The Effects of Total Parenteral Nutrition on Immunodepression Due to Malnutrition

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An experimental study was performed in 16 dogs to investigate the effects of sub-acute malnutrition on humoral and cellular immunity and phagocytic functions and, subsequently, to investigate the ability of total parenteral nutrition (TPN) to restore abnormal immunological variables. Deficiencies of IgG, C3, primary immune response to sheep red blood cells (SRBC), lymphocyte counts, lymphocyte response to phytohemagglutinin (PHA), and neutrophil chemotaxis were found to be caused by malnutrition. Nutritional repletion by means of TPN resulted in a return to normal or supranormal serum concentrations of IgG, IgM, and C3, and the primary immune response to SRBC was prompter and higher. Moreover, TPN resulted in restoration of normal neutrophil chemotactic responses. TPN did not improve lymphocyte response to PHA in these experiments. The study demonstrates that subacute malnutrition results in broad based deficiencies of the immunological response of the type that predispose to infection and that the proper use of TPN can correct most of these abnormalities.

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THE INTERACTION of infection and malnutrition is well recognized on the basis of clinical observations and epidemiologic data.[23,47] When protein deprivation reaches the point ot inanition and hypoproteinemia, non-specific resistance to infections is affected.[4] Several investigations have been performed showing numerous and sometimes conflicting immunologic defects associated with malnutrition. One of the limitations has been that most of the clinically related studies have been performed in children affected by Kwashiorkor, which is often characteristically complicated with infection. This could explain the frequent elevation of all three major classes of immunoglobulins (IgG, IgM and IgA) observed in these cases. However, infants less than 1 year of age with protein-calorie malnutrition (PCM) do not usually have elevated immunoglobulins; these infants often have low levels of immunoglobulins which remain depressed even after nutritional correction.[9] Antibody responses to injected antigens are either normal (to live attenuated measles vaccine, poliomyelitis vaccine, smallpox vaccine)[36] or somewhat diminished (e.g., to typhoid, influenza, yellow fever and diphtheria vaccines).[42,46] When depressed, the antibody response reverts rapidly to normal when an adequate diet is instituted.[41,42]

Cellular immunity in malnutrition is generally depressed as shown by clinical and experimental studies revealing lymphoid hypoplasia and lymphopenia,[54] involution of thymus,[51] decrease in the per cent of the E rosette forming lymphocytes,[13] and reduction of lymphocyte proliferative responses as evidenced by decreased rates of incorporation of tritiated thymidine into lymphocyte DNA after phytohemagglutinin stimulation.[51]

Other abnormalities accompanying malnutrition have included intracellular bactericidal and chemotactic defects of granulocytes,[48,49] although these immunological functions seem to behave differently in acute starvation. Experimental studies in rats have shown that during acute starvation and stress, the ability of neutrophils to ingest and kill bacteria is improved in the majority of instances.[3]

Total serum hemolytic complement levels and individual serum complement components, with the exception of C4, are markedly depressed in PCM in children and may result in abnormal opsonization. Repair of these

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TABLE 1. *Schedule of Progressive Dietary Restriction for the Induction of Subacute Malnutrition*

Day of Malnutrition	Quantity of Dog Food Administered
1–5	50% of measured basal dietary requirement
6–10	30% of measured basal dietary requirement
11–15	20% of measured basal dietary requirement
16–21	Starved

deficiencies can be influenced by the quantity of dietary protein and caloric intake.[50]

Alexander[1,3] has recently presented two excellent reviews of nosocomial and surgical infections and emphasizes that correction of nutritional deficiencies can result in the restoration of immunological competence. His group has been able to demonstrate an improvement in survival and a significant decrease in septic episodes by improving the nutritional status with dietary supplementation.[5,30]

The use of parenteral hyperalimentation for the nutritional repletion of malnourished surgical patients as proposed by Dudrick and his colleagues[20] and more recently by Duke and Dudrick[22] seems to be the best available tool to break the vicious circle of malnutrition and infection, and recent clinical studies on surgical patients undergoing total parenteral nutrition (TPN) provide evidence that prolonged TPN can improve host defense mechanisms, positively influencing both humoral and cellular immunity.[14,17,18,27] Although these clinical studies have the merit of direct relevance to therapy, they have the limitations of multiple uncontrollable variables, due in part to the heterogeneity of the patient population, the presence of coexisting infections and the use of drugs of unknown effects on the immunological system. For these reasons, the use of an animal model should allow a more precise measurement of the effect of protein-calorie malnutrition on immunological functions.

This study was designed, therefore, to investigate the effects of sub-acute malnutrition on humoral and cellular immunity and phagocytic functions in controlled experimental conditions and, subsequently, to investigate the ability of nutritional repletion by exclusive intravenous diets of amino acids and glucose to restore abnormal immunological variables.

TABLE 2. *Composition of 1000 ml of TPN Solution*

Calories	1,170
Dextrose	250 g
Aminoacids	42.5 g
Electrolytes	
Na	50 mEq
K	40 mEq
Ca	5 mEq
Mg	4 mEq
HPO_4	8 mEq
Cl	80 mEq
SO_4	4 mEq
Vitamins*	
C	125 mg
Niacin	50 mg
B_1	20 mg
B_2	2 mg
B_6	5 mg
B_{12}	125 μg
Folic acid	0.3 mg
K	1 mg

* Fat-soluble vitamins daily administered i.m.: A–5,000 USP Units; D–500 USP Units; E–25 I.U.

Materials and Methods

Animal Groups

Conditioned male pure-bred Beagle dogs, 6–8 months of age and weighing 5.7–7.2 kg were used for this study. Before initiating the studies the animals were housed in metabolic cages for stabilization and observation for two weeks and fed a standard dog diet[35] ad libitum in order to evaluate the daily mean caloric intake of each dog.

Sixteen dogs were studied and divided into three groups according to the following schedule: I. *Malnourished and TPN treated*: This group (8 dogs) was progressively malnourished for 21 days following a diet restriction schedule (Table 1), which lead to a weight loss of 24–31% and a level of serum albumin of 2.7–3.1/100 ml. At the end of the malnutrition period TPN was promptly started and carried out for 21 days through an indwelling catheter surgically inserted in the superior vena cava. II. *Normal controls*: This group (4 dogs) was maintained on regular standard diet ad libitum. III. *Malnourished controls*: This group (4 dogs) was progressively malnourished for 21 days following the same diet schedule of Group I and then used to study the primary immune response to sheep red blood cells (SRBC), the animals being refed a highly reduced diet (20% the basal caloric intake).

TPN Solution and Administration

The TPN fluids consisted of synthetic L-aminoacid preparations (8.5%) (Fre Amine®) mixed with 50% anhydrous dextrose in water in a 1:1 ratio. One thousand milliliters of this solution provided 6.25 g of nitrogen and 1000 non-protein calories. Minerals and vitamins were added to the base solution prior to use as summarized in Table 2. A vinyl catheter was surgically inserted under general anesthesia (Ketamine 2 mg/kg i.v.) with aseptic techniques into an external jugular vein and threaded into the superior vena cava; its proximal end was brought out with a trocar in the skin between the scapulas.[19] A stainless steel support assembly similar to

one previously described[21] was secured to the dog's back in order to maintain the catheter firmly connected with the delivery apparatus but allowing the animal freedom of movement in his cage.

The nutrient solutions were delivered by a roller pump (Holter TM Roller Pump, mod. 912) at the rate of 100 cal/kg/day for the first 48 hours and then 180 cal/kg/day for the remaining period of treatment. In preliminary experiments we found that the rate of 180 cal/kg/day is the minimal in order to achieve weight gain and positive nitrogen balance in 6–8-month-old malnourished beagles.

Laboratory Tests

The following laboratory tests were performed on a routine basis: 1) Blood sugar, BUN, serum and urine electrolytes determined daily; 2) Urine clinitest, pulse, respiration and temperature twice per day; 3) Input (parenteral feeding)—output record and weight determination daily; 4) Complete blood count, hematocrit, serum proteins, liver function tests, blood cultures once per week.

Assessment of Immunologic Competence

Serum IgG, IgM, and C3 were measured by the single radial diffusion in agar method.[32]

The primary immune response to an insoluble T dependent antigen was studied by an intravenous injection of 5 ml of a suspension containing 5×10^5 sheep red blood cells (SRBC) cells/ml (Istituto Sieroterapico Milanese). Hemagglutination (HA) titers were performed using standard techniques with doubling dilutions of sera and taken as the highest dilution in which HA occurred and expressed as the $\log_2$ of the reciprocal dilution. Sera for HA titers were obtained every day for 9 days after immunization, then every other day. Primary response to SRBC was studied in all three groups of animals; in Group I (malnourished and TPN treated) immunization was performed in 4 animals at the end of the parenteral feeding treatment; in Group II (normal controls) immunization was performed in all the animals in basal condition in order to evaluate the normal immune response; in Group III (malnourished controls) the SRBC suspension was administered at the end of the malnutrition period.

Cell mediated immunity was evaluated by absolute blood lymphocyte counts and by lymphocyte response to PHA *in vitro*. The reactivity of peripheral lymphocytes to PHA was assessed by culturing 1×10^5 isolated lymphocytes and measuring the incorporation of ^{3}H-thymidine into DNA. The technique for lymphocyte separation and *in vitro* culture has been described previously in detail.[16] In all experiments 10% fetal calf serum was used in the cell culture medium.

Leukocyte function was evaluated by testing leukocyte random migration,[37] latex stimulated and unstimulated nitroblue tetrazolium (NBT) dye reduction by neutrophils,[29] and neutrophil chemotaxis. In the studies on chemotaxis leukocyte suspensions containing approximately 85% PMN were prepared from heparinized venous blood (20 U/ml) by employing standard techniques of dextran sedimentation and hypotonic lysis of erythrocytes. Perspex chambers of the Boyden design were used to study neutrophil chemotaxis across a millipore filter having a 5 μ pore size and 13 mm diameter which was interposed between the upper and lower compartments. Two milliliters of chemotactic agent (*E. coli* lipopolysaccharide—fetal calf serum, equal volumes) was placed in the lower chamber. A 1.8 ml aliquot of the washed neutrophils (5×10^6) in Hank's solution was placed in the upper chamber. The chambers were then incubated for 3 hours at 37° and the filter was then removed and stained with Wright-Giemas stain.

The results of these experiments were expressed as a functional chemotactic index (F.C.I.).[52] The cells which had migrated through the membrane (lower chamber) were counted, as were the cells remaining in the upper chamber. A total of 200 cells were counted. A chemotactic index (C.I.) was calculated for each chamber as follows:

$$\text{C.I.} = \frac{\text{number of PMNS in lower chamber side}}{\text{number of PMNS in upper chamber side}} \times 100$$

The C.I. determined during the whole period of study in the animals of Group I were compared with the average C.I. (16 determinations in duplicate) of the normal control dogs and a functional chemotactic index (F.C.I.) calculated as percent of C.I. of control PMNS.

$$\text{F.C.I.} = \frac{\text{C.I. of Group I animals}}{\text{C.I. of normal control animals}} \times 100$$

Baseline determinations of the parameters listed above were obtained from each animal in basal conditions at least twice before undergoing malnutrition and once weekly during the malnutrition period and the TPN treatment period.

Statistical analysis of means was accomplished by the Student's t test.

Results

Serum Immunoglobulins and Complement

The variations of serum IgG, IgM and C3 concentrations are shown in Figs. 1, 2, and 3. The average of the IgG levels slowly and constantly decrease during the malnutrition period, reaching a significant ($P < 0.01$) low concentration two days after beginning TPN, the animals being still malnourished. During TPN, IgG progres-

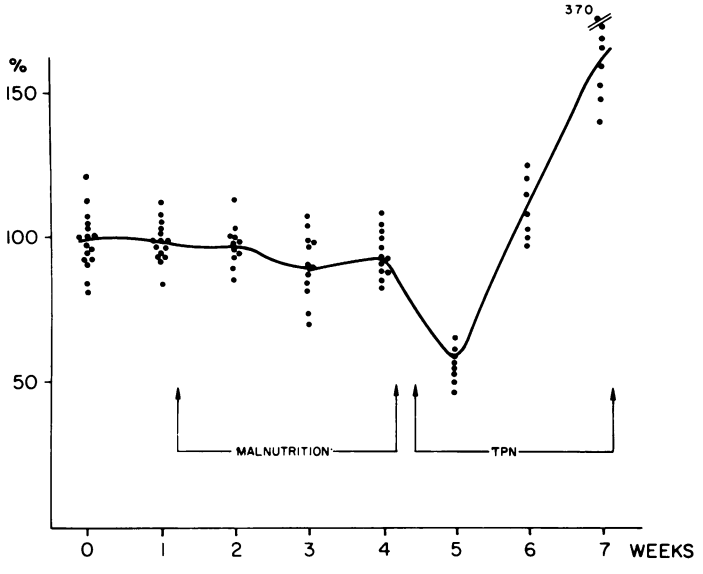

FIG. 1. Variations of serum IgG concentration during malnutrition and TPN. In this figure and in the following ones the values are expressed in arbitrary units since standard dog IgG, IgM and C3 solutions were not available.

sively increases, and by the end of the treatment period it rises significantly ($P < 0.05$) towards levels higher than normal (163%).

Malnutrition caused a statistically insignificant reduction in IgM concentration, but subsequent TPN therapy caused supranormal elevation of its levels (146%) ($P < 0.05$). No septic episodes or other abnormalities were clinically present which could account for the increase of serum immunoglobulins. Levels of the third component of complement C3 showed a marked fall, to 61% of baseline values ($P < 0.01$). After 3 weeks of TPN therapy, the mean C3 levels returned to normal (103%; $P < 0.01$).

Hemagglutination Titers

The results are shown graphically in Fig. 4. The average of the titers in the three groups of animals did not show any difference for the first 4 days after immunization. On the fifth day, HA titers in the TPN treated group were significantly higher ($P < 0.05$) than those of the malnourished group and slightly higher than the normal control group.

The titers of the malnourished group remained lower than those of the other two groups for the whole study period, the difference being statistically significant from the TPN treated group on days 6, 7, 8, and 9 ($P < 0.05$), and on days 13, 16, and 18 when compared to normal controls. The titers of the TPN treated group were slightly higher than those of the normal animals until the ninth day, when the highest titers were recorded at the same levels in both groups. Titers of the TPN treated group were not significantly different from the control group in the following period.

Lymphocyte Counts

The mean absolute lymphocyte count was significantly lower at the end of the malnutrition period compared with normal values ($P < 0.01$) (Table 3). Severe lymphopenia (lymphocyte counts below 1000 cells/mm^3) was present in 45% of the malnourished animals at the end of the starvation period. During TPN, peripheral blood lymphocytes remained at low levels, and no significant improvements were induced by the treatment.

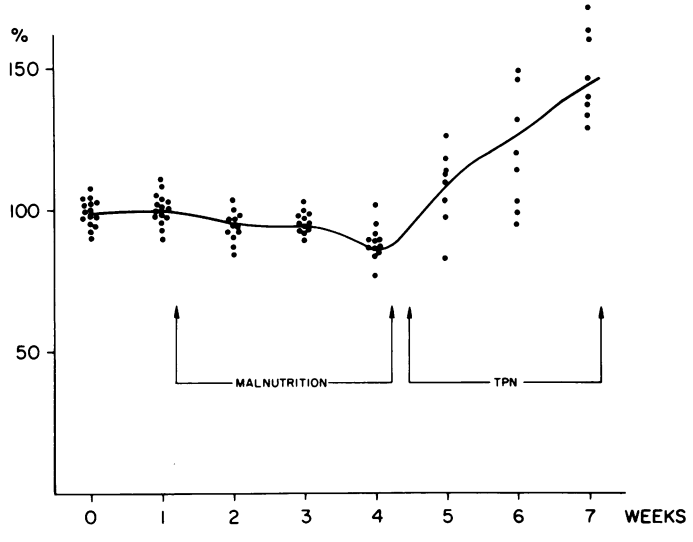

FIG. 2. Variations of serum IgM concentration during malnutrition and TPN.

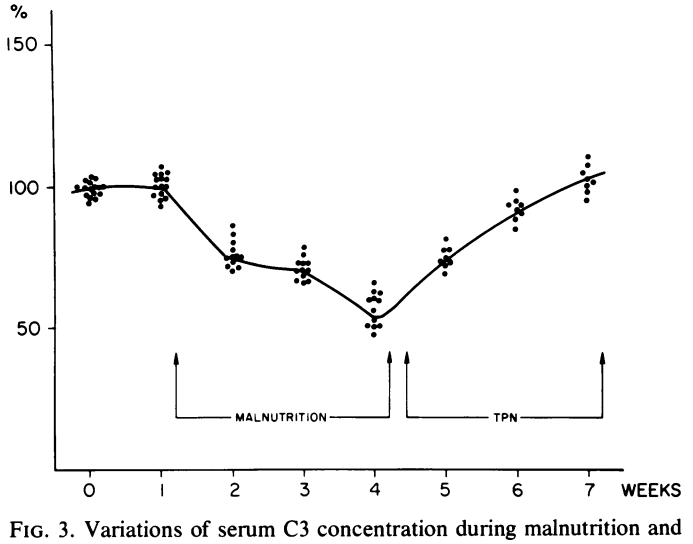

FIG. 3. Variations of serum C3 concentration during malnutrition and TPN.

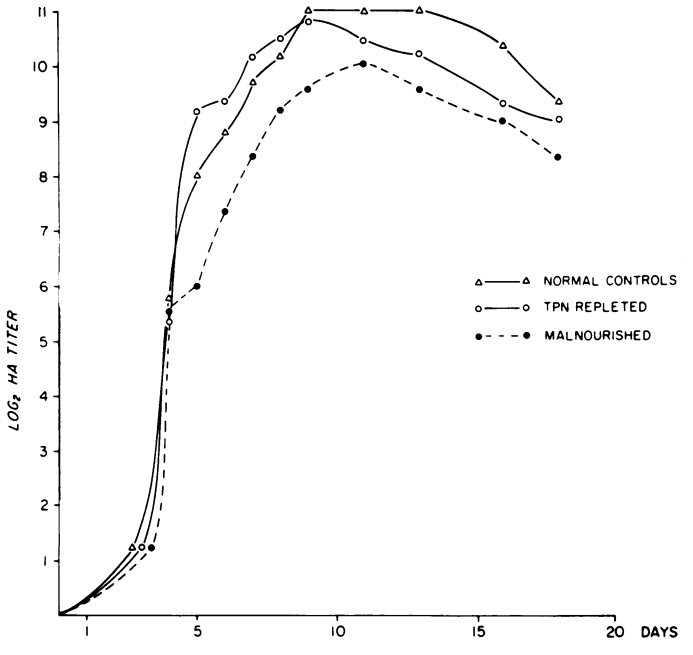

FIG. 4. Primary immune response to SRBC. HA titers were taken as the highest dilution in which HA occurred and expressed as the $\log_2$ of the geometric mean of the reciprocal dilution.

Lymphocyte Response to PHA in Vitro

Results of [^{3}H] thymidine uptake by PHA-stimulated lymphocytes are presented in Table 3. Sequential determinations of lymphocyte activation in basal condition showed mean stimulation values of 75. During the malnutrition period, the level of response fell gradually to 63 cpm. No improvement was observed during TPN treatment; in fact, the lowest mean value of PHA stimulation was recorded after 21 days of parenteral feeding.

Neutrophil Function Tests

Circulating neutrophil counts showed a decrease during malnutrition which was not corrected by TPN. No major variations of random leukocyte migration were observed throughout the experiments (Table 3).

Phagocytic function assessed by reduction of NBT by non stimulated or latex-stimulated blood PMN demonstrated non significant fluctuations within the normal range throughout the course of the experiments (Table 3).

Neutrophil Chemotaxis

The results of these experiments are shown in Fig. 5. The average F.C.I. curve suggests that neutrophil chemotaxis is depressed during the malnutrition period, reaching the lowest levels at the end of the malnutrition period and at the beginning of nutritional repletion. After 21 days of TPN, the average F.C.I. is superior to that observed in basal condition before the progressive reduction of diet. Statistical comparison revealed a significant difference between the mean F.C.I. at the end of TPN and at the end of malnutrition ($P < 0.01$).

Discussion

It has long been recognized that there is a relationship between malnutrition and infectious diseases, suggesting that some aspects of host resistance are functionally impaired. It is thought that malnutrition can either increase host susceptibility to infection or that infection can precipitate malnutrition[47] but still contradicting results have been reported in examining several host factors of resistance that are altered by changes in nutritional status. Since studies of nutritional immunological relationships in man are difficult, mainly because of the great number of uncontrollable variables, our studies have been carried out in an experimental animal under controlled conditions in order to evaluate which of the host defense factors are more affected by subacute malnutrition and to determine if it is possible to improve the impaired immunocompetence by nutritional repletion by TPN.

In this study, malnutrition induced a significant decrease in serum IgG but did not greatly affect IgM levels. Our results seem to be in contradiction to most

TABLE 3. *Immunologic Parameters Not Improved by TPN Treatment*

	Normal	(a)	Malnourished	(b)	TPN repleted
PHA lymphocyte stimulation (^{3}H-thymidine uptake, cpm/tube × 10^3)	75 ± 44	(N.S.)	63 ± 32	(N.S.)	39 ± 36
Peripheral blood lymphocytes (cell/mm^3)	3270 ± 1430	($P < 0.01$)	1790 ± 550	(N.S.)	1700 ± 540
Leukocyte migration (mm/5h)	1.3 ± 0.7	(N.S.)	1.3 ± 0.5	(N.S.)	1.1 ± 0.5
NBT dye reduction capacity (% NBT positive cells)					
Latex stimulated	42 ± 26	(N.S.)	23 ± 16	(N.S.)	25 ± 12
Unstimulated	9 ± 5	(N.S.)	11 ± 4	(N.S.)	7 ± 4

Mean values ± standard deviations are given. Significance of differences was tested (Student's t test) comparing: (a) normal dogs to malnourished; (b) malnourished dogs to TPN repleted.

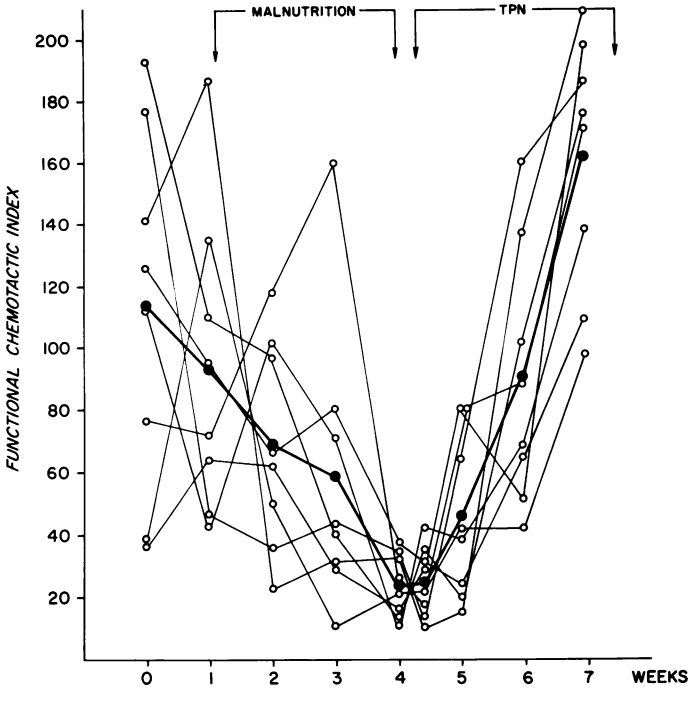

FIG. 5. Functional chemotactic index during malnutrition and TPN.

of the clinical studies performed in malnourished children. Marasmic infants reportedly have elevated levels of IgM,[38] but children with Kwashiorkor may have low,[9] normal,[43] or high[33] immunoglobulin G. A recent study[39] of the immunologic responses in children with protein-calorie malnutrition showed that the three major immunoglobulins (IgG, IgM, IgA) and IgE were elevated. It has been suggested that high immunoglobulin levels in children with severe malnutrition may be in response to infections or may reflect intestinal parasitism.[39,53]

Levels of the third component of complement were significantly reduced in the malnourished beagles. This is in agreement with the work of others[26,39,50,51] and may be explained by diminished protein synthesis by the liver.[7] In an extensive clinical study, Sirisinha[50] measured levels of seven complement components in malnutrition and found reduction in all components except for C4. This might suggest that the alternate pathway of complement is activated in malnutrition, possibly by bacteria and their endotoxins, which would lead to breakdown of C3 and later components without affecting C1, C2 and C4. However, Sirisinha noted that C1q, and C1s were reduced as well as C3. The reduction of C3 concentration in the malnourished animals is in our opinion one of the principal findings of this study since impairment of the complement system increases susceptibility to gram-negative sepsis.

In our study, nutritional supplementation by means of TPN corrected the depressed serum concentrations of IgG, IgM, and C3, and showed a rebound type of "supranormal" response, confirming the significant finding of Law et al.,[28] who recorded supranormal responses in all immune parameters tested after protein repletion of protein depleted rats.

Persons suffering from severe malnutrition are not always able to produce circulating antibodies in response to certain bacterial and viral antigens.[10,11] The capacity to synthesize certain antibodies is restored when proteins are incorporated into the diet.[34] In our study the antibody response to SRBC was not abolished in the malnourished animals, but it was constantly lower than the controls and the TPN treated dogs, confirming that antibody response is defective in experimental malnutrition[25,40] and can be effectively restored by TPN. However, it is our impression that further studies are needed for a better evaluation of specific antibody response during severe malnutrition since this variable type of immunological response depends on the physical and biological state of the antigen, the type of adjuvant, the route of immunization and the relative T or B-cell dependence of antigens.

Cellular immunity is depressed in malnourished children whose thymus gland and lymphoid tissues present anatomic and functional changes typical of depressed cellular immunity.[13,51] Diminished cellular function during the malnutrition period was manifested in this study by lymphopenia and diminished *in vitro* lymphocyte response to PHA. This is in agreement with other studies,[12,39,51] but in contrast with the report of Jose and Good[24] who demonstrated enhanced cell-mediated immunity in protein deficiency states. The depressed cellular immunity during malnutrition could also be a result of other mechanisms such as increased levels of cortisol from the stress of malnutrition.[6] The presence of low serum albumin levels also contributes to increased levels of unbound active cortisol.[44] This depressed cell mediated immunological response is generally acknowledged to contribute to a susceptibility to viral, fungal, parasitic, gram-negative bacteria, and mycobacterial infections.

Nutritional repletion of the animals by TPN was not able to restore an efficient T-cell function or a normal peripheral lymphocyte count. This is in contrast with previous clinical reports[18,27] in which *in vitro* proliferative responses to mitogens did change significantly after TPN treatment. It is possible that the lack of positive results in the animal model could be either because of the relative short period of treatment (21 days), or the persistence of a stressful situation (the animal being constantly restricted in the metabolic cage). Also, since more information is necessary before accepting the response of lymphocyte *in vitro* to PHA as an accurate indicator of T-cell function in the dog, studies of other indices of dog T lymphocyte function such as rosette formation and skin tests during malnutrition and nutritional repletion should be undertaken in order to define more accurately the specific lesion of host defense mechanisms and the possibility of reversion.

There have been few systematic investigations on

the phagocytic activity under various conditions of nutritional stress, and the reports that are available are conflicting, possibly because of the varied procedures employed for assaying phagocytic activity and the degree and modality of malnutrition. Our study using the NBT test (a measure of hexose monophosphate shunt activity) did not show any significant difference throughout the course of the experiments, the values remaining in the normality range. This confirms the isolated observation of Altay et al.[8] who, using the same technique, did not find any difference in the phagocytic activity between malnourished children and control groups. Phagocytic cells from patients with malnutrition have been shown to have impairment in bacterial killing capacity compared with controls[15,45] and this defect seems to be reversible following recovery from malnutrition by oral hyperalimentation.[2] In recent clinical investigations[29,30] Lennard et al. showed a correlation between leukocyte zinc content and bacterial phagocytic and killing capacity. Preliminary laboratory data[31] of neutrophil function tests from animal fed diets which were different in either zinc or manganese indicate a slowly developing decrease in neutrophil function compared to control animals.

Leukocyte chemotaxis in vitro is the unidirectional migration of leukocytes in response to a diffusing gradient of chemical attractant; it is an important biologic phenomenon, which derives the energy necessary to permit the chemotactic responses of leukocytes from glycolytic pathways, as is also the case with phagocytosis. Leukocyte chemotaxis in malnutrition has not been previously evaluated. Our studies showed that malnutrition induced defects of chemotactic activity and that TPN markedly improved this activity to a "supranormal" level. It is interesting to observe the correlation we found between the serum concentration of C3 and neutrophil chemotaxis. Since some components of the complement system (C3 and C5) constitute a major pathway for the production of chemotactic factors, deficiences would logically be expected to have important consequences. Whether or not the more serious problem during malnutrition induced C3 deficiency is from the impaired production of chemotactic factors or opsonic factors still needs to be determined. Both chemotactic and opsonic factors derive from the same molecules, and both are essential in resistance to infections. If either or both chemotactic factors and opsonic factors are lacking, protection against bacterial invaders is seriously impaired.

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