INFLUENCE OF INGESTION OF SINGLE AMINO ACIDS ON THE BLOOD LEVEL OF FREE AMINO ACIDS*

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Our studies of normal and abnormal subjects have demonstrated that the postabsorptive levels of the free amino acids of the plasma remain within rather narrow normal limits except in very extreme terminal conditions. This investigation was made to determine whether the levels of all of the amino acids in the plasma are maintained by the same mechanism and whether there are any cross-relationships between the various amino acids. By feeding a large dose of a single amino acid, the plasma level of that amino acid is raised much above normal. This disturbance of the normal blood pattern gives some indication of the interrelationships existing between the free amino acids of the plasma.

Earlier workers have made similar studies of the effect of single amino acids on the blood amino nitrogen level, but no studies have been made of the effect of ingestion of one amino acid on the level of the other individual amino acids in the blood.

Seth and Luck (1) fed by gavage various amino acids in water to rabbits in amounts equivalent to 560 mg. of amino nitrogen per kilo. They found that glycine, alanine, and histidine caused marked increases in amino nitrogen, whereas leucine, tryptophan, glutamic acid, aspartic acid, and cystine caused very slight if any increases. In a later study with rats Luck (2) found that when glycine or alanine was given to rats there was a marked increase in amino nitrogen, whereas the dicarboxylic acid fraction, the hexone base fraction, and the monoamino acid fraction from casein caused no increase in amino nitrogen. Bang (3) found no increase in the blood amino nitrogen level of rats fed leucine by mouth. Johnston and Lewis (4) working with rabbits found increased blood amino nitrogen levels following glycine, DL-alanine, and glutamic acid feeding but no change following aspartic acid, arginine, and lysine feeding. Shambaugh, Lewis, and Tourtellotte (5) gave 1 gm. of tyrosine and 0.91 gm. of phenylalanine per kilo to rabbits and found no rise in the amino acid nitrogen of the blood at any period, although an increase in phenol content was noted. King and Rappaport (6) injected tyrosine intravenously and found that 95 to 98 per

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cent disappeared within 5 minutes. They indicated that this rapid decrease was due to disappearance into the tissues, since no increase in blood urea or urinary nitrogen sufficient to explain the loss was found. Friedberg and Greenberg (7) recently showed that intravenously administered amino acids are rapidly removed from the blood plasma, but at different rates. They also found that the injected amino acids were concentrated at different rates by the various tissues examined.

EXPERIMENTAL

Method

Four dogs were used for the series of experiments. Dog 1, a female, weighed 9.0 kilos, Dog 2, male, weighed 12 kilos, Dog 3, male, weighed 12 kilos, and Dog 4, male, weighed 8.7 kilos. The dogs were fasted for 24 hours previous to the experimental day. A 45 cc. sample of blood was removed from the femoral artery with a syringe containing heparin. The amino acid was then given by stomach tube in a neutralized solution or suspension. At intervals of 60, 180, 300, and 1440 minutes, additional blood samples were removed. A 2 week rest interval was allowed before the dog was used again. The doses were given at the levels indicated in Tables I to VI. All experiments were done at least twice, although only typical data from one experiment are reported.

The plasma was analyzed for the various amino acids by the procedures previously described for tryptophan, leucine, isoleucine, valine, arginine, phenylalanine, tyrosine, histidine, and lysine (8). Threonine and methionine were determined with *Streptococcus faecalis* with the media of Stokes *et al.* (9). Cystine was determined with *Leuconostoc mesenteroides* P-60 with Medium D described by Dunn *et al.* (10).

Observations

The plasma levels of leucine, isoleucine, threonine, and valine are found to rise rapidly following ingestion of the dose, reaching a maximum in about 60 minutes, as shown in Tables I and II. After 24 hours the plasma levels are only slightly above normal for these amino acids. When the leucine level is high, there is a fall in the level of all of the other amino acids except cystine, histidine, lysine, and tryptophan. When isoleucine is fed, the plasma level of arginine falls slightly, probably not significantly, while that of phenylalanine, tyrosine, and valine falls significantly. The levels of the other amino acids show no marked change. The high plasma level of threonine and valine reached following ingestion of these amino acids does not appear to affect the level of the other amino acids in the plasma.

TABLE I

Effect of Ingestion by Dogs of Leucine and Isoleucine on Free Amino Acids of Blood

The values are reported in micrograms per cc. of plasma. Throughout the tables the bold-faced figures emphasize the blood level of ingested amino acids.

Time after dose	Argi-nine	Cys-tine	Histi-dine	Iso-leucine	Leucine	Lysine	Methio-nine	Phenyl-alanine	Threo-nine	Trypto-phan	Tyro-sine	Valine
Dog 2. L-Leucine, 0.28 gm. per kilo												
min.												
0	35.1	11.7	17.4	19.4	**39.2**	29.4	14.3	25.0	31.2	14.1	17.7	40.5
60	28.8	9.6	13.8	9.6	**334**	27.6	7.7	13.0	26.7	12.0	7.5	27.0
180	16.2	9.0	12.3	8.6	**168**	20.9	5.3	11.9	17.7	11.1	6.0	16.0
300	15.9	10.5	12.6	11.7	**86.0**	20.6	7.5	15.2	18.8	13.2	7.8	20.1
1440	22.5	12.6	12.6	22.7	**61.1**	30.6	10.5	14.1	25.0	12.6	10.8	38.9
Dog 4. DL-Isoleucine, 0.56 gm. per kilo												
0	45.9	6.6	12.6	**14.3**	20.6	29.7	8.6	16.7	18.0	10.5	9.3	24.2
60	38.4	8.1	11.4	**305**	15.5	23.1	7.0	9.0	15.8	8.7	3.3	17.1
180	36.9	6.9	9.6	**183**	13.4	25.7	5.9	10.5	15.2	9.6	4.2	14.0
300	33.3	7.8	9.3	**115**	13.9	28.8	6.5	11.4	14.0	9.3	3.9	14.7
1440	33.0	9.0	9.6	**29.3**	25.7	31.7	8.9	14.3	18.2	9.9	6.0	32.0

TABLE II

Effect of Ingestion by Dogs of Threonine and Valine on Free Amino Acids of Blood

The values are reported in micrograms per cc. of plasma.

Time after dose	Argi-nine	Cys-tine	Histi-dine	Iso-leucine	Leucine	Lysine	Methio-nine	Phenyl-alanine	Threo-nine	Trypto-phan	Tyro-sine	Valine
Dog 3. DL-Threonine, 0.56 gm. per kilo												
min.												
0	24.6	5.4	10.5	19.4	23.3	21.6	10.5	12.6	**18.5**	8.7	10.5	21.0
60	21.0	5.7	10.2	14.3	19.8	15.0	9.2	9.9	**653**	8.1	7.5	19.8
180	19.2	6.9	10.2	13.8	21.5	17.6	9.2	10.5	**268**	7.2	9.0	19.7
300	21.6	6.6	9.9	14.8	21.9	20.4	9.9	11.4	**193**	7.5	10.2	18.2
1440	22.8	7.8	8.5	16.0	22.5	24.2	8.9	10.2	**54.0**	5.7	8.7	20.4
Dog 4. DL-Valine, 0.56 gm. per kilo												
0	44.1	7.8	9.9	18.2	32.4	28.6	8.4	12.5	23.0	10.8	9.6	**28.8**
60	42.9	7.5	9.8	14.7	27.8	25.9	8.2	10.5	19.4	10.2	7.2	**623**
180	37.5	6.6	9.0	14.6	25.8	20.3	8.0	8.7	23.3	9.3	6.3	**548**
300	38.7	7.5	8.4	19.5	30.8	21.3	8.4	9.6	20.0	10.5	6.7	**488**
1440	38.7	9.0	7.1	24.0	33.3	29.3	9.2	9.6	18.0	9.6	7.2	**72.2**

Table III shows the results of feeding L-arginine, L-histidine, and DL tryptophan. When arginine is ingested, the tyrosine level may be seen to fall slightly, while no other amino acid is affected, even though the plasma level of arginine increases about 17 times. When the same quantity of histidine is given, the histidine level rises about 100 times. Despite this

TABLE III

Effect of Ingestion by Dogs of Arginine, Histidine, and Tryptophan on Free Amino Acids of Blood

The values are reported in micrograms per cc. of plasma.

Time after dose	Argi-nine	Cys-tine	Histi-dine	Iso-leucine	Leucine	Lysine	Methio-nine	Phenyl-alanine	Threo-nine	Trypto-phan	Tyro-sine	Valine
				Dog 1. L-Arginine, 0.56 gm. per kilo								
min.												
0	**17.7**		12.3	13.7	16.7	19.7		10.4	32.9	6.3	6.8	23.0
60	**297**		11.1	10.6	12.2	23.3		8.6	34.8	6.3	4.2	23.4
120	**183**		12.3	13.9	17.2	18.0		9.2	30.3	7.0	5.1	25.5
300	**78.0**		10.0	15.8	17.7	18.0		8.7	28.5	5.0	4.2	27.5
1440	**25.0**		10.0	20.0	21.8	22.3		10.9	38.1	5.7	6.9	34.4
				Dog 1. L-Histidine, 0.56 gm. per kilo								
0	24.6		**10.8**	15.8	16.4	16.4		9.2	27.6	5.7	6.9	21.0
60	28.8		**1160**	17.6	19.8	26.6		8.9	40.5	7.5	6.3	23.9
120	18.6		**338**	16.4	16.5	15.5		9.0	31.7	8.0	5.7	33.2
300	21.0		**97.5**	19.4	20.9	19.5		10.5	33.6	6.3	6.0	29.9
1440	26.4		**13.2**	24.8	27.5	25.0		12.0	40.7	6.9	6.3	23.0
				Dog 3. DL-Tryptophan, 0.56 gm. per kilo								
0	34.5	8.1	9.8	11.8	12.0	22.2	8.0	13.0	11.7	**8.7**	7.8	15.5
60	28.2	5.1	9.6	11.4	12.2	19.8	8.0	9.8	11.3	**110**	6.9	16.0
120	28.5	6.0	8.7	15.2	15.2	19.8	7.4	11.5	10.8	**154**	8.4	18.0
300	27.9	5.4	8.7	13.0	15.3	18.3	7.7	9.5	10.5	**95**	7.2	17.1
1440	32.7	7.2	8.4	14.6	15.5	28.7	8.3	12.6	12.5	**10.5**	7.8	18.0

increase in the plasma histidine, there is no effect on the level of any other amino acid. Tryptophan ingestion, similarly, is without effect on any other amino acid level, although the tryptophan content of the plasma is increased about 10 times.

When DL-methionine is given, as shown in Table IV, the level of methionine in the plasma goes up and remains high, even after 24 hours, which is not found to be the case with the other amino acids. As a result of the high plasma level of methionine, there is a lowering of the plasma level of iso-

TABLE IV

Effect of Ingestion by Dog of Methionine and Cystine on Free Amino Acids of Blood

The values are reported in micrograms per cc. of plasma.

Time after dose	Arginine	Cystine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Tyrosine	Valine
					Dog 1. DL-Methionine, 0.56 gm. per kilo							
min.												
0	42.6	14.7	14.4	17.7	33.5	24.3	**16.4**	19.2	31.2	24.3	12.0	27.2
60	39.0	20.4	15.3	9.3	20.0	20.6	**756**	11.3	33.3	19.8	10.7	19.5
180	32.1	15.0	14.1	7.5	14.1	18.9	**700**	11.3	27.9	16.5	8.7	16.2
300	32.9	13.5	13.8	9.0	20.1	25.0	**597**	11.7	29.1	15.3	8.4	16.0
1440	37.2	23.1	11.4	14.7	20.0	34.5	**429**	13.5	28.9	15.3	9.3	22.4
					Dog 1. L-Cystine, 0.56 gm. per kilo							
0	38.7	**12.7**	19.5	21.0	35.4	35.7	15.6	11.4	25.5	14.1	20.1	28.0
60	31.6	**12.9**	18.7	20.1	34.1	31.2	14.8	11.0	22.1	12.0	19.1	27.5
180	32.3	**13.8**	16.8	22.2	34.7	29.7	14.8	11.6	20.9	9.0	16.8	29.0
300	26.1	**13.8**	16.5	19.8	28.5	27.0	13.2	9.6	18.9	7.5	14.7	27.9
1440	27.0	**19.5**	15.3	26.0	41.6	34.3	15.4	13.7	27.0	12.0	23.4	28.7

TABLE V

Effect of Ingestion by Dogs of Phenylalanine and Tyrosine on Free Amino Acids of Blood

The values are reported in terms of micrograms per cc.

Time after dose	Arginine	Cystine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Tyrosine	Valine
					Dog 4. DL-Phenylalanine, 0.56 gm. per kilo							
min.												
0	52.2	9.0	13.8	19.0	12.0	22.5	12.9	**16.0**	22.7	7.8	14.2	24.0
60	48.3	10.5	15.3	14.6	13.0	20.1	12.3	**49.2**	22.2	6.9	71.4	28.4
180	33.6	10.8	15.0	21.9	16.8	18.9	13.4	**28.4**	16.8	8.1	24.6	27.0
300	29.1	9.6	14.7	27.0	14.0	18.3	13.7	**24.5**	16.9	10.8	16.5	21.2
1440	48.0	9.0	12.8	32.0	12.9	24.0	14.1	**19.1**	27.9	9.0	12.3	23.2
					Dog 1. L-Tyrosine, 0.56 gm. per kilo							
0	47.1	4.7	15.0	22.0	34.8	28.8	11.3	15.2	22.0	17.4	**16.5**	35.9
60	45.9	5.1	14.7	22.1	25.4	28.8	11.7	12.2	21.6	20.4	**78.0**	30.6
180	44.1	5.4	15.3	24.5	28.3	30.8	12.2	12.6	18.6	19.2	**97.5**	32.3
300	36.9	5.4	15.0	26.8	28.9	34.9	10.8	12.3	20.7	17.1	**60.0**	33.8
1440	45.9	6.5	12.3	24.9	30.0	35.5	11.1	14.1	18.3	18.5	**12.9**	31.2

leucine, phenylalanine, tyrosine, and valine. Arginine and tryptophan show more variable changes. Despite the high level of methionine, there is no increase in the cystine level.

The same dog given the same amount of L-cystine as DL-methionine demonstrates no marked rise in the plasma cystine level. The levels of the other amino acids also do not appear to be affected. This is also shown in Table IV.

Table V demonstrates the results of feeding DL-phenylalanine and L-tyrosine. When phenylalanine is given, the phenylalanine plasma level rises rapidly, while simultaneously there is an increase in the tyrosine level. No other amino acids are affected. When tyrosine is given, the tyrosine level rises without affecting that of phenylalanine or any other amino acid.

DISCUSSION

The plasma level of all of the amino acids given, except cystine, was found to rise significantly following ingestion of the corresponding amino acid. Usually the maximum level was reached in about 60 minutes and was followed by a decline. After 24 hours the level was usually very close to normal. Following methionine ingestion, however, even after 24 hours, the level was still many times above normal.

When the leucine, isoleucine, and methionine plasma level rose owing to ingestion of the respective amino acid, the plasma level of certain other amino acids fell. Table VI shows the results of assay of a 24 hour sample of urine excreted by a dog following a dose of L-leucine compared to the assay of a previous sample of urine after a 24 hour fast. There is no increased excretion of any amino acid except leucine; so that increased loss in the urine does not appear to be the explanation for the drop in the plasma amino acid levels of arginine, isoleucine, methionine, phenylalanine, threonine, tyrosine, and valine. It may be that the same amino acid oxidase or deaminase is involved and that when the organism metabolizes the large amount of leucine, for example, the other amino acids are also removed from the plasma. It is also possible that certain equilibria must be maintained which are disturbed by the increased amount of the single amino acid, resulting in a shift of the amino acids from the plasma to the tissues. Studies with tissue slices would probably help to answer this question.

Phenylalanine when ingested causes a rise in the plasma tyrosine level simultaneously with the increased phenylalanine level, indicating the rapidity with which this well recognized conversion occurs. Tyrosine, as might be expected, does not have any effect on the phenylalanine level.

Ingestion of methionine, which is presumably one source of cystine in the body, does not cause an increase in the plasma level of cystine. This is

not surprising, however, when it is noted that ingestion of a large dose of cystine does not cause the plasma level of cystine to rise. Presumably, the organism can rapidly remove the cystine from the plasma either by excretion, conversion, or storage. Methionine, on the other hand, appears to be difficult to metabolize, since the plasma level remains high for a much longer period following methionine ingestion than with any other amino acid studied.

Brown and Lewis (11), using rabbits, found that the inorganic sulfate content of plasma ultrafiltrates increased more rapidly after ingestion of cystine than after methionine. The organic sulfur reached a higher level and remained high longer after methionine than after cystine. Pirie (12) observed that methionine forms sulfate more slowly than cystine when incubated with tissue slices. The rate of absorption from the intestinal tract is

TABLE VI

Effect of 0.56 Gm. per Kilo of L-*Leucine on Urine Excretion of Amino Acids (Dog 3)*

The values are reported in terms of mg. excreted per 24 hours.

	Arginine	Cystine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Tyrosine	Valine	Urine volume
													cc.
Preceding dose	16.5	26.4	2.0	0	1.5	6.0	3.0	2.3	4.0	15.0	1.5	0.13	195
Following dose	16.2	21.3	1.6	0	3.0	3.9	2.5	1.4	3.0	11.8	1.1	0.13	175

probably not a factor in explaining the difference in behavior between cystine and methionine since Chase and Lewis (13) found the rates to be approximately the same for both substances.

SUMMARY

The plasma level of arginine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine rose when the corresponding amino acid was given by gavage to dogs. Leucine, isoleucine, and methionine when at a high level in the plasma caused a fall in the level of certain other amino acids. The tyrosine level was found to rise in the plasma when phenylalanine was ingested, while the reverse did not occur. Although the methionine level of the plasma rose to a marked extent after feeding methionine and was maintained for at least 24 hours, the cystine plasma did not increase because of this. In fact, ingestion of an equivalent amount of cystine caused no rise in the cystine plasma level.

BIBLIOGRAPHY

1. Seth, T. N., and Luck, J. M., *Biochem. J.*, **19,** 366 (1925).
2. Luck, J. M., *J. Biol. Chem.*, **77,** 13 (1928).
3. Bang, I., *Biochem. Z.*, **74,** 278 (1916).
4. Johnston, M. G., and Lewis, H. B., *J. Biol. Chem.*, **78,** 67 (1928).
5. Shambaugh, N. F., Lewis, H. B., and Tourtellotte, D., *J. Biol. Chem.*, **92,** 499 (1931).
6. King, F. B., and Rappaport, D., *Am. J. Physiol.*, **103,** 288 (1933).
7. Friedberg, F., and Greenberg, D. M., *J. Biol. Chem.*, **168,** 411 (1947).
8. Hier, S. W., and Bergeim, O., *J. Biol. Chem.*, **163,** 129 (1946).
9. Stokes, J. L., Gunness, M., Dwyer, I. M., and Caswell, M. C., *J. Biol. Chem.*, **160,** 35 (1945).
10. Dunn, M. S., Shankman, S., Camien, M. M., Frankl, W., and Rockland, L. B., *J. Biol. Chem.*, **156,** 703 (1944).
11. Brown, B. H., and Lewis, H. B., *J. Biol. Chem.*, **138,** 717 (1941).
12. Pirie, N. W., *Biochem. J.*, **28,** 305 (1934).
13. Chase, B. W., and Lewis, H. B., *J. Biol. Chem.*, **101,** 735 (1933).

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