Excretion of N^1-Methylnicotinamide and the 6-Pyridone of N^1-Methylnicotinamide in Urine of Human Subjects.* (20591)

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The major excretory metabolites of nicotinamide in human subjects are N^1-Methylnicotinamide (N^1-Me) and the 6-pyridone of N^1-Methylnicotinamide (pyridone)(1-4). After a test dose of nicotinamide, pyridone is excreted in larger quantity than N^1-Me and in persons receiving diets low in niacin and its precursors, pyridone excretion decreases more rapidly than N^1-Me. Subjects with pellagra, experimentally induced by diets low in niacin and tryptophan, excrete no detectable pyridone or only a trace of this metabolite, but continue to excrete a small amount of N^1-Me. Thus, pyridone appears to be the more important end product in nicotinamide metabolism(5). Holman and deLange(6) have studied the rate of N^1-Me and pyridone excretion during 24 hours following the ingestion of nicotinamide. The major increase in N^1-Me excretion occurred within the first 3-hour period and values fell to control levels after 9 hours. The rate of excretion of pyridone, however, was maximum during the second 3-hour period and decreased slowly toward the control level during the remainder of the 24 hours. There is a paucity of information concerning the quantitative excretion of pyridone and N^1-Me in subjects maintained on relatively constant diets of known composition. It is the purpose of this report to present data on the urinary excretion of these metabolites by human subjects on such diets and following the ingestion of a test dose of nicotinamide. Diets A and B used in this study were designed to provide minimal amounts of nicotinic acid and tryptophan. Addition of tryptophan to the diets has been shown to result in increased excretion of N^1-Me(7-11) and of pyridone(5,12) in man. In the present experiments, dietary tryptophan was maintained at a constant level, and the changes in the excretion of N^1-Me and pyridone are associated with the administration of nicotinamide.

Methods and materials. Adult human subjects of both sexes between 25 and 57 years of age, who were found to be essentially free of organic disease, and 2 subjects with pellegra were used in the study. They were maintained in a metabolism ward on standardized diets high in either corn (Diet A) or wheat (Diet B) as previously described(5). Analysis of the diets showed a daily intake ranging from 4.3 to 6.0 mg nicotinamide and 180 to 230 mg of tryptophan. In one series of experiments, a standardized diet of better nutritional value (Diet C) was used which supplied approximately 10 mg of nicotinamide and 1 g of tryptophan daily. Urine samples were collected in dark bottles containing 5 ml glacial acetic acid. A portion of each sample was neutralized (pH 6.9 $\pm$ 0.1) and frozen until analyzed. Fluorometric methods for the determination of N^1-Me(13) and pyridone(14) were modified for use in this laboratory. Creatinine determinations using alkaline picrate reagent and total nitrogen by the macro Kjeldahl procedure were performed to check completeness of urine collections. When oral supplements of nicotinamide were administered, they were given with breakfast and subjects were permitted to eat at regular mealtimes.

Results. Six normal subjects were maintained on Diet A and 3 normal subjects on Diet B for 2 to 4 days in order to obtain basal excretion values prior to supplementation. Following this, a 50 mg test dose of nicotinamide was administered and urines were collected for 24 hours. In 4 of the subjects, the

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TABLE I. Excretion of N^1-Methylnicotinamide and 6-Pyridone of N^1-Methylnicotinamide Following Ingestion of 50 mg Nicotinamide by Human Subjects.

Subject	No. of determinations	Diet	N^1-Methylnicotinamide (mg/day) Control	N^1-Methylnicotinamide (mg/day) Nicotinamide	6-Pyridone (mg/day) Control	6-Pyridone (mg/day) Nicotinamide
E.	1	Corn (Diet A)	3.7	10.5	4.9	35.5
K.	3	"	2.9	8.0	7.6	25.7
T.	1	"	4.3	12.9	4.6	36.0
O.	1	"	2.2	6.6	3.3	23.4
M.	3	"	3.1	7.3	10.0	22.7
C.	3	"	1.6	5.9	2.4	27.1
D.	2	Wheat (Diet B)	1.3	8.1	1.7	23.8
P.C.	1	"	2.4	6.8	5.0	32.1
L.	1	"	4.2	9.2	3.2	25.2
	Avg ± S.E.*		2.9 ± .36	8.4 ± .73	4.7 ± .91	28.1 ± 1.74

* S.E. (stand. error) $= \sqrt{\frac{\Sigma d^2}{N\,(N-1)}}$. This formula refers to all such data in this report.

TABLE II. Excretion of N^1-Methylnicotinamide and 6-Pyridone of N^1-Methylnicotinamide During the First 4 Hours and Subsequent 20 Hours Following Ingestion of 50 mg Nicotinamide by Human Subjects.

Subjects	No. of determinations	Diet	N^1-Methylnicotinamide (mg) 4 hr	N^1-Methylnicotinamide (mg) 20 hr	6-Pyridone (mg) 4 hr	6-Pyridone (mg) 20 hr
M.	6	Corn (Diet A)	4.2	3.4	11.5	15.7
K.	5	"	4.2	2.8	10.0	16.2
H.	1	"	3.6	5.5	6.5	19.6
C.	3	"	3.8	2.1	11.1	16.0
M.M.	1	"	1.7	2.0	6.7	15.1
D.	1	"	5.2	3.7	11.6	16.5
T.	1	"	6.7	6.2	12.0	24.0
B.	1	"	5.6	4.4	8.0	19.0
O.	1	"	3.3	3.3	8.4	14.9
H.	2	"	3.0	2.9	6.2	11.8
V.D.	2	Wheat (Diet B)	4.3	3.5	6.6	9.0
P.C.	1	"	3.2	3.5	13.4	18.7
	Avg ± S.E.		4.1 ± .37	3.6 ± .37	9.3 ± .71	16.4 ± 1.10

tests were carried out 2 or 3 times. The values obtained in these repeated tests agreed well and were averaged to obtain the values for the individual subjects (Table I). Average excretion on the basal diet was 2.9 mg of N^1-Me and 4.7 mg of pyridone per day. Following administration of a 50 mg test dose of nicotinamide there was a 4 to 8 mg increase in the excretion of N^1-Me (average 5.5 mg) and a 13 to 31 mg increase in the excretion of pyridone (average 23.4 mg) above basal levels. When calculated on an equivalent basis, the average excretion of N^1-Me represented 10%, and of pyridone, 39% of the nicotinamide test dose, during the 24-hour period.

The rate of excretion of N^1-Me and pyridone during the first 4 hours, and subsequent 20 hours of the 24-hour period following a 50 mg test dose of nicotinamide, was determined in 12 subjects maintained on Diets A and B (Table II). Data for 6 of these subjects were included in Table I. In some subjects more than one determination was carried out. The excretion of N^1-Me averaged 4.1 mg during the first 4 hours and 3.6 mg during the succeeding 20 hours; the excretion of pyridone was 9.3 mg during the first 4 hours and 16.4 mg during the following 20 hours. These data indicate that the administration of nicotinamide is followed by a rapid increase in the excretion of N^1-Me during the first 4 hours after the test dose. Excretion of pyridone,

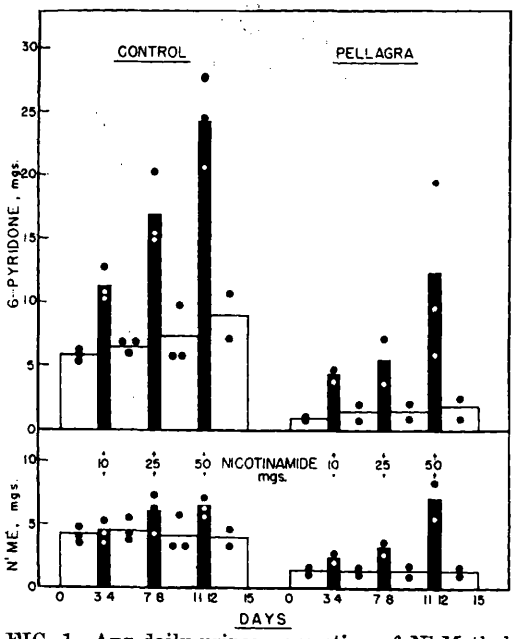

FIG. 1. Avg daily urinary excretion of N¹-Methylnicotinamide and 6-pyridone of N¹-Methylnicotinamide in 3 normal subjects and 2 subjects with pellagra on Diet C and following the administration of 10, 25 and 50 mg nicotinamide.

on the other hand, increases more slowly and smaller amounts are excreted in the initial 4 hours than in the subsequent 20 hours. The body stores of the subjects in the experiments appear to be lower than those previously found in 51 control subjects(15) who excreted 8.0 mg of N¹-Me in 4 hours following a 50 mg test dose of nicotinamide, as contrasted with 4.1 mg found in the present study. However, the excretion is similar to that found in 14 miscellaneous hospital patients who excreted 5.1 mg in 4 hours.

The excretion of N¹-Me and pyridone following graded doses of 10, 25 and 50 mg of nicotinamide was studied in 3 control female subjects and 2 female subjects with pellagra. The subjects were maintained on the basal diet (Diet C) throughout the test, allowing 3-day periods between successive test doses. This diet contained about twice as much nicotinamide and 5 times as much tryptophan as did Diets A or B. After each test dose, the 24-hour urine sample was collected and analyzed. The daily basal excretion of the control subjects on Diet C averaged 4.2 mg N¹-Me and 5.9 mg pyridone (Fig. 1). These basal values are significantly greater than those found for nine subjects on Diets A or B, who excreted 2.9 mg of N¹-Me and 4.7 mg of pyridone, daily (Table I). The subjects with pellagra, although maintained on Diet C, excreted only 1.3 mg of N¹-Me and 0.9 mg of pyridone. This excretion is much less than that of control subjects on any of the basal diets which were very low in nicotinamide and tryptophan.

The average excretion of N¹-Me by the control subjects following a 10 mg test dose of nicotinamide increased only slightly above the basal level, but that of pyridone increased by almost 5 mg (Fig. 1). Following doses of 25 and 50 mg, the excretion of both metabolites increased significantly above basal levels. The pyridone excretion, however, increased more than did N¹-Me. Subjects with pellagra excreted considerably less N¹-Me and pyridone following a 10 or 25 mg dose of nicotinamide than did control subjects. Following a 50 mg dose, excretion of N¹-Me was similar in the 2 groups while excretion of pyridone was lower in one pellagrin, and essentially the same as the control subjects in the other. In the latter subject, pellagra was relatively mild. These findings suggest that the 50 mg dose of nicotinamide may be too large to be useful in detecting borderline cases of niacin deficiency. Determinations of N¹-Me and pyridone excretion on standard diets and following a 10 or 25 mg dose of nicotinamide, however, appear to be valuable procedures in evaluating niacin nutrition.

It may be noted that following administration of graded doses of nicotinamide, the basal excretion of pyridone increased slightly while that of N¹-Me tended to remain constant. With large doses of nicotinamide, there is probably an increase in pyridone excretion beyond the first 24-hour period, which is reflected in the values given for the 3-day basal periods.

Summary. Essentially normal subjects maintained for short periods on diets low in nicotinamide and tryptophan excrete approximately 3 mg of N¹-Me and 5 mg of pyridone per day. Following an oral 50 mg test dose of nicotinamide, the average increase in excretion of N¹-Me is 5.5 mg and that of pyridone, 23.4 mg. The excretion of N¹-Me fol-

lowing the test dose rises rapidly during the first 4 hours and decreases to basal levels within 24 hours; pyridone is excreted more slowly the first few hours but appears in large amounts later in the day. After graded doses of nicotinamide, the excretion of pyridone increases more rapidly than does that of N^1-Me. In subjects with pellagra, the excretion of these metabolites is lower both on standard diets and following administration of small doses of nicotinamide. These data suggest procedures that may be of use in evaluating niacin nutrition.

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Observations on Antibiotic Treatment of Bacterial Infections of Cortisone-Treated Mice. (20592)

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Numerous reports on the action of cortisone in promoting progression of various types of infection in man and in experimental animals have appeared in recent years, and in addition, observations on alteration of cellular response to various inflammatory stimuli have been described(1). In previous studies(2) in which *Mycobacterium tuberculosis* infection was induced in mice by the intraperitoneal route, it was observed that mice treated with large doses of isoniazid and cortisone showed much better therapeutic response than did mice treated with isoniazid alone Mice treated with large doses of cortisone and streptomycin did not give such a good response. The work was extended to gain further information on the relationship between invading bacteria and the defense mechanisms of the host; studies have been made in which experimental infections induced in mice by other bacteria were treated with appropriate antibiotics and with or without cortisone.

Methods. CF_1 male mice (18-22 g) were used in all experiments. Mice were infected by the intraperitoneal injection of cultures which had been incubated at 37°C for 24 hours. The strain of streptococcus used in most experiments (*S. zooepidemicus*—Group C) was originally isolated from a casaeus lymphnode of a guinea pig and was maintained on blood agar slants. The culture of *Streptococcus haemolyticus* (Group A) used was obtained through the courtesy of Dr. Fred B. Traub. Cultures for infecting mice were prepared in