4680

Calcification of Teeth and Bones on Rachitic and Non-Rachitic Diets.*

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Changes in teeth as a result of deficient diets have been reported by a number of investigators. McCollum, Simmonds and Kinney[1] produced a number of defects in the teeth of rats by feeding rachitic diets. Orban,[2] by means of histological examination, found poor calcification in the incisor teeth of rats on a number of deficient diets. Mellanby[3, 4] reported poor calcification in the teeth of dogs and rabbits on rachitic diets. Toverud[5] obtained a small reduction in the ash, calcium, and phosphorus in the teeth of rats on a diet deficient in calcium. Perlzweig[6] found decreases in calcium and phosphorus in the incisor teeth of rats on diets low in either calcium or phosphorus, the low calcium diet giving a greater decrease than the low phosphorus diet.

The object of this investigation was to determine whether the calcium, phosphorus, and ash of the incisor teeth of rats would undergo changes similar to those produced in bone by a rachitic diet known to produce marked changes in bone. The Steenbock diet as modified by Epstein[7] was employed. This consists of the following and will be referred to as the basal diet:

Yellow corn meal 66%, wheat gluten 20%, egg albumin 10%, calcium carbonate 3%, sodium chloride 1%, spinach 10 gm. per rat per day, water *ad lib.*

Excluding the spinach, this diet contains 1.22 gm. Ca and 0.09 gm. P. (ratio 13.5:1) in 100 gm. of the ration and produces marked rickets accompanied by good growth.[7] The addition of 2% KH_2PO_4 or 2% cod liver oil to this diet prevents rickets.[7]

* This work has been conducted with the aid of a grant from the Chemical Foundation for research in biochemistry.

[1] McCollum, Simmonds, Kinney and Grieves, *Johns Hopkins Hosp. Bull.*, 1922, xxxiii, 202.

[2] Orban, B., *J. Am. Dental Assn.*, 1927, xiv, 1619.

[3] Mellanby, M., *Biochem. J.*, 1926, xx, 902.

[4] Mellanby, M., *British Dent. J.*, 1923, xliv, 1031.

[5] Toverud, G., *J. Biol. Chem.*, 1923, lviii, 583.

[6] Perlzweig, W. A., *J. Allied Dental Soc.*, 1916, xi, 70.

[7] Epstein, N., Dissertation, Columbia University, 1928.

The teeth and bones (femur) were compared on 2 groups of diets as follows:

Group I—(1) Basal; (2) Basal + 2% KH_2PO_4; (3) Basal + 2% KH_2PO_4 + 2% cod liver oil.

Group II—(2) as in group I; (3) as in group I; (4) Basal supplemented after 30 to 45 days by 2% KH_2PO_4; (5) Basal supplemented after 30 to 45 days by 2% cod liver oil.

After 30 to 45 days the rats in group I were killed with chloroform, and the incisor teeth and femurs were removed. The pulp and marrow (after splitting the bone) were removed by means of a dental pulp extractor. In several series the pulp and marrow were not removed. Rats in group II were killed after 64 to 80 days. In order to equalize conditions the rats in each comparative group were of the same litter and sex. There were at least 6 rats on each diet; 12 comparisons were made between the basal (rachitic) diet and the basal diet + KH_2PO_4. Analyses were made on the alcohol-ether extracted material by 2 methods: (1) the method of Shear and Kramer[8] in which the calcium and phosphate are removed from the tooth and bone powder by digestion with 1*N* HCl for 10 minutes (three to four times the weight of the sample specified in the method was employed); (2) after ashing.

We have been able to produce only slight variations in calcium, phosphorus, or ash of the teeth on any of the diets. Even when the bone calcium on the basal diet was reduced to 46% and the bone phosphorus to 38% of that on the basal diet + KH_2PO_4 + cod liver oil, the tooth calcium was practically unchanged and the phosphorus was reduced to the extent of only 4% of the total. In most experiments even this slight variation was not obtained.

The averages and average deviations of calcium and phosphorus of the teeth and bones on diets (1), (2) and (3) are given in the table. The figures represent the percentage of the dry weight of the alcohol-ether extracted material.

TABLE I.

Diet	Teeth		Bone	
	% Ca	% P	% Ca	% P
1 Rachitic	26.7 ± 0.6	13.8 ± 0.5	11.3 ± 1.2	5.9 ± 0.8
2 Rachitic + KH_2PO_4	26.2 ± 0.8	13.8 ± 0.6	20.3 ± 0.7	9.7 ± 0.4
3 Rachitic + KH_2PO_4 + cod liver oil	26.5 ± 0.5	14.0 ± 0.1	21.8 ± 0.7	10.5 ± 0.5

[8] Shear and Kramer, *J. Biol. Chem.*, 1928, lxxix, 105.

Tooth growth, as measured by the weight of the freshly extracted tooth was as good on the rachitic as on the non-rachitic diets and varied approximately with the variations in the growth of the rats. The percentage of bone calcium, phosphorus and ash on the basal diet $+KH_2PO_4$ + cod liver oil was about 8% higher than that on basal diet + KH_2PO_4.

In the recovery experiments when KH_2PO_4 or cod liver oil was added after the rats had been on the rachitic diet for 30-45 days, the calcium and phosphorus of the bones were increased to about the same level in each case, but fell somewhat short of the calcium and phosphorus of the bones of rats that were on the basal diet + KH_2PO_4 from the outset of the experiment. The latter, however, gave slightly lower values than the basal diet + KH_2PO_4 + cod liver oil.

The rats grew well on the rachitic diet, but in most cases growth was not as good as on the rachitic diet + KH_2PO_4. The latter, however, did not give as good growth as the rachitic diet $+KH_2PO_4$ + cod liver oil.

4681

Liver Extract Therapy in Splenectomized Anemic Rats.

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The therapeutic effect of liver extract on splenectomized anemic rats was investigated with the principal object of inquiring into the possibility of developing a biological assay for liver extracts. Great similarity between this severe rat anemia and human pernicious anemia had been noted by several observers, Lauda going so far as to name the disease "perniziöse Anämie" in rats.[1] At the time of our research we were unaware of the recently completed work of Vedder, who found that liver extract therapy was of no value in the treatment of this anemia.[2]

Our experiments confirmed this finding. In a series of 40 splenectomized rats (averaging 40 gm. in weight, the experimental animals being fed from 0.0125 gm. to 0.4 gm. of Lilly's liver extract No.

[1] Lauda, *Klin. Woch.*, 1925, ii, 1587.
[2] Vedder, A., *Nederl. Tijdschr. v. Geneesk.*, 1928, ii, 4411.