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Fat Deposition in Rat Tibia Due to Lysine Deficiency.* (24200)

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Recent publications(1-5) have shown that amino acid composition of the diet may have a decided effect on bone structure. Bavetta *et al.*(1,2) described pathological changes in bones and teeth of rats fed diets deficient in lysine or tryptophan. Specific effects should also be evident in the chemical composition of bone. Harris *et al.*(6) previously observed an abnormal, fatty bone marrow in histological sections from rats fed lysine-deficient gliadin. Quantitative changes in fat content of the tibiae of rats fed diets containing various amounts of lysine are described in this report.

Methods. Rats reared on stock diet(7) were weaned at 21 days with average weight of about 50 g. They were caged individually and fed the experimental diets, using litter mate distribution with 3 males and 3 females for each diet. At termination of the 5-week feeding period the animals in each group were weighed, killed, and their tibiae analyzed. For this purpose, the tibiae were dissected from tissues of right hind legs. After cleaning, the raw weight for the pooled group of 6 tibiae was obtained. All analyses were calculated on the basis of this weight. For moisture determination the bones were dried at 100°C. For fat determination the bones were then boiled in 95% alcohol under reflux for 24 hours and extracted with ether in a Soxhlet for same period of time. The bones were dried again and fat content calculated by difference. Finally the dried fat-free bones were ashed at 800°C. Having established amount of water, fat and ash present, the remaining constituents of raw bone have been called "organic residue" by Chick *et al.* (8). The basal diet used consisted of 90% commercial bread, dried at room temperature, supplemented with vitamins, minerals and fat. The bread contained 4% non-fat milk solids. This diet is low in lysine and was previously used by Rosenberg and Rohdenburg(7) to investigate improvement in growth and protein efficiency attainable by supplementation with lysine. An additional group of rats was fed the stock diet to study normal bone composition.

The results of adding increasing amounts of L-lysine monohydrochloride† to the bread diet are summarized in Table I. Fat content of tibiae of rats fed the unsupplemented bread diet was greater than that observed when stock diet was fed. Addition of lysine to the bread diet improved both growth and bone composition. With increasing lysine supplementation there was a decrease in fat content

* Our attention was originally called to this problem by Dr. Theodore F. Zucker of Columbia University, to whom we wish to express our appreciation.

† Du Pont L-lysine monohydrochloride, containing 95% L-lysine monohydrochloride and 5% D-lysine monohydrochloride.

TABLE I. Effect of Lysine on Rat Growth and Composition of Tibiae.

Diet	% L-lysine in diet	5-wk gain (g)	Pooled tibiae analysis (6 rats) Raw tibia wt (g)	% water	% fat extracted	% bone ash	% organic residue	% protein (N × 6.25)
Control bread	.29	84	1.7600	38.03	4.39	36.59	20.99	19.31
Bread + .2% L-lysine monohydrochloride	.45	143	2.1852	38.59	3.12	36.88	21.41	20.68
Bread + .5% L-lysine monohydrochloride	.69	186	2.5693	39.50	2.39	36.59	21.52	21.31
Bread + 1.0% L-lysine monohydrochloride	1.09	187	2.5566	39.11	2.70	36.20	21.99	20.12
Stock diet	1.10	204	2.7750	40.06	2.32	36.09	21.53	19.87

of the tibia. The per cent of bone ash and organic residue was constant with all 5 diets, but amount of moisture varied inversely with fat content. Thus lysine deficiency produced a fatty marrow with low moisture in the bones.

Protein content of the raw bones of each group was determined by Kjeldahl analysis of the tibiae from the left hind legs. It is evident that the organic residue is composed almost exclusively of protein.

These observations concerning changes in lipid content of tibiae of rats fed bread diets have been confirmed in experiments with other diets of low lysine content. Details of these experiments, as well as observations on the fat content of the tibiae of normal animals over their life-span, will be published separately.

Summary. Results with lysine-supplemented experimental diets and with stock diet show that the lipid content of the tibia is relatively low when sufficient lysine for optimum growth is available to young rats. With the lysine-deficient diet, growth is retarded and bone lipids are increased. Interference with production of normal bone marrow appears to be a definite indication of lysine deficiency.

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An *in vitro* Study of Fatty Acid Absorption.*† (24201)

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The form in which lipids are absorbed has been the subject of a number of investigations (1). Evidence has been accumulated in support of each of the 2 major theories of fat absorption, partition or lipolytic. The 2 theories have in common the necessity of free

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