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# Effect of Enterectomy on Synthesis of Niacin in the Rat.*

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The limited interchangeability of tryptophan and niacin in the nutrition of experimental animals[1] receiving low protein rations has indicated the formation of niacin from this amino acid.[2-4] This synthesis through the intermediate, kynurenine, has been shown to occur in *Neurospora*.[5] Investigations of Ellinger *et al.*,[6] and the marked effect of the character of the carbohydrate on the growth of rats receiving a niacin-low diet containing 9% casein[1] suggested a possible transformation of tryptophan to niacin or its derivatives by intestinal bacteria. The chief arguments against this site of synthesis were the prompt excretion of niacin derivatives in the urine following parenteral administration of tryptophan[7] and more recently the evidence that injection of free tryptophan into the egg results in increased niacin in the chick embryo.[8] A study of the effect of removal of the major portion of the gastro-intestinal tract on the capacity of the animal to form niacin derivatives and excrete them in the urine appeared to be a direct and plausible approach to this problem.

Surgical procedure and ease of catheterization for collection of urine specimens for short periods made the dog preferable to the rat. It was found, however, that the responses to tryptophan in the dog were small compared to those in the rat. N'Methyl nicotinamide was elevated 2-4 fold following the administration of a large dose of tryptophan to the dog, but the acetone-fluorometric method[9] was not suitable in our hands for accurate determination of this metabolite. The amount present was small necessitating the use of large samples, which resulted in high blanks and poor recoveries. Small increases were noted in the nicotinic acid values for dog urine following trytophan administration. The largest increase was noted in the niacin released by acid hydrolysis,[8] but the increase was never more than 100%. Increases up to 100 fold were found in this fraction in rat urine following administration of L-tryptophan either orally or parenterally. The dog appears to have a limited capacity for "converting" tryptophan to niacin derivatives, which is in agreement with the finding that black tongue can be produced with rations containing 19% casein.[10]

*Experimental.* Male, Sprague-Dawley rats weighing 235-360 g were employed. After receiving stock ration for several weeks, the

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TABLE I.
Effect of Enterectomy on Excretion of Nicotinic Acid and Its Derivatives by Rats Following Tryptophan Administration.

| | Urinary excretion, μg per 24 hr per 100 g body weight | | | | | |
|---|---|---|---|---|---|---|
| | Free nicotinic acid | | Total nicotinic acid following acid hydrolysis | | N'methyl nicotinamide | |
| | Avg | Range | Avg | Range | Avg | Range |
| Enterectomized rats | | | | | | |
| A. Amigen | 3.7 | 1.08- 8.1 | 8.1 | 2.24- 14.7 | 110 | 19- 240 |
| B. Amigen + *l*-tryptophan | 27 | 5.9 - 91 | 390 | 55 - 620 | 1300 | 250-3800 |
| Intact rats | | | | | | |
| C. Amigen | 7.5 | 7.3 - 7.8 | 15.1 | 12.7 - 16.7 | 120 | 41- 270 |
| D. Amigen + *l*-tryptophan | 71 | 42 -100 | 1570 | 1270 -2020 | 1090 | 300-1700 |

rats were fasted for 24 hours before operating. The gastro-intestinal tracts from the duodenum to the anus were removed from half of the animals, and mock surgery was performed on the remainder. Morphine and light ether anaesthesia were used. After ligating and severing the superior and inferior mesenteric arteries, ligatures were placed on the small intestine, one approximately four inches from the pylorus, just below the pancreas, and another as near the rectum as possible. The gastrointestinal tract between these ligatures was removed by sectioning the gut and all attachments. Animals so treated lived from one to 4 days, when nourished by subcutaneous alimentation as described below. Secretory accumulations were removed from the stomach at 4 hour intervals with a catheter tube and syringe.

The mock surgery on the control animals resembled the enterectomy in that the viscera were handled in the same manner and then replaced intact. The medial incisions were closed with gut sutures for the muscle wall and silk thread for the skin.

The rats were then divided into 4 groups as follows: A. Enterectomized control (4 animals), B. Enterectomized plus added tryptophan (5 animals), C. Intact control (3 animals), and D. Intact animals receiving added tryptophan (3 animals). Within 1-3 hours after surgery the rats were placed in individual metabolism cages, and each received 5 ml of a sterile, modified Amigen.‡ This subcutaneous alimentation was repeated at 4 hour intervals during the entire urine collection period. The control animals in Group A and C received Amigen supplemented with 0.3% sucrose and 0.3% sodium chloride. Groups B and D received the same preparation containing 10 mg of L-tryptophan per ml. During the 24 hour period a total of 6 injections were given for a total dosage of 30 ml for each rat. Autopsy following the experiment showed that the solution was completely absorbed from beneath the skin of the back and neck of each animal.

At the end of the 24 hour collection period the urines were made to volume, filtered, and stored at 5°C under toluene until analyzed. N'Methyl nicotinamide was determined by the acetone-fluorometric method.[9] While this method gave reproducible results in previous studies with urine from normal rats, the results were more variable with these urines, particularly when large quantities of tryptophan were given.

Another portion of each urine sample was neutralized, diluted and analyzed for nicotinic acid by the microbiological assay.[11] The value obtained was termed "free" niacin. A third portion of the urine was hydrolyzed by autoclaving for one hour at 15 lb pressure with an equal volume of 2 N HCl. After neutralizing, "total" niacin was determined microbiologically.[11] The identity and signifi-

‡ Mead Johnson preparation containing 5% enzymatic digest of casein and 5% glucose.

[11] Krehl, W. A., Strong, F. M., and Elvehjem, C. A., *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 471.

cance of the substance(s) which gives rise to niacin activity for *L. arabinosus* during acid hydrolysis is unknown.[3] The activity of a number of niacin related compounds for *L. arabinosus* have been reviewed by Snell.[12]

*Results and discussion.* The N'methyl nicotinamide content of the urines (Table I), though variable, was approximately 10 times as high for the rats receiving tryptophan. Enterectomy had no significant effect on this response to tryptophan administration. The average daily excretion of "free" niacin was also increased following tryptophan administration from 3.7 to 27 $\mu$g per 100 g of body weight for the enterectomized rats and from 7.5 to 71 $\mu$g for the intact animals. Except for one animal whose "free" niacin excretion was 91 $\mu$g all of the rats in Group B excreted less than 17 $\mu$g, while the intact animals in Group D excreted 42 to 100 $\mu$g.

The "total" niacin values increased 100 fold in the intact animals and approximately 50 fold in the enterectomized rats. It should be pointed out that the Amigen administered contains a small amount of tryptophan. The added L-tryptophan, however, resulted in a 15 to 20 fold increase in the total tryptophan intake. At the low tryptophan level (Groups A and C) the N'methyl nicotinamide excretion was much greater than total nicotinic acid. The effect of massive doses of tryptophan was most marked in the latter fraction, resulting in a greater excretion of "total" niacin than N'methyl nicotinamide in Group D. Less than 1% of the administered tryptophan appeared in the urine as the end products measured.

It is evident that the increase in "free" and "total" niacin in the urine following tryptophan administration is somewhat less marked in the enterectomized animals than in intact controls. Whether this difference is a result of the action of bacteria, possibly on tryptophan which has passed into the gut, or of removal of intestinal tissue which participates in the formation of these metabolites, or of the effect of surgical trauma on the metabolic activity of other tissues is not known. It is evident, however, that significant though slightly limited formation of nicotinic acid end products occurred in the enterectomized animals. It seems unlikely that the microbial population in the stomach and a few inches of the intestine could contribute much to urinary nicotinic acid derivatives. Some growth did occur, however, since the stomach contents had a pH of 8-8.5, and there was evidence of putrefactive changes after 2-3 days. It is interesting that enterectomy did not affect the N'methyl nicotinamide excretion, since it has been found to be synthesized in the liver of the rat.[13] The data presented suggest that the tissues, and not the intestinal microflora are the primary sites of formation of these compounds in response to the administration of large amounts of tryptophan. The possibility of the formation of small, though physiologically significant, amounts of niacin derivatives in the tract has not been eliminated.

*Summary.* 1. The effect of removal of the intestinal tract below the pancreas on the ability of the rat to form niacin derivatives in response to tryptophan administered subcutaneously has been determined.

2. The response in urinary N'methyl nicotinamide was not affected by enterectomy.

3. "Free" and "total" urinary niacin as measured by microbiological assay before and after acid hydrolysis respectively were increased in enterectomized animals although the increase was not as great as in intact animals.

4. The results suggest that the tissues are the primary site of the formation of niacin derivatives in the rat in response to large doses of tryptophan.

[12] Snell, E. E., *Biological Symposia*, 1947, **12**, 183.

[13] Perlzweig, W. A., Bernheim, M. L. C., and Bernheim, F., *J. Biol. Chem.*, 1943, **150**, 401.