

Determination of Amino Acids and Polyamines in Human Erythrocytes Part 2. Clinical Application^{*)}

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ABSTRACT

- 1) A rapid and high sensitive method for quantitation of free amino acids and polyamines in human erythrocytes using high performance liquid chromatography (HPLC) was devised by the author and his colleagues.
- 2) Measurement of free amino acids in erythrocytes was performed on 12 patients on our ward. Marked change in amino acid pattern was observed, and there are 2 in whom the prognosis of disease was not favorable. The amino acid pattern was generally constant in the remaining 10 patients.
- 3) The polyamine levels in erythrocytes of 15 patients who had undergone surgical operation were determined, and it was noted that the value began to increase from immediately after surgery in those whose postoperative course was good, and peaked 7 to 10 days after the operation, after which it gradually reverted to the preoperation level. In those who developed complications after operation, the pattern was different from Pattern 1.
- 4) No difference in polyamine value could be demonstrated between 9 cancer patients and 7 non-cancer patients.
- 5) A correlation between polyamine values in erythrocytes and erythrocyte aging was observed with the value being higher in young erythrocytes, i. e. reticulocytes, and low in those about to break-down. Thus, it was considered that if hematopoietic action was accelerated due to bleeding during and after operation, the polyamine values would be elevated, but if hematopoiesis was not active, the polyamine values would be low.
- 6) The Spm value in leucocytes was comparatively higher than that in erythrocytes, and this finding was also confirmed in clinical cases.

INTRODUCTION

Amino acids are used as material for protein synthesis, and is administered by intravenous and enteral hyperalimentation. On the other hand, polyamines are synthesized from ornithine and s-adenosyl-methionine, but there is a correlation in volume between them and nucleic acid-protein synthesis in tissue. It has been suggested that polyamines serve as a priming agent for cell proliferation, and increase in their

values precede increase of RNA and cell division in actively proliferating tissue.

Russel et al. (1971) and Uehara et al. (1980) contended that determination of polyamines in urine and in erythrocytes of cancer patients was helpful in the establishment of diagnosis. Uehara et al. also claims that their erythrocyte value decreased after surgical operation. Therefore, it is considered to be an important substance from the clinical viewpoint. In our previous report¹⁹⁾, we described our analytical meth-

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od for free amino acids and polyamines in erythrocytes by HPLC using the capillary sampling system (C-S system). In this report, we have reviewed the quantitative analysis of free amino acids in erythrocytes and changes in erythrocyte polyamine values prior to and after surgical operation in cancer and non-cancer patients.

METHOD AND SUBJECTS

1) Method of measurement

The details have been described in the previous paper¹⁹⁾. Erythrocytes were treated by destroying the membrane and deproteinizing the cells with 1% Triton X-100 D and 10% trichloroacetic acid (TCA) using the C-S system. The supernatant was injected into HPLC (Toyo Soda Co Ltd., 801 type) and fluoresced with Orthophthal aldehyde (OPA) and analyzed by peak-height ratio method.

2) Subjects

(1) Free amino acid determination

Esophageal varices with liver cirrhosis
2 cases

(2) Polyamine determination in erythrocytes

[1] Cancer patients 9 cases
Non-cancer patients 7 cases

[2] Pre- and postoperative cases

Cancer patients (esophagus 1, lung 2, stomach 2, pancreas head 1, ileocecum 1, retroperitoneum 1) 8 cases
Gastric ulcer 3 cases
Chronic renal failure 3 cases
Cholelithiasis 1 case

Blood was drawn from the cubital vein in fasting state early in the morning, and treated within 3 hr after collection. The brief clinical course of the 2 cases who showed changes in the free amino acids and 15 cases with changes in polyamines was follows.

(1) Free amino acids in plasma and erythrocytes

Case 1. 54-year-old, male

He underwent esophageal transection under the diagnosis of liver cirrhosis and esophageal varix. The patient's NH_3 value increased to 477.1 mg/dl in serum from the 2nd day after operation, and he lapsed into hepatic coma. Lacturose (disaccharide preparation, Nikken Kagaku KK), Argi-

mate (Hyperammonemia preparation, Morishita Seiyaku KK), GO-80 (transfusion for hepatic failure, Ootsuka Seiyaku KK) were administered, and he regained consciousness 4 days later. His serum NH_3 was decreased. His condition subsequently became aggravated and marked jaundice developed. He lost consciousness and died 4 months later.

Case 2. 51-year-old, female

The patient underwent splenectomy and perigastric devascularization under the diagnosis of liver cirrhosis and esophageal varix. Preoperative total protein (T. P.) and A/G ratio were 7.3 mg/dl and 1.03 respectively, and the same on the 1st day after operation were 5.8 mg/dl and 1.23. Intravenous hyperalimentation (Including 10% total amino acid infusion 200 ml/day) was administered from the 7th day after operation because of persistent symptoms of peritonitis. Laparotomy was performed under the diagnosis of perforation of gastric wall on the 12th day after operation, but her general physical condition failed to improve and she died on the 13th day.

(2) Polyamine values in erythrocytes

Case 1. 70-year-old, male

His chief complaint was ileocecalgia. Family history and past history were not remarkable. He received right hemicolectomy and ileum-transverse colostomy, end to end, under the diagnosis of cancer of ileocecal region (stage II). Blood loss during surgery was 200 ml, but transfusion was not performed. Oral administration of FT-207 300 mg/day began from the 8th postoperative day. His postoperative course was good, and he was discharged on the 15th day after operation.

Case 2. 59-year-old, female

She consulted our hospital with a 7-year history of right hypochondralgia. She was diagnosed as cholelithiasis by oral cholecystography and echography. And underwent normograde cholecystectomy. Blood loss was 200 ml, and no transfusion was performed. As her postoperative course was good, she was discharged on the 10th day.

Case 3. 48-year-old, female

She had experienced occasional epigastralgia, but had left it unattended. Abnormal-

ity of the stomach was pointed out on mass gastric examination. Her family and past history were not remarkable. She underwent Gastrectomy (Billroth I method, Stage II) under the diagnosis of gastric cancer (Borrmann II type). Blood loss amounted to 550 ml, but transfusion was not administered. As her condition improved, she was discharged on the 15th day after operation.

Case 4. 65-year-old, male

An abnormal density was pointed out in the left lung field on chest X-ray. History revealed total laryngectomy had been performed for cancer of the larynx six months prior to this visit. Family history was not remarkable. Left upper lobectomy was carried out under the diagnosis of left metastatic pulmonary cancer. Volume of bleeding was 600 ml, but transfusion was not performed. His postoperative course was uneventful, and he was discharged on the 24th day after operation.

Case 5. 48-year-old, male

He was without subjective symptoms, but an abnormal density was pointed out in the stomach on mass G.I. examination. Family and past history were not remarkable. Cardiectomy, esophago-gastrostomy and pyloroplasty were performed under the diagnosis of gastric cancer (Borrmann II type). Volume was bleeding 1300 ml, and 1400 ml of blood was transfused. Chemotherapy was initiated from the 18th day after operation. His postoperative course was uneventful, and he was discharged on the 29th day after operation.

Case 6. 65-year-old, male

He visited the hospital with the chief complaint of upper abdominal distention. He had a past history of pulmonary tuberculosis when 30. Adenocarcinoma of stomach had been pointed out by gastroendoscopy. Gastrectomy (Billroth I method) was performed and the histopathology diagnosis of chronic peptic ulcer was made on the basis of excised specimens. Volume of bleeding during operation was 500 ml, and transfusion of 600 ml was made. His postoperative course was good, and he was discharged on the 12th day after operation.

Case 7. 43-year-old, male

His chief complaint was persistent epigastralgia of 5 years' standing. A diagnosis of duodenal ulcer was made and selective proximal vagotomy and pylorotomy were performed. Volume of bleeding was 200 ml, and No transfusions were given. He was discharged on the 12th day after operation.

Case 8. 41-year-old, male

He visited our hospital with the complaint of epigastralgia from 3 years ago. Family and past history were not remarkable. He received selective vagotomy and antrectomy (Billroth I method) under the diagnosis of gastric ulcer. Blood loss was 450 ml, and transfusion of 400 ml was made. His postoperative course was good, and he was discharged on the 16th day after operation.

Case 9. 34-year-old, male

He had been diagnosed as having chronic renal failure when 26 years old, and at age 30 had also received cholecystectomy for cholelithiasis. Living renal transplantation was performed, and he was recovering uneventfully, when complication of the ileus developed on the 40th day after operation, necessitating removal of the ileus.

Case 10. 31-year-old, male

Proteinuria and hematuria had been noticed at age 19. When 24 years old, hemodialysis (HD) was commenced under the diagnosis of chronic renal failure, and was receiving HD 2 times a week. When 25 years old, he received living renal transplantation, and the postoperative course was smooth, but signs of rejection appeared from about 30 years old. HD was resumed once a week. He subsequently received his second living renal transplantation.

Case 11. 24-year-old, female

She had been diagnosed as nephrosis at age 6, and was receiving medication. At age 19, she began receiving hemodialysis under the diagnosis of chronic renal failure. She underwent living renal transplantation, but because of poor urine elimination after the operation, obstruction was suspected, and she received a second operation for 6 hr later. A stenosis in the uretero-bladder anastomosis was discovered and an external A-V shunt was made, but renal function could not be obtained. Thus, the renal

graft was removed on the 13th day after operation. Her condition improved and she was discharged 51 days after the 2nd operation.

Case 12. 52-year-old, male

He had complaints of abdominal distention and mass formation in the left hypogastrium. Family and past history were not remarkable. Laparotomy was performed under the diagnosis of retroperitoneal tumor (malignant lymphoma). On the 5th day after operation, Fluid with a foul stench

was drained from under the diaphragm, and on the 10th day, he received his second laparotomy, at which time colostomy was performed because of panperitonitis and perforation of anastomosis of large intestine. Volume of bleeding was 2500 ml, and blood transfusion of 2400 ml was administered at the time of the 1st operation. His condition improved and he was discharged on the 81st day after his second operation.

Case 13. 62-year-old, male

The patient's chief complaint was epigastric

Table 1. Subjects of free amino acid and polyamine determination

Case	Diagnosis	Operation	Bleeding (ml)	Anesthesia			time after operation
				Transfusion (ml)	time	Operation time	
(1) 70y. M.	Ileocecal tumor	right hemicolectomy	250	(-)	3°50'	2°50'	15 days
(2) 59y. F.	Gallstone	cholecystectomy	200	(-)	3°10'	2°15'	10 days
(3) 48y. F.	Gastric cancer	Gastrectomy (Bill-1)	550	(-)	4°30'	3°40'	15 days
(4) 65y. M.	Metastatic pulmonary cancer	Left upper lobectomy	600	(-)	4°00'	3°00'	24 days
(5) 48y. M.	Gastric cancer	Gastrectomy (Bill-1)	1300	1400	5°20'	4°20'	29 days
(6) 65y. M.	Gastric ulcer	Gastrectomy (Bill-1)	512	600	3°50'	3°00'	12 days
(7) 43y. M.	Duodenal ulcer	Selective proximal vagotomy +Pylorotomy	210	(-)	3°50'	3°05'	12 days
(8) 41y. M.	Gastric ulcer	Vagotomy Antrectomy (Bill-1)	450	400	4°10'	3°40'	16 days
(9) 34y. M.	Chronic renal failure	Renal Transplantation	150	(-)	7°05'	5°10'	46 days
(10) 31y. M.	1st: Chronic renal failure 2nd: Ileus	1st: Renal transplantation 2nd: Removal	250	(-)	7°50'	6°10'	38 days
(11) 24y. F.	Chronic renal failure	1st: Renal transplantation 2nd: Extirpation of graft	250	(-)	8°30'	6°20'	51 days
(12) 52y. M.	1st: Retroperitoneal tumor 2nd: Perforation of colon, peritonitis	1st: Extirpation 2nd: Colostomy	1st 2500	2400	5°20'	4°15'	81 days
(13) 62y. M.	Esophageal cancer	1st: Esophagogastrostomy 2nd: Resection	300	(-)	2nd 6°16'	2nd: 2°40'	61 days (died)
(14) 74y. M.	Metastatic pulmonary cancer Diabetes mellitus	Right middle lobectomy	650	600	4°10'	3°05'	18 days (died)
(15) 69y. M.	Cancer of pancreas head Obstructive jaundice	Cholecystojejunostomy	460	420	3°55'	2°50'	12 days

discomfort. After various tests, he was diagnosed as cancer of the esophagus (Ei, squamous cell carcinoma). After a pre-operative course of 3000 rad of Co irradiation, he received esophagogastrostomy and esophagocolostomy as the 1st operation. and 40 days later, the remnant of the esophagus was extirpated. Volume of bleeding was 300 ml, for which blood transfusion of 600 ml was made. He became anuric on the 5th day after operation and received hemodialysis from the 6th day.

Leukemoid reaction was noted on peripheral blood test, and he expired 61 days after operation.

Case 14. 74-year-old, male

He visited the hospital with the chief complaint of bloody sputum. His family history was not remarkable, but he had received colostomy under the diagnosis of cancer of rectum 8 years ago. He also had diabetes mellitus which was under control with dietary therapy. Right middle lobectomy was performed under the diagnosis of right metastatic pulmonary cancer, but he developed pneumonia of the right upper lobe 2 or 3 days after operation which was complicated by non-ketotic diabetes mellitus. Continuous injection of insulin was carried out from the 12th day after operation, but was to no effect, and he died on the 18th day.

Case 15. 69-year-old, male

The chief complaints were generalized fatigue, itchiness and jaundice. Past history revealed gastrectomy for gastric cancer 12 years ago, but family history was not remarkable. Laparotomy was performed under the diagnosis of cancer of the pancreas head, but as resection of the tumor was not possible, only cholecystojejunostomy was carried out. Volume of bleeding was 460 ml, and blood transfusion of 600 ml was administered. His postoperative course was good, and jaundice gradually decreased. As his condition improved, he was discharged on the 12th day. These cases are summarized in Table 1.

RESULTS

- (1) Free amino acids in plasma and erythrocytes

Table 2. Free amino acids in plasma & erythrocytes (Case 1)

54-year-old, male (nmol/ml)

	Plasma	Erythrocytes	E/P
Tau	64.9	56	0.9
Met-SO		2576	
Asp	5.9	60	10.2
Thr+Gln	1522.2	864	0.6
Ser+Asn	217.1	314	1.4
Glu	66.1	306	4.6
Gly	292.6	332	1.1
Ala	439.0	340	0.8
Val	159.3	4	0.03
Met	67.3		
Ile	46.0	2	0.04
Leu	67.3	2	0.03
Tyr	126.3	44	0.3
Phe	96.8	6	0.1
His	83.8	74	0.9
Orn	99.1	438	4.4
Lys	257.2	256	1.0
NH ₃	312.7	156	0.5
E. A.	14.2	2	0.1
Trp	129.8		
Arg	231.3	62	0.3
Total	4098.3	5888	1.4

Case 1. 54-year-old, male (Table 2)

Table 2 shows the state of free amino acids in plasma and erythrocytes 3 months after operation. The Thr+Gln, Gly, Met, Tyr, Phe, Orn, Lys and Arg in plasma were higher than normal, and total amino acids in plasma were also 1.5 times greater than normal, while the Leu value was decreased. In erythrocytes, Met-SO, Thr+Gln, Ser+Asn, Orn, Lys and Arg were increased, and the total amino acid value was about 2 times higher than normal.

Case 2. 51-year-old, female (Table 3)

On the 11th day after operation, Asp, Gly and Val were found to be elevated in plasma, while Met-SO, Asp, Thr, Val, Tyr and Arg were decreased in erythrocytes.

(2) Polyamine in erythrocytes

- 1) The polyamine values elevated from immediately after the operation and peaked on the 7th to 10th day.

Case 1 : The polyamine values peaked on the 10th day after operation. The erythrocyte count was lowest on the 4th day after

Table 3. Free amino acids in plasma & erythrocytes (Case 2)

51 year old Woman (n mol/ml)

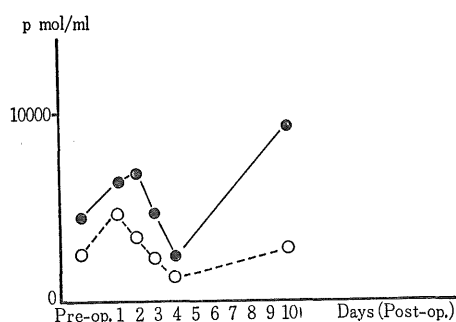
	Post- Operative Day						
	3 P	4 P	11 P	2 E	3 E	4 E	11 E
Tau.	32.9	39.5	57.6	90.7	51.6	39.2	52.6
Met-SO	—	—	—	928.6	922.4	697.6	644.9
Asp.	11.0	12.6	16.8	181.3	157.0	126.4	72.2
Thr.	402.6	399.7	331.2	754.6	354.8	289.9	313.7
Ser.	141.5	167.5	128.4	242.6	189.2	183.1	169.3
Glu.	57.3	74.3	96.0	345.5	292.4	300.8	288.8
Gly.	164.7	194.3	214.8	539.0	372.0	348.8	458.2
Ala.	331.8	366.6	321.6	543.9	324.7	320.5	336.2
Val.	224.5	262.3	100.8	129.9	96.8	133.0	39.8
Met.	42.7	42.7	30.0	24.5	12.9	13.1	2.5
Ile.	75.6	101.1	54.0	34.3	19.4	43.6	12.5
Leu.	135.4	183.3	91.2	61.3	30.1	65.4	24.9
Tyr.	107.4	113.8	67.2	122.5	88.2	89.4	54.8
Phe.	103.7	115.3	91.2	58.8	25.8	39.2	22.4
His.	122.0	143.8	85.2	105.4	178.5	187.5	149.4
Orn.	106.1	128.0	115.2	227.9	223.6	211.5	201.7
Lys.	273.3	273.3	154.8	193.6	163.4	122.1	87.2
Trp.	40.3	30.0	6.0	—	—	—	—
Arg.	64.7	74.3	46.8	19.6	12.9	24.0	12.5

P : Plasma

E : Erythrocyte

operation, but gradually increased thereafter. Polyamine values in erythrocytes gradually increased (Fig. 1).

Case 2 : The polyamine values peaked on



BW (kg)	66	62
WBC	7000	4800
RBC ($\times 10^4$)	412	365
TP	6.4	5.7
A/G	1.29	1.11
GOT	13	25
GPT	17	17
BUN	119	18
Creatinine	0.9	1.9

Fig. 1. Changes of polyamine values in erythrocytes prior to and after operation, 70-year-old, male

the 10th day after operation and the erythrocyte count was lowest on the 4th day after operation (Fig. 2).

Case 3 : Peaking of the polyamine values was noted on the 7th day after operation, and the erythrocyte count was lowest on that day also (Fig. 3).

Case 4 : The polyamine values peaked on the 10th day after operation, while the erythrocyte count was lowest on the 7th day (Fig. 4).

Case 5 : The polyamine values peaked on the 8th day after operation, and the erythrocyte count was lowest on the 14th day (Fig. 5).

2) Although the polyamine value became elevated from immediately after the operation, it did not peak during the 7th to 10th day after operation.

Case 6 : The polyamine values peaked on the 5th day after operation, and the erythrocyte count gradually increased from after surgery (Fig. 6).

Case 7 : The polyamine values peaked on the 1st day after operation and showed a

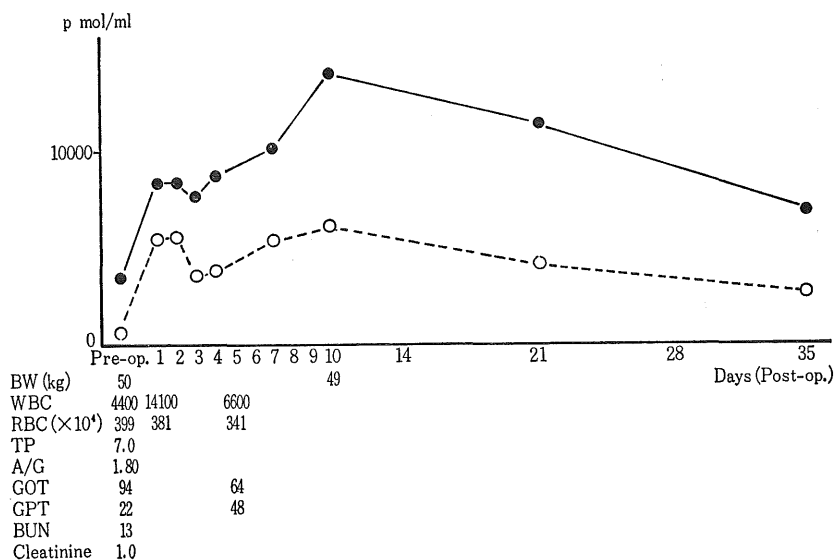


Fig. 2. Changes of polyamine values, 59-year-old, female

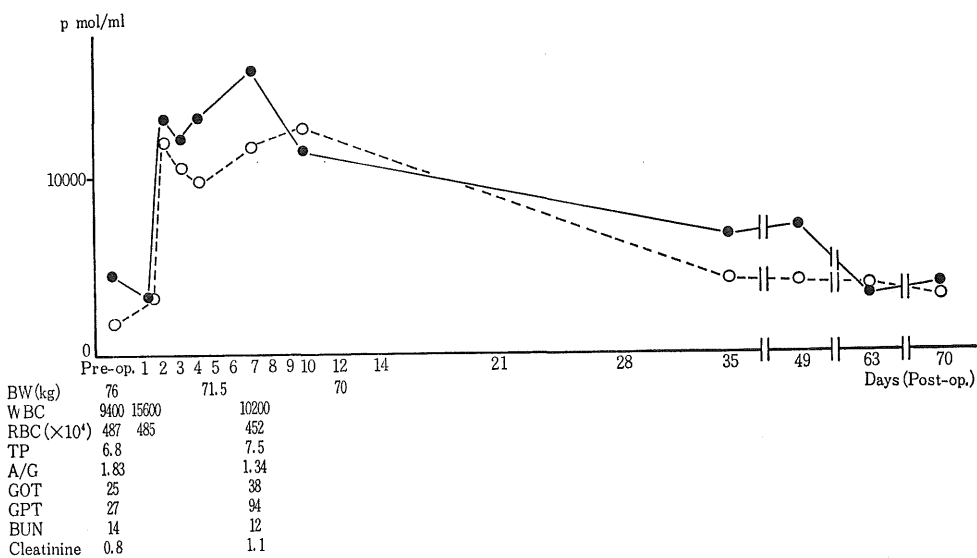


Fig. 3. Changes of polyamine values, 48-year-old, female

second peak on the 10th day. The erythrocyte count decreased on the 7th day (Fig. 7).

Cases 8 : The polyamine values peaked on the 3rd day after operation, and then decreased by the 10th day after operation (Fig. 8).

3) Renal transplantation cases

Case 9 : The polyamine values decreased from immediately after operation, but began to

increase from the 13th day, and became 7 times the preoperative level on the 49th day (Fig. 9).

Case 10 : The polyamine values peaked on the 28rd day after operation, but decreased to one half of this value on the 53rd day (Fig. 10).

Case 11 : The polyamine values were high after operation, but began to fluctuate thereafter (Fig. 11).

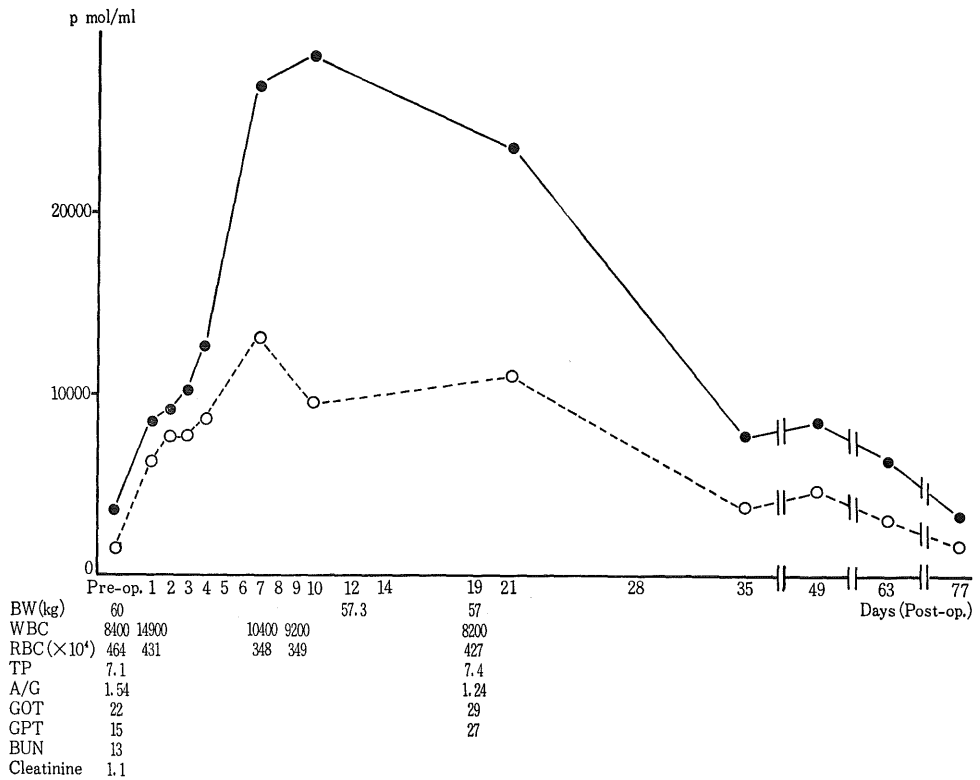


Fig. 4. Changes of polyamine values, 65-year-old, male

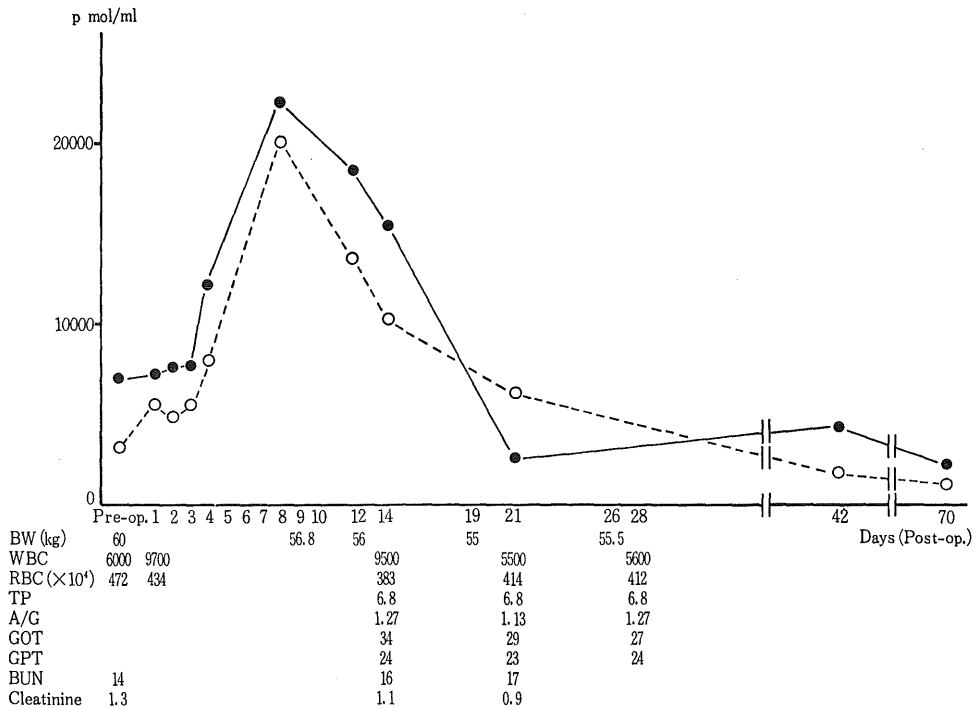


Fig. 5. Changes of polyamine values, 48-year-old, male

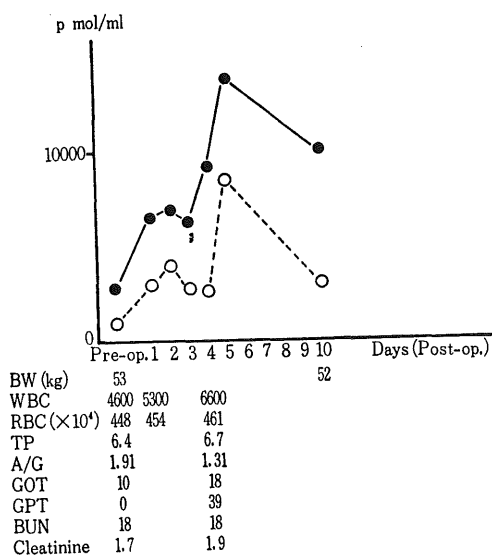


Fig. 6. Changes of polyamine values, 51-year-old, male

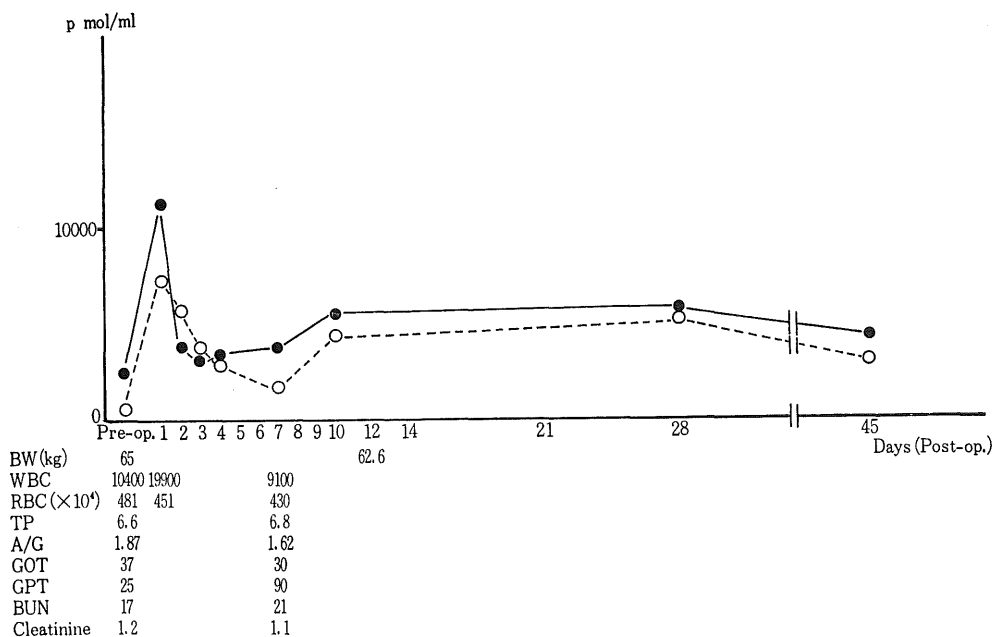


Fig. 7. Changes of polyamine values, 43-year-old, male

Case 15 : The polyamine values decreased from immediately after operation (Fig. 15).

DISCUSSION

(1) Free amino acids in erythrocytes

It was reported in Part I that some free amino acids in erythrocytes readily pass

4) Cases which developed complication of postoperative infection

Case 12 : The polyamine values in erythrocytes were elevated from the 1st day after operation, with the Spm value being higher than the Spd value, but decreased on the 10th day. However, the Spm value remained higher than the Spd value (Fig. 12).

Case 13 : The polyamine values peaked on the 5th day after operation, and the Spm value was higher than the Spd value. The leucocyte count was also increased (Fig. 13).

Case 14 : The polyamine values in erythrocytes was not so high as in Case 10, but the Spm value was higher than the Spd value (Fig. 14).

5) Case which received operation to alleviate jaundice

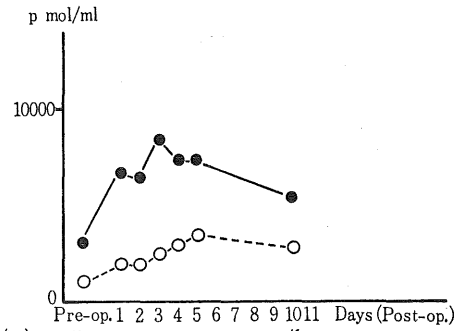
through the erythrocyte membrane. The state of free amino acid prior to and after operation were studied by the irrigation method in patients with liver cirrhosis and esophageal varix. The 1st case was in hepatic coma and the 2nd case had hypercatabolism. In both case Met-SO,

Thr+Gln, Ser, Asn, Orn and Lys were increased. In the 2nd case, the free amino acids in erythrocytes decreased as the catabolic state progressed, and the total protein became 6.0 mg/dl and A/G ratio was 0.62 on the 1st day after operation, which made the authors feel that serum protein decreases as a consequence of generalized progression of catabolism. From this, it was confirmed that the free amino acids in erythrocytes constitute one of the amino acid pools. However, in general, free amino acids were usual, and disturbance of the free amino acid pattern excluding the branched chain amino acids was noted in only the abovementioned 2 cases. As disturbance of the free amino acid pattern was not generally observed in the majority of cases, these findings fail to suggest that the free amino acid value in erythrocytes could serve as an index of the condition of the whole body.

(2) Polyamines in erythrocytes

On the other hand, polyamines are said to be present widely throughout nature.

Their discovery dates back to the beginning of the 17th century. However, they did not attract attention until the report of Russell¹²⁾ et al. in which it was pointed out that the polyamine values in urine



BW (kg)	42.5				41
WBC	9500	11600	6900	5900	5000
RBC ($\times 10^4$)	451	454	390	397	388
TP	6.7		6.1	6.2	
A/G	1.68		1.54	1.70	
GOT	15		11	17	41
GPT	21		22	19	59
BUN	10	8		16	13
Creatinine	1.7	1.9		1.8	1.5

Fig. 8. Changes of polyamine values, 41-year-old, male

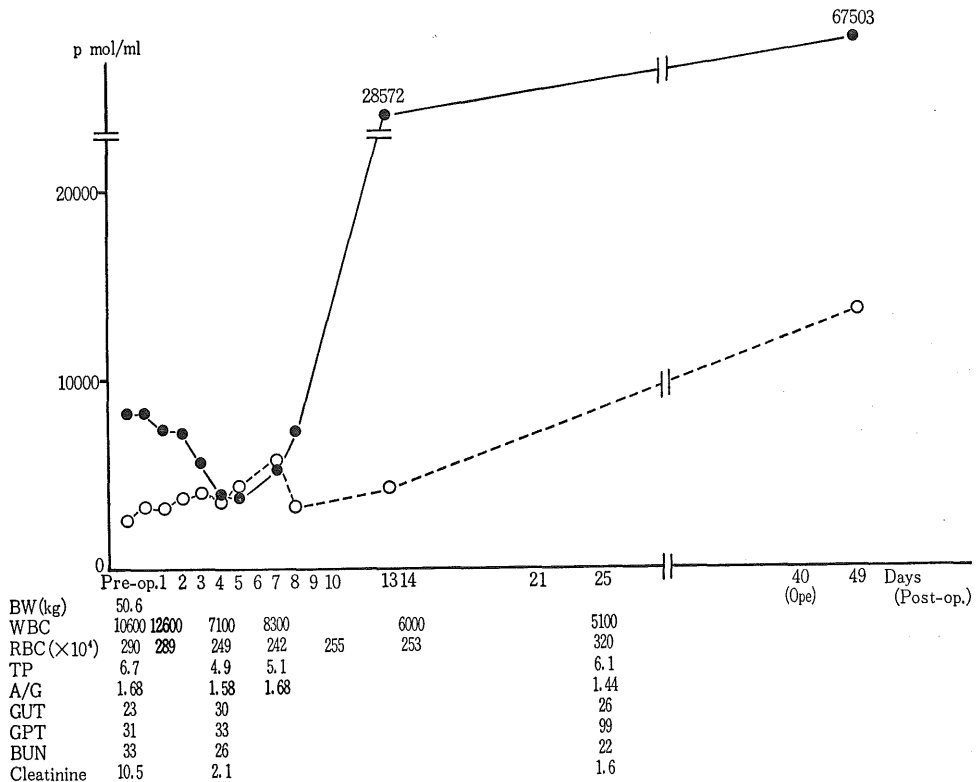


Fig. 9. Changes of polyamine values, 34-year-old, male

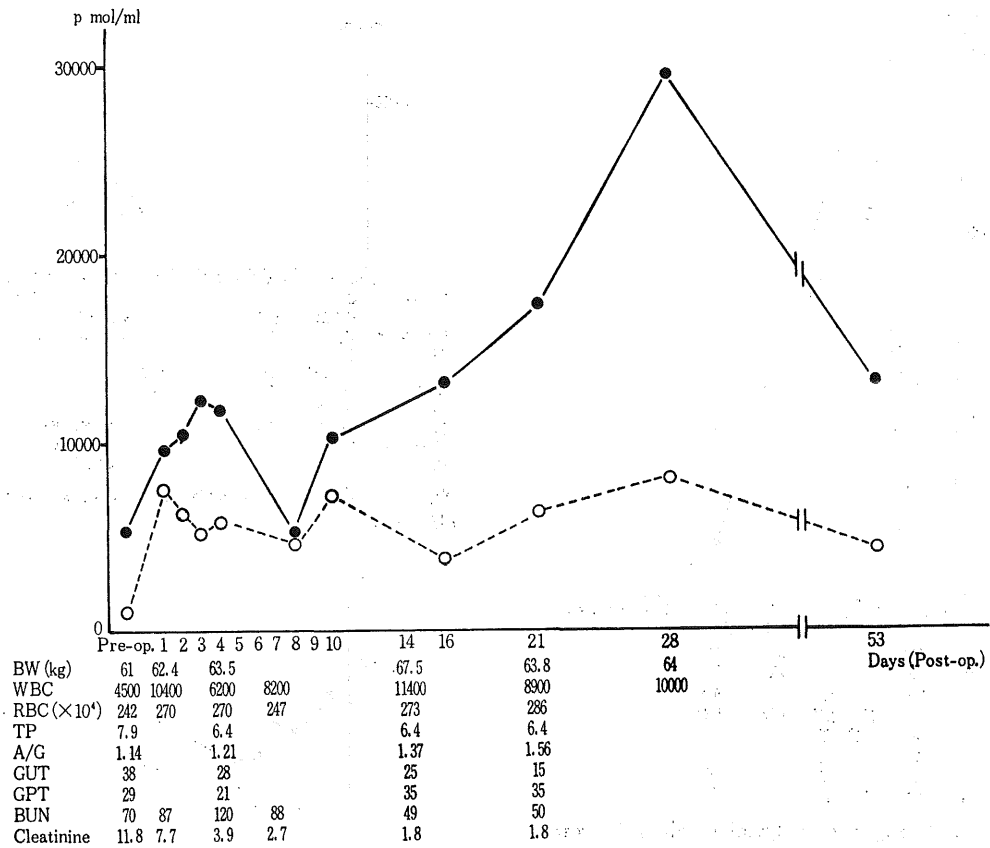


Fig. 10. Changes of polyamine values, 31-year-old, male

serve as an index of cancer. Urine of both cancer patients and controls collected over a 24-hr period were hydrolyzed with 6M HCl and then analyzed with high voltage electrophoresis. In cases of post-operative ovarian teratoma, the Put value in urine was decreased, but still higher than in controls. Spd and Spm were both 50 times higher than the control values. Spd in inoperable tumor cases was reported to be several times higher than that of the controls.

According to polyamine determination in erythrocytes by the authors, the standard deviation (SD) of Spd and Spm were 6660 ± 2430 p mol/ml and 2760 ± 1750 p mol/ml ($n=7$) respectively in preoperative non-cancer patients, whereas the SD of the same in preoperative cancer patients were 5150 ± 2050 p mol/ml and 2580 ± 1720 p mol/ml ($n=9$) respectively, while the SD in healthy adults were 5590 ± 2500 p mol/ml and 1730 ± 830 p

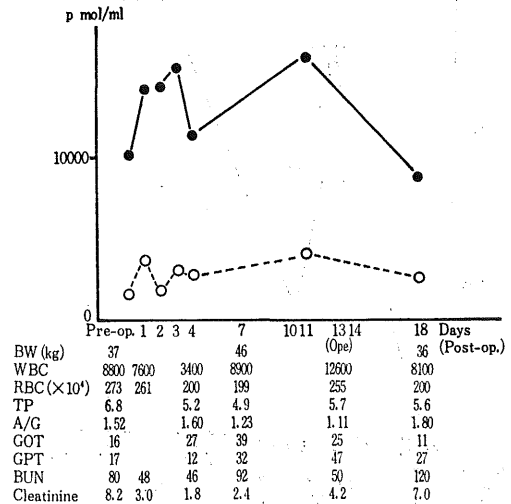


Fig. 11. Changes of polyamine values, 24-year-old, female

mol/ml ($n=17$) respectively. Thus, a marked differences among the three groups could not be demonstrated (Figs. 16 and 17). However,

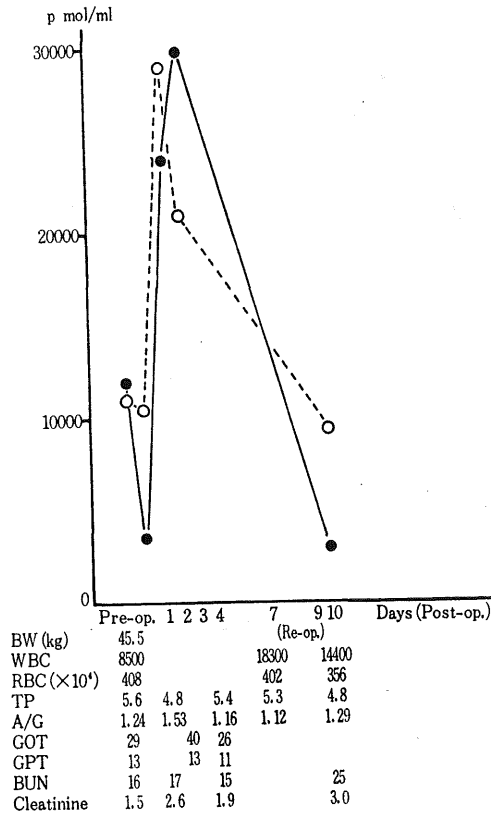


Fig. 12. Changes of polyamine values, 52-year-old, male

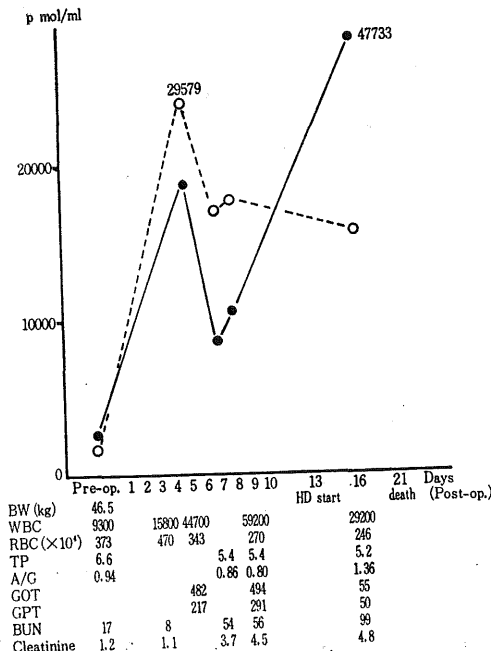


Fig. 13. Changes of polyamine values, 62-year-old, male

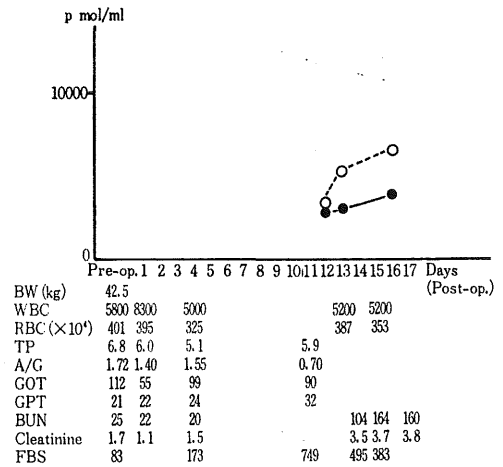


Fig. 14. Changes of polyamine values, 74-year-old, male

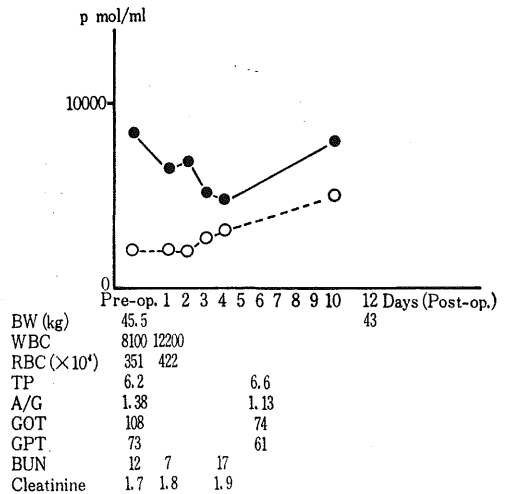


Fig. 15. Changes of polyamine values, 69-year-old, male

according to reports by many workers such as Otsuji et al.¹¹⁾ (1973) who measured polyamine values in whole blood of 80 cancer patients, found that Spd and Spm values which exceeded the upper limits of normal were noted in 18 cases (22.5%) and 21 cases (26.3%) respectively. Saeki et al.¹³⁾ (1978) reported that the polyamine values in erythrocytes of malignant disease patients were 3-10 times higher than normal. Takami et al.¹⁷⁾ (1979) reported that polyamine concentration in 108 patients with solid tumors was elevated in the blood cells in 89% and in plasma in 39%. Uehara et al.²⁰⁾ (1980) announced that Spd and Spm values in packed erythrocytes of 69 advanced cancer patients

was more increased than those of controls. Changes of polyamine values in erythrocytes other than cancer patients were reported in patients with blood dyscrasia and renal failure. Chun et al.¹⁾ (1976) contended that polyamine values in erythrocytes of 2 cases with sickle cell anemia were 5-6 times greater than normal. Cohen et al.²⁾ (1976) reported that the Spd value in erythrocytes of 13 cases of cystic fibrosis increased to 150% of that of controls while the Spm value decreased, and the Spd/Spm ratio was 2.3 as compared to 1.5 in controls. Cooper et al.³⁾ (1978) advocated that when there was cell proliferation the Spm/Spd ratio was greater than that of the controls, but was decreased in hereditary elliptocytosis and sickle cell anemia. Swendseid et al.¹⁰⁾ (1980) reported that the Spd value in erythrocytes of advanced renal failure (serum creatinine value over 6 mg%) patients not receiving hemodialysis was increased, but the Spm value was unchanged. The Spd value in erythrocytes of patients undergoing maintenance hemodialysis was high, because polyamine excretion in renal failure was decreased with retention of Spd in erythrocytes. The polyamine values in erythrocytes are summarized in Table 4.

As reported in the previous paper, the Spd concentration in erythrocytes was greater than Spm. Many workers considered this to be attributable to the increase of immature erythrocytes. The differences between these reports and our data are due to the following.

- ① The general condition of cancer patients, especially peripheral blood examination results, were not recorded. A correlation exists between polyamine values in erythrocytes and cell aging. If immature erythrocytes increase in peripheral blood in anemia, the polyamine values in erythrocytes will also increase¹⁸⁾. The effects of blood transfusions are similar. In general, different from fresh blood, in preserved blood more than 1 or 2 days have elapsed since drawing. As reticulocytes lose their network structure within 4-5 days after being drawn, it is considered that reticulocytes are either markedly reduced or non-existent. Therefore, it was felt that the effect of blood transfusion on fraction 6 was small.
- ② The erythrocyte layer is collected by using

packed cells or packed cells plus high speed centrifugation.

- ③ Samples were analyzed by high voltage electrophoresis, amino acid analyzer (AAA) or HPLC.

Thus, the normal range and mean \pm SD of polyamine values differ by reporter depending upon the procedure used under (2) and (3) above.

- ③ Changes of the pattern of polyamine value

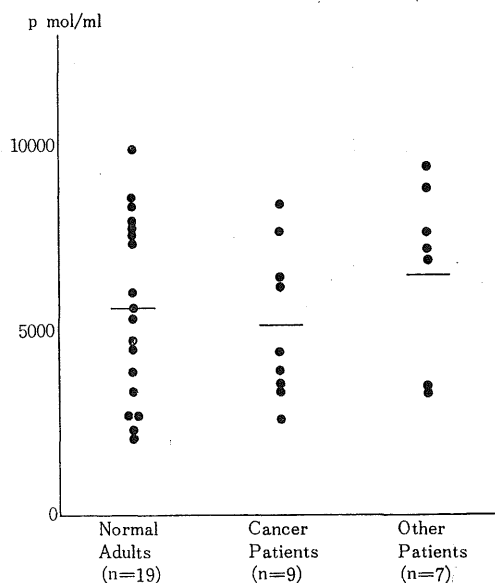


Fig. 16. Spermidine level in preoperative patients

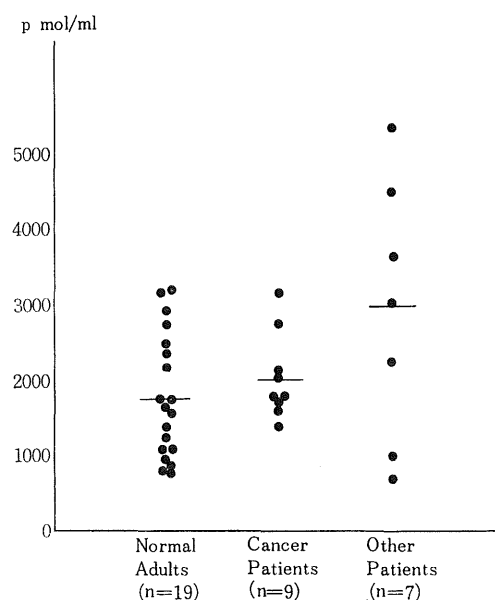


Fig. 17. Spermine level in preoperative patients

Table 4. Many reports on polyamine determination in human erythrocytes

References	Method of Determination	Treatment of specimen	No. of cases	Unit	Normal range
Otsuji ⁹⁾ (1973)	cellulose-acetate electrophoresis	packed erythrocyte	cancer patients: 80 healthy adults: 50	mcg/ml	spd. 1.5 ± 0.68 spm. 3.2 ± 1.00
Chun ⁶⁾ (1976)	HPLC (Durrum D-500)	10% sulfosalicylic acid	sickle cell anemia: 2 controls: 9	n mol/10 ⁹ (cells)	put. 0.007– spd. 1.39 ± 0.46 spm. 0.9 ± 0.27
Cohen ⁷⁾ (1976)	AAA	10 ml whole	blood cystic fibrosis: 13	n mol/10 ⁹ (cells)	spd. 1.02 ± 0.08 spm. 0.89 ± 0.28
Cooper ⁸⁾ (1978)	AAA (Durrum D-500)	washed 5 times	patients: 24 controls: 17	n mol/10 ⁹ (cells)	put. 0.065 ± 0.01 spd. 0.815 ± 0.07 spm. 0.48 ± 0.04
Saeki ⁴⁾ (1978)	HPLC (Hitachi 634 Model)	packed erythrocyte washed 5 times 10% perchloric acid	controls: 27 cancer patients: 10	n mol/ml (packed erythrocytes)	spd. 14.1 ± 3.1 spm. 8.4 ± 2.8
Takami ⁵⁾ (1979)	AAA (Durrum D-500 type)	5 % TCA	solid tumor patients: 108 controls: 37	n mol/ml (packed erythrocytes)	put. 0.083–0.367 spd. $8.54-20.90$ spm. $3.66-15.18$
Shipe ¹⁸⁾ (1980)	HPLC (OPA method)	10% TCA	healthy adults: 25	n mol/10 ⁹ (cells)	put. 0.04 (0.01–0.07) spd. $1.50 (0.6-2.4)$ spm. $0.81 (0.4-1.22)$
Swendseid ⁹⁾ (1980)	AAA (Beckman 121 HP, ninyhydrin method)	10 ml whole blood 10% TCA	controls: 6 chronic renal failure: 18 continuous hemodialysis: 6	n mol/ml (packed erythrocytes)	spd. 24.8 ± 6.3 spm. 12.4 ± 3.4 spd./spm. 2.1 ± 0.5
Uehara ²⁾ (1980)	HPLC	packed erythrocyte 10% perchloric acid	patients: 69 controls: 37	n mol/10 ¹⁰ (cells)	spd. 15.04 ± 3.63 spm. 8.82 ± 3.12 spm. 1.81 ± 0.82
Moulinoux ¹⁹⁾ (1981)	thin layer chromatography	blood-10 ml washed 3 times HClO ₄	chronic renal failure	n mol/10 ⁹ (cells)	spd. 1.32 ± 0.17 spm. 0.54 ± 0.08
Shideler ¹⁷⁾ (1981)	AAA (Durrum DC-4A)	packed erythrocyte 10% TCA Triton X-100D	head-neck patients: 29 controls: 36	n mol/10 ¹⁰ (cells)	put. 0.04 (0.01–0.07) spd. $1.50 (0.6-2.4)$ spm. $0.81 (0.4-1.22)$

in erythrocytes prior to and after operation. The variation in polyamine values prior to and after operation was followed closely for 70 days.

1) The characteristics of Cases 1 to 5 in whom there was elevation of values from immediately after operation and reached a peak on the 7th to 10th day after operation:

- ① After the polyamine values peaked, it took 2 to 3 months before they reverted to their preoperative value.
- ② Erythrocyte count decreased on the 7th

to 14th day after operation in these cases

- ③ Loss of body weight was recognized from the 10th to the 12th day after operation.
 - ④ A relationship between the volume of blood transfusion and liver and renal functions was not observed.
- 2) Characteristics of Cases 6 to 8 in whom the elevated values immediately after operation did not peak on the 7th to 10th day after operation:
- ① The polyamine values in erythrocytes

did not peak on the 7th to 10th day after operation.

- ② A relationship between volume of blood transfusion and bleeding and liver and renal function was not observed.
- 3) Characteristics of Cases 9 to 11 who underwent renal transplantation:
 - ① As bone marrow function is decreased in chronic renal failure, it was considered erythropoiesis was inadequate from immediately after operation.
 - ② After the renal graft took and erythropoietin^{9,10} production increased, young erythrocytes gradually increased and anemia improved, which brought about an increase in the polyamine values.
- 4) Characteristics of Cases 12 to 14 in whom complication with postoperative infection developed:

when there is complication with infectious diseases, the peripheral leucocyte count increases. The fact that Spm which is considered to be high in leucocytes^{1,3,13}, was found to actually be higher than Spd, agrees well with the increase in leucocytes.
- 5) Characteristics of Case 15 who received choledochoduodenostomy. There are reports of increase in peripheral blood reticulocytes in jaundice^{4,5}, and it was considered that the polyamine values would decrease with diminishing of jaundice.

In general, the theory of Moore et al.⁷ on postoperative living body reaction, especially metabolic modality, is widely accepted by most surgeons. According to this, surgical intervention or Stage I surgical stress brings about negative balance, cell destruction and catabolization of proteins (period 2-4 days).

At Stage II protein metabolism changes from catabolism to anabolism for which 1-2 days are required (period 3-7 days). In Stage III progress is seen in protein repair mechanism and muscular strength is regained (period 2-5 weeks). In Stage IV fat is accumulated and body weight increases (many months to many years).

Madden et al.⁹ (1970) reported that there was a relationship between rate of collagen synthesis and deposit and tensile strength of wound site during wound healing. The rate was calculated using rate of conversion of radioactive labelled proline to labelled hydroxypro-

line. Results showed that the rate of collagen synthesis did not increase until after 48 hr, reaching its peak between 7 to 21 days after operation, after which it slowly decreased. But the rate continued to be elevated for 8 weeks after operation.

The polyamine values in erythrocytes generally become elevated from immediately after operation and peaked at 7 to 10 days after operation, after which they decreased and reverted to the preoperative values.

This elevation coincides with Stage II and III of Moore's theory and Madden's period of collagen deposit in the wound.

This suggests some kind of acceleration of protein metabolism during this time phase. Elevation of polyamine values in erythrocytes after about one month following renal transplantation is due to increase of reticulocytes in peripheral blood as a result of bone marrow function recovery. It is considered that in the future, check of bone marrow function should be made by polyamine determination in erythrocytes in addition reticulocyte count. Uehara et al.¹⁹ (1980) reported that Spd and Spm in erythrocytes decreased in 7 of 32 cancer patients within 2 weeks after surgical operation, and advocated the possibility of using polyamine determination in erythrocytes of cancer patients as a marker of the disease activity. Shideler et al.¹⁷ (1981) reported that there was a relationship between tumor mass and Spd concentration in erythrocytes in 29 head and neck cancer patients, and that the concentration of Spd in blood samples drawn 1 to 3 days after surgical operation and also after radiation therapy was decreased. However, as their findings were based on packed erythrocytes, it is felt that they did not always measure the polyamine values in young erythrocytes.

As a result of our long term observation of changes in polyamine values in erythrocytes of individual cases prior to and after operation, it was learned that they could be classified into five patterns, particularly many cases in whom the postoperative prognosis was good demonstrated pattern 1.

Polyamine determination in erythrocytes can be performed by use of the same tubes used for other tests such a hematocrit determination. As the sample is readily available and the procedure for determinations is comparatively sim-

ple, it is a useful method for observing the postoperative course of surgical operation.

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