Effect of Food Intake on Amino Acids in Human Plasma

By E. Staten Wynne, ph.d.,* and Cora L. Cott, m.a.

In any investigation of free amino acids in human plasma, knowledge of the possible importance of the post-prandial status is desirable. If ordinary eating has an appreciable effect, blood must of course be collected following a relatively constant post-prandial period. Such a limitation is somewhat inconvenient; moreover, experience has demonstrated that fasting status cannot invariably be determined from statements of experimental subjects.

No published data are available on the influence of fasting on the concentration of individual amino acids in *untreated* human plasma. With microbiological assays of protein-free filtrates of plasma from children, Waisman and associates[1] reported no appreciable difference in levels of amino acids before and after eating. However, no details of the procedure were furnished, nor were any data given. In a study of seven amino acids in protein-free filtrates of whole blood from six adults, Charkey and co-workers[2] claimed that a 48-hour fast increased the levels of leucine and valine, but decreased those of arginine, lysine, methionine, threonine, and tryptophan. Inspection of their data, however, reveals that with methionine only two individuals exhibited a decrease on fasting, while three showed an increase. Likewise, arginine and lysine levels were increased after fasting in only two of the six individuals studied. Of the five amino acids reported to be decreased as a result of fasting, two failed to show any increase six hours after termination of the fast. Most of the amino acids showed a rather marked variation in levels among individuals, and apparently the data were not accorded statistical treatment.

Using microbiologic assays of 13 amino acids in unhydrolyzed rat plasma, Henderson, Schurr, and Elvehjem[3] claimed that the concentrations of most of the amino acids increased as the fasting period increased and reached maximum values in from 9 to 12 hours following the last food intake. However, control studies were not made on animals before, nor immediately after, they had been deprived of food; the earliest assay was carried out after three hours of fasting. Inherent variability among the groups of rats receiving the different treatments was stated to be of the order of ±10 per cent; but these data were not accorded adequate statistical analysis. It is difficult to interpret the findings of these workers, but it would seem that short-term fasting did result in an appreciable increase in the concentration of arginine, glycine, leucine, and lysine.

In this paper, studies are reported on microbiologic assays of amino acids in unhydrolyzed human plasma from blood collected both following an overnight fast and after subsequent food intake.

METHODS

Blood was secured from healthy volunteers before, and one and three hours after, food intake. At the time of collection of the first specimen, no food of any kind had been taken for at least nine hours. Meals were composed of two eggs; ham, bacon, or sausage; half a cup of grits; two slices of buttered toast; jelly; and milk or coffee. At least five in-

From the Section of Experimental Pathology, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Tex.

* Research Bacteriologist, University of Texas M. D. Anderson Hospital and Tumor Institute; Associate Professor of Bacteriology, University of Texas Postgraduate School of Medicine.

This investigation was supported in part by field investigation grant CS-9152 from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

dividuals were used in the study of each amino acid. In some cases assays were made of plasma samples collected from the same individual on two or three occasions.

Blood was obtained aseptically by venous puncture and mixed immediately with sterile 0.1 M sodium oxalate in the ratio of 9 to 1. After separation of the plasma by centrifugation, appropriate amounts were incorporated into tubes of the previously sterilized amino acid assay medium of Henderson and Snell.[4] For each plasma sample, assays were conducted at one concentration level in triplicate or at two levels in duplicate. The final volume was 5 ml per tube in all cases. Arginine was assayed with *Streptococcus faecalis* ATCC 8043; tryptophan with *Lactobacillus plantarum* ATCC 8014; and glycine, histidine, isoleucine, and tyrosine with *Leuconostoc mesenteroides* P-60 ATCC 8042. The inoculum consisted of one drop per tube of a 1 to 20 dilution of a washed 24-hour culture from Difco micro inoculum broth, and incubation was for 48 hours at 30° C. Growth was estimated from turbidity measurements with a Klett-Summerson photoelectric colorimeter at a wave length of 655 mμ. By reference to standard curves constructed from data obtained with tubes containing known amounts of amino acid and incubated simultaneously with the samples, results were expressed as μg of amino acid per ml of plasma. It is recognized that simpler peptides as well as free amino acids may have contributed to the responses of the micro-organisms used.[5]

The data were examined for statistical significance by the *t* test and by analysis of variance. Differences were regarded as significant only when the value of P was less than 0.01.

RESULTS

As seen from the data in Table I, the intake of food had no significant effect on plasma tryptophan, isoleucine, tyrosine, and glycine. Furthermore, statistical evaluation of the data by the *t* test revealed that of these four amino acids, only tyrosine exhibited significant variation ($P < 0.01$) in the fasting level from one individual to another. Analysis of variance confirmed the significance ($P < 0.01$) of differences in plasma tyrosine among individuals.

Analysis of the histidine data by the *t* test indicated that a significant increase following food intake was found only once in 13 experiments on eight individuals (Table I). The subject exhibiting this increase did so in only 1 of 3 experiments. However, analysis of variance failed to indicate significance (at the 1 per cent level) of food consumption on plasma histidine, or of variation of histidine level among individuals.

In 10 of 11 experiments on the same eight subjects, there was some elevation of plasma arginine after eating, although the increase was statistically significant by the *t* test in only four cases (Table I). Of these four instances, two occurred in the same individual (G. D.). However, on a third occasion this person failed to show a significant rise in arginine following food intake. Three hours after breakfast the overall mean plasma level for the 11 arginine experiments was increased over the fasting mean with $0.02 > P > 0.01$. Analysis of variance revealed the trend toward elevation of arginine following eating to be highly significant ($P < 0.001$), and also indicated that arginine, like tyrosine, exhibited a significant variation ($P < 0.01$) in level among individuals.

DISCUSSION

The findings just detailed for arginine are in essential agreement with those of Charkey *et al.*,[2] who reported increases in this amino acid in three of six individuals six hours after termination of a 48-hour fast. On the other hand, our failure to detect any significant effect of food intake on tryptophan is contrary to the findings of these workers, who reported a decrease in this amino acid on fasting and an increase following termination of the fast. However, it should be pointed out that both fasting and post-prandial periods were different in the two studies. Furthermore, protein-free filtrates of whole blood were used by Charkey *et al.*,[2] while untreated plasma was employed in the present investigation.

In rats, Henderson *et al.*[3] found that levels of certain amino acids generally reached peak concentrations in from 9 to 12 hours after the last food intake. Though fasting periods

TABLE I

Microbiologically Available Amino Acids in Human Plasma[a] before Breakfast, and One and Three Hours after Breakfast

	Tryptophan[b]			Isoleucine[b]			Tyrosine[b]			Glycine[c]				Histidine[d]				Arginine[d]			
Subject	Before	1 hr	3 hr	Before	1 hr	3 hr	Before	1 hr	3 hr	Before	1 hr	3 hr	P	Before	1 hr	3 hr	P	Before	1 hr	3 hr	P
F.S.	19.1	20.1	19.2	6.2	6.4	9.2	8.8	11.8	11.5	24.5	18.4	20.8	<.02	20.7	19.5	20.9	>.10	26.3	39.3	34.5	<.01
G.D.	17.7	20.4	16.8	5.3	7.1	7.5	9.1	10.1	13.4	21.0	22.5	25.5	>.10	20.8	17.8	19.6	>.10	17.6	22.0	29.5	<.01
							12.5	13.2	12.0	21.8	18.5	25.2	>.10	20.1	17.6	17.5	>.10	14.3	25.4	24.1	<.01
							13.0	14.0	15.2					17.9	18.6	21.2	>.10	17.6	26.1	25.0	>.10
L.R.	17.1	19.6	18.6				12.3	8.8	8.7	26.2	20.1	18.6	<.05	16.9	15.5	16.1	>.10	15.1	15.8	25.5	<.01
E.S.W.	15.5	14.5	18.8				9.9	6.8	12.2	24.0	23.5	22.5	>.10	21.3	17.8	20.9	>.10	18.7	15.2	26.1	>.05
C.L.G.	20.9	20.5	18.8	7.8	5.1	7.9	10.8	8.0	10.4	21.4	17.6	19.4	<.05	21.5	19.5	18.5	>.10	30.3	35.0	35.5	>.10
							15.6	18.2	16.9					18.6	17.8	24.6	<.05	22.0	22.4	26.4	>.10
N.M	17.4	16.7	17.1											16.8	14.5	17.3	>.10	15.9	18.9	25.1	<.05
M.T.				6.6	6.9	5.9	17.5	14.3	16.2					5.3	13.4	15.4	<.01	13.6	10.3	12.5	>.10
														24.0	23.6	26.5	>.10				
														17.2	14.2	14.4	>.10				
P.F.							17.5	22.3	18.9					20.0	18.0	17.6	>.10	16.3	21.5	19.1	>.10
E.S.				5.0	4.6	7.2															
Mean	17.9	18.6	18.2	6.2	6.0	7.5	12.7	12.8	13.5	23.1	20.1	22.0	>.10	18.5	17.5	19.3	>.10	18.9	22.9	25.7	<.02
Standard Error	0.75	1.0	0.37	0.49	0.41	0.53	0.92	1.36	1.0	0.82	1.0	1.19		1.32	0.75	0.98		1.7	2.74	2.1	

[a] As μg/ml of plasma, including free amino acid plus microbiologically available peptide.
[b] P >0.10 in all cases.
[c] P calculated from fasting value vs lowest value.
[d] P calculated from fasting value vs highest value.

in rats are not necessarily equivalent to those in humans, the fasting interval in the present investigation was also about 9 to 12 hours. If human subjects react as rats do, one might expect amino acid levels to be higher in the fasting state than after eating. As seen in Table I, a trend toward a decrease from a fasting peak was actually observed with glycine; however, in no instance did the *t* test show this decrease to be significant at the level of P = 0.01. Furthermore, analysis of variance failed to indicate a significant trend toward decrease of glycine after eating.

The findings reported here are not at variance with published information on the effect of ordinary eating on total amino acid nitrogen of human blood. Thus Hammett[6] found essentially no difference in amino acid nitrogen of blood drawn before or $3^1/_2$ hours after breakfast. Edgar[7] reported the average level of amino acid nitrogen in whole blood of 77 fasting diseased children to be 8.23 mg per 100 ml, while the average of 40 non-fasting diseased children was 8.97 mg per 100 ml, an increase of only 9 per cent. In the studies of Folin and Berglund,[8] amino acid nitrogen in plasma of fasting adults was 5.2 mg per 100 ml, while $5^1/_2$ hours after eating the level was 6.4 mg per 100 ml, a rise of 23 per cent.

The present data show rather marked variation in the levels of some amino acids among different individuals, and in the same individual tested at different times. In this connection it is of interest to point out the finding of Hammett[6] that amino acid nitrogen in one individual ranged from 3.6 to 6.1 mg per 100 ml, with values in different subjects from 3.1 to 7.2 mg per 100 ml. Analogous data of Edgar[7] revealed a range of 5.13 to 8.14 mg per 100 ml for one individual and 6.08 to 9.52 mg per 100 ml for different individuals.

SUMMARY

Levels of microbiologically available glycine, isoleucine, tryptophan, and tyrosine in unhydrolyzed human plasma were similar in blood obtained in the fasting state or after food intake. In 1 of 13 experiments histidine was significantly increased ($P < 0.01$) following eating. In 4 of 11 experiments on eight individuals, arginine was significantly elevated ($P < 0.01$) after food intake. Of the six amino acids studied, analysis of variance indicated significant variation ($P < 0.01$) among individuals for tyrosine and arginine only.

REFERENCES

1. Waisman, H. A., Pastel, R. A., and Poncher, H. G.: Amino acid metabolism in patients with acute leukemia. *Pediat.* 10: 653, 1952.
2. Charkey, L. W., Kano, A. K., and Hougham, D. F.: Effects of fasting on blood non-protein amino acids in humans. *J. Nutrition* 55: 469, 1955.
3. Henderson, L. M., Schurr, P. E., and Elvehjem, C. A.: The influence of fasting and nitrogen deprivation on the concentration of free amino acids in rat plasma. *J. Biol. Chem.* 177: 815, 1949.
4. Henderson, L. M., and Snell, E. E.: A uniform medium for determination of amino acids with various microorganisms. *J. Biol. Chem.* 172: 15, 1948.
5. Krehl, W. A., and Fruton, J. S.: The utilization of peptides by lactic acid bacteria. *J. Biol. Chem.* 173: 479, 1948.
6. Hammett, F. S.: Studies of variation in the chemical composition of human blood. *J. Biol. Chem.* 41: 599, 1920.
7. Edgar, S. H.: Amino-acid content of the blood of children in health and in disease. *Biochem. J.* 22: 168, 1928.
8. Folin, O., and Berglund, H.: The relation and distribution of amino acids with special reference to urea formation. *J. Biol. Chem.* 51: 395, 1922.