an index of lathyrogenicity. The administration of BAPN and BHPN together appeared to give rise to saline-soluble collagen levels higher than those produced by either compound alone.

None of this series of BHPN compounds gave rise to ruptured aortae, nor were any gross aneurysms found in any of the sacrificed animals. However, occasional microscopic sections of aortae were found which showed disorganized or fragmented elastic fibers in the media (Hornowski stain). This was true of some animals fed each of the BHPN compounds tested, indicating that each of these substances can cause at least mild aortic damage.

Not tested was the methyl-substituted BHPN having a methyl group on the terminal nitrogen atom of the hydrazino group. This compound would be of particular interest in view of the hypothesis that osteolathyrogenic agents act by blocking aldehydic crosslinking in collagen(5).

Summary. A new series of compounds, β-hydrazinopropionitrile (BHPN) and certain of its methyl derivatives, have been found to have osteolathyrogenic properties for the rat. They cause skeletal deformities (spinal curvatures, periosteal new bone formation), increased saline-soluble collagen levels in sponge biopsy tissue, and occasional mild damage to the aortic media. Neither ruptured aortae nor gross dissecting aneurysms were observed. The most potent lathyrogens of this series are BHPN itself and α-methyl-BHPN. They appear to be comparable in lathyrogenicity to BAPN, but are more toxic than the latter. Less potent was β-methyl-BHPN with N^1-methyl-BHPN being the least potent. N^2-methyl-BHPN (methyl on terminal N of hydrazino group) was not tested.

Addendum. Although we are still unable to prepare any N^2-alkyl-BHPN's, we have recently prepared the N^2-acetyl-BHPN by acetylating BHPN with either acetyl chloride or with acetic anhydride. Chemical tests and IR spectra confirm acetylation on the terminal hydrazino nitrogen. Treatment with HCl converts this compound to aminopyrazoline hydrochloride with the loss of acetic acid. The N^2-acetyl-BHPN is lathyrogenic in our tests and seems to have roughly the same potency as the N^1-methyl-BHPN described above.

We are grateful to Dr. R. V. Milliser of our Dept. of Pathology for confirming our microscopic observations; to Dr. Charles L. Bell, College of Pharmacy, Univ. of Illinois, for nuclear magnetic resonance and infrared spectral analyses; to Abbott Laboratories, North Chicago, Ill., for generous gifts of BAPN fumarate; and to Ezra Chavez for conscientious technical assistance. Elemental analyses were made by Micro-Tech Laboratories, Skokie, Ill.

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Received November 20, 1964. P.S.E.B.M., 1965, v118.

Anserine, A Source of 1-Methylhistidine in Urine of Man.* (29950)

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The amount of 1-methylhistidine excreted in human urine is not influenced by administration of histidine(1) but may be increased when large quantities of meat protein are ingested(2,3,4). When rabbit meat was fed to 2 human subjects an increased excretion of anserine and/or methylhistidine resulted(5).

* Supported in part by Nutrition Foundation, Inc. Grant 319 and by U.S.P.H.S. Grant R01-AM-08780-01 GM.

The 2 compounds were not separated by the paper chromatographic method employed. These results suggested anserine (beta-alanyl-1-methyl-L-histidine) as a possible dietary source of urinary 1-methylhistidine. The amount of anserine found in animal muscles which form part of the diet of man varies widely. This dietary variation may be responsible for the wide ranges of urinary 1-methylhistidine reported for normal subjects (2,3,4). To determine the influence of dietary constituents on 1-methylhistidinuria in man, the effects of feeding chicken, which contains high concentrations of anserine(6), and of feeding anserine were studied.

Methods. Four men and seven women without abnormality of intestinal or renal function were studied. Two of the women were in the last trimester of pregnancy, a period associated with an aminoaciduria. In the nonpregnant, menstruating women, collections were made during the first 12 days of the cycle, thus avoiding the post-ovulation aminoaciduria(7). Urine was collected initially for 24 hours during which unregulated diets were eaten. Following this control period, each subject ate chicken, without regard either to quantity or to proportion of white or dark meat, as the meat source for the evening meal for 3 days. Another urine sample was collected for 24 hours starting the morning of the third day of the chicken diet.

An adult male subject was fed, on separate days, meals consisting of 250 g of broiled beef, 210 g of broiled beef, 210 g of broiled white chicken meat, or 1 g vegetable protein. The calculated protein contents of 250 g of beef and of 210 g of chicken were equal(8). Urine was collected for 5 hours following each of the test meals. An aliquot of the chicken fed to this subject was broiled, extracted with perchloric acid, and assayed chromatographically for anserine content(6). In the same subject the effects of feeding anserine nitrate,† 0.5 g in a single dose, was also tested.

Urines were preserved during collection with 10 cc of 6 N HCl per specimen and between time of collection and analysis by freezing at —10°C. Urinary basic amino

† General Biochemical Co. (chemically pure).

TABLE I. Effect of a Chicken Diet on Urinary Excretion of 1-Methylhistidine, Other Basic Amino Acids, Anserine, Total and Alpha-Amino Nitrogen in 11 Human Subjects.

Subject*	Surface area (M^2)	1-Methylhistidine† (mg/24 hr)		3-Methylhistidine† (mg/24 hr)		Histidine† (mg/24 hr)		Anserine† (mg/24 hr)		Lysine† (mg/24 hr)		Total nitrogen† (g/24 hr)		Alpha-amino nitrogen† (mg/24 hr)	
1	2.0	48	310	92	145	159	194	131	166	53	41	14.2	12.4	246	379
2	2.0	125	338	109	56	160	97	+	+	50	35	15.0	15.1	258	313
3	1.8	42	207	101	92	106	134	100	83	37	47	12.8	13.4	252	278
4	1.9	111	164	18	44	207	81	127	80	43	21	15.7	7.6	259	312
5	1.5	19	111	45	46	78	120	55	89	19	21	8.4	8.7	229	248
6	1.4	22	134	36	87	43	92	37	64	26	56	6.2	8.6	206	214
7	2.0	21	116	56	50	61	58	68	86	22	20	7.5	8.9	205	194
8	1.8	71	167	76	70	127	113	84	123	20	24	6.7	9.2	281	235
9	2.0	64	128	38	44	77	92	54	115	17	27	7.0	7.9	171	232
10	1.8	12	187	58	85	150	210	69	60	54	78	8.0	11.2	297	337
11	1.6	122	277	77	105	427	307	+	+	178	98	11.5	9.8	381	336
Mean		60	194	64	75	145	136	81	96	47	42	10.3	10.3	252	280
± S.D.		±41	±76	±28	±32	±111	±74	±33	±32	±46	±25	±3.6	±2.5	±55	±60
p value		<.001		>.1		>.1		>.1		>.1		>.1		>.1	

* Subjects 1-4—males, ages 30-56; 5-9—females, ages 22-49; 10-11—females, 29 in last trimester of pregnancy.
† Nonchicken diet data followed by chicken diet data. ‡ Anserine not determined due to technical difficulties.

TABLE II. Effect of Various Diets, Anserine, and Fasting on Urinary Excretion of 1-Methylhistidine, Other Basic Amino Acids, Anserine, and Total and Alpha-Amino Nitrogen in One Subject.

Diet	1-Methyl-histidine (mg/5 hr)	3-Methyl-histidine (mg/5 hr)	Histidine (mg/5 hr)	Anserine (mg/5 hr)	Lysine (mg/5 hr)	Total nitrogen (g/5 hr)	Alpha-amino nitrogen (mg/5 hr)
210 g chicken protein	176	71	42	27	26	3.5	65
.5 g anserine nitrate	68	9	23	18	7	3.6	57
210 g beef protein	20	38	25	30	4	3.2	53
250 g beef protein	33	28	50	29	34	2.7	36
1 g vegetable protein	8	13	34	40	12	2.4	36
Fasting	6	8	13	5	5	5.2	36

TABLE III. Effect of Ingestion of Anserine Nitrate and of Fasting on Urinary Excretion of 1-Methylhistidine, Other Basic Amino Acids, Anserine, and Total and Alpha-Amino Nitrogen in One Subject for 24 Hours.

Diet	1-Methyl-histidine (mg/24 hr)	3-Methyl-histidine (mg/24 hr)	Histidine (mg/24 hr)	Anserine (mg/24 hr)	Lysine (mg/24 hr)	Total nitrogen (g/24 hr)	Alpha-amino nitrogen (mg/24 hr)
.5 g anserine nitrate	172	68	97	62	29	10.1	213
Fasting	12	24	31	16	15	8.6	194

acids were separated by ion exchange column chromatography(9) and determined by the technique of Rosen(10). Urinary total nitrogen was determined by micro-Kjeldahl analysis and alpha-amino nitrogen estimated by a modification of the ninhydrin method of Meyer(11).

Results. The amounts of basic amino acids, anserine, total nitrogen and alpha-amino nitrogen excreted by 11 subjects during the periods when chicken was ingested and while an *ad libitum* diet containing no chicken was eaten are shown in Table I. Excretion of histidine, 3-methylhistidine, lysine, anserine, alpha-amino and total urinary nitrogen varied among the subjects, but the chicken diet produced no consistent changes in these parameters. The chicken diet resulted in an increased excretion of 1-methylhistidine in the urine in each subject. The mean increase was significant ($p<0.001$)(12).

Chicken, fed as a single meal to one subject, resulted in an increased excretion of 1-methylhistidine compared with feeding either the same amount of beef or feeding an amount of beef containing a similar quantity of protein (Table II). The anserine content of an aliquot of broiled chicken was 30 micromoles per gram, the 1-methylhistidine content was 1.2 micromoles per gram, and no 3-methylhistidine was detected. The calculated anserine content in 210 g of chicken ingested as the test meal was 1504 mg, containing 1059 mg of 1-methylhistidine. The total available 1-methylhistidine excreted in the urine in 5 hours following the chicken meal thus approximated 16% of the available 1-methylhistidine.

Anserine nitrate, fed as a single meal to the same subject, resulted in an increased excretion of 1-methylhistidine compared with a period of fasting (Table II). The anserine content of 0.5 g of anserine nitrate was 397 mg of which 280 mg was 1-methylhistidine. The additional 1-methylhistidine excreted in the urine in 5 hours following the anserine nitrate meal amounted to 22% of the available 1-methylhistidine. The urines collected for 24 hours were also analyzed following ingestion of anserine nitrate and during fasting (Table III). The increase in urinary 1-methylhistidine after anserine ingestion together with the increase in urinary 3-methylhistidine and histidine expressed as 1-methylhistidine was 274 mg. This compared with the 280 mg of 1-methylhistidine available in the ingested anserine.

Discussion. Chicken fed to human subjects resulted in a significant 1-methylhistidinuria. The anserine contained in muscle appears to be the primary source of 1-methylhistidine in the urine since feeding of pure anserine

resulted in a similar 1-methylhistidinuria. Dietary variation in the anserine content of foods thus may be responsible for the reported variations in amounts of 1-methylhistidine excreted. Control of anserine content of diets is of importance in studies of aminoaciduria or of 1-methylhistidinuria.

The mechanisms whereby anserine is digested, absorbed, and excreted are unknown. No studies of anserinase activity of human gut are available, although a muscle anserinase has been detected in other species(13). The almost stoichiometric excretion of 1-methylhistidine as urinary 1-methylhistidine, 3-methylhistidine, and histidine following ingestion of anserine by one subject suggests either that the 1-methylhistidine does not enter protein metabolism other than for demethylation or isomerization, or that it displaces histidine moieties from the amino acid pool.

One of the components of anserine, beta-alanine, is not transported by hamster intestine(14). Thus, anserine appears to be a dipeptide made up of one poorly absorbed amino acid, beta-alanine, and one amino acid, 1-methylhistidine, which is absorbed and excreted rapidly. Studies of anserine digestion should aid in elucidating various aspects of the absorption of peptides and amino acids.

Summary. Chicken fed to men and women resulted in significant increases in the amount of 1-methylhistidine excreted in the urine. The source of the 1-methylhistidine is the anserine (beta-alanyl-1-methyl-L-histidine) contained in chicken muscle.

The authors wish to acknowledge the technical assistance of Miss Lillian Tybuszewski.

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Received November 23, 1964. P.S.E.B.M., 1965, v118.

Presence of Neutralizing Antibody for Myxovirus Parainfluenza 3 In Sheep Sera.* (29951)

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Since 1954, when the first of the parainfluenza viruses was described, the members of this group have increased to form 4 main serologic groupings, and knowledge of their distribution in nature has expanded. Chanock *et al*(1) have reviewed the antigenic types and some of the natural hosts of these viruses. Types 1-4 all infect man, while only types 1-3 seem to possess animal analogues. Type 1 (HA-2) has a related strain, Sendai virus, which infects the pig and mouse. Type 2 (CA) has 2 related strains infecting monkeys, SV-5 and SV-41 viruses. Type 3 (HA-1) has a related strain infecting cattle, SF-4 virus. Ditchfield *et al*(2) claim the isolation of a type 3 strain from horses.

* This project was supported in part by funds from Johns Hopkins University International Center for Medical Research and Training.

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