The Effect of Dietary Fluoride on Plasma Free Amino Acid Concentrations in the Rat[1] (35963)

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Diets containing increased amounts of inorganic fluoride have long been known to be toxic, but, although many enzyme systems are known to be inhibited by fluoride *in vitro* (1), the underlying biochemical mechanisms responsible for the toxicity of fluoride in the whole animal remain to be elucidated. With the exception of increased concentrations of blood and liver citrate, we have previously shown that most of the changes observed in carbohydrate (2–4) and fatty acid metabolism (5) in rats fed diets containing 450 or 600 ppm F are due to the effect of dietary fluoride on food consumption: fluoride ingestion depresses food intake, and alters the pattern of ingestion by increasing the length of time spent eating each meal (3).

Fluoride inhibits *in vitro* protein synthesis by interfering with the initiation of new peptide synthesis on the ribosomes (6, 7). In the intact rat, large doses of fluoride have been shown to produce degenerative histological changes that were associated with the inhibition of protein synthesis in hepatocytes (8) and ameloblasts (9, 10). Kruger (11) has recently shown that ^{3}H-proline uptake over the ameloblast is reduced in rats injected with 7 mg of F/kg of body weight. However, there have been no studies which assess the effect of fluoride on amino acid metabolism in the whole animal when the fluoride is presented in the diet. The experiments reported below were undertaken to assess the effects of dietary fluoride on amino acid metabolism in the intact rat.

Materials and Methods. Female Holtzman rats (150 or 200 g) were maintained in individual screen bottom cages in a room with automatic temperature and lighting conditions (12 hr light—12 hr dark). The animals were fed a low fluoride, semipurified diet (4) with, or without, the addition of NaF. Distilled water was offered *ad libitum*. In the programmed-feeding experiments the rats were fed the control diet in pelleted form by a pellet dispenser (4), which delivered pellets in either of two preset intake patterns. The tape A pattern delivered control diet to the rats in both the depressed amount and the altered pattern of food intake of rats fed fluoride. Tape B fed the control diet in the altered pattern of intake, but offered normal amounts of diet. In the pair-feeding experiments, 6.6 g of control diet was given to the rats at 8:00 a.m. This was the average daily food consumption of the fluoride-fed rats the first 3 days on the diet (4).

Blood was collected from the abdominal aorta of etherized rats, and after pooling the plasma from at least five rats, 1 ml of 15% sulfosalicylic acid (Fisher A-297) was stirred into 9 ml of the plasma sample. Precipitated protein was removed by centrifugation and the pellet was resuspended in 2 ml of sulfosalicylic acid and recentifuged. The two supernatants were combined and a 4 ml sample was used for amino acid analyais. Amino acids were determined on a Beckman-Spinco

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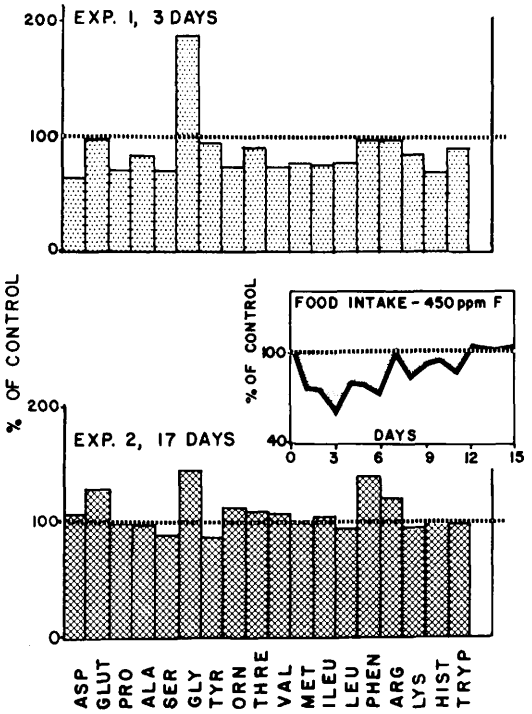

FIG. 1. Effect on dietary fluoride on the concentrations of plasma free amino acids in rats: (inset) the effects of fluoride on food consumption during these two experiments: The control values (μmoles/100 ml of plasma) in Expt. 1 were: Asp, 55; Glu, 20; Pro, 69; Ala, 77; Ser, 96; Gly, 11; Tyr, 16; Orn, 8; Thr, 47; Val, 25; Met, 12; Ile, 17; Leu, 27; Phe, 8; Arg, 16; Lys, 83; His, 10; Try, 12. The controls for Expt. 2 were similar.

Model 120 amino acid analyzer by a slight modification of the procedure of Spackman *et al.* (12). Acidic and neutral amino acids were eluted from a 145 mm × 0.9 cm column of Spinco 50A equivalent resin. After 11 hr, the original 0.2 *N* sodium citrate eluting buffer (pH 3.28) (30°) was changed to a 0.2 *N* sodium citrate buffer (pH 4.25) at 50°. Basic amino acids were determined in a separate run on a 55 mm × 0.9 cm column of Spinco 50 A equivalent resin. Elution was accomplished by the use of a 0.38 *N* sodium citrate buffer (pH 4.26), and the starting column temperature of 30° was changed after 10 hr to 50°.

Results. Expts. 1 and 2. Effects of dietary F on the concentrations of plasma amino acids. Substantial changes in the plasma free amino acid pattern (Fig. 1) were observed in rats fed 450 ppm F for 3 days (Expt. 1). Most amino acid levels were depressed, except for glycine, which was elevated 90% above control values. In contrast to this, after the diet containing fluoride was fed to rats for 17 days (Expt. 2), the pattern of plasma amino acids in the fluoride animals more closely resembled the amino acid pattern in the control group. It appeared that plasma amino acid levels were corrected back towards the normal levels.

We previously reported (13) that food intake is markedly reduced at 3 days by dietary fluoride, but that by 14 days, food intake has returned to normal (see inset Fig. 1). It was noted in the present experiments

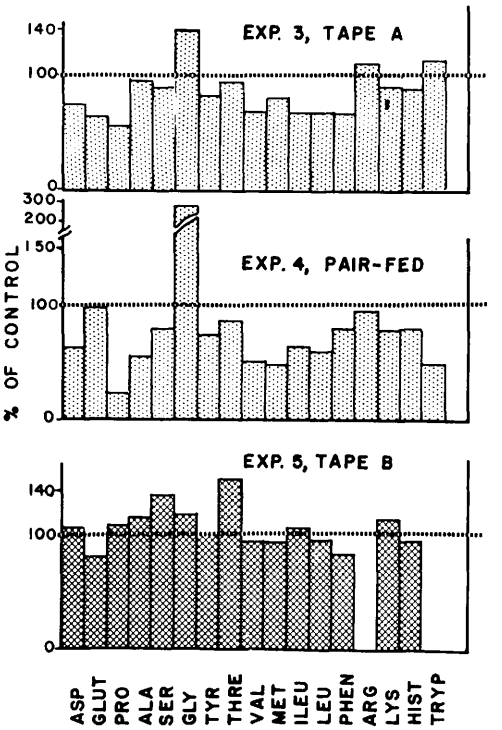

FIG. 2. Effects of changes in the amounts and pattern of intake of control diet on the concentrations of plasma free amino acids in rats compared to *ad libitum* controls: The control values (μmoles/100 ml of plasma) in Expt. 3 were: Asp, 72; Glu, 14; Pro, 80; Ala, 74; Ser, 83; Gly, 14; Tyr, 13; Thr, 51; Val, 37; Met, 10; Ile, 16; Leu, 25; Phe, 9; Arg, 16; Lys, 83; His, 8; Try, 10. The control values for Expts. 4 and 5 were similar.

that the correction in plasma amino acids occurred roughly at the same time as the correction in food intake.

Expts. 3, 4, and 5. Effect of pattern of food intake on the concentrations of plasma amino acids. In a group of rats program-fed the control diet in the depressed amounts and altered pattern of food intake of the fluoride-fed rats (Tape A, Expt. 3), many amino acids were again depressed, except for glycine which was elevated (Fig. 2). Although the alteration in glycine concentration was not as great, the pattern of plasma amino acids in the Tape A programmed-fed rats was similar to the pattern in the fluoride-fed rats at 3 days. These data suggest that the changes in plasma amino acids observed in the fluoride fed rats at 3 days were not due to a direct effect of fluoride on amino acid metabolism but were secondary to the fluoride-induced changes in food intake. This secondary response could have been due either to the decreased amounts of diet ingested by these animals, or their altered pattern of food intake (increased length of time spent eating each meal).

Control rats of this size consumed 13 g of diet/day and fluoride-fed rats, 6–7 g. When a group of rats were pair-fed 6.6 g of control diet in one meal each day for 3 days (Expt. 4), most plasma amino acids, with the exception of glycine, were depressed (Fig. 2). Although they were more accentuated, these changes in the pattern of plasma amino acids in the pair-fed rats closely resembled the depressed pattern observed in the fluoride-fed rats. The pattern of food intake however was markedly different between these two groups. The pair-fed rats were continuously hungry, and consumed their one meal immediately, while the fluoride-fed rats voluntarily ate many meals over a period of 12 hr (4).

In a fifth experiment, the pellet dispenser was set (Tape B) so that animals were exposed to the same pattern of intake, but not the amount of food as was eaten by the fluoride-fed rats. The concentrations of amino acids (Fig. 2) in these animals did not resemble the plasma amino acid concentrations seen in the rats receiving the fluoride diet. Taken together, these last two experiments indicated that the amount of food eaten, and not the pattern, was responsible for the fluoride-induced changes in the concentrations of plasma amino acids.

Discussion. These studies indicated that when rats are fed diets containing 450 ppm F for 3 days, the concentrations of most plasma free amino acids were depressed, except for an elevation in glycine. Additional experiments indicated that the fluoride-induced decrease in the amount of food eaten but not the altered pattern of food intake was the cause of the depressed pattern of plasma free amino acids at 3 days. After 17 days, at which time the food consumption of the fluoride-fed rats returned to nearly normal, plasma amino acid levels were also corrected towards normal. The depressed pattern of plasma amino acids could be reproduced in animals program-fed the control diet in the depressed amounts and altered pattern of food intake of the fluoride-fed rats, and also in animals pair-fed the control diet. These changes could not be reproduced in rats when only the characteristic eating pattern of fluoride-fed rats (increased meal times) was imposed.

Decreases in the concentrations of plasma free amino acids (14, 15), as well as numerous other changes in carbohydrate and lipid metabolism, have been observed in animals on restricted or altered patterns of food intake (15). In those cases, as well as in these experiments, decreases in the concentrations of plasma amino acids probably reflect the fact that there is an increased shunting of amino acids into the tricarboxylic acid cycle for energy production. The fact that pair-feeding technique as well as the programmed-feeding technique duplicated these effects of fluoride ingestion on plasma amino acid levels in the present experiments was surprising. Suttie has shown previously (4) that completely opposite alterations in pentose cycle enzymes are observed when these two types of intake are imposed as controls for fluoride-fed rats. Plasma amino acid levels appear to be more dependent on amount of food eaten rather than the pattern of intake.

The corrected pattern of plasma amino acids which occurred at 17 days when food

intake was corrected was interesting because this adaptation phenomenon is similar to the correction in the concentrations of glycolytic and some tricarboxylic acid cycle intermediates observed after long-term feeding of diets containing fluoride (2). Harper *et al.* (16) have presented evidence that food intake in rats can be regulated by altered patterns of plasma amino acids, and Anderson *et al.* (14) have demonstrated that adaptive enzymatic changes in serine dehydratase accompany the subsequent correction in food intake. The present data would indicate that the changes in the pattern of plasma amino acids seen in fluoride-fed rats were a result of, rather than a cause of, reduced food intake. It is possible, however, that a metabolic alteration in some other pathway caused the depressed food intake at 3 days, and this is corrected by metabolic adaptation by 17 days. These changes in the concentrations of plasma amino acids must be added to a growing list of characteristic metabolic changes induced by dietary fluoride ingestion which influence the pentose cycle (17), carbohydrate (2, 3, 18) and fatty acid metabolism (5).

Summary. When diets containing 450 ppm F are fed to rats for 3 days, the concentrations of most amino acids are decreased, except for glycine which is elevated. By 17 days, the plasma amino acid concentrations are corrected towards normal. Fluoride ingestion causes a decreased food intake and an alteration in the pattern of food intake. The altered pattern of plasma amino acids at 3 days has been shown to be due to the decrease in amount of diet consumed and not the altered pattern of food intake.

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