AN ABSTRACT OF THE THESIS OF

Ien-Lan Wei   for the degree of   Master of Science

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Title: Bioavailability of Niacin from Tuna Fish, Peanut Butter and

        Whole Wheat Bread

Abstract approved: _____
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The bioavailability of niacin from tuna fish, peanut butter
and whole wheat bread was estimated in eight men aged 21 to 30 years.
Following a 10-day adjustment period, each test food, which was add-
ed to a constant basal diet, was fed for 14 days; the order in which
these three foods were eaten was randomized. Of the total dietary
niacin intake, tuna fish supplied 68 percent of the preformed nia-
cin and 35 percent of the tryptophan, peanut butter supplied 70 per-
cent of preformed niacin and 47 percent of the tryptophan intake,
while whole wheat bread supplied only 34 percent of preformed nia-
cin and 23 percent of tryptophan intake.   The bioavailability of
niacin was assessed by comparing the total intake of niacin equiva-
lents to the total excretion of N'-methylnicotinamide (N'-Me) and

N'-methyl-2-pyridone-5-carboxamide (2-pyridone) in 24-hour urine specimens collected on days 12 and 14 of each experimental period. The differences in bioavailability among three test foods were statistically significant ($p < 0.05$). Expressed as percent of total niacin intake, the bioavailability of niacin in tuna fish, peanut butter and whole wheat bread was 60 ± 7, 28 ± 6 and 36 ± 13, respectively. The results suggest that the niacin in tuna fish is readily available, while niacin in peanut butter and whole wheat bread are inadequately available for humans. These results are in agreement with other studies on niacin bioavailability carried out in humans and rats.

Bioavailability of Niacin from Tuna Fish,
Peanut Butter and Whole Wheat Bread

by

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INTRODUCTION

Niacin is the generic term for nicotinic acid and nicotinamide.
Niacin is a component of two coenzymes, nicotinamide adenine di-
nucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate
($NADP^+$). The major roles of NAD^+ and $NADP^+$ are in glycolysis, fat
synthesis and cellular respiration. Nicotinic acid is found pri-
marily in plant foods, while nicotinamide is found mainly in
animal tissues [1].

The amino acid tryptophan is converted to niacin in the liver
[2]. The term "niacin equivalent" was introduced to calculate the
combined effect of dietary niacin and tryptophan in satisfying the
requirements for this vitamin. It is accepted that 60 mg of trypto-
phan is equivalent to one mg of niacin [3]. However, this relation-
ship varies from person to person [4,5].

The richest sources of preformed niacin are animal products,
for instance, liver, kidney, meat, poultry and fish. Milk and
eggs are low in preformed niacin, but because they are high in
tryptophan they contribute considerable amounts of niacin to the
diet [6,7]. Cereals contain appreciable amounts of preformed
niacin, but they are considered poor sources because niacin is in a
bound form [8,9,10]. Ghosh, Sarkar and Guha [1] reported that 85
to 90 percent of the total niacin in cereals and about 40 percent

of the total niacin in peanuts are bound. Measuring bioavailability by rat assay, Carter and Carpenter [11] reported less than 40 percent bioavailability of niacin in cooked wheat, corn, barley, rice and potatoes. For peanut flour, the bioavailability of total niacin was 46 percent.

The purpose of the research reported in this thesis is to compare in men the bioavailability of niacin from an animal source and two plant products. Bioavailability of niacin was determined by measuring two niacin metabolites, N'-Me and 2-pyridone, in urine. Since these metabolites reflect niacin intake and tryptophan conversion to niacin, we assumed that the bioavailability of niacin can be determined by measuring these two urinary metabolites.

REVIEW OF LITERATURE

Chemical Properties of Niacin

There are two forms of niacin.[1] Nicotinic acid, or pyridine-3-carboxylic acid, is a non-hygroscopic, stable, white crystalline solid. Nicotinamide, also called niacinamide or niotinic acid amide, is the amide of pyridine-3-carboxylic acid. It is a white, crystalline, odorless powder. The chemical structures of these compounds are shown in Figure 1. Niacin is the most stable of the B-complex vitamins. Because it is water-soluble, some niacin may be lost in cooking water and in meat drippings. Freezing, dehydration, canning and food storage result in little destruction of niacin [12].

The major metabolites of niacin excreted in urine are N'-methylnicotinamide (N'-Me) and N'-methyl-2-pyridone-5-carboxamide (2-pyridone), which are also shown in Figure 1. Two-pyridone is an oxidation product of the N'-Me. The latter forms a strongly fluorescent compound on treatment with alkali [13].

Physiological Function

Niacin is a component of the two coenzymes, nicotinamide adenine dinucleotide (NAD$^+$) and nicotinamide adenine dinucleotide

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[1]In the United States the custom is to use "niacin" as a generic term for both nicotinic acid and nicotinamide. This practice will be followed in this thesis unless specified otherwise.
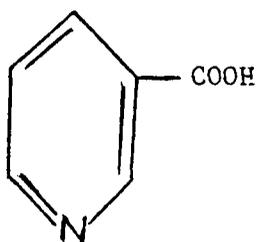
Nicotinic acid

Nicotinamide

N'-Methylnicotinamide

(N'-Me)
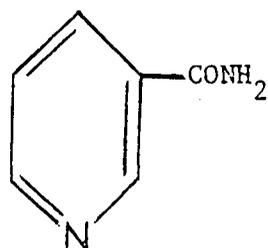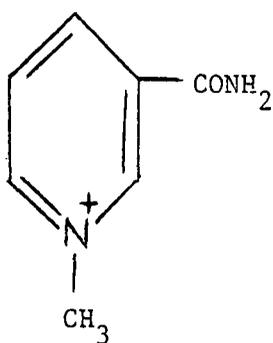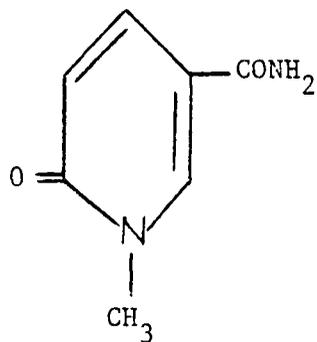
N'-Methyl-2-pyridone-5-carboxamide

(2-pyridone)

FIGURE 1. Formulas of nicotinic acid, nicotinamide,
N'-Methylnicotinamide and N'-Methyl-2-
pyridone-5-carboxamide

phosphate (NADP$^+$). These structures are shown in Figure 2. In
animal cells there is usually more NAD$^+$ than NADP$^+$. In the liver
about 60 percent of the total NAD$^+$ is present in the mitochondria
and the remainder is in the cytoplasm. The NADP$^+$ content of cells
is proportional to their biosynthetic activity. NAD$^+$ and NADP$^+$
function mainly as coenzymes in dehydrogenase reactions. In
general, these pyridine nucleotides are bound relatively loosely
to the dehydrogenase protein during the catalytic cycle and, there-
fore, serve more as substrates than as prosthetic groups. NAD$^+$ and
NADP$^+$ act as election acceptors during the enzymatic removal of
hydrogen atoms from specific substrate molecules. One hydrogen
atom from the substrate is transferred as a hydride ion to the
nicotinamide portion of the oxidized forms of these coenzymes,
NAD$^+$ and NADP$^+$, to yield the reduced coenzymes NADH and NADPH; the
other hydrogen atom from the substrate becomes a hydrogen ion [14].

NAD-linked dehydrogenases serve primarily in cellular respira-
tion and in the transfer of electrons from substrates toward
oxygen, e.g., in glycolysis and fatty acid degradation. NADP-
linked dehydrogenases serve primarily to transfer electrons from
intermediates of catabolism to those of biosynthesis, e.g., from
pentose phosphate pathway to fatty acid synthesis [15].

Historical Perspective

In 1867, Huber first prepared nicotinic acid as a pure sub-
stance by oxidizing nicotine. Later, Funk [16,17] isolated
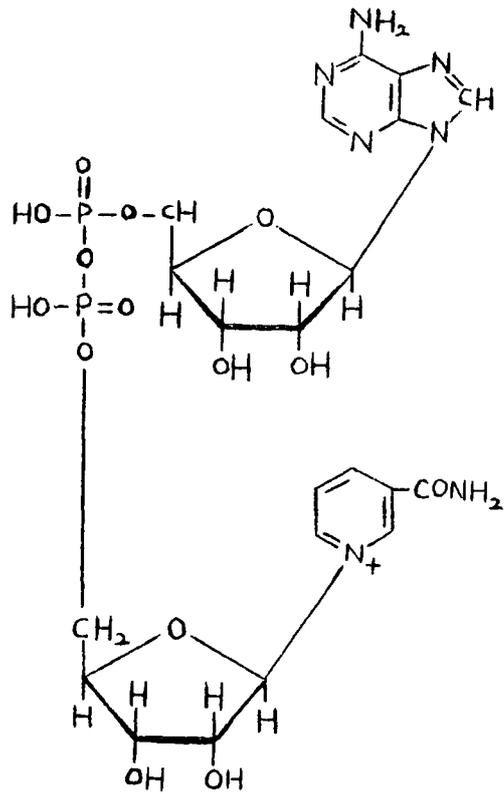
Nicotinamide adenine dinucleotide
(NAD^+)
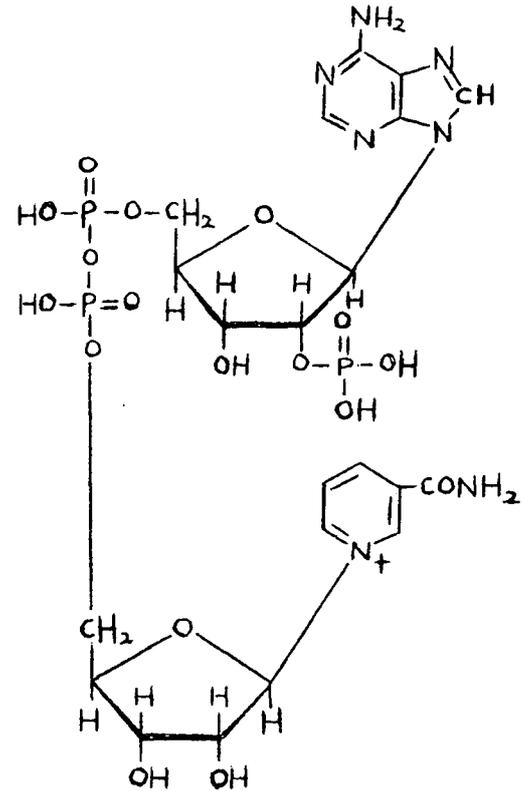
Nicotinamide adenine dinucleotide phosphate
($NADP^+$)

FIGURE 2. Coenzymes nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$).

nicotinic acid from rice polishings and yeast. At that time, Funk
was attempting to isolate an anti-beriberi factor, but found that
nicotinic acid had no effect in curing this condition. He did not
suspect that he had isolated a vitamin that was able to cure
pellagra, a different deficiency disease.

The history of pellagra was reviewed recently by Roe [18].
Pellagra, recognized as a disease of maize-eating populations, was
first observed in Europe during the eighteenth century. Caspar
Casal, a Spanish physician, found a strange and terrible new ail-
ment among poor peasants of Asturias. The sick told him that this
disease was "Mal de la rasa" because of the red discoloration of
their skin. The term pellagra, which had been used by Italian
peasants, was published by Dr. Franscio Frapolli in 1771. Many
people suffered from the physical ailments and mental disturbances
of this malady when pellagra spread rapidly across Europe in the
nineteenth century. In the early part of the twentieth century
pellagra was prevalent in southern United States. The early
symptoms of the disease are weakness, anorexia and indigestion.
These are followed by the three D's: dermatitis, diarrhea and
dementia [18].

For a long time, pellagra was believed to be an infectious
disease. Starting in 1916, Goldberger carried out a series of
studies to determine whether or not this disease was caused by
infection or by a dietary deficiency. He noticed that canine
black tongue was the analogue of pellagra in man. This observation

led to the use of dogs as an experimental model in the intensive study of food factors which could prevent or cure both pellagra and canine black tongue [18].

Goldberger and his co-workers introduced the use of yeast and liver extracts in the treatment of pellagra. Large amounts, however, had to be administered to make this form of treatment successful. Attempts to isolate the pellagra-preventing factor led to observation that this factor in liver and yeast was heat stable, and soluble in water and alcohol [18].

Finally in 1937, Elvehjem isolated nicotinamide from a liver extract, and demonstrated that it and crystalline nicotinic acid cured canine black tongue. Soon after this important report, nicotinic acid and nicotinamide were used widely for the treatment of human pellagra. It has been accepted that pellagra is a disease of niacin deficiency [18].

Metabolic Pathway of Tryptophan to Niacin

In 1945, Krehl, Teply and Elvehjem [19] found that niacin or casein corrected the growth depression in rats fed niacin-free diets high in maize. That same year Krehl and his co-workers discovered that protein or tryptophan, in particular, had a profound effect on the niacin requirement of rats [20]. These discoveries greatly stimulated the study of tryptophan conversion to niacin.

That tryptophan is a niacin precursor was soon established in

animals [21,22] and humans [23]. In human subjects, the urinary

excretion of niacin metabolites N'-Me and 2-pyridone increased

after the ingestion of L or DL-tryptophan [23]. The metabolic

pathway of the conversion of tryptophan to niacin (Fig. 3) was re-

viewed by Price, Brown and Yess [2].

Vivian, Chaloupka and Reynolds [24] investigated the metabolism

of tryptophan in human subjects receiving a semi-synthetic diet low

in niacin (2.5 mg) and supplemented with increasing levels of

L-tryptophan. Vivian and co-workers observed that tryptophan was

used first to maintain nitrogen balance and then for the synthesis

of blood pyridine nucleotides. Further increases in tryptophan (up

to 810 mg) resulted in an increased urinary excretion of the niacin

metabolites, N'-Me and 2-pyridone.

The term "niacin equivalent" was introduced to calculate the

combined effects of dietary niacin and tryptophan. It is generally

accepted that 1 mg of niacin is derived from 60 mg of tryptophan

[3]. This relationship, however, is variable; the range of tryp-

tophan converted to niacin varies widely among humans. Horwitt

et al. [4], who measured the amount of N'-Me excreted by 15 subjects

receiving different levels of nicotinamide and tryptophan, showed

that approximately 60 mg of tryptophan (range 46 to 86) were

equivalent to 1 mg of niacin. Goldsmith, Miller and Unglaub [5]

also found that the percentage of tryptophan converted to niacin

varied considerably among subjects: from 33.7 to 86.3 mg, with a

mean of 55.8 mg, of tryptophan were equivalent to 1 mg of niacin.

Tryptophan → Anthranilic Acid

Kynurenine ⟷ PLP → Kynurenic Acid

NADPH

3-Hydroxy-Kynurenine —PLP→ Xanthurenic Acid

? → 3-Methoxy-Kynurenine

Picolinic Acid ← PC ← 3-hydroxy-Anthranilic Acid —→ CO_2 + NH_3

PLP

QPRT

Quinolinic Acid

1-(5'-Phosphoribosyl) Nicotinic Acid

Nicotinamide Adenine Dinucleotide

N'-Methylnicotinamide (N'-Me)

N'-Methyl-2-Pyridone-5-Carboxamide (2-ptridone)
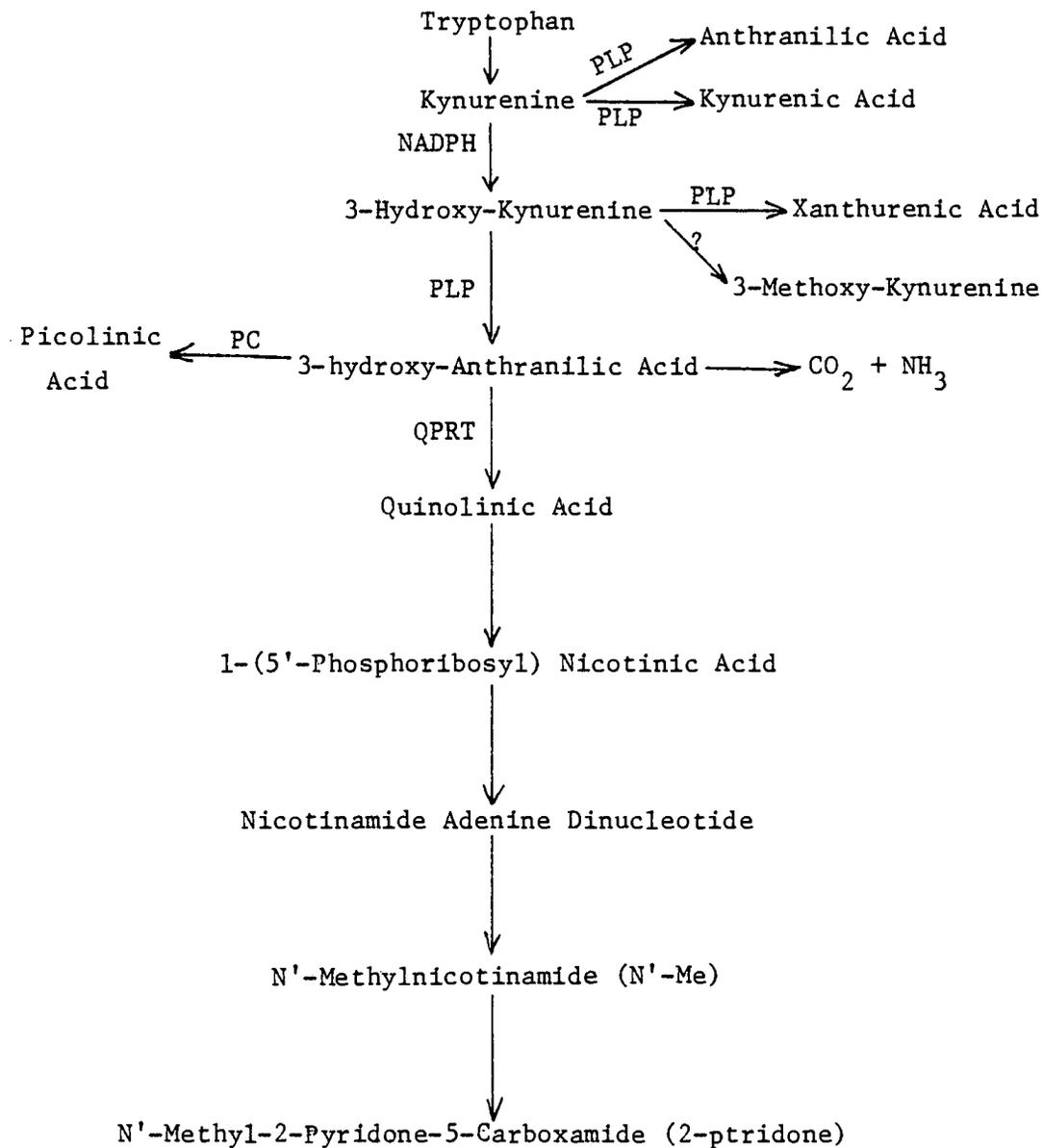
FIGURE 3. Metabolic pathway of tryptophan to niacin. Steps requiring pyridoxal phosphate and nicotinamide adenine dinucleotide phosphate as coenzymes are indicated by PLP and NADPH, respectively.

PC: picolinate carboxylase

QPRT: quinolinate phosphoribosyl transferase

Patterson et al. [25] observed that the tryptophan to niacin conversion ratio decreased with increasing tryptophan intake, which was also observed by Hakagawa et al. [26].

Factors Affecting the Conversion
of Tryptophan to Niacin

Vitamin B-6 is necessary for the conversion of tryptophan to niacin (Fig. 3), and in vitamin B-6 deficiency this conversion is impaired [27,28]. In response to a loading dose of tryptophan, vitamin B-6 depleted animals [27] and humans [28-31] excreted abnormal amounts of xanthurenic acid [27,28], kynurenine [28], 3-hydroxykynurenine [28] and quinolinic acid [29,30] in urine. The urinary metabolites of niacin, on the other hand, were markedly reduced [29,30]. Ranke, Ranke and Chow [32] observed that prolonged deprivation of vitamin B-6 in rats reduced vitamin B-12 reserves to such an extent that the function of this vitamin as a methylating agent was impaired, as indicated by a decreased N'-Me excretion following a dose of niacin.

Amino acid imbalance may adversely affect the conversion of tryptophan to niacin. Niacin-free casein diets that contain low levels of gelatin, acid-hydrolyzed protein or additional amounts of certain amino acids caused growth depression in rats which could be corrected by niacin or tryptophan. Niacin was more effective, however, in correcting this problem than tryptophan per se, indicating that amino acid imbalances affect the efficient conversion

of tryptophan to niacin [33]. The amino acid that most markedly affects the tryptophan-niacin pathway is leucine. High levels of leucine added to a diet low in protein or of poor quality protein can diminish the conversion of tryptophan to niacin [25,34]. This inhibition was first described by Gopalan and Srikantia [35] who administered leucine to humans receiving a diet low in protein which was derived from jowar. Pellagra had been observed in populations consuming diets high in this grain, which contains biologically available preformed niacin, and is high in leucine. A diet containing 65 percent jowar induced black tongue in dogs [36].

Administration of leucine to normal subjects and pellagrins produced a significant increase in urinary quinolinic acid and 2-pyridone. When tryptophan was given, leucine had similar effects on the urinary excretion of quinolic acid and 2-pyridone. The presence of niacin, however, reduced this increase [37]. According to the results of a rat study [38], the increase in urinary quinolinic acid in response to a high leucine diet may be due to the low activity of quinolinate phosphoribosyl transferase (QPRT) and the high activity of picolinate carboxylase, resulting in a decrease in nicotinic acid nucleotide formation from tryptophan (Fig. 2). A study by Raghuramulu et al. [39] showed that the nicotinamide adenine dinucleotide content in erythrocytes of pellagrins was not different from that observed in normal healthy subjects. The administration of 10 g of L-leucine daily for five days, however, depressed the nicotinamide nucleotide synthesizing

ability of erythrocytes, both in normal subjects and in pellagrins.

Sources of Niacin

Preformed niacin is found in both animal and plant tissues.
It occurs in animal tissues mainly in the amide form and in plant
tissues as the acid [1]. The richest readily available source of
niacin is yeast [7]. Dried brewers' yeast contains 38 mg per 100 g
and torula yeast contains 44 mg per 100 g. Kidney and liver contain
more niacin than muscle, which is also a good source of niacin.
On the whole, fish is a good source of preformed niacin. Saltwater
fish, especially tuna, halibut and swordfish, are rich in preformed
niacin. Milk and eggs are low in niacin, but since they are high in
tryptophan, they contribute considerable amounts of potential niacin
to the diet. Fruits are low in both niacin and tryptophan. Vege-
tables vary in preformed niacin content. In general, legumes are
better sources of preformed niacin and tryptophan than leaves, stems,
and roots of plants. Almonds and chestnuts, respectively, contain
3 mg and 1.2 mg of preformed niacin per 100 g [7]. Peanuts, a
legume, contain 15 mg of preformed niacin per 100 g, of which about
40 percent is in the bound form [1].

Cereals contain appreciable amounts of preformed niacin which
is unavailable for absorption. Bound forms of niacin are found in
wheat bran [8], rice [9] and corn [10]. Present evidence shows that
bound niacin in wheat is not a single substance, but is a part of a
number of substances of different molecule weights [40]. Two types

of bound niacin [41] have been described in wheat: in one, niacin is linked to a polysaccharide, and is called niactin; in the other, niacin is attached to peptides and called niacinogens. Since such molecules are resistent to hydrolysis by digestive enzymes, niacin in these macromolecules is not available for absorption.

Although treating corn with alkali or heat makes niacin more available, treating with alkali releases more niacin in a short time than does heat [42]. This may explain the low incidence of pellagra in Central American countries where lime-treated corn is used to make tortillas. The complete availability of niacin in tortillas has been confirmed [11].

Carter and Carpenter [43] reported the bioavailability of bound niacin, alkali-treated bound niacin and authentic nicotinic acid. Bound niacin was extracted from wheat bran with aqueous enthanol, dialyzed and dried on starch. One part, prepared as bound niacin, was steamed; the other was heated with alkali (calcium oxide) to release the bound niacin. Three subjects who were on standard diets which provided about 20 mg niacin equivalents per day, received doses of bound niacin and alkali-treated bound niacin, each containing 35 mg of niacin, and 24 mg of authentic nicotinic acid. Urinary N'-Me and 2-pyridone collected following these doses was equivalent to 24 percent ingested niacin after the dose of bound niacin and 62 percent after the alkali-treated bound niacin.

Metabolism

Preformed niacin is absorbed readily from the intestinal
tract, as is tryptophan. The fate of preformed niacin in the body
is shown in Figure 4. Some evidence indicates that niacin is syn-
thesized by microflora in the intestinal tract and that some of
this niacin is available to animals [45]. In man, however, this
does not appear to be an important source of niacin.

Niacin stores in the body are not large. High concentrations
are found in the liver, muscle and kidney for the performance of
metabolic functions. In man, a decrease in niacin intake is
followed by a decrease in the concentrations of NAD^+ and $NADP^+$ in
striated muscles, but not in erythrocytes [46]. Amounts in excess
of niacin need are disposed of mainly by the excretion of N'-Me and
2-pyridone in urine [47,48].

Urinary Excretion of Niacin Metabolites

In humans, the two major metabolic end-products of niacin ex-
creted in urine are N'-Me and 2-pyridone (Fig. 1). Healthy persons
receiving an adequate diet excreted about 3 to 6 mg of N'-Me and
4 to 17 mg of 2-pyridone daily [49]. Both Horwitt [4] and Gold-
smith [5,50] and their associates have reported the relationship
of these urinary metabolites to the tryptophan-niacin ratio. They
found that when large amounts of niacin and/or tryptophan are
eaten, more 2-pyridone is excreted than N'-Me.

NICOTINAMIDE-N'-OXIDE

NICOTINAMIDE

NICOTINIC ACID

NICOTINURIC ACID

methionine
ATP

N'-METHYLNICOTINAMIDE

NICOTINAMIDE ADENINE DINUCLEOTIDE

N'-RIBOSYL-2-PYRIDONE-5-CARBOXAMIDE

O_2
aldehyde oxidase

N'-METHYL-2-PYRIDONE-
5-CARBOXAMIDE
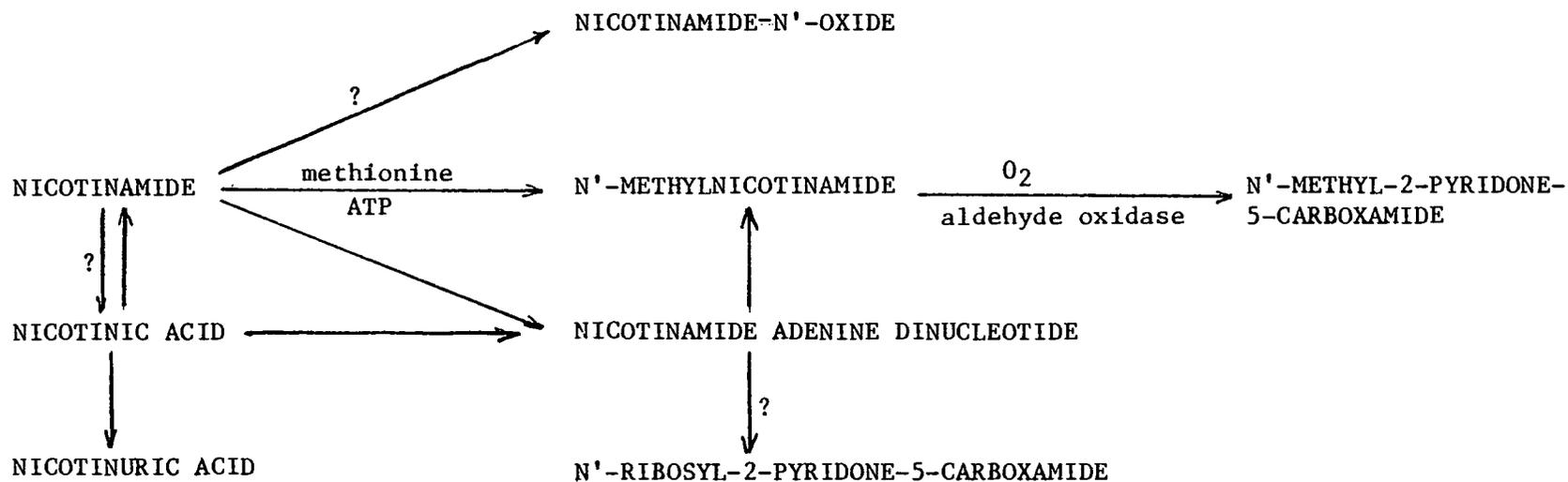
FIGURE 4. The major metabolic pathway for preformed niacin and its metabolites
(adapted from Mrochek, J. E., et al. [44]).

Pathways marked with question marks are speculative.

From their studies in man, Goldsmith et al. [5] observed that
the equivalent of only 10 to 30 percent of a test dose of nico-
tinamide was recovered as in urine as N'-Me, whereas the equivalent
of 70 to 90 percent was recovered as 2-pyridone. Urinary excretion
of niacin metabolites seem to be dependent in part on body stores
of niacin and also on the metabolic characteristics of the indivi-
dual, including his niacin requirement. The ratio of 2-pyridone to
N'-Me in urine does not change greatly, even when the body receives
excess amounts of niacin or tryptophan [49]. Subjects with an opti-
mal or high intake of niacin appear to excrete urinary 2-pyridone
and N'-Me in the ratio of 1.3 to 4 [51]. This range may be con-
sidered as an indication of body saturation with niacin. The excre-
tion of 2-pyridone is decreased in niacin deficiency, whereas the
excretion of N'-Me is never reduced, even in pellagra [49]. Urinary
excretion of N'-Me and 2-pyridone are generally used for judging
niacin nutritional status. A ratio of 2-pyridone to N'-Me below
one is evidence of niacin intake inadequacy [49].

Dietary Allowances

Because the coenzymes NAD^+ and $NADP^+$ are associated in cellu-
lar respiratory enzyme systems, the niacin requirement is related
to caloric intake. The amount of niacin recommended is also in-
fluenced by the amount and kind of dietary protein intake, i.e.,
tryptophan intake. In the study of Horwitt et al. [4,52], the
minimal requirement for niacin and tryptophan to prevent pellagra

was 4.4 mg niacin equivalents[2] per 1000 Kcal per day, or 9 mg niacin

equivalents per day for adults on caloric intakes of less than

2000 Kcal. A 50 percent margin of safety above the minimum require-

ment was added to allow for variations in individual needs as well

as for differences in conversion of tryptophan to niacin. The niacin

allowance recommended by the Food and Nutrition Board [53] in 1980

for all age groups is 6.6 mg niacin equivalents per 1000 Kcal. The

recommended allowance for infants up to six months of age is 8 mg

niacin equivalents per 1000 Kcal, of which about two-thirds will

irdinarily come from tryptophan. Although there is an increased

conversion of tryptophan to niacin during pregnancy, an additional

2 mg niacin equivalents per day are recommended for pregnant women

because of increased caloric intake. During lactation, an addi-

tional intake of 5 mg niacin equivalents per day is recommended.

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[2]Niacin equivalent was discussed earlier under "metabolic path-
way from tryptophan to niacin."

MATERIALS AND METHODS

This research was a part of an investigation in which the bioavailability of vitamin B-6 and selenium in tuna fish, peanut butter and whole wheat bread was measured in young men. Results of these investigations are published elsewhere.

Subjects

Nine men between the ages of 21 to 30 years participated in this study. Their body weights were within the normal ranges for their heights [54]. Descriptive data on the subjects are presented in Table 1. The subjects were in good health and free from any known metabolic disease as determined by a questionnaire. Each subject exhibited a normal response to a 5-gram D-xylose test dose, which indicates normal intestinal absorption [55]. All subjects completed the study except subject 6 who dropped out during the first experimental period for personal reasons. Approval of this research was obtained from the Oregon State University Human Subjects Committee. Before participating in this investigation, the subjects signed an informed consent form which was approved by this committee.

While participating in the study, the subjects did not take any drugs or vitamin supplements, or drink any alcoholic beverages. They maintained their normal physical activities, but did not engage in any strenuous physical exercise.

TABLE 1. Physical description of subjects

| Subject | Age | Height | Weight | |
			Beginning	End
No.	Yrs	cm	Kg	Kg
1	24	172.7	68.9	70.9
2	29	175.3	83.4	85.4
3	28	190.5	89.6	89.8
4	27	172.7	63.7	65.1
5	21	170.2	66.2	66.5
7	30	177.8	66.9	69.7
8	21	180.3	70.1	70.2
9	21	172.7	68.9	68.4
$\bar{X} \pm SD$	25.1 ± 3.8	176.5 ± 6.5	72.2 ± 9.2	73.3 ± 9.1

Experimental Design

The purpose of this investigation was to estimate the bio-availability of niacin from tuna fish, peanut butter and whole wheat bread which were added to a constant basal diet. Three 3x3 Latin square designs (Table 2) were used to assign the order in which these three foods were administered to the nine subjects during three experimental periods of 14 days each. This investigation was preceded by a 10-day adjustment period. The purpose of this period was to allow subjects to become acquainted with the experimental regime and their responsibilities, and to allow them to adjust to a constant metabolic status.

Diets

The subjects' meals were prepared, served and eaten under the supervision of nutritionists in the metabolic unit of Oregon State University Department of Foods and Nutrition. The adjustment (Table 3) and three experimental diets (Table 4) were nutritionally adequate. The nutrient composition of these diets is given in Table 5.

The dietary level of niacin in the three experimental diets met the recommended allowance for niacin for human adults. According to the Food and Nutrition Board of the Nutrition Research Council [53], adult men should have an intake of 6.6 mg of niacin equivalents per 1000 Kcal.

TABLE 2. Experimental Design

Subject No.	Adjustment	Period		
		I	II	III
	10 days	14 days	14 days	14 days
8		WWB'	T'	PB'
2		T	PB	WWB
6[3]		PB	WWB	T
4	Adjustment Diet[2]	WWB	PB	T
5		T	WWB	PB
7		PB	T	WWB
9		WWB	T	PB
3		T	PB	WWB
1		PB	WWB	T

[1]WWB = whole wheat bread

   T = tuna fish

  PB = peanut butter

[2]Presented in Table 3.

[3]Subject 6 dropped out of the study during the
first experimental period.

TABLE 3. Adjustment Diet

Food	Amount
Breakfast	g
Orange juice, frozen reconstituted	250
Rice cereal, ready-to-eat	35
Bread,[2] white enriched	25
Milk, 22.5 g dry nonfat reconstituted	240
Additional[1]	variable
Lunch	
Cheese, cheddar	70
Bread, white enriched	25
Milk, 22.5 g dry nonfat, reconstituted	240
Carrots, raw	80
Raisins	56
Pears, canned	100
syrup	20
Additional	variable
Dinner	
Bread, white enriched	25
Casserole[3]	
Ground beef, raw	270
Rice, long-grain, raw	45
Tomato paste, canned	100
Water	30 ml
NaCl, 10% solution	10 ml
Green beans, canned	65
brine	10
Corn, canned whole kernel	35
Celery, raw	80
Peaches, canned	100
syrup	20
Ice cream, vanilla	80
Cookies, vanilla sandwich	3 each
Additional	variable

[1]Subjects maintained their weights by consuming additional free foods, which included honey, margarine, hard candies, sugar and 7-up. Coffee and tea were also allowed. They recorded the amounts of these foods that they consumed.

[2]Toasted white bread was allowed only at breakfast.

[3]Preheat oven at 350°F. Place all ingredients in 1-quart casserole, mix, cover, and bake for 45 minutes.

TABLE 4. Experimental Diets (constant basal diet plus test foods)

Food	Amount
	g
Breakfast	
Orange juice, frozen reconstituted	250
Rice cereal, ready-to-eat	35
Bread,[2] white enriched	25
Milk, 22.5 g dry nonfat reconstituted	240
Additional[1]	variables
Lunch	
Bread, white enriched	25
Milk, 22.5 g dry nonfat reconstituted	240
Carrots, raw	80
Raisins	56
Pears, canned	100
Pears, canned	20
syrup	
Additional.	20
	Variables
Dinner	
Bread, white enriched	25
Casserole[3]	
Rice, long grain	45
Tomato paste canned	100
Water	30 ml
NaCl, 10% solution	10 ml
Green beans, canned	65
Brine	10
Corn, canned, whole kernel	35
Celery, raw	80
Peaches, canned	100
syrup	20
	80
Cookies, vanilla sandwich	3 each
Additional	variables
In addition to above diet,	
tuna, canned, oil-packed[4]	293
or	
peanut butter,[5] smooth,	252
yeast supplement[6]	1 capsule
or	
whole wheat bread[7]	465
each added for 14 days	

[1]Subjects maintained their weights by consuming additional "free" foods, which included honey, margerine, hard candies, sugar and 7-up. They were also allowed coffee and tea. They recorded the amounts of foods as well as the coffee and tea that they consumed.

[2]Toasted white bread was allowed only at breakfast.

[3]Casserole was covered and baked at 350°F for 45 minutes.

[4]The tuna was from a regular plant run made up of extra small yellow fin. It was brine frozen from a purse seiner, unloaded in Astoria. The fish were from five to eight pounds in size. The cans were packed with five percent vegetable broth and soybean oil to fill and 1.2 percent salt added.

[5]The peanuts blanched at room temperature. Roasting temperaures were 300-310°F for 18 minutes. To the finished product was added three percent peanut oil, 6-3/4 to 7-1/2 percent corn syrup, one percent salt and 1-1/2 percent stabilizer. The peanut butter was stored at 0°F 100 percent spanish peanut creamy style.

[6]Selenized yeast (225 μg/day) was given to the subjects when they received peanut butter experimental diet.

[7]The whole wheat bread was made from a blend of whole wheat flours. The flours were the North Dakota Hard Red Spring from Dallas, S.D., and a soft white and Hard Red wheat blend from Corvallis. The bread was baked in a commercial bakery in Corvallis, OR.

TABLE 5. Nutrient content of adjustment and experimental diets

Nutrient	Adjustment Diet Amount[1]	Experimental Diet Amount[1] W/T	W/WWB	W/PB
Energy (Kcal)	2390	2222	2509	2853
Protein (g)	108	113	85	112
Fat (g)[2]	93	73	25	138
Carbohydrate (g)[2]	287	285	514	329
Calcium (g)	1522	994	1361	1123
Phosphorus (g)	1759	1689	2184	2029
Iron (mg)	20	18	23	18
Vitamin A (IU)	14477	13686	13452	13452
Thiamin (mg)	2	2	3	2
Riboflavin (mg)	2	2	2	2
Vitamin C (mg)	188	188	188	188
Niacin[3] (mg)	21	45	22	48
Vitamin B-6[4] (mg)	1.48	1.52	1.56	1.56
Selenium[5] (μm)	92.3	331.5	354.5	346.6

[1]Calculated from Agriculture Handbook No. 8 Composition of Foods [6], unless stated otherwise.

[2]Daily subjects consumed calories, fat and carbohydrate which came from free foods were not included here. Daily subjects' total caloric intakes were 2609 ± 133 on adjustment diet, 2652 ± 240 on tuna fish experimental diet, 3180 ± 299 on peanut butter diet and 3364 ± 314 on whole wheat bread diet.

[3]Determined by the colorimetric method of AOAC (1980)

[4]Determined by Houssein Kabirmeidanshan, using a microbiological method with Saccharomyces uvarum (AOAC 1980).

[5]Determined by Ann Alexander.

The dietary intake of tryptophan was also considered. The term niacin equivalent was used to facilitate the calculation of the combined effects of niacin and tryptophan in diet. The amount of tryptophan chosen to be equivalent to 1 mg of niacin was 60 mg [3,4]. In this thesis, dietary tryptophan and preformed niacin were expressed on the molar basis; thus, 36 μmoles of tryptophan were equivalent to 1 μmole of niacin. The niacin and tryptophan content of the adjustment and constant basal (experimental) diets, and canned tuna fish, peanut butter and whole wheat bread which were added to the constant basal diet are given in Table 6. Niacin was determined by a colorimetric method. Tryptophan was calculated, based on nitrogen content determined in the three test foods and individual foods in the constant basal diet, from the Table of Amino Acid Content of Foods [56].

The amount of leucine in the adjustment and experimental diets is also given in Table 6. Leucine can affect the conversion of tryptophan to niacin [37]. However, in this study we did not have to worry about leucine effects because they occur only in people who consume low-protein diets which contain an excessive amount of leucine [34]. In this diet study, the diet was adequate in all nutrients and consisted of normal foods.

Tuna fish and whole wheat bread were stored frozen ($-5°C$) and thawed at the time of serving. Peanut butter was stored at $4°C$. Toasted bread was allowed only at breakfast. Margarine, honey,

TABLE 6. Niacin, tryptophan and leucine content of the adjustment diet, canned tuna fish, peanut butter and whole wheat bread and basal experimental diet.

Item	Niacin[1]	Tryptophan[2]	Leucine[2]
	mg	mg	mg
Adjustment diet	20.5	2001.9	9591.6
Basal diet	14.3	973.5	2348.7
Tuna fish (293 g)[3]	31.0	519.5	3954.9
Peanut butter (252 g)	33.9	848.7	4674.0
Whole wheat bread (459 g)	7.3	296.5	1607.0

[1]Niacin was determined by the colorimetric method of AOAC (1980).

[2]Tryptophan and leucine content in foods were calculated based on nitrogen content, from the Table of Amino Acid Content of Foods [56].

[3]Amount administered.

7-Up,[3] hard candies and sugar were available to the subjects. Variable amounts of these foods were eaten by the subjects to maintain a constant body weight throughout the study. In addition, coffee[4] and tea were allowed. Daily consumption of these additional items was recorded.

Procedure

During the study the subjects maintained their normal activities, which they reported daily on provided forms. The subjects recorded their body weights every morning before breakfast. Blended food composites were prepared weekly and stored at (-15°C) until analyzed for vitamin B-6, selenium, and nitrogen content. Portions of frozen food composites, as well as of the test foods, were freeze-dried and analyzed for niacin content. Nitrogen was also determined in the individual foods of each diet.

The subjects collected all of their urine. Their 24-hour urine specimens were stored under toluene in the refrigerator (4°C) and were measured and mixed each morning. Completeness of urine collections was monitored by the determination of creatinine. Portions of

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[3]7-Up is distributed by Seven-Up Enterprises, St. Louis, Missouri 63105.

[4]Only subjects 4, 7 and 9 consumed coffee. Their coffee intake was relatively constant throughout the study. Subject 4 drank 2 to 3 cups daily; subject 7, 2 cups and subject 9, 2 to 3 cups. MJB Instant Coffee (distributed by MJB Co., San Francisco, CA 94107) was served.

urine of the last and third last day of each period were stored

frozen until analyzed for the two niacin metabolites, N'-Me and

2-pyridone. Urinary vitamin B-6, 4-pyridoxic acid and selenium

were also determined and are reported elsewhere. A fecal marker,

50 mg F.D. and C. Brilliant blue dye No. 1 mixed with 200 mg

methylcellulose, was given to the subjects in a gelatin cap-

sule every 4 or 5 days during the study. Subjects collected all

of their feces in plastic cartons. Fecal samples were frozen until

they were combined into 4- or 5-day composites. Feces were ana-

lyzed for vitamin B-6, selenium and nitrogen content.

Blood was drawn from the subjects' antecubital vein at regular

intervals and at the end of each dietary period by a registered

medical technologist. Blood samples were analyzed for plasma

pyridoxal phosphate, plasma total vitamin B-6, selenium, hemoglobin

and hematocrit. Results of vitamin B-6 and selenium bioavailability

will be published elsewhere.

<u>Laboratory Analyses</u>

<u>N'-Methylnicotinamide (N'-Me)</u>

The procedure was essentially the fluorometric method of Huff

and Perlzweig [13], except that N'-Me was first purified by ion-

exchange chromotography [2]. Dowex 1-(acetate) removes most of the

anions from the urine. As reported by Gassmann and Scheunert [57],

N'-Me, which does not stay on an anion-exchange resin, may be chroma-

tographed on the strong cation-exchange resin Dowex-50 W (H^+). The

intensity of fluorescence is proportional to the concentration of N'-Me in the sample. Data are presented in μmoles per 24 hours.

N'-Methyl-2-pyridone-5-carboxamide (2-pyridone)

Two-pyridone is one of the few constituents of normal human urine which does not stay on either anion- or cation-exchange resins [2]. After this niacin metabolite had been separated by ion-exchange chromatrography, its concentration was determined spectrophotometrically. One percent of the 24-hour urine sample was passed through the ion-exchange column which was packed with alternating layers of Dowex 1 (OH$^-$) and Dowex 50 W (H$^+$) resins. The effluent from the columns was then read against distilled water at 238, 295, 310 and 350 nm. Ordinarily, the values for the readings at 258 nm were high. The samples were read at 350 nm because at this wavelength the readings should be correctible to zero. The quantity of 2-pyridone in the 24-hour urine sample was calculated from the readings at 295 and 310 nm and are presented as μmoles per 24 hours.

Total Nitrogen

Total nitrogen of the individual foods in the adjustment and experimental diets as well as the three test foods was measured by the boric acid modification of the Kjeldahl method by Scales and Harrison [58].

Dietary Niacin and Tryptophan

Niacin content in the food composites and test foods was determined by the AOAC colorimetric method [59]. The pyridine group of niacin was cleaved by cyanogen bromide and reacted with sulfanilic acid to form a yellow complex, which was then read in a spectrophotometer. Niacin in cereal products was extracted with calcium hydroxide before color development.

Tryptophan content of the diets and test foods was calculated, based on nitrogen content, from the published table, Amino Acid Content of Foods [56].

Creatinine

Daily urinary creatinine was determined on a Technicon Autoanalyzer[5] by an automated modification of the Jaffe reaction [60].

Statistical Analysis

Because one subject dropped out of the study, the data were statistically analyzed by analysis of variance (ANOVA) for the randomized block design using the Statistical Interactive Programming System (SIPS). Differences in bioavailability among the test foods were determined by t-test. The five percent level was used to measure significant differences among the three test foods.

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[5]Technicon Autoanalyzer, Technicon Corporation, Terrytown, New York.

RESULTS

In order to allow the subjects to adapt to each of the three diets, N'-Me and 2-pyridone were determined in 24-hour urine specimens that were collected on days 12 and 14 of each experimental period. Since the differences in urinary N'-Me (Table 7) and 2-pyridone (Table 8) on days 12 and 14 of each period were not statistically significant, the values from these two days were averaged for each subject. Because the amounts of niacin and tryptophan in the three test foods differed (Table 6), urinary N'-Me and 2-pyridone executed in response to each experimental diet were reported as percent of total niacin equivalents intake.

Urinary N'-Me Excretion During the Three Experimental Periods

The mean urinary N'-Me, expressed as percent of total dietary niacin equivalents, was 16 ± 4 on the tuna fish diet, 8 ± 3 on the peanut butter diet and 11 ± 5 on the whole wheat bread diet (Fig. 5). These differences were significantly different from each other.

There was much individual variation ($p < 0.05$) in the amount of urinary N'-Me excreted by the subjects in response to the three experimental diets (Table 7). All subjects except subject 2 excreted the highest percent of dietary niacin equivalents as N'-Me when receiving the tuna fish diet. Subject 2 excreted the highest percent of N'-Me on whole wheat bread diet. Each subject excreted the lowest percent of the niacin equivalents as N'-Me while receiving

TABLE 7.   Urinary N'-Methylnicotinamide (μmoles/24 hr).

Subject No.	Adjustment		Experimental Diet					
			Tuna		Peanut Butter		Whole Wheat Bread	
	a	b	a	b	a	b	a	b
1	44.0	47.7	87.9	84.8	41.6	45.8	26.3	28.5
2	67.4	80.6	114.9	91.9	89.5	89.9	75.1	74.5
3	31.6	40.6	116.6	106.6	60.8	46.3	47.0	55.1
4	41.6	37.7	128.2	106.6	35.6	37.2	21.0	26.9
5	36.9	34.3	46.9	52.0	52.1	45.1	21.9	26.1
7	54.9	45.7	81.9	96.3	42.5	53.2	29.2	39.8
8	31.2	37.9	86.6	123.6	36.3	48.1	36.9	36.9
9	51.9	39.4	47.5	61.8	32.9	40.6	15.3	28.2
$\bar{X}$ + SD	50.8±23.3	45.5±14.8	88±30.5	90±23.9	48.9±18.9	50.8±16.5	34.1±19.3	39.5±17.2

a = The third from the last day of each period.

b = The last day of each period.

TABLE 8. Urinary N'-Methyl-2-pyridone-5-carboxamide (μmoles/24 hr).

Subject No.	Adjustment		Experimental Diet					
			Tuna		Peanut Butter		Whole Wheat Bread	
	a	b	a	b	a	b	a	b
1	111.2	124.3	250.7	245.1	126.3	119.4	71.4	81.1
2	197.0	185.4	312.8	248.0	157.9	185.5	149.0	160.9
3	69.1	87.8	239.9	230.5	112.5	95.0	88.7	99.2
4	160.0	152.6	313.5	270.6	120.1	103.9	77.7	68.1
5	135.5	128.6	205.5	289.8	136.8	133.9	79.7	69.9
7	160.5	127.0	236.4	243.9	132.9	139.5	72.0	83.6
8	105.3	133.9	233.2	284.5	112.5	125.3	85.2	83.9
9	141.8	136.9	232.9	265.5	94.5	135.9	65.5	68.1
$\bar{X}$ + SD	135.1±39.6	134.6±27.5	253.1±39.2	259.7±21.1	124.2±19.1	129.8±27.4	86.2±26.5	89.4±30.8

a = The third from the last day of each period.

b = The last day of each period.
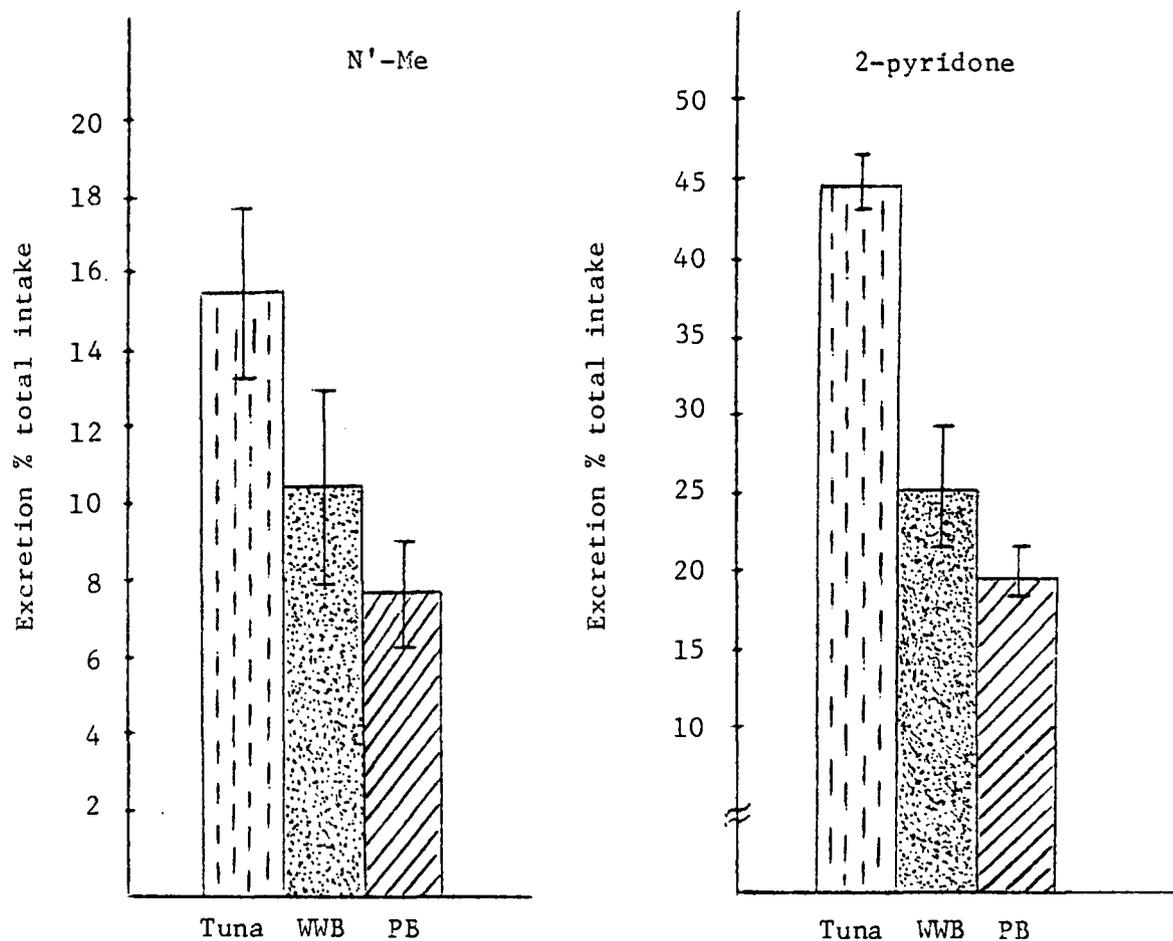
FIGURE 5. Mean ± S.D. percent of dietary tryptophan and
niacin excreted as N'-Me and 2-pyridone. N'-Me
and 2-pyridone were determined in 24-hour urinary
specimens collected on days 12 and 14 of each 14-
day period.

the peanut butter diet, except subject 5 who excreted the lowest per-

cent of niacin equivalents as N'-Me while on the whole wheat bread

diet.

Urinary 2-Pyridone Excretion During the Three Experimental Periods

Expressed as percent of total intake of niacin equivalents,

the mean 24-hour urinary 2-pyridone excretion was 45 ± 4, 20 ± 3 and

25 ± 8 on the tuna fish, peanut butter and whole wheat bread diets,

respectively (Fig. 5). The percent of dietary niacin equivalents

excreted as 2-pyridone on the tuna fish diet were significantly

higher than that on the peanut butter and whole wheat bread diets.

The difference between whole wheat bread and peanut butter diets in

urinary 2-pyridone was also statistically significant.

Individual variation in urinary 2-pyridone excretion among

subjects (Table 8) was statistically significant. All subjects

excreted the highest percent of the dietary niacin equivalents as

2-pyridone when receiving the tuna fish diet and the lowest percent

on the peanut butter diet.

The excretion of urinary 2-pyridone was higher than that of

N'-Me in each subject throughout the study. An average of 73 per-

cent (range 64 to 85) was present in the urine as 2-pyridone and

27 percent (range 19 to 36) as N'-Me. The 2-pyridone to N'-Me

ratios, which are presented in Table 9, ranged from 1.8 to 5.6.

TABLE 9. The ratio of 2-pyridone to N'-Me

Subject No.	Adjustment		Experimental Diet					
			Tuna		PB		WWB	
	a	b	a	b	a	b	a	b
1	2.5	2.6	2.9	2.9	3.0	2.6	2.7	2.8
2	2.9	2.3	2.7	2.7	1.8	2.2	2.0	2.2
3	2.2	2.2	2.1	2.2	1.9	2.1	1.9	1.8
4	3.8	4.0	2.4	2.5	3.4	2.8	3.6	2.5
5	3.7	3.7	4.4	5.6	2.6	3.0	3.6	2.7
7	2.9	2.8	2.9	2.5	3.1	2.6	2.5	2.1
8	3.4	3.5	2.7	2.3	3.1	2.6	2.3	2.3
9	2.7	3.5	4.9	4.3	2.9	3.3	4.3	2.4

a = the third from the last day of each period.

b = the last day of each period.

Percent Bioavailability of Niacin

Percent bioavailability was calculated by dividing the total excretion of N'-Me and 2-pyridone, in μmoles, by total niacin equivalents intake, in μmoles, and then times 100 (Table 10). Accordingly, niacin bioavailability (Table 11) was 60 ± 7 percent, 28 ± 6 percent and 36 ± 13 percent in the experimental diets in which tuna fish, peanut butter and whole wheat bread, respectively, furnished a large portion of niacin equivalents. Analysis of variance of these data for bioavailability revealed significant differences among the three experimental diets and among subjects. For all eight subjects the niacin in the tuna fish diet was the most biologically available and that in the peanut butter diet the least.

Bioavailability of Niacin in Beef and Cheese Compared to Tuna Fish Diet

The mean urinary excretion of N'-Me and 2-pyridone, expressed as percent of total intake of niacin equivalents, was 41 ± 12 (range 23 to 60) and 41 ± 9 (range 29 to 60) on days 8 and 10 of the adjustment period, respectively (Table 12). The reader is reminded that the adjustment diet (Table 3) without the beef and cheese is the same as the constant basal diet for the experimental diets (Table 4). Thus, results from the adjustment period can give an indication of the bioavailability of niacin equivalents from beef and cheese as compared to any of the three experimental diets.

TABLE 10. Summary of the calculations on percent bioavailability of niacin

Total Intake – from basal diet and test food	Diet		
	Tuna Fish	Peanut Butter	Whole Wheat Bread
Niacin (μmoles)	370 (68%)[a]	394 (70%)	176 (34%)
Tryptophan (as niacin equivalents[b])	7319 (35%)[c]	8932 (47%)	6226 (23%)
	36[c]	36	36[c]
Total (μmoles niacin equiv.)	573	642	349
Total Excretion, moles/24 hr			
N'-Me	90 ± 26[d]	50 ± 17	37 ± 18
2-pyridone	256 ± 20	127 ± 12	88 ± 28
Total	346	177	125
% bioavailability[e] from diet	60 ± 7	28 ± 6	36 ± 13

[a]Percent of total dietary niacin from test food.

[b]36 μmoles of tryptophan = 1 μmole of niacin.

[c]Percent of total dietary tryptophan from test food.

[d]Mean ± S.D.

TABLE 11. Percent bioavailability of niacin from tuna fish, peanut butter and whole wheat bread experimental diets.

Subject No.	Diet		
	Tuna fish	Peanut Butter	Whole Wheat Bread
1	58.3	26.0	29.7
2	67.0	40.7	65.8
3	60.5	24.5	41.6
4	71.5	23.1	27.8
5	51.9	28.7	28.3
7	57.5	28.7	32.2
8	63.2	25.1	34.8
9	53.0	23.7	25.4
% bioavaility[1]	60.4 ± 6.7	27.5 ± 5.7	35.7 ± 13.2

[1]N'-Me + 2-pyridone/niacin equivalents x 100.

TABLE 12.   Comparison of niacin bioavailability between adjustment and tuna fish experimental diets.

Total intake from diet and test food	Diet	
	Adjustment[1]	Tuna Fish
Niacin (μmoles)	167 (30%)	370 (68%)
Tryptophan as niacin equiv.	$\frac{9813}{36}$ (51%)	$\frac{7319}{36}$ (35%)
Total (μmoles niacin equiv.)	440	573
Total Excretion, μmoles/24 hr		
N'-Me	45 ± 13	90 ± 26
2-pyridone	135 ± 33	256 ± 20
Total	180	346
% bioavailability from diet	41 ± 10	60 ± 7

[1]The subjects received the same basal diet except beef and cheese were added to the basal diet during the adjustment period and tuna during the tuna fish experimental period.

DISCUSSION

Since this research was an offshoot of another investigation on nutrient bioavailability, the three experimental diets were different in niacin and tryptophan content (Table 6). The three test foods furnished a large portion of the total dietary niacin: tuna fish supplied 68 percent of the total pre-formed niacin and 35 percent of the total tryptophan intake, peanut butter, 70 percent of the niacin and 47 percent of the tryptophan, and whole wheat bread only 34 percent of the niacin and 23 percent of the tryptophan intake. In spite of this variation among the three diets, the bioavailability of niacin from these three foods can still be estimated because each food was added to a constant basal diet. Eleven days' adaptation to each diet was sufficient for the subjects to adjust to each diet. Carter and Carpenter [43] reported that six days were sufficient for the urinary niacin metabolites to return to baseline values after a loading dose of niacin had been administered, while in Goldsmith's study [5], five days were allowed for adaptation.

The niacin bioavailability was determined by urinary excretion of N'-Me and 2-pyridone. Individual variation in the excretion of urinary N'-Me (Table 7) and 2-pyridone (Table 8) was large. This had been observed also by Goldsmith [5] who stated, "Excretion [of N'-Me and 2-pyridone] is dependent in part on bodily stores of niacin and presumably also metabolic characteristics of the individual, including his niacin requirement." According to the

subjects' caloric intake, the amount of niacin equivalents provided by the diets in this study was more than adequate (Table 5). In this study, the subjects' 2-pyridone to N'-Me ratio ranged between 1.8 to 5.6,which indicates all subjects had received adequate dietary niacin. The range of 1.3 to 4.0 for the 2-pyridone : N'-Me ratio is an indication of tissue saturation with niacin [51]. Another explanation for the variation in urinary niacin metabolites observed in the present study is the difference among individuals in their conversion of tryptophan to niacin [4,5].

The results in our study demonstrated that the bioavailability of niacin from the tuna fish diet was superior to that from the peanut butter and whole wheat bread diets. On the tuna fish diet the recovery of dietary niacin equivalents as urinary N'-Me and 2-pyridone was 60 percent. This rate of recovery is close to that obtained from other investigators who measured the recovery of oral doses of crystalline nicotinamide. Mrochek et al. [44] and Goldsmith et al. [5] both recovered a mean of 57 percent of crystalline nicotinamide administered orally as urinary N'-Me and 2-pyridone. Carter and Carpenter [43], on the other hand, recovered 89 percent of oral doses of authentic nicotinic acid and 62 percent of alkali-treated bound niacin. The results of these investigations on crystalline niacin suggest that most of the niacin equivalents supplied by tuna fish and the basal constant diet were highly available.

Bound forms of niacin are mostly unavailable for the human. Ghosh, Sarka and Guhu [1] reported that cereals contain 85 to 90

percent of their total niacin in the bound form; in legumes, e.g., peanuts, 40 percent of the total niacin is bound. In peanut flour, the ratio of available to total niacin was only 46 percent [11]. An unexpected result in our study is that the niacin in the whole wheat bread diet was more available than that in the peanut butter diet; the recoveries of urinary metabolites corresponded to 36 percent and 28 percent, respectively. An explanation for this may be that whole wheat bread supplied only 34 percent of the total preformed niacin intake, while peanut butter supplied 70 percent of the total. Carter and Carpenter [43] demonstrated in a human study that 24 percent of the niacin in loading doses of bound niacin was available as judged by urinary excretion of N'-Me and 2-pyridone. Carter and Carpenter [11] also reported that rat assays showed less than 40 percent of the niacin in mature cooked wheat, corn, barley, rice and potatoes was bioavailable. These values, which were derived from loading doses of bound niacin given to humans and a rat bioassay, are very close to our results.

From our study, we see that niacin in animal products is readily available, while that in plant products is less biologically available. A person consuming a mixed western diet can easily achieve normal niacin status. A possible exception are vegetarians, especially those who do not consume milk, eggs and cheese. These three foods are low in niacin, but because they are high in tryptophan, they contribute considerable amounts of potential niacin to the diet. A study on Trappist monks [61], who were lacto-ovo

vegetarians, showed that most of the 16 subjects had low niacin
intakes, ranging between 34 to 86 percent of recommended dietary
allowances. The caloric (2465 to 3836 Kcal) and protein[6] (51 to
148 gm) intakes, however, were adequate. Improvement in the niacin
status for those vegetarians who do not consume any animal products
could be achieved only by greater consumption of legumes, nuts and
grains; however, the bulk necessary for achieving nutritional ade-
quacy may produce discomfort for humans.

The adjustment diet (Table 3) in our study was composed of
ground beef and cheese in addition to the basal diet which was used
also during the experimental periods (Table 4). The recoveries of
urinary niacin metabolites on days 8 and 10 corresponded to 41 ± 12
percent and 41 ± 9 percent, respectively. Cheese, ground beef and
tuna fish are all animal sources. In comparison to the niacin
bioavailability from the tuna fish diet, however, the correspond-
ing values from the adjustment diet seem low. This is probably
because cheese and ground beef supplied more tryptophan (51%)
than niacin (30%) to the adjustment diet, whereas tuna fish supplied
more niacin (68%) than tryptophan (35%). Most likely, this differ-
ence in niacin bioavailability is due to the tryptophan to niacin
conversion.

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[6]Tryptophan content (niacin equivalent) depends on type of
protein.

CONCLUSION AND SUMMARY

The bioavailability of niacin from tuna fish, peanut butter and whole wheat bread was estimated in eight men, aged 21 to 30 years. Following a 10-day adjustment period, each test food which was added to a constant basal diet was fed for 14 days; order in which these three foods were eaten was randomized. Of the total dietary niacin intake, tuna fish supplied 68 percent of the preformed niacin and 35 percent of the tryptophan, peanut butter, 70 percent of niacin and 47 percent of tryptophan, and whole wheat bread only 34 percent of niacin and 23 percent of tryptophan.

Bioavailability of niacin was determined by measuring the niacin metabolites, N'-Me and 2-pyridone, in 24-hour urine specimens collected on the last day and the third last day of each period. Individual variation in the excretion of N'-Me and 2-pyridone in this study was large. The subjects' 2-pyridone to N'-Me ratios ranged between 1.8 to 5.6 which indicated all subjects received adequate niacin. Because the amounts of niacin and tryptophan in the three test foods differed, urinary N'-Me and 2-pyridone excreted in response to each experimental diet were represented as percent of total niacin equivalent intake. The differences in bioavailability of niacin among three test foods were statistically significant. Niacin bioavailability was 60 ± 7 percent, 28 ± 6 percent and 36 ± 13 percent of the experimental diets in which tuna fish, peanut butter and whole wheat bread, respectively, furnished a large

portion of niacin equivalents. The unexpected result in our study is that the niacin in whole wheat bread diet was more available than that in the peanut butter diet. An explanation for this may be that whole wheat bread supplied only 34 percent of the total preformed niacin intake, while peanut butter supplied 70 percent of the total.

The results from our study suggest that the niacin in tuna fish is readily available, while that in peanut butter and whole wheat bread is poorly available for humans. These results are in agreement with other studies on niacin bioavailability carried out on humans and rats.

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