TOLERANCE TO AMINO ACID MIXTURES AND CASEIN DIGESTS GIVEN INTRAVENOUSLY

Glutamic Acid Responsible for Reactions*

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Amino acids and protein digests are being used to provide more adequate nutrition to many patients. We report here tolerance tests to the intravenous injection into dogs of certain amino acid and digest preparations. Parenteral feeding of protein-building materials has been shown valuable for those who cannot take sufficient protein orally (5, 3, 1). For greatest benefit such solutions should be well tolerated and convenient to administer as well as well utilized.

We find that *certain mixtures of crystalline amino acids are well tolerated*, whereas the protein digests tested must be given fairly slowly to avoid such reactions as vomiting. We have shown in an earlier report that the amino acid solutions are fully as well utilized on rapid injection as are the digest solutions upon slow injection (6). The saving in inconvenience to patient and doctor by more rapid injection is therefore at no cost in utilization. Such amino acid solutions have proven their value in human patients (1, 10).

We find that one amino acid, namely *glutamic acid*, is particularly likely to induce *vomiting* if included in the amino acid mixtures. The usual digests of casein possess a large content of glutamic acid, which may largely account for their lower tolerance. It is of further interest that addition of *glycine* to certain mixtures of the ten growth essential (Rose) amino acids *improves* tolerance.

Good utilization of certain amino acid mixtures for blood plasma and hemoglobin formation, as well as positive nitrogen balance, has been previously reported (6, 11, 7). Certain protein digests have also been shown to be well utilized when given parenterally (4, 8, 11, 7), but their lower tolerance calls for improvement. Serious reactions apparently seldom if ever occur during the use of proper mixtures of amino acids or of protein digests, in contrast to the toxicities reported (2, 9) to injection of unnatural or large quantities of certain individual amino acids.

* We are indebted to Merck and Company, Inc., for the crystalline amino acids used in these experiments; to Mead Johnson and Company for the Amigen, and to Frederick Stearns and Company for the Amino Acids Stearns. The latter two products have been modified for improvement since the data of this report were obtained.

Methods

Healthy adult dogs immunized against distemper were given single daily injections of the amino acid mixtures listed in Tables **1** and 4, as recorded in Tables 2, 3, 5, and 6. The amino acids were dissolved in distilled water just removed from boiling, in nitrogen concentrations ranging from 0.6 to 2.2 per cent. The amino acid solutions were usually near pH 5 without adjustment, except for those containing glutamic acid or aspartic acid. These were adjusted to near pH 5 by the addition of sodium carbonate, except for a few given unadjusted near pH 3.5. The presence of glutamic acid proved important in producing reactions; the degree of acidity did not.

Amigen, an enzymic hydrolysate of proteins prepared by the controlled digestion of a mixture of casein and pork pancreas, was given in the 10 per cent solution (in distilled water)

TABLE 1

Mixtures of Crystalline Amino Acids

		VII	VIIa	VIIb	VIIc	VIId	VIIe	Vm	Vj
1	*dl*-Theonine	13.0	13.2	14.0	7.9	8.9	9.0	7.4	5.8
2	*dl*-Valine	13.0	13.2	14.0	12.1	13.8	14.0	11.1	12.4
3	*dl*-Leucine	15.5	15.9	16.9	23.7	14.8	15.0	22.3	24.8
4	*dl*-Isoleucine	10.3	10.6	11.3	11.1	12.3	12.5	10.4	11.6
5	*dl*-Lysine·HCl·H_2O	15.5	15.9	16.9	12.1	13.8	14.0	11.1	12.4
6	*l*(−)-Tryptophane	4.1	2.1	4.5	4.2	4.4	4.0	3.7	3.3
7	*dl*-Phenylalanine	5.2	5.3	5.6	12.1	13.8	14.0	11.1	8.3
8	*dl*-Methionine	13.0	13.2	5.6	6.3	6.9	6.0	5.9	4.9
9	*l*(+)-Histidine·HCl·H_2O	2.6	2.7	2.8	4.2	4.4	4.5	3.7	4.1
10	*l*(+)-Arginine·HCl	7.8	7.9	8.4	6.3	6.9	7.0	5.9	4.1
11	Glycine							7.4	8.3
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Nitrogen, *per cent*	12.8	12.8	13.1	12.5	12.6	12.7	13.0	12.9

provided by the manufacturer, having a nitrogen concentration of 1.2 per cent. Amino Acids Stearns, an acid hydrolysate of casein, fortified with tryptophane, was given after diluting with distilled water to a nitrogen concentration of 1.27 per cent the solution provided by the manufacturer.

The injections were made into the leg veins and the rate controlled by regulating a screw clamp below a drip bulb, sometimes with a pressure pump supplementing gravity. The injections were always given before feeding, some dogs a stock diet, others a low protein basal diet.

Vomiting was taken as the most readable sign of reaction, salivation being much less reliable. Injections were often continued after vomiting reactions occurred, but except as noted in Table 3 all data given are for injections without vomiting or up to the time of vomiting.

EXPERIMENTAL OBSERVATIONS

The data recorded in Table 2 show that injections of *certain mixtures of the ten essential amino acids* produced vomiting 8 out of 10 times, whereas similar mixtures *with glycine added* were injected 10 out of 11 times with *no vomiting.* Mixtures with and without glycine were given on alternate days, not consecutively as might be suggested by the table. The rates of injection are comparable even when the glycine nitrogen is excluded from the comparison, as has been done in Table 2. The daily injections, moreover, were sufficient to maintain a positive nitrogen balance for the 3 weeks of Table 2 (Table 2-*a*, reference 6).

TABLE 2

Tolerance to Intravenous Administration of Solutions of Essential Amino Acids with and without Glycine

Dog 41-187, weight 10.6 kg.

Test No.	Mixture	Description	Nitrogen* total	Nitrogen* rate	Vomiting
			mg./kg.	*mg./kg./min.*	
1	VII	Ten essential amino acids	234	2	–
2	VII		234	4	+
3	VIIa	VII with tryptophane halved	159	6	+
4	VIIb	VII with methionine reduced 60 per cent	193	6	+
5	VIIc	VII in proportions of Vm	224	9	–
6	VIIc		160	7	+
7	VIId	VIIc with leucine reduced	242	12	+
8	VIIe	VIId slightly modified	179	12	+
9	VIIe		240	8	+
10	VIIe		240	8	+
11	Vm	Essentials + glycine	147	3	–
12	Vm		147	6	–
13	Vm		147	6	–
14	Vm		147	10	–
15	Vm		221	8	–
16	Vp	VII + glycine, 15.5 per cent	187	6	+
17	Vq	VIIb + glycine, 16.9 per cent	220	5	–
18	Vq		220	7	–
19	Vs	VIIe + glycine, 15 per cent	240	10	–
20	Vs		240	11	–
21	Vs		240	9	–

* Exclusive of glycine nitrogen.

The injections of *casein digests* as recorded in Table 3 produced vomiting even though done at much slower rates than the amino acid injections of Table 2. There is some suggestion that the enzymatic digest is better tolerated, but the difference is slight and vomiting usually occurred with injection rates of 2 to 5 mg. nitrogen/kilo/minute. Tolerance was not improved by mixing the acid hydrolysate half and half with a well tolerated amino acid mixture—tests 29 and 30, Table 3.

The synthetic mixtures of amino acids listed in Table 4 were tested as recorded in Tables 5 and 6. The most significant correlation exists between the

TABLE 3

Tolerance to Casein Digests Given by Vein

Dog 41-187, weight 10.6 kg.

Test No.	Mixture	Description	Nitrogen total	Nitrogen rate	Vomiting
			mg./kg.	*mg./kg./min.*	
1	Amigen	Enzymatic digest of casein and pork pancreas	141	5	+
2			189	2	+
3			282	2	−
4			189	2	+
5			282	4	−
6			282	3	−
7			282	3	−
8			282	4	−
9			282	4	+*
10			282	3	+*
11			282	4	+*
12			282	4	+*
13			282	2	−
14			282	4	+*
15	Amino Acids Stearns	Acid hydrolysate of casein fortified with tryptophane	150	2	+
16			300	2	+*
17			300	2	−
18			300	1	−
19			300	1	−
20			300	2	+*
21			300	2	−
22			300	2	−
23			300	2	+*
24			300	1	+*
25			300	1	+*
26			300	2	+*
27			300	2	+*
28			300	3	+*
29	Amino Acids Stearns	Plus mixture Vj (Table 1) in equal parts of nitrogen	300	3	+*
30			300	4	+*

* Vomiting 1 to 3 times during steady injection, time of first vomiting not recorded.

inclusion of glutamic acid in the mixtures and the occurrence of vomiting. Vomiting occurred during 19 of 29 injections with glutamic acid, whereas it occurred only 4 times in 31 injections without glutamic acid. The 31 injections all contained glycine and include the last 11 of Table 2.

Certain items are of interest although a close statistical analysis may not be justifiable. When glutamic acid of less than 100 mg./kilo was given, vomiting occurred in only 40 per cent of 10 injections. Above 100 mg./kilo vomiting occurred in 79 per cent of 19 injections. Only one of the seven dogs failed to

TABLE 4

Mixtures of Crystalline Amino Acids

		X	XIa	XIII	XIIIa	XV
1	*dl*-Threonine	4.0	7.0	5.2	5.8	4.8
2	*dl*-Valine	8.6	15.0	11.2	12.4	10.4
3	*dl*-Leucine	12.6		16.4		
4	*l*(−)-Leucine		10.9		9.1	7.7
5	*dl*-Isoleucine	5.8	9.9	7.5	8.3	7.0
6	*l*(+)-Lysine·HCl·H_2O	6.3	10.9	8.2	9.1	7.7
7	*dl*-Tryptophane	1.7	3.0	2.2	2.5	2.1
8	*dl*-Phenylalanine	5.8	9.9	7.5	8.3	7.0
9	*dl*-Methionine	3.4	6.0	4.5	5.0	4.2
10	*l*(+)-Histidine·HCl·H_2O	2.9	5.0	3.7	4.1	3.5
11	*l*(+)-Arginine·HCl	2.9	5.0	3.7	4.1	3.5
12	Glycine	5.8	9.9	7.5	8.3	7.0
13	*dl*-α-Alanine	2.3	4.0	3.0	3.3	2.8
14	*dl*-Serine	1.1	2.0	1.5	1.6	1.4
15	*dl*-Aspartic acid					4.2
16	*dl*-Glutamic acid	23.0				
17	*l*(+)-Glutamic acid					13.9
18	*l*(−)-Proline	11.5		14.9	16.5	10.4
19	*l*(−)-Hydroxyproline	0.3		0.4	0.4	0.7
20	*l*(−)-Cystine	0.3	0.5	0.4	0.4	0.3
21	*l*(−)-Tyrosine	1.7	1.0	2.2	0.8	0.7
22	*dl*-Norleucine					0.7
	Total	100.0	100.0	100.0	100.0	100.0
	Nitrogen, *per cent*	12.7	13.4	12.9	13.2	12.5

XI = X minus glutamic acid minus prolines.
XII = X minus prolines.
XIIa = XIa plus *dl*-glutamic acid, 9.9 per cent.
XIIb = XIa plus *dl*-glutamic acid, 29.9 per cent.
XIId = XIa plus *l*(+)-glutamic acid, 9.9 per cent.
XIIe = XIa plus *l*(+)-glutamic acid, 29.9 per cent.
XIIf = XIa plus *dl*-glutamic acid, 14.9 per cent.
XIIg = XIa plus *l*(+)-glutamic acid, 14.9 per cent.
XVI = XV minus glutamic acid.

vomit when first given a solution containing glutamic acid and this dog received a small quantity, 24 mg. glutamic acid/kilo (dog 40–73, Table 6). The quantities which first induced vomiting in the six dogs were 18, 72, 94, 141, 158, and 219 mg./kilo.

From the standpoint of *rate of total nitrogen* injection all but 6 of the 60 injections containing glycine were given more rapidly than 5 mg. nitrogen/kilo/min-

TABLE 5

Tolerance to Solutions of Amino Acids by Vein

Vomiting Increased by Inclusion of Glutamic Acid

Test No.	Mixture (Table 4)	Nitrogen total	Nitrogen rate	Amino acid rate	Glutamic acid rate	Glutamic acid total	Vomiting
Dog 42-893, weight 12.2 kg.							
		mg./kg.	*mg./kg./min.*	*mg./kg./min.*	*mg./kg./min.*	*mg./kg.*	
1	X	50	5	41	9	94	+
2	XI	121	6	46	0	0	−
3	XI	121	7	54	0	0	−
4	XIa	110	64	480	0	0	−
5	XII	95	7	56	15	205	+
6	XIIa	118	10	79	7	82	−
7	XIIb	78	11	89	12	143	+
8	XIIb	134	13	107	25	246	+
9	XIIb	67	19	153	35	123	+
10	XIII	142	6	43	0	0	−
11	XIII	142	5	41	0	0	−
12	XIIIa	131	11	86	0	0	−
13	XIa	332	18	130	0	0	−
14	XIIa	86	19	149	11	49	+
Dog 40-122, weight 11.4 kg.							
15	XI	130	6	44	0	0	−
16	XIa	118	30	224	0	0	−
17	XII	102	6	49	13	219	+
18	XIIa	126	17	129	12	88	−
19	XIIb	143	13	104	24	265	+
20	XIIb	143	14	114	26	265	−
21	XIIb	119	24	190	44	219	+
22	XIII	139	7	51	0	0	+
23	XIII	145	7	53	0	0	+
24	XIIIa	140	11	82	0	0	−
25	XIa	355	22	160	0	0	−
26	XIIa	202	20	155	14	140	+
27	XIa	118	34	282	0	0	−
28	XIIf	131	15	112	15	131	+
29	XIIg	131	14	107	14	131	+
30	XIIg	125	14	108	14	126	+
31	XIIe	140	12	93	22	258	−

ute. The 4 instances of vomiting without glutamic acid occurred at rates from 6 to 9 mg. nitrogen/kilo/minute, although 14 of the remaining 27 injections were given at rates greater than 9 mg. nitrogen/kilo/minute.

No good correlation is found between the vomiting associated with solutions *containing glutamic acid* and the rate of total nitrogen injection. The nitrogen rate of these solutions varied from 4 to 31 mg./kilo/minute and 9 of the 10 instances without vomiting lay in the middle range from 10 to 20 mg.

TABLE 6

Tolerance to Solutions of Amino Acids by Vein

Vomiting Increased by Inclusion of Glutamic Acid

Test No.	Mixture (Table 4)	Nitrogen total	Nitrogen rate	Amino acid rate	Glutamic acid rate	Glutamic acid total	Vomiting
Dog 41-187, weight 10.6 kg.							
		mg./kg.	*mg./kg./min.*	*mg./kg./min.*	*mg./kg./min.*	*mg./kg.*	
1	XIa	127	20	146	0	0	−
2	XIIf	140	10	81	11	141	+
3	XIIg	140	16	121	16	141	−
4	XIIg	146	14	105	14	147	−
5	XIIe	88	8	67	15	161	+
6	XV	136	23	180	25	151	+
7	XVI	152	17	130	0	0	−
Dog 37-151, weight 20.9 kg.							
8	XIa	194	9	63	0	0	+
9	XIa	194	10	72	0	0	−
10	XIId	26	5	42	4	18	+
Dog 32-396, weight 37.7 kg.							
11	XIa	107	8	61	0	0	−
12	XIId	104	4	31	3	72	+
13	XIId	42	19	143	13	29	−
14	XIIa	38	16	126	11	27	−
Dog 40-73, weight 37.0 kg.							
15	XIId	35	18	141	14	24	−
16	XIIa	39	31	240	22	27	−
Dog 42-1283, weight 10.1 kg.							
17	XV	143	24	190	26	158	+
18	XVI	159	13	102	0	0	−

Aspartic acid when present as 4 to 5 per cent of the total mixture did not produce vomiting (Table 6, tests 7 and 18). This concentration is similar to that in casein. A glutamic acid concentration about 14 per cent, which is less than that in casein produced vomiting when given with the mixture containing aspartic acid (Table 6, tests 6 and 17).

Acidity did not appear to contribute to reactions in the range tested. Amino acid mixtures at pH 6.5 were no better tolerated than at pH 5, and were better tolerated than Amigen (pH 6.5). Moreover, reaction to solutions with glutamic acid occurred just as readily at pH 5 as when given unadjusted at pH 3.5. The tests of mixtures XV and XVI in Table 6, for example, were all done at pH 5, with the expected reaction to glutamic acid.

Either the natural or the racemic glutamic acid is very likely to provoke reaction. A slightly higher percentage of injections containing *dl*-glutamic acid produced vomiting but the data are not sufficient to warrant a conclusion on this point.

DISCUSSION

Much more can be done in tolerance testing. The data presented merely skim the surface. There is the suggestion from Table 2 that mixture VII is less tolerable than VIIe, for the addition of glycine to VII did not eliminate vomiting (test 16). If not a chance variation why should there be this difference? This and many other questions must be answered by future experiment.

Poorer utilization of protein digests than of amino acid mixtures has been reported in the dog (7). Poorer tolerance with associated obvious disturbance may be responsible for this difference. No such difference between an amino acid mixture and Amigen was found in man during one brief comparison done without clinical disturbance (10).

The impression has been gained by users of protein digests (containing glutamic acid) that tolerance improves upon repeated injection. We have a similar impression although the data presented here are inconclusive. Other differences in reaction in the same and between different animals we must for the time being simply call biological variations.

Of physiological interest is the suggestion that *glutamic acid* is less well tolerated than any of the amino acids tested, that is, less well tolerated when included in amino acid mixtures in concentrations similar to its concentration in certain common food proteins. Aspartic acid, another dicarboxylic amino acid, produced no disturbance in the concentration tested. This concentration, while in the range of that in natural proteins, is much lower than that of glutamic acid. A comparison of these two amino acids should be made at the same concentration. The process by which glutamic acid injection leads to vomiting is open to speculation and investigation.

It is suggested by the experiments of Tables 5 and 6 that attention might well be given to the preparation of an inexpensive protein digest of very low glutamic acid content. If this proves impossible it may be that appropriate mixtures of pure amino acids can be made available at increasingly low cost. Some material suitable and convenient for parenteral use as a source for blood and body protein construction should have an important, often life-saving, part in medical care.

SUMMARY

Several synthetic mixtures of natural and racemic crystalline amino acids suitable for the daily nitrogen requirement are tested in dogs for their tolerance upon intravenous injection.

Certain mixtures of the ten essential amino acids plus non-essential amino acids exclusive of glutamic acid are accepted without any obvious sign of disturbance even at rates above 10 mg. nitrogen per kilo per minute for quantities greater than 300 mg. per kilo. One such mixture consists in parts per 100 of *dl*-threonine 7, *dl*-valine 15, *l*(−)-leucine 10.9, *dl*-isoleucine 9.9, *l*(+)-lysine·HCl·H_2O 10.9, *dl*-tryptophane 3, *dl*-phenylalanine 9.9, *dl*-methionine 6, *l*(+)-histidine·HCl·H_2O 5, *l*(+)-arginine·HCl 5, glycine 9.9, *dl*-α-alanine 4, *dl*-serine 2, *l*(−)-cystine 0.5, and *l*(−)-tyrosine 1. In addition other well tolerated mixtures included the prolines.

When *glutamic acid*, natural or racemic, is included in similar mixtures vomiting reactions frequently occur at nitrogen rates above 4 mg. per kilo per minute. Vomiting almost always occurs on the first daily injection containing glutamic acid and usually on any subsequent injection containing more than 100 mg. glutamic acid per kilo unless given very slowly.

Upon the addition of *glycine* certain mixtures of the ten essential amino acids show an improved tolerance.

Two *casein digests* tested usually produced vomiting at injection rates above 2 mg. nitrogen per kilo per minute, probably because of their glutamic acid content.

No serious reaction has ever occurrred to any mixture of amino acids or casein digest tested. Elimination of minor reactions such as vomiting appears possible and desirable for greater usefulness of these solutions in parenteral feeding.

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