Table 2. The Composition of the Diet 1

| | Breakfast | Lunch | Dinner | Total |
|---|---|---|---|---|
| Energy (kcal) | 402 | 689 | 617 | 1708 |
| Protein (g) | 19.5 | 23.8 | 25.2 | 68.5 |
| Fat (g) | 15.7 | 25.5 | 9.6 | 50.8 |
| carbohydrates (g) | 46.0 | 85.8 | 104.4 | 236.2 |
| Fat-soluble vitamins | | | | |
| Vitamin A (μg) | 150 | 309 | 419 | 878 |
| Vitamin D (μg) | 1 | 0 | 2 | 3 |
| Vitamin E (mg) | 1.1 | 2.1 | 2.4 | 5.6 |
| Vitamin K (μg) | 8 | 204 | 98 | 310 |
| Water-soluble vitamins[1] | | | | |
| Vitamin $B_1$ (mg as thiamin) | 0.35 | 0.17 | 0.07 | 0.59 |
| Vitamin $B_2$ (mg as riboflavin) | 0.47 | 0.20 | 0.25 | 0.92 |
| Vitamin $B_6$ (mg as pyridoxine) | 020 | 0.36 | 0.68 | 1.24 |
| Vitamin $B_{12}$ (μg as cyanocobalamin) | 0.7 | 0.5 | 6.2 | 7.4 |
| Niacin equivalent[2](mg) | 7.0 | 8.4 | 14.9 | 30.3 |
| Pantothenic acid (mg) | 2.0 | 4.2 | 3.1 | 9.3 |
| Folic acid (μg as pteroylmonoglutamic acid) | 52 | 134 | 44 | 230 |
| Biotin (μg) | 21 | 20 | 26 | 67 |
| Vitamin C (mg as L-ascorbic acid) | 34 | 34 | 50 | 118 |
| Minerals | | | | |
| Na (mg) | 794 | 1175 | 850 | 2819 |
| K (mg) | 592 | 601 | 625 | 1818 |
| Ca (mg) | 249 | 142 | 85 | 476 |
| Mg (mg) | 47 | 71 | 74 | 192 |
| P (mg) | 380 | 293 | 317 | 990 |
| Fe (mg) | 0.8 | 3.4 | 2.6 | 6.8 |
| Zn (mg) | 1.8 | 3.7 | 2.5 | 8.0 |
| Cu (mg) | 0.15 | 0.44 | 0.43 | 1.02 |

[1]Water-soluble vitamins except for vitamin $B_{12}$ are measured. Other nutrients are calculated by using the Standard Tables of Food Composition in Japan, Fifth Revised and Enlarged Edition -2005-, Report of the Subdivision on Resources, The Council for Science and Technology, Ministry of Education, Culture, Sports, Science and Technology, Japan.
[2]The niacin equivalent intake was calculates as follows: The average tryptophan content in food protein is 1.1 % and the 1/60 (in weight basis) of tryptophan taken was converted into niacin in the body.

ジエチルエーテルで抽出後，HPLCで同時定量する方法を用いた[13]．

パントテン酸

定量の標準品として使用したパントテン酸カルシウムは和光純薬工業株式会社(大阪)から購入した．尿中のパントテン酸定量は，尿を直接試料検体として，*Lactobacillus plantarum* ATCC 8014を用いる微生物学的定量方法で行った[14]．

葉　酸

定量の標準品として使用したプテロイルモノグルタミン酸は和光純薬工業株式会社(大阪)から購入した．尿中の葉酸定量は，尿を直接試料検体として，*Lactobacillus casei* ATCC 27773を用いる微生物学的定量方法で行った[15]．

ビオチン

定量の標準品として使用したD(+)-ビオチンは和光純薬工業株式会社(大阪)から購入した．尿中のビオチン酸定量は，尿を直接試料検体として，*Lactobacillus plantarum* ATCC 8014を用いる微生物学的定量方法で行った[16]．

ビタミンC

定量の標準品として使用したL-アスコルビン酸は和光

Table 3. The Composition of the Diet 2

| | Breakfast | Lunch | Dinner | Total |
|---|---|---|---|---|
| Energy (kcal) | 463 | 549 | 606 | 1618 |
| Protein (g) | 19.6 | 21.4 | 20.5 | 61.5 |
| Fat (g) | 22.3 | 12.8 | 10.0 | 45.1 |
| carbohydrates (g) | 46.1 | 85.6 | 105.5 | 237.2 |
| Fat-soluble vitamins | | | | |
| Vitamin A (μg) | 294 | 144 | 444 | 882 |
| Vitamin D (μg) | 1 | 0 | 0 | 1 |
| Vitamin E (mg) | 2.7 | 0.6 | 2.9 | 6.2 |
| Vitamin K (μg) | 12 | 98 | 100 | 210 |
| Water-soluble vitamins[1] | | | | |
| Vitamin $B_1$ (mg as thiamin) | 0.35 | 0.09 | 0.02 | 0.46 |
| Vitamin $B_2$ (mg as riboflavin) | 0.47 | 0.18 | 0.17 | 0.82 |
| Vitamin $B_6$ (mg as pyridoxine) | 0.20 | 0.35 | 0.31 | 0.86 |
| Vitamin $B_{12}$ (μg as cyanocobalamin) | 0.7 | 0.3 | 10.3 | 11.3 |
| Niacin equivalent[2](mg) | 7.0 | 8.1 | 9.7 | 24.8 |
| Pantothenic acid (mg) | 2.0 | 3.7 | 3.6 | 9.3 |
| Folic acid (μg as pteroylmonoglutamic acid) | 52 | 125 | 105 | 282 |
| Biotin (μg) | 21 | 12 | 20 | 53 |
| Vitamin C (mg as L-ascorbic acid) | 34 | 25 | 53 | 112 |
| Minerals | | | | |
| Na (mg) | 833 | 1237 | 1080 | 3150 |
| K (mg) | 594 | 851 | 615 | 2060 |
| Ca (mg) | 250 | 173 | 96 | 519 |
| Mg (mg) | 47 | 113 | 96 | 256 |
| P (mg) | 381 | 253 | 317 | 951 |
| Fe (mg) | 0.8 | 6.2 | 3.2 | 10.2 |
| Zn (mg) | 1.9 | 2.8 | 4.2 | 8.9 |
| Cu (mg) | 0.15 | 0.33 | 0.47 | 0.95 |

[1]Water-soluble vitamins except for vitamin $B_{12}$ are measured. Other nutrients are calculated by using the Standard Tables of Food Composition in Japan, Fifth Revised and Enlarged Edition -2005-, Report of the Subdivision on Resources, The Council for Science and Technology, Ministry of Education, Culture, Sports, Science and Technology, Japan.
[2]The niacin equivalent intake was calculates as follows: The average tryptophan content in food protein is 1.1 % and the 1/60 (in weight basis) of tryptophan taken was converted into niacin in the body.

純薬工業株式会社（大阪）から購入した．尿中のビタミン C 定量は Kishida ら[17]の方法に従った．

## 結　果

被験者に，第 1 週目は規定食のみを食べさせ，その時の尿に排泄されるビタミン量を対照値とした（Table 5-Data 1）．第 2 週目は食事摂取基準に示された推奨量あるいは目安量の約 6 倍量の水溶性ビタミン混合を規定食に付加した時の尿を集め分析を行った（Table 5-Data 2）．そして，クリアランスを調べるために，ビタミン混合の付加を中止した 1 週間後の尿を集め分析を行った（Table 5-Data 3）．

食事摂取基準に示された推奨量あるいは目安量の約 6 倍量の水溶性ビタミン混合剤を 1 週間投与し続けた時の尿中への水溶性ビタミンの排泄量（Data 2）は，ビタミン $B_1$ では対照値（Data 1）の約 20 倍であった．被験者のビタミン $B_1$ の第 1 週目の摂取量は 0.53 mg/ 日，第 2 週目の摂取量は 4.42 mg/ 日であったことから，8 倍の摂取量の増加に対し，尿中への排泄量は約 20 倍に増加したことになる．ビタミン $B_2$ では，摂取量が 0.87 mg/ 日から 6.61 mg/

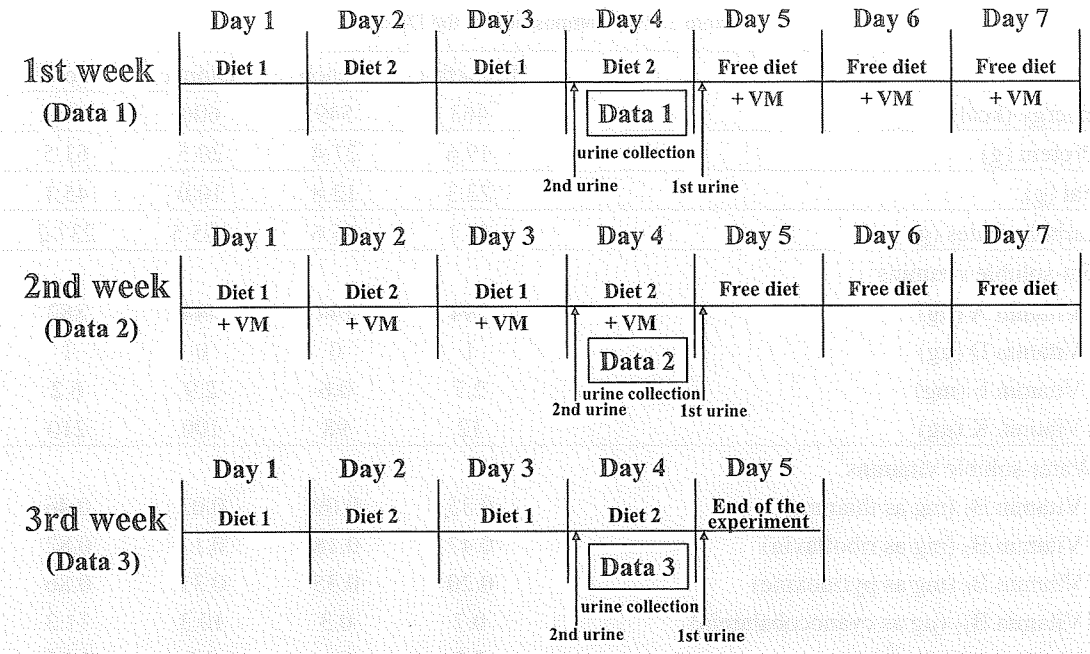


Fig. 1. The Scheme of the Experimental Design

The subjects were given the diet shown in Table 2 and 3 from day 1 to day 4 in each week. Six-fold of the water-soluble vitamin mixture (VM) based on Dietary Reference Intakes for Japanese, 2005[2], was given to the subjects from day 5 of week 1 to day 4 of week 2. The 24-h urine samples were collected from the second urinary excretion on day 4 to the first excretion on day 5 in each week. Composition of vitamin mixtures is shown in Table 4.

Table 4. The Composition of the Water-soluble Vitamin Mixture

| Vitamins | Content in the mixture | Intake per day[a] |
|---|---|---|
| Thiamin | 1.30 mg | 3.9 mg |
| Riboflavin | 1.91 mg | 5.73 mg |
| Pyridoxine | 2.20 mg | 6.60 mg |
| Nicotinamide | 22.5 mg | 67.5 mg |
| Pantothenic acid | 10.5 mg | 31.5 mg |
| Pteroylmonoglutamic acid | 0.45 mg | 1.35 mg |
| D-Biotin | 0.061 mg | 0.183 mg |
| L-Ascorbic acid | 200 mg | 600 mg |

[a] The subjects were taken the mixture at breakfast, lunch, and dinner, respectively.

日へと8倍に増加すると，尿中排泄量は約20倍に増大した．ビタミン$B_6$では，摂取量が1.05 mg/日から7.66 mg/日へと7倍に増加すると，ビタミン$B_6$異化代謝産物である4-ピリドキシン酸の尿中排泄量は約8倍に増大した．ナイアシンでは，摂取量が27.6 mgNE/日から95.0 mgNE/日へと3.5倍に増加すると，総ニコチンアミド異化代謝産物(MNA+2-Py+4-Py)の尿中排泄量は約5倍に増大した．パントテン酸では，摂取量が9.3 mg/日から40.7 mg/日へと4倍に増加すると，尿中排泄量は約5倍に増大した．葉酸では，摂取量が0.26 mg/日から1.60 mg/日へと6倍に増加すると，尿中排泄量は約12倍に増大した．ビオチンでは，摂取量が60 μg/日から242 μg/日へと4倍に増加すると，尿中排泄量は約4倍に増大した．ビタミンCでは，摂取量が115 mg/日から715 mg/日へと6倍に増加すると，尿中排泄量は約10倍に増大した．

以上のように，ビタミン$B_{12}$を除く水溶性ビタミンの摂取量の増大により，尿中排泄量は摂取量と同等もしくはそれ以上の倍率で増大した．ビタミン$B_{12}$の主要な排泄経路は尿ではないため，今回は測定対象とはしなかった[18]．

メルクインデックスで調べたこれら水溶性ビタミンの尿中排泄量は単なる水への溶解度と対応しないが，参考として，水溶性ビタミンの水への溶解度を以下に示す[19]．チアミン塩酸塩は1 gが約1 mLの水に溶ける，リボフラビンは結晶形により異なるが，1 gが3000～15,000 mLの

Table 5. Urinary Excretion of Water-soluble Vitamins Before Administration (Data 1), During Administration (Data 2), and One Week After Stopping Administration of the Water-soluble Vitamin Mixture (Data. 3).

| Vitamins | Data 1 | Data 2 | Data 3 |
|---|---|---|---|
| Thiamin (nmol/day) | 288 ± 74 | 6095 ± 1058 | 1259 ± 403 |
| Riboflavin (nmol/day) | 283 ± 73 | 5569 ± 1215 | 972 ± 609 |
| 4-Pyridoxic acid (μmol/day) | 3.44 ± 0.41 | 27.96 ± 2.69 | 6.81 ± 2.60 |
| Nicotinamide metabolites (μmol/day) | 85.6 ± 10.8 | 560.4 ± 78.3 | 89.2 ± 22.7 |
| Pantothenic acid (μmol/day) | 14.6 ± 2.0 | 71.2 ± 11.0 | 34.9 ± 7.3 |
| Folic acid (nmol/day) | 21.9 ± 8.5 | 830.1 ± 235.8 | 68.6 ± 11.6 |
| Biotin (nmol/day) | 74.5 ± 12.0 | 315.0 ± 30.0 | 51.6 ± 15.0 |
| Ascorbic acid (μmol/day) | 294 ± 82 | 4514 ± 954 | 459 ± 408 |

Values are means ± SD for 6 subjects.

水に溶ける，ピリドキサミン塩酸塩は 1 g が約 4.5 mL の水に溶ける，ニコチンアミドは 1 g が約 1 mL の水に溶ける，パントテン酸カルシウムは 1 g が 2.8 mL の水に溶ける，プテロイルモノグルタミン酸は 1 g が約 625,000 mL の水に溶ける，ビオチンは 1 g が約 4,500 mL の水に溶ける，アスコルビン酸は 1 g が約 3 mL の水に溶ける．

## 謝 辞

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資 料

# パンを主食とした食事中に含まれる水溶性ビタミンの遊離型ビタミンに対する相対利用率

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# Relative Availability of Water-Soluble Vitamins in a White Bread Diet to Free Vitamins

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The relative availability of water-soluble vitamins to free vitamins in a white bread diet consumed by Japanese male and female subjects was determined. The subjects, 9 female Japanese college students in experiment 1, and 7 male Japanese and 5 female Japanese college students in experiment 2, consumed the test diet with or without a water-soluble vitamin mixture for five consecutive days, and the water-soluble vitamin levels in a 24-h urine sample were measured. The ratio of the urinary excretion rate for each water-soluble vitamin in the test diet to that in the water-soluble vitamin mixture was determined as the relative availability. The relative availability of each vitamin was as follows: $B_1$, 55%; $B_2$, 50%; vitamin $B_6$, 85%; niacin, 60%; pantothenic acid, 70%; folate, 50%; biotin, 85%; and C, 95%.

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**Keywords:** bioavailabitily 生体利用率, B vitamins B群ビタミン, bread パン, diet 食事.

## 1. 緒 言

現在の日本では，食パンは主要な糖質・たんぱく質供給源であると同時に，B群ビタミン，特にビタミン$B_1$，ビタミン$B_2$，ナイアシン，パントテン酸，葉酸の供給源ともなっている．五訂増補日本食品標準成分表[1]の値は，食品中に含まれる結合型のB群ビタミンを *in vitro* で化学的あるいは酵素的処理を行うことによりすべて遊離型にした後，測定した値である．したがって，消化率・吸収率および体内利用率を考慮した値ではなく，食品中に含まれる栄養素を資源的な見方から記載した数値である．一方，日本人の食事摂取基準（2005年版）において，ビタミン$B_6$，ビタミン$B_{12}$，葉酸の食事摂取基準は生体利用率を考慮して策定された[2]．「日本食品標準成分表」と「食事摂取基準」との間の整合性を保つことは，国民に質の高い健康・栄養をもたらすために必要不可欠なことである．

我々は，最近，一般的な食事を被験者に摂取させ，遊離型B群ビタミンを付加したときとしなかったときの尿中B群ビタミン排泄量を測定することにより，遊離型B群ビタミンに対する食事中のB群ビタミンの相対利用率を簡便に決定する方法を確立した[3]．数多くのメニューに対してこの方法を用い，データを蓄積していけば，日本人が摂取する食事中のビタミンの平均的な利用率を明らかにすることができる．本研究では，食パンを主食としたときの水溶性ビタミンの相対利用率を決定したので，資料として報告する．

## 2. 実験方法

### (1) 被 験 者

被験者は，予め実験内容の説明を受け，書類にて，実験への参加を希望した者である．実験1の被験者は22～25歳の成人女性9名であり，年齢は22.7±1.2歳（平均値±SD），身長は157±4 cm，体重は49.4±5.6 kg，BMIは20.0±1.7であった．実験2の被験者は，

Table 1. The composition of test diets for female subjects in Experiment 1

| | Breakfast | Lunch | Dinner | Snack | Total |
|---|---|---|---|---|---|
| Energy (kcal) | 388 | 527 | 545 | 325 | 1,785 |
| Protein (g) | 9.7 | 19.5 | 27.0 | 8.6 | 64.8 |
| Fat (g) | 19.2 | 16.3 | 13.5 | 3.6 | 52.6 |
| Carbohydrate (g) | 44.9 | 74.3 | 83.3 | 67.3 | 269.8 |
| Water-soluble vitamins[*1] | | | | | |
| Vitamin $B_1$ (mg as thiamin chloride) | 0.28 | 0.19 | 0.47 | 0.17 | 1.11 (3.30 μmol) |
| Vitamin $B_2$ (mg as riboflavin) | 0.09 | 0.14 | 0.33 | 0.04 | 0.60 (1.60 μmol) |
| Vitamin $B_6$ (mg as pyridoxine) | 0.12 | 0.20 | 0.58 | 0.10 | 1.00 (5.92 μmol) |
| Vitamin $B_{12}$ (μg as cyanocobalamin) | 0.1 | 0.2 | 6.6 | 0 | 6.9 (5.09 nmol) |
| Niacin equivalent[*2] (mg) | 5.2 | 6.8 | 11.0 | 2.8 | 25.8 (210 μmol) |
| Pantothenic acid (mg) | 0.9 | 2.1 | 4.8 | 1.8 | 9.6 (43.8 μmol) |
| Folic acid (μg as pteroylmonoglutamic acid) | 11 | 35 | 99 | 16 | 161 (365 nmol) |
| Biotin (μg) | 11.1 | 9.9 | 15.8 | 4.2 | 41.0 (168 nmol) |
| Vitamin C (mg as L-ascorbic acid) | 16 | 7 | 45 | 36 | 104 (591 μmol) |

[*1] Water-soluble vitamins except for vitamin $B_{12}$ were measured. Other nutrients were calculated by using the Standard Tables of Food Composition in Japan. [*2] The niacin equivalent intake was calculated as follows: the average tryptophan content in food protein is 1.1% and the 1/60 (in weight basis) of tryptophan taken was converted into niacin in the body.

19～25 歳の成人男性 7 名，21～22 歳の成人女性 5 名であった．男性被験者の年齢は 21.6±2.4 歳，身長は 172±6 cm，体重は 64.5±7.1 kg，BMI は 21.7±1.7 であり，女性被験者の年齢は 21.8±0.5 歳，身長は 159±4 cm，体重は 52.5±9.4 kg，BMI は 20.5±2.9 であった．いずれも，喫煙，飲酒の習慣がなく，朝食など規則正しい食習慣をもつ者であった．被験者の本研究は，滋賀県立大学倫理審査委員会において承認を受け，ヘルシンキ宣言の精神に則って行われた．

(2) 食　事

18～29 歳の男性および女性の食事摂取基準に従い，男性に対してはエネルギー摂取量が約 2,400 kcal，女性に対しては約 1,800 kcal となるような献立を作成し，被験者に摂取させた．献立は 1 日 3 回の主食をパンと，パン食に適した主菜と副菜とした．具体的な規定食の内容は，朝食は食パン，マーガリン，野菜ジュース，ミニトマト，ハム，ゆで卵，昼食は食パン，イチゴジャム，ツナ，おろしハンバーグ，キャベツ，夕食は食パン，マーガリン，ホタテのトマトスープ，ほうれん草炒め，温州ミカン，間食は食パン，ブルーベリージャム，グレープフルーツジュースとした．用いた規定食中の栄養素量を Table 1～3 に示した．食品成分表に基づいて計算すると，実験 1 において，全食品に対するパンのエネルギー比率は 53.3%，たんぱく質摂取量に対する比率は 51.7%，脂質では 30.2%，炭水化物では 62.3%であった．パン由来の水溶性ビタミンについては，実測値に基づくと，ビタミン$B_1$では 22.1%，ビタミン$B_2$では 23.3%，ビタミン$B_6$では 8.3%，ナイアシン当量では 39.7%，パントテン酸では 23.1%，葉酸では 24.7%，ビオチンでは 14.7%，ビタミン C が 0%であった．実験 2 の男性に対しては，全食品に対するパンの比率は，エネルギーでは 52.9

Table 2. The composition of test diets for male subjects in Experiment 2

| | Breakfast | Lunch | Dinner | Snack | Total |
|---|---|---|---|---|---|
| Energy (kcal) | 604 | 625 | 736 | 431 | 2,396 |
| Protein (g) | 25.2 | 28.3 | 35.8 | 13.0 | 102.2 |
| Fat (g) | 22.2 | 22.8 | 26.6 | 5.6 | 77.2 |
| Carbohydrate (g) | 74.9 | 74.1 | 91.8 | 84.6 | 325.5 |
| Water-soluble vitamins[*1] | | | | | |
| Vitamin $B_1$ (mg as thiamin chloride) | 0.32 | 0.17 | 0.40 | 0.17 | 1.35 (4.01 μmol) |
| Vitamin $B_2$ (mg as riboflavin) | 0.28 | 0.32 | 0.52 | 0.06 | 1.17 (3.11 μmol) |
| Vitamin $B_6$ (mg as pyridoxine) | 0.16 | 0.21 | 0.53 | 0.10 | 1.01 (5.98 μmol) |
| Vitamin $B_{12}$ (μg as cyanocobalamin) | 0.7 | 0.8 | 7.5 | 0 | 9.0 (6.64 nmol) |
| Niacin equivalent[*2] (mg) | 7.8 | 6.9 | 11.0 | 3.7 | 29.5 (240 μmol) |
| Pantothenic acid (mg) | 2.3 | 2.8 | 4.8 | 1.7 | 11.7 (53.3 μmol) |
| Folic acid (μg as pteroylmonoglutamic acid) | 36 | 54 | 139 | 30 | 249 (565 nmol) |
| Biotin (μg) | 13.7 | 22.0 | 26.0 | 4.2 | 65.9 (270 nmol) |
| Vitamin C (mg as L-ascorbic acid) | 52 | 23 | 40 | 46 | 162 (920 μmol) |

[*1] Water-soluble vitamins except for vitamin $B_{12}$ were measured. Other nutrients were calculated by using the Standard Tables of Food Composition in Japan. [*2] The niacin equivalent intake was calculated as follows: the average tryptophan content in food protein is 1.1% and the 1/60 (in weight basis) of tryptophan taken was converted into niacin in the body.

%，たんぱく質では43.7%，脂質では27.4%，炭水化物では68.9%，ビタミン$B_1$では24.2%，ビタミン$B_2$では15.9%，ビタミン$B_6$では11.0%，ナイアシン当量では46.3%，パントテン酸では25.3%，葉酸では21.3%，ビオチンでは12.2%，ビタミンCでは0%であった．実験2の女性に対しては，全食品に対するパンの比率は，エネルギーでは50.1%，たんぱく質では44.3%，脂質では26.6%，炭水化物では62.5%，ビタミン$B_1$では20.8%，ビタミン$B_2$では17.4%，ビタミン$B_6$では9.4%，ナイアシン当量では42.0%，パントテン酸では26.1%，葉酸では18.3%，ビオチンでは15.6%，ビタミンCでは0%であった

(3) 実験計画

実験計画の概略をFig. 1に示した．実験開始日をDay 1とした．1日のスケジュールは6時起床，6時30分～7時に朝食，13時～13時30分に昼食，18時～18時30分に夕食，23時に就寝とし，間食の摂取時間は自由にさせた．水はミネラルウォーターを自由摂取とした．Table 1に示した規定食をDay 1～Day 5およびDay 8～Day 12に摂取させた．Day 6とDay 7は被験者の負担軽減を考慮し，自由食とした．Day 6以降は，水溶性ビタミン混合を食後3回服用させた．1日当りの水溶性ビタミン混合の摂取量は，チアミン塩酸塩1.98 mg/日（7.12 μmol/日），リボフラビン3.45 mg/日（9.18 μmol/日），ピリドキシン2.94 mg/日（17.4 μmol/日），ニコチンアミド56.4 mg/日（459 μmol/日），パントテン酸19.0 mg/日（86.8 μmol/日），プテロイルモノグルタミン酸570 μg/日（1.29 μmol/日），ビオチン69 μg/日（283 nmol/日），アスコルビン酸90 mg/日（511 μmol/日）である．

Day 5の2回目の尿から翌日のDay 6の1回目までの尿を蓄尿し，これをDay 5の1日尿とした．Day

Table 3. The composition of test diets for female subjects in Experiment 2

| | Breakfast | Lunch | Dinner | Snack | Total |
|---|---|---|---|---|---|
| Energy (kcal) | 499 | 427 | 644 | 326 | 1,896 |
| Protein (g) | 21.5 | 16.8 | 28.0 | 9.3 | 75.6 |
| Fat (g) | 20.4 | 14.9 | 20.4 | 3.8 | 59.6 |
| Carbohydrate (g) | 56.3 | 55.2 | 91.7 | 65.9 | 269.1 |
| Water-soluble vitamins*1 | | | | | |
| Vitamin $B_1$ (mg as thiamin chloride) | 0.38 | 0.14 | 0.48 | 0.18 | 1.18 (3.50 μmol) |
| Vitamin $B_2$ (mg as riboflavin) | 0.26 | 0.13 | 0.36 | 0.05 | 0.80 (2.13 μmol) |
| Vitamin $B_6$ (mg as pyridoxine) | 0.14 | 0.16 | 0.50 | 0.09 | 0.89 (5.27 μmol) |
| Vitamin $B_{12}$ (μg as cyanocobalamin) | 0.7 | 0.2 | 6.9 | 0 | 7.8 (5.76 nmol) |
| Niacin equivalent*2 (mg) | 6.9 | 4.9 | 9.8 | 2.8 | 24.4 (198 μmol) |
| Pantothenic acid (mg) | 2.1 | 1.4 | 3.6 | 1.4 | 8.5 (38.8 μmol) |
| Folic acid (μg as pteroylmonoglutamic acid) | 25 | 40 | 131 | 21 | 217 (492 nmol) |
| Biotin (μg) | 12.8 | 7.9 | 15.1 | 2.9 | 38.7 (159 nmol) |
| Vitamin C (mg as L-ascorbic acid) | 52 | 23 | 40 | 46 | 162 (920 μmol) |

*1 Water-soluble vitamins except for vitamin $B_{12}$ were measured. Other nutrients were calculated by using the Standard Tables of Food Composition in Japan. *2 The niacin equivalent intake was calculated as follows: the average tryptophan content in food protein is 1.1% and the 1/60 (in weight basis) of tryptophan taken was converted into niacin in the body.

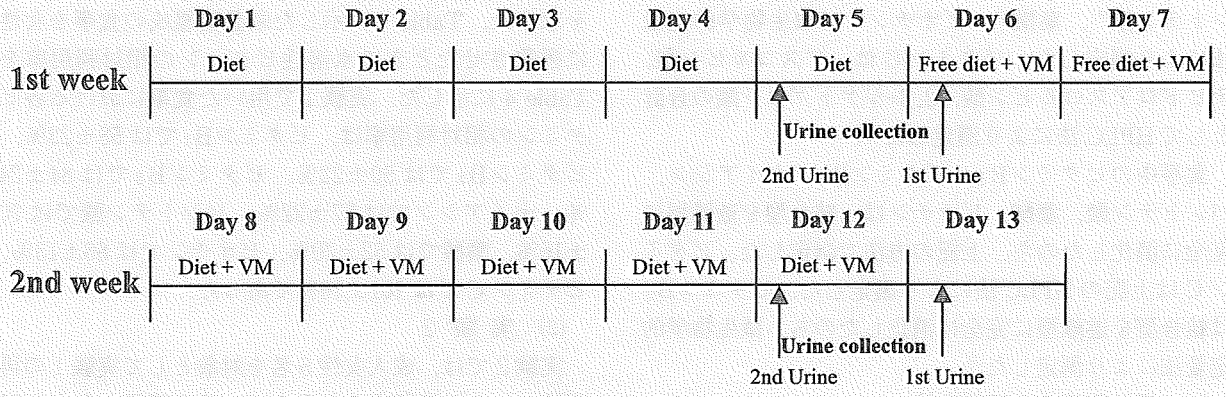


Fig. 1. Experimental design

The subjects were given the diet shown in Tables 1, 2 and 3 from Day 1 to Day 5 and from Day 8 to Day 12. The subjects freely took self-selected diet at Day 6 and Day 7. Water-soluble vitamin mixture (VM) containing 1.98 mg/d (7.12 μmol/d) of thiamin chloride, 3.45 mg/d (9.18 μmol/d) of riboflavin, 2.94 mg/d (17.4 μmol/d) of pyridoine, 56.4 mg/d (459 μmol/d) of nicotinamide, 19.0 mg/d (86.8 μmol/d) of pantothenic acid, 570 μg/d (1.29 μmol/d) of pteroylmonoglutamate, 69 μg/d (283 nmol/d) of biotin and 90 mg/d (511 μmol/d) of ascorbic acid was given to the subjects from Day 6 to Day 12. The 24-h urine samples were collected from the second urinary excretion on Day 5 and Day 12 to the first excretion on Day 6 and Day 13, respectively.

Table 4. Urinary excretions and relative availability of water-soluble vitamins in Experiment 1

| Vitamins | Data 1 (μmol/day) | Data 2 (μmol/day) | Data 3 (μmol/day) | Urinary excretion rate for vitamins in the test diet (%) | Urinary excretion rate for free vitamins (%) | Relative availability (%) |
|---|---|---|---|---|---|---|
| Vitamin $B_1$ | 0.698±0.372 | 3.437±1.556 | 2.628±1.198 | 21.4±11.4 | 44.7±20.4 | 58±23 |
| Vitamin $B_2$ | 0.515±0.228 | 6.414±1.041 | 5.899±0.947 | 32.3±14.3 | 64.0±10.3 | 50±22 |
| Vitamin $B_6$ | 2.33±0.52 | 10.70±1.05 | 8.37±1.11 | 39.3±8.7 | 48.2±6.4 | 84±24 |
| Niacin | 88.7±37.7 | 438.0±91.5 | 349.3±77.3 | 42.3±18.0 | 76.2±16.9 | 57±22 |
| Pantothenic acid | 17.6±2.6 | 68.6±9.0 | 51.0±8.7 | 40.1±5.9 | 59.2±10.1 | 70±16 |
| Folate | 0.019±0.004 | 0.159±0.047 | 0.140±0.045 | 5.2±1.1 | 10.8±3.5 | 52±20 |
| Biotin | 0.098±0.024 | 0.289±0.046 | 0.191±0.25 | 58.6±14.0 | 67.5±8.7 | 86±13 |
| Vitamin C | 475±56 | 879±57 | 408±40 | 79.9±9.5 | 79.7±7.8 | 101±16 |

Data 1: The values are urinary excretions of vitamins when only the diet is fed to the subjects. Data 2: The values are urinary excretions of vitamins when the diet and vitamin mixtures are fed to the subjects. Data 3: The values are calculated "Data 2" − "Data 1." Values are means±SD for 9 female subjects.

12の2回目の尿から翌日のDay 13の1回目までの尿を蓄尿し，これをDay 12の1日尿とした．

(4) 分析方法

尿中のチアミン[4]，リボフラビン[5]，ビタミン$B_6$代謝産物4-ピリドキシン酸（4-PIC）[6]はHPLC法により測定した．ニコチンアミド（Nam）[7]，$N^1$-メチルニコチンアミド（MNA）[8]，$N^1$-メチル-2-ピリドン-5-カルボキサミド（2-Py）[7]，$N^1$-メチル-4-ピリドン-3-カルボキサミド（4-Py）[7]はHPLC法により測定し，これらの合計を総ニコチンアミド代謝産物とした．パントテン酸[9]，葉酸[10]，ビオチン[11]は微生物学的定量法により測定した．ビタミンCは，アスコルビン酸，デヒドロアスコルビン酸，2,3-ジケトグロン酸の合計としてHPLC法により測定した[12]．

食事中のビタミン$B_1$，ビタミン$B_2$，ナイアシン，パントテン酸，葉酸，ビオチンは，結合型を遊離型に完全に消化したのち，上記の方法で測定した．ビタミンCは上記のHPLC法により測定した．ビタミン$B_6$は結合型を遊離型に完全に消化したのち，微生物学的定量法により測定した[13]．

(5) 相対利用率の計算方法

相対利用率の計算方法は前報に記載した[3]．簡略に記すと，規定食摂取時の水溶性ビタミン排泄量（データ1）を規定食中の水溶性ビタミン量で割り，規定食摂取時の水溶性ビタミン排泄率を求めた．遊離型水溶性ビタミン付加時の水溶性ビタミン排泄量（データ2）とデータ1から，遊離型水溶性ビタミン付加による増加分（データ3）を求めた．データ3を遊離型水溶性ビタミン量で割り，遊離型水溶性ビタミンの排泄率を求めた．規定食中の水溶性ビタミンの相対利用率は，規定食摂取時の水溶性ビタミン排泄率を遊離型水溶性ビタミンの排泄率で割って求めた．

## 3. 結　果

(1) 実験1

実験1では，成人女性9名を対象として，パンを主食とした食事に含まれる水溶性ビタミンの相対利用率を求めた．Table 1に示した栄養素組成の食事を女性に摂取させたときの水溶性ビタミンの相対利用率をTable 4に示した．実験1で用いた食事における各ビタミンの相対利用率は，ビタミン$B_1$では58±23%，ビタミン$B_2$では50±22%，ビタミン$B_6$では84±24%，ナイアシンでは57±22%，パントテン酸では70±16%，葉酸では52±20%，ビオチンでは86±13%，ビタミンCでは101±16%であった．

(2) 実験2

実験2では，成人女性5名を対象として実験1の再現性について確認するとともに，成人男性9名をも対象とすることにより相対利用率の男女間の比較を行った．Table 2に示した栄養素組成の食事を男性に，Table 3に示した食事を女性に摂取させたときの水溶性ビタミンの相対利用率をTable 5に示した．実験2で用いた食事における各ビタミンの相対利用率は，ビタミン$B_1$は男性で48±20%，女性で55±10%，ビタ

Table 5. Urinary excretions and relative availability of water-soluble vitamins in Experiment 2

| Vitamins | | Data 1 (μmol/day) | Data 2 (μmol/day) | Data 3 (μmol/day) | Urinary excretion rate for vitamins in the test diet (%) | Urinary excretion rate for free vitamins (%) | Relative availability (%) |
|---|---|---|---|---|---|---|---|
| Vitamin $B_1$ | Male | 0.554±0.207 | 2.654±0.480 | 2.100±0.422 | 13.9±5.2 | 29.5±5.9 | 48±20 |
| | Female | 0.731±0.101 | 3.474±0.569 | 2.743±0.535 | 20.9±2.9 | 38.5±7.5 | 55±10 |
| | Total | 0.628±0.188 | 2.995±0.650 | 2.368±0.558 | 16.8±5.5 | 33.2±7.8 | 51±17 |
| Vitamin $B_2$ | Male | 0.605±0.347 | 4.036±1.612 | 3.431±1.313 | 19.5±11.2 | 37.4±14.3 | 51±17 |
| | Female | 0.394±0.117 | 4.563±1.770 | 4.170±1.674 | 18.5±5.5 | 45.4±18.2 | 43±9 |
| | Total | 0.517±0.288 | 4.256±1.622 | 3.739±1.450 | 19.1±8.9 | 40.8±15.8 | 47±14 |
| Vitamin $B_6$ | Male | 3.48±0.24 | 14.53±1.14 | 11.05±1.07 | 58.2±4.0 | 63.6±6.2 | 92±10 |
| | Female | 2.84±0.54 | 13.48±1.11 | 10.64±0.76 | 53.9±10.3 | 61.2±4.4 | 88±16 |
| | Total | 3.21±0.50 | 14.09±1.21 | 10.88±0.94 | 56.4±7.2 | 62.6±5.4 | 90±12 |
| Niacin | Male | 64.4±12.8 | 261.9±19.0 | 197.5±11.1 | 26.9±5.3 | 43.1±2.4 | 62±11 |
| | Female | 47.9±7.9 | 243.1±47.8 | 195.2±47.1 | 24.1±4.0 | 42.6±10.3 | 59±18 |
| | Total | 57.5±13.6 | 254.1±33.5 | 196.5±29.6 | 25.7±4.8 | 42.9±6.4 | 61±14 |
| Pantothenic acid | Male | 20.2±2.3 | 65.7±3.5 | 45.5±2.2 | 38.0±4.4 | 52.5±2.6 | 72±9 |
| | Female | 13.9±2.4 | 63.9±7.5 | 50.0±5.6 | 35.8±6.2 | 57.6±6.4 | 62±8 |
| | Total | 17.6±4.0 | 65.0±5.3 | 47.4±4.4 | 37.1±5.1 | 54.6±5.1 | 68±10 |
| Folate | Male | 0.033±0.006 | 0.212±0.092 | 0.179±0.089 | 5.8±1.0 | 13.8±6.9 | 51±22 |
| | Female | 0.034±0.005 | 0.255±0.079 | 0.221±0.082 | 6.8±0.9 | 17.1±6.3 | 47±23 |
| | Total | 0.033±0.005 | 0.230±0.086 | 0.196±0.085 | 6.3±1.1 | 15.2±6.6 | 49±21 |
| Biotin | Male | 0.144±0.019 | 0.350±0.085 | 0.206±0.079 | 53.4±7.2 | 71.6±28.5 | 81±22 |
| | Female | 0.093±0.011 | 0.294±0.052 | 0.201±0.050 | 58.7±7.0 | 70.9±17.6 | 86±21 |
| | Total | 0.123±0.031 | 0.326±0.076 | 0.204±0.066 | 55.6±7.3 | 71.3±23.5 | 83±21 |
| Vitamin C | Male | 823±89 | 1,316±121 | 493±98 | 89.5±9.7 | 96.4±19.3 | 96±22 |
| | Female | 844±54 | 1,356±97 | 512±65 | 91.7±5.9 | 100.2±12.8 | 93±11 |
| | Total | 832±74 | 1,332±109 | 501±83 | 90.4±8.1 | 98.0±16.3 | 95±18 |

Data 1: The values are urinary excretions of vitamins when only the diet is fed to the subjects. Data 2: The values are urinary excretions of vitamins when the diet and vitamin mixtures are fed to the subjects. Data 3: The values are calculated "Data 2" − "Data 1." Values are means±SD for 7 for male subjects, 5 for female and 12 for total.

ミン$B_2$は男性で51±17%，女性で43±9%，ビタミン$B_6$は男性で92±10%，女性で88±16%，ナイアシンは男性で62±11%，女性で59±18%，パントテン酸は男性で72±9%，女性で62±8%，葉酸は男性で51±22%，女性で47±23%，ビオチンは男性で81±22%，女性で86±21%，ビタミンCは男性で96±22%，女性で93±11%であった．実験2の女性の相対利用率は，実験1で得られた値と近似したものであった．いずれの水溶性ビタミンにおいても，相対利用率に性差は認められなかった．男女計12名の相対利用率は，ビタミン$B_1$は51±17%，ビタミン$B_2$は47±14%，ビタミン$B_6$は90±12%，ナイアシンは61±14%，パントテン酸は68±10%，葉酸は49±22%，ビオチンは83±21%，ビタミンCは95±18%であった．

## 4. 結　論

本研究では，成人男性7名（19～25歳）および成人女性のべ14名（21～25歳）を対象とし，以下の結果を得た．パンを主食としたときの水溶性ビタミンの相対利用率は，概ね次のような値であった．ビタミン

$B_1$は55%，ビタミン$B_2$は50%，ビタミン$B_6$は85%，ナイアシンは60%，パントテン酸は70%，葉酸は50%，ビオチンは85%，ビタミンCは95%であった．なお，ビタミン$B_{12}$については，主要な排泄経路が尿ではなく腸管であることから[14]，本法では相対利用率を測定することはできないので，他の方法を考案しなければならない．

これらの値は，前報[3]のめしを主食としたときの相対利用率と近似した値であった．

なお，米国人を被験者とし，脂肪エネルギー含量が40%程度の食事ではビタミン$B_6$が75%，パントテン酸が50%程度と報告されている[15]．また，葉酸の生体利用率に関しては，50%程度と報告されている[16]．

本研究は，平成16年度～18年度厚生労働科学研究費補助金・循環器疾患等生活習慣病対策総合研究事業・日本人の食事摂取基準（栄養所要量）の策定に関する研究（主任研究者　柴田克己），および平成19年度厚生労働科学研究費補助金・循環器疾患等生活習慣病対策総合研究事業・日本人の食事摂取基準を改定するためのエビデンスの構築に関する研究―微量栄養素と多量栄養素摂取量のバランスの解明―（主任研究者　柴田克己）を受けて行ったものである．関係各位に謝意を表する．

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# Comparison of the Nicotinamide Catabolism among Rat Strains

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**We discovered markedly differing catabolism of nicotinamide among rat strains. We compared the catabolism of nicotinamide and also that of the other tryptophan-nicotinamide and water-soluble vitamins among the four strains, Wistar, Sprague-Dawley (SD), August-Copenhagen Irish (ACI) and Fischer 344. The major urinary catabolite of nicotinamide was $N^1$-methyl-4-pyridone-3-carboxamide in Wistar, SD and ACI, and $N^1$-methylnicotinamide in Fischer rats. This phenomenon was attributed to the enzyme activity involved in the reaction of $N^1$-methylnicotinamide to $N^1$-methyl-4-pyridone-3-carboxamide being much lower in Fischer than in the other three strains. With the water-soluble vitamins, this specific phenomenon was only observed in the catabolism of vitamin $B_6$; the urinary catabolite, 4-pyridoxic acid, was much lower too. It was found for the first time that the activities of oxidase were lower in Fischer than in the other strains. This study showed that Wistar, SD, ACI strains had similar water-soluble vitamin metabolism including nicotinamide catabolism.**

**Key words:** nicotinamide; rat strain; urine; vitamin $B_6$; water-soluble vitamin

Nicotinamide (Nam) is a unique vitamin because mammals including humans can synthesize it from the essential amino acid, tryptophan. The Trp-Nam metabolic pathway (see Fig. 1) is very important, because over 400 enzymes need NAD or NADP as coenzymes, and elucidation of the mechanism regulating the pathway would provide useful information for maintaining the best health. For example, NAD and NADP are involved in numerous cellular reactions, and are a substrate for the reaction of poly(ADP-ribosyl)ation.[1] Poly(ADP-ribosyl)ation catalyzed by chromatin-associated poly(ADP-ribose)polymerase (PARP-1; EC 2.4.2.30) is related to the regulation of gene expression, cellular differentiation, apoptosis, DNA replication and repair.[2] The intermediates involved on the Trp-Nam pathway are excreted to the urine, and the amount of these substances reflects the changes in the activities of enzymes involved along the pathway. For example, the urinary excretion of $N^1$-methylnicotinamide (MNA), $N^1$-methyl-2-pyridone-5-carboxamide (2-Py) and $N^1$-methyl-4-pyridone-3-carboxamide (4-Py), which are nicotinamide catabolites, can serve as surrogate biological markers of an adequate amino acid intake.[3] The urinary ratio of (2-Py + 4-Py)/MNA is high when rats eat an adequate amino acid diet, and the ratio is low when they eat an inadequate diet, because the activity of liver MNA oxidases decrease with an excess and lack of essential amino acids.[3]

Studies on Trp-Nam metabolism are often performed on humans, but when we cannot use humans as subjects, we almost exclusively use the Wistar strain of rats. We have believed that the findings obtained when using the Wistar strain of rats would be common to all strains of rat. We discovered in this study that the catabolism of nicotinamide markedly differed among rat strains. We therefore investigated whether the urinary excretion of the metabolites involved on the Trp-Nam pathway and the metabolism of water-soluble vitamins differed among strains or not. We measured the urinary excretion of the Trp-Nam metabolites and water-soluble vitamins by the four strains of rat, and compared the metabolism.

## Materials and Methods

*Chemicals.* $NAD^+$ was purchased from Sigma Chemical Company (St. Louis, MO, USA). Vitamin-free milk casein, sucrose, and L-methionine were purchased from Wako Pure Chemical Industries (Osaka, Japan). Corn oil was purchased from Ajinomoto (Tokyo, Japan). Gelatinized cornstarch, the mineral mixture (AIN-93M)[4] and the vitamin mixture (AIN-93-VX containing choline bitartrate)[4] were obtained from Oriental Yeast Co. (Tokyo, Japan).

Thiamin hydrochloride (vitamin $B_1$, $C_{12}H_{17}ClN_4OS$–HCl = 337.27), riboflavin (vitamin $B_2$, $C_{17}H_{20}N_4O_6$ = 376.37), cyanocobalamin (vitamin $B_{12}$, $C_{63}H_{88}CoN_{14}O_{14}P$ = 1355.40), nicotinamide (Nam, $C_6H_6N_2O$ = 122.13), calcium pantothenate (PaA-Ca, $C_{18}H_{32}N_2O_{10}$–Ca = 476.54), folic acid (FA, $C_{19}H_{19}N_7O_6$ = 441.40), D(+)-biotin ($C_{10}H_{16}N_2O_3S$ = 244.31), L(+)-ascorbic acid (AsA, $C_6H_8O_6$ = 176.13), anthranilic acid (AnA, $C_7H_7O_2N$ = 137.14) and quinolinic acid (QA, $C_7H_5O_4N$ = 167.13) were purchased from Wako Pure Chemical Industries. 4-Pyridoxic acid (4-PIC, a vitamin $B_6$ catabolite, $C_8H_9NO_4$ = 183.16) was made by ICN Pharmaceuticals (Costa Mesa, CA, USA) and obtained through Wako Pure Chemical Industries. Xanthurenic acid (XA, $C_{10}H_7O_4N$ = 205.17), kynurenic acid (KA, $C_{10}H_9O_4N$ = 207.19), 3-hydroxyantranilic acid (3-HA, $C_7H_7O_3N$ = 154.14) and MNA chloride ($C_7H_9N_2O$–HCl = 159.61) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). 2-Py ($C_7H_8N_2O_2$ = 152.15) and 4-Py ($C_7H_8N_2O_2$ = 152.15) were synthesized by the methods of Pullman and Colowick[5] and Shibata *et al.*,[6] respectively. All other chemicals used were of the highest purity available from commercial sources.

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*Abbreviations*: Nam, nicotinamide; PARP-1, poly(ADP-ribose)polymerase; MNA, $N^1$-methylnicotinamide; 2-Py, $N^1$-methyl-2-pyridone-5-carboxamide; 4-Py, $N^1$-methyl-4-pyridone-3-carboxamide; PaA, pantothenate; FA, folic acid; AsA, L(+)-ascorbic acid; AnA, anthranilic acid; QA, quinolinic acid; 4-PIC, 4-pyridoxic acid; XA, xanthurenic acid; KA, kynurenic acid; 3-HA, 3-hydroxyantranilic acid; SD, Sprague-Dawley; ACI, August-Copenhagen Irish; F344, Fischer 344; HPLC, high-perfomance liquid chromatography

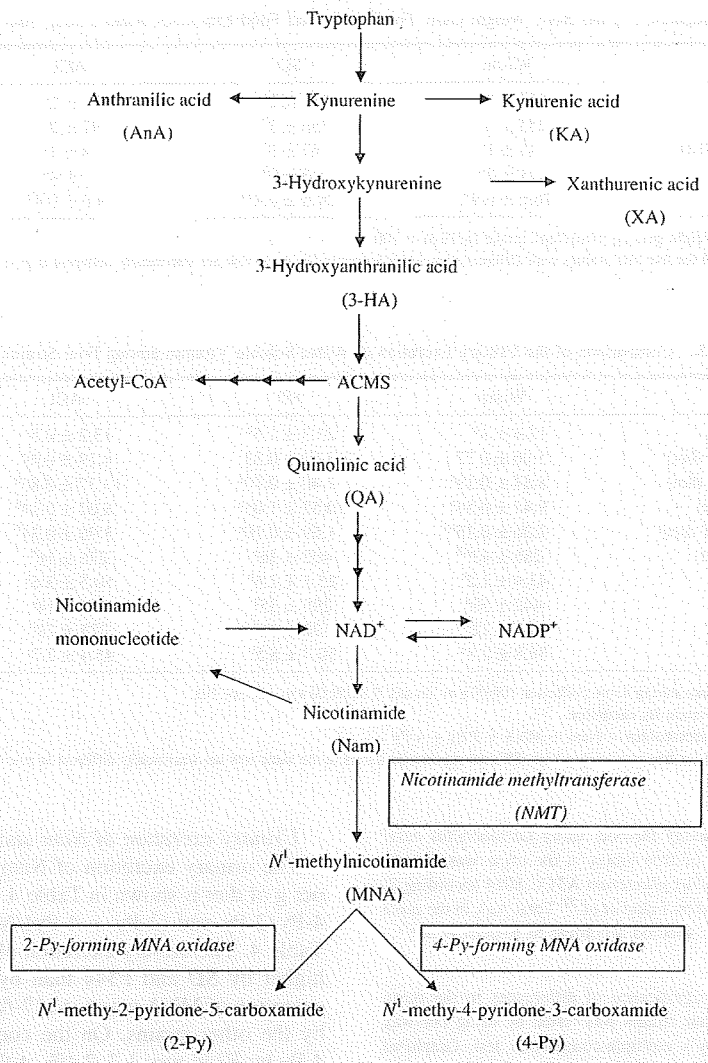


**Fig. 1.** Metabolic Pathway of Tryptophan to Nicotinamide.

**Table 1.** Composition of the Diet

| | (g/100 g of diet) |
|---|---|
| Vitamin-free milk casein | 20.0 |
| L-Methionine | 0.2 |
| Gelatinized cornstarch | 46.9 |
| Sucrose | 23.4 |
| Corn oil | 5.0 |
| Mineral mixture (AIN-93M) | 3.5 |
| NiA-free vitamin mixture (AIN-93-VX containing chorine bitartrate) | 1.0 |

*Animals and diets.* The care and treatment of the experimental animals conformed to The University of Shiga Prefecture guidelines for the ethical treatment of laboratory animals.

The animal room was maintained at a temperature of around 22 °C and at about 60% humidity with a 12-h light (06:00–18:00)/12-h dark (18:00–06:00) cycle. Body weight and food intake were measured daily at around 09:00 a.m., and the diet and water were renewed daily.

Five male rats each of the Wistar, Sprague-Dawley (SD), August-Copenhagen Irish (ACI) and Fischer 344 (F344) strains (8 weeks old each) were obtained from Clea Japan (Tokyo, Japan) and immediately placed in individual metabolic cages (CT-10; Clea Japan). They had free access to the diet (Table 1) for 11 d. Urine samples on the last day (09:00 a.m.–09:00 a.m.; 24-h urine) were collected in amber bottles with 1 ml of 1 M HCl and stored at −20 °C until needed for use. The rats were killed by decapitation after the last urine samples had been collected, a 10-μl sample of blood was taken from the carotid artery for measuring NAD and NADP, and the liver was removed for measuring the enzyme activities involved in the Nam catabolism.

*Analysis.* The contents of Nam, 2-Py, and 4-Py in the urine were simultaneously measured by the high-performance liquid chromatographic (HPLC) method of Shibata *et al.*,[6] while the content of MNA in the urine was measured by the HPLC method of Shibata.[7] The contents of KA,[8] XA,[9] 3-HA,[9] AnA,[10] and QA[11] in the urine were measured by using the HPLC methods.

The concentrations of NAD ($NAD^+ + NADH$) and NADP ($NADP^+ + NADPH$) in the blood were respectively measured by the colorimetric method of Shibata and Murata,[12] and by the method of Shibata and Tanaka.[13]

Thiamin in the urine was measured by the HPLC post-labeled fluorescence method of Fukuwatari *et al.*[14]

The urinary concentration of riboflavin was analyzed according to the method of Ohkawa *et al.*[15] The urinary excretion of 4-PIC, which is a catabolite of vitamin $B_6$, was determined according to the method described by Gregory and Kirk.[16] The cyanocobalamin concentration in the urine was assayed by the microbiological method with *Lactobatillus delbueckii* subsp. *lactis* ATCC 7830.[17] The content of free PaA in the urine was directly measured by using *Lactobacillus plantarum* ATCC 8014.[18] The concentrations of urine folates were

**Table 2.** Comparison of the Body Weight Gain, Food Intake and Food Efficiency Ratio among Four Strains of Rats

| | Wistar | SD | ACI | F344 |
|---|---|---|---|---|
| Initial body weight (g) | $210 \pm 2^{a}$ | $289 \pm 2^{b}$ | $173 \pm 2^{c}$ | $143 \pm 1^{d}$ |
| Final body weight (g) | $255 \pm 3^{a}$ | $356 \pm 3^{b}$ | $197 \pm 2^{c}$ | $184 \pm 2^{d}$ |
| Body weight gain (g/10 d) | $45 \pm 1^{a}$ | $67 \pm 3^{b}$ | $24 \pm 1^{c}$ | $41 \pm 2^{a}$ |
| Food intake (g/10 d) | $171 \pm 2^{a}$ | $223 \pm 9^{b}$ | $127 \pm 4^{c}$ | $133 \pm 3^{c}$ |
| Food efficiency ratio* | $26.6 \pm 0.9^{a}$ | $29.8 \pm 0.4^{a,c}$ | $19.0 \pm 0.4^{b}$ | $31.0 \pm 1.9^{c}$ |

*Food efficiency ratio, body weight gain (g/10 d)/food intake (g/10 d) × 100

Each value is the mean ± SEM for five rats; values with different superscript letters in the same row are statistically different at $p < 0.05$, as determined by Tukey's multiple-comparison test.

**Table 3.** Comparison of the Urinary Excretion of Water-Soluble Vitamin among Four Strains of Rats

| | Wistar | SD | ACI | F344 |
|---|---|---|---|---|
| Food intake* (g/d) | $17.4 \pm 1^{a}$ | $23.3 \pm 1.5^{b}$ | $13.2 \pm 0.5^{c}$ | $15.4 \pm 0.3^{a,c}$ |
| Vitamin $B_1$ (nmol/g of diet) | $5.06 \pm 0.77$ | $3.69 \pm 0.33$ | $4.19 \pm 0.49$ | $4.59 \pm 0.20$ |
| Vitamin $B_2$ (nmol/g of diet) | $8.64 \pm 0.19^{a}$ | $7.40 \pm 0.70^{a}$ | $13.37 \pm 0.83^{b}$ | $6.88 \pm 0.24^{a}$ |
| 4-PIC** (nmol/g of diet) | $9.42 \pm 0.54^{a}$ | $10.52 \pm 1.05^{a}$ | $8.02 \pm 0.74^{a}$ | $2.27 \pm 0.39^{b}$ |
| Vitamin $B_{12}$ (pmol/g of diet) | $2.26 \pm 0.15^{a,c}$ | $1.69 \pm 0.10^{a}$ | $3.96 \pm 0.34^{b}$ | $2.87 \pm 0.13^{c}$ |
| SUM*** (nmol/g of diet) | $286 \pm 43^{a,b}$ | $402 \pm 36^{a}$ | $276 \pm 16^{b}$ | $343 \pm 20^{a,b}$ |
| PaA (nmol/g of diet) | $43.4 \pm 1.2^{a}$ | $25.1 \pm 2.6^{b}$ | $50.5 \pm 3.6^{a}$ | $50.8 \pm 4.0^{a}$ |
| FA (pmol/g of diet) | $442 \pm 50^{a}$ | $295 \pm 35^{b}$ | $156 \pm 14^{c}$ | $87 \pm 6^{c}$ |
| Biotin (pmol/g of diet) | $207 \pm 12^{a,b}$ | $190 \pm 16^{a,b}$ | $223 \pm 28^{b}$ | $138 \pm 17^{a}$ |
| AsA (nmol/g of diet) | $38.8 \pm 2.8^{a}$ | $50.7 \pm 8.5^{a}$ | $45.4 \pm 2.7^{a}$ | $21.3 \pm 1.1^{b}$ |

*This value is for the food intake during urine collection (09:00 a.m. on day 9 to 09:00 a.m. on day 10).

**4-PIC, 4-pyridoxic acid, a vitamin $B_6$ catabolite

***SUM, nicotinamide and its metabolites = Nam + MNA + 2-Py + 4-Py

Each value is the mean ± SEM for five rats; values with different superscript letters in the same row are statistically different at $p < 0.05$, as determined by Tukey's multiple-comparison test.

determined by the microbioassay method, using *Lactobacillus casei* ATCC 7469.[19] The content of free biotin in the urine was directly measured by using *Lactobacillus plantarum* ATCC 8014 according to the agar plate assay developed by Fukui *et al.*[20] Total AsA in the urine was determined by the HPLC method according to Kishida *et al.*[21]

*Statistical analysis.* The significance of differences in the mean concentration between the four strains was tested by using one-way ANOVA followed by Tukey's multiple-comparison test. GraphPad Prism (version 4; obtained from GraphPad Software, San Diego, CA, USA) was used for all statistical analyses.

## Results

*Body weight and food intake*

Table 2 shows the initial and final body weights, body weight gain, food intake and food efficiency ratio during experiments with each strain. The initial body weight differed among the strains, and up to the final day. Since the size differed among the four strains, the food intake, body weight gain and food efficiency ratio differed too. Therefore, in this study the urinary excretion was corrected to 1 g of food intake.

*Urinary excretion of vitamins*

The urinary excretion of vitamin $B_1$, vitamin $B_2$, 4-PIC, vitamin $B_{12}$, Nam and its metabolites (SUM), PaA, FA, biotin and AsA per g of diet, respectively, is shown in Table 3. The urinary excretion of vitamin $B_1$ did not differ among the strains. The urinary excretion of vitamin $B_2$ was significantly higher by ACI than by the other strains. The excretion of both 4-PIC and AsA was significantly lower by F344 than by the other strains. The excretion of PaA was significantly lower by SD than by the other strains. The urinary excretion of vitamin $B_{12}$, FA, biotin and SUM differed among some strains.

*Urinary excretion of Nam and its metabolites*

The urinary excretion of Nam, MNA, 2-Py and 4-Py per g of diet is shown in Table 4. The excretory ratios of 4-Py/2-Py and (2-Py + 4-Py)/MNA are also shown in Table 4. The urinary excretion of Nam was significantly higher by SD and F344 than by ACI and Wistar. The excretion of MNA was 6- to 17-fold higher by F344 than by the other strains. On the contrary, the excretion of 4-Py by F344 was 1.7–2.4% of that by the other strains. The excretion of 2-Py was significantly higher by ACI and SD than by F344 and Wistar.

The results for the Wistar strain showed 4-Py to be the most abundant metabolite, accounting for 83.7% of the urinary excretion of SUM, and was followed by MNA, 6.0%; 2-Py, 5.5%; and Nam, 4.8%. For the SD strain, 4-Py was the most abundant metabolite, accounting for 73.6% of SUM, and followed by MNA, 12.1%; Nam 7.8%; and 2-Py, 6.5%. For the ACI strain, 4-Py was the most abundant metabolite, accounting for 76.4%, and followed by 2-Py, 10%; MNA, 8.6%; and Nam, 5.0%. For the F344 strain, MNA was the most abundant metabolite, accounting for 85.1%, and followed by Nam, 8.0%; 2-Py, 5.4%; and 4-Py, 1.5%. Consequently, for F344, the excretory ratios of (2-Py + 4-Py)/MNA, which is an index of amino acid adequacy and possibly an index of liver MNA oxidase activity, and 4-Py/2-Py were significantly lower than for the other strains.

*Urinary excretion of upper metabolites on the Trp-Nam pathway*

The urinary excretion of AnA, KA, XA, 3-HA and QA per g of diet are shown in Table 4. The order of amount of AnA in urine of each strain was ACI, Wistar, F344, and SD, and a significant difference was observed between ACI and SD. The urinary excretion

**Table 4.** Comparison of the Urinary Excretion of the Metabolites on the Tryptophan-Nicotinamide Pathway among Four Strains of Rats

| | Wistar | SD | ACI | F344 |
|---|---|---|---|---|
| AnA (nmol/g of diet) | $3.99 \pm 0.10^{a,b}$ | $2.94 \pm 0.22^{a}$ | $4.63 \pm 0.57^{b}$ | $3.49 \pm 0.13^{a,b}$ |
| KA (nmol/g of diet) | $78.9 \pm 5.0^{a}$ | $58.9 \pm 2.4^{b}$ | $34.7 \pm 4.6^{c}$ | $57.2 \pm 2.7^{b}$ |
| XA (nmol/g of diet) | $52.5 \pm 1.7^{a}$ | $59.3 \pm 5.9^{a}$ | $24.2 \pm 3.4^{b}$ | $35.3 \pm 1.0^{b}$ |
| 3-HA (nmol/g of diet) | $3.98 \pm 0.32^{a}$ | $1.72 \pm 0.35^{b}$ | $5.05 \pm 0.53^{a}$ | $6.72 \pm 0.39^{c}$ |
| QA (nmol/g of diet) | $28.8 \pm 2.1^{a,b}$ | $42.7 \pm 8.0^{a}$ | $15.3 \pm 1.9^{b}$ | $27.2 \pm 5.2^{a,b}$ |
| Nam (nmol/g of diet) | $13.7 \pm 1.7^{a}$ | $31.5 \pm 4.6^{b}$ | $13.8 \pm 0.7^{a}$ | $27.5 \pm 1.7^{b}$ |
| MNA (nmol/g of diet) | $17.3 \pm 2.0^{a}$ | $48.5 \pm 10.9^{a}$ | $23.7 \pm 2.0^{a}$ | $292.2 \pm 17.4^{b}$ |
| 2-Py (nmol/g of diet) | $15.6 \pm 2.4^{a}$ | $26.0 \pm 1.5^{b}$ | $27.7 \pm 1.8^{b}$ | $18.4 \pm 1.3^{a}$ |
| 4-Py (nmol/g of diet) | $239 \pm 37^{a}$ | $296 \pm 34^{a}$ | $211 \pm 12^{a}$ | $5 \pm 1^{b}$ |
| 4-Py/2-Py | $15.3 \pm 0.4^{a}$ | $11.3 \pm 0.8^{b}$ | $7.6 \pm 0.1^{c}$ | $0.3 \pm 0.1^{d}$ |
| (2-Py + 4-Py)/MNA | $14.58 \pm 0.60^{a}$ | $8.70 \pm 2.30^{b}$ | $10.17 \pm 0.37^{a,b}$ | $0.08 \pm 0.01^{c}$ |
| SUM* (μmol/d) | $4.84 \pm 0.42$ | $9.29 \pm 0.71$ | $3.64 \pm 0.14$ | $5.30 \pm 0.36$ |
| Trp intake** (μmol/d) | $193 \pm 11^{a}$ | $258 \pm 16^{b}$ | $147 \pm 6^{a,c}$ | $171 \pm 3^{c}$ |
| Conversion ratio of Trp-Nam*** (%) | $2.58 \pm 0.38^{a,b}$ | $3.63 \pm 0.32^{a}$ | $2.49 \pm 0.14^{b}$ | $3.10 \pm 1.77^{a,b}$ |

*SUM, Nam + MNA + 2-Py + 4-Py
**Trp intake (μmol/d), food intake (g/d) $\times 0.2 \times 0.875 \times 0.013 \times 1/204 \times 10^{6}$
***Conversion of Trp-Nam (%), SUM (mol/d)/Trp intake (mol/d) $\times$ 100
Each value is the mean ± SEM for five rats; values with different superscript letters in the same row are statistically different at $p < 0.05$, as determined by Tukey's multiple-comparison test.

**Table 5.** Comparison of the NAD and NADP Content in Whole Blood among Four Stains of Rats

| | Wistar | SD | ACI | F344 |
|---|---|---|---|---|
| $NAD^+ + NADH$ (nmol/ml of whole blood) | $72.9 \pm 2.6$ | $70.0 \pm 1.1$ | $74.7 \pm 2.2$ | $73.5 \pm 1.1$ |
| $NADP^+ + NADPH$ (nmol/ml of whole blood) | $11.4 \pm 0.5^{a}$ | $11.7 \pm 0.8^{a}$ | $8.4 \pm 0.7^{b}$ | $11.4 \pm 0.5^{a}$ |

Each value is the mean ± SEM for five rats; values with different superscript letters in the same row are statistically different at $P < 0.05$, as determined by Tukey's multiple-comparison test.

of KA was significantly higher from Wistar than from the other strains, and significantly higher from SD and F344 than from ACI. The excretion of XA was significantly higher from SD and Wistar than from F344 and ACI. The 3-HA excretion was significantly higher from F344 than from the other strains, and higher from ACI and Wistar than from SD. The order of amount of excreted QA was SD, Wistar, F344, ACI, and a significant difference was observed between SD and ACI.

*Conversion ratio of Trp to Nam*

The conversion ratio of Trp to Nam is shown in Table 4. It was calculated from the Trp intake and urinary excretion of SUM. A significant difference was observed between the SD and ACI strains.

*Concentration of NAD and NADP in whole blood*

The concentrations of NAD ($NAD^+ + NADH$) and NADP ($NADP^+ + NADPH$) in whole blood are shown in Table 5. The concentration of NAD did not significantly differ among the strains. The concentration of NADP was significantly lower in ACI than in the other strains, but the total concentration of NAD and NADP did not significantly differ among the strains.

*Enzyme activities involved in Nam catabolism*

The enzyme activities of nicotinamide methyltransferase (NMT), 2-Py-forming MNA oxidase and 4-py-forming MNA oxidase in the liver are shown in Table 6. The enzyme activity of NMT was significantly higher in ACI than in the other strains. The activity of 2-Py-forming MNA oxidase was significantly higher in ACI than in the other strains, and in Wistar and SD than in F344. The activity of 4-Py-forming MNA oxidase in F344 was approximately 1/160 to 1/110 as high as in the other strains.

## Discussion

We have previously studied the metabolism of the Trp-Nam pathway by using the Wistar strain of rats obtained from Clea Japan. Generally speaking, Nam is catabolized to 2-Py and 4-Py *via* MNA in rat liver, the most abundant catabolite being 4-Py which accounts for around 80% in the sum of Nam and its metabolites.[3,22] We discovered by accident that the catabolism of Nam extremely differed among rat species. We chose the four strains of Wistar, SD, ACI and F344 rats to represent normal rats, because these strains are commonly used for experimental purposes. We therefore investigated the metabolic characteristics of vitamins including Nam in the four strains of normal rats, Wistar, SD, F344 and ACI, obtained from Clea Japan.

We recognized that only the F344 rat had characteristic properties: the most abundant catabolite was MNA, accounting for 85%, and the amount of 4-Py was lower than that of 2-Py (middle part of Table 4). In fact, as shown in Table 6, the two MNA oxidase activities in F344 were extremely low compared with the case for

**Table 6.** Comparison of the Enzyme Activities in Nicotinamide Catabolism

| | Wistar | SD | ACI | F344 |
|---|---|---|---|---|
| Liver weight (g) | $11.5 \pm 0.2^a$ | $16.1 \pm 0.6^b$ | $7.1 \pm 0.1^c$ | $7.8 \pm 0.1^c$ |
| NMT* (nmol/h/g of liver) | $77.5 \pm 8.8^a$ | $73.4 \pm 5.4^a$ | $125.2 \pm 5.7^b$ | $77.3 \pm 4.3^a$ |
| 2-Py-forming MNA oxidase (nmol/h/g of liver) | $930 \pm 95^a$ | $753 \pm 186^a$ | $1421 \pm 42^b$ | $107 \pm 55^c$ |
| 4-Py-forming MNA oxidase (nmol/h/g of liver) | $5047 \pm 564^a$ | $3327 \pm 1004^a$ | $4717 \pm 327^a$ | $31 \pm 25^b$ |

*NMT, nicotinamide methyltransferase

Each value is the mean ± SEM for five rats; values with different superscript letters in the same row are statistically different at $p < 0.05$, as determined by Tukey's multiple-comparison test.

the other three strains. Furthermore, the activity ratio of 4-Py-forming MNA oxidase/2-Py-forming MNA oxidase was also much lower in F344. This meant that the MNA oxidases in F344 were not only lower overall than in the other strains, but also that the 4-Py-forming MNA oxidase activity was lower than that of 2-Py-forming MNA oxidase. A similar phenomenon was observed with LEC rats[23] and the Wistar rats fed with an inadequate diet such as low in protein.[3,24] We had already clarified that the activity of 4-Py-forming MNA oxidase was affected by various nutrients.[25–27] So, we carried out a preliminary experiment on F344 rats fed with a high protein diet (40% casein) for 10 d to learn whether the activity of 4-Py-forming MNA oxidase increased or not. We had already shown that the activity increased when Wistar rats were fed with an appropriate diet such as 20% casein.[3] However, the result of the preliminary experiment was negative, and the activity of 4-Py-forming MNA oxidase in F344 rats did not increase even when fed with a 40% casein diet, and the urinary excretory ratio of (2-Py + 4-Py)/MNA did not change either. This result means that the catabolism of Nam in F344 would be rigidly controlled. In this connection, the urinary excretion of 4-PIC, a metabolite of vitamin $B_6$ pyridoxine and pyridoxal, was much lower in F344 than in the other three strains (Table 3). This result can be explained by the fact that the formation of 4-PIC is catalyzed by pyridoxal oxidase. The urinary excretion of AsA tended to be lower, which implies that L-gulonolactone oxidase would be lower in F344 than in the other strains. Several reports have supported the oxidases in F344 being lower. Duclos *et al.*[28] have reported that the cytochrome c oxidase activity in F344 was lower than that in Lewis rats, and Levy *et al.*[29] that the acyl-CoA oxidase activity in F344 was lower that than SD rats. These observations indicate that several oxidases in F344 were much lower than in the other strains of rats. This is characteristic of F344 rats.

Other metabolic characteristics in the water-soluble vitamins of F344 were not apparent, although trivial statistical differences were observed (Table 3). However, we do not disregard the phenomenon that the urinary excretion of folic acid was lower from F344 than from the other three strains.

Although the catabolic fate of Nam was extremely different between F344 and the other three strains, the sum of Nam and its metabolites was almost the same (Table 3). We therefore furthermore investigated the upper part of Nam metabolism, namely, the metabolites on the Trp-QA pathway. Such metabolites as AnA, KA, XA, 3-HA, and QA were marginally different, although this was trivial, because the maximum value for each did not differ 4-fold more than the minimum value. We therefore concluded that the metabolism of Trp-QA among the rats did not differ. In fact, the conversion ratio of Trp to Nam among the strains was no different (lower part of Table 4) and nor were the concentrations of NAD and NADP in the blood (Table 5).

**Table 7.** Comparison of the Urinary Excretory Pattern of Nam and Its Metabolites among Humans and the Four Strains

| | Humans* | Wistar | SD | ACI | F344 |
|---|---|---|---|---|---|
| Nam (%) | N.D.** | 4.8 | 7.8 | 5.0 | 8.0 |
| MNA (%) | 35.2 | 6.0 | 12.1 | 8.6 | 85.1 |
| 2-Py (%) | 56.3 | 5.5 | 6.5 | 10.0 | 5.4 |
| 4-Py (%) | 8.5 | 83.7 | 73.6 | 76.4 | 1.5 |

*This data was taken from ref. 30.

**N.D., not detected

Each value is expressed as a percentage over the sum of Nam and its metabolites (Nam + MNA + 2-Py + 4-Py) in each animal.

Each value is the mean for eleven humans or five rats.

We compared the catabolism of Nam among humans and the four rat strains. Table 7 shows the urinary excretory pattern of Nam and its metabolites over the sum of them (Nam + MNA + 2-Py + 4-Py). In humans, Nam metabolized to MNA, and much of the MNA oxidized to 2-Py, the rate being approximately 65%.[30] In the Wistar, SD, and ACI strains, Nam was almost completely oxidized to 4-Py *via* MNA. In F344, Nam was catabolized to MNA, and a little of the MNA was catabolized into such pyridones as 2-Py and 4-Py, differing from the other strains. In the Wistar, SD, and ACI strains, MNA was efficiently oxidized into the pyridones like in humans, but the ratio of 2-Py/4-Py was the reverse. It was therefore found that the three strains apart from F344 would be suitable models for Trp-Nam metabolism instead of humans.

We further compared the metabolism of water-soluble vitamins among the Wistar, SD and ACI strains. Although trivial differences were apparent, nor essential distinction was observed (Tables 3–5). We concluded that the metabolism of water-soluble vitamins among the strains did not differ.

In summary, the Wistar strain, which our laboratory has usually used, had common metabolism and would reflect the result for the metabolism of the strains, expect for the Nam catabolism in F344 rats. The enzyme activities of various oxidases in F344 rats were extremely low compared with such other strains as Wistar, SD, and ACI. The reactions of MNA to 2-Py, MNA to 4-Py, and pyridoxal to 4-PIC were extremely weak in F344.

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# Effects of UVA Irradiation on the Concentration of Folate in Human Blood

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**Although it is well known that ultraviolet A (UVA) irradiation destroys folate, no definite conclusion for the biological degradation has yet been drawn. In the present study, we determined the effects of UVA exposure on the blood folate concentration *in vitro* and *in vivo*. UVA irradiation reduced the synthesized folate pteroylmonoglutamic acid (PGA) content in the blood, but not 5-methyltetrahydrofolate, a major folate form in the blood stream. Exposure to sunlight also decreased the plasma folate concentration in human subjects who took PGA prior to the exposure, but not in subjects who did not take PGA. These results suggest that UVA exposure destroyed PGA but not 5-methyltetrahydrofolate in human blood *in vivo*.**

**Key words:** folate; ultraviolet A (UVA); pteroylmonoglutamic acid; 5-methyltetrahydrofolate; humans

Folate is a vitamin; therefore, folate deficiency, or impairment of the folate metabolism in humans, leads to several diseases such as megaloblastic anemia,[1] neural tube defects,[2] and increasing the risk of development of cardiovascular diseases.[3] It is well known that the requirement of folate increases in pregnant women.[4] Supplementation with folate during pregnancy is strongly recommended in many countries. Pteroylmonoglutamic acid (PGA) is a synthetic form of folate (Fig. 1) and is the oxidized and most stable form of the folates. Therefore, PGA is commonly used as a folate supplement. However, there is a deficit in masking vitamin $B_{12}$ deficiency by PGA supplementation which is a more severe deficiency than folate deficiency.[5] Vorobey *et al.*[6] have reported that PGA in an aqueous solution was degraded by UVA. Furthermore, Der-Petrossian *et al.*[7] have reported that extracorporeal exposure of plasma to UVA during extracorporeal photophoresis led to photodegradation of folate. We have reported that the folate level in serum was higher in young Japanese women than in young men who had been given a vitamin mixture containing PGA.[8] We discussed that the phenomenon that a lower folate level in men would be a result of bathing in a lot of sunlight compared with women. On the other hand, the serum folate concentration of subjects exposed to UVA has been reported as unchanged.[9] No definite conclusion about the possibility of folate photodegradation *in vivo* has yet been drawn.

*In vivo*, folates exist mainly in the reduced form, for instance as 5-methyltetrahydrofolate (5-MTHF) (Fig. 1).[10] When PGA is taken, some appears in the blood stream in the form of PGA itself,[11] although a part of PGA is converted to 5-MTHF in the tissues of the small intestine. Staindal *et al.*[12] have reported that 5-MTHF absorbed less UVA when compared with PGA, and that 5-MTHF was not destroyed by UVA irradiation, but was by UVB and UVC. However, as UVB and UVC do not penetrate the atmosphere and reach the human skin, 5-MTHF in the blood is not destroyed by exposure to sunlight.

We have the hypothesis that PGA in blood would be destroyed, but not 5-MTHF, by exposure to sunlight. If humans do not take PGA, the blood folate concentration would not be decreased by exposure to sunlight, because the form of the blood folate is mainly 5-MTHF. On the contrary, if humans take PGA, the blood concentration would be decreased by exposure to sunlight, because the PGA taken appears as PGA itself in the blood stream. We conducted an experiment to prove this hypothesis, and as we were able to achieve some valid results.

## Materials and Methods

*Subjects.* Healthy Japanese college students aged from 21 to 24 years old participated in the present experiments. They did not have regular use of medications or dietary supplements, or any habitual alcohol or cigarette consumption. This study was reviewed and approved by The Ethical Committee of the University of Shiga Prefecture.

*Chemicals.* PGA and 5-MTHF calcium salt were purchased from Wako Pure Chemical Industries (Osaka, Japan), and from Schircks Laboratories (Jona, Switzerland), respectively.

*Experiment 1 (Change of PGA in an aqueous solution by UVA irradiation).* An aqueous solution of 49 μM PGA was made, 200 μl of the solution was put into the wells of a microtiter plate (Sumilon multiwell plate, MS-8496F, 0.4 ml × 96 wells, flat bottom), and the plate was irradiated with UVA light (EBF-140L/J, Spectronics Corporation; the wavelength was 365 nm) for 0, 30, 60, 90, or 120 min at room temperature. The UVA dose in this study was 0, 800, 1600, 2400, and 3200 mJ/cm$^2$, respectively. The respective residual amount of PGA was measured by an HPLC method and microbiological assay recently described.

*Experiment 2 (Changes of 5-MTHF in an aqueous solution by UVA or UVB irradiation).* An aqueous solution of 20 μM 5-MTHF was made, 200 μl of the solution was put into wells of a microtiter plate

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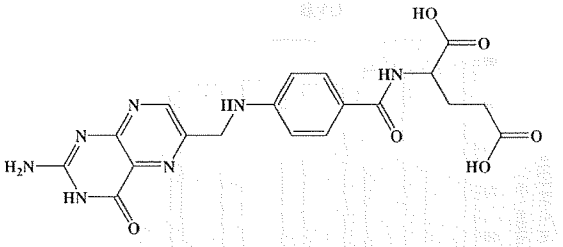
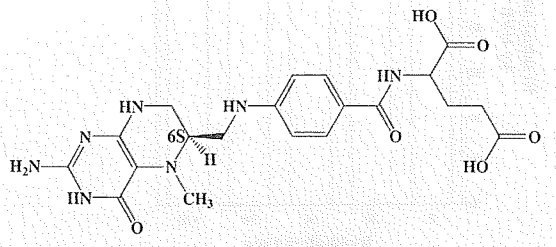
*Abbreviations*: PGA, pteroylmonoglutamic acid; 5-MTHF, 5-methyltetrahydrofolic acid; UVA, ultraviolet A; UVB, ultraviolet B; UVC, ultraviolet C



Folic acid = pteroylmonoglutamic acid = $C_{19}H_{19}N_7O_6$ = 441.4



5-Methyltetrahydrofolic acid = $C_{20}H_{23}CaN_7O_6$ =497.5

**Fig. 1.** Structures of Folic Acid and 5-Methyltetrafolic Acid.

(Sumilon multi-well plate, MS-8496F, 0.4 ml × 96 wells, flat bottom), and the plate was irradiated with UVA light (EBF-140L/J, Spectronics Corporation; the wavelength was 365 nm) or UVB light (EBF-260C/J, Spectronics Corporation; the wavelength was 312 nm) for 0, 30, 60, 90, or 120 min at room temperature. The UVA dose in this study was 0, 800, 1600, 2400, and 3200 mJ/cm$^2$, respectively, and the UVB dose was 0, 900, 1800, 2700, 3600 mJ/cm$^2$, respectively. The respective residual amount of 5-MTHF was measured by an HPLC method.

*Experiment 3 (Change of the folate concentration in blood by UVA irradiation* (in vitro *experiment)).* Blood was taken from the venous vain at 09:00 before breakfast from Japanese college students (4 males and 7 females), who ate freely, but had not taken any PGA supplements, by using a syringe coated with EDTA. A 3-ml amount of the blood in a plate (Sumilon dish, $\phi$60 × 15 mm) was irradiated with UVA light (EBF-140L/J, Spectronics Corporation; the wavelength was 365 nm) for 120 min at room temperature. The UVA dose was 3200 mJ/cm$^2$. As a control, the dish was placed in the dark. The folate was measured by a microbioassay. To correct the vaporization under processing, the amount of protein in the blood was measured.

*Experiment 4 (Change of the folate concentration in blood, to which PGA or 5-MTHF has been added, by UVA irradiation* (in vitro *experiment)).* Blood was taken from the venous vein at 09:00 before breakfast from Japanese college students (7 males and 4 females), who ate freely, but had not taken any vitamin supplements, by using a syringe coated with EDTA. One milliliter of 110 μM PGA or 110 μM 5-MTHF was added to 6 ml of the blood taken and mixed well. Three milliliter of each sample was withdrawn from the PGA- or 5-MTHF-added blood, and the sample in a plate (Sumilon dish, $\phi$60 × 15 mm) was irradiated with UVA light (EBF-140L/J, Spectronics Corporation; the wavelength was 365 nm) for 120 min at room temperature. The UVA dose was 3200 mJ/cm$^2$. As a control, the dish was placed in the dark. The folate was measured by a microbioassay. To correct the vaporization under processing, the amount of protein in the blood was measured.

*Experiment 5 (Comparison of the folate concentrations in blood between male and female young adults who ate freely).* The subjects were 23 male and 32 female students who ate freely. Blood was taken from the venous vein before lunch at around 12:00. The folate concentration was measured by a microbioassay.

*Experiment 6 (Change of the folate concentration in blood, withdrawn from the subjects who took no PGA supplements, by sunlight exposure* (in vivo *experiment)).* The subjects were 9 male (average (± SD) age, height, body weight, and BMI were 23.6 ± 2.7 years, 173.7 ± 4.6 cm, 69.2 ± 8.7 kg, and 22.9 ± 2.7 kg/m$^2$) and 14 female (average (± SD) age, height, body weight, and BMI were 21.8 ± 2.4 years, 160.0 ± 4.0 cm, 51.7 ± 4.4 kg, and 20.0 ± 1.3 kg/m$^2$) students who ate freely. Blood was taken from the venous vein before and after sunlight exposure at 11:00 and 13:00, respectively. The subjects with short trousers and the tank tops were exposed to sunlight from 11:00 to 13:00 in the summer. The dose of UVA was about 19,000 mJ/cm$^2$. The folate concentration was measured by a microbioassay.

*Experiment 7 (Change of the folate concentration in blood, withdrawn from the subjects who took PGA, by sunlight exposure* (in vivo *experiment)).* The subjects were 7 female students. Their

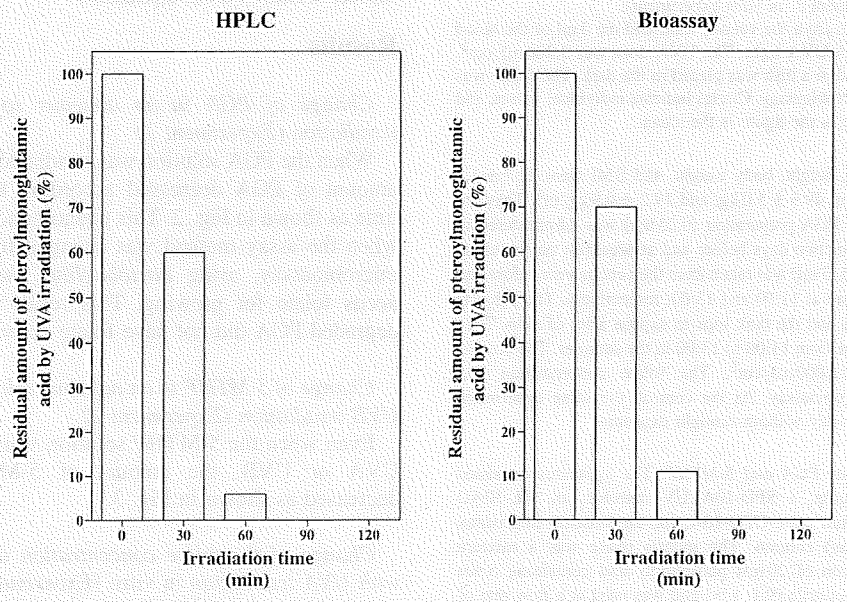



**Fig. 2.** Change of PGA in an Aqueous Solution by UVA Irradiation (Experiment 1).
A 200-μl amount of 49 μM PGA was irradiated with UV light for 0, 30, 60, 90, and 120 min. The respective residual amount of PGA was measured by an HPLC method and a microbiological assay. Each value is the mean of two separate experiments.

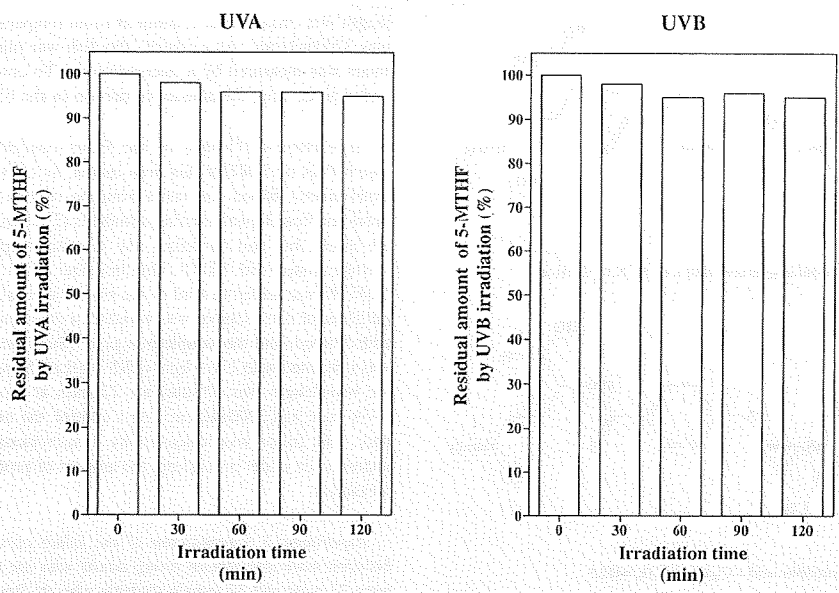



**Fig. 3.** Changes of 5-MTHF in an Aqueous Solution by UVA and UVB Irradiation (Experiment 2).
A 200-μl amount of 20 μM 5-MTHF was irradiated with UVA or UVB light for 0, 30, 60, 90, and 120 min at room temperature. The UVA dose in this study was 0, 800, 1600, 2400, and 3200 mJ/cm$^2$, respectively, and the UVB dose was 0, 900, 1800, 2700, and 3600 mJ/cm$^2$, respectively. The respective residual amount of 5-MTHF was measured by an HPLC method. Each values is the mean of two separate experiments.

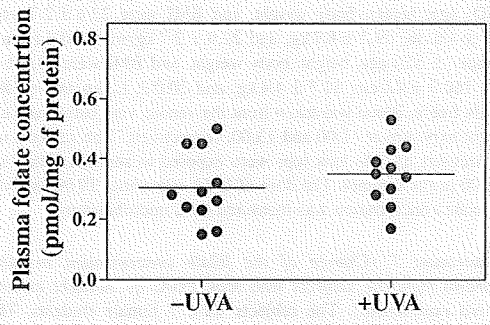



**Fig. 4.** Change of the Folate Concentration in Human Blood by UVA Exposure (Experiment 3, *in vitro* experiment).
Blood was taken from the venous vein, and the 3 ml of the blood was irradiated with UVA light. The UVA dose was 3,200 mJ/cm$^2$. As a control, blood in a dish was placed in the dark. The folate was measured by a microbioassay. Circles indicate individual values, and the horizontal line in the figure is the mean.

average (± SD) age, height, body weight, and BMI were 22.7 ± 1.8 years, 160.9 ± 5.5 cm, 49.9 ± 5.0 kg, and 19.2 ± 0.9 kg/m$^2$. They ate freely, however, the PGA preparation (0.25 mg) was administered to them at each meal for two days before and at breakfast on the blood collection day. Blood (3 ml) was taken from the venous vein before and after sunlight exposure at 11:00 and 13:00, respectively. The subjects put on short trousers and the tank tops to expose a lot of skin. They bathed in the sunlight from 11:00 to 13:00 in the summer. The dose of UVA was about 12,000 mJ/cm$^2$. The folate concentration was measured by a microbioassay. As the control, the same experiment was done on another day without sunlight exposure.

*HPLC methods for PGA and 5-MTHF.* The apparatus consisted of an LC-10AD pump, a SPD-10A UV detector, an SIL-10AD auto-injector, a column oven CTO-10A, and an Shiseido Superiorex ODS ($\phi$4.6 × 250 mm) column. The mobile phase was a mixture of a degassed solution of 20 mM phosphoric acid containing 5 mM hexanesulfonate-acetonitrile (9:1, v/v) and was used at a flow rate of 1.0 ml/min. The column temperature was maintained at 40 °C, and the UV detector was set at 280 nm. The HPLC system was interfaced with a Shimadzu Chromatopac C-R8A instrument for data processing.

*Microbioassay.* Plasma was obtained from EDTA-treated blood by centrifuging at 3,000 × *g* for 5 min at 4 °C. The plasma obtained was directly used for a microbioassay using *Lactobacillus rhamnosus* ATCC 27773.[13]

*Protein determination.* Protein concentration was determined by a BioRad protein assay, with bovine serum albumin as the standard.

*Statistical analysis.* The computer program, GraphPad Prism version 4.03 (GraphPad Software, San Diego, USA) was used for data analysis. The D'agostino and Pearson omnibus normality test showed that the blood folate concentration in experiments 3, 4, 5 and 6 was normally distributed, and the Shapiro-Wilk normality test showed this in experiment 7. Statistical significance was assessed by two-tailed paired Student's *t* test in experiments 3, 4, 6 and 7, and by a two-tailed unpaired Student's *t* test in experiment 5.

## Results

*Change of PGA in an aqueous solution by UVA irradiation (Experiment 1)*

When the PGA solution was irradiated with UVA, the amount of PGA decreased according to the exposure time as shown in Fig. 2. This phenomenon was observed when the assay method was changed from HPLC to a microbioassay, using *Lactobacillus rhamnosus* which needs folate for growing. This result means that the degraded PGA did not have folate activity.

*Change of 5-MTHF in an aqueous solution by UVA or UVB irradiation (Experiment 2)*

Even when the 5-MTHF solution was irradiated with UVA or UVB, the amount of 5-MTHF was not decreased as shown in Fig. 3.

*Change of the folate concentration in human blood with UVA irradiation* in vitro *(Experiment 3)*

The blood was withdrawn from college students, and the EDTA-treated blood was directly exposed to UVA for 120 min. However, the folate concentra-