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Influence of Intestinal Bacteria on Synthesis of Nicotinic Acid from Tryptophan.

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Several investigators have presented suggestive but not conclusive evidence that intestinal bacteria play an important role in synthesizing nicotinic acid, making this vitamin available to the host. This subject has been reviewed elsewhere.[1]

Since it has been shown that tryptophan can substitute for nicotinic acid in the diet of most species,[2-7] and since tryptophan acts by increasing the synthesis of nicotinic acid,[8-16] it has been a natural assumption that the synthesis of nicotinic acid from tryptophan may involve the intestinal bacteria.

Ellinger and Abdel Kader[14] have reported that succinylsulfathiazole greatly reduced the urinary output of N'-methylnicotinamide when tryptophan was administered. From this and other evidence they concluded that the intestinal bacteria were involved in the conversion of tryptophan to nicotinic acid. However, Spector[12] in a somewhat similar type of experiment was led to the opposite conclusion. Junqueira and Schweigert[17] found that succinylsulfathiazole would not interfere with the conversion of tryptophan to nicotinic acid under certain conditions but would under others.

Dann and Handler[18] and Levy and Young[19] have shown that the bacteriologically sterile chick embryo can synthesize nicotinic acid. Schweigert *et al.*[20] have shown that tryptophan will increase the nicotinic acid content of the chick embryo. These experiments demonstrate that at least under certain conditions, nicotinic acid can be synthesized by the tissues independent of bacterial action.

It is the purpose of this communication to present evidence demonstrating that synthesis of nicotinic acid from tryptophan is not dependent on the intestinal bacteria in the rat.

Experimental methods. Male rats weighing approximately 250 g were maintained on a purified "nicotinic acid free" diet consisting of 12% casein, 81% sucrose, 3% corn oil, 4% minerals and the usual vitamins except nicotinic acid as described elsewhere.[10]

After at least one week on this diet, 24 hour urine specimens were collected from each rat and the basal output of N'-methly-

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4 Wooley, J. G., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 315.

5 Singal, S. A., Sydenstricker, V. P., and Littlejohn, Julia M., *J. Biol. Chem.*, 1948, **176**, 1051.

6 Schweigert, B. S., Pearson, P. B., and Wilkening, M. C., *Arch. Biochem.*, 1947, **12**, 139.

7 Lucke, R. W., McMillen, W. N., Thorp, F., Jr., and Tull, C., *J. Nutr.*, 1947, **33**, 251.

8 Singal, S. A., Sydenstricker, V. P., and Littlejohn, J. M., *J. Biol. Chem.*, 1946, **166**, 573; *ibid.*, 1947, **171**, 203.

9 Rosen, F., Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.*, 1946, **163**, 343.

10 Hundley, J. M., *J. Nutr.*, 1947, **34**, 253.

11 Schweigert, B. S., and Pearson, P. B., *J. Biol. Chem.*, 1947, **168**, 555.

12 Spector, H., *J. Biol. Chem.*, 1948, **173**, 659.

13 Sarrett, H. P., and Goldsmith, G. A., *J. Biol. Chem.*, 1947, **167**, 293.

14 Ellinger, P., and Abdel Kader, M. M., *Nature*, 1947, **160**, 675.

15 Perlzweig, W. A., Rosen, F., Levitas, N., and Robinson, J., *J. Biol. Chem.*, 1947, **167**, 511.

16 Heidelberger, C., Abraham, E. P., and Lepkovsky, S., *J. Biol. Chem.*, 1948, **176**, 1461.

17 Junqueira, P. B., and Schweigert, B. S., *J. Biol. Chem.*, 1948, **175**, 535.

18 Dann, W. J., and Handler, P., *J. Biol. Chem.*, 1941, **140**, 935.

19 Levy, M., and Young, N. F., *J. Biol. Chem.*, 1948, **176**, 185.

20 Schweigert, B. S., German, H. L., and Garber, M. J., *J. Biol. Chem.*, 1948, **174**, 383.

nicotinamide determined. Each rat was then given subcutaneously 100 mg l (–) tryptophan dissolved in 10 cc of 0.85% sodium chloride solution and the urinary N′-methylnicotinamide output in the following 24 hours determined. All solutions were sterilized by boiling.

The animals were then matched in control and experimental groups according to their weight and to the amount of N′-methylnicotinamide excreted in response to tryptophan. The control animals were subjected to the same surgical and other procedures as the experimental animals, except that they received only saline subcutaneously while the experimental animals received the same amount of saline containing the tryptophan. A rest period of at least one week was allowed between the test dose of tryptophan and the operative procedures.

In some of the animals the entire intestine, excluding the stomach, was removed. In the remainder, only the stomach was excised. Clean but not aseptic technic was observed. The bowel stumps were simply ligated. No effort was made to anastomose esophagus and duodenum in the gastrectomized rats. Intraperitoneal sodium pentobarbital (4.5 mg/100 g) was used for anesthesia.

Immediately following operation, each rat was given either saline or saline and tryptophan subcutaneously in the same amounts as used in the control period and the urinary N′-methylnicotinamide excretion in the following 24 hours determined. Neither water nor food was allowed during this period. At the end of the urine collection all animals were carefully autopsied. N′-methylnicotinamide was determined using the method of Huff and Perlzweig.[21]

Results. The mortality rates were rather high in all the operated animals. It was found early that the rats having their intestines removed but with the distally ligated stomach remaining, would universally develop a gastric ulcer, usually with perforation and peritonitis. 3 cc of aluminum hydroxide gel placed in the stomach by stomach tube immediately after operation, prevented the complication completely and was consequently used in all rats reported here, except of course, the gastrectomised group. Only rats surviving the 24 hour post-operative period in good condition and showing no evidence of peritonitis or hemorrhage were used.

The results in the various groups are summarized in Table I. On the basal diet alone the rats excreted 120 γ of N′-methylnicotinamide per 100 g of rat per day. 100 mg l(—) tryptophan caused a 10-fold increase in the same unoperated rats.

The operative procedure itself caused some increase in the basal excretion level, but the magnitude of the tryptophan response was so great that this was of no consequence.

In the group having all their intestine removed, the N′-methylnicotinamide output with saline alone was 246 γ per 100 g of rat per day; with tryptophan there was a 9-fold increase, the absolute response being considerably in excess of the response to tryptophan in the control period. This, we believe, demonstrates that the intestinal bacteria are not necessary for the synthesis of nicotinic acid from tryptophan.

Several investigators[22-24] have advanced evidence that the stomach is involved in some way in the nicotinic acid deficiency state. Accordingly, it seemed appropriate to determine whether the stomach might be necessary in converting tryptophan to nicotinic acid.

Gastrectomized rats (Table I) receiving saline only, excreted 182 γ of N′-methylnicotinamide per 100 g of rat per day while those receiving tryptophan showed a 10-fold increase, indicating that the stomach was not necessary for this process.

All of the rats, both in the control and operated periods, showed wide fluctuations in their N′-methylnicotinamide output as can be seen from the ranges listed in Table I. However, the output level seemed to be rather

[21] Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.*, 1947, **167**, 157.

[22] Sydenstricker, V. P., Armstrong, E. S., Derrick, C. J., and Kemp, P. J., *Am. J. Med. Sci.*, 1936, **192**, 1.

[23] Petri, S., Norgaard, F., Trautner, K., and Klaer, W., *Acta Med. Scand.*, 1944, **117**, 90.

[24] Gillman, T., and Gillman, J. J., *J. Am. Med. Assn.*, 1945, **129**, 12.

TABLE I.
Influence of Excision of Stomach and Intestine on Synthesis of Nicotinic Acid from Tryptophan.

No. of rats	Wt Avg	Wt Range g	Operative procedure	Treatment	N'-methylnicotinamide output μg/100 g of rat/24 hr Avg	N'-methylnicotinamide output μg/100 g of rat/24 hr Range
26	243	170-312	None	Basal diet only	120	20-413
25	244	170-312	"	100 mg L (—) tryptophan in 10 cc saline	1274	154-2821
6	257	231-312	Intestine removed	10 cc saline	246	41-512
8	247	218-310	" "	100 mg L (—) tryptophan in 10 cc saline	2156	457-4840
6	244	217-289	Stomach removed	10 cc saline	182	22-568
6	223	170-285	" "	100 mg L (—) tryptophan in 10 cc saline	1864	682-2976

characteristic for each rat, *i.e.*, if its output was low during the control period; it would also be low in the operated period and vice versa. This correlation also held when comparing the response to tryptophan in control and operated periods. Every rat* showed a significant increase in N'-methylnicotinamide output in response to tryptophan, and the response after operation was quantitatively similar to that before operation in each individual rat.

Discussion. While the experiments reported here seem to show clearly that intestinal bacteria are not required for the synthesis of nicotinic acid from tryptophan, they cannot be interpreted to mean that under normal conditions these bacteria may not produce some of the host's supply of this vitamin. It is well known that many of the intestinal bacteria can and do synthesize nicotinic acid. It remains to be proven, however, that any of this nicotinic acid is absorbed and used by the host. The experiments of Ellinger and co-workers[1] suggest that this may occur but some of their findings have not been confirmed by others.[25]

Conclusion. Rats deprived of their intestinal bacteria showed no impairment in their ability to convert tryptophan to N'-methylnicotinamide indicating that the synthesis of nicotinic acid from tryptophan does not occur in the gastrointestinal tract.

Since this paper was prepared for publication Henderson and Hankes (PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 26) have reported a study of the conversion of tryptophan to nicotinic acid using a technic similar to that described here. The results of these two studies are in essential agreement.

* We have observed an occasional rat in other experiments which will methylate neither nicotinic acid nor the nicotinic acid formed from tryptophan.

[25] Najjar, V. A., Holt, L. E., Jr., Johns, G. A., Mediary, G. C., and Fleischman, G., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 371.