PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 165, 457–461 (1980)

Growth Stimulating Effect of Picolinic Acid Added to Rat Diets[1] (41004)

GARY W. EVANS AND ELAINE C. JOHNSON

United States Department of Agriculture, Science and Education Administration Human Nutrition Laboratory, Grand Forks, North Dakota 58202

Abstract. Rats fed either a soybean protein-based diet or a casein-based diet supplemented with picolinic acid gained significantly more weight than rats fed unsupplemented diets. The zinc concentration of the kidneys from the rats fed the diets supplemented with picolinic acid was significantly greater than that of the rats fed the unsupplemented diets. Rats fed a pyridoxine-free diet formulated with vitamin-free casein and supplemented with picolinic acid gained significantly more weight than rats fed an unsupplemented pyridoxine-deficient diet. The zinc concentration of the kidneys from the rats fed the pyridoxine-deficient diet supplemented with picolinic acid was significantly greater than that of the rats fed the unsupplemented diet. Dietary zinc absorption and food efficiency were increased when rats were fed supplemental picolinic acid. The results suggest that picolinic acid may increase the bioavailability of dietary zinc and perhaps other divalent cations which results in increased food efficiency.

The pyridine-2-carboxylic acid commonly known as picolinic acid is a product of tryptophan metabolism (1, 2) but to date the biological function of this metabolite has not been delineated. By use of a technique referred to as modified gel filtration chromatography (3), investigators in our laboratory have discovered that picolinic acid is a zinc-binding ligand present in human milk (4), rat intestine (5), and bovine pancreatic extracts (6). Because picolinic acid is a strong bidentate metal chelating ligand (7), we have begun investigating the possibility that this metabolite may function in facilitating the absorption of divalent cations such as zinc, iron, and copper.

To determine whether or not picolinic acid facilitates intestinal absorption of some of the essential metals we began testing the effect of this ligand on dietary zinc absorption. Three different types of experiments have been conducted to date. Zinc absorption was measured in rats fed a 5% casein diet supplemented with either tryptophan or picolinic acid (8). The results of these experiments suggested that exogenous and endogenous picolinic acid facilitates absorption of dietary zinc. Zinc absorption was measured in rats fed varying levels of vitamin B_6 which is a cofactor for at least one of the enzymes in the pathway from tryptophan to picolinic acid (1, 2). The results of these experiments also suggested that exogenous and endogenous picolinic acid facilitate zinc absorption (9). The third type of experiment we conducted was a series of biological assays in which the growth rate of rats fed dietary supplements of picolinic acid was measured. Since zinc is required to maintain growth in animals (10) we hoped to obtain indirect evidence of the effect of picolinic acid on zinc absorption by comparing growth rates in rats given a picolinic acid supplement with growth rates in unsupplemented rats. The results reported in this paper describe the effect of supplemental picolinic acid on growth rates in rats fed a soy protein-based diet, a casein-based diet, and a pyridoxine-free diet formulated with vitamin-free casein.

Materials and methods. Weanling, male Long–Evans rats age 21 days that had been born and reared in our laboratory were used in the experiments. The rats were housed in suspended stainless-steel cages and were fed food and water *ad libitum*.

The rats which weighed 45–52 g were divided into six groups of six rats each and were fed one of the diets described in Table I. Diets 1 and 2 contained 5 ppm zinc. The rats fed these diets were given water that

[1] Supported in part by the USDA Cooperative Agreement No. 12-14-3001-294 with the University of North Dakota.

0037-9727/80/110457-05$01.00/0

TABLE I. COMPOSITION OF DIETS

Ingredient	Grams per kilograms			
	Diet 1	Diet 2	Diet 3 Diet 5[a]	Diet 4 Diet 6[a]
Soybean protein[b]	200.0	200.0	—	—
Vitamin-free casein[b]	—	—	200.0	200.0
Celluflour[c]	40.0	40.0	30.0	30.0
Salt mix	26.9[d]	26.9[d]	50.0[e]	50.0[e]
Choline chloride[f]	1.5	1.5	1.5	1.5
Vitamin mix[e]	15.0	15.0	15.0	15.0
Methionine[f]	2.0	2.0	—	—
Corn oil[g]	90.0	90.0	90.0	90.0
Picolinic acid[h]	—	0.2	—	0.2
Sucrose[i]	624.6	624.4	613.5	613.3

[a] Pyridoxine·HCl was omitted from the vitamin mix of Diets 5 and 6.
[b] ICN Pharmaceuticals, Inc., Cleveland, Ohio.
[c] Whatman CF11, W. and R. Balston Ltd., London, England.
[d] Zinc-free Bernhart–Tomarelli (13) mineral mix prepared by Teklad Test Diets, Madison, Wisconsin.
[e] See Ref. (11).
[f] Grand Island Biological Company, Grand Island, New York.
[g] Mazola, Best Foods, Englewood Cliffs, New Jersey.
[h] Sigma Chemical Company, St. Louis, Missouri.
[i] Jack Frost, National Sugar Refining Company, Philadelphia, Pennsylvania.

contained 8 ppm zinc in the form of zinc acetate. Diets 3–6 contained 16.5 ppm zinc. The rats fed these diets were given deionized water. Pyridoxine·HCl was omitted from Diets 5 and 6.

The rats in each group were weighed weekly for a period of 6 weeks. Thereafter the rats were decapitated and the kidneys and intestines were removed for analyses. The tissues were wet ashed in a mixture of nitric and sulfuric acids after which the concentration of zinc was determined by atomic absorption spectrometry (Varian, Model 1250).[2]

In a second series of experiments, designed to determine the mechanism whereby picolinic acid stimulates growth, rats were divided into three groups of six rats each. One group of rats was fed Diet 3 and deionized water *ad libitum* and a second group of rats was fed Diet 4 *ad libitum* as described above. The third group of rats was also fed Diet 4 but the quantity of food given each day was limited to that consumed on the previous day by the rats fed Diet 3. After 4 weeks, each of the rats were injected intramuscularly with 10 μCi carrier-free ^{65}Zn. Nine days later quantitative collection of feces and urine was carried out for a period of 5 days. The rats were then killed and the kidneys and small intestine were removed. True, daily absorption of zinc was determined by the isotope dilution technique (11).

Results. Food consumption of rats fed the soy protein-based diets (Diets 1 and 2) could not be measured because, for reasons we cannot explain, the rats wasted much of the diet and an accurate measure of intake could not be obtained. For this reason we could not conduct a pair-feeding experiment with this group of rats. Food consumption of the rats fed the casein-based diet supplemented with picolinic acid (Diet 4) was significantly greater than the food consumption of rats fed the unsupplemented diet (Diet 3). During the last 2 weeks of the experiment, rats fed Diet 4 ate 19.1 ± 2.4 g/day while rats fed Diet 3 ate 15.3 ± 1.4 g/day. Food consumption of the rats fed the pyridoxine-deficient diets

[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

(Diets 5 and 6) did not vary significantly between the groups. The rats fed Diet 5 ate 10.0 ± 2.2 g/day during the course of the experiment while the rats fed Diet 6 ate 8.7 ± 1.5 g/day during the course of the experiment. Thus, there was no need to conduct a pair-feeding experiment with these two groups of rats.

As illustrated in Fig. 1, rats fed a soy protein-based diet supplemented with picolinic acid (Diet 2) gained significantly ($P < 0.05$) more weight than rats fed the unsupplemented soy-based diet (Diet 1). Rats fed the diet formulated from vitamin-free casein and supplemented with picolinic acid (Diet 4) gained significantly ($P < 0.05$) more weight than rats fed the unsupplemented casein-based diet (Fig. 2). In addition, rats fed the pyridoxine-deficient diet supplemented with picolinic acid (Diet 6) gained significantly ($P < 0.05$) more weight than the rats fed the unsupplemented pyridoxine-deficient diet (Fig. 2).

The zinc concentration of the kidneys from rats fed the soy protein-based diet supplemented with picolinic acid was significantly greater ($P < 0.05$) than the zinc concentration of the kidneys from rats fed the unsupplemented soy protein-based diet (Table II). The zinc concentration of the kidneys from rats fed the diet formulated with vitamin-free casein was significantly greater ($P < 0.05$) than that of rats fed the unsupplemented casein-based diet (Table II). The zinc concentration of the kidneys from rats fed the pyridoxine-deficient diet supplemented with picolinic acid was significantly greater ($P < 0.05$) than that of the rats fed the unsupplemented casein-based diets (Table II). The zinc concentrations of the intestines did not vary significantly among the six groups.

The results listed in Table III demonstrate that zinc absorption and zinc retention were significantly increased ($P < 0.05$) when rats were fed the casein-based diet supplemented with picolinic acid. True, daily zinc absorption and daily zinc retention were both increased when the supplemented diet (Diet 4) was pair-fed to the unsupplemented diet (Diet 3). In addition, both the daily weight gain and the food efficiency (g gained/g food consumed) determined during the last week of the experiment were greater when rats were pair-fed Diet 4 than when rats were fed Diet 3 *ad libitum*.

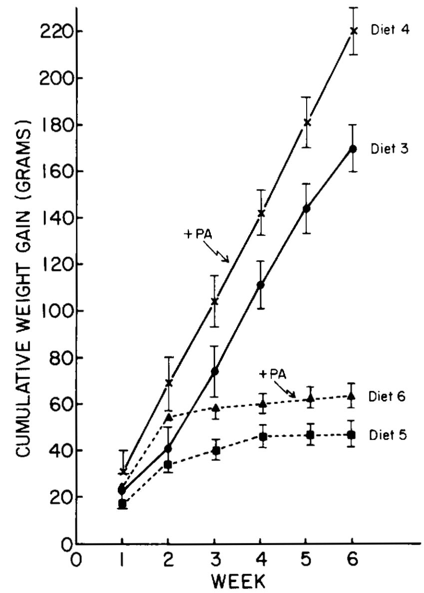

FIG. 2. Cumulative weight gain of rats fed pyridoxine-deficient and pyridoxine-adequate diets with and without picolinic acid (PA). Each point represents the mean ± SD of six rats. For details on composition of diets, see Table I.

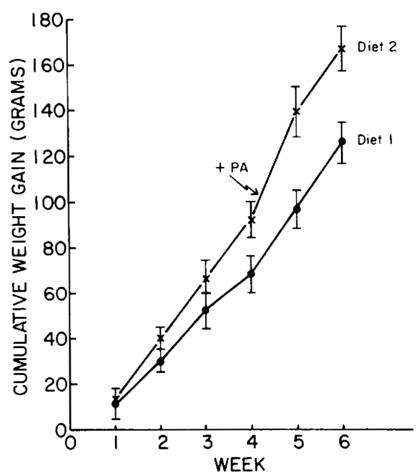

FIG. 1. Cumulative weight gain of rats fed a soybean protein-based diet with and without picolinic acid (PA). Each point represents the mean ± SD of six rats. For details on composition of diets, see Table I.

TABLE II. ZINC CONCENTRATIONS OF INTESTINE AND KIDNEYS FROM RATS FED THE SIX EXPERIMENTAL DIETS

Diet	Zinc concentration (μg/g dry wt) Kidney	Intestine
1	77.1 ± 11.9[1,a]	93.6 ± 15.8[1]
2	90.9 ± 7.7[2]	93.4 ± 6.8[1]
3	83.6 ± 6.9[1,2]	86.3 ± 23.8[1]
4	132.4 ± 14.7[3]	92.7 ± 13.8[1]
5	83.0 ± 17.6[1,2]	94.3 ± 17.9[1]
6	126.5 ± 10.8[3]	118.4 ± 14.0[1]

[a] Values are mean ± SD of six animals. Values not followed by the same superscript are significantly different ($P < 0.05$).

As shown in Table III, daily zinc intake, zinc retention, true daily zinc absorption, food efficiency, and daily weight gain were significantly greater ($P < 0.05$) when rats were fed Diet 4 *ad libitum* than when rats were fed Diet 3 *ad libitum*. These results demonstrate that food efficiency was increased when rats were fed supplemental picolinic acid and the results suggest that the increased efficiency resulted in part from increased absorption and retention of dietary zinc.

Discussion. The results illustrated in Figs. 1 and 2 demonstrate that supplemental picolinic acid promotes growth in rats fed either soy protein-based diets or diets formulated with vitamin-free casein. To our knowledge, this is the first report describing any beneficial effect derived from this tryptophan metabolite. The fact that picolinic acid stimulated growth in rats fed a pyridoxine-deficient diet was particularly interesting because one of the enzymes in the pathway from tryptophan to picolinic acid requires pyridoxine as a cofactor (1, 2).

For several years, investigators have suspected that low-molecular-weight chelating ligands facilitate transport of metal ions through the intestinal cells. Picolinic acid forms stable complexes with several cations including the essential elements zinc, iron, and copper (7). The association constants for zinc picolinate are log $K_1 = 5.75$ and log $\beta_2 = 10.01$. The association constants for ferrous picolinate are log $K_1 = 4.9$ and log $\beta_2 = 9.0$. The association constant for ferric picolinate is log $\beta_2 = 12.8$ and the association constants for cupric picolinate are log $K_1 = 7.95$ and log $\beta_2 = 14.95$. Metal complexes with picolinic acid are formed by coordination between the pyridine nitrogen and the carbonyl oxygen on the adjacent carboxylic acid group (12). Picolinic acid could very readily chelate cations within the intestinal lumen and/or cells and facilitate their transport into the portal blood.

The increased growth rate observed in rats fed supplemental picolinic acid resulted at least in part from an increased absorption and retention of dietary zinc. As described in Table II, the zinc concentration of kidneys from rats fed supplemental picolinic acid was significantly greater than that from rats fed the unsupplemented basal diets. Moreover, as shown in Table III daily dietary zinc absorption was significantly increased in rats fed supplemental picolinic acid. We have not yet examined the effect

TABLE III. ZINC ABSORPTION AND FOOD EFFICIENCY IN RATS FED A BASAL DIET AND A BASAL DIET SUPPLEMENTED WITH PICOLINIC ACID

Diets	Daily Zn intake (μg)	Daily Zn retention (μg)	True daily Zn absorption[a] (μg)	Daily wt gain[b] (g)	g gain/ g food[b]
3 *ad lib.*	256 ± 39[c,1]	47 ± 15[1]	120 ± 23[1]	3.7 ± .2[1]	0.237[1]
4 pair-fed	256 ± 39[1]	72 ± 5[2]	161 ± 14[2]	4.5 ± .2[2]	0.290[2]
4 *ad lib.*	318 ± 17[2]	89 ± 10[3]	198 ± 18[3]	5.6 ± .3[3]	0.291[2]

[a] True, daily zinc absorption determined by isotope dilution technique (see Ref. (11)).
[b] Last 7 days of experiment.
[c] Values are mean ± SD of six rats in each group. Values not followed by the same superscript are significantly different ($P < 0.05$).

of picolinic acid on the absorption of other divalent cations. However, since zinc is required to maintain growth (10), the growth stimulating effect of picolinic acid must have been due in part to an increased bioavailability of dietary zinc which resulted in increased food efficiency and a concomitant increase in growth.

1. Mehler, A. H., J. Biol. Chem. **218**, 241 (1956).
2. Mehler, A. H., and May, E. L., J. Biol. Chem. **223**, 449 (1956).
3. Evans, G. W., Johnson, P. E., Brushmiller, J. G., and Ames, R. W., Anal. Chem. **51**, 839 (1979).
4. Evans, G. W., and Johnson, P. E., Pediat. Res. **14**, 876 (1980).
5. Evans, G. W., Johnson, P. E., and Johnson, E. C., J. Nutr. **109**, 32 (1979).
6. Krieger, I., and Evans, G. W., J. Pediat. **96**, 32 (1980).
7. Sillen, L. G., and Martell, A. E., "Stability Constants of Metal Ion Complexes," Special Pub. No. 17, p. 496, The Chemical Society, London (1964).
8. Evans, G. W., and Johnson, E. C., J. Nutr. **110**, 1076 (1980).
9. Evans, G. W., and Johnson, E. C., Clin. Res. **27**, 682A (1979).
10. Underwood, E. J., "Trace Elements in Human and Animal Nutrition," 4th ed., p. 196, Academic Press, New York (1977).
11. Evans, G. W., Johnson, E. C., and Johnson, P. E., J. Nutr. **109**, 1258 (1970).
12. Paul, R. C., Chopra, R. S., Bhambri, R. K., and Singh, G., J. Inorg. Nucl. Chem. **36**, 3703 (1974).
13. Bernhart, F. W., and Tomarelli, R. M., J. Nutr. **89**, 495 (1966).

Received December 7, 1979. P.S.E.B.M. 1980, Vol. 165.