accuracy (A_2 or B_2 value) and by using these values the pharmacokinetical analysis might be possible.

TABLE VI. Recovery of DKA-9 and Its Metabolites in Human Urine according to GLC Separative Determination Method Established ($n=4$)

	Added (μg/ml)	Found (μg/ml±S.D.)
DKA-9	5.0	5.08±0.32
DKA-24	5.0	5.10±0.17
DKA-24S	20.0	19.71±1.18
DKA-24OG	10.0	9.50±0.78
DKA-9G[a)]	50.0	48.28±1.52
DKA-24G[a)]	10.0	9.68±2.16

a) Recovery experiments were carried out according to the method of alkaline hydrolysis.

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Seasonal Variation in Urinary Excretion of Aminopyrine and Its Metabolites in Man[1)]

TSUYOSHI GOROMARU,[2a)] KENJI MATSUYAMA, ATSUKO NODA,[2)] and SADAO IGUCHI[2b)]

Faculty of Pharmaceutical Sciences, Kyushu University[2)]

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The data of urinary excretion of aminopyrine and its metabolites were collected for two years using eight of male healthy volunteers. In conclusion, individual differences among subjects were remarkable. Besides, seasonal variations were demonstrated statistically.

Keywords——aminopyrine and its metabolites; human urinary excretion; individual difference; seasonal variation; data collected for 2 years

Since 1974, we have examined the fate of aminopyrine (AM) in man by means of gas chromatography (GC) for measuring concentration of AM and its metabolites in cooperation with male healthy volunteers in our laboratory. The previous papers indicated the individual variability for urinary and plasma levels of AM and its metabolites.[3,4)]

The present study provides the additional supporting evidence for inter- and intraindividual variations in AM metabolism and preliminary observations relative to a genetic factor in acetylation and/or environmental factor in oxidative metabolism. The data in the present report are very valuable from the standpoint of clinical pharmacy and pharmacology in order to establish a proper dosing schedule for a patient.

1) This work was partly presented at 8th Symposium of Drug Metabolism and Action, Hiroshima, November 1976.

2) Location: *Maedashi 3-1-1, Higashi-ku, Fukuoka, 812, Japan*; *a*) Present address: *Tokyo College of Pharmacy, 1432-1, Horinouchi, Hachioji-shi, Tokyo, 192-03, Japan*; *b*) To whom communications should be directed.

3) T. Goromaru, A. Noda, K. Matsuyama, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **24**, 1376 (1976).

4) T. Goromaru, K. Matsuyama, A. Noda, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **26**, 33 (1978).

Materials and Methods

Chemicals——Aminopyrine (AM), 4-aminoantipyrine (AA), 4-monomethylaminoantipyrine (MAA), 4-acetylaminoantipyrine (AcAA), 4-formylaminoantipyrine (FAA) and N,O-bistrimethylsilylacetamide (BSA) used in this report were obtained as described in the previous paper.[3]

Human Experiment——Eight male healthy volunteers in our laboratory, who were distributed between the ages of 24 and 54, participated in these investigations. They were permitted to live as usual except for taking other drugs and drinking alcohols. They were given 100 mg of AM in the form of aqueous solution orally in the morning after 12 hr fasting. The ingestion of breakfast was permitted at 2 hr after drug administration. After obtaining a baseline sample, urine was collected at 0—2, 2—4, 4—6, 6—8, 8—12, 12—24, 24—36 and 36—48 hr. The subsequent administration was not performed within at least 2 months.

Gas Chromatography (GC)——AM and its metabolites were assayed on a glass column (1 m × 3 mm inner diameter) packed with 1.5% OV-17 on Shimalite W (80—100 mesh) in Shimadzu Model GC-4BM-PF gas chromatograph equipped with a hydrogen flame ionization detector. Temperature of the injector, detector, and column oven was set at 250°, 250° and 225°, respectively. Carrier gas was nitrogen (flow rate: 20 ml/min).

Assay Procedure——Being the same method in the previous paper,[3] the assay procedure was abbreviated in the present paper.

Results and Discussion

AM and its metabolites in urine specimens from eight healthy male volunteers were measured by GC method from 1974 to 1976. The amounts of urinary products, intact AM, MAA, AA, AcAA and FAA, excreted during the 48 hr period after oral administration of AM are summarized in Table I. Since 100 mg of AM was administered, the excreted amount also indicates the percent excretion of the amount of each product to the administered dose.

As shown in Fig. 1 which is a bar chart of Table I, the variable amounts of AM and its metabolites indicated the characteristic behavior in AM metabolism. From the excreted amount of intact AM which is less than 1% of the dose in all subjects' urine, it was concluded that AM is easy to accept oxidative metabolism, *i.e.* demethylation and was independent of

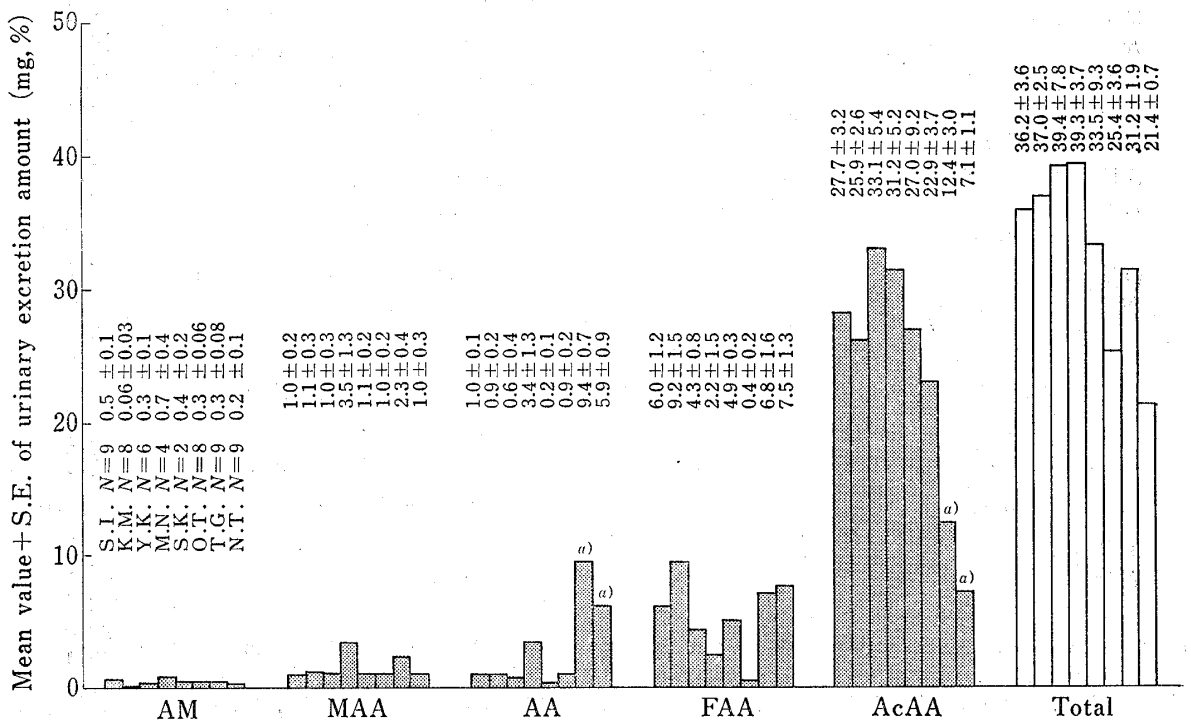

Fig. 1. Individual Differences of Urinary Excretion Amount of Each Metabolite during 48 hr following the Oral Administration of AM 100 mg

a) Slow acetylator.

Table I. Urinary Excretion Amount of AM and Its Metabolites during 48 hr following the Oral Administration of AM (100 mg)

Subject		Excreted amount (mg)					Total (mg, %)
		AM	MAA	AA	FAA	AcAA	
S.I. (54)	A	0.6	1.6	1.3	2.5	13.6	19.6
$N=9$		0.2	0.5	1.1	9.7	21.7	33.2
	B	0.8	1.2	1.0	6.7	38.1	47.8
		0.9	0.4	0.6	8.4	25.0	35.3
	C	0.0	1.1	1.0	2.4	43.1	47.6
		0.3	0.2	0.4	7.5	33.7	42.1
		0.6	2.0	1.9	11.9	31.6	48.0
	D	0.2	1.4	1.1	2.4	20.9	26.0
		0.8	0.4	0.7	2.8	21.8	26.5
	Mean±S.E.	0.5±0.1	1.0±0.2	1.0±0.1	6.0±1.2	27.7±3.2	36.2±3.6
K.M. (24)	A	0.0	2.1	1.4	4.0	20.1	27.6
$N=8$		0.0	0.5	0.6	7.4	19.8	28.3
	B	0.0	1.7	1.6	0.0	36.2	39.5
		0.2	0.7	0.4	8.7	26.5	36.5
	C	0.1	0.8	0.5	7.4	31.3	40.1
		0.1	0.8	0.4	14.4	33.8	49.5
	D	0.0	0.9	0.7	24.4	15.2	41.2
		0.1	1.5	1.2	6.9	23.9	33.6
	Mean±S.E.	0.06±0.03	1.1±0.3	0.9±0.2	9.2±1.5	25.9±2.6	37.0±2.5
Y.K. (27)	A	0.2	1.1	0.8	6.3	14.6	23.0
$N=6$	B	0.1	2.2	0.9	0.0	54.4	57.6
		0.4	0.3	0.5	3.0	28.1	32.3
	C	0.2	0.5	0.3	7.7	36.9	45.6
	D	0.9	1.1	0.5	5.9	32.5	40.9
		0.2	0.9	0.4	3.1	32.1	36.7
	Mean±S.E.	0.3±0.1	1.0±0.3	0.6±0.4	4.3±0.8	33.1±5.4	39.4±7.8
M.N. (24)	A	0.6	1.5	1.4	6.4	19.6	29.5
$N=4$	B	0.0	6.6	1.2	0.0	32.0	39.8
	C	2.0	1.0	0.0	0.0	44.6	47.6
	D	0.1	4.8	4.2	2.4	28.6	40.1
	Mean±S.E.	0.7±0.4	3.5±1.3	3.4±1.3	2.2±1.5	31.2±5.2	39.3±3.7
S.K. (40)	B	0.2	1.3	0.3	4.6	17.8	24.2
$N=2$	C	0.6	0.9	0.1	5.1	36.1	42.8
	Mean±S.E.	0.4±0.2	1.1±0.2	0.2±0.1	4.9±0.3	27.0±9.2	33.5±9.3
O.T. (32)	A	0.6	1.4	0.8	1.6	13.0	17.4
$N=8$		0.5	0.5	0.7	0.4	11.9	14.0
	B	0.2	1.3	0.7	0.0	12.7	14.9
		0.2	0.6	0.9	0.3	25.6	27.6
	C	0.1	0.9	0.5	0.0	26.2	27.7
		0.5	0.3	0.2	0.0	24.3	25.3
	D	0.2	1.8	1.7	0.6	26.5	30.8
		0.2	0.8	1.3	0.4	42.6	45.3
	Mean±S.E.	0.3±0.06	1.0±0.2	0.9±0.2	0.4±0.2	22.9±3.7	25.4±3.6
T.G. (32)	A	0.4	4.7	10.7	7.3	3.6	26.7
$N=9$		0.1	1.9	11.9	7.2	6.7	27.8
	B	0.1	2.6	8.3	0.0	31.1	42.1
		0.7	2.6	7.9	6.2	4.6	22.0
	C	0.2	0.9	6.4	5.4	19.2	32.1
		0.0	2.0	10.4	6.0	15.8	34.2
	D	0.4	1.0	7.7	18.0	5.8	32.9
		0.2	3.3	8.3	6.9	10.7	29.4
		0.6	1.7	12.7	3.9	14.4	33.3
	Mean±S.E.	0.3±0.08	2.3±0.4	9.4±0.7	6.8±1.6	12.4±3.0	31.2±1.9

Subject		Excreted amount (mg) AM	MAA	AA	FAA	AcAA	Total (mg, %)
N.T. (23)	A	0.9	0.5	3.9	12.0	6.2	23.5
$N=9$		0.1	0.9	10.2	6.1	2.1	19.4
	B	0.3	3.0	5.5	3.2	9.2	21.2
		0.1	0.9	4.2	3.9	10.9	20.0
	C	0.0	0.4	2.3	8.3	9.6	20.6
		0.1	0.4	3.0	12.0	4.9	20.4
		0.2	0.5	6.0	12.3	5.4	24.4
	D	0.1	1.8	7.9	3.9	11.0	24.7
		0.2	0.4	7.6	6.1	4.5	18.8
	Mean±S.E.	0.2±0.1	1.0±0.3	5.9±0.9	7.5±1.3	7.1±1.1	21.4±0.7

a) A; Jan., Feb., Mar. B; Apr., May, Jun. C; Jul., Aug., Sep. D; Oct., Nov., Dec.
b) ( ) the age as of May, 1974.

the individual difference. On the contrary, intra- and interindividual variations were remarkable on the excretion of FAA which was derived from MAA by oxidation.[5] As to AA and AcAA, there is a relationship between them, *i.e.* two subjects (N.T., T.G.) excreted more amount of AA and less amount of AcAA, while six subjects excreted less amount of AA and more amount of AcAA. The total amounts of urinary products during 48 hr were affected by the amount of AcAA, because AcAA was dominant in AM metabolites and the total amount were less than 50% of the administered dose.

In order to examine a genetic factor of acetylation, isoniazid (INAH, 100 mg) was orally administered in the form of aqueous solution to five volunteers. The results shown in Table II indicated that the degree of AA acetylation was parallel to that of INAH acetylation controlled by the genetic factor. From the metabolic data of AM and INAH, it is deduced that N.T. and T.G. may be slow acetylators.

Table II. Urinary Excretion of Acetyl Metabolite of INAH following the Oral Administration

Subject	Excreted Amount (mg) INAH	AcINAH	Total (INAH+AcINAH)	Recovery (%, 24 hr)	AcINAH/Total (%)	AcAA/AA+AcAA (%)[b]
K.M.	6.5	50.5	57.0	57.0	88.6	91.5
S. I.	9.5	61.8	71.3	71.3	86.7	92.4
T.G.	34.5	39.8	74.3	74.3	53.6	30.7
N.T.	42.0	16.0	58.0	58.0	27.6	24.0
O.T.	23.3	26.0	49.3	49.3	52.7	85.0

a) Dose amount: INAH 100 mg. All values were determined by calculation from the converted value into INAH.
b) These data were already reported in the previous paper.[3]

The data of seasonal variation in the urinary excretion of AM and its metabolites collected for two years are shown in Fig. 2 as a bar chart. For convenience' sake, one year was devided into four terms (A: January to March, B: April to June, C: July to September, D: October to December).

As to each metabolite except intact AM, seasonal variation was observed. The excretion of AcAA is of particular interest, because there seems to be a relationship between excreted

5) A. Noda, T. Goromaru, N. Tsubone, K. Matsuyama, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **24**, 1502 (1976).

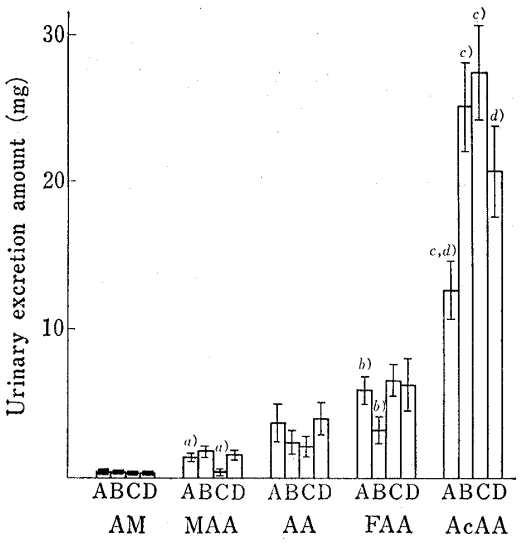

Fig. 2. Classification of AM and Its Metabolites periodically after the Oral Administration of AM 100 mg

A: Jan., Feb., Mar. (N=12)
B: Apr., May, Jun. (N=14)
C: Jul., Aug., Sep. (N=16)
D: Oct., Nov., Dec. (N=14)
a) $p<0.02$ (A *vs.* C)
b) $p<0.05$ (A *vs.* B)
c) $p<0.005$ (A *vs.* B, A *vs.* C)
d) $p<0.05$ (A *vs.* D)

amount of AA and that of AcAA. The same tendency was observed not only in seasonal variation but also in interindividual difference mentioned above. Therefore, acetylation seems to be controlled both by genetic factor and environmental factor. Seasonal variation was also observed in the excretion of MAA and FAA.

According to *t*-test, the excreted amount of AcAA in term A was significantly smaller than that in term B ($p<0.005$), term C ($p<0.005$) or term D ($p<0.05$), respectively. The excreted amount of MAA in term A was significantly larger than that in term C ($p<0.02$). As for FAA, significant variation was not observed each other in the cases of term A, term C and term D, but the excreted amount only in term B was significantly less ($p<0.05$). However, it is quite interesting that the value of MAA, precursor of FAA, increased in term B.

The data collected for two years demonstrated that the individual variations of AM metabolism depend on the enzyme activity on acetylation of AA to AcAA and/or oxidation of MAA to FAA. The hepatic microsomal enzyme metabolizing system is particularly sensitive to many environmental and genetically controlled factors. Further investigations are in progress to assess the importance of "temperature" as one of the environmental factors. The results will be reported in the nearest future.