Excretion of 1- and 3-Methylhistidine by Human Subjects After Oral Administration of L-Histidine.* (29359)

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Histidine and its methylated forms, 1-methylhistidine and 3-methylhistidine, are normal constituents of human urine(1-3). According to Stein *et al*(4), the amount of protein ingested influences the amount of 1-methylhistidine excreted, but otherwise little is known of the primary sources of these two methylated histidine compounds in human urine. In the following study subjects were fed various amounts of L-histidine for different lengths of time, and amounts of urinary histidine, 1-methylhistidine, 3-methylhistidine, anserine, and carnosine determined.

Methods. Subjects and diets. Five normal adult males between 20 and 30 years of age were fed diets containing protein from beef, milk, vegetables, and fruit; although protein intake varied among subjects, ranging from 0.9 to 1.3 g per kilo, it was constant for each subject. After a dietary stabilization period that varied in length with each subject, 3 were fed single doses of 10 g of L-histidine (free base) dissolved in orange juice with their morning meal; a fourth received 7.5 g. After a second period of stabilization on the basic diet, one of the above subjects was fed 1.7 g of L-histidine, divided into 2 daily doses for 3 days; another received a like amount for 6 days. The fifth subject received 3.4 g of L-histidine, divided into 2 daily doses, for 7 days. *Urine collections and analysis.* Continuous 24 hour collections of urine were made. Phenol and HCl were added to the collection bottles and the urine was kept refrigerated. Aliquots were prepared for assay by the method of Stein(2) and analyzed for histidine, 1-methylhistidine, 3-methylhistidine, anserine, and carnosine by the technique of Spackman *et al*(5).

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Results. Table I gives the amounts of histidine, 1-methylhistidine, 3-methylhistidine, anserine, and carnosine excreted by the 5 subjects fed various amounts of L-histidine.

Ingestion of free L-histidine resulted in an increase in histidine excretion ranging from 0.6% to 6.3% of the administered dose except for one subject, F, who showed no increase in excretion while receiving 1.7 g of histidine daily for 3 days. He did, however, excrete 2.9% of the histidine given him as a single 10 g dose (Table I).

No consistent effect on the excretion of 1-methylhistidine was noted when histidine was given either as a single large dose for one day or in smaller doses over a period of days.

3-Methylhistidine excretion was depressed in subjects K, F, and S by the 10 g single dose of histidine. No consistent effect was noted when smaller amounts were fed over a period of days.

Excretion of the β-alanyl-1-methylhistidine dipeptide, anserine, by subjects K and F remained essentially the same when they were given a 10 g single dose of histidine; subject P, who received 3.4 g daily for 7 days showed a decrease. Urinary excretion of the unmethylated dipeptide, β-alanyl-histidine or carnosine, showed an increase ranging from 3 mg to 17 mg daily in 5 of 7 instances after histidine administration (Table I).

Discussion. Brown *et al*(6) fed uniformly labeled L-histidine-C^{14} to human subjects and found 0.01% of the administered activity excreted in the 1-methylhistidine fraction within 5 hours. The low amount of activity would indicate that this substance is not a major urinary end-product of histidine metabolism. In the present experiment approximately one-half of the subjects excreted more 1-methylhistidine after ingesting L-histidine,

TABLE I. Excretion of Histidine and Some Histidine Containing Compounds After Oral Ingestion of Histidine by Human Subjects Fed Constant Diets.

Subject	Histidine fed, g	Compound excreted, mg per 24 hr				
		Histidine	1-Methyl-histidine	3-Methyl-histidine	Anserine	Carnosine
K	— *	92	50	54	16	35
	10 †	246	45	47	15	42
	— ‡	94	42	46	11	33
F	—	122	47	58	44	44
	10 †	412	50	38	42	42
	—	260	46	44	40	39
S	—	128	76	68	—‖	27
	10 †	425	59	57	—	30
	—	105	62	58	—	20
Sc	—	268	72	54	—	20
	7.5†	897	96	54	—	37
	—	651	82	66	—	34
P	—	102	38	26	32	18
	3.4§	156	40	44	8	32
	—	109	39	46	60	22
K	—	67	41	54	—	12
	1.7§	76	39	47	—	21
	—	72	42	47	—	30
F	—	101	43	50	—	23
	1.7§	100	43	53	—	22
	—	102	41	48	—	31

* Day before histidine ingestion.
† Given in one dose, for one day only.
‡ Day after histidine ingestion.
§ Given daily, in 2 divided doses, for 7, 3, and 6 successive days, respectively, for subjects P, K, and F. Average excretion values for these periods are given.
‖ Either could not be detected on chromatogram or was not calculated for technical reasons.

the other half excreted less. The lack of constancy in results cannot be explained by variation in diet as all subjects were eating weighed diets constant in protein intake. If urinary 1-methylhistidine arises from histidine, the 10 g single dose of this amino acid given to 3 of the subjects should have been sufficient to increase the amount of the methylated amino acid excreted, but 2 showed a decrease and the third an increase of only 3 mg. Thus the data confirm the conclusion drawn from the study of Brown *et al*(6).

Neither would 3-methylhistidine seem to be a major end-product of histidine metabolism. As with 1-methylhistidine, excretion was erratic from subject to subject and the only constant relationship to the oral ingestion of histidine noted was a depressed excretion in the 3 subjects given the 10 g single dose of histidine.

McManus(7) showed that DL-histidine-2-C^{14} was well utilized for carnosine synthesis in the muscle of rabbits. In the present study histidine ingestion increased the amount of carnosine excreted, and it is possible this may be one mechanism used by the human being for excreting unwanted histidine, although from the slight increases noted it is an inefficient one. On the other hand, anserine, a dipeptide in which the histidine is methylated, was decreased slightly in the urine of 2 subjects, and substantially decreased in a third by feeding histidine. Because of technical difficulties inherent in the analytical method used, the effect, unfortunately, could not be determined for all of the subjects.

The results indicate that, in general, human beings do not respond to single large doses of histidine, or to repeated smaller ones, by increases in urinary methylated forms of the amino acid, either free or combined in the dipeptide anserine.

Orally ingested histidine does influence the amount of histidine excreted but the percentage of the ingested dose appearing in the urine seems to be more dependent on the individual subject than on the amount of the amino acid ingested, *e.g.*, the subject receiving 7.5 g of histidine excreted 6.3%, while the 3 subjects receiving 10 g excreted 3% or less.

Studies of the urinary content of other imidazoles after histidine administration to human subjects are in progress.

Summary. Oral L-histidine given to human subjects fed weighed diets constant in protein content had no consistent effect on the urinary content of 1-methylhistidine and 3-methylhistidine; the amounts of carnosine excreted increased somewhat, while anserine excretion decreased. The methylated forms of histidine, either free or combined in the dipeptide anserine, do not appear to be primary urinary end-products of histidine metabolism in human beings.

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Antibody Response in Splenectomized Monkeys.* (29360)

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Previous studies from this laboratory(1) and elsewhere(2-4) showed that antibody response in splenectomized humans after subcutaneous inoculation of particulate bacterial antigens was usually not different from that in non-splenectomized controls. Rowley(5), however, observed a depressing effect of splenectomy on hemolysin production in humans inoculated intravenously with sheep erythrocytes. Similarly, intravenous inoculations of heterologous erythrocytes in splenectomized rabbits(6,7) and rats(8,9) were followed by poor antibody response. This led to the assumption that splenectomy may depress antibody response after intravenous, but not after subcutaneous inoculation of antigen(10).

This report compares antibody response of intact and splenectomized monkeys after intravenous inoculation of typhoid vaccine and sheep erythrocytes, and after subcutaneous inoculation of tularemia and typhoid vaccines.

Materials and methods. Young pre-pubertal monkeys (*Macaca mulatta*) were splenectomized under pentothal-ether anaesthesia, and search made for accessory spleens.† Antigens used were triple-washed 2% or 20% sheep erythrocytes, triple typhoid vaccine (Wyeth) diluted to contain 25 or 50 million typhoid bacilli per ml, and Foshay phenolized tularemia vaccine containing 7.5 billion organisms per ml.† Intravenous inoculations were made into the saphenous vein, and subcutaneous inoculations in the lateral thigh. Blood for serologic study was drawn from the femoral vein before inoculation, and at 12 weekly intervals. Tularemia agglutination tests were performed as previously described(11). Typhoid "H" and

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