PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 166, 369–373 (1981)

Decreased Absorption of Orally Administered Ammonia by Clinoptilolite in Rats (41076)

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Abstract. Sprague–Dawley growing rats were used in four experiments to determine the effect of a natural zeolite mineral, clinoptilolite, on portal blood ammonia concentration following oral administration of $(NH_4)_2CO_3$. Stomach tubing rats with 315, 472.5, 630, or 945 mg clinoptilolite/100 g body wt reduced portal vein blood ammonia concentrations of rats orally administered, simultaneously, 45 or 90 mg $(NH_4)_2CO_3$/100 g body wt. The reduction was greater at the higher clinoptilolite concentration at each level of $(NH_4)_2CO_3$ administration (i.e., 472.5 mg clinoptilolite was more effective than 315 mg at a dosage of 45 mg $(NH_4)_2CO_3$/100 mg body wt, and 945 mg clinoptilolite was more effective than 630 mg at a dosage of 90 mg $(NH_4)_2CO_3$/100 mg body wt). Inclusion of 5% clinoptilolite in a diet containing 4% $(NH_4)_2CO_3$ did not decrease portal blood ammonia after a 30-min meal, but it did reduce portal blood ammonia concentration compared with that of rats fed the basal diet not containing clinoptilolite. It is concluded that clinoptilolite has the capacity to bind free ammonia in the gastrointestinal tract and that the degree of binding is predictable from its known ion-exchange capacity. The ammonia binding may be related to the improved efficiency of feed utilization reported in animals fed diets containing clinoptilolite.

Clinoptilolite, a naturally occurring zeolite (crystalline, hydrated aluminosilicates of alkali and alkaline earth cations, having infinite three-dimensional structures) has been reported, as reviewed by Mumpton and Fishman (1), to improve efficiency of feed utilization of swine and poultry when used as a dietary supplement. The mineral has the theoretical formula $(Na_4K_4)(Al_8Si_{440}O_{96}) \cdot 24\ H_2O$, a cation-exchange capacity of about 2.54 mEq/g, and its selectivity for NH_4^+ has been exploited (2, 3) in the development of an ion-exchange process to remove ammonia-N from municipal sewage. Ammonia is a recognized toxin in higher animals (6, 7). Intestinal microflora contribute significantly to the concentration of portal blood ammonia through deamination of ingested protein and urea hydrolysis (4, 5). A suggested mode of action of the growth promotion by subtherapeutic levels of dietary antibiotics in animals is the reduced bacterial ammonia production in the gastrointestinal lumen (8, 9). The purpose of the present experiments was to determine whether the oral administration of clinoptilolite to rats inhibited ammonia absorption in the gastrointestinal tract, thereby mimicking the effect of antibiotics in promoting animal growth.

Materials and Methods. Growing or young adult male Sprague–Dawley rats were housed individually in stainless-steel wire-bottom cases in a well-ventilated room, with temperature controlled thermostatically at 20°, and were fed a standard commercial rat pellet diet *ad libitum* except in experiment 4 in which a corn–soybean meal-based diet fortified with vitamins and minerals was used. In experiments 1–3, animals were fasted for 12 hr before receiving $(NH_4)_2CO_3$ (20% aqueous solution) or clinoptilolite (20% aqueous suspension) or both by stomach tube. Clinoptilolite from Buckhorn, New Mexico crushed to pass a 50 mesh/in. screen (designated −50 mesh) was used. To obtain blood samples, animals were anesthetized with diethyl ether, a midline ventral incision was made from the rectum to the rib cage to expose the peritoneal cavity, and blood (0.4–0.8 ml) was drawn from the portal vein into a heparinized syringe through a 26-gauge 5/8-in. needle for determination of portal blood ammonia concentration (10). Protein-free filtrates were prepared of all blood samples immediately after sampling (11). In experiment 4, the animals were fasted for 12 hr and then sacrificed, as

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above, immediately after a 30-min meal consisting of a basal corn–soybean meal diet fortified with vitamins and minerals or the basal diet supplemented with $(NH_4)_2CO_3$, or clinoptilolite or both.

Experiment 1. Thirty-one rats weighing an average of 280 g were randomly assigned to the following four treatments: (i) control (sacrificed after a 12-hr fast), six rats; (ii) oral dose of $(NH_4)_2CO_3$, 45 mg/100 g body wt., and sacrificed 15 or 30 min after dosing (five and four rats, respectively); (iii) same as (ii), plus clinoptilolite, (315 mg/100 g body wt) (four rats sacrificed at each time interval) and (iv) same as (ii), plus clinoptilolite, 472.5 mg/100 g body wt (four rats sacrificed at each time interval).

Experiment 2. Twenty rats weighing an average of 74 g were randomly assigned to the following three treatments: (i) control (sacrificed after a 12-hr fast), four rats; (ii) oral dose of $(NH_4)_2CO_3$, (90 mg/100 g body wt) and sacrificed 10, 20, or 30 min after dosing (three, two, and three rats, respectively, at each time interval); (iii) same as (ii) plus clinoptilolite, 630 mg/100 g body wt (two, three, and three rats, respectively, at each time interval).

Experiment 3. Twenty-eight rats weighing an average of 200 g were randomly assigned to the following four treatments: (i) control (sacrificed after a 12-hr fast), four rats; (ii) oral dose of $(NH_4)_2CO_3$, 90 mg/100 g body wt, and sacrificed 15 or 30 min after dosing (four rats at each time interval); (iii) same as (ii) plus clinoptilolite, 630 mg/100 g body wt (four rats at each time interval), and (iv) same as (ii), plus clinoptilolite, 945 mg/100 g body wt (four rats at each time interval).

Experiment 4. Twenty-four rats weighing an average of 93 g were randomly assigned to the four diets (six rats/diet) as follows: (i) basal (B); (ii) B + 4% $(NH_4)_2CO_3$; (iii) B + 5% clinoptilolite; (iv) B + 4% $(NH_4)_2CO_3$ + 5% clinoptilolite. All rats were fasted for 12 hr, then offered their respective diets *ad libitum* for 30 min. After 30 min, they were sacrificed immediately to determine portal blood ammonia concentrations. Feed consumed by each rat during the 30-min meal was recorded.

Results. *Experiment 1.* The portal blood ammonia concentration (Fig. 1) was reduced from 5.2 to 3.9 and 3.3 mg/dl by clinoptilolite at 315 and 472.5 mg/100 g body wt 15 min after $(NH_4)_2CO_3$ administration (45 mg/100 g body wt). After 30 min, the portal blood ammonia concentration had declined to 3.1 mg/dl in rats given only $(NH_4)_2CO_3$ and to 3.2 and 2.3 mg/dl, respectively, in rats given 315 and 472.5 mg clinoptilolite/100 g body wt. The portal blood ammonia concentration of rats sacrificed 30 min after receiving ammonia and the higher dosage of clinoptilolite was similar to that of control rats (2.3 vs 1.9 mg/dl).

Experiment 2. The administration of 90 mg of $(NH_4)_2CO_3$/100 g body wt increased the portal blood ammonia concentration (Fig. 2) at 10 and 15 min after dosing to values nearly twice those obtained after 15 min following the administration of 45 mg $(NH_4)_2CO_3$/100 g body wt (experiment 1). The portal blood ammonia concentration increased further to 12.5 mg/dl after 30 min when clinoptilolite was not present, but declined progressively from a peak of 7.6 mg/dl 10 min after dosing to 5.5 mg/dl after 20 min and 4.4 mg/dl after 30 min when

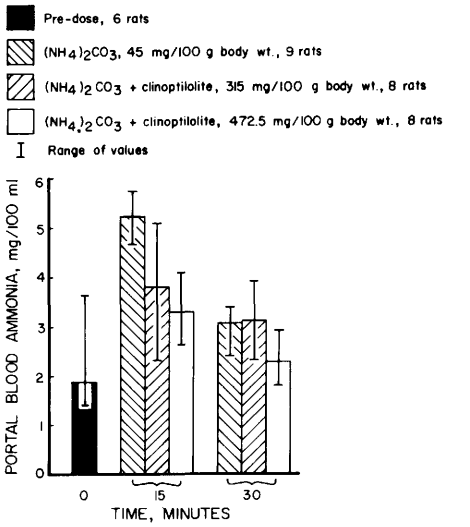

FIG. 1. Effect of zeolite level on portal blood ammonia of weanling rats dosed orally with $(NH_4)_2CO_3$ (45 mg/100/g body wt). Significant effect of clinoptilolite ($P < 0.01$). SD = 0.79.

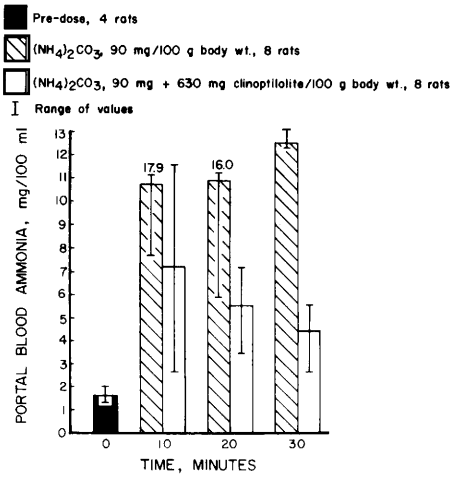

FIG. 2. Effect of zolite on portal blood ammonia of weanling rats dosed orally with $(NH_4)_2CO_3$ (90 mg/100 g body wt). Significant effect of clinoptilolite ($P < 0.05$). SD = 3.7.

clinoptilolite was administered simultaneously (630 mg/100 g body wt) with $(NH_4)_2CO_3$.

Experiment 3. The rise in portal blood ammonia concentration was inversely related to the level of clinoptilolite administered with $(NH_4)_2CO_3$ (Fig. 3) at 15 min after dosing. The dramatic rise from a basal level of 1.4 mg/dl to 16.4 mg/dl 15 min after $(NH_4)_2CO_3$ administration in the absence of clinoptilolite was lower with 630 mg clinoptilolite/100 g (10.0 mg/100 ml) and still lower (6.3 mg/dl) with 945 mg/100 g. After 30 min, the concentration had declined to 5.4, 9.1, and 3.5 mg/dl in rats given $(NH_4)_2CO_3$ alone, $(NH_4)_2CO_3$ + 630 mg clinoptilolite, and $(NH_4)_2CO_3$ + 1.5 times as much clinoptilolite, respectively. The concentration of ammonia in the portal blood of rats receiving the higher level of clinoptilolite was significantly less than that in rats in other treatment groups at 15 and at 30 min, but had not returned to the basal level of 1.4 mg/100 ml even after 30 min.

Experiment 4. Ingestion of feed containing 4% $(NH_4)_2CO_3$ increased the portal blood ammonia concentration with or without 5% clinoptilolite in the feed (Fig. 4). The presence of clinoptilolite in the basal diet without added $(NH_4)_2CO_3$ was associated with a lower portal vein ammonia concentration than in the absence of clinoptilolite. Feed consumption during the

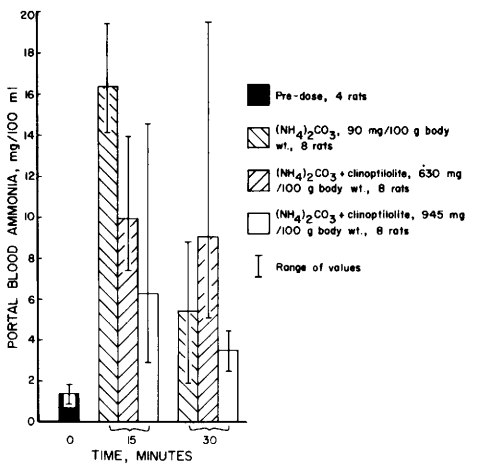

FIG. 3. Effect of level of zeolite on portal blood ammonia of weanling rats dosed orally with $(NH_4)_2CO_3$ (90 mg/100 g body wt). Significant effect of clinoptilolite ($P < 0.01$). SD = 3.9.

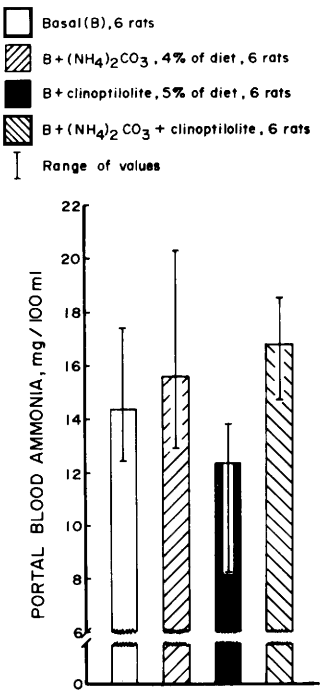

FIG. 4. Effect of zeolite in the diet on portal blood ammonia of weanling rats 30 min after a meal containing 4% $(NH_4)_2CO_3$. Significant effect of $(NH_4)_2CO_3$ ($P < 0.05$). SD = 2.2.

30-min feeding period preceding the test doses of $(NH_4)_2CO_3$ and clinoptilolite together and alone was similar (approximately 2 g) for all rats, with no apparent effect of diet on feed intake.

Discussion. The rise in the portal blood ammonia concentration in response to oral administration of $(NH_4)_2CO_3$ at a level of 45 to 90 mg/100 g body wt observed in the present study was considerably greater than that observed in peripheral systemic blood in pigs given 15 mg $(NH_4)_2CO_3$/100 g body wt intraperitoneally (12); peripheral blood ammonia of rats given orally 45 to 90 mg $(NH_4)_2CO_3$/100 g body wt in the present study (data not reported) rose to a similar value as that of pigs given 15 mg $(NH_4)_2CO_3$/100 g body wt intraperitoneally (12). Systemic blood glucose and lactate, although not reported here, were elevated slightly by ammonia administration in rats in the present study in agreement with earlier reports with rats (13–15) and pigs (12). The administration of 90 mg $(NH_4)_2CO_3$/100 g body wt induced hyperirritability and coma in approximately 10% of the rats in each experiment; rats that died in coma were discarded from the experiment, although portal blood obtained at death was not noticeably higher in ammonia than that observed in surviving rats. The incidence of coma in rats given clinoptilolite plus $(NH_4)_2CO_3$ appeared to be less, but the small number of observations does not permit a firm conclusion.

Clinoptilolite in all cases reduced the concentration of portal blood ammonia attained in response to $(NH_4)_2CO_3$ administration. The effect was greater at a clinoptilolite:$(NH_4)_2CO_3$ ratio of 10.5 than at a ratio of 7, both when 45 and 90 mg $(NH_4)_2CO_3$/100 g body wt were given. The ion-exchange capacity of clinoptilolite of about 2.5 mEq/g suggests that a clinoptilolite:$(NH_4)_2CO_3$ ratio greater than 7 but less than 10.5 is sufficient to bind the ammonia in the gastrointestinal tract and prevent its absorption into the portal blood. The failure to inhibit the rise in portal blood ammonia completely after $(NH_4)_2CO_3$ administration indicates that binding was not complete.

The failure of dietary clinoptilolite in experiment 4 to prevent a rise in portal blood ammonia concentration of rats given a meal containing 4% $(NH_4)_2CO_3$ also indicates the incomplete binding of dietary ammonia by clinoptilolite. However, the lower ammonia concentration in the portal blood of rats fed a diet containing 5% clinoptilolite than in those fed the same basal diet without clinoptilolite indicates that some of the ammonia released by deamination of ingested protein and urea hydrolysis was bound by the clinoptilolite. The clinoptilolite:$(NH_4)_2CO_3$ ratio of 1.25 selected for the diet supplemented with both compounds may have been too low to demonstrate an ion-exchange effect at the level of 4% added $(NH_4)_2CO_3$. Holtzman and Visek (16) obtained a growth response in rats in experiments employing an ion-exchange resin capable of binding ammonia in the gastrointestinal tract. It has been suggested (17) that ion-exchange resins and antibiotics inhibit the production of ammonia or bind it, as has been suggested (18) for bentonite.

The data from the present experiments show clearly that clinoptilolite binds free ammonia in the gastrointestinal tract of rats to a degree sufficient to reduce the concentration of portal vein ammonia significantly in rats dosed orally with ammonia. The beneficial effects of clinoptilolite and other zeolites reported in the literature on efficiency of feed utilization of animals may therefore be directly related to their capacity to bind ammonia in the intestinal tract. The results provide, for the first time, a suggested physiological explanation for the improved growth and efficiency of feed utilization (1) and increased protein and energy retention (19, 20) reported in animals fed diets supplemented with clinoptilolite. Further work is needed to determine the effect of clinoptilolite from other geographic sources and of different particle size on ammonia binding capacity in the gastrointestinal tract. Mumpton and Fishman (1) reported that some clinoptilolites are more silicified than others and that mixtures of clinoptilolite with other zeolites occur.

We thank Robert Lee, Bruce Larson, and Susan Reece for technical assistance and Peggy Green for stenographic work.

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Received September 1, 1980. P.S.E.B.M. 1981, Vol. 166.