

trations of mercuric ions. Both mercuric and cadmium ions inhibited l-epinephrine; mercuric, but not cadmium, ions inhibited angiotensin II. Zinc ions were inert. Silver, cupric, cuprous, barium, mercurous, and vanadyl ions induced contractions but only in concentrations greater than  $10^{-6}$  molar. Relatively high concentrations of two sulfhydryl-binding agents inhibited contractions induced by the mercuric ion; whereas low concentrations of a chelating agent markedly augmented them.

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### Effect of Cortisone on the Lipids of Bone Matrix in the Rat.\* (31771)

T. SAKAI AND R. L. CRUESS (Introduced by I. Clark)

*Orthopaedic Research Laboratories, Royal Victoria Hospital, Montreal, Quebec*

Recent studies have shown that lipids are present in significant amounts in the matrix of bone and that they vary upon the administration of exogenous agents which alter the state of mineralization of bone(1,2). Because of the known effect of cortisone on bone(3,4) and because cortisone is known to alter systemic lipid metabolism(5) the following experiments were carried out in order to determine whether the lipids of bone matrix are changed after cortisone administration and whether the osseous changes brought about by cortisone can be related to lipid changes.

*Material and methods.* 150 g male R.V.H. strain rats were divided into 4 groups of 10 each, as follows: (1) control, (2) adrenalectomized, (3) cortisone treated, and (4) adrenalectomized and cortisone treated.

The adrenal glands of the rats from Groups 2 and 4 were removed under light ether anaesthesia through a posterior approach. One-half of Groups 1 and 3 were subjected to a false operation in which all steps were carried out except removal of the gland. The

remainder of the animals of these groups served as an absolute control.

Six hours after operation 2 mg of cortisone acetate per .2 ml (Cortone, Merck, Sharp Dohne Ltd.) per 100 g body weight was injected subcutaneously to Groups 3 and 4. The same amount of the suspension base was given to the rats of Groups 1 and 2. Injections were given every day at 24-hour intervals for 10 days. Each cage of experimental animals consisted of 5 rats. They were allowed to eat Purina Labena and to drink tap water *ad libitum* except for the rats of Group 2, to which 1% NaCl solution was given. Body weight, and food and water consumption per 24 hours were recorded. Twenty hours after the last injection all animals were anaesthetized with intraperitoneal nembutal and blood was collected from the abdominal aorta. Complete removal of the glands was ascertained at this time. After sacrifice, the bones were removed and weighed.

Serum calcium concentration was determined according to the method of Yanagisawa (6). The humeri, femora, and tibiae were removed from all animals and the soft tissues

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TABLE I. Growth and Nutrition.

No. of rats	Group 1 9	Group 2 10	Group 3 10	Group 4 10
Initial body wt	165 ± 2.4	165 ± 2.4	170 ± 2.5	167 ± 2.7
Body wt at sacrifice	208 ± 5.2	206 ± 3.6	154 ± 2.8 *	132 ± 2.0 *
Gain	43 ± 3.8	43 ± 2.6	-16 ± 2.0 *	-33 ± 2.1 *
Food intake (mean), g/100 g b.w./24 hr	9.6 ± .09	8.7 ± .33*	7.3 ± .24*	7.6 ± .39*
Water intake (mean), g/100 g b.w./24 hr	14.5 ± .17	18.3 ± 1.04*	15.1 ± .66	20.1 ± .97*

\* P &lt; .01

† P &lt; .05

± standard error

removed. The bones were broken at the mid-shaft and the epiphyses were pried off and discarded. The marrow was flushed out with distilled water under pressure through a fine needle. The bones were lyophilized at  $-50^{\circ}\text{C}$ , with a vacuum of  $50 \mu$  and were ground to a powder in a Spex grinder for 1 minute in the cold. Lipids were extracted and purified according to the method of Folch *et al*(7). Total lipids were determined gravimetrically and total cholesterol and total fatty acids(8), phospholipids(9), and triglycerides(10) were determined according to methods reported previously(2). All values are expressed as milligrams per gram of lyophilized bone.

*Results.* No significant differences between the two cages within the same group or between the absolute controls and those animals which had been subjected to false operation were observed.

1. Body weight changes are shown in Table I. The mean weights of the control and adrenalectomized rats (Groups 1 and 2) were the same at time of sacrifice. The cortisone treated rats (Groups 3 and 4) showed a continuous regression in their rate of gain, with the adrenalectomized rats more severely affected than those in which the adrenal gland was present.

2. Food and water intake: As seen in Table I the food intakes of the rats of Groups 2, 3 and 4 were significantly less than those of Group 1, ( $P < 0.01$ ). The mean amounts of food consumed per 100 g of body weight of the rats of Groups, 2, 3 and 4 during the experimental period were 91%, 76% and 78% of the amount of the control groups respectively. The water intake of the rats without adrenalectomy (Groups 1 and 3) was sig-

nificantly less than that of those which had been adrenalectomized (Groups 2 and 4) ( $P < 0.01$ ). There were no differences between Groups 1 and 3 or between Groups 2 and 4.

3. Serum calcium concentration: There was no significant difference in the concentration of serum calcium among Groups 1, 2 and 3 while the concentration in Group 4 was lower than that of the others ( $P < 0.05$ ) (Table II).

4. Ash weight: The ash content of Groups 3 and 4 was significantly greater than that of Group 1 but there were no significant differences in ash content between these 2 groups (Table II).

5. Lipids of bone matrix: There was an absolute decrease in total lipids following adrenalectomy whether expressed as mg/g of bone or % of the organic fraction. Cortisone greatly increased the total lipids no matter how expressed.

There were no significant changes in the phospholipids of bone except in Group 4 in which the total lipid was also markedly increased. Adrenalectomy decreased triglyceride, cholesterol, and total fatty acid, while administration of cortisone increased all of these values with a greater increase in the adrenalectomized (Group 4) than in the non-adrenalectomized (Group 3) rats. Values calculated as percent of the organic matter demonstrated the same tendencies. In Group 2 the percentage values of total lipid and triglycerides were decreased significantly ( $P < 0.01$ ). In Groups 3 and 4 the percentage values of each lipid component except for phospholipid were increased significantly ( $P < 0.01$ ) when compared with that of the control, but there were no significant differences between these two groups.

TABLE II

No. of rats	Group 1		Group 2		Group 3		Group 4	
	9		10		10		9	
	mg/g of bone	% org. fr.	mg/g of bone	% org. fr.	mg/g of bone	% org. fr.	mg/g of bone	% org. fr.
Total lipid	17.74 ±1.21	4.22 ±.32	10.38* ±.56	2.74* ±.15	36.59* ±3.56	11.90* ±6.52	61.79* ±9.35	16.80* ±2.31
Phospholipid	1.54 ±.13	.36 ±.02	1.57 ±.11	.41 ±.02	1.54 ±.11	.42 ±.02	1.79 ±.11	.49* ±.02
Total fatty acid	4.52 ±.54	1.09 ±.31	2.13* ±.54	.56† ±.13	7.43* ±1.06	2.39* ±.43	10.75* ±2.27	2.93* ±.60
Triglyceride	1.17 ±.15	.28 ±.03	.50 ±.11	.16* ±.03	7.83* ±2.01	1.95* ±.96	13.35† ±5.39	3.77* ±1.57
Total cholesterol	.52 ±.05	.12 ±.01	.32* ±.03	.08† ±.01	1.12* ±.19	.31† ±.05	1.38* ±.28	.39* ±.08
Serum calcium	10.0 ± .20		10.0 ± .17		9.7 ± .12		9.3 ± .16†	
Ash, %	57.63 ± 1.43		61.78 ± 1.04		63.75 ± 1.32*		63.61 ± .84*	
Organic fr., %	42.37 ± 1.49		38.22 ± 1.03		36.25 ± 1.32*		36.39 ± .84*	

\* P &lt; .01

† P &lt; .05

± standard error

*Discussion.* The body weight changes demonstrate that a systemic response to adrenalectomy and to administration of cortisone was achieved.

The effect of cortisone on serum calcium concentration is unclear. Conway and Hingerty(11) showed that adrenalectomy decreased serum calcium level while Pincus *et al*(12) reported that cortisone diminished serum calcium. Taylor and Cavin(13) and Leemka *et al*(14) reported contradictory results. In the present experiment, cortisone caused a slight but significant decrease in serum calcium in the adrenalectomized animal. As cortisone is known(15) to have an effect on intestinal calcium transport as well as on urinary and fecal excretion, the results may reflect secondary changes only. In any event, it is evident that the serum calcium level was maintained and that the changes in the lipids are not due in Groups 2 and 3 to serum calcium changes. As the serum calcium level is maintained by alterations in parathyroid activity, the bone changes could be merely a reflection of parathyroid effect. However, we have not found similar changes in bone lipids after parathyroidectomy or treatment with parathyroid extract (unpublished data). The percent recovery of the lipids is between 50 and 60. This has been reported previously(2) and is thought to represent a non-lipid frac-

tion which is extracted with the lipids. Work is now in progress in an attempt to characterize this material which is believed to be a protein.

The increase in total lipid, triglyceride, total cholesterol and fatty acids following cortisone administration and their decrease following adrenalectomy are all the more significant in view of the absence of or small alteration in the phospholipids. It is thus not a non-specific increase in all of the lipids of bone matrix, but a selective change which has been found. Similar alterations in lipid metabolism in other organs have been reported (16). It is apparent that the changes in bone are similar to those reported for liver which have recently been attributed to increased autogenous synthesis(17) and that the level of enzymes responsible for synthesis of triglycerides and other lipids is increased by cortisone treatment(18).

The osteoporosis(3) produced in the bones of man and the changes produced in those of the rat by cortisone(4) have been well recognized and the effect of cortisone on fat metabolism has also been demonstrated(5). It is axiomatic that any qualitative or quantitative change in osseous tissue must result from changes in cellular function. It has not been demonstrated that changes in the lipids lead to an alteration in the amount of bone

present, but a real biochemical difference has been found between the bones of normal animals and those with alterations in circulating cortisone. This is not in keeping with the usual concept of such bone. It has previously been thought that this bone was qualitatively normal but that synthesis was decreased(15).

Histochemical studies have suggested that lipids play an important role in the process of calcification. Johnson(19) has recently proposed a mechanism for calcification of collagen which involves a sequential series of chemical events involving lipids. Until recently there was no information available on variations in lipids in pathological states. Paloyan *et al*(20) have reported that in the dog parathyroidectomy decreases serum cholesterol while the administration of parathormone causes an increase in serum total lipid and cholesterol. We have found alterations in cholesterol in the bone matrix of animals with altered amounts of circulating parathyroid hormone. The concept which seems most reasonable is that exogenous agents which are capable of altering lipid metabolism in the body are also capable of changing lipid metabolism in the bones. These changes must reflect alterations in cellular metabolism. It is conceivable that the changes found in the physical and chemical state of bones following administration of exogenous agents such as cortisone are a result of changes in lipid metabolism. It is not clear whether this represents a selective action or merely a change in the metabolic pattern of the involved tissues.

The statistical significance of the differences be-

tween means was calculated by using Fisher's F-test and refers only to differences between the treated groups and the control.

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