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Complement Activity in Carcinoma of the Digestive Tract

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SUMMARY

The function of the complement and its affection by operative insults were evaluated in patients with carcinoma of the digestive tract in comparison with 7 benign diseases and 4 critical patients. The C_4 levels in the patients undergoing major operative insult and exposed prolonging operation time were significantly reduced. There was no tendency toward a certain alteration in C_3, Factor B activity, CH_{50} and Factor H. whereas ACH_{50} was reduced until the 3rd day of operation. It was suggested of delayed recovery in alternativee pathway. Moreover, the protease inhibitor (α_1-Antitrypsin, α_2-Macrogloburin), which acts as an inhibitor of complement activity, and the ω-amino-acid (Arg, Lys) were markedly increased when a nutritional condition in cancer patient had been improved in help of TPN. It, however, was not statiscally significant. The complex release activity of the complement was increased according to advancing disease stage in gastric cancer. In contrast, it was much depressed in hepatic cancer in relation to liver function of C_3 production.

In conclusion, the complement activity related to surgery in carcinoma of the digestive tract was variably influenced by the degree of an operative insult and the staging of cancer disease including the nutritional status.

INTRODUCTION

There are few reports concerning the function of complement and its affection of the operative insult in patients with carcinoma of the digestive tract. Numerous investigations have attempted to identify the contributing factors to immunologic responses which

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were represented in hemolytic activity, passing through either the classical or alternative pathway. It is well known that a large number of the parameters to asses the complement activity have been developed such as immune complex, plasmin, c-reactive protein, ω -aminoacid (Arg, Lys), RNA-virus, vit B_{12}, antibiotics, Metrizamide, protein A, Methylprednisolone until recently. In general, surgical resection has been offered to cancer patients with the promising prognosis. It, however, is well recognized that immunological response of the tumor-bearing host, especially including the complement activity may be much inhibited by surgical stress. Advances in laboratory techniques to evaluate the complement activity have shed some light on production and metabolism of each complement component. In 1978, PEDERSON[1] clarified the alteration of C_3, C_4, C_5 during the 9 days of postoperative periods, and elucidated the reduction of C_3 in serum. And also LEWIS[2] in 1982 made a quantitative analysis of C_1 to C_9 during a period of operation. These results demonstrated a reduction of C_3 and C_5, suggesting activation of alternative pathway. The activity of the complement component has been evaluated only on the first day of surgery by KIN and OGATA[3] in spite of few reports on the following days of surgery. On the other hand, it is obscure as to whether or when the complement activity impeded by the operative insults may be returned to the preoperative level or not. At present, little information is available for the activity of functional C_3 proactivator, ACH_{50}, control protein of Factor H, immunecomplex, complex release activity of complement. This study was undertaken to clarify the complement activity in relation to operative insults in patients with carcinoma of the digestive tract.

MATERIAL AND METHOD

This study was based on 40 patients with carcinoma of the digestive tract compared with 7 benign diseases and 4 critical illness, in whom surgical treatment was done during a period from Oct 1984 to Oct 1985 in the First Department of Surgery, Nagasaki University School of Medicine. The ages ranged from 28 to 82 with a mean of 60.8 ± 8.6 years. They were 26 men and 25 women, and consisted of 18 gastric cancers, 13 colon cancers, 5 hepatomas, 4 pancreatic carcinomas including 7 benign diseases and 4 critical illness (2 hemorrhagic shock, 1 liver failure, 1 renal failure).

Blood sampling and urinalysis; 10 ml of blood samples were drawn and the serum was separated for 10 min at 3000 rpm. The sera were frozen in aliquots at 10 min at $-80°C$ until analyzed. Re-frozen sera was not used to assay. The values of C_3, C_4, CH_{50}

factor B activity, and ACH_{50} were assayed periodically in the patients subjected to this study. The measurement was performed at preoperative day, 1, 3, 7, 10 and 14th day after operation as a general rule. Factor H which regulate complement system, α_1-Antitrypsin and α_2-Macroglobulin which play a key role in depressing, rapid turnover protein which represents one of the nutritional indice the urinary level of nitrogen and 3-methylhistidine, and aminoacids in the peripheral blood were also assayed.

Measurement of complement system and other examinations; (Tab. 1)

(1) C_3, C_4; Single radial immunodiffusion method. (Hechst coop)

(2) CH_{50}; Mayer's 1/2.5 method. (Denkaseiken coop)

(3) ACH_{50}; The hemolytic activity of complement was measured by NAGAKI's[5] method founded on the study of PLATTS-MILLS[4] and ISHIZAKA. The blood was drawn from the auricular vein of rabbit and stored in the Alsever's solution. The red blood cell of rabbit was washed and used under the *EGTA-MG-GGVB* buffer solution. 1 : 41 dilution of patient' sera was added to the buffer at 37°C for 60 min. After the addition of 2ml of buffer, the samples were centrifuged and the absorbance at O. D. 450 nm of the supernatant was measured with use of Hitachi 557 photospectrometer. ACH_{50} was calculated according to the same way with Mayer's method.

(4) Factor B activity; Guinea pig RBC hemolysis[6] method in the agarose gel was used in this study. The guinea pig RBC, which was drawn by the heart puncture, was washed and ajusted to 50% v/v in the *Mg-EGTA-GVB*. This ajusted solution was added to the 1% agarose contained Factor B removed sera. 10 μl of patient' sera was added into the small hole of 2.5mm in diameter in the agarose gel. After 18 hours

Table 1 Measurement used for this study

(1) C_3, C_4; SRID plate (Hechst Coop)
(2) CH_{50}; Mayer's 1/2.5 method (Denkaseiken Coop)
(3) ACH_{50}; Nagasaki's method
(4) Functional C_3 activator; Guinea pig RBC hemolysis in the agarose plate
(5) Factor H; Rocket immunoelectropheresis
(6) Circlating immune complex; Polyethyleneycol precipitation Complement consumtpion test
(7) Complex-release activity of Complement; Amano's method
(8) Study of the cases received postoperatve TPN
  i) Daily nitrogen balance
  ii) Urinary output of 3-Mehis
  iii) Rapid turnover protein (Prealbumin, Retinol-bindlng protein, Transferrin)
  iv) Aminogram
  v) α_1-Antitrypsin
    α_2-Macrogloburin

at 4°C, the gel plate was incubated for 4 hours at 37°C. And the hemolytic ring was measured with the use of Messhapron (Behring institute, West Germany). Factor B activity was expressed as the percentage to the control sera.

(5) The measurement of Factor H ; The anti-Factor H serum (Behring coop.) was used for the rocket[7] immunoelectrophoresis. (Laurell's method)

(6) The measurement of circulating immune complex[8] (CIC) ; 50 μl of borate buffer, 50 μl of 0.2M EDTA, and 0.1ml of 12.5% polyethylenglycol 6000 (PEG) were added to 0.3ml of test or control serum, and left at 4°C for 90 min. The tubes were centrifuged at 1700G for 10 min and the pellets were washed in 1ml of 2.5% PEG, followed spun again at 1700G for 15 min at 4°C. The supernatant were discarded, 30 μl of GVB^{2+} and 10 μl of normal human serum were added to the sediment and incubated at 37°C for 30 min. After the addition of 1ml of 1.5×10^8/ml EA, the tubes were maintained at 37°C for 60 min. 6.5ml of cold isotonic saline was added and centrifuged, followed the examination of absorbance at O. D. 450 nm by the spectrophotometry. PEG—CC% = (1 − sample hemolysis/control hemolysis) × 100

(7) Complex-release activity of complement (CRA) ; Amano's[9] method.

i) Preparation of precipitable immune complex. Horseradish peroxidase (Wako-Junyaku, Osaka) and diluted anti-PO rabbit IgG (Cappel Lab, USA) were incubated at equivalence for 1h at 37°C. The test tubes were left overnight at 4°C, and 0.2% 5-aminosalicylic acid (ASA) containing 0.016% H_2O_2 washed out. Antigen-antibody ratio was found by the minimal absorbance at O. D. 450 nm. Precipitable immune complex was prepared at this Ag-Ab ratio.

ii) CRA assay. 0.2ml of PO-IC and 0.1ml of sample sera were mixed for an hour incubation at 37°C. After the addition of PBS(+), the test tubes were centrifuged and 0.5ml of supernatant was added to ASA, followed incubation for an hour at 37°C. The reaction was discontinued by the addition of 0.5ml of 1N NaOH. The absorbance at O. D. 450 nm was the value of CRA.

iii) ACRA assay. Instead of PBS(+) in the CRA assay, 0.03M-EGTA-Mg^{2+} PBS was used as a buffer. The absorbance at O. D. 450 nm expressed the value of ACRA.

(8) Quantitative measurement of nitrogen-balance, 3-Mehis, rapid turnover protein, protease inhibitor (α_1AT, α_2 MG), amino acid, activity of complement. TPN (35 ~45 kcal/kg/day) was performed until the 14th day post op, and daily nitrogen balance was calculated. 3-Mehis in the daily urine, blood level of rapid turnover protein, protease-inhibitor, aminoacid and the activity of complement were assessed in comparison with those of non-TPN patients as the control.

The blood level of aminoacid and the urine level of 3-Mehis were measured by the autoanalyzer. (JNC-200A, Nippondenshi coop.) Nitrogen in the urine was measured with the use of MT 1600. (Yanagimoto coop.) Protease inhibitor was measured with the use of Paltigen plate. (Hechist coop.)

The data was analyzed separately for the diseases, operative method, operation time (group A ; less than 3 hours of the operation time, group B ; more than 4.5 hours of the operation time), and nutritional supplementation. Each data was expressed as mean±SD and analyzed statistically by student-t-test.

RESULTS

Comparison in C_3 and C_4 between benign diseases and gastric and colon cancers except for stage I and II cancer. (9 gastric cancers and 7 colon cancers) ;
On the first day of surgery, the levels of C_3 and C_4 remained higher in patients with benign diseases as shown in Fig. 1. In contrast, these were low in cancer patients. It, however, was not statistically significant in either group. Three days later following surgery, the C_3 and C_4 values increased and reached higher levels than the preoperative one at 14 days after surgery.

Comparison in changes in CH_{50}, ACH_{50} values and Factor B activity ;
These were raised in benign diseases. Meanwhile, CH_{50} values somewhat decreased and also Factor B activity reduced from 101.6±11.3% in preoperative period to 86.0±17.8% on

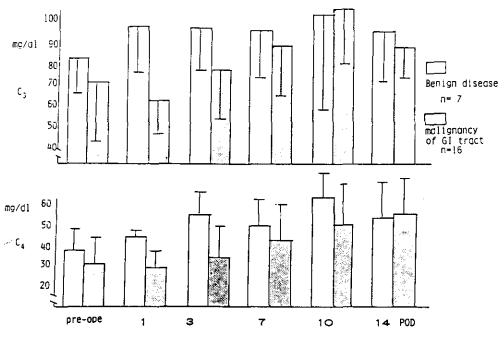

Fig. 1. Changes in plasma C_3 C_4 levels of carcinoma of the digestive tract by comparing with those of benign diseases.

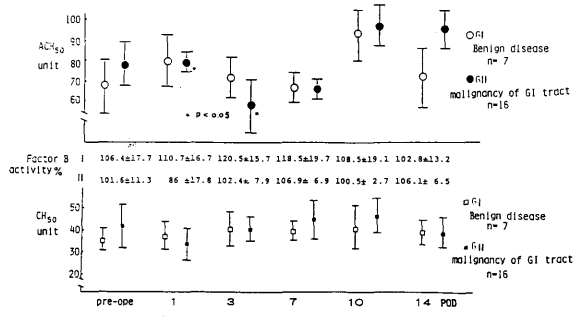

Fig. 2. Assessment of the influence on classical and alternative pathways in patients with digestive tract cancer in comparion with benign diseases.

day 1. The ACH_{50} levels remained constant from preoperative period to day 1 but these were not significantly reverted to the preoperative level, ranged from 78.1 ± 10.0 on day 1 to 56.3 ± 11.2 unit on day 3 ($P < 0.05$) as shown in Fig. 2 whereas CH_{50} and Factor B activity resumed the preoperative levels.

Comparison in C_3, C_4, CH_{50} ACH_{50} and Factor B activity between operation time of whithin 3 hours (group A) and over 4 hours and 30 min (group B);

The C_3 and C_4 levels were compared between 9 cases whithin 3 hours and 6 cases over 4 hours and and 30 min of operation time respectively. The C_3 levels in group A began to increase on day 1. In contrast, those in group B declined on day 1, and then increased on day 3. The C_4 levels in group B were reduced from $34.8 + 3.9$mg/dl in preoperative level to 20.2 ± 13.8mg/dl on day 1. It was statistically significant ($P < 0.05$). The variation of C_4 levels were very small in group A and almost the same as in either group. (Fig. 3)

The CH_{50} levels in group A did not vary and those in group B were low on day 1 and high on day 3 to 7, subsequently on day 10 to 14 returned to a similar level with those in group A. On observation of Factor B activity, it did not remarkably vary in group A whereas low in group B on day 1 to 10. The ACH_{50} levels in group A was elevated on day 1 and later showed the same increase as shown in group B (Fig. 4).

Comparison in C_3 and C_4 between the loss of blood during surgery;

The C_3 levels in case with the blood loss of less than 300ml continued to increase on day 1 to 10. These in case with the blood loss of more than 1500ml slightly fell down on day 3 and continued to be elevated until on day 10 (Fig. 5).

Influence of operative insult on C_3, C_4 and CH_{50} levels;

The C_3, C_4 and CH_{50} levels were compared in 8 patients with partial gastrectomy and 5 with total gastrectomy. The C_3 levels were reduced on day 1 in both cases and gradually increased. The C_4 levels significantly reduced from 27.3 ± 16.3mg/dl in the preoperative period to 11.2 ± 2.5mg/dl on day 1. It was statistically significant ($P < 0.05$). On day 3 to 14, these showed a similar variation in the both groups with partial and total gastrectomy. The CH_{50} values showed a slight reduction on day 1, gradual increase on day 7 to 10 and the same as indicated in the preoperative period on day 14 (Fig. 6).

The postoperative alteration of Factor H, the regulator of the alternative pathway; Fig 7 showed the postoperative alteration of Factor H in patient undergoing total gastrectomy for the treatment of gastric cancer, 76-year old man. The ACH_{50} value of the patient fluctuated 83.0 on day 1, 53.9 on day 3, 70.1 on day 7 as compared with 76.6 on the day before surgery.

Fig. 8 showed the photograph of the electrophoresis in the normal serum as the control.

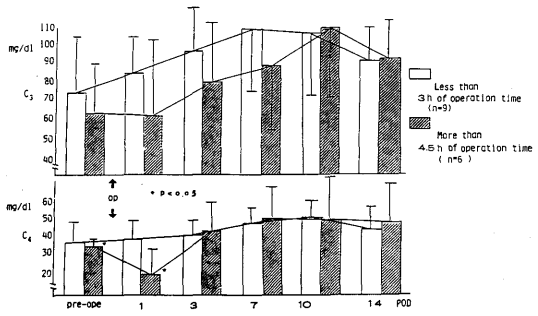

Fig. 3. Changes in plasma C_3, C_4 in relation to the operation time.

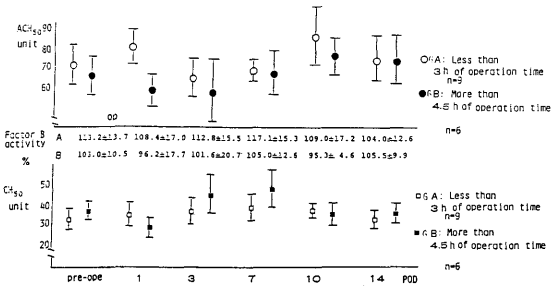

Fig. 4. Influence of the operative time on classical and alternative pathway.

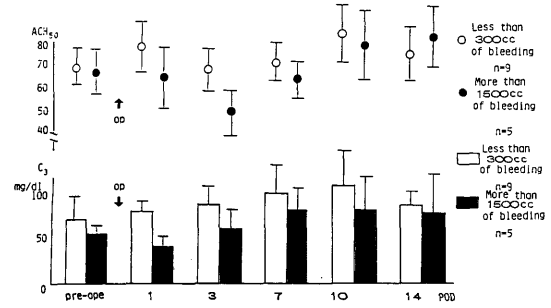

Fig. 5. Changes in ACH_{50} and C_3 in alternative pathway in relation to blood loss during surgery.

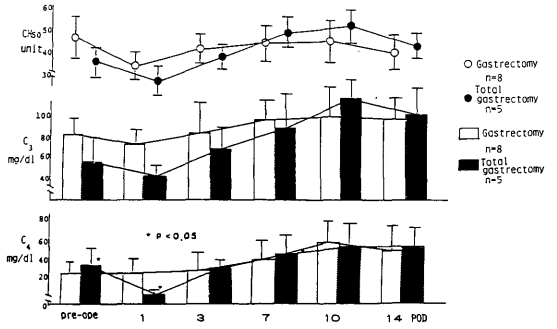

Fig. 6. Changes in C_3 C_4 and CH_{50} levels in terms of an operative insult.

The magnitude of the dilusion of control serum is1×, 2×, 4× and 8×. Moreover even in case with varying variety of ACH_{50} values, Factor H values were stable. It means that the complement activity is more likely to be affected by extrinsic factors such as nutritional condition and operative insult rather than does the intrinsic inhibition of Factor H.

Comparison in the CRA and ACRA values between various digestive diseases; In gastric and colon cancer patients, the CRA and ACRA values remained high but in hepatic cancer patients these were significantly lower, compared with those in gastric cancer patients (Fig. 9). According to an analysis in disease stages, the CRA values were higher with advancing the disease stage (Fig. 10). The ACRA levels did not show certain tendency. In a patient with serious illness of traumatic liver injury, the ACRA level was significantly depressed.

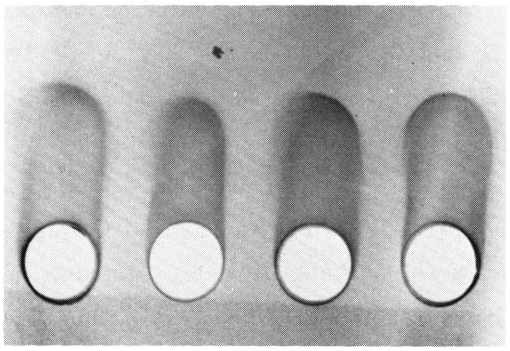

Fig. 7. Changes in Factor H levels in a 76 year old with gastric cancer prior surgery, on Day 1, 3 and 7.

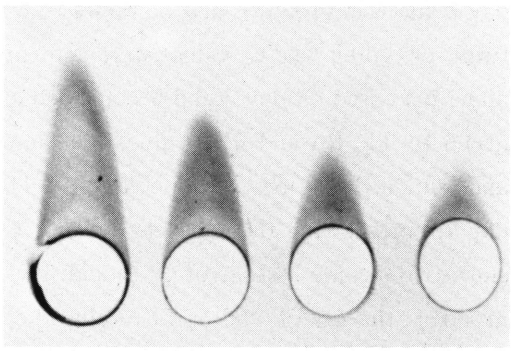

Fig. 8. Changes in Factor H to control serum (1×, 2×, 4×, 8× from the left side)

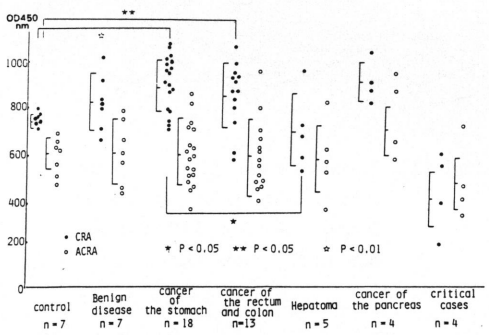

Fig. 9. Changes in CRA and ACRA in carcinoma of the digestive tract.

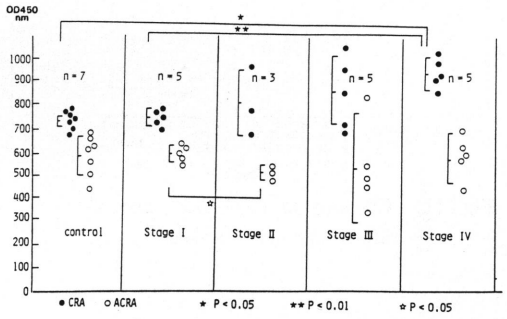

Fig. 10. Changes in CRA and ACRA according to gastric cancer stage.

Circulating immune complex in various digestive diseases;

Fig. 11 showed alteration of PEG-CC% in these diseases, declining the high detection rate in patients with tumor-bearing and serious illness patients in comparison with those in benign diseases.

Nutritional states and complement activity;

Nutritional states of the patients subjected to the present study were evaluated as the indice of N-balance and urinary excretion of 3-Mehis on day 7 after surgery. In group I treated with TPN, N-balance was positive as being +4.7g whereas in group II not treated with TPN, it was negative as being −40.8g. The 3-Mehis excretion in urine increased on

day 3 and 6 in group I and on day 4 to 6 in group II (Fig. 12). The variation of rapid turnover protein and C_3 values were compared between group I and II. In group I, TF alone increased on day 3 and it coincided closely with great increase in C_3 on day 3. In group II, TF, PA and RBP values were lowered on day 3, comparing with those on day 1 and remained still lower even on day 7. The C_3 levels resumed an increasing tendency on day 7, not showing the recovery on day 1 and 3 (Fig. 13). The variation of C_4 values showed the same as that of C_3 though this was not remarkable. (Fig. 14) Moreover, in group I, the α_1-AT and α_2-MG values kept high level on day 3 whereas in group II the α_1-AT values showed almost the same as in group I and the α_2-MG values were lowered

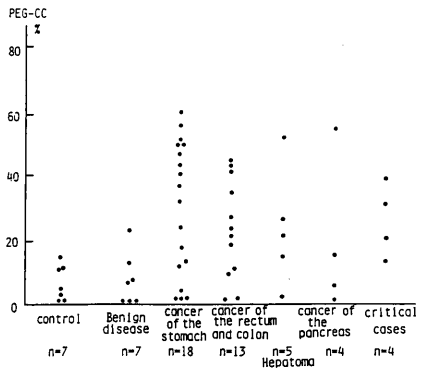

Fig. 11. Changes in PEG-CC% according to various diseases.

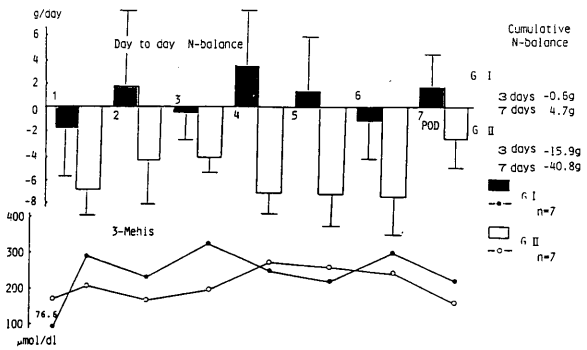

Fig. 12. N-balance and urimary excretion of 3-Mehis on day 7 after surgery betwwen TPN (G_1) and non-TPN groups (G_2).

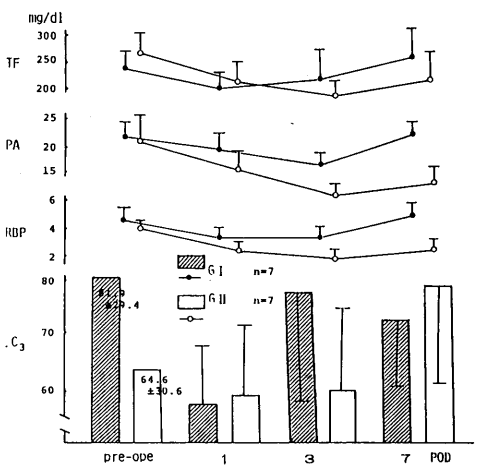

Fig. 13. Changes in rapid turnover protein (TF, PA, RBP) and C_3 after surgery between TPN (G_1) and non-TPN groups (G_2).

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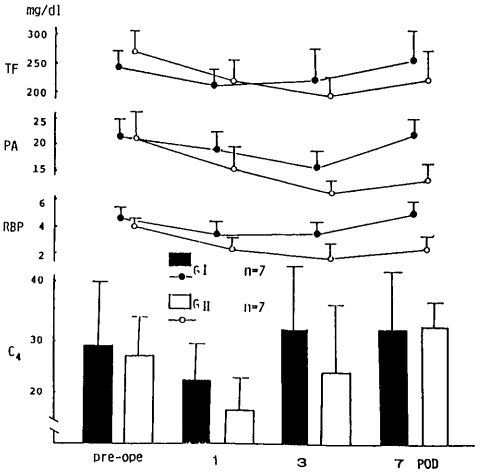

Fig. 14. Changes in rapid turnover protein and C_4 between TPN (G_1) and non-TPN groups (G_2).

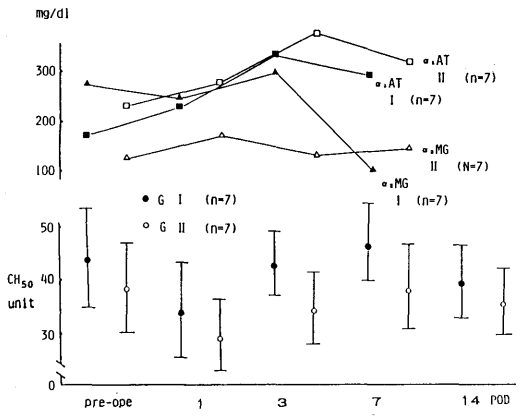

Fig. 15. Correlation of CH_{50} level with α_1-AT and α_2-MG levels between TPN (G_1) and non-TPN (G_2) groups.

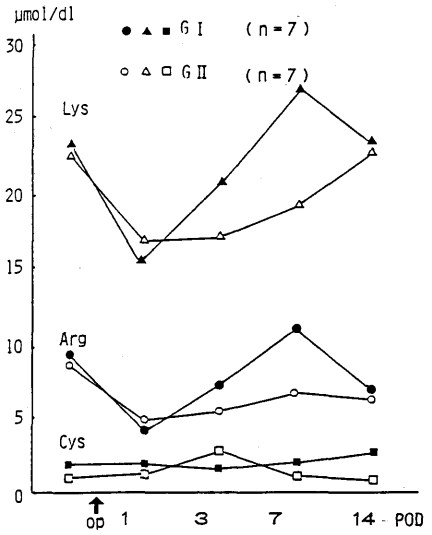

Fig. 16. Changes in concentration of amino-acid in blood between TPN (G_1) and non-TPN (G_2) groups.

until on day 3. The CH_{50} values in group I were somewhat higher than those in group II on day 3 to 7 but it was not statistically significant (Fig. 15). The Lys and Arg contents in sera, acting as an inhibitor of complement activity were also evaluated. Those in group I were higher than those in group II on day 3 to 7 (Fig. 16).

DISCUSSION

LEWIS, CRUSE and RICHEY[2] reported in 1982 that C_3 and C_5 of complement proteins are reduced by anesthesia and operative insult. In their report, it is clarified that a decrease in C_3 and C_5 values in the pre-and post operative periods is not only based on dilusion but also activation on the classical and alternative pathways of complement. It is difficult to search for the mechanism of activation of complement as to which pathway is main or not unless the hemolytic activites to all the complement components would have been evaluated. In the present study, the activation of alternative pathway in early stage was evaluated from the study on Factor B activity with use of Guinea pig erythrocyte as well as ACH_{50} activity with use of rabbit erythrocyte. NISHIOKA[10] and KAWAMURA cited that poor immunoresponses in tumor-bearing hosts have led to high activity of the complement system. It is interesting to elucidate how the surgical resection of the tumor mass acts on the

complement activity in tumor-bearing host. In this study, C_4, which means an early appearance of the complement component in classical pathway and CH_{50}, which imply all the complement activity did not significantly vary. C3, Factor B activity and ACH_{50}, which were associated with the activity of alternative pathway were much more altered with varying variety in comparison with those in benign diseases. Base on this result, it has been become clear that surgery for patients with carcinoma of the digestive tract brings depression and delayed recovery of the complement activity which lasts for at least 1 week after surgery, and variations of C_3 and C_4 are preceded by an alteration in Factor B activity, ACH_{50} and CH_{50}, demonstrating that recovery of the complement concentration is serum in not necessarily consistent with that of the functional activity. The major contributing factor to variation of the complement activity by surgery is considered to be not so much the control system inside the body as the extrinsic affections related to operative insult, such as the operation times, the loss of blood during surgery, the degree of operative insult and the nutritional status, the level of ciruclating immunocomplex in blood and protease inhibitors such as α_1-AT and α_2-MG. Those influential factors were clinically evaluated in the present study. The longer the operation time, the more the catabolic process is accelerated. A decrease in the C_4 level immediately after surgery is not consistent with the changes in the CH_{50} level. In view of the loss of blood during surgery, the greater the loss of blood, the more the C_3 and ACH_{50} levels fall down, demonstrating that surgical stress has selectively depressed the alternative pathway activity which is more likely to be influenced by extrinsic affection such as anemia, blood transfusion, hypoxia, fever and hepatic function failure. It is believed that these contribute to catabolism of complement proteins, their activities and production. FUKAYAMA[29] reported that the complement component is producing at the sites of the epithelial cells of the gut (C_1), and the parenchymal cell of the liver and the peritoneal macrophage (C_3). The peritoneal macrophage[11] also releases C_2, C_3, C_4 and Factor B properties. It is easy to anticipate a role of the alveolar macrophage under anesthetic circumstances. In the abdominal surgery, an operative insult for the gut and the liver is directly contributable to production of the complement component. Therefore, gastric cancer patients were subjected to this study, taking it into consideration that surgery would minimize the impared production of the complement component, excluding direct operative procedures to the gut and liver. In the patients undergoing partial and total gastrectomies for the treatment of gastric cancer, variation of the complement components was basically similar except for C_4.[12]

MORRISON[13] identified that the bile tract is the excretion route of C_3, C_4, and Factor

B. It is said that the liver is the production site of the components related to complex release activity of complement.[14] When cancer develops in the liver, CRA is easily influenced. The result in this study indicated that the CRA values remained high in gastric and colon cancer patinets with well functioning liver. In contrast, those in hepatic cancer were lower, demonstrating a close relationship between C_3 production and impared hepatic functin. According to the disease staging in gastric cancer, the CRA levels were elevated. It means that growing cancer contributes to the elevation of CRA on the basis of the mechanism concerning higher C_3 production in the healthy liver rather than in liver cancer.

In view of nutritional status SAKAMOTO[15] described that following fact that starvation in infant does not necessarily impair the complement activity. It is considered that surgical stress easily leads to negative N-balance in patients with poor nutritional condition so that the complement activity may be reduced. KONDO[16] identified that the amount of plasma protein related to the complement accounts for 5 to 10% of the total of plasma protein and its turnover is very rapid, showing almost half is newly replaced every day. To clarify the relationchip of the complement activity to nutritional status, rapid turnover protein (RBP, TF, PA), aminoacids in serum, N-balance and urinary excretion of 3-Mehis[17] were assessed in the postoperative period. The amount of urinary excretion of 3-Mehis correlated closely with that of aminoacid given intravenously as cited by NEUHAUSER[18] and also the elevation of serum *PA*[19],*PBP*[20], *TF*[21] also coincided with improvement of N-balance. Needless to say, in the present study, TF only coincided closely with improved N-balance state, demonstrating reduced PA and RBP. The variations of C_3, C_4 and RTP also reflected the nutritional status in the circumstances without postoperative complications. It is well known that protease inhibitor such as α_1-AT[22] and α_2-MG[23] inhibits coenzymic activity which converts C_1 by interaction of plasmin. TPN in the Present study was beneficial to hold high α_1-AT and α_2-MG so that the complement activity might be able to remain high. Serum aminoacid levels tended to alter easily in case of administration of a large amount of aminoacids. TAKADA[24] experimentally evidenced that aminoacids such as Arg, Lys and Cys inhibit the conversion of C_1s to $C_1\bar{s}$. High Arg and Lys induced by TPN is useful in maintaining high activity of the complement system. The high aminoacid level experimentally reported by TAKADA[25], which was useful to sustain high activity of the complement, is too excessive to apply for clinical use. Moreover, it is said that the immunecomplex[26] acts as an activator of the classical pathway and appears much more frequently in serum of cancer-bearing patients.[27] In this study the most high frequency accounted for 60% in carcinoma of Digestive tract. The clearance of the immunecomplex was made in the Kupffer-cell in the liver. While the

liver function is impaired, it seems that clearance of the immunecomplex may be impeded. In this study PEG-CC% was not significantly raised in hepatic cancer patients. At present it is impossible to measure the immune complex specific for cancer quantitively. Even the polyethylenglycol method is not specific for cancer. High detection rate of the immune complex in cancer patients of the stomach and colon is not frequently disregard for cancer. And also the immunecomplex acts as an activator of the complement. As far as the optimum activity of the complement may be concerned except in SLE, production of the complement is facilitated. Consequently an increasing production of the complement results in activation of the complement system.

FUKAYAMA[29] and KAWAMOTO state that it is difficult to assess the variation of the complement activity due to rapidly cyclic changes in production, activation and excretion of the complement. When the nutritional state is depressed by surgery, the complement production is activated as cited by IKUYAMA[28] who is indicating as an overreaction after large amount of consumption of the complement. It is sure that surgical stress such as the factors of the operation time, the loss of blood during surgery and operative insult leads to inhibition of the complement activity. The activation of the alternative pathway was revealed with varying variation of C_3, Factor B, ACH_{50} related to its pathway activity in the present study. The reason does that the alternative pathway is not require the existence of antibody and response the integrity of the complement system.

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