Metabolic Fate of the Precursors of *N*-Nitroso Compounds (III)

Urinary Excretion of Nitrate in Man

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Urinary excretion rate of nitrate in man was studied by determining the amount of nitrate ingested with foods and beverages and that excreted into urine for 24 hr and 48 hr.

The mean nitrate concentration $\pm$ S.D. in the urine ($n=33$) which were collected from 11 subjects at random was 74.4 ± 42.5 ppm. The excretion amounts of nitrate in the 24 hr urine obtained from 9 subjects who ingested conventional diet containing from 44.1 to 864.2 mg of nitrate was from 45.6 to 236.9 mg, indicating a remarkable and individual differences of urinary excretion rate of nitrate (27.4 to 133.8%). The excretion amount of urinary nitrate every 2 hr in two subjects was less than 10 mg when only the low-nitrate diet containing a small amount of nitrate was ingested, but increased more than 25 mg every 2 hr by the ingestion of 200 mg of sodium nitrate or 100 g of salted Chinese cabbage, containing 75.1 mg of nitrate, with the low-nitrate diet. The range of the urinary excretion rates of nitrate for 48 hr was from 78.2 to 86.2% ($n=3$). No nitrite was detected in any urine specimens, and neither nitrate nor nitrite was detected in any feces ($n=6$).

1. Introduction

Epidemiological studies have revealed that there was a very high incidence of stomach cancer among the residents at Worksop in the United Kingdom,¹⁾ at Narino in Colombia²⁾ and at Talca in Chile,³⁾ where there were high levels of nitrate in drinking water. Elevated risk of stomach cancer among Japanese in Hawaii was known for Issei and Nisei who were users of pickled vegetables, especially salted Chinese cabbage, and dried/salted fish.⁴⁾ The most frequent consumers of those showed the highest risk. Salted Chinese cabbage contains high level of nitrate,⁵⁾ and fish and its products contain dimethylamine.^{6,7)}

For producing the carcinogenic *N*-nitroso compounds, the reduction of nitrate to nitrite and the reaction of nitrite with dimethylamine or other nitrosatable compounds are required. Nitrate ingested was reduced to nitrite in human saliva,⁸⁻¹¹⁾ and also in human stomach¹²⁾ and bladder^{13,14)} infected with nitrate-reducing

microorganisms. Nitrate may also be reduced to nitrite in human intestines if it arrives.

In this paper the authors described the estimation of urinary excretion rate of nitrate in men by determining the amount of nitrate ingested with foods and beverages and that excreted into urine.

2. Experimental

2.1 Low-nitrate diet

Low-nitrate diet was prepared with food-stuffs containing small amounts of nitrate. During the experimental period and on the day before the experimental day, only the low-nitrate diet was taken to keep the concentration of nitrate in urine at low and constant level.

2.2 Ingestion of nitrate

On the experimental day, 200 mg of sodium nitrate or 100 g of salted Chinese cabbage containing 75.1 mg of nitrate was ingested with the low-nitrate diet at the breakfast.

2.3 Collection of foods and beverages to be ingested and urine and feces excreted

The foods and the beverages to be ingested for 24 hr (from 7 or 8 a.m. to the same time on next day) and the urine and the feces excreted for 24 hr on the same day were collected in each bottle containing a small amount of toluene.

2.4 Determination of nitrate and nitrite

a) Diet: Nitrate and nitrite in the diet were extracted by the method of Harada.^{*3} The mixture of the foods and the beverages collected for the 24 hr study was homogenized with a definite volume of water. To 10 g of the homogenate, 0.5 g of diastase (from *A. oryzae*, Tokyo Kasei Co., Ltd.) and 50 ml of water were added. After the incubation at 70°C for 30 min, 5 ml of 12% zinc sulfate solution and 5 ml of 2% sodium hydroxide solution were added. Then the reaction mixture was heated at 80°C for 10 min. After cooling, 10 ml of 10% ammonium acetate solution which was adjusted to pH 9.0 was added and the volume was made to 100 ml with water. The reaction mixture was filtered through a filter paper. Nitrate and nitrite in the filtrate were determined by the method described by Harada, *et al.*⁸⁾

b) Urine: Nitrate and nitrite in urine were determined by the method for the determination of those in saliva described by Ishiwata, *et al.*⁹⁾

c) Feces: Feces were homogenized with a definite volume of water. Nitrate and nitrite in 2 g of the homogenate were determined by the method described in urine.

3. Results

3.1 Concentration of urinary nitrate

The urine specimens were collected three times from six men and five women on different days. The menu of diet on the day when urine was collected was not restricted. The results are shown in Table 1.

The mean value and S.D. of the concentrations of urinary nitrate ($n=33$) was 74.4 ± 42.5 ppm, and the maximum and minimum concentrations were 170.8 and 12.6 ppm, respectively. No nitrite was detected in any

Table 1. Concentration of Urinary Nitrate

Subject	Sex	Age	Urinary nitrate (ppm)		
A	M	33	170.8	96.0	97.9
B	M	37	70.0	52.5	33.1
C	M	41	89.1	112.9	129.1
D	M	49	104.6	128.4	121.5
E	M	50	71.7	33.6	52.4
F	M	51	159.8	76.3	47.1
G	F	27	126.2	106.2	30.2
H	F	27	20.4	27.3	47.6
I	F	30	56.4	76.8	63.5
J	F	30	36.3	16.2	12.6
K	F	31	64.6	20.9	102.9
Mean $\pm$ S.D.			74.4 $\pm$ 42.5		

No nitrite was detected in any urine specimens.

urine specimens.

The concentrations of urinary nitrate shown in Table 1 were higher than those of the residents in the area where the nitrate concentration in the drinking water supply was less than 4 ppm nitrate-nitrogen, but lower than those of the residents in the area where the drinking water contained 21 ppm nitrate-nitrogen when compared with the data reported by Hawksworth and Hill.¹⁵⁾

3.2 Urinary excretion rate of nitrate during the ingestion of conventional diet

The amounts of nitrate ingested with foods and beverages and that excreted into urine for 24 hr in 9 subjects are shown in Table 2. The ranges of ingestion and excretion amounts of nitrate were from 44.1 to 864.2 mg and from 45.6 to 236.9 mg, respectively, and that of urinary excretion rates of nitrate was from 27.4 to 133.8%, indicating remarkable and individual differences of urinary excretion rate of nitrate. No nitrite was detected in any urine specimens.

3.3 Change of the urinary excretion amount of nitrate after the ingestion of sodium nitrate

At the breakfast on the experimental day, 200 mg of sodium nitrate was ingested by a 33-year old man. The low-nitrate diet was ingested on both the experimental and the control days. The urine specimens were separately collected every 2 hr except for sleeping

*3 Harada, M.: unpublished

Table 2. Daily Ingestion and Urinary Excretion of Nitrate

Subject	Age	Sex	Ingestion		Excretion		Disap- pearance	Rate		Ingestion
			Food ^{a)} (kg)	Nitrate (mg)	Urine (L)	Nitrate (mg)	Nitrate (mg)	Excretion (%)	Disap- pearance (%)	Nitrite (mg)
A	37	M	1.98	44.1	1.01	59.0	-14.9	133.8	-33.8	N.D. ^{b)}
B	41	M	2.35	86.9	1.00	53.3	33.6	61.3	38.7	1.3
C	50	M	2.73	90.6	1.23	57.8	32.8	63.8	36.2	0.4
D	52	M	1.93	121.2	1.10	80.1	41.1	66.1	33.9	N.D.
E	25	F	1.71	87.5	0.75	45.6	41.9	52.1	47.9	N.D.
F	27	F	1.80	864.2	0.83	236.9	627.3	27.4	72.6	N.D.
G	30	F	2.24	405.1	0.98	146.1	259.0	36.1	63.9	1.0
H	30	F	1.50	99.1	0.86	52.7	46.4	53.2	46.8	N.D.
I	31	F	1.75	165.4	1.05	150.5	14.9	91.0	9.0	0.6

^{a)} Foods and beverages^{b)} Less than 0.1 mg

No nitrite was detected in any urine specimens.

Fig. 1. Change of urinary excretion amount of nitrate after the ingestion of sodium nitrate

↑: ingestion of 200 mg of sodium nitrate with low-nitrate diet

↑: ingestion of low-nitrate diet

↓: collection of feces

The subject was a 33-year old man. The amount of urinary nitrate during sleeping time was shown as 2-hr equivalent. No nitrite was detected in any urine specimens, and neither nitrite nor nitrate was detected in any feces.

time.

The urinary excretion amount of nitrate increased by the ingestion of sodium nitrate (Fig. 1). In contrast, no increase was observed on the control days. The amounts of nitrate ingested were 311.1 mg on the former experimental period (1st and 2nd days) and 325.8 mg on the latter experimental period (3rd and 4th days), and those excreted into urine were 243.5 mg and 281.0 mg, respectively. The ex-

cretion rates of nitrate were 78.3% on the former and 86.2% on the latter (Table 3). No nitrite was detected in any urine specimens, and neither nitrate nor nitrite was detected in any feces for 4 days.

3.4 Change of the urinary excretion amount of nitrate after the ingestion of salted Chinese cabbage

At the breakfast on the experimental day, 100 g of salted Chinese cabbage was ingested with the low-nitrate diet by a 33-year old man and a 31-year old woman.

The excretion patterns of nitrate into the urine of both subjects (Fig. 2) were almost similar to that of a man who ingested 200 mg of sodium nitrate (Fig. 1). The amount of nitrate ingested for the experimental period (2 days) in the male subject was 250.6 mg and that excreted was 192.7 mg. No nitrite was detected in any urine specimens, and neither nitrate nor nitrite in any feces was detected for 2 days. Similar result was observed in the female subject. In this case, sampling of feces was omitted.

4. Discussion

Hill *et al.*¹⁾ estimated that a weekly intake of nitrate was 980 mg (140 mg/day) for the residents at Worksop, an area of high incidence of stomach cancer, and 440 mg (63 mg/day) for the residents at the control towns. White¹⁰⁾ estimated that a daily intake of nitrate was

Table 3. Urinary Excretion Rates of Nitrate after Ingestion of Sodium Nitrate

Day	Nitrate (mg)			Rate (%)	
	Ingestion	Excretion	Disappearance	Excretion	Disappearance
1st ^{a)}	231.4	169.2	62.2	73.1	26.9
2nd ^{b)}	79.7	74.3	5.4	93.2	6.8
3rd ^{a)}	238.5	189.2	49.3	79.3	20.7
4th ^{b)}	87.3	91.8	-4.5	105.2	-5.2
Former period ^{c)}	311.1	243.5	67.6	78.3	21.7
Latter period ^{d)}	325.8	281.0	44.8	86.2	13.8

The conditions were as described in Fig. 1.

^{a)} Low-nitrate diet plus 200 mg of sodium nitrate (experimental day)

^{b)} Low-nitrate diet (control day)

^{c)} Total of 1st and 2nd days

^{d)} Total of 3rd and 4th days

No nitrite was detected in any urine specimens, and neither nitrate nor nitrite was detected in any feces.

Fig. 2. Change of urinary excretion amount of nitrate after the ingestion of salted Chinese cabbage

—: male, 33-year old

···: female, 31-year old

↑: ingestion of 100 mg of salted Chinese cabbage with low-nitrate diet

↑: ingestion of low-nitrate diet

↓: collection of feces (only male subject)

The amount of urinary nitrate during sleeping time was shown as 2-hr equivalent. No nitrite was detected in any urine specimens, and neither nitrite nor nitrate was detected in any feces.

99.8 mg in the U.S. The established daily intake of nitrate was 293.3 mg for the residents in Tokyo.^{*4} In the present study, the amount

of daily ingestion of nitrate was from 44.1 to 864.2 mg.

It was also reported that the mean value of urinary nitrate for the residents at Paddington in London, a low-nitrate area, was 62 ppm, and in contrast that for the residents at Worksop, a high-nitrate area, was 161.2 ppm.¹⁷⁾ The urinary excretion rates of nitrate intubated by a stomach tube in four rats were 90% in two rats and 63 and 42% in other two rats within 8 hr.¹⁵⁾ Hill and Hawksworth¹⁷⁾ also reported the time course of the urinary excretion of nitrate in guinea-pigs. In human subjects as shown in this paper, the time course of the urinary excretion of ingested nitrate was almost similar to the results obtained in guinea-pigs.¹⁷⁾ The excretion rates of nitrate in 48-hr urine in a subject who ingested 200 mg of sodium nitrate with the low-nitrate diet were 78.3 and 86.2%. Almost the same excretion patterns were observed in two subjects who ingested 100 g of salted Chinese cabbage with the low-nitrate diet. Although the excretion rates varied when the subjects ingested conventional diet, most of nitrate disappeared might be reduced to and/or *via* nitrite because nitrate was not excreted into feces.

It is suspected that there is a few affecting factors on the urinary excretion rate of nitrate, namely, sweat excretion of nitrate^{*5} and conversion of nitrite to nitrate in the stomach.¹⁸⁾

*4 The Research Group on the Ingestion of Nitrate and Nitrite: unpublished.

*5 Ishiwata, H: unpublished.

Table 4. Urinary Excretion Rates of Nitrate after Ingestion of Salted Chinese Cabbage

Subject	Day	Nitrate (mg)			Rate (%)	
		Ingestion	Excretion	Disappearance	Excretion	Disappearance
Male	1st ^{a)}	147.0	139.8	7.2	95.1	4.9
	2nd ^{b)}	103.6	52.9	50.7	51.1	48.9
	Total ^{c)}	250.6	192.7	57.9	76.9	23.1
Female	1st ^{a)}	272.2	231.8	40.4	85.2	14.8

The conditions were as described in Fig. 2.

^{a)} Low-nitrate diet plus 100 g of salted Chinese cabbage (experimental day)

^{b)} Low-nitrate diet (control day)

^{c)} Total of 1st and 2nd days

No nitrite was detected in any urine specimens, and neither nitrate nor nitrite was detected in any feces.

The concentrations of nitrate in sweat specimens ($n=2$) were 9.2 and 5.8 ppm, and those in urine simultaneously collected from the same subject were 100 and 112 ppm, respectively.*⁵ The ratios of the concentrations of nitrate in sweat to those in urine were 9.3 and 5.2%. On the contrary, a portion of nitrite intubated was changed to nitrate in the stomach contents of guinea-pigs,¹⁸⁾ but the ingested amount of nitrite was too small as compared with that of nitrate. Therefore the excretion rate of nitrate in to urine may be not so affected by these positive and negative factors, because these factors would be comprised in the variation of the excretion rates. Then the available nitrate in human body may be estimated in the rate ranging from 13.8 to 23.1% of the ingested amount. The organs in which nitrate is reduced are considered to be the mouth, especially saliva,^{9-11,19)} the small intestines, and the large intestines if nitrate arrives.²⁰⁾ Additionally, nitrate is reduced in human stomach¹²⁾ and bladder^{13,14)} which are infected with bacteria. The excretion rate of nitrate may decrease in these cases.

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