);
(b) thinning of epiphyseal plates in long bones;
(c) increased density of metaphyseal bone;
(d) decreased bone resorption;
(e) increased thickeness of trabeculae.

Some of these findings were quite opposite to the effects of cortisone in man, and Storey (8) attributed the differences to the efficient calcium metabolism of the rat. He assumed that the cortisone effect in man is in some part due to inhibition of calcium absorption, and the rat could overcome the effect because of its ability to absorb almost all of the calcium consumed in its food. Storey (10, 11) experimenting on the effect of variations in dietary calcium and phosphorus, found that rats fed a diet of low or unbalanced levels of calcium and phosphorus developed rarefied bone. He further noted that cortisone administration produced an even more severe osteoporosis in rats on such a diet. He concluded that under these conditions the cortisone-induced osteoporosis was similar to that seen in other animals and in Cushing's Syndrome in man.

The present study was designed to establish a model system of steroid-induced osteoporosis, evaluated by (a) bone density and (b) calcium content.

MATERIALS AND METHODS:

Animals: Young male albino rats from 38 to 45 days old at commencement of experiment, initially weighing 125-195 grams were used. The normal diet

groups were fed Purina laboratory chow, and the low calcium diet groups a ration accoeding to Kenny and Muson (12). Water was fed al libidum.

Drugs: Experiment 1. Prednisolone sodium succinate powder was mixed with distilled water and administered intraperitoneally at a dose level of 1.5 mg per 100 grams of initial body weight. An equal volume of distilled water was injected daily. This was the only experiment in which the animals received a prescribed dose of drug, which remained the same throughout the experiment and was identical for all animals.

Experiment 2. Animals in the experimental group received daily a dose of prednisolone equivalent to 3.0 mg/100 g body weight. Control animals received an equal volume of distilled water daily by the intraperitoneal route.

Experiment 3. The experimental animals were divided into two groups: (a) received Cortisone Acetate as saline suspension at a dose level 4.5 mg/100g body weight: (b) received prednisolone (1.5 mg/100g), dissolved in saline of similar strength.

Experiment 4. Once again the experimental animal were divided into two groups (a) receiving Cortisone Acetate (b) Hydrocortisone Acetate at the following dose levels: Cortisone 4.5 mg/100g; Hydrocortisone 4.5 mg/100g. Control animals received sterile saline.

Experiment 5. Prednisolone and cortisone were administrated as in ex periment 3.

Analysis of Bone and Tooth Calcium: Animals were sacrificed by ether and the following bones and teeth were removed: left femur; left tibia-fibula; left side of mandible; left mandibular incisor; left mandibular molars.

The head and left hind leg of each animal was removed, wrapped in aluminium foil and cooked for 35 minutes in an autoclave at 125°c and 15-20 lbs. pressure. The bones and teeth were then defleshed and soaked for 24 hours in 5 cc of hydrochloric acid. The samples were diluted and the calcium content determined by EDTA titration, according to the method of Copp (13).

Volumetric Determination: was made according to a method described by Robinson and Elloit (14).

Experimental Design: In each experiment the animals were divided into 2 groups: (a) fed normal diet (b) low calcium diet. The animals were further divided into control and experimental subgroups where one drug was being tested; where two drugs were utilized the animals on any one diet were equally divided into 3 subgroups, i.e., control, drug A, drug B.

administered over the following periods of time:

Experiment	1a	(prednisolone:	1.5 mg/100g):	2 weeks
Experiment	1b	(prednisolone:	4.5 mg/100g;	23 days
Experiment	2	(prednisolone:	3.0 mg/100g):	2 weeks
Experiment	3	(cortisone:	4.5 mg/100g;	
		(prednisolone:	1.5 mg/100g):	2 weeks
Experiment	4	(cortisone:	4.5 mg/100g):	
		hydrocortisine:	4.5 mg/100g;	2 weeks
Experiment	5	(corisone:	4.5 mg/100g):	
		(prednisolone:	1.5 mg/100g):	4 weeks.

RESULTS:

The results of preliminary experiments 1 to 3 are found in the tables 1 to 7. The results of experiments 4 and 5 are outlined below:

Experiment 4. Effect of cortisone and hydrocortisone administered over a period of 14 days.

NORMAL DIET:

Body Weight: Body weight gain was reduced by cortisone and ceased altogether when hydrocortisone was injected.

Bone Weight: Mandibular weight was affected by cortisone, but hydrocortisone reduced it slightly. The weight of the femur was unchanged.

Bone Volume: Cortisone did not reduce mandibular volume, but hydrocortisone caused a marked reduction. Femur volume was reduced although cortisone had less effect than hydrocortisone.

Bone Density: In the mandible cortisone had no effect whereas hydrocortisone increased the density. Both drugs caused a notable increase in femur density.

Calcium Concentration: Mandibular calcium concentration was unchanged; in the femur the concentration of calcium was increased to a greater extent by cortisone.

Table 1 Experiment 1 - a weeks
Normal Diet

	CONTROL	PREDNISOLONE: 1.5+	Effect of Drug
IBW	178	175	
FBW	275	238	
Tibia			
Weight	271	255	
(Ca)/mg	22.2±.30 (4)	22.8±.30 (4)	
Femur			
Weight	285	269	
(Ca)/mg	23.3±.46 (4)	22.2±.46 (4)	°
Mandible			
Weight	207	198	
(Ca)/mg	25.2±.51 (4)	24.7±.51 (4)	°
Incisor			
Weight	50	50	
(Ca)/mg	26.8±.32 (4)	26.4±.32 (4)	°
Molars			
Weight	34	31	
(Ca)/mg	27.6±.51 (4)	27.5±.59 (3)	

Numbers in parentheses = numbers of rats
° no significant difference
Values are means ± SE.
+ Dose level in mg/100 g body weight.

LOW CALCIUM DIET:

Body Weight: The diet had no significant effect on growth. Cortisone reduced the gain and hydrocortisone prevented growth completely.

Bone Weight: Both the mandible and femur were reduced in the control and hormone group. The mandible remained unchanged by cortisone but increased when hydrocortisone was injected. The femur was not affected by either hormone.

Bone Volume: Mandibular volume was reduced to a greater degree than was the femur volume, which was hardly affected by a low calcium diet.

Bone Density: Cortisone and hydrocortisone increased the density of the mandible; hydrocortisone alone produced an increase in femur density.

Calcium Concentration: Mandible and femur both contained less calcium in all groups of rats fed low calcium diet, as compared with rats fed normal diets. Administration of the hormones caused a reduction in the weight of the lower central incisors. Cortisone increased calcium concentration.

Experiment 5. Effect of cortisone and prednisolone administration over a period of 4 weeks.

NORMAL DIET:

Body Weight: Cortisone reduced growth by approximately 50 %, Prednisolone reduced weight gain by about 13 %.

Bone Weight: The weight of the mandible was reduced by cortisone. Femur weight was reduced by both hormones.

Bone Volume: Cortisone alone induced reduction. Femur volume was reduced by cortisone.

Bone Density: The only significant change was an increase in the density of the mandible due to cortisone.

Calcium Concentration: No change in either mandibular or femoral calcium concentration could be detected.

LOW CALCIUM DIET:

Body Weight: Both hormones reduced body growth: the effect of cortisone was greater causing a 50 % reduction in weight gain, whereas prednisolone only reduced body growth by about 30 %.

Bone Weight: The hormones caused an increase in mandibular weight, significant in the cortisone group. Femur weights remained unaffected.

Bone Volume: Decreases in mandibular volume was caused by the hormones. Femur volume was also decreased more by cortisone.

Bone Density: Both cortisone and prednisolone caused an increase in mandibular density. Only the administration of cortisone resulted in a denser femur.

Calcium Concentration: Cortisone caused increases in calcium concentration in both mandible and femur.

Effect on Teeth: Incisor weight, but not calcium concentration was reduced by the hormones. The molars were unaffected.

Effect on Alveolar Bone: No bone loss nor defect of any kind was observed due to either low calcium or steroid administration.

DISCUSSION

The work of Bundgerd-Jorgensen (15), Labelle and Schaffer (16) and Bernick and Ershoff (17) indicate that steroids cause microscopic changes in the peridontium of rats. However, these investigators also noted that the epithelial attachment remained at the cemento-enamel junction. The studies of Zipkin (18) Stahl and Gerstner (19), and Applebaum and Seelig (20) indicate that steroids do cause destruction of alveolar bone in rats. These conflicting reports on the effect of steroids upon the periodontium suggest that no specific disease syndromes can be attributed to these hormones.

Cortisone produced a diminished rate of growth; this depression of growth was greater than that noted following prednisolone administration. Hydrocortisone alone caused a decrease in body weight over the experimental period.

In all instances in which low calcium diet was used, the bone weight of both femur and mandible was significantly reduced. The steroids to cause a disruption of the growth of long bones, but that this disruption is not acute enough to become manifest in a two week experiment.

In those rats fed normal diet, the administration of steroids tended to cause a decrease in mandibular growth, and this decrease was related in severity to the steroid potency as evaluated by effect by body growth. When rats were fed low calcium diet, both cortisone and hydrocortisone caused increases in mandibular weight relative to the controls.

The comparison between the effects of low calcium diet and steroids on body and bone growth indicates that, although steroids affect skeletal growth, the skeletal effects of a deficiency of calcium in the diet are far more striking. The steroid hormones caused a consistent depression of body growth.

According to Harrison and Fraser (21), the rarefying effect of diets deficient in calcium is due largely to the great increase in resorptive activity. This osteoporotic effect results from the regulatory function of the parathyroid hormone as documented by McLean and Urist (22), in which the hemostatasis of body calcium is maintained by parathyroid hormone. The osteoporotic effect of low calcium diets is mediated through bone resorption than through deposition, which may be in the normal range. In the present study there was no consistent pattern of volume decrease either from steroid administration or feeding on low calcium diet.

Analysis of the tibia, femur, and mandible showed that in all instances, both in the control and steroid groups, low calcium diet caused a statistically significant depletion of bone calcium.

The calcium immobilization may be related to steroid potency, since hydrocortisone which had the most marked effect on body weight also caused the most frequent increase in calcium content.

In teeth the effect of both low calcium diet and steroids was nil except in one instance; cortisone caused a significant reduction in the calcium content of the incisors of another group.

A higher calcium concentration and the higher Ca/P ratio but unchanged ash content found in steroid-treated bones may be a reflection of decreased resorption and apposition. The decreased turnover has thus resulted in a higher hydroxyapatite content or "older" bone.

All the drugs used caused growth suppresion as measured by body weight. At identical dose levels hydrocortisone had a far greater suppresive effect on growth than cortisone, and at one third of this dose, prednisolone had a lesser suppressive effect. Cortisone and hydrocortisone were injected as saline suspensions of the acetate compound, which may be more slowly absorbed intraperitoneally than the succinate solution of prednisolone. If allowance is made for the possibility of different absorption rates, it can be stated that the drugs caused similar patterns of bone and body weight changes.

The purpose of this study was to design an experimental model of steroid-induced osteoporosis to utilize in testing the ability of some new drugs to inhibit the effect of the hormones. However, under the prescribed conditions, the hormones caused an increased density of bones and a tendency towards calcium immobilization, rather than the expected osteoporosis.

The present study showed that a diet low in calcium (normal phosphorus) caused osteoporotic changes in bone, and that the effects of steroid administration were the same whether the animals were fed normal or low calcium diets. Harrison and Fraser (21) investigated osteoporosis induced in rats by a calcium deficient diet. They concluded that the osteoporosis occuring in these circumstances must be due to an increase in the rate of bone resorption. Albright (23) put forward the concept that adrenal corticosteroids transformed bone structure by antianabolic activity rather than catabolic. Instead of an active breakdown of bone, a slower process took place whereby less bone was formed.

There is agreement also that corticosteroids inhibit the synthesis of collagen as demonstrated by Castor and Muirden (24) in tissue culture and

by Kivirikko et al .(25), and Smith and Allison (26) in rats by the use of 14c proline and 14c glycine respectively. Clark (27), using radioactive nitrogen, showed decreased protein synthesis in rats following cortisone administration. Other studies show that steroids decrease bone formation in rats. This was demonstrated by Kahn and Skoryna (28) utilizing radioactive strontium and its incorporation into bone tumors, when cortisone was included in the diet. Simmons and Kunin (29) found that the osteoblast population had decreased in the experimental animals, and the number of osteoclasts had remained the same as in the controls. Cell division of osteoblasts was hardly affected where as the turnover rate of precursor cells was significantly lower . Since the precursor cells are more involved in reproduction than osteoblasts, inhibition of their turnover would explain the decrease in osteoblast cell count. Simmons and Kunin (29) also pointed out that precursor cells may be responsible for formation of vascular tissue, and it seems reasonable that depression of bone formation might be associated with reduced vascularity.

The experimental literature thus contains a large body of evidence indicating that steroid therapy in rats has an anti-anabolic action on bone. After four weeks of cortisone therapy in the present study, the mandible was significantly lighter in weight, and smaller in volume than the mandibles of control animals in the normal diet, but the density and calcium content were unchanged. These findings agree with the concept that steroids cause decreased bone deposition and thus decreased bone growth. In the four week experimental rats fed on low calcium diet and injected with cortisone, the mandibles were actually heavier, although again smaller, and with significantly higher calcium content. The mandible of a rat treated with cortisone and fed low calcium diet is smaller due to decreased bone deposition; it is heavier and higher in calcium content because the steroids have effectively inhibited the calcium withdrawal and resorption caused by the diet alone.

If these are the results of short term corticosteroid administration, it seems likely that osteoporosis would develop if the experimental period was

lengthened. In a short experiment the decrease in resorption is the dominant effect. Eventually, however, the lower rate of bone formation should case an overall diminution of bone mass, and the density of bone observed in the experimental animals of this study would be transformed into osteoprosis seen in the long term experiment of Kahn and Skoryna (28).

Table 2 Experiment 1 2 weeks
Low Calcium Diet

	CONTROL	PREDNISOLONE: 1.5+	Effect of Drug	Effect of Diet	
				Control	P
IBW	181	180			
FBW	254	229			
Tibia					
Weight	198	206			
(Ca)/mg	21.0±.30 (4)	20.9±.30 (4)	°	*	**
Femur					
Weight	209	214			
(Ca)/mg	20.4±.46 (4)	20.2±.46 (4)	°	***	*
Mandible					
Weight	125	119			
(Ca)/mg	22.2±.51 (4)	22.5±.51 (4)	°	**	*
Incisor					
Weight	51	50			
(Ca)/mg	26.6±.32 (4)	26.8±.32 (4)	°	°	°
Molars					
Weight	34	33			
(Ca)/mg	28.2±.51 (4)	27.4±.51 (4)	°	°	°

Numbers in parentheses = numbers of rats
* $p < .05$
** $p < .01$
*** $p < .001$
° no significant difference
Values are means ± SE.
+ Dose level in mg/100 g body weight
P = Prednisolone

Table 3 Experiment 1 23 days
Normal Diet

	CONTROL	PREDNISOLONE: 1.5+	Effect of Drug
IBW	180	179	
FBW	289	267	
Tibia			
Weight	323	308	
(Ca)/mg	23.1 ± .33 (4)	23.4 ± .33 (4)	°
Femur			
Weight	353	340	
(Ca)/mg	23.3 ± .57 (4)	22.8 ± .57 (4)	°
Mandible			
Weight	232	223	
(Ca)/mg	25.6 ± .58 (4)	25.5 ± .58 (4)	°
Incisor			
Weight	55	52	
(Ca)/mg	26.9 ± .56 (4)	26.9 ± .56 (4)	°
Molars			
Weight	36	36	
(Ca)/mg	29.2 ± .59 (4)	28.4 ± .59 (4)	°

Numbers in parentheses = numbers of rats
° no significant difference
Values are means ± SE.
+ Dose level in mg/100 g body weight

Table 4 Experiment 1 23 days
Low Calcium Diet

	CONTROL	PREDNISOLONE: 1.5+	Effect of Drug	Effect of Diet	
				Control	P
IBW	184	183			
FBW	287	248			
Tibia					
Weight	229	216			
(Ca)/mg	20.8±.33 (4)	21.4±.33 (4)	°	***	***
Femur					
Weight	237	226			
(Ca)/mg	19.8±.57 (4)	20.5±.57 (4)	°	***	*
Mandible					
Weight	111	109			
(Ca)/mg	218±.58 (4)	22.6±.58 (4)	°	***	**
Incisor					
Weight	59	58			
(Ca)/mg	26.9±.56 (4)	26.5±.56 (4)	°	°	°
Molars					
Weight	36	35			
(Ca)/mg	28.7±59 (4)	29.7±.59 (4)	°	°	°

Numbers in parentheses = numbers of rats
* $p < .05$
** $p < .01$
*** $p < .001$
° no significant difference
Values are means ± SE.
+ Dose level in mg/100 g body weight
P = Prednisolone

Table 5 Experiment 2-2 weeks
Normal Diet

	CONTROL	PREDNISOLONE: 1.5+	Effect of Drug
IBW	150	150	
FBW	250	226	
Tibia			
Weight	246	244	
(Ca)/mg	22.5±.29 (6)	22.1±.29 (6)	°
Femur			
Weight	255	261	
(Ca)/mg	21.9±.25 (5)	21.8±.22 (6)	°
Mandible			
Weight	186	181	
(Ca)/mg	24.1±.22 (6)	23.9±.22 (6)	°
Incisor			
Weight	45	45	
(Ca)/mg	25.4±.80 (5)	26.2±.80 (5)	°
Molars			
Weight	34	35	
(Ca)/mg	27.3±.96 (6)	26.6±.96 (6)	°

Numbers in parentheses = numbers of rats
° no significant difference
Values are means ± SE.
+ Dose level in mg/100 g body weight.

Table 6 Experiment 2-2 weeks
Low Calcium Diet

	CONTROL	PREDNISOLONE: 3.0±	Effect of Drug	Effect of Diet Control	Effect of Diet P
IBW	151	152			
FBW	242	190			
Tibia					
Weight	177	168			
(Ca)/mg	20.4±.29 (6)	21.1±.29 (6)	°	***	*
Femur					
Weight	179				
Mandible		180			
(Ca)/mg	19.7±.22 (6)	20.0±.22 (6)	°	***	***
Weight	104				
Incisor		102			
(Ca)/mg	22.6±.22 (6)	23.2±.22 (6)	°	***	*
Weight	49				
Molars		46			
(Ca)/mg	25.6±.73 (6)	27.0±.89 (6)	°	°	°
Tibia					
Weight	33	33			
(Ca)/mg	28.8±.96 (6)	29.4±.96 (6)	°	°	°

Numbers in parentheses = numbers of rats
* $p<.05$
*** $p<.001$
° no significant difference
Values are means ± SE.
+ Dose level in mg/100 g body weight.
P = Prednisolone

Table 7 Experiment 3-2 weeks
Normal Diet

	CONTROL	CORTISONE: 4.5 +	Effect of Drug	PREDNISOLONE: 1.5 +	Effect of Drug
IBW	173	170		171	
FBW	268	220		247	
Femur					
Weight	267	248		260	
(Ca)/mg	23.5 ± .30 (8)	24.4 ± .32 (7)	°	23.5 ± .30 (8)	
Mandible					
Weight	309	76		298	
(Ca)/mg	23.0 ± 26 (8)	24.2 ± .26 (8)	**	23.1 ± .29 (8)	
Incisor					
Weight	205	193		49	
(Ca)/mg	24.3 ± .35 (8)	25.2 ± .35 (8)	°	26.0 ± .32	
Molars					
Weight	36	48		35	
(Ca)/mg	26.7 ± .47 (7)	25.7 ± .30 (8)	°	27.0 ± .44 (8)	

Table 8 Experiment 3–2 weeks
Low Calcium Diet

	CONTROL	CORTISONE: 4.5+	Effect of Drug	PREDNISOLONE: 1.5+	Effect of Drug	Effect of Diet Control	C	P
IBW	173	174		227				
FBW	251	292		180				
Femur								
Weight	incomplete	188		198				
(Ca)/mg	20.4±.30 (0)	21.4±.30 (8)	*	21.1±30 (8)	°	***	***	***
Mandible								
Weight	incoplete	212		228				
(Ca)/mg	19.6±.26 (8)	20.3±.26 (8)	°	19.9±26 (8)	°	***	***	***
Incisor								
Weight	incoplete	118		117				
(Ca)/mg	22.5±.35 (8)	23.3±35 (8)	°	226±35 (8)	°	***	***	***
Molars								
Weight	52	48		51				
(Ca)/mg	26.4±.30	25.9±.32 (7)	°	26.0±.30 (8)	°	*	°	°
Tibia								
Weight	36	35		35				
(Ca)/mg	27.4±.44 (8)	27.3±.44 (8)	°	27.9±.44 (8)	°	°	°	°

Numbers in parentheses = numbers of rats
* $p<.05$
*** $p<.001$
° no significant difference
Values are means ± SE.
\+ Dose level in mg/100 g body weight.
C = Cortisone
P = Prednisolone

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