# Effectiveness of Enteral Nutrition in Restoring on Immunocompetence Comparing with Naturl Oral Feeding in Malnourished Rats

Motoko SAKAMOTO, Sohko ISHII, Sachiko KOBAYASHI and Yumiko KAWANOBE

## ABSTRACT

A protocol for nutritional restoration with estimated amount of nutrients from malnourished state was set up by increasing protein intake at a week interval. Based on this protocol, recovery of hematological, biochemical and immunological parameters in rats nutritionally restored with per os natual diet (PD) and enteral tube feeding of Clinimeal (EN) was described. When these two nutrients were compared, body weight gains showed somewhat delay in EN group but Hb, Ht, and serum total protein level, C3 level, macrophage function and PPD skin reactivity were restored more rapidly and effectively in the group restored with EN than that with PD. From these points, it is preferable to restore the severe malnourished state with enteral nutrient, Clinimeal.

## INTRODUCTION

Nutritional restoration from malnourished state is an important problem in malignancies or aged people in hospitalized patients. It is urgently required to restore nutritional state before surgical operation or after follow up treatment in such cases. For this purpose, central parenteral nutrition or enteral nutrition have been employed (1,2) and central parenteral nutrition has been widely used before surgical operation (3,4,5).

As for the cumulative nitrogen balance (6,7), whole body utilization of calorie, energy balance and urinary nitrogen excretion, there is no essential difference between central parenteral nutrition and enteral nutrition. On the other hand, it has been reported that in case with central parenteral nutrition, mineral supply such as Mg or P

essentially required for construction of lean body mass was not appropriate (8,9) and less effective than enteral nutrition (10,11).

Recently much attention has been paid on nutritional restoration through enteral route.

Therefore, it is required to set up experimental model to give estimated nutrient supply through enteral route for nutritional restoration from malnourished state. In addition to this, to understand the state of recovery of immunological parameters after nutritional restoration is required for elevation of host resistance.

For this purpose, we set up a protocol for the experimental model of nutritional restoration from malnourished state by enteral route and compared the result with natural per os diet (PD) and that with one of the enteral feeding diets, Clinimeal.

## MATERIALS AND METHODS

Two hundred twenty male, 4 weeks old averaging 80g in weight, Sprague-Dawley rats (SPF) were purchased from Charles River Breeding Company, Japan, and housed in individual cages located in an airconditioned room at 24± 2 °C, 50±10% humidity with lighting regulated to provide 12 h intervals of light and darkness. The rats were divided into four groups; (1) natural per os diet (PD) fed with 18% protein group as a well nourished control, (2) 3% protein diet group as a malnourished control, (3) recovery from malnourished state with PD and (4) recovery from malnutrition with Clinimeal (Eisai Pharmaceutical Co. Ltd. Tokyo, Japan) by enteral tube feeding (EN).

The PD was consisted of corn flour, wheat flour, fish meal, skim milk, corn oil, salt mixture, and vitamin mixture. One hundred g of EN is consisted of 18g of protein (milk casein and hydrolysis of soybean protein), 58.4g of carbohydrate (dextrin and saccharose), 14g of fat (corn oil and coconut oil), minerals and vitamins. Total energy of 100g EN is 450 kcal and energy balance is consisted of 15.9% of protein, 56.2% of carbohydrate and 27.9% of fat and for clinical use in patients, EN was initially administered at the concentration of 1kcal/ml (osmotic pressure 300mOsm/1) at the rate of 30ml/h. Eighty nine gram of EN was diluted with 200ml of warm water to make 1.5kcal/ml and administered to the rats by metal tube feeding. Contents of energy, protein, fat and carbohydrates in 100g of EN and PD (18% protein and 3% protein) are shown in Table 1. The rats offered a limited diet supplying to control the protein intake levels, 6, 9,

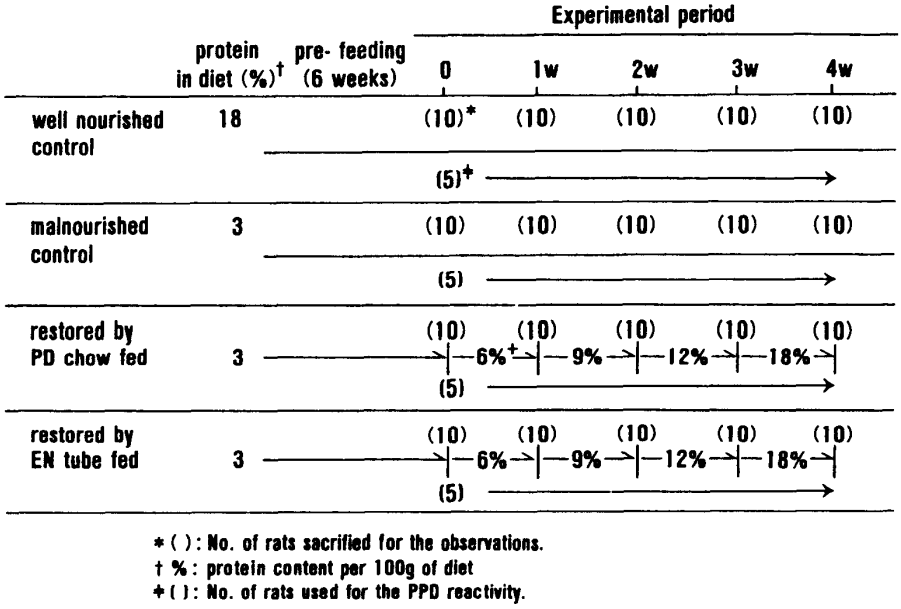
**Table 1 Diet composition of enteral nutrients and natural per os 18% and 3% protein diet.**

| | EN/100g | PD/100g | |
|---|---|---|---|
| | | 18% cont. | 3% cont. |
| energy (kcal) | 452 | 420 | 380 |
| protein (g) | 18.0 | 18.0 | 2.7 |
| fat (g) | 14.0 | 14.7 | 10.6 |
| carbohydrate (g) | 63.4 | 54.5 | 67.6 |

12, and 18% in the diet. The rats in well-nourished control group were given 20g/day/rat according to the recommended dietary allowance in rats (14). The rats in malnourished control group were given 10g/day/rat containing 3% protein and the rats in recovery from 3% protein malnourished state were given limited diets such as 7g, 10g, 13g, and 20g/day/rat to give 6, 9, 12, and 18% protein levels respectively at a week interval. Water was available at all time.

**Experimental protocol**

Three percent protein diet were fed for 6 weeks to establish a malnourished state with low dietary protein. For recovery from malnourished state, the diets of PD and EN with




* ( ): No. of rats sacrified for the observations.
† %: protein content per 100g of diet
+ ( ): No. of rats used for the PPD reactivity.

**Fig. 1 Experimental protocol for recovery from malnourished state.**

increasing protein concentration, 6, 9, 12 and 18% protein were given for a week period respectively as shown in Fig. 1.

Well-nourished and malnourished control groups were fed by 18% and 3% protein diets respectively throughout the experiment. The daily food intake was recorded and body weight gain was measured two times per week. During the experiment, on day 0, before the experiment started, and on the days after 1, 2, 3 and 4 weeks, ten rats from each groups were sacrificed for the measurements of hematological changes, complement activity, immunoglobulins, number of macrophage, phagocytic activity and T lymphocyte subpopulation (Helper and Suppressor). Delayed type hypersensitivity (PPD skin reactivity) was tested (on the days after 1, 3 and 4 weeks).

**Hematological analysis**

The blood was withdrawn from the axilla vein. Erythrocyte (RBC) and leucocyte (WBC) counts and hemoglobin (Hb) were carried out by an Automatic Blood Cell Counter (MEK 1100, Nihon Kooden, Japan) and serum total protein (TP) was measured by refractometry.

**Immunological parameters**

Blood serum was separated in a low temparature centrifuge and at −80°C immediately after separation. As an indicator of the complement system, comlement hemolytic activity (CH50) was determined by Mayer's method (15) by 50% hemolysis of sensitized sheep erythrocytes in the gelatin veronal buffer ($GVB^{++}$) with $Ca^{++}$ and $Mg^{++}$ with incubation at 20°C for 30min (16). After centrifugation, supernatants were separated and oxidized hemoglobin was measured spectorophotometrically at 541nm. The third component of complement (C3) was measured by single radial immunodiffusion (SRID). Goat anti-rat C3 anti-serum (United States Biochemical Corporation, Ohio, USA) was diluted 13 times with gelatin veronal buffer (GVB) and mixed with the same amounts of 2% agarose and poured into SRID plates. Eight $\mu$l of the rat serum were poured in wells on the plates and diffusions were read after 48h. Mean value of C3 concentration of serum samples taken from the well-nourished rats was determined by SRID and expressed as 100%.

Immunoglobulins such as IgG and IgM were measured with a SRID kit (Hoechst, West Germany).

Macrophages were collected peritoneally and phagocytic activity of rat peritoneal macrophages to ingested polystyrene latex beads (SIGMA Chemical Co., MO, USA) was observed.

One hour prior to sacrifice the rats were injected intraperitoneally with 5ml of sterile saline to induce peritoneal macrophage accumulation. The abdominal cavity was opened aseptically and the peritoneal macrophages were collected by washing the peritoneal cavity with 6ml of Eagle MEM and washed 3 times with MEM and adjusted the volume to 5ml with MEM. Number of the cells was counted microscopically. Polystyrene latex beads were added to cell in MEM at approximately 400 : 1 ratio and poured on a cover glass seated in Petri dish and incubated for 2h at 37°C in moist 5% $CO_2$ in air. The cells on the cover glass were washed carefully at 4°C with phosphate-buffered saline (PBS) at least 3 times and stained with Giemsa and dried. Numbers of macrophage (M$\phi$) and the phagocytosis percent of M$\phi$ and intracellular numbers of polystyrene latex beads was calculated microscopically (17).

As the indicator of the cell-mediated immunity, purified protein derivative of tuberculin (PPD) skin reactivity was measured. Heat killed tuberculin bacilli, Aoyama B strain, 0.15mg in 0.05ml of liquid paraffin, were injected into the foot pad of the rats in order to sensitize them. After time elapse longer than 12days after the injection, 25$\mu$g of PPD in 0.1ml of PBS (pH 7.36) was injected intradermally into back skin of the rats. After 24h, skin reaction was observed and the diameter of induration was measured and erythema was scored as a reference. More than 5mm of the diameter of induration was considered positive (18).

Peripheral blood mononuclear cells were isolated from heparinized venous blood by Ficoll-hypaque dencity gradient centrifugation and total number of lymphocytes were measured microscopically. Lymphocyte subsets were measured using monoclonal antibodies (Sera-Labo, England) CLONE W3/13-HLK with specificities for T-lymphocytes, CLONE W3/25 for T-helper cells and CLONE OX8 for non-helper cells by laser flowcytometry system using Ortho Spectrum 3 (Ortho Diagnostic Systems Inc. Raritan, NJ, USA). Non-helper T-cells were considered to be suppressor T-cells.

**Statistical analysis**

The singnificance of difference between two means was evaluated using a student t-

test.

## RESULTS

### Food intake and body weight

Average food intake and nutrients intake per rat during the experiment and changes in body weight were shown in Table 2 and 3 respectively. The nutrients intake of the rats in PD and EN groups during the recovery were nearly the same amount in each stage. The influence of the PD and EN diets on the gain of body weight of rats was highly dependent on the protein and energy intake. Throughout the experiment, the body weight increase was stopped in malnourished group. After nutritional restoration with PD or EN, recovery of body weight increase is significantly elevated ($p<0.001$) after 3 to 4 weeks restoration. When the rate of recovery with PD and EN was compared, the recovery of body weight was more effective in rats restored with PD and showed significantly different $p<0.01$ on the week 1 and $p<0.001$ on the week 2 and 3. However on the week4, no significant difference was observed between the two groups. However the body weight did not recover to the well-nourished control level.

### Hematological observations

The patterns of RBC, WBC, Hb, Ht, and TP showed significant recovery after PD and EN administration from malnourished state. The rate of recovery both groups were compared in each items as shown in Fig. 2. RBC in EN group recovered more

**Table 2 Average weight of food and nutrients intake by PD and EN per rat per day.***

| levels of protein | malnourished control (3%) | wellnourished control (18%) | 6% | | 9% | | 12% | | 18% | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | PD | EN | PD | EN | PD | EN | PD | EN |
| food intake (g) | 6.9 ±0.3 | 18.9 ±0.6 | 2.2 ±0.2 | 2.7 ±0 | 7.0 ±0 | 7.0 ±0 | 13.0 ±0 | 13.0 ±0 | 19.9 ±0.7 | 20.3 ±0.5 |
| energy (kcal) | 26 | 79 | 10 | 12 | 29 | 31 | 55 | 64 | 84 | 94 |
| protein (g) | 0.2 | 3.6 | 0.4 | 0.5 | 1.3 | 1.3 | 2.5 | 2.6 | 3.8 | 3.8 |
| carbohydrate (g) | 4.7 | 10.7 | 1.4 | 1.7 | 4.0 | 4.4 | 7.4 | 8.9 | 11.4 | 13.0 |
| fat (g) | 0.7 | 2.5 | 0.3 | 0.4 | 0.9 | 1.0 | 1.7 | 2.0 | 2.7 | 3.0 |

* Intake of the nutrients was calculated from the average food intake in each groups.

**Table 3 Effect of dietary protein level on body weight during the experiment**

| | Experimental week | | | | |
|---|---|---|---|---|---|
| | 0w | 1w | 2w | 3w | 4w |
| well nouriwhed control (18%) | 278.4±4.6* | 311.7±6.4 | 345.1±6.7 | 374.6±8.9 | 399.6±9.1 |
| malnourished control (3%) | 83.4±1.2 | 83.3±1.5 | 82.2±1.7 | 86.1±2.9 | 87.3±1.8 |
| level of protein | 3% | 6% | 9% | 12% | 18% |
| PD | 84.7±1.6 | 98.8±2.5$^{\circ\circ}$ | 124.6±3.5$^{\circ\circ\circ}$ | 170.2±3.4$^{\circ\circ\circ}$ | 245.4±3.6$^{\circ\circ\circ}$ |
| EN | 84.7±1.6 | 83.1±3.0$_{++}$ | 99.9±3.1$_{+++}^{\circ\circ}$ | 136.6±8.2$_{+++}^{\circ\circ\circ}$ | 244.0±5.6$^{\circ\circ\circ}$ |

$^{\circ}$ $P<0.05$ $^{\circ\circ}$ $P<0.01$ $^{\circ\circ\circ}$ $P<0.001$ significantly different from malnourished control.
$^{+}$ $P<0.05$ $^{++}$ $P<0.01$ $^{+++}$ $P<0.001$ significantly different between PD and EN.
$^{*}$ The values are indicated gram, $\bar{X}\pm SEM$

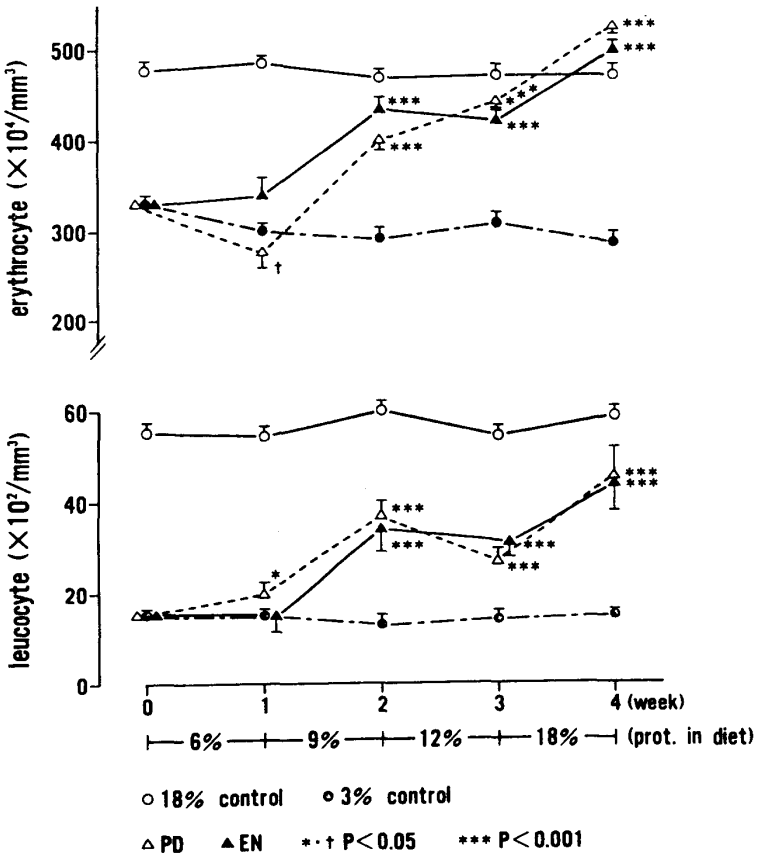



**Fig. 2 Recovery of numbers of erythrocyte and leucocyte during nutritional restoration with PD and EN. The values indicate $\bar{X}\pm SEM$.**

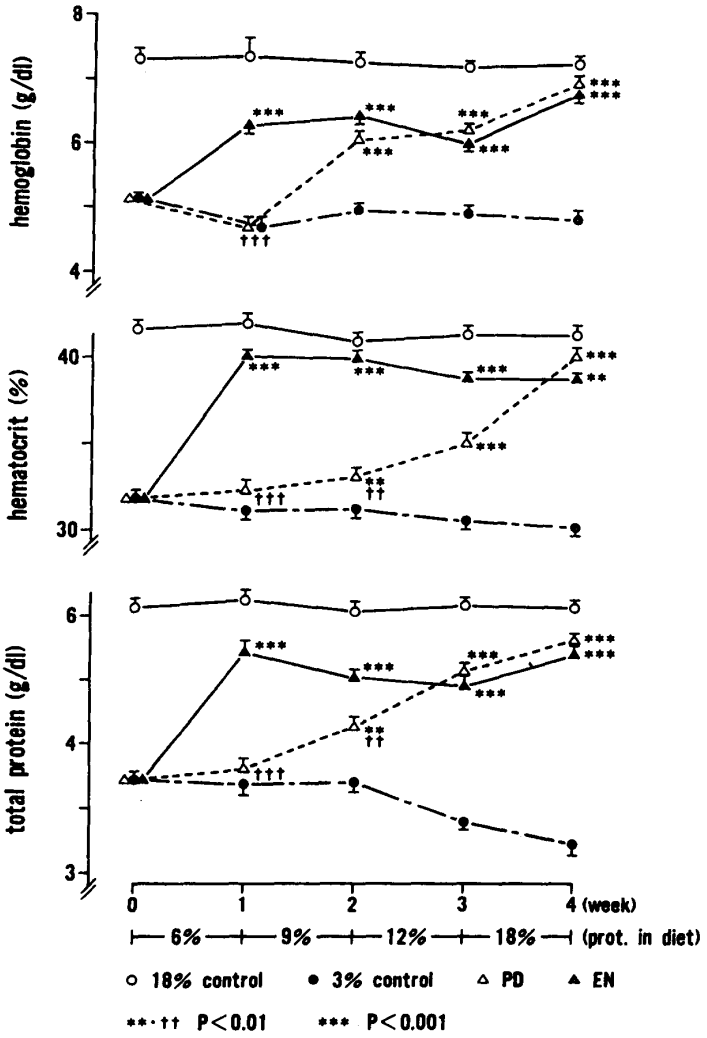



**Fig. 3 Recovery of Hb, Ht and TP in serum during nutritional restroration with PD and EN. The values indicate $\bar{X}\pm SEM$.**

effectively than in PD on the weeks 1 ($p<0.05$) and 2 after the diet tranfer. On the week 4, 18% protein stage, RBC in both groups recovered over the normal control level. After the week 2, the RBC in both groups recovered significantly effective ($p<0.01$) throughout the experiment. WBC recovered significantly effective ($p<0.001$) from the malnourished stage in a similar way in response to restoration with both PD and EN. Nevertheless, the levels did not reach to the normal level at the end of the experiment.

As shown in Fig. 3, Hb, Ht and TP were recovered singnificantly ($p<0.001$) up to close to the normal level on the week 1 in EN group, and maintained the high level during the experiment. On the other hand these levels in PD group were recovered slowly and reached to the levels of EN group on the week 2 in Hb, 3 in TP and 4 in Ht respectively.

**Immunological factors**

As shown in Fig. 4 , the complement system, CH50 was nearly the same irrespective of whether PD or EN was administered during the course of recovery. The tendency of recovery was gradual and titers reached to the well nourished control level at the week 4. C3 recovered more rapidly than that of the other immunological parameters. On the week 2, C3 level recovered significantly ($p<0.05$) as compared to the prior level in both PD and EN groups. On the week 3, C3 in EN recovered to the normal level with significant difference ($p<0.001$) from malnourished state and maintained that level throughout the experiment. C3 in PD recovered slowly than that in EN but reached to the normal level on the week 4 with significant difference ($p<0.001$) as compared to the prior level.

IgG and IgM did not show consistent tendency throughout the experiment in both PD and EN groups.

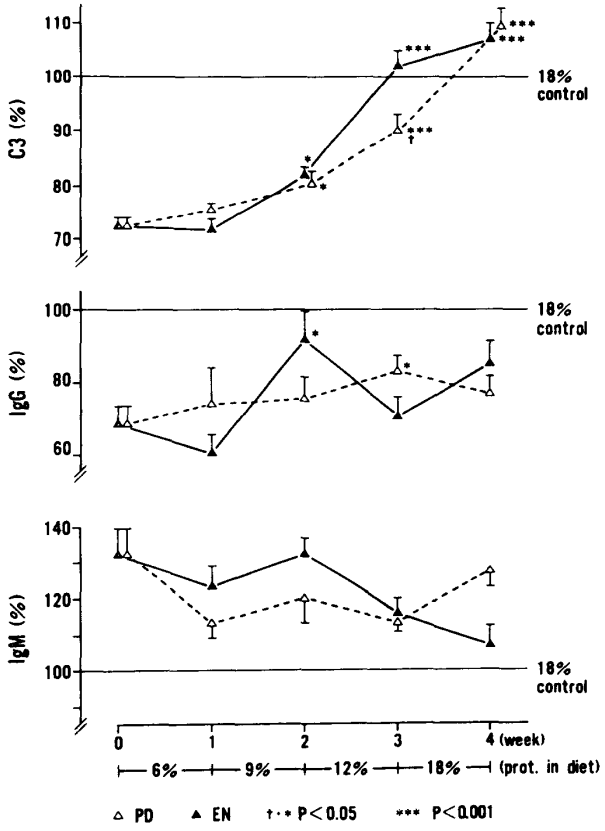



**Fig. 4 Recovery of C3, IgG and IgM in serum during nutrition restoration with PD and EN. The values indicate $\bar{X}\pm SEM$.**

As for the indicator of cell mediated immunity, the changes of number of Mφ and phagocytosis with latex beads were observed. Figure 5 described the changes of number of Mφ and intracellular number of latex beads phagocytized per cell during the restoration. The number of Mφ in PD recovered significantly on the weeks 1 ($p<0.05$), 2 ($p<0.001$) and 3 ($p<0.05$) as compared to the prior level. On the other hand the number of Mφ in EN showed less effective recovery but on the week 2 the significant increase level ($p<0.001$) was shown and decreased again on the week 3 but still significant ($p<0.01$) level as compared to the malnourished state. The recovery of phagocytic activity as measured by the intracellular numbers of beads per Mφ showed significantly more in EN than in PD. On the week 1, the number of beads was signifcantly high ($p<0.05$), and that on the week 2 ($p<0.001$) and that on the week 3 ($p<0.001$) respectively. On the week 3 the value was over the level of well-nourished control group. Table 4 showed the effect of restoration with PD and EN on PPD skin reactivity. On the week

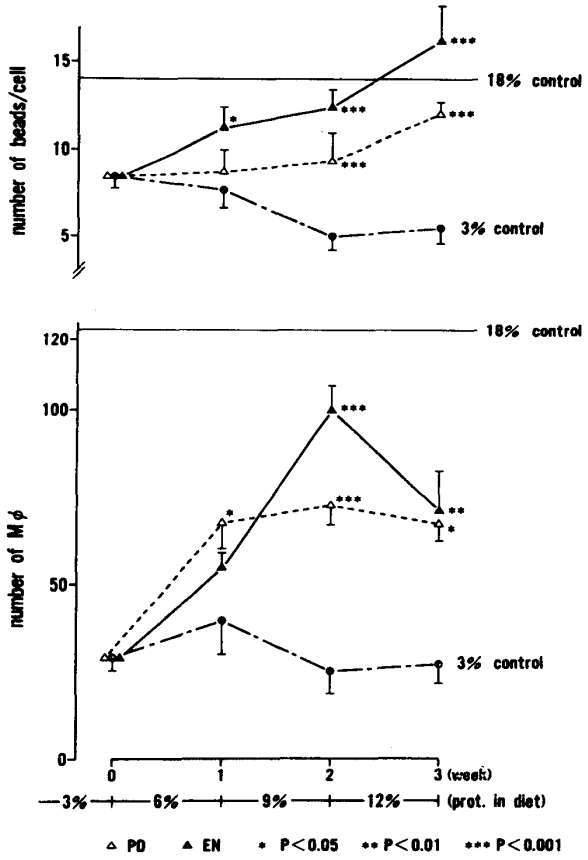



**Fig. 5 Recovery of number of Mφ and phagocytic activity with intracellular numbers of polystyrene latex beads in macrophage cell. The values are indicated by $\bar{X}\pm SEM$.**

**Table 4 Effect of diets during the recovery on PPD skin reactivity**

| Experimental week (w) | | 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|
| Well nourished control | D* | 5.5 | 7.5 | ND‡ | 8.0 | 8.0 |
| | P† | 4/5 | 5/5 | ND | 5/5 | 5/5 |
| Malnourished control | D | 0 | 0 | ND | 0 | 0 |
| | P | 0 | 0 | ND | 0 | 0 |
| Level of dietary protein (%) | | 3% | 6% | 9% | 12% | 18% |
| Restored by PD | D | 0 | 0 | ND | 5.0 | 5.0 |
| | P | 0 | 0 | ND | 1/5°°°§ | 2/5°°° |
| Restored by EN | D | 0 | 0 | ND | 7.0 | 6.0 |
| | P | 0 | 0 | ND | 3/5°‖ | 3/5° |

* D : Diameter (mm) of the positive induration.
† P : Number of rats giving positive reaction/number of rats tested.
‡ ND : not done
§ ° $P<0.05$ °°° $P<0.001$ significantly different from well nourished control.
‖ ++ $P<0.01$ significantly different between PD and EN.

2 in the experiment, the PPD skin reactivity was all negative. The data represent mean diameter (in mm) showed positive in response to 25$\mu$g dose of PPD. All animals in well nourished control group showed always positive ratio (100%) except the first week and there were a little variation in the diameter of induration. The rats in malnourished group showned negative throughout the experiment. The rats restored in PD and EN groups exhibited measurable cutaneous response on the weeks 3 and 4 after the diet transfer but the size of the reaction was less than that of the well nourished control.

The rats fed EN showed 60% positive on the weeks 3 and 4, on the other hand, the rats in PD showed only 20% and 40% positive ratio on the same period. The reaction was not strong as well-nourished control but the size of induration in EN was somewhat larger than that in PD. The positive ratios of PPD reaction were significantly different in PD ($p<0.001$) and in EN ($p<0.05$) from well nourished control on both the weeks 3 and 4. Comparing the groups restored with PD and EN, EN showed significantly effective recovery ($p<0.01$) on the weeks 3 and 4.

Number of lymphocytes and, ratio of T-lymphocyte subsets to examine the effects of restoration with PD and EN during the nutritional recovery, lymphocyte number, ratio of T-lymphocytes and T-lymphocyte subsets were measured on the weeks 1, 2, and 4. The total number of lymphocytes, ratio of T-lymphocytes and helper T and suppressor

T-cell subsets of well nourished and malnourished controls and the groups restored with PD and EN during the recovery were shown in Table 5. There wes an absolute reduction in the total number of lymphocytes in malnourished state. After the diet transfer in both PD and EN, total number of lymphocytes did not recover up to the week 1 showing one tenth of control. At the weeks 2 and 4, they recovered but remaining one third of normal value and the values did not recover to the the level of well nourished control at the end of the experiment. Percentage of T-helper in PD did not differ from well-nourished control group during the experiment. In EN group, the percentage of helper cell was less as compared to control of the week 1 and constantly recovered to the level of well nourished control group thereafter. Percentage of suppressor T-cell subset in PD showed higher as compared to the control on the week 1 and decreased thereafter. Percentage of suppressor T-cell in EN showed similar recovery tendency as that in PD. The helper/suppressor ratio in both PD and EN groups were similar as control before the diet transfer and rose thereafter. EN group showed slightly higher helper/suppressor ratio than PD, but among the control, PD and En restored groups, percentage of T-lymphocytes, helper and suppressor did not show significant difference. Helper/suppressor ratio showed the value over 1.3 on all the cases throughout the experiment.

**Table 5 T lymphocyte subset during dietary recovery with PD and EN**

| | | Reactivity with monoclonal antibodies (%) | | | | | | H/S ratio | | Total Number of lymphocyte | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | T-lymphocyte | | T-helper | | T-suppressor | | | | | |
| Levels of protein in diet | Exp. week | PD* | EN† | PD | EN | PD | EN | PD | EN | PD | EN |
| 6% | 1 | 68.5 ±3.4 | 56.0 ±3.7 | 38.5 ±4.1 | 32.0 ±4.2 | 31.0 ±3.5 | 24.3 ±4.5 | 1.3 ±0.2 | 1.9 ±0.4 | 1045 ±330 | 1167 ±232 |
| 9% | 2 | 58.7 ±2.7 | 64.7 ±4.3 | 35.4 ±4.0 | 42.7 ±5.1 | 24.2 ±1.2 | 20.9 ±1.3 | 1.9 ±0.5 | 2.2 ±0.2 | 3632 ±433 | 2607 261 |
| 18% | 4 | 61.0 ±3.1 | 62.0 ±1.8 | 44.7 ±2.4 | 46.0 ±2.2 | 20.3 ±2.0 | 18.7 ±1.0 | 2.4 ±0.3 | 2.5 ±0.2 | 3135 ±333 | 2624 ±261 |
| Well nourished control | | 62.6 ±2.5 | 40.3 ±1.7 | 21.2 ±0.9 | 1.9 ±0.1 | 10717 ±1177 | | | | | |

* PD : natural per os diet
† EN : enteral nutrients by tube feeding

## DISSCUTION

The purpose of using parenteral nutrients for the hospitalized patients who will not or cannot eat is a therapeutic means to assist clinical recovery of the patients and also hightened resistance against opportunistic infections which commonly occured in the host with lowered immune competence due to malnutrition. Although the efficiency of parenteral and enteral nutrients for restoration of nutrition has been investigated, little has been mentioned on the benefit and failure about the recovery of immune competence. This investigation focused on this point and also evaluated the nutritional restoration effect with natural per os chow diet (PD) and enteral nutrients by tube feeding (EN) on nutritional as well as immunological parameters during the recovery process from malnutrition, especially on sequence of recovery process of various immunological parameters.

A state of malnutrition can be induced by feeding SD male rats, 4 week old, averaging 80g in weight, with 3% protein diet for 6 weeks. Hematological and immunological factors were significantly lowered (18). After the state of malnutrition was defined with our previous observations (18) in body weight and further confirmed by hematological and biochemical and immunological parameters, the diet for the rats in experimental groups were transferred to EN (Clinimeal) and PD which contained very similar nutritional values as EN. This was consisted of corn flour. wheat flour, fish meals, corn oil, mimerals and vitamins mixture. The levels of protein in diets were increased 6%, 9%, 12% and 18% at a week interval for 4 weeks.

The efficiency of nutrients was assessed by measuring body weight, food intake, RBC, WBC, Ht, Hb and TP. As an immunological parameters, complement activity, C3, IgG, IgM, macrophages, phagocytic activity, T-lymphocyte, T-helper cell, T-suppressor cell and PPD skin reactivity were measured every week.

Our result showed the significant recovery of body weight showing subtle difference in the rats fed PD in spite of giving nearly the very similar amount of energy, carbohydrate, and protein. One possible influencing factor may have been the hurness of the rats for enteral nutrition that this was a stress which affects normal body weight gain or of limited value in improving lean body mass as observed in rats or in human fed

parenterally in the reports of other investigators (3, 4) because of less intake of trace minerals such as Mg or P or other minerals.

RBC and WBC recovered in a similar way whether the nutrients was given by EN or PD.

Hb, Ht and TP in serum were already significantly higher on the week 1 after diet transfer in EN than in PD suggesting very rapid net utilization of chemically defined nutrients in EN.

As for sequence of recovery of each immunological parameters, level of C3 recovered to normal level in EN on the week 3. This is much faster than the recovery of the number of lymphocytes, T-cells and macrophage and the recovery of PPD skin reactivity related to T-lymphocyte activity.

PPD skin reactivity did not show any positive reaction on the rats fed with 3% protein level diets.

The recovery of PPD reactivity was observed with weak positive on the week 3 in 20% of rats in PD and 60% of rats in EN and on the week 4, 40% in PD 60% in EN. The recovery of PPD reactivity in EN was more effective than that in PD. Total number of lymphocytes in malnourished rats was reduced (19) to 1/10 of that of well-nourished control and recovered to one third at the weeks 3 and 4, but never came up to the normal level. This may be related to the recovery of PPD skin reactivity.

Although total number of lymphocytes decreased, T-helper and T-suppressor cells did not show any significant changes as compared with that of well-nourished control. These results showed different tendecy in the study of malnourished human (13). This may be due to the state of malnourishment is more severe in our experiment than in human cases or due to species differences of lymphocyte systems in human and rats.

T-helper and T-suppressor rations (H/S) were always over 1.3 and did not show inverted ratio.

In the course of recovery, the recovery of T-helper cell in EN was effective than in PD. Especially H/S ratio in EN on the week 1 showed 1.9 and the ratio increased thereafter. This may indicate that the utilization of EN under the condition of very low lymphocyte level is more effective than that of PD.

Number of macrophage recovered significantly as compared to the level of malnouri-

shed control group but the level did not reach up to the level of well-nourished group.

On the other hand as shown in Fig. 6 , phagocytic activity of macrophage to ingest latex beads showed significant recovery on the week 3 and the phagocytic activity in EN was over the level of well-nourised control.

The relation of recovery of number of macrophages, phagocytic activity as shown by number of latex beads ingested per cell and C3 level was shown in Fig. 6 . These indicate the parallel recovery of C3 level with that of phagocytic activity of macrophages. As a role of macrophage in host defence, phagocytic activity will be the most important parameter. Therefore, more rapid recovery of C3 will be more effective to enhance the macrophage activity.

These experiments described above may offer a protocol to evaluate the efficiency of nutritional recovery from malnourished state. Here we compared immunological and nutritional parameters in the course of nutritional restoration with PD and EN tube

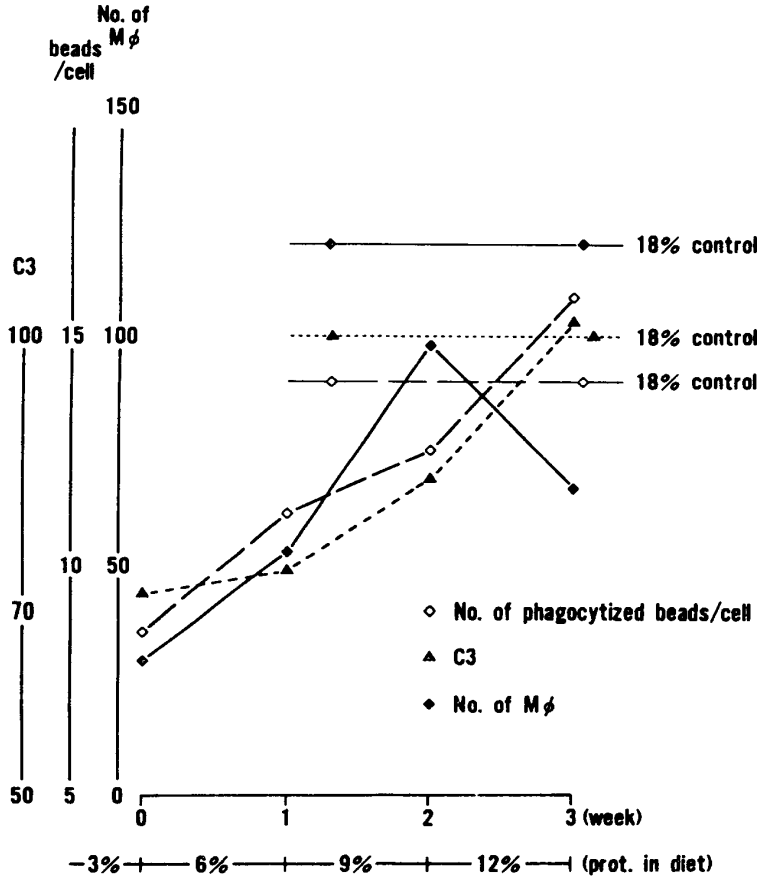



**Fig. 6 Level of C3, Mφ and phagocytic activity with latex beads in the course of nutritional restoration with enteral nutrients by tube feeding.**

feeding. The nutritional recovery due to EN showed less weight gain in early stage but the utilization of proteins recovered somewhat earlier. This may suggest the use of EN is more effective in the stage of severe clinical stage of malnutrition.

For the immunological factors, the recovery of C3 with EN was significantly rapid in parallel with phagocytic activity and this may stimulate phagocytic activity of macrophage cells due to hightened C3 level.

In this experiment, nutritional state in the rats was much severe as compared with clinical cases in human and total lymphocyte count was extremely lowered. Therefore the observations on changing pattern of T-lymphocytes, T-helper and T-suppressor cells were not analysed appropriately during this experimental period. In comparison of EN showed more effective results than by PD.

## ACKNOWLEDGEMENTS

We are obliged to Dr. K. Nishioka, Vice Director of the Japanese Red Cross Blood Center for valuable scientific advices and are acknowledged to Mr. N. Yahagi, Ortho Diagnostic Co. for his collaboration in flowcytometry with Spectrum 3.

## REFERENCES

1. Rickard KA, Kirsey A, Baehner RL, et al. Effectiveness of enteral and parenteral nutrition in the nutritional management of Wilms' tumors. Am J Clin Nutr 1980 ; 33 : 2622-9.
2. Rickard KA, Foland BB, Detamore CM, et al. Effectiveness of central parenteral nutrition versus peripheral parenteral nutrition plus enteral nutrition in reversing protein-energy malnutrition in children with advanced neuroblastoma and Wilms' tumor: a prospective randomized study. Am J Clin Nutr 1983; 38: 445-56.
3. Shizgal HA, Forse RA. Protein and calories requirements with total parenteral nutrition. Ann Surg 1980; 192: 562-9.
4. Nixon DW, Lawson DH, Kutner M, et al. Hyperalimentation of the cancer patient with protein-calorie under nutrition.
Cancer Res 1981; 41: 2038-45.
5. Lauza-Jacoby S, Sitren HS, Stevenson NR. Comparative adaptation to intragastric and intravenous feeding with a hypertonic alimentation diet. Surg Forum 1980; 31: 101-3.
6. McArdle AH, Palmason C, Morency I, Brown RA. A rationale for enteral feeding as the

preferable route for hyperalimentation. Surgery 1981; 90: 616-23.

7. Benngard K, Lindmark L, Wickstrom I, et al. A comparative study of the efficiency of intragastric and parenteral nutrition in man. Am J Clin Nutr 1984; 40: 752-7.

8. Nixon DW, Moffitt S, Lawson DH, et al. Total parenteral nutrition as an adjunct to chemotherapy of metastatic colorectal cancer. Cancer Treat Rep 1981; 65 (suppl 5): 121-8.

9. Levine AS, Brennan MF, Ramu A, Fisher RI, Pizzo PA, Glanbiger DL. Controlled clinical trials of nutritional intervention as an adjunct to chemotherapy, with a comment on nutrition and resistance. Cancer Treat Rep 1982; 42 (suppl): 774s-8ls.

10. Sitren HS, Fisher H, Ali R. Comparison of two amino acid solutions for total parenteral nutrition of normal and tranumatized rats. J Nutr 1975; 105: 1318-25.

11. Cameron IL, Pavlat WE, Urban E. Adaptive responses to total intravenous feeding. J Surg Res 1974; 17; 45-52.

12. Dionigi R, Dominioni L, Gnes F, Ballabio A. The effects of total parenteral nutrition on immunodepression due to malnutrition. Ann Surg 1977; 185: 467-74.

13. Chandra RK, Gupta S, Singhe H. Inducer and suppressor T cell subsets in protein-energy malnutrition: Analysis by monoclonal antibodies. Nutr Res 1982; 2: 21-6.

14. The National Research Council. Nutrient Requirements of Laboratory Animals. 3rd revised ed. No. 10, NAS, Washington, Washington D. C. 1978.

15. Mayer MM. Experimental immunochemistry, 2nd ed. Charles C. Thomas, Springfield, I11. 1979; 298-315.

16. Sakamoto M. Study on rat complement I. Immune adherence and immune hemolysis activity of rat serum. Japan J Exp Med 1975; 45: 183-91.

17. Shimura K, Ito H, Hibasami H, Shiomi T, Hidaka H, Nishioka K. Enhancement of antibody-independent phagocytosis by a lowmolecular weight compound I. Experiment with N-(6-amino-hexyl)-5-chloro-1-naphthalene sulfonamide (W-7). Immuno Communications 1983; 24 (4): 363-74.

18. Sakamoto M, Nishioka K, Shimada K. Effect of malnutrition and nutritional rehabilitation on tuberculin reactivity and comlement level in rats. Immunology 1979; 38: 413-20.

19. Petro TM. Effect of reduced dietary protein intake on regulation of murine in vitro polyclonal T lymphocyte mitogenesis. Nutr Res 1985; 5: 263-76.

坂本 元子 (本学教授)
石井 荘子 (本学助教授)
小林 幸子 (本学教授)
川野辺由美子 (本学助手)