Role of Intestinal Bacteria in Aromatization of Quinic Acid in Man and Guinea Pig.* (25860)

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Conversion of non-aromatic cyclic hydrocarbons to aromatic substances by mammals is poorly understood. Aromatization of cyclohexane-carboxylic acid and a small number of related compounds was observed by Friedman(1) and by Bernhard(2,3), and confirmed by Dickens(4). Beer *et al.*(5) demonstrated that slices of liver and kidney, as well as the intact animal, could aromatize several cyclohexanecarboxylic acids. Recently it has been observed that hippuric acid is bacteriostatic to common bacterial pathogens of the urinary tract(6). The major recognized source of urinary hippuric acid is benzoic acid. However, not all of the hippuric acid found in urine can be accounted for on the basis of benzoic acid content of ordinary diets. Quinic acid (1,3,4,5 tetrahydroxy*cyclo*hexanecarboxylic acid), which is present in many fruits and vegetables, has been suggested as a major non-aromatic dietary precursor to hippuric acid(7,8).

In man, Lauteman(9), Quick(10), Beer *et al.*(6), and Bernhard(11), have demonstrated conversion of ingested quinic acid to hippuric acid. In guinea pigs, Beer *et al.*(5) found little or no conversion of quinic acid to hippuric acid, whereas Bernhard(11) observed that guinea pigs excreted about 60% of ingested quinic acid as benzoic or hippuric acids. In experiments conducted by Beer *et al.*, in which little or no aromatization was observed, quinic acid was given subcutaneously. Davis (12) has demonstrated that coliform bacteria can aromatize quinic acid.

Material and methods. Healthy male volunteers ingested their usual diets. Their urine was refrigerated immediately after collection, and aliquots from 24 hour specimens were stored at –20°C. Stool specimens were obtained at least once daily. Male guinea pigs, weighing approximately 400 g, were maintained in pairs in metabolic cages, on diet of cabbage given *ad lib.* Bacterial multiplication in urine of guinea pigs was inhibited by adding 4 ml of 4N HCl to each container. Quinic acid was obtained from A. D. Mackay Inc. and for human studies was packed in gelatin capsules, each containing 0.5 g. For guinea pigs, quinic acid was dissolved in water, adjusted to pH 7.4 by addition of small amounts of alkali, and administered as single dose of 7 ml by stomach tube or intraperitoneal injection. Neomycin† was given as 0.5 g tablets. Hippuric acid in urine was estimated by paper chromatographic method of Gaffney(13), as modified by Armstrong (14). Organic acids were determined by modification of the method of Van Slyke and Palmer(6). For bacteriologic examination of the stool, aliquots of each specimen were suspended in 10 times their volume of broth, and shaken to make an even suspension. Serial dilutions were then made in nutrient broth, plated in meat infusion agar and colony counts made after aerobic incubation at 37°C for 24 hours. A loopful of each specimen was spread over surface of sheep-blood agar plate for determinative purposes.

Results. Each of 2 volunteers ingested 6 g of quinic acid daily for 3 days, after 2 days of control observation. On fourth day of administration of quinic acid, neomycin was added and given until stool cultures had fallen to 10^3 colonies/g or below. This required 3-4 days in each of the 2 volunteers studied. The third volunteer ingested quinic acid for 3 days, then after an interval of 9 days took quinic acid and neomycin for 4 days. Fig. 1 gives results for one volunteer. Data in the other 2 were comparable. In all

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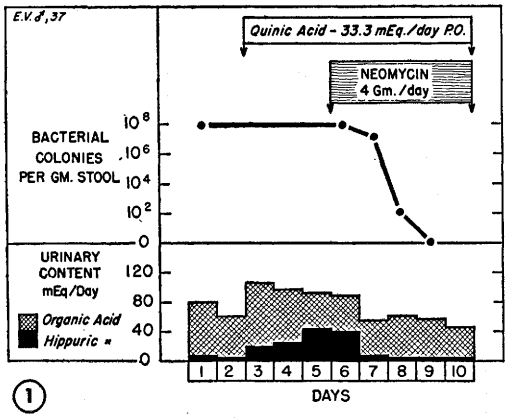

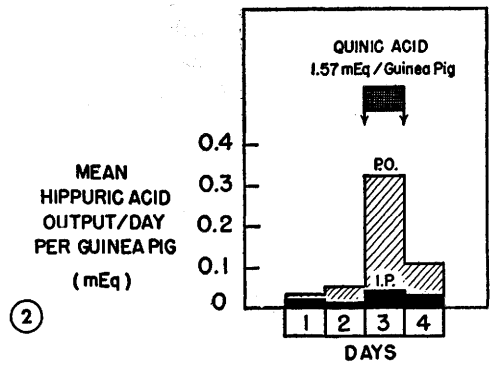

FIG. 1. Effect of neomycin on conversion of quinic to hippuric acid by intestinal flora in man.
FIG. 2. Effect of oral and parenteral administration of quinic acid on hippuric acid excretion.

3 ingestion of quinic acid was followed by recovery of about 70% of administered acid as hippuric acid in urine. Addition of neomycin to the diet led to prompt fall in excretion of hippuric acid to control levels, or to levels below control values, despite continued ingestion of quinic acid.

In the guinea pigs, oral administration of quinic acid also led to prompt excretion of amounts of hippuric acid sufficient to account for 30% of ingested quinic acid (Fig. 2). When quinic acid was given intraperitoneally no significant increase in excretion of hippuric acid was found, but organic acid content of urine rose in correspondence with amount of quinic acid injected.

Discussion. That inhibition of bacterial multiplication in intestinal tract is accompanied by failure of this conversion to occur argues strongly that aromatization is accomplished by the intestinal bacteria. Although the possibility of direct action of neomycin on absorption or aromatization cannot be completely eliminated, it seems unlikely. The malabsorption syndrome occasionalliy reported after administration of neomycin(15) occurs with larger doses, given for longer period, and does not lead to complete absence of absorption from the intestinal tract. The volunteers experienced slight epigastric discomfort but no changes in frequency or character of the stool suggestive of malabsorption. Furthermore, the clear differences in response to intraperitoneal as opposed to oral administration of quinic acid in the guinea pig indicate that only the intestinal bacteria achieve aromatization of quinic acid in this animal species.

Summary and conclusions. When quinic acid is given orally to man and to guinea pigs, it is aromatized and appears in urine as hippuric acid. Neomycin, in doses sufficient to inhibit bacterial multiplication in the intestinal tract, prevents conversion of quinic to hippuric acid in man. When quinic acid is given parenterally to guinea pigs, it is not converted to hippuric acid. It is concluded that aromatization of quinic acid in man and in guinea pig is achieved by intestinal bacteria.

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Demonstration of Thiosemicarbazide-Induced Convulsions in Rats with Elevated Brain Levels of γ-Aminobutyric Acid.* (25861)

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The brain and spinal cord of vertebrate organisms contain high levels of GABA† (1). Physiological experiments have shown GABA to have inhibitory properties in invertebrate and vertebrate nervous systems (2,3). When TSC was injected into rats, it lowered cerebral levels of GABA probably by preferential inhibition of glutamic acid decarboxylase and increased susceptibility of these animals to convulsions (4). When NH_2OH was injected into rats, cats or monkeys, it elevated cerebral levels of GABA in all 3 species (5) presumably through inhibition of γ-amino-butyric acid-α-ketoglutaric acid transaminase (6). At the same time it was noted that rats were protected against Metrazol-induced convulsions after NH_2OH administration and that in the cat and monkey sensitivity of the cortex to electrical stimulation was decreased (7,8). These and similar observations suggested that certain types of convulsions and resistance to them may be related to levels of GABA in the brain of mammals (9,10). The experiments presented here demonstrate that the physiological effects of TSC and NH_2OH cannot be attributed solely to their effect upon the mechanisms which regulate synthesis and degradation of GABA and that a greatly elevated level of GABA can exist in most brain areas of rats undergoing thiosemicarbazide-induced convulsions.

Method. Twenty-eight male Sprague-Dawley rats weighing 190 to 210 g each, were selected for this experiment and divided into 6 groups. Rats in 5 groups were injected intraperitoneally either with $NH_2OH \cdot HCl$ (75 mg/kg neutralized) or with TSC (20 mg/kg) or with both at varying time intervals as indicated in Table I. Animals injected with $NH_2OH \cdot HCl$ only, were decapitated at 90 to 120 minutes after injection. *All* animals receiving TSC had convulsions within 90 to 135 minutes after injection. Pretreatment with NH_2OH tended to shorten the time period between injection of TSC and onset of convulsions. All TSC-treated animals were sacrificed after showing one maximal tonic extensor convulsion. Nine areas were removed from each brain and analyzed for content of GABA by an enzymatic procedure described previously (5).

Results. TSC alone lowered levels of GABA while NH_2OH alone elevated them in all areas (Table I), in agreement with previous observations (11,5).

In the 3 groups of animals receiving both TSC and NH_2OH, levels of GABA were significantly elevated in most of the areas examined. Several areas showed normal levels, but none were depressed at time of maximal convulsion. In comparing the results of the 3 groups of rats receiving both TSC and NH_2OH, it was found that level of GABA was elevated to a greater extent in the group receiving NH_2OH prior to TSC. This effect was especially noticeable in the colliculi, diencephalon and midbrain tegmentum, areas of brain with the highest levels of glutamic acid decarboxylase (Baxter and Roberts, unpublished).

Discussion. The above results do not ex-

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† Abbreviations: GABA: γ-aminobutyric acid; TSC: thiosemicarbazide; NH_2OH: hydroxylamine.