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Free Amino Acid Patterns of Serum of the Patients with Gastric Cancer, Eespecially on their Control by Infusions of Amino Acid Solution*

Amino Acid Fractions in Patients with Gastric Cancer and in Patients with Gastric Ulcer

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Introduction

Among the factors contributing greatly to the enlargement of the circle of operable patients and the improvement of the results of operation is the pre- and postoperative nutritional control, especially the preoperative improvement of free proteins. Many studies have been conducted on protein metabolism before and after operative insult since old times, after Elmann's[1,2] study mainly from the standpoint of nitrogen balance.

Most of these studies were focused on the relationship between the total amino acid nitrogen and the operative insult. According to Kuwabara, Mann and Albanese[3,4], total amino acid nitrogen not only reflects the reaction of the individual to an operative insult with a high sensitivity, but also is intimately related to the nutritional state of the individual, as an excellent index of protein metabolism. On the other hand, the development of mixed amino acid solution in which the amount of each amino acid may be regulated at will has promoted studies on individual amino acid component as the constituents of the protein by many investigators. Despite a rapid development of studies on amino acid since Rose[5~8] in the field of biochemistry and nutrition with a great deal of work, total amino acid nitrogen has been used as the only criterion of amino acid metabolism in the field of clinical medicine. Among the factors inhibiting the progress of such study in clinical medicine are, the extremely difficult and time consuming method of determining individual amino acid and a large amount of sample required for determination, making the clinical application almost impossible. Moreover, observation in time course is mandatory.

As part of the studies on the pre- and postoperative protein metabolism being carried out in our department, we have conducted studies on amino acid metabolism in relation to gastrectomy as the fundamental operative insult on the digestive tract, using amino acid autoanalyzer produced by Technicon in patients with gastric and duodenal ulcer as wells as gastric cancer. Deviations from normal values, relationship with the degree of progress of the cancer, and the relationship with the albumin level were

* Part of this work was presented at the following annual meetings: The 62nd, 65th and 66th Meetings of Japan Society of Surgery; The 26th and 27th Meetings of Japan Society of Practical Surgery; The 2nd Meeting of Japan Society of M. E.; The 6th and 7th Meetings of Hokkaido Society of Surgery; and The 4th Meeting of Japan Society for Cancer Therapy.

mainly studied. Some new information obtained are hereby reported.

Experimental Methods

1. *Experimental Subjects*

Twenty-one cases of gastric cancer and 11 cases of gastric ulcer, the total of 32 cases were selected for study. Only patients with normal liver, kidney, and adrenocortical functions were selected. As controls, 11 male and 11 female hospital workers without remarkable past history ranging in age from 21 to 35 years were selected.

2. *Objects of Determination and Methods*

Since the successful separation and qualitative determination of amino acids with paper chromatography by Consden[9] in 1944, many reports[10~28] are available on the attempt of quantification of amino acids. This method is still widely used and many reports on the use of this method have been published in the field of surgery[29].

Although this method is simple, requiring only a small amount of sample and a rather short time for the number of samples to its advantage, uncertainty in the reproducibility, lack of objecting in the determination of spots for quantitative estimation, and a considerable doubt on the accuracy may be cited as the disadvantage according to many investigators[30].

On the other hand, a remarkable progress of gas chromatography has recently accomplished especially in the field of biochemistry. The advantage of this method consists in the fact that a very small amount of sample, the analysis is accomplished in a very short period, and the evaluation and recording of the state of separation is quite easy[31~34,55].

Although the studies on amino acids by Meister[35] and Zlaktis[36,37] are highly evaluated, the most difficult problem before preparing the sample for determination or "how to change the amino acid for determination into corresponding volatile compound" has not yet been solved. Gas chromatography has therefore not yet been used in the field of surgery.

On the other hand, high voltage filter paper electrophoresis also has the advantage of requiring a small amount of sample and a short time for determination[38~51]. We used this method in the initial stage for studies on amino acid metabolism in the field of surgery[52~54]. The largest drawback of this method is represented by the overlap of 6 neutral fractions, requiring re-extraction of this part and paper chromatography. Satisfactory results have not readily been obtained.

Although the method using microorganism is able to separate individual amino acid with certainty, the preservation of the bacterial stock presents a rather difficult problem and the procedure of quantification is rather complex, requiring a long time, so that this method may not be readily used as a general method[56~64]. However, Everson's[65] report for the first time on the separation and quantification of 10 amino acids by a method using microorganism and the demonstration of the low values of all fractions in patients with malnutrition in the field of surgery is noteworthy.

In 1958 Spackman[66] and Moore[67] successfully automatized the determination of amino acids using ion exchange column chromatography. Although this is said to be

the most accurate and reliable method, the complexity of the apparatus and a long time required for analysis prevented it from being used generally.

However, recently constructed autoanalyzer produced by Technicon covered these drawbacks. This amino acid autoanalyzer uses a kind of automatic liquid chromatography, an apparatus for separating each component through liquid chromatography, measuring it with an adequate method of detection, and automatically recording it on a chromatogram.

The autoanalyzer produced by Technicon used a method entirely different from the original method of Stein and Moore[68,69], except for the method of colorimetry in which a modification of Stein and Moore's ninhydrin method was employed. As the method of chromatography, Peiz and Moore's[70] entirely different method was employed.

This method characteristically uses a single thin column, measuring all amino acids at once within a short time, using a minute amount of the sample.

An apparatus for amino acid analysis may be divided into two parts: the part for chromatography and the part for photoelectric colorimetry for detection and quantification.

i) Contents of the column: A single column measuring 133 cm×6.3 mm was used for separation of 21 acidic, neutral and alkaline amino acids.

The content of the column is a resin with minute particles, chromobeads developed by Technicon, with a completely spherical form with diameters of 17±1 μ.

ii) Automatic pH regulator: This apparatus made it possible to supply the buffer ranging in pH from 2.2 to 9.8 continuously.

iii) Photoelectric colorimeter: The color was developed with ninhydrin. The developed color (DYDA) was subjected to colorimetry at the maximum wavelength of absorption, 570 mμ.

The range of the error in the wavelength of the filter was adjusted to±3 mμ. The colorimeter was combined with a recorder and the voltage of the power supply was adjusted to 100 to 125 volts. Fluctuations in the voltage within this range were abjuted to cause changes less than 0.5% on the recording paper.

Materials

Blood samples were obtained in the early morning in a fasting state. One mℓ of the serum was deproteinized with exactly 1 mℓ of 2% sulfosalicylic acid and centrifuged at 4,000 r.p.m. for 10 minutes. The supernatant was again centrifuged at 4,000 r.p.m. for 10 minutes for the total of 3 times. The sample was kept in a cold place for 24 hours and the pH was adjusted exactly to 2.1 with sodium citrate. As the sample, 0.5 mℓ of this mixture was used.

According to Oepen[94], Gerok, Knrauff, and Higashiizumi, the conventional amino acid autoanalyzer was unable to separate serine and threonine forming a single peak and lysine and ornithine eluated in an identical part, in addition to the complexity of the method of deproteinization and pretreatment. (The high value of lysine is due to this factor.)

Various reports are available concerning the recovery of various amino acids. Sasaki and Takeda of our department used this autoanalyzer and obtained almost 100%

recovery from samples obtained from living organism, so that the values obtained from analysis were used without further treatment.

We have used 570 mμ. filter for determination. Since proline has the maximum wavelength at 440 mμ, proline was excluded from the results of determination.

NH_3 and ornithine were also excluded due to a specific changes on account of the equilibrium and a sensitive response to an insult itself according to Takeda[71]. The fractions to be analyzed were finally composed of eight essential and ten non-essential amino acids. (Table 1)

Each amino acid is referred in abbreviation as indicated in the Table.

Table 1

Threonine	○ Thr	Cysteic Acid	Cys・COH
Valine	○ Val	Serine	Ser
Methionine	Met	Glutamic Acid	○ Glu
Isoleucine	▲ Ileu	Glycine	○ Gly
Leucine	▲ Leu	Alanine	○ Ala
Phenylalanine	▲ Phe	Cystine	○ Cys
Lysine	Lys	Tyrosine	▲ Tyr
Trypophane	Try	Histidine	His
Aspartic Acid	○ Asp	Arginine	Arg

○ Glucogenic Aminoacid ▲ Ketogenic Aminoacid

2) Determination of total protein and protein fractions

Total protein was determined with Hitachi's proteinometer and protein fractions by electrophoresis according to Tiselius. The ratio was calculated from the results.

Experimental Results

1. *Amino Acid Fractions in Normal Subjects*

Tables 2 and 3 summarize the results of measurement of 18 fractions, the range of fluctuations and average values. Many amino acids showed a distinct difference between males and females. glutamic acid, Isoleucine, and leucine were high in males, while aspartic acid, glycine, cystine, methionine, lysine, histidine, and arginine predominated in females. However, no sex difference was evident in cysteic acid, serine, alanine, valine, phenylalanine, tryptophane, and threonine. The concentration of essential amino acids followed the decreasing order of threonine, lysine, valine, leucine, phenylalanine, isoleucine, methionine, and tryptophane, as the average between males and females. Non-essential amino acids followed the decreasing order of alanine, glycine, serine, glutamic acid, arginine, tyrosine, histidine, cystine, aspartic acid, cysteic acid in males, and alanine, glycine, serine, arginine, glutamic acid, tyrosine, histidine, cystine, aspartic acid, and cysteic acid in females. As shown in Fig. 1, range of fluctuation followed the decreasing order of lysine, threonine, glycine, alanine, and valine, while aspartic acid, glycine, methionine, isoleucine, tyrosine, phenylalanine, tryptophane, histidine, arginine, and cysteic acid showed minor fluctuations.

Table 2 *Amino Acid Fractions in Normal Males Adults* (r/mℓ)

Name	H. H	K. K	N. O	H. T	T. I	H. U	Y. N	O. K	S. W	Y. T	S. F	Mean r/cc	Mean μmol/ℓ
Cys・NH_2	0.15	0.13	0.13	0.26	0.24	0.27	0.18	0.20	0.28	0.26	0.11	0.20	1.18
Asp	0.32	0.29	0.31	0.16	0.28	0.44	0.23	0.36	0.21	0.28	0.23	0.28	2.12
Thr	19.55	39.25	22.80	21.08	39.96	24.00	39.26	16.27	33.58	31.05	29.31	28.74	241.3
Ser	16.60	11.90	12.50	15.41	11.36	13.71	13.73	15.39	11.01	11.34	12.83	13.25	126.1
Glu	7.50	5.56	5.70	8.74	9.91	6.16	5.19	9.69	7.06	6.41	5.89	7.07	42.8
Gly	23.70	12.90	7.20	27.92	15.57	15.60	8.40	6.46	19.22	15.33	11.50	14.89	198.3
Ala	18.65	16.41	19.80	39.18	33.80	10.82	14.69	18.39	29.87	28.50	22.18	22.94	257.4
Cys	1.84	2.93	3.20	3.00	7.42	1.67	2.93	1.38	1.50	5.61	1.60	3.01	50.2
Val	5.71	9.84	13.70	25.34	29.67	13.29	11.00	6.78	16.27	15.70	15.90	14.84	126.7
Met	1.38	1.84	3.80	3.98	4.66	2.40	1.92	2.07	1.95	3.00	2.55	2.69	18.0
Ileu	3.61	8.31	9.00	8.70	9.79	2.71	5.40	4.11	4.97	6.84	4.89	6.21	47.3
Leu	5.16	10.35	12.80	17.11	10.48	13.45	12.47	9.49	10.79	10.62	9.33	11.09	84.6
Tyr	5.97	7.11	9.90	4.48	2.78	2.76	2.37	3.95	4.54	9.37	5.39	5.33	29.2
Phe	5.23	6.08	8.90	6.06	9.80	3.58	4.79	6.17	7.42	6.63	7.51	6.56	39.7
Lys	11.07	20.26	12.50	39.59	10.10	9.00	14.50	13.57	25.45	25.46	30.92	19.31	105.7
His	2.81	1.94	3.19	5.87	5.36	2.49	2.42	2.51	4.17	3.39	3.80	3.44	16.4
Try	1.86	2.68	2.68	4.89	1.94	1.36	1.67	2.52	2.10	2.32	2.49	2.41	10.0
Arg	7.15	4.65	5.48	7.81	7.04	2.64	2.74	6.90	5.14	7.41	6.34	5.75	27.3
Total	138.26	162.25	153.59	239.58	210.16	126.35	143.89	126.21	185.51	189.52	172.77	168.01	145.50

Table 3 *Amino Acid Fractions in Normal Females Adults* (r/mℓ)

Name	N. K	S. Y	N. I	T. S	S. K	N. T	Y. M	N. A	K. K	I. Y	R. M	Mean r/cc	Mean μmol/ℓ
Cys・NH_2	0.44	0.19	0.21	0.35	0.36	0.38	0.27	0.41	0.22	0.22	0.35	0.31	1.8
Asp	0.42	0.23	0.92	0.80	0.92	0.83	0.93	0.37	0.47	0.19	0.65	0.62	4.7
Thr	36.08	24.07	29.16	30.23	35.79	26.90	27.23	25.53	22.09	27.87	29.87	29.52	247.9
Ser	11.03	11.39	10.46	12.12	14.07	16.20	16.10	13.50	17.20	17.61	12.92	13.87	132.0
Glu	5.18	8.16	4.99	7.25	7.90	7.10	4.71	4.28	4.59	4.83	4.28	5.75	54.7
Gly	20.35	20.99	18.65	19.80	18.74	21.70	18.40	23.90	18.20	20.75	13.20	19.35	257.7
Ala	33.21	41.05	23.52	37.89	29.75	20.31	15.02	12.17	15.70	25.42	13.88	24.36	279.6
Cys	9.42	2.51	4.91	4.51	3.01	3.76	4.89	3.51	2.51	5.98	6.69	4.70	78.2
Val	24.11	25.18	13.82	17.52	15.17	13.65	14.11	15.02	14.10	10.04	6.21	15.36	131.0
Met	2.07	3.08	2.83	2.78	2.70	1.23	3.40	8.90	2.10	1.54	2.70	2.39	22.7
Ileu	5.56	9.71	3.34	6.29	6.68	2.53	2.17	3.25	3.25	6.50	2.44	4.70	35.8
Leu	10.70	16.33	9.72	11.40	12.96	3.30	4.04	4.90	5.19	13.69	5.02	8.84	67.4
Tyr	4.09	4.10	9.46	5.77	6.81	2.76	2.67	3.55	3.26	5.92	2.67	4.64	25.6
Phe	7.24	11.99	9.60	7.70	10.12	2.82	4.02	3.22	4.02	8.94	3.49	6.59	39.9
Lys	42.37	46.16	31.52	37.77	42.99	14.00	12.04	27.50	17.88	26.30	12.72	28.30	154.9
His	6.24	5.11	3.59	6.60	3.10	6.46	2.37	2.53	1.63	4.85	2.35	4.07	19.4
Try	4.31	3.80	2.49	2.47	1.70	2.12	2.73	2.12	4.23	3.57	2.46	2.91	12.1
Arg	8.43	9.47	8.99	10.59	7.40	8.96	9.65	9.90	9.31	4.20	7.30	8.53	40.5
Total	231.25	243.52	186.38	210.62	220.17	155.01	154.75	159.56	145.95	188.42	929.20	184.80	

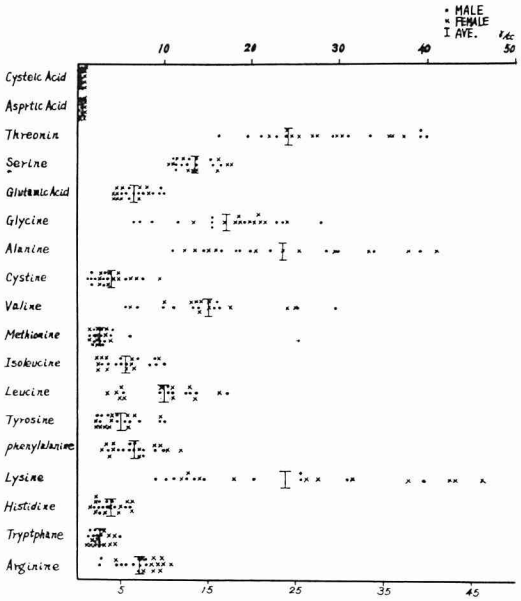


Fig. 1 Amino acid fractions in normal adults.

Table 4 *Amino Acid Fraction in Normal Adults* (Mole/ℓ)
Various reports in Japan and abroad

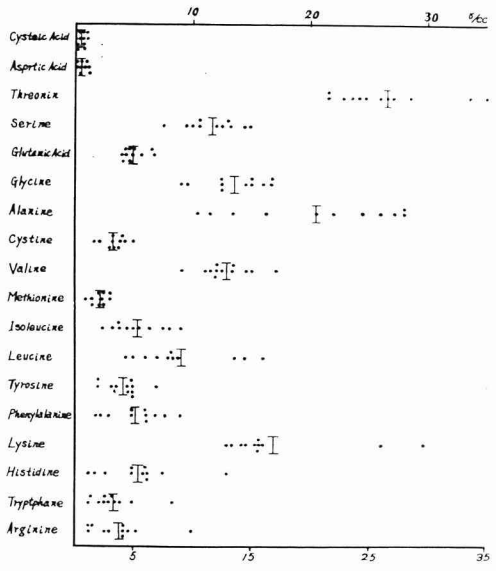
Re-porter	Author	Tosen	Stein	Cus-worth	Lol	Oepeu	Acker-mann	Do-olan	Nishi-hara	Ariti	Mc-Menamy	Mi-ura
Year Number	1967 (22)	1966 (18)	1954 (5)	1960 (4)	1963 (14)	1963 (10)	1964 (33)	1955 (4)	1958 (10)	1959 (10)	1957 (15)	1959 (10)
Methode	Ion exchange column chromatography							Microbiological method paper chrometography				
Asp	1–6	0–5	1–5	7	—	16–27	15–24	—	—	—	—	—
Thr	164–336	228–683	102–145	110–170	98–190		124–171	126	144	89	100–180	196
Ser	99–168		96–119	114–133	73–168	—	88–131	143	—	—	150	186
Glu	41–95	3–60	29–78	40–90	14–83	51–107	59–84	—	—	—	0–25	—
Pro		38–153	160–290	150–230	148–337	152–247	178–228	148	—	—	100–180	139
Gly	86–372	55–306	179–231	240–290	149–442	229–317	196–270	267	—	—	140–290	370
Ala	121–461	93–428	338–472	260–400	310–662	298–427	289–364	281	—	—	200–450	515
Cys	23–156	5–39	90–108	—	37–135	—	59–84	90	—	—	—	29
Val	49–254	102–219	203–317	200–280	141–316	174–201	215–291	248	260	196	110–220	284
Met	8–31	5–26	22–29	—	15–36	18–24	21–33	34	29	28	10–45	
Ileu	17–75	20–59	53–98	47–68	36–97	62–72	70–90	92	125	76	35–90	178
Leu	25–130	49–125	108–176	90–130	75–168	124–152	112–146	145	174	114	50–105	
Tyr	13–55	13–65	45–80	35–49	39–87	43–55	59–78	44	—	—	35–90	—
Phe	17–73	18–55	42–58	48–55	43–78	40–55	58–76	94	125	46	35–75	77
Try	6–20	6–41	7r	—	—	15–48	—	6	67	50	14–26	—
Lys	49–153	172–388	172–207	144–164	134–238	97–162	134–176	164	242	133	105–190	—
His	8–31	47–79	51–96	77–97	48–106	59–100	82–108	88	—	42	60–110	—
Arg	13–50	25–98	70–111	60–140	58–120	55–63	68–97	161	—	78	50–96	—

Upon comparison of these values with various reports in Japan and abroad[69,72~77] (Table 4), a considerable difference is noted from the results of Moore and Stein. Threonine, serine, and glycine showed higher values while other amino acid showed lower values.

A considerable difference is also seen between the present result and those obtained by microbiological method or paper chromatography, especially in threonine and histidine[64,75,76]. The low value of lysine in the author's method of ion-exchange chromatography is due to the separation from ornithine as stated before.

2. *Amino Acid Fractions in Patients with Gastric Ulcer*

The amino acid fractions in 11 cases of gastric ulcer upon admission are summarized in Table 5 and Figure 2.



I average values in normal adults

Fig. 2 Amino acid fractions in patients with gastric ulcer.

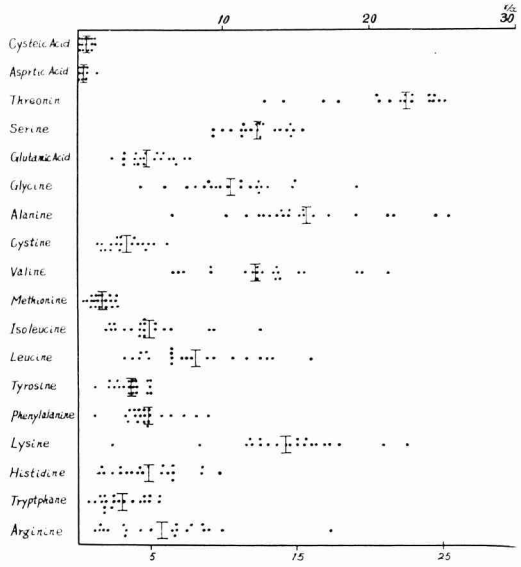
Table 5 *Amino Acid Fractions in Patients with Gastric Ulcer* (r/mℓ)

Name	T. H	S. K	S. K	T. S	H. Y	S. Y	S. T	S. Y	K. D	K. I	K. T	Mean r/cc	Mean mole/ℓ
Cys · NH_2	0.49	0.24	0.26	0.12		0.59	0.15	0.44	0.68	0.45	0.64	0.41	2.4
Asp	0.31	0.80	0.65	0.06			0.74	0.90	0.12	0.30	0.28	0.46	3.5
Thr	22.81	23.34	26.50	21.50	21.68	33.59	28.58	26.90	27.60	24.60	35.00	26.55	222.9
Ser	12.50	13.20	10.51	13.00	9.43	12.08	14.80	14.40	7.30	10.50	10.00	11.61	110.5
Glu	5.70	6.70	4.80	3.90	4.18	6.32	4.31	4.60	5.10	4.50	4.00	4.92	29.8
Gly	9.00	14.36	16.85	16.00	12.58	12.39	16.70	15.10	12.60	9.50	15.00	13.64	181.7
Ala	16.20	27.83	25.95	27.20	11.58	13.47	24.60	21.80	10.40	20.50	28.00	20.68	232.1
Cys	4.20	3.22	1.67	4.00	3.21	4.80	3.84	3.75	3.08	3.20	2.00	3.36	55.9
Val	11.40	14.56	13.29	12.10	12.07	13.45	17.10	15.20	12.30	11.50	9.00	12.91	110.2
Met	2.38	2.85	2.92	2.40	2.18	1.39	2.31	1.50	2.15	2.28	1.00	2.12	14.2
Ileu	9.00	4.94	4.54	3.80	5.44	2.32	6.30	3.20	3.70	7.50	8.00	5.34	40.7
Leu	16.00	8.76	8.27	7.00	13.67	4.38	14.40	6.10	8.20	8.00	5.00	9.07	69.1
Tyr	4.90	4.89	1.97	4.50	6.81	4.81	4.99	3.50	2.00	4.90	3.20	4.22	23.3
Phe	4.89	2.86	1.79	6.20	6.07	6.18	7.70	4.90	1.80	9.00	6.80	5.22	31.6
Lys	15.63	26.04	27.20	15.60	15.16	14.69	15.50	12.80	13.90	14.26	13.26	16.91	92.6
His	7.50	12.99	4.85	1.44	4.96	6.24	5.83	6.00	1.70	6.20	2.62	5.48	26.2
Try	3.79	2.78	8.33	1.14	2.67	2.14	4.82	3.41	1.33	3.26	2.65	3.30	13.7
Arg	4.16	4.66	2.85	2.66	4.19	9.95	1.21	1.41	1.16	4.10	5.23	3.78	17.9
Total	150.85	175.02	163.20	142.62	135.88	148.79	173.88	145.91	117.12	144.55	150.88	149.98	

In patients with gastric ulcer, threonine, histidine, tryptophane, and cysteic acid were slightly higher than the average in normal subjects, while lysine, arginine, pheylalanine, tyrosine, leucine, methionine, valine, cystine, alanine, glycine, glutamic acid, and serine were slightly lower than the normal average. Isoleucine and aspartic acid were not much different from normal average.

3. *Amino Acid Fractions in Patients with Gastric Cancer*

In 21 patients with gastric cancer, the amino acid fractions upon admission are shown in Table 6 and Figure 3. cysteic acid, histidine, and tryptophane were higher than the



I average values in normal adults

Fig. 3 Amino acid fractions in patients with gastric cancer.

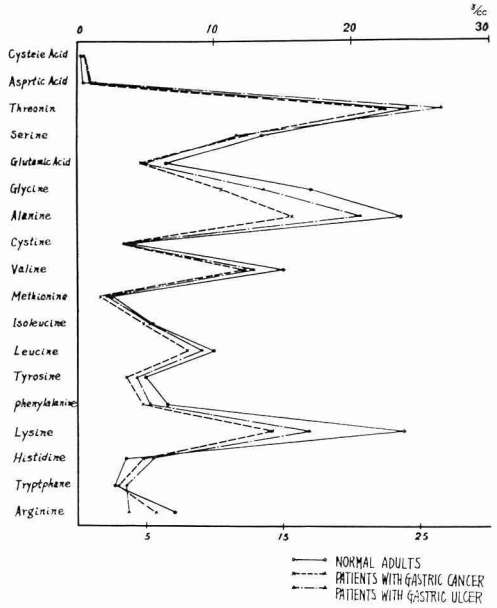


Fig. 4 Amino acid fractions in normal adults and patients with gastric cancer and gastric ulcer. (Average values)

normal average, while aspartic acid, threonine, serine, glutamic acid, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, and arginine were lower than the normal average.

Upon comparison of the values between gastric ulcer and gastric cancer, aspartic acid, threonine, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, pheylalanine, lysine, and histidine were higher in ulcer, whereas serine, cysteic acid, and arginine were higher in cancer. Glutamic acid, cystine, and tryptophane showed no difference between the two groups (Fig. 4).

4. *The Degree of Progress of Gastric Cancer and Amino Acid Fractions*

In 21 cases of gastric cancer the degree of progress was classified according to the classification of Japanese Cancer Society together with calculation of A/G ratio as shown in Table 7. Two patients were in stage 1°, 4 in 2°, 8 in 3°, and 7 in 4°. Amino acid fractions and their correlation with the stage are shown in Fig. 6. Among the

Table 6 *Amino Acid Fractions in*

Name	T. M	M. Y	I. A	T. M	T. T	K. T	K. M	S. T	K. F	Y. S	G. T
Cys·NH_2	0.24	0.97		0.52	0.89		0.39	0.12	0.76	0.10	0.24
Asp	0.31	0.31	0.62	0.12	0.20	0.24	0.15	0.40	0.51	0.03	0.31
Thr	22.46	28.70	22.89	24.77	24.23	17.85	22.50	22.96	24.41	21.40	20.70
Ser	14.40	13.80	11.05	14.60	12.37	11.46	14.60	14.80	12.55	10.50	12.50
Glu	5.21	5.70	2.24	4.45	3.85	6.22	5.40	4.00	3.08	4.60	5.90
Gly	9.53	6.00	12.03	4.31	14.79	8.06	9.70	14.90	10.34	11.80	11.20
Ala	14.11	14.40	13.21	6.45	17.21	12.56	21.60	19.20	15.28	10.20	15.40
Cys			2.67	2.94	1.67	4.80	4.60	2.33	2.71	4.40	1.30
Val	9.10	13.70	13.64	13.51	6.56	19.19	9.10	15.50	13.68	12.70	12.30
Met	1.38	2.50	0.86	0.69	1.38	2.40	1.90	1.40	1.59	1.05	1.08
Ileu	9.00	9.15	1.85	2.08	4.52	5.36	3.60	3.20	4.52	4.30	4.50
Leu	6.40	16.00	3.72	4.89	6.41	13.22	7.70	11.60	8.89	12.50	3.20
Tyr	4.90	4.90	2.16	3.56	4.94	3.71	3.79	4.00	2.42	2.80	4.90
Phe	4.45	3.89	5.70	4.34	4.89	3.12	1.12	7.20	3.91	4.50	4.80
Lys	11.88	12.50	15.57	15.02	12.50	17.92	13.06	16.90	8.37	20.90	22.50
His	5.77	2.89	8.48	6.14	4.04	6.59	4.30	5.89	8.52	1.70	2.89
Try	4.80	1.89	4.49	4.51	3.79	2.41	4.36	2.46	5.56	0.76	1.89
Arg	6.40	6.65	17.32	4.30	6.65	8.91	1.70	1.58	7.44	2.00	4.99
Total	130.34	143.95	138.50	117.20	130.89	144.02	129.57	148.44	133.82	126.24	130.60

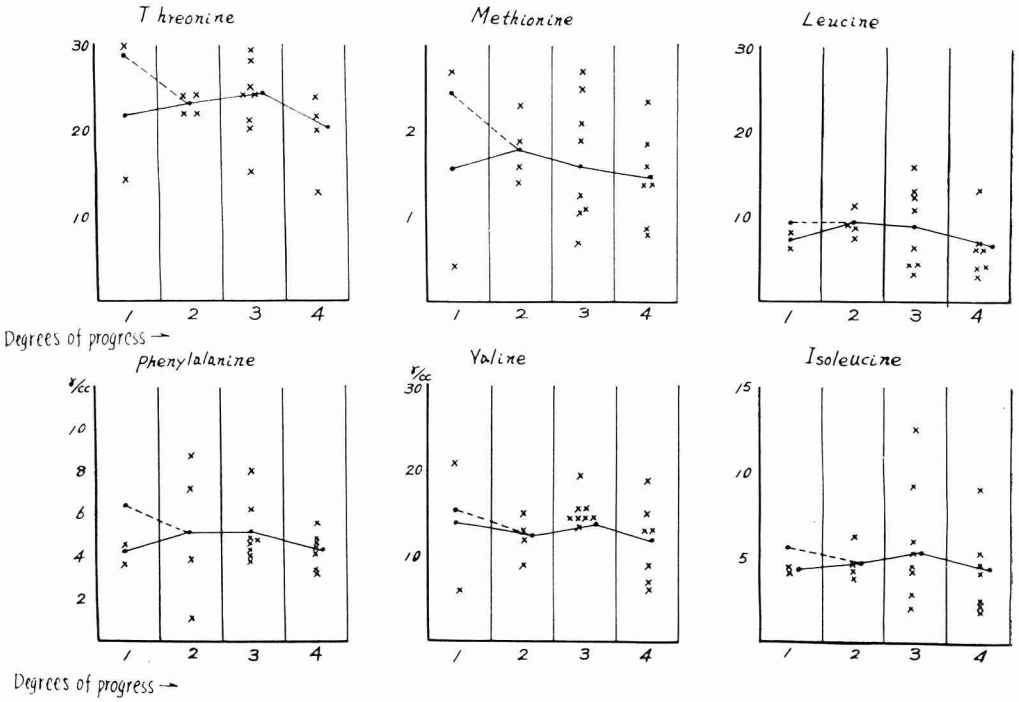


Fig. 5-a Degrees of progress of gastric cancer and amino acid fractions.

Patients with Gastric Cancer (r/mℓ)

T. T	K. N	S. F	T. I	S. O	Y. S	T. Y	S. S	N. I	K. S	Mean	
										r/cc	μmol/ℓ
0.73	1.09			1.02	0.34	0.12	0.16		0.99	0.54	3.2
1.23	0.03	0.13		1.20	0.13	0.68	0.31		0.28	0.38	2.8
14.10	42.14	29.69	20.56	16.94	29.40	12.80	25.17	22.20	24.16	22.48	188.7
10.00	12.60	9.23	11.20	9.30	11.30	11.19	15.40	12.30	13.60	12.32	117.2
2.90	3.14	7.32	3.22	4.31	6.50	6.70	4.50	7.60	4.00	4.66	28.2
12.40	13.02	12.41	8.77	19.22	11.20	12.52	9.20	9.00	7.50	10.85	144.5
15.40	21.31	24.54	14.09	11.63	25.40	16.20	13.72	14.67	12.70	15.68	176.0
2.84	5.15	6.01	4.12	3.78	1.65	1.81	2.01	2.80	5.90	3.34	55.6
6.80	19.49	21.27	13.75	12.07	12.30	15.28	11.51	7.25	12.40	12.24	104.5
0.40	2.69	2.72	1.85	2.11	1.25	1.65	1.91	0.80	2.30	1.61	10.8
4.50	5.24	4.25	2.56	2.03	12.50	2.49	5.90	4.10	6.30	4.85	37.0
6.40	6.41	8.76	4.21	4.76	13.00	4.34	10.60	7.06	9.30	8.07	61.5
4.90	3.99	1.07	2.08	3.99	3.02	3.72	3.90	2.67	3.50	3.57	19.7
4.50	4.02	3.70	4.01	8.04	4.75	4.68	6.30	3.49	8.90	4.78	28.9
15.60	11.88	14.54	13.64	2.30	14.32	16.66	11.63	16.13	15.08	14.23	77.9
2.31	1.65	6.57	4.80	3.23	6.40	9.76	3.69	1.53	3.40	4.79	22.8
1.89	17.0	1.11	2.71	5.62	1.50	4.09	1.89	2.31	3.03	2.99	12.4
3.33	1.53	9.19	8.67	6.61	8.50	7.76	3.16	1.19	3.24	5.69	27.0
111.23	139.42	162.51	120.24	109.76	163.46	132.45	130.96	115.10	136.28	149.98	

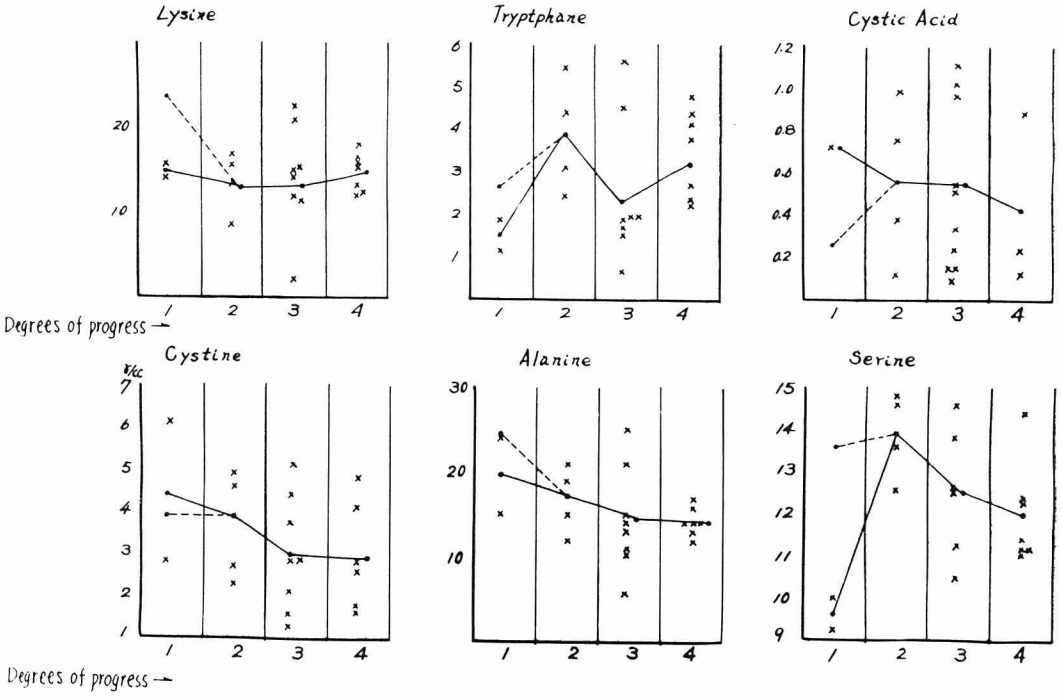


Fig. 5-b Degrees of progress of gastric cancer and amino acid fractions.

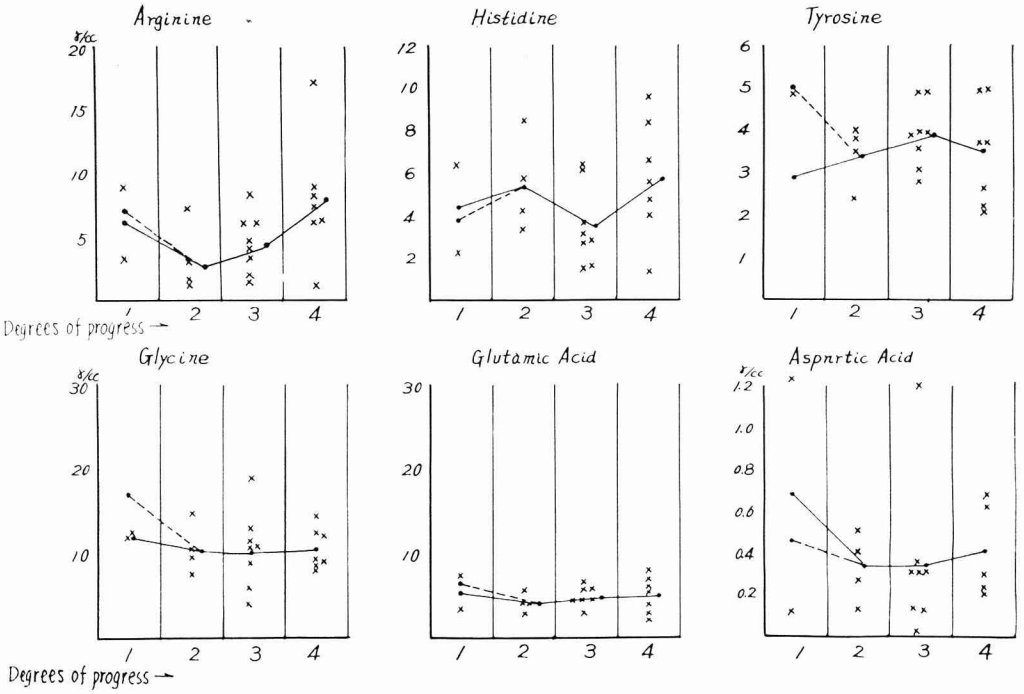


Fig. 5-c Degrees of progress of gastric cancer and amino acid fractions.

Table 7

Name	Age	Sex	Bpdy Weight (kg)	Op. Time	Anes thesia	Op. Me.	R	P.	H.	N.	S.	Stage	Bo.	A/G
S. F	43	♂	50	110	GOF	BII	1	P0	H0	N0	S0	I	2	1.18
T. T	55	♀	47	60	GOF	BI	1	P0	H0	N0	S0	I	1	1.71
S. T	49	♂	48.5	50	GOF	BI	2	P0	H0	N0	S1	II	3	1.06
K. M	56	♂	39	70	GOF	BI	2	P0	H0	N0	S1	II	3	1.28
K. F	58	♂	57	105	GOF	BII	3	P0	H0	N1	S0	II	3	1.76
K. S	70	♂	49	70	GOF	BI	1	P0	H0	N0	S1	II	2	1.23
K. N	43	♂	58	60	TH	BI	2	P1	H0	N2	S1	III	1	0.86
Y. S	45	♂	49.5	80	GOF	BI	1	P0	H0	N2	S2	III	3	0.47
M. Y	51	♂	50	105	GOF	BI	3	P1	H0	N3	S2	III	3	1.38
S. O	58	♂	50	100	GOF	BII	3	P1	H0	N1	S2	III	2	1.34
T. M	66	♀	33	90	GOF	BI	1	P1	H0	N1	S0	III	3	0.68
S. S	68	♂	51	150	GOF	BII	2	P1	H0	N2	S2	III	2	1.60
G. T	74	♂	55.5	70	GOF	BI	1	P1	H0	N1	S1	III	2	1.42
Y. S	76	♂	38	60	GOF	BII	2	P1	H0	N2	S2	III	3	2.09
N. I	31	♀	51.5	60	GOF	BII	3	P2	H0	N4	S0	IV	3	1.58
T. I	40	♂	52.5	110	GOF	BII	2	P1	H0	N2	S3	IV	2	1.36
T. Y	52	♂	48	100	GOF	BII	3	P2	H0	N4	S3	IV	3	1.43
T. M	54	♀	46	90	GOF	BII	3	P2	H1	N3	S1	III	1	1.48
I. A	56	♂	48	90	GOF	BII	3	P1	H0	N3	S3	IV	3	1.11
T. T	57	♂	47	90	GOF	BII	3	P1	H2	N3	S2	IV	3	1.30
K. T	67	♀	36	60	T Th	BI	1	P1	H1	N1	S1	IV	2	1.30

P: Metastasis of peritoneum H: Metastasis of hematogenic
N: Metastasis of lymphnode S: Metastasis of serosa

essential amino acids, threonine and methionine showed a tendency of decrease as the stage progressed. Valine and leucine showed similar tendencies, while isoleucine, phenylalanine, and tryptophane showed scarcely any difference.

However, upon comparison with the normal average, the values in 4° showed a tendency of decrease except for tryotophane.

Among non-essential amino acids, cystine and alanine decreased as the disease progressed to 4°, cysteic acid and serine gradually desreased, and glycine and glutamic acid were unchanged. Upon comparison of the values at 4° with the normal average, tyrosine, cystine, alanine, glycine, glutamic acid, and serine were decreased, whereas cysteic acid, arginine, and histidine showed an increase. In some cases at 2° and 3°, valine, isoleucine, tryptophane, and leucine among the essential amino acids showed higher values than normal. Among non-essential amino acids, histidine, arginine, and serine occasionally showed higher values than normal at 2° and 3°.

5. *A/G and Amino Acid Fractions*

Fig. 6 shows the relationship between amino acid fractions and A/G ratio.

Among essential amino adids, methionine, isoleucine, and leucine showed a tendency of decrease as A/G decreased, while tryptophane showed a tendency of decrease in patients with gastric cancar. Valine, on the contrary, showed a tendency of increase as the A/G decreased. Threonine, phenylalanine, and lysine were unrelated with A/G.

Among non-essential amino-acids; arginine, tyrosine, glycine and aspartic acid showed a tendency of decrease, while cystine showed a tendency of increase and alanine, glutamic acid, serine and cysteic acid were unrelated.

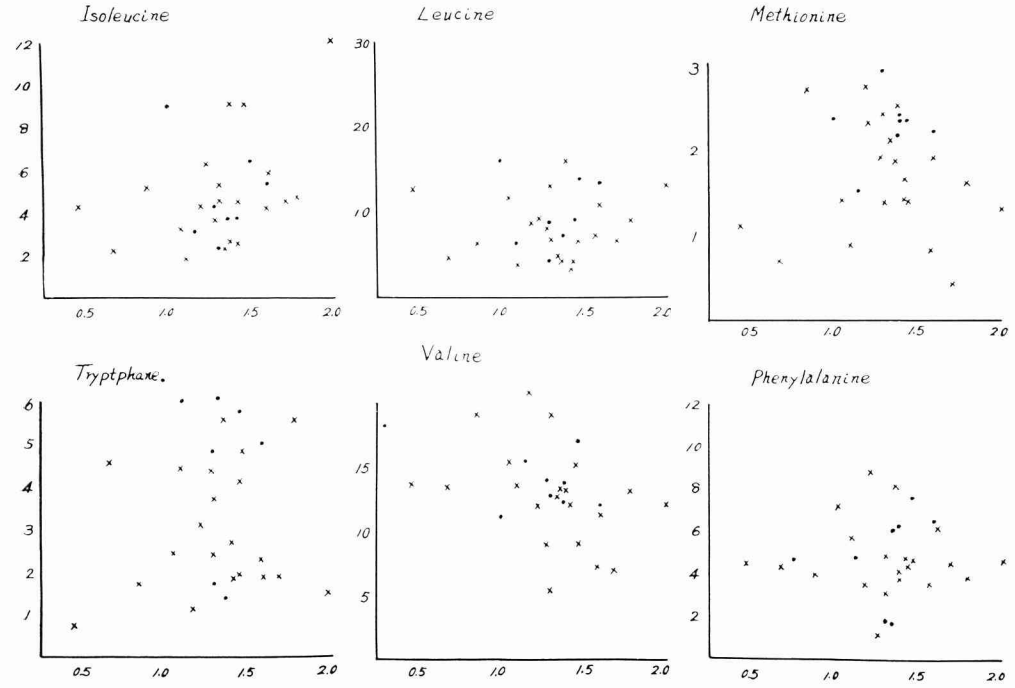


Fig. 6-a A/G ratio and aminoacid fractions.

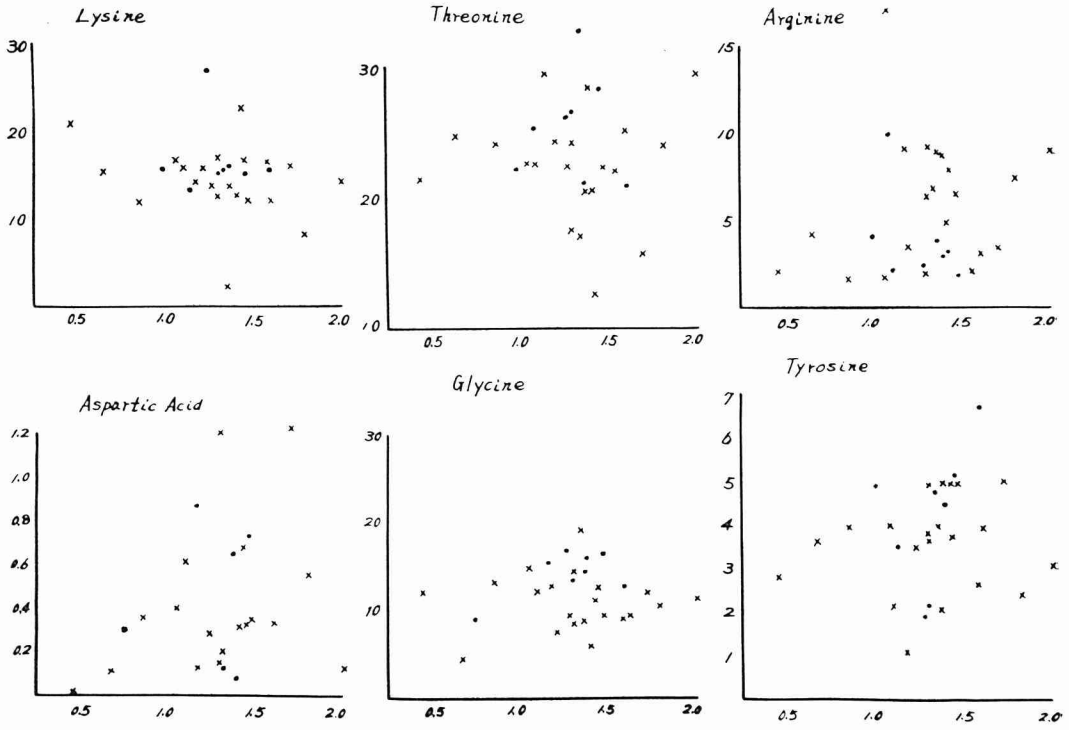


Fig. 6-b A/G ratio and aminoacid fractions.

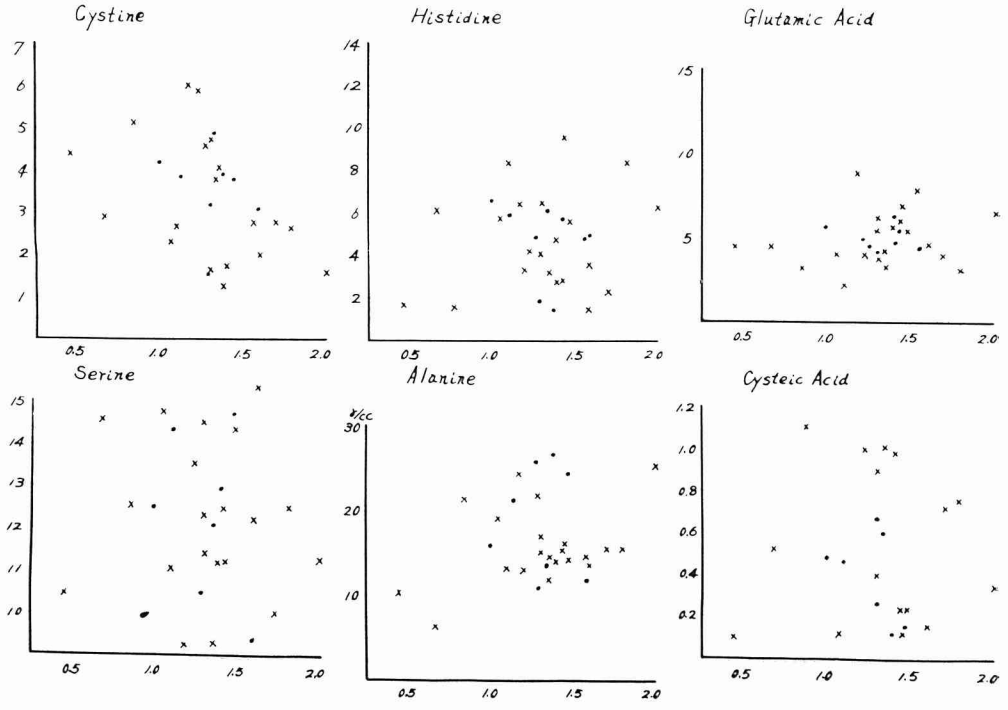


Fig. 6-c A/G ratio and aminoacid fractions.

6. *Changes in Amino Acid Fractions due to Glucose Loading*

The effect of infusion of glucose solution on serum amino acid fractions was studied. In the early morning and at the fasting state, 5% glucose solution was infused and a blood sample was obtained 4 hours later in 3 patients before gastrectomy immediately after admission, showing A/G within normal limits. The amount of infusion was regulated to 1/5 of the amount of extracellular fluid.

The amount of infusion was regulated to 1/5 of the amount of extracellular fluid.

Following the infusion of glucose solution most of the amino acids decreased. (Table 8, Fig. 7)

The rate of decrease was inconstant, but higher in non-essential amino acids than in essential amino acids.

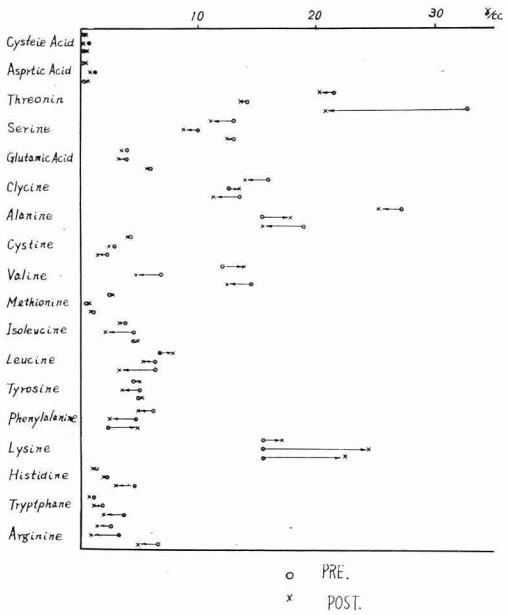


Fig. 7 Changes in amino acid fractions due to glucose loading.

Table 8 *Amino Acid Fraction in Glucose Loading* (r/mℓ)

Name	T. S			T. T			G. T		
	Pre.	Post.	C.V. %	Pre.	Post.	C.V. %	Pre.	Post.	C.V. %
Cys・NH_2	0.12	0.12	0	0.73	0.15	20.54	0.26	0.24	92.31
Asp	0.06	0.05	83.33	1.23	0.99	80.48	0.31	0.31	0
Thr	21.50	20.20	93.95	14.10	13.40	82.92	32.80	20.70	63.10
Ser	13.00	11.00	81.62	10.00	8.40	84.00	13.00	12.50	96.15
Glu	3.90	3.70	94.87	3.90	3.10	79.48	5.95	5.90	99.16
Cly	16.00	14.00	87.50	12.40	13.60	109.67	13.60	11.20	82.35
Ala	27.20	25.20	92.64	15.40	17.80	115.58	19.00	15.40	81.05
Cys	4.00	4.00	0	2.84	2.45	86.27	4.20	1.30	30.95
Val	12.10	11.40	94.21	6.80	4.10	60.29	14.60	12.30	84.25
Met	2.40	2.40	0	0.40	0.54	135.00	1.10	1.08	98.18
Ileu	3.80	3.40	89.47	4.50	2.20	48.88	4.50	4.50	0
Leu	7.00	7.80	111.42	6.40	5.30	82.81	6.40	3.20	50.00
Tyr	4.50	4.90	108.88	4.90	3.50	71.43	4.90	4.90	0
Phe	6.20	4.80	77.42	4.50	2.50	55.55	2.34	4.89	208.97
Lys	15.60	17.00	108.97	15.60	24.50	157.05	15.60	22.50	80.13
His	1.44	1.27	88.19	2.31	2.15	93.07	4.62	2.89	62.55
Try	1.14	0.76	66.66	1.89	1.06	56.08	3.79	1.89	49.86
Arg	2.66	1.33	12.40	3.33	0.75	22.52	6.65	4.99	75.03
Total amino acid	142.62	133.33	93.486	111.23	84.44	75.914	153.62	130.69	85.07
Essential amino acid	69.74	67.76	97.16	54.19	53.60	98.91	81.13	71.06	87.59
non essential amino acid	72.88	65.57	89.96	57.04	30.84	54.06	72.49	59.63	82.26

Summary and Discussion

Upon reviewing the history of the determination of free amino acids in serum, we cannot help being profoundly surprised by each method of quantitative determination established by the efforts of previous investigators.

In the initial stage, determination of individual amino acids such as Sakaguchi and Diazo reaction was carried out, utilizing the property of each amino acid. Through the classical method of determination of amino acid nitrogen by Van Slyke[79], the age of chromatography arrived. Paper chromatography, gaschromatography, and starch column chromatography became available[77,78]. DNP-amino acid chromatography is frequently used in various clinical fields at present, since the apparatus is simple and of low cost and the procedure takes only a short period of time. This method was also questioned and became uncertain in view of the results which were considerably different from those obtained by microbioassay, utilizing the specificity of microorganism, known to be the most accurate but time consuming method. As one of the sources of such variations and uncertainties in the results, inconsistencies in the preparation of the sample especially the process of deproteinization may be cited. For deproteinization, deproteinizing agents such as sodium tangstate[80~82], trichloracetic acid[83,84], picric acid[85,86], and sulfosalicylic acid are used for the pretreatment.

Although the protein and large peptide become insoluble and precipitated at this stage, the borderline between precipitable and nonprecipitable peptides is not clear at present[87,88]. Recent progress in peptide autoanalyzer might be able to solve the problem in the near future.

The variable results according to the deproteinizing agents therefore definitely present a problem.

The desalting procedure following deproteinization is also troublesome and difficult, deeply influencing the result of determination.

When Technicon autoanalyzer is used, these deproteinizing and desalting procedure is entirely different from those in the conventional procedure and extremely simple. By using sulfosalicylic acid and keeping the sample 24 hours thereafter, we have successfully solved this problem, preparing all samples under identical conditions.

Although the conventional method requires 3 to 7 days to analyze the whole amino acids in one sample, column chromatography requires only 8 to 24 hours and the Technicon autoanalyzer only 4 hours and 45 minutes. The accuracy in the Technicon autoanalyzer method allowed the error of only $100\pm2\%$.

Generally speaking, the normal values in adults are difficult to obtain even with an excellent apparatus, since the serum amino acid level is delicately influenced not only by serum protein, but also by the dietary intake such as sugar, fat, and vitamins as well as by various physiological condition. Even a slight difference in dietary intake might give different levels of normal values. Racial or geographical difference such as the location in the tropical or temperate zone might also influence the result. Lob[73] also emphasized the difficulty in determining the normal value due to the individual difference or difierence within the individual between summer and winter.

According to our determination, lysine, threonine, glycine, alanine, and valine showed a wide individual variation, while other amino acids were relatively free from such fluctuations.

According to Takada[89] of our department, plasma amino acid concentration was higher in infants than in children, and that of children higher than in adults. Andrews[90] and Seligson[91] obtained similar results. Yoshimoto[92] of our department obtained lower values in aged subjects and Ackermann[93] obtained similar results.

The normal values obtained by us were determined in subjects between 20 and 30 years of age as long as it is possible, by blood sampling after some period of rest in the early morning in a fasting state. Somewhat lower values were obtained than the result of Moore and Stein used as the standard reference by many investigators in Japan or other reports by European and American workers. Although the same method of column chromatography was used, there was some difference between our results and those of Job[73], Oepen[94], Ackermann[93], and Cusworth[72] in histidine and lysine.

Concerning the difference between males and females, Ackerman[93] reported the larger values in females and Oepen[94] claimed a slightly higher values in males. However, according to our results, a significant difference was noted between males and females, females generally showing higher values than in males.

Soupart[95] pointed out the relationship between serum amino acid fractions and menstrual cycle and the decrease of threonine, serine, alanine, and lysine during the luteal phase. According to our results obtained regardless of these reports, these 4 kinds of amino acid showed a considerably high value. The relationship with the menstrual cycle is to be determined in the future. However, Tosen[77] found no difference between males and females at all.

Concerning the amino acid fractions in patients with gastric ulcer, threonine, histidine, tryptophane, and cysteic acid were higher than normal, lysine, arginine, phenylalanine, tyrosine, leucine, methionine, valine, cystine, alanine, glycine, glutamic acid, and serine were lower than normal, and isoleucine and aspartic acid showed values similar to the normal level according to our results. Tosen[77] pointed out that tryptophane and histidine were slightly low but without significant difference. Ubukata reported an increase in phenylalanine, alanine, histidine, tryptophane, threonine, aspartic acid, alanine, and tyrosine compared with the normal subjects, while, valine, methionine, and glycine showed a decrease[96].

Serum free amino acid levels in patients with gastric ulcer showed an increase in lysine, histidine, and tryptophane and a decrease in most of the amino acid such as aspartic acid, threonine, serine, glutamic acid, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, and arginine as compared with normal subjects.

No reports are available in Europe and America concerning the amino acid fractions in patients with gastric cancer, except for that of Everson[65] who reported low valued of isoleucine and leucine. In Japan, Inao[97] stated that most of the amino acids including threonine, tryptophane, and lysine showed higher values, Arichi[64] also pointed out higher values of most of the amino acids as compared with normal subjects, Ubukata reported

the increase of tyrosine, glutamic acid, aspartic acid, phenylalanine, alanine, leucine, isoleucine, histidine and tryptophane as well as the decrease of valine and methionine[96]. Kasaoka[98] reported the higher values of threonine, glutamic acid, valine, isoleucine, leucine, lysine, and arginine, and Hirai[29] reported lower values of threonine, lysine, and cystine and higher values of other amino acids. With the method of high voltage filter paper electrophoresis during the last few years, we have reported the increase in isoleucine and leucine and the decrease in glycine, lysine, and histidine[52~54]. Tosen[77] reported a slightly higher value of glutamic acid and lysine and a slightly lower value of tryptophane, histidine, and arginine without statistical significance, denying the presence of a characteristic change in cancer.

Although there are many contradictions in these reports, these date should not be directly compared each other, especially in view of the fact that the amino acid metabolism of the whole living organism could not be reasonably discussed unless the amino acid metabolism of the cancer itself is considered first. The author has therefore studied the relationship between the degree of progress of cancer and amino acid metabosism and found certain relationship. Most of the amino acids decreased as the cancer progressed to the fourth degree, especially easential amino acids such as threonine, methionine, lysine, valine, leucine, isoleucine, and phenylalanine, and non-essential amino acids such as cystine, alanine, tyrosine, glycine, glutamic acid, and serine.

However, among essential amino acids such as valine, leucine, isoleucine, and tryptophane showed a temporary increase at the second and third degree over the normal level, and similar tendency was noted in non-essential amino acids such as histidine, cystine, arginine, and serine.

It appears therefore without significance to discuss the amino acid fractions without considering the difference in the nutritional state and the degree of progress of the cancer lesion.

Although we have selected only cases with normal liver function for the determination, Watanabe[99] in our department studied cases of gastric cancer with disturbance of liver function to compare the degree of progress of gastric cancer and amino acid fractions. A distinct relationship was found, indicating a marked decrease in amino acids along with the progress of gastric cancer.

We have studied the relationship between serum amino acid fractions and albumin value in gastrectomized patients for the first time together with the problem of the progress of cancer. As the albumin concentration decreased, essential amino acids such as leucine, isoleucine, glycine, and non-essential amino acids such as arginine, tyrosine, glycine, and aspartic acid showed a decrease, while valine showed a tendency of increase. The occurrence of anemia and hypoproteinemia in patients with gastric cancer is a well known fact[100,101].

As Kurosawa and many others have pointed out, hypoproteinemia is mostly due to hypoalbuminemia[102~107].

Many investigators have agreed on the role of the decrease of protein synthesis in the liver as the source of protein synthesis as the cause of hypoalbuminemia in cancer carrying organism. Galchr, Zettel, Wuhrmann, Wundery, Leuteher, Osawa, Fujita, Akai,

Yoshida, Miyoshi, and Yamaguchi, on the other hand, pointed out the relationship between the changes in albumin concentration on one hand, and the size of the tumor, degree of metastasis, and the reaction of the lymphocytes on the other. A considerable effect is apparently exerted on serum albumin from the intense anabolism and catabolism of the cancer tissue and its metastasis. Cancer tissue apparently takes up more albumin from the blood of the host as its component. The energy source is probably obtained from glycolysis and polysaccharides and glycoprotein which increases α-globulin are released as the metabolite.

The cause of the decrease in amino acid especially the free amino acid in serum as the component of albumin in patients with gastric cancer seems to be as described above.

As we found a decrease in many of the essential amino acids along with the decrease in albumin, Everson[65] also reported the decrease in isoleucine, lysine, and histidine in patients with malnutrition subjected to surgery.

There are only few reports on the decrease in serum free amino acid fractions in response to glucose loading. In an experiment using isotopes, Munro[108,109] demonstrated the uptake of serum free amino acid into the muscles upon glucose loading. Folin[110], Berglund, and Tamura[111] confirmed the decrease in serum free amino acid and amino acid nitrogen following sugar administration at the fasting state, reporting an excellent agreement between serum free amino acid fractions and amino acid fractions in human muscle. A correlation was found between these.

Although we have demonstrated the decrease of most of the amino acid fractions in response to glucose loading in the fasting state in the early morning in patients with gastric cancer and those with gastric ulcer, no correlation was found between the rate of decrease and the amino acid fractions in human muscle.

According to the study of Takeda of our department, the rate of decrease of serum free amino acid might be used as the measure of the amino acid requirement of the body. This problem deserves further study[71].

Conclusion

No definite informations are yet available concerning the protein metabolism in the field of surgery despite the recent establishment of the method of determination of amino acids leading to studies of individual amino acid.

As a fundamental study on the amino acid metabolism in the field of surgery, plasma free amino acid fractions were determined in normal males and females, patients with gastric cancer. The relationship between the degree of progress of gastric cancer and amino acid fractions and between A/G ratio and amino acid fractions as well as the decrease in amino acid fractions in response to glucose loading was studied.

Following conclusions were obtained.

1) Serum free amino acid fractions in 11 normal males and females showed a distinct sex difference, males generally giving somewhat lower values than in females. However, males showed higher values of glycine, methionine, isoleucine, leucinet, tyrosine. A distinct difference was also noted in glycine, glutamic acid, isoleucine, leucine, lysine, and arginine.

2) Serum total amino acid concentration followed the decreasing order from normals, patients with gastric ulcer, and those with gastric cancer.

3) The amino acid fractions in patients with gastric ulcer was higher than normal in threonine, histidine, tryptophane, and cysteic acid, and lower than normal in lysine, arginine, phenylalanine, tyrosine, leucine, methionine, valine, cystine, alanine, glycine, glutamic acid, and serine.

4) Most of the amino acid fractions were decreased in patients with gastric cancer than in normals.

5) As the gastric cancer progressed, most of the amino acid fractions decreased except for a temporary increase at certain stage.

6) As the serum albumin concentration decreased, amino acids such as leucine, isoleucine, methionine, tryptophane, arginine, tyrosine, glycine and aspartic acid showed a tendency of decrease.

7) Upon glucose loading, most of the amino acid showed a decrease.

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