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Studies on the *in vivo* Metabolism of α- and β-Aspartylglycine-1-C^{14}*

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Human subjects were given 4-mg intravenous doses of α-L-aspartylglycine-1-C^{14}, β-L-aspartylglycine-1-C^{14}, or an equivalent molar amount of free glycine-1-C^{14} and unlabeled aspartic acid. Much more radioactivity appeared in the expired carbon dioxide, in the urinary hippuric acid, and in the glycine and serine of plasma protein when the α rather than the β peptide was given. The results with free glycine and the α peptide were similar. When the β peptide was administered most of the radioactivity promptly appeared in the urine in the same chemical form but mixed with a larger quantity of endogenous β-aspartylglycine.

The accompanying report from this laboratory (Buchanan *et al.*, 1962) describes the isolation and identification of a number of β-aspartyl and γ-glutamyl oligopeptides from human urine. The most abundant of these, β-aspartylglycine, was shown not to arise by isomerization of α-aspartylglycine (John and Young, 1954; Swallow and Abraham, 1958; Byrant *et al.*, 1959) during the isolation procedure, and it is likely that the other β-aspartyl oligopeptides found are actually excreted in the β form. The present paper reports studies on the metabolic fates of synthetic α- and β-aspartylglycines given intravenously to human subjects. The results support a hypothesis (Buchanan *et al.*, 1962) that may explain the presence of β-aspartyl peptides in urine.

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METHODS

The α-and β-L-aspartylglycines were prepared from carbobenzoxy-L-aspartic acid and glycine-1-C^{14} by a slight modification of the procedure of LeQuesne and Young (1952), separated chromatographically, and recrystallized from alcohol (Buchanan *et al.*, 1962). The specific radioactivity of crystalline peptides was 2.5 μc per mg.

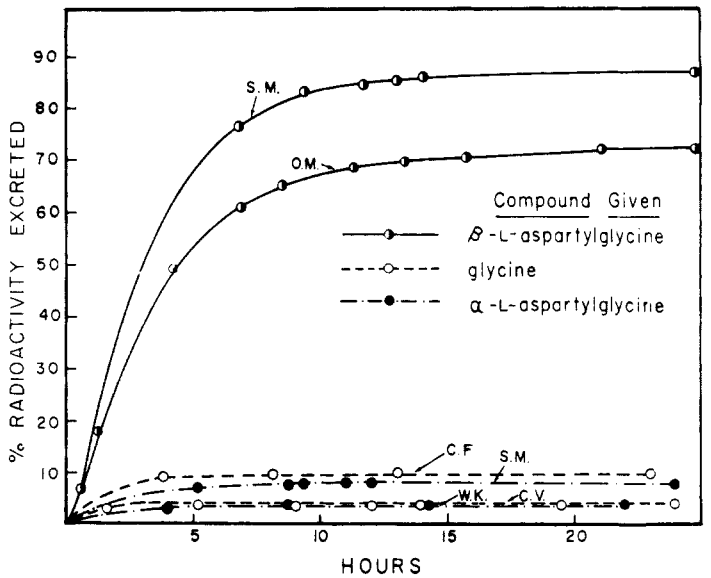

FIG. 1.—Specific radioactivity of expired carbon dioxide after intravenous administration of α-aspartylglycine-1-C^{14}, β-aspartylglycine-1-C^{14}, or glycine-1-C^{14} to humans.

Because autoclaving causes isomerization and hydrolysis of these compounds, doses were prepared for intravenous injection by weighing 4 mg of peptide, freshly recrystallized from alcohol, into autoclaved vials and then adding 5 ml of sterile saline. Doses of equivalent molar quantities of free unlabeled aspartic acid and glycine-1-C^{14} of the same specific radioactivity as that in the peptides were autoclaved after the addition of the saline solution. Aliquots of the uninjected residue of all doses were subjected to paper chromatography and the radioactivity measured with a strip scanner.

Human subjects were given no breakfast, and at 8:00 a.m. either α- or β-L-aspartylglycine-1-C^{14} or an equivalent quantity of a mixture of the constituent amino acids was administered intravenously. Food was withheld until noon. Carbon dioxide samples were taken at frequent intervals for 6 hours by having the patient inflate toy balloons and then allowing these to deflate slowly through 5 ml of carbonate-free N NaOH.

To increase urinary secretion of hippuric acid, sodium benzoate (1 g) was given orally either at the time of injection or in three equal doses at 4-hour intervals beginning with the injection. One patient (C.F., Table I) swallowed the first dose 20 minutes before the injection. Urine specimens were collected as voided and in most experiments aliquots were taken for determination of radioactivity before the urine was pooled into 24-hour collections. Venous blood (10 ml) was drawn 30 to 32 hours after the injection, and the plasma proteins were precipitated with 5% trichloroacetic acid and washed with alcohol, acetone, and ether. Glycine and serine were isolated from plasma protein by hydrolysis in 6 N HCl and carrier displacement chromatography (Buchanan, 1957).

The aspartylglycines were isolated chromatographically (Buchanan *et al.*, 1962). The β peptide was purified in each case, but because of the small quantity of the α peptide present the radioactivity of the crude fraction was determined in most of the experiments. The radioassay of CO_2 was performed by proportional gas counting, and the radioactivity of solid compounds was determined either by sealed tube combustion and proportional gas counting (Buchanan and Corcoran, 1959) or with a gas-flow counter after the material had been plated on aluminum planchets.

RESULTS

Figure 1 is a graph of the specific radioactivity of expired carbon dioxide from patients given one or the other of the aspartylglycines or free glycine. Although there is a considerable difference between the two curves obtained with free glycine, perhaps because of the earlier administration of sodium benzoate in one instance (C.F.), it is clear that α-aspartylglycine is catabolized about as rapidly as the free amino acid but that β-aspartylglycine is not. In two experiments on the same patient the elimination of radioactive carbon dioxide during the 6-hour period was 27% of the total dose when the α peptide was given but only 2.3% with the β peptide. Because these percentages are based on an estimation of total carbon dioxide output, the ratio between them is more reliable than the absolute values.

The cumulative excretion of radioactivity in the urine is graphed in Figure 2. With α-aspartylglycine only 4.4 and 8.8% of the total radioactivity administered was recovered in the urine, compared with 4.4 and 10.2% when free glycine was given. The higher value with glycine

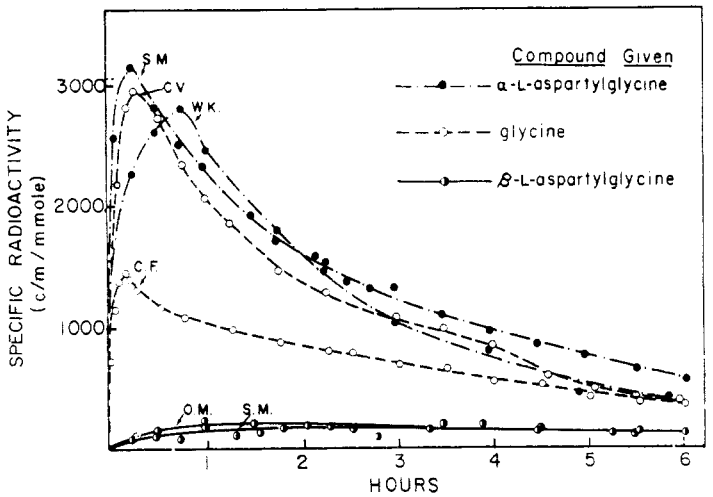

FIG. 2.—Cumulative urinary excretion of radioactivity after intravenous administration of α-aspartylglycine-1-C^{14}, β-aspartylglycine-1-C^{14}, or glycine-1-C^{14}

TABLE I

SPECIFIC RADIOACTIVITIES OF URINARY COMPOUNDS AND PLASMA PROTEINS AFTER INTRAVENOUS ADMINISTRATION OF LABELED ASPARTYLGLYCINES OR AN EQUIVALENT MIXTURE OF THE FREE AMINO ACIDS

Patient	Compound[a] Administered	Specific Radioactivities[b] α-asp-gly (cpm/mg)	β-asp-gly (cpm/mg)	Hippuric Acid (cpm/ mmole C)	Plasma Protein (cpm/ mmole C)
W.C.	α-L-asp-gly-1-C^{14}	11,500	1,050		99
S.M.		6,800	2,230	12,600	77
W.K.		236,800	1,520	4,730	60
C.V.	Aspartic acid[c] plus	—	—	4,660	91
C.F.	glycine-1-C^{14}	—	—	21,630	80
D.B.[d]	β-L-asp-gly-C^{14}	970	159,400	—	67
S.M.		400	117,500	460	13

[a] 4 mg (10 μc) intravenously. [b] Urinary compounds isolated from 24-hour urine collection following the injection. Plasma sample taken 30-32 hours after injection. [c] Equimolar mixture equivalent to peptide dose. [d] 8% of administered radioactivity found to occur in α-aspartylglycine.

was obtained with the patient who took sodium benzoate before the injection. When β-aspartylglycine was injected, 89 and 72% of the radioactivity was recovered from the urine during the first day in two patients. In the case of the patient with the lower recovery an unknown quantity of urine was inadvertently discarded at the first voiding. In each case most of the radioactivity was excreted during the first few hours.

The specific radioactivities of urinary α and β-aspartylglycines and hippuric acid from subjects receiving either of the peptides or free glycine are given in Table I. Except in one experiment (W.K.), the α peptide was isolated only as a crude fraction, and for this reason the specific radioactivities given are minimal values. So little radioactivity was found in the crude peptide fractions from the patients given free glycine that no attempt was made to isolate the aspartylglycines.

When α-aspartylglycine was given the same peptide isolated from the urine was considerably more active than the β form. Had the α peptide in each instance been free of impurities the difference would probably have been more striking—as it was in the one instance (W.K.). When the α peptide was given the specific radioactivity of the same compound isolated from urine was much higher than that of the α-peptide and all but a small fraction of the total urinary radioactivity was present as β-aspartylglycine. The 24-hour excretion of this peptide calculated by isotope dilution in one instance (S.M., Table I) was 72 mg. This amount, which includes the 4 mg administered, was from a subject from whom 32 mg of the pure compound had been obtained (Buchanan *et al.*, 1962).

Urinary hippuric acid contained a variable but significant amount of radioactivity after the administration of α-peptide or glycine, and the patient who took sodium benzoate 20 minutes

before getting the labeled glycine had the highest value. Hippuric acid radioactivity was found in much smaller amount after the β-peptide had been given. The values in Table I for hippuric acid and plasma protein were obtained by proportional gas counting and may be converted to the units used for the peptides by dividing by 34 for hippuric acid and 40 for protein.

Even with gas counting the radioactivity of the whole plasma protein was too low to permit reliable comparisons, so the whole plasma proteins from each group were pooled and glycine and serine isolated from each and their radioactivity determined (Table II). Again glycine and the α-peptide gave similar results, but a lesser amount of radioactivity was found in these amino acids when β-aspartylglycine was given.

TABLE II

SPECIFIC RADIOACTIVITIES OF GLYCINE AND SERINE FROM HYDROLYSATES OF POOLED PLASMA PROTEINS[a] OF PATIENTS GIVEN LABELED ASPARTYLGLYCINES OR GLYCINE INTRAVENOUSLY

Compound Administered	Specific Radioactivities[b] Glycine (cpm/ mmole C)	Serine (cpm/ mmole C)
α-L-asp-gly-1-C^{14}	1720	815
β-L-asp-gly-1-C^{14}	514	251
Aspartic acid plus glycine-1-C^{14}	1410	908

[a] See Table I. [b] Proportional gas counting.

DISCUSSION

The data presented show that the glycine of α-aspartylglycine enters into the metabolic reactions studied to about the same extent as free glycine, suggesting that most of the peptide is rapidly hydrolyzed. The presence of some activity in the β peptide is consistent with slight isomerization either *in vivo* or after excretion. However, if all of the β-aspartylglycine had originated from the α form *after* excretion its specific radioactivity would have been as high as that of the α peptide or higher because of the greater purity of the β peptide. This again demonstrates that β-aspartylglycine is not an artifact brought about during the isolation procedures (Buchanan *et al.*, 1962).

The fact that most of the β-aspartylglycine is quickly excreted in the urine indicates that enzymes capable of splitting the β-aspartyl linkage are not as effective as those that act on the α-aspartyl peptide bond and that the kidney has little capacity to retain the molecule. It is not possible to state whether the small quantity of the glycine of β-aspartylglycine that is converted to carbon dioxide or incorporated into hippuric acid and protein is the result of direct hydrolysis or conversion to the α-form with subsequent hydrolysis.

Some α- and β-aspartyl peptides interconvert under acid conditions, and the β structure is more stable (John and Young, 1954; Swallow and Abraham, 1958; Bryant *et al.*, 1959). Unpublished data show that some interconversion occurs with the aspartylglycines even in water at 37°. The hypothesis (Buchanan *et al.*, 1962) that α-aspartylpeptides to a slight extent spontaneously or perhaps catalytically isomerize *in vivo* to the β-aspartylpeptides, which then are not as susceptible to the action of peptidases and are more prone to renal excretion, is strengthened by the data presented here.

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