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Skeletal Storage of Fluorine in the Growing Rat Fed Bone Meals of Varying Fluorine Content.*

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In a previous experiment the authors reported that the bones of bovine fetuses and of veal calves were low in fluorine as compared to adult cattle bones.[1] This would seem to offer a means of obtaining a low fluorine bone meal which could be used for certain dietary and therapeutic purposes, especially in view of the fact that commercial bone meals are obtained from bones of cattle from all parts of the country, and may vary therefore in their fluorine content.

To test this suggestion veal calf bones (metatarsal) were obtained from a local packing plant at Madison. The bones were prepared by boiling them to remove the excess trimmings, crushing and extracting with alcohol to remove the fat, and then grinding to a fairly fine powder. This bone meal was analyzed and found to contain 20 parts of fluorine per million. A bone meal was prepared in the same manner from metatarsal bones obtained from University of Wisconsin cattle whose dietary history was known. This bone meal was quite coarse and contained 474 parts of fluorine per million. Two commercial bone meals were obtained. One of these was a special steamed bone meal used for livestock feeding, and the other was supposed to be a higher quality bone meal. They contained 550 and 529 parts of fluorine per million respectively.

Rats were used to test these bone meals, since Ellis and Maynard[2] have shown that the storage of fluorine in the bones of the rat is roughly proportional to the fluorine fed in the ration whether it is present as sodium fluoride or as a constituent of bone meal. The basal ration used had a fluorine content of 4.8 parts per million. It had the following composition:

Yellow corn	76
Crude casein	15
Yeast	3

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1 Evans, R. J., Phillips, P. H., and Hart, E. B., *J. Dairy Science*, 1938, **21**, 81.

2 Ellis, G., and Maynard, L. A., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 12.

Cod liver oil	1
Iodized salt	1

This basal ration was supplemented with the respective bone meals to be tested on the basis of equal amounts of ash. Five lots of 8 weanling rats each were fed as follows:

Lot I—Basal ration + 5.9 parts of veal calf bone meal.
II—Basal ration + 5.3 parts of a bone meal prepared from bones of University of Wisconsin herd cattle.
III—Basal ration + 4.0 parts of a high quality commercial bone meal.
IV—Basal ration + 4.4 parts of a commercial grade of special steamed bone meal.
V—Basal ration + 2.2 parts of a commercial grade of special steamed bone meal.

The fluorine content of these rations is given in Table I.

TABLE I.
Fluorine Content of Rations Expressed in Parts Per Million.

	Lot I Veal	Lot II U. of W.	Lot III High quality bone meal	Lot IV 4.4% commercial bone meal	Lot V 2.2% commercial bone meal
Fluorine in bone meal	20	474	529	550	550
Fluorine added to the ration by the bone meal	1.2	25.1	21.2	24.2	12.1
Total fluorine in ration	6.0	29.9	26.0	29.0	16.9

The rats weighed from 30 to 50 g each when they were placed on the experimental ration, and were kept thereon for 8 weeks. At the end of this period they were sacrificed, the femurs were removed and analyzed for fluorine. The method of analysis used was a modification of the micro-method of Armstrong.[3] The bones were finely ground in an iron mortar. Duplicate samples of 0.5 g each were weighed out and ashed as previously described.[4] The fluorine was titrated in aqueous solution with 0.02 N thorium nitrate, care being taken to keep the volume of the titrated solution small and its fluorine contents between 5 and 30 micrograms. This method of analysis was found to be very satisfactory for the levels of fluorine encountered in this experiment.

The rats grew well on all of the supplements and showed no gross symptoms of fluorine toxicity or of fluorine deficiency. No noticeable bleaching of the incisors occurred in any group. There was no significant difference in the growth rates of the rats in the different lots.

[3] Armstrong, W. P., *Ind. and Eng. Chem.*, Anal. Ed., 1936, **8**, 384.

[4] Chang, C. Y., Phillips, P. H., Hart, E. B., and Bohstedt, G., *J. Dairy Science*, 1934, **17**, 695.

The femurs of the rats receiving the veal calf bone meal (Lot I) contained on the average 44 parts of F per million (Table II). Attention is called to the fact that this ration contained *in toto* only 6.0 parts of F per million. On the other hand, Lots II to V were fed rations containing from 3 to 5 times as much fluorine on the basis of total fluorine expressed in parts per million, and yet the skeletal storage as indicated by fluorine in the femur was 7 times greater, or approximately 300 p.p.m. This would seem to indicate that the veal calf bone meal contained less physiologically effective fluorine.

TABLE II.
Fluorine Content of Rat Femurs Expressed in Parts Per Million.

	Lot I Veal calf bone meal	Lot II Bone meal U. of W.	Lot III High quality commercial bone meal	Lot IV 4.4% commercial bone meal	Lot V 2.2% commercial bone meal
	40	306	256	327	285
	38	285	269	352	309
	54	314	313	359	303
	49	280	320	338	293
	46	310	316	350	298
	36	272	252	316	352
	41	265	316	333	305
	50	268	275	313	276
Avg.	44	288	288	336	293

It will be noted from the data in Table I that most of the fluorine in the lot I ration was furnished by the basal ration. This would indicate that much of the fluorine present in veal calf bone meal was apparently combined in such a way that it was not available to the rat for storage in the skeleton.

It was previously indicated that the commercial special steamed bone meal was fed at 2 different levels. It was deemed advisable to feed one bone meal at a lower level, since the 4.4% level is much higher than the bone meal content of practical rations. This was justified as indicated in Table II, for the rats receiving the higher level of bone meal stored very slightly more fluorine in their femurs than did those receiving half this level of bone meal. This would indicate an incomplete absorption or utilization of the bone meal for skeletal growth at the higher level.

The fluorine content of the rations of lot II and lot IV were practically the same. However, the rats of lot IV stored significantly more fluorine than did those of lot II. As was pointed out earlier, the bone meal fed to the lot II rats was quite coarse. Therefore it may have been less readily absorbed by the rats and the fluorine

would thus have been less available to the rat. On the other hand, this bone meal contained more organic material and a part of the fluorine may have been bound up in this organic portion and was perhaps less available to the rat for bone storage.

Summary. It was demonstrated again that veal calf bones were low in fluorine. This experiment demonstrated clearly that bone meal prepared from veal calf bones produced femurs with an exceptionally low fluorine content, thus indicating a calcium and phosphorus carrier of high desirability insofar as the fluorine content is concerned. Commercial bone meals were found in this experiment to be responsible for the deposition of considerable amounts of fluorine in the femurs of the growing rat.

These data, further, suggest that the high levels of bone meal feeding in the case of the growing rat resulted in a limited utilization of the minerals as indicated by the storage of fluorine in the femurs and that the fluorine present in bone meal is not selectively absorbed.

Some of these data seemed to indicate that a part of the fluorine in veal calf bones was there in a form not readily available for absorption and storage in the skeleton of the rat.

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Comparison of Pregnancy Urine Injection and Coitus as Stimuli for Ovulation in the Rabbit.*

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Rabbit ovulation, as has been known since 1905,[7] usually follows successful mating by 9-14 hours. The stimulus of coitus is ineffective after transection of the pituitary stalk.[1] Ovulation may also follow electrical stimulation of the central nervous system[10, 6] and of the superior sympathetic ganglia[3] by 18-24 hours. Finally ovulation in the intact animal follows intravenous injection of gonadotropic hormone from either the anterior pituitary or from pregnancy

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7 Heape, W., *Proc. Roy. Soc.*, (London), 1905, B**76**, 260.

1 Brooks, C. McC., *Am. J. Physiol.*, 1935, **113**, 18.

10 Marshall, F. H. A., and Verney, E. B., *J. Physiol.*, 1936, **86**, 327.

6 Harris, G. W., *Proc. Roy. Soc.*, (London), B**122**, 374.

3 Friedgood, H. B., and Pincus, G., *Endocr.*, 1936, **19**, 710.