Influence of Riboflavin on Fluoride Metabolism in the Rat

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In spite of the practice of adding fluoride (F) to vitamin preparations for pediatric supplements, there is little information available concerning the influence of various vitamins on F metabolism. This report summarizes the findings of two studies concerning the influence of riboflavin on skeletal F accretion in the rat.

Study 1 was done to determine the effect of various amounts of riboflavin supplements on F retention in rats using modified pair-feeding conditions. Male Wistar rats[a] (148, weighing 90 to 100 gm each) were separated into six equal groups according to body weight. The rats were housed in pairs in raised wire cages in an air-conditioned room. All rats were tube-fed at about 12 hour intervals throughout the study and distilled water was provided ad libitum. Composition of the diet and method of preparation and intubation have been described previously (Scow, *Endocrinology* 60: 359, 1957). The semisynthetic diet for all groups was of the same composition, except for a variation in riboflavin content (provided as riboflavin-5-phosphate). Riboflavin doses representing 0.5, 1.0, and 2.0 minimal daily requirements (MDR) for rats, or 12.5, 25, and 50 μg per day, were provided as a constituent of the diet intubated to the rats in groups *A* and *D*, *B* and *E*, and *C*, *F*, respectively. In addition, the rats in groups *D*, *E*, and *F* were provided, by oral intubation, 1.0 mg F daily as an aqueous solution (2.21 gm NaF per liter) at 24 hour intervals, about six hours before or after diet intubation. During days 0 to 2, 29 to 31, and 58 to 60 of the experimental period, 12 rats from each group were placed in individual metabolism cages and urine and feces were collected. Urine and feces samples were prepared with CaO as a F fixative, ashed, and analyzed for F by steam distillation from $HClO_4$ and titration with thorium nitrate. After a 60 day study period, the rats were killed by chloroform inhalation, the femurs and the remaining carcass were ashed independently and the pulverized ash was analyzed for F.

Study 2 was designed to determine the influence of varying amounts of riboflavin on the retention of F when the diet was provided ad libitum. Weanling male Wistar rats, 112, were separated into 14 equal groups according to body weight. The rats were provided distilled water and the riboflavin-deficient, semisynthetic diet ad libitum. Riboflavin was provided as an aqueous solution of riboflavin-5-phosphate by daily oral intubation with doses of 0, 5, 10, 12, 20, 25, and 50 μg. These doses were selected to include a deficiency (0 μg/day) and an excess of about two minimal daily requirements (50 μg/day). Each riboflavin dose was provided to two comparable groups of rats, one of which received an additional daily supplement of 1.0 mg F as aqueous NaF. The rats were maintained on these regimens for 30 days, at which time they were killed. Femurs and carcasses were analyzed for F as described in study 1.

The growth data in study 1 indicated that although all rats were provided an identical amount of food during the experimental period, the rats that received the fluoride plus either 0.5 or 2.0 MDR riboflavin (but not 1.0 MDR) gained significantly ($P < 0.05$) less weight than the corresponding controls. The metabolism data showed no significant differences in F excretion associated with the varying levels of riboflavin doses during the initial and 30 day periods. After 60 days, the rats provided 2 MDR riboflavin plus F (group *F*) excreted significantly more fluoride in the feces and less in the urine than rats provided lesser riboflavin doses. The net F retention data (carcass plus femur, less appropriate values) indicated that the rats provided 0.5, 1.0, and 2.0 MDR riboflavin retained 57.27, 56.57, and 52.81% of the F, respectively. The differences in fluoride retention between the lowest and highest amounts of riboflavin supplementation were statistically significant ($P < 0.05$). However, neither of the latter values differed significantly from the net percent F retention value observed in the rats provided the optimal amount of riboflavin (1.0 MDR).

In study 2, the daily administration of 10 μg of riboflavin or less resulted in a significant decrease in body weight gain and the simultaneous administration of F in the presence of these suboptimal riboflavin doses resulted in a further adverse effect on growth. The net percent F retention values were 68.96, 63.12, 70.48, 66.90, 64.39, 65.33, and 61.92 for the rats provided daily riboflavin doses of 0, 5, 10, 15, 20, 25, and 50 μg, respectively. Only the differences in F retention associated with the extremes of the range of riboflavin administration were statistically significant.

Collectively, these data suggest that only with an extremely high or low level of ingestion does riboflavin influence the skeletal retention of F. In such instances the retention of F in the rat appears to be related inversely to the amount of riboflavin ingested; riboflavin deficiencies are associated with modest increases in F retention and vice versa. However, at near optimal riboflavin doses, this vitamin does not appear to alter F metabolism or retention in the rat.

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