THE NUTRITIONAL ASSESSMENT OF PATIENTS RECEIVING PARENTERAL NUTRITION

A Thesis
Submitted to the Faculty of Graduate Studies
of the University of Manitoba

In Partial Fulfillment
of the Requirements for the Degree of
Master of Science

by
Lilian Patricia Phillips
Winnipeg, Manitoba
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ABSTRACT

Total parenteral nutrition (TPN) is now utilized in hospitalized patients with a broad range of disease conditions. To derive maximal benefit from TPN, it is essential that its use be accompanied by a reliable means of assessing nutritional changes, and a wide variety of methods have been recommended for this purpose. The aim of the present study was to evaluate many of these proposed nutritional markers in hospitalized patients receiving TPN.

Tests of nutritional status were carried out in sixteen adult patients. These tests included anthropometric measurements, serum albumin, prealbumin and transferrin, creatinine-height index, and urine and serum levels of creatinine, zinc and ribonuclease. In addition, some patients received tests of immune competence, including skin testing for delayed cutaneous hypersensitivity, and measurement of immunoglobulins, complement and T and B lymphocytes.

Those tests found to be most useful were the ratio of urine ribonuclease to serum ribonuclease, the urine ribonuclease concentration, triceps skinfold thickness, serum prealbumin, serum transferrin and weight. Serum albumin, urine zinc concentration and the creatinine-height index did not appear to be useful in nutritional assessment except in cases of severe, long-term malnutrition. The remaining biochemical measurements showed no clear relationship to nutritional status. Levels of complement C3 appeared to be

related to changes in nutritional status, but the remaining immunological tests failed to show any promise as nutritional indicators.

Discriminant analysis of the more promising of the measurements selected four parameters which enabled the patients to be classified as either nutritionally improved or non-improved following a course of TPN.

It was concluded that several parameters, particularly the ratio of urine to serum ribonuclease, the triceps skinfold thickness, serum prealbumin and serum transferrin, were useful in evaluating changes in the nutritional status of a group of hospitalized patients receiving TPN.

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TABLE OF CONTENTS

INDEX OF TABLES

INDEX OF FIGURES

INTRODUCTION

A. Introduction to the Study

Total parenteral nutrition (TPN) is the intravenous administration, usually by means of a central vein, of amino acids, glucose and other essential nutrients in quantities sufficient to maintain or restore adequate nutritional status. Since its introduction into clinical practice little more than ten years ago, TPN has become a recognized mode of therapy for a wide range of medical and surgical conditions.

TPN is used primarily in patients with abnormalities of the gastrointestinal tract, such as short bowel syndrome, enterocutaneous fistulae, acute pancreatitis, and inflammatory bowel disease including Crohn's disease and ulcerative colitis. It is sometimes employed as adjunctive therapy in a variety of conditions such as cancer, major burns, trauma, cardiac diseases, renal failure and hepatic failure (Grant, 1980). In addition, TPN has been used pre-operatively and/or post-operatively, since improvement in nutritional status appears to be associated with less post-operative morbidity and mortality (Mullen et al., 1979). Recently, partial parenteral nutrition (PPN) by means of a peripheral vein has been shown to be useful for many patients in whom TPN is not feasible or not required. Parenteral feeding, if properly employed in suitable patients, can lead to a marked reduction in patient morbidity and mortality.

An essential aspect of the proper use of parenteral nutrition is an adequate and on-going assessment of the patient's nutritional status. In the past several years, a

variety of parameters have been employed for this purpose. These include anthropometric measurements, extensive biochemical testing and tests of immune function. Ideally, such nutritional "markers" should be reliable, safe for the patient, inexpensive and relatively simple to perform. Furthermore, they should permit prompt assessment of a change in nutritional state. There is as yet insufficient data available for thorough evaluation of many of the parameters recommended for this purpose, and there has not been universal agreement as to which of them are the most reliable.

This study was undertaken to evaluate a variety of these proposed nutritional markers with regard to their dependability and their usefulness in a clinical setting. The information obtained will contribute to the development of a revised protocol for nutritional assessment, which, it is hoped, will provide a reliable indication of changes in a patient's nutritional status while avoiding unnecessary tests and causing minimal inconvenience to the patient.

The study was conducted at the St. Boniface General Hospital, a 867-bed teaching hospital situated in Winnipeg, Manitoba. Most patients were drawn from the hospital's gastrointestinal or surgical wards, although suitable adult patients on any ward were eligible for inclusion. Suitable patients were those who were eligible to receive a standard St. Boniface General Hospital TPN regimen (Appendix I) for a minimum of seven days. Adjustment of the quantity of electrolytes in the standard solution was permitted.

Criteria for exclusion from the study included any of the following:

1) inability or unwillingness to give informed consent;
2) a planned duration of TPN therapy of less than seven days;
3) use of non-standard TPN regimens;
4) severe complications, such as renal or hepatic failure, which would preclude reliable data collection; and
5) inability to tolerate loss of sufficient blood for the required tests.

Patients in whom parenteral nutrition was to be administered *via* the subclavian vein were preferred. However, some patients in whom a peripheral vein was used were included if this procedure was expected to continue for a prolonged period and if central TPN (*via* subclavian) was not considered feasible in that patient.

The majority of patients also received oral feeding at some time during the course of their parenteral nutrition regimen. For this reason, the term "TPN" should be interpreted here as parenteral nutrition, which is serving as the primary but not necessarily the sole source of nutrients.

The following parameters were to be evaluated:

1) anthropometric measurements - weight, triceps skinfold thickness, mid-upper arm circumference and mid-upper arm muscle circumference;
2) biochemical parameters - serum albumin, prealbumin, transferrin, zinc and ribonuclease;

urinary excretion of creatinine, zinc, ribonuclease and 3-methylhistidine;

creatinine - height index, skin surface lipids, and total body water calculated from ethanol dilution;

3) tests of immune function — serum immunoglobulins (IgG, IgM, IgA), serum complement (C3 and C4), T and B lymphocyte detection, lymphocyte transformation and skin testing for delayed hypersensitivity.

Some of the above tests could not be performed, chiefly due to problems with the methods of assay. Those parameters for which results are not available are 3-methylhistidine, skin surface lipids, total body water and lymphocyte transformation.

B. Anthropometric Measurements

Anthropometry deals with the measurement of the size, weight and proportions of the human body. Weight, especially in relation to height, is the most widely used anthropometric measurement, and in many subjects it provides a useful estimate of changes in nutritional status (Butterworth and Blackburn, 1975). However, weight changes are unreliable "markers" in edematous or obese patients. The latter may lose weight without suffering from protein depletion (Young et al., 1978). Edema, resulting from drug therapy or a disease state, can produce weight gain which does not correspond to a change in the patient's nutritional state (Butterworth and Blackburn, 1975).

Numerous investigators (Gurney and Jelliffe, 1973; Frisancho, 1974; Durnin and Womersley, 1974; Bray, 1978; Faintuch et al., 1979) have examined the use of skinfold thicknesses and mid-upper arm circumference measurements as nutritional markers. These measurements are relatively simple to perform, inexpensive and non-invasive, and the results are immediately available. Waterlow and Harper (1975) have commented that these anthropometric measurements are relatively insensitive compared to body weight, but agree that the measurements are helpful when weighing is impractical.

1. Triceps Skinfold

Skinfold measurements provide an estimate of subcutaneous fat, and have been used to give an approximation of total body fat (Ward et al., 1975). They are primarily useful for indicating changes in the patient's status rather than for determining absolute values (Waterlow and Harper, 1975). According to Blumenkrantz et al. (1980), most observers find a good correlation between subcutaneous fat and total body fat. However, the subject's age, sex and the presence of obesity must be taken into consideration. As discussed by Durnin and Womersley (1974), it appears that the proportion of subcutaneous fat to total body fat decreases with age, and also is lower in women than in men. In obese patients, a larger proportion of the body fat may be situated subcutaneously.

The skinfold thicknesses most frequently measured are those of the triceps, biceps, subscapular and supra-iliac sites. In a study of obese patients, Bray et al. (1978) reported that the subscapular skinfold correlated better with weight loss than did the triceps skinfold. However, this site is less accessible in severely debilitated patients. In the present study, it was decided to evaluate only the triceps skinfold, which is readily accessible even in bedridden patients, and therefore likely to be of particular use in patients for whom weighing is difficult or impractical.

2. Mid-upper Arm Circumference

The circumference of the upper arm has been suggested as a possible indicator of lean body mass (Kaminski and Jeejeebhoy, 1979). However, it has also been claimed that changes only become apparent in the more severe forms of protein-calorie malnutrition (Grant, 1979). This measurement is primarily recommended as a means of calculating the mid-upper arm muscle circumference.

3. Mid-upper Arm Muscle Circumference

The calculation of arm muscle circumference has been used to estimate muscle mass, and as an indirect indicator of protein reserve (Jelliffe, 1966). The calculation is made using the triceps skinfold thickness and the mid-upper arm circumference (see Method). This calculation is clearly only an approximation, since it does not take into account the bone diameter, and it assumes that the arm is perfectly cylindrical (Frisancho, 1974).

C. Biochemical Measurements

1. Serum Albumin

Albumin is a protein with a molecular weight of approximately 69,000 (Harper et al., 1979). It maintains osmotic pressure in the plasma and also serves as a carrier for a large number of substances including metals, ions and enzymes (Grant, 1980). It is synthesized in the liver, and has a half-life of about 20 days in a non-stressed individual (Kaminski and Ruggiero, 1979). The normal range of serum concentration is 3.5-5.0 gm/100 ml (Grant, 1980).

A reduction in the concentration of serum or plasma albumin has been used as "the classic 'test' for protein deficiency" (Waterlow and Harper, 1975), and albumin levels are widely used in monitoring TPN patients. Albumin synthesis has been shown to be markedly decreased by protein restriction (Kelman et al., 1972; James and Hay, 1968). However, there are several limitations to the use of serum albumin as a measure of nutritional state.

A major drawback is that, because of the relatively long half-life of albumin, a change in status may take some time to become apparent. A further limitation is the existence of compensatory mechanisms which come into effect when serum albumin concentrations fall. These mechanisms include a decreased rate of catabolism, and a shift from the extravascular to the intravascular compartment (James and Hay, 1968). In addition, James et al. (1976) reported that in

children placed on a low-protein diet, the decrease in rate of albumin synthesis was greater in malnourished than in well-nourished subjects. Due to the compensatory mechanisms, decreases in serum albumin levels are apparent only after "significant protein depletion" has occurred (Dudrick et al., 1979). For this reason, serum albumin is not a reliable indicator of a marginal impairment of nutritional status.

A number of factors other than malnutrition have been associated with hypoalbuminemia. These include chronic loss due to nephrotic syndrome or burns, impaired synthesis due to cirrhosis, exudative enteropathy (excessive loss into the gastrointestinal tract) (Harper, 1979), chronic inflammatory bowel disease such as ulcerative colitis and Crohn's disease (Jensen and Jarnum, 1976), neoplastic diseases and leukemia (Krupp and Chatton, 1978). Impaired albumin synthesis has also been associated with acute stress (due to surgery, trauma, burns or infection) and hypothyroidism (Rothschild et al., 1972). These factors tend to complicate the use of serum albumin levels as a measure of nutritional status.

2. Serum Prealbumin

Prealbumin is a glycoprotein which is synthesized in the liver. Its molecular weight has been reported as 73,000 (Oppenheimer et al., 1965), 50,000 (Raz and Goodman, 1969) or 60,000 (Harris and Kohn, 1974). Its name was derived from the fact that it migrates ahead of albumin in polyacrylamide gel electrophoresis (Helen et al., 1975). Prealbumin is

active in the binding and plasma transport of L-thyroxine (Raz and Goodman, 1969). It also forms a complex with retinol-binding protein, the carrier protein for vitamin A (Kanai et al., 1968).

Various reports of normal serum concentrations in adults are shown in Table 1.

Table 1. Normal Serum Prealbumin Concentrations

Concentration (mg %; mean ± SD)	Subjects	Reference
27	normal adults	Ingenbleek et al., 1972
28.4 ± 5.2	normal adults	Young and Hill, 1978
10 - 40	normal adults	Harris and Kohn, 1974
15 - 45	normal adults	Hutchinson et al., 1980
21.5 ± 2.24	men	Stabilini et al., 1968
28 ± 6	men	Rossi et al., 1970
18.2 ± 3.57	young women	Stabilini et al., 1968
19.7 ± 4.92	postmenopausal women	Stabilini et al., 1968
22 ± 5	non-pregnant women	Rossi et al., 1970

The plasma half-life of prealbumin has been reported as 2.67 ± 0.53 days (Socolow et al., 1965) and as 1.9 days in normal subjects (Oppenheimer et al., 1965). Due to the short half-life, any change in the rate of synthesis or catabolism of prealbumin will be rapidly apparent. This observation has led in recent years to the suggestion that serum prealbumin

may be a more useful indicator of changes in protein status than is serum albumin, and a number of authors have recommended the use of prealbumin levels as an indicator of nutritional status. Ingenbleek et al. (1972) and Smith et al. (1973) reported abnormally low serum prealbumin levels in malnourished children and increased levels associated with an improved nutritional state. In the former study, mean prealbumin plasma levels doubled after one week of dietary therapy and tripled after two weeks. A recent study (Ogunshina and Hussain, 1980) found that prealbumin served to distinguish between various grades of protein-energy malnutrition, and was also useful in distinguishing children with mild protein-energy malnutrition from normal subjects, when other measurements were not sufficiently sensitive to do so. However, there was considerable individual overlapping of values between the mildly malnourished group and the normal controls.

Harris and Kohn (1974) reported that in 4846 normal subjects, only 0.3% showed abnormally low serum prealbumin concentrations. Evaluation in their study was by visual inspection of an electrophoretic strip.

Shetty et al. (1979) observed that prealbumin and retinol-binding protein were very sensitive to alterations in protein and energy intake in obese subjects, while neither albumin nor transferrin responded to short-term energy or protein restriction. The authors concluded that prealbumin and retinol-binding protein may be useful both in the early diagnosis of subclinical malnutrition and as indicators of response to treatment.

Prealbumin appears to be the first plasma protein to be significantly decreased, in apparently healthy children, during borderline protein intake (Ingenbleek et al., 1975). Furthermore, children with kwashiorkor who were fed a good-quality protein diet showed a significant rise in prealbumin levels by the second day of treatment, with the increase in marasmic children occurring by the seventh day (Large et al., 1980).

As with other plasma proteins, the interpretation of prealbumin results in nutritional assessment may be complicated by the presence of other factors affecting serum levels. Since prealbumin is synthesized in the liver, it is to be expected that its concentration might be decreased in hepatic disease, as has been found in infectious hepatitis and liver cirrhosis (Helen et al., 1975). In fact, prealbumin has been recommended as a sensitive indicator of impaired hepatic function (Hallen and Laurell, 1972; Hutchinson et al., 1980). Other conditions associated with low levels of prealbumin include pregnancy (Stabilini et al., 1968; Rossi et al., 1970) and surgery (Surks and Oppenheimer, 1964; Young and Hill, 1978). Harris and Kohn (1974) found low prealbumin levels in patients suffering from hepatic disease, inflammatory disorders (including collagen disorders, bacterial infections, ulcerative colitis and Crohn's disease), malignant disease, congestive heart failure and burns. In all groups except the burn patients, approximately one-quarter to one-third of these patients had normal

serum albumin levels.

The effect of surgery on prealbumin levels was measured in six patients by Surks and Oppenheimer (1964). The mean maximal decrease in prealbumin concentration was 46% (range 44-54%), commencing on the day of surgery, with lowest levels reached 2 to 5 days postoperatively.

3. Serum Transferrin

Transferrin is a beta globulin which serves as the iron transport protein in human plasma (Awai and Brown, 1963). Its exact molecular weight is uncertain but Morgan (1974) suggests from a literature review a value of approximately 76,000 daltons, although values of 66,000 to 95,000 have been reported. The plasma half-life has been reported as 8.8 days in normal males, with a range of 8.0 to 10.4 days (Awai and Brown, 1963). This is similar to the 8.7 days calculated by Jarnum and Lassen (1961), and the 7 to 9 days observed by Morton et al. (1976) in normal subjects.

Normal serum concentrations in adults have been reported as 187-312 mg% (Tsung et al., 1975), 283 $\pm$ 41.7 mg% (Heidland and Kult, 1975), and 230-380 mg%, with the mean values for women being slightly lower than for men (Kreutzer, 1976). Rajamäki et al. (1979) found values of 200-350 (mean 270) mg% in men and 200-330 (mean 260) mg% in women.

The plasma half-life of transferrin is approximately one-half that of albumin. A study of the metabolism of these two plasma proteins has similarly shown that the relative turnover

rate of transferrin is approximately twice that of albumin (Jarnum and Lassen, 1961). Therefore, it has been suggested that this protein, like prealbumin, may be preferable to albumin as an indicator of changes in protein status. This was investigated by McFarlane et al. (1969), who concluded that serum transferrin, unlike albumin, provided a useful measurement of nutritional state in children with protein malnutrition, and that changes in transferrin levels appeared useful in assessing response to treatment, particularly for subjects with kwashiorkor. Similarly, a correlation was observed between serum transferrin levels and clinical severity of kwashiorkor in infants (Gahr et al., 1971) and in children (Reeds and Laditan, 1976). The latter also found below-normal transferrin concentrations in marginally malnourished children.

In an assessment of nutritional status in children with cancer, transferrin levels underwent a significant increase, to within the normal range, after 9 to 14 days of TPN, while notable improvement in albumin, weight and skinfold measurements were seen after 28 days of TPN (Rickard et al., 1979). Recently, several authors have recommended the use of serum transferrin together with albumin and immune competence, to assess visceral protein depletion (Dudrick et al., 1979; Kaminski and Jeejeebhoy, 1979; Blackburn and Kaminski, 1980) and as a prognostic index of postoperative morbidity and mortality (Mullen et al., 1979).

Serum transferrin concentrations can be measured directly

by radial immunodiffusion, which involves an antigen-antibody interaction as described under the method for prealbumin determination. However, an estimate of transferrin levels can be obtained from the total iron-binding capacity (TIBC), which is a measure of the total amount of iron which can be carried by transferrin in the blood. Normally, transferrin is 30-40% saturated with iron (Harper et al., 1977). Transferrin levels were derived using the following formula (Blackburn et al., 1977):

$$\text{serum transferrin (mg\%)} = [0.8 \times \text{TIBC } (\mu g\%)] - 43$$

Other formulas have also been used (Morgan, 1974; Tsung et al., 1975; Grant, 1979).

The normal TIBC is about the same in both sexes and has been reported as 300-360 µg%, with a range of 250-410 µg% depending on the procedure used (Harper et al., 1977).

Von der Heul et al. (1972) reported that actual transferrin content in sera of normal subjects is about 20% lower than that calculated from the TIBC. On the other hand, fairly good correlations were found between serum transferrin and TIBC by Rajamäki et al. (1979) and Forster et al. (1979). A review by Morgan (1974) concluded that there is close agreement by the two methods in both normal and iron-deficient subjects, although actual transferrin levels were lower than the calculated values in patients with kwashiorkor, liver cirrhosis or iron overload.

The determination of transferrin levels by radial immunodiffusion (Tsung et al., 1975; Rajamäki et al., 1979)

is simpler than use of the TIBC, more direct and less susceptible to analytical problems such as lack of specificity. However, it is more expensive, requires more time (up to 48 hours of incubation) to obtain accurate results, and is not routinely available at this hospital. Since determination of TIBC is readily available, this method was chosen for the study.

Besides protein deficiency, a number of conditions are associated with altered transferrin and TIBC levels. Decreased levels of transferrin and/or TIBC have been reported in hepatic disease, nephrotic syndrome (Ritchie, 1979), chronic infection, pernicious anemia (Harper et al., 1977) and protein-losing enteropathy (Beutler, 1980) as well as in malignancy, chronic inflammatory conditions and iron overload (Woo et al., 1979). In addition, TIBC is reported to decrease with age, with values of around 250 µg% in subjects over 70 years compared to values of 300 to 360 µg% in healthy younger adults (Woo et al., 1979). Iron deficiency is the most common cause of elevated serum transferrin. However, increased levels have also been associated with use of oral contraceptives (Ritchie, 1979), the third trimester of pregnancy and with hypoxia (Beutler, 1980).

4. Creatinine and Creatinine-height Index

Creatinine is a metabolic by-product of the irreversible dephosphorylation of creatine phosphate (Kaplan and Szabo, 1979). Creatine is located mainly in the muscle cells, and

the body content of creatine is proportional to the muscle mass; consequently, creatinine production is also directly related to muscle mass (Woo et al., 1979). Creatinine is not reutilized in the body but is excreted in the urine. Normal creatinine excretions have been reported as 21-26 mg/kg/24 hours for males, and 16-22 mg/kg/24 hours for females (Woo et al., 1979). Normal creatinine values are dependent on normal renal function.

Daily creatinine excretion is considered to be reasonably constant for a given individual; however, problems of variability are common and will be discussed later. Serum concentrations of creatinine are relatively constant, assuming normal renal function, and need not be considered further.

Creatinine excretion has been used for many years as a measurement of relative muscle mass (Arroyave and Wilson, 1961). Consequently, it was suggested that creatinine excretion might also help to evaluate the response to protein intake in malnourished subjects. One study found that 24-hour creatinine excretion was markedly decreased in acutely marasmic children and gradually increased towards normal values with recovery (McLaren et al., 1970). Barac-Nieto et al. (1978) observed that daily creatinine excretion was progressively lower in severely malnourished than in intermediate or in mildly malnourished subjects. Schiller et al. (1979) state that creatinine excretion "is elevated in the early stages of muscle wasting diseases and falls below normal levels when the musculature has become atrophic".

Dudrick et al. (1979) comment that in patients with chronic wasting disease, unlike in normal adults, creatinine excretion is reduced and "no longer correlates well with weight". Therefore, a more useful parameter than the usual measurement of daily creatinine excretion per kilogram of body weight appears to be the creatinine-height index (CHI). The CHI is defined as the 24-hour creatinine excretion of the subject divided by the 24-hour creatinine excretion of a normal subject of the same height (Viteri and Alvarado, 1970). These authors found, in protein-calorie malnourished children, CHI's ranging from 0.25 to 0.85, with the lower values occurring in severely malnourished, edematous subjects. Following treatment, mean values reached the normal range in the fully recovered group. The authors concluded that the CHI, if properly obtained, could serve to indicate the degree of protein depletion and repletion in hospitalized children.

Bistrian et al. (1975a) observed that the initial mean CHI for malnourished surgical patients ranged from 0.36 to 0.64, and was approximately one-half that of the controls. The CHI appeared to be a more sensitive indicator of malnutrition than serum albumin, nitrogen balance or weight for height (the ideal weight of a normal subject of a given height). Fluid retention, which altered weight, did not affect an abnormally low CHI. Similar results were obtained by Gross et al. (1976) in a group of surgical patients, and Kaminski et al. (1977) recommended the CHI as a valuable addition to a nutritional assessment profile. Schiller et al.

(1979) found that CHI's were "diminished in malnourished patients, normal in well nourished patients and increased in those with strong catabolic stresses".

A major problem in evaluations based on excretion of creatinine is variability. Not only are there often wide variations between subjects of the same sex, weight and height, but also there are variations within the same subject. The extent of this individual day-to-day variability in creatinine excretion has been reported as 10 to 25% in normal males (Albanese and Wangerin, 1944). Chattaway et al. (1969) found daily creatinine excretion to be reasonably constant in some subjects; however, others showed "considerable variation". These authors also observed a correlation between 24-hour urine volume and creatinine output in some subjects but not in others. Rich et al. (1977) found that marked day-to-day variations in creatinine excretion affected the usefulness of the CHI.

Creatinine excretion is known to diminish with age. Rowe et al. (1976) found a progressive linear decline in creatinine clearance from age 30 to about age 65, after which the rate of decline increased. Although the serum creatinine levels also increased somewhat in the older subjects, values remained within the normal range. It appears, therefore, that there is an actual age-related decline in creatinine excretion, which is "probably secondary to declining muscle mass" (Rowe et al., 1976). Using the data from the above study and a variation of the definition of CHI, Driver and McAlevy (1980)

estimated the decline in CHI with age as 20% by age 65 to 74 years and almost 30% by age 75 to 84 years.

Diet must be considered in creatinine measurements, since creatinine excretion is increased on a high meat intake (Chattaway et al., 1969). However, most patients on TPN receive little or no oral intake, and very few receive meat products.

One factor which has been associated with increased creatinine excretion is increased muscular exercise (Srivastava et al., 1967; Kaminski and Ruggiero, 1979). Furthermore, fluctuations in creatinine excretion have been observed during periods of altered emotional stress (Schottstaedt et al., 1956). Chattaway et al. (1969) also found lowered creatinine excretion during the depressed state in manic-depressive patients, but commented that this might be accounted for by diminished physical activity.

5. Zinc

Zinc is a micronutrient which is involved in a large number of essential functions, primarily through incorporation into enzymes. These zinc metalloenzymes participate in a wide range of metabolic processes, including synthesis or degradation of carbohydrates, lipids, proteins and nucleic acids (Riordan and Vallee, 1976).

Some reports of normal serum or plasma levels in adults are shown in Table 2. Serum and plasma levels appear similar, although according to Foley et al. (1968), the zinc content

Table 2. Normal Zinc Concentrations in Adults

In Serum	
Concentration (μg/ml; mean ± SD)	Reference
1.15 (range .89 - 1.43)	Henzel et al., 1970
.96 (range .90 - 1.18)	Falchuk, 1977
.96	Lindeman et al., 1977
.97 ± .13	Sullivan et al., 1979

In Plasma		
Concentration (μg/ml; mean ± SD)	Subjects	Reference
.98	men	Dawson & Walker, 1969
.96 ± .13	men	Halsted & Smith, 1970
.96	women	Dawson & Walker, 1969
.97 ± .11	women	Halsted & Smith, 1970

Table 3. Normal 24-hour Urine Zinc Excretion

24-hour excretion (in μg)	Reference
492 (range 286 - 642)	Henzel et al., 1970
496 ± 197 (SD)	Cuthbertson et al., 1972
484 (7.4 ± 2.9 μmoles)	Mills and Fell, 1979
641 ± 60 (SEM)	Lindeman et al., 1977
585 (range 263-817) in men	Dawson and Walker, 1969
414 (range 276-702) in women	Dawson and Walker, 1969

of serum is about 16% higher than that of plasma. Zinc levels in adults do not change significantly with age (Davies et al., 1968; Halsted and Smith, 1970).

Under normal circumstances, most of the dietary intake of zinc is excreted in the feces, while the urinary excretion is relatively small (Spencer et al., 1976). In a review of the literature, Halsted et al. (1974) reported that in normal subjects, means for urinary excretion ranged from 400 to 600 µg per 24 hours. Other reported values are shown in Table 3. Lindeman et al. (1977) found no correlation between serum zinc concentration and urine zinc excretion in patients or in control subjects.

During the 1960's, several studies of children with kwashiorkor revealed abnormally low serum or plasma zinc levels, which increased when the clinical state improved (Hambidge and Walravens, 1976). More recently, mean plasma zinc levels of malnourished infants, especially those with kwashiorkor, were found to be significantly lower than those of normal controls (Khalil et al., 1974; Golden and Golden, 1979). Mean plasma levels following treatment were similar to those of the controls (Khalil et al., 1974).

Less work has been done regarding urine zinc levels in malnourished subjects; however, there is evidence that the measurement may be useful. Fell et al. (1973), using ^{65}Zn in two patients, found that urine zinc excretion increased markedly following surgery, peaking at eight to ten days postoperatively. The authors suggest that the process of

skeletal muscle catabolism following injury includes the breakdown of zinc metalloproteins, leading to increased urine zinc excretion. Therefore, urine zinc might serve as a useful supplementary indicator of the catabolic state.

Since many patients requiring TPN are severely catabolic, high urine zinc levels might be expected. Kay et al. (1976) report that such patients frequently show daily urinary losses of 4000 to 8000 µg, with a loss of 22,600 µg recorded in one patient. These authors concluded that since the urine zinc excretion decreases when the catabolic state is corrected, zincuria appears to be "a useful index of metabolic control". Severe plasma zinc depletion was not observed in these patients during catabolism but was seen during a subsequent phase of anabolism and weight gain. This depletion appeared to be due to a rapid tissue demand for zinc during anabolism (Kay et al., 1976). It appears from this report that urine zinc excretion is more reliable than plasma zinc as an indicator of metabolic status.

Situations of metabolic stress which have been associated with increased urine zinc excretion include acute starvation (Spencer et al., 1976), major surgery, burns (Henzel et al., 1970), active ulcerative colitis (Mills and Fell, 1979) and acute viral hepatitis (Henkin and Smith, 1972). Increased excretion has also been observed in cirrhosis (Prasad et al., 1965), nephrotic syndrome (Lindeman et al., 1977), sickle cell anemia (Prasad et al., 1975), diabetes (Pidduck et al., 1970) and some forms of porphyria (Roman, 1969).

Many disease conditions have been associated with below-normal serum zinc levels. Sullivan et al. (1979) found mean values to be significantly lower than those of controls in cirrhosis, malignancy, infection, chronic obstructive pulmonary disease, hypertension, arthritis, diabetes, pancreatitis, arteriosclerotic heart disease, ulcer and psychoses. Other conditions associated with low serum or plasma zinc levels include nephrotic syndrome (Lindeman et al., 1977), Crohn's disease (Main et al., 1980), acute myocardial infarction (Lindeman et al., 1976; Lekakis and Kalofoutis, 1980), pregnancy, use of oral contraceptives (Halsted et al., 1968), active tuberculosis, uremia (Halsted and Smith, 1970), acute viral hepatitis (Henkin and Smith, 1972) and porphyria (Roman, 1969). However, Davies et al. (1968) did not observe abnormal plasma zinc levels in diabetes or congestive heart failure, or following myocardial infarction. Falchuk et al. (1977) found that serum zinc levels of almost all their acutely ill patients, irrespective of the disease, were lower than those of convalescent patients, whose values did not differ significantly from controls. A potential major problem with use of zinc levels in nutritional assessment appears to be the many conditions which could alter zinc concentration.

6. Ribonuclease

The ribonucleases, which catalyze the hydrolysis of ribonucleic acid, occur widely in human tissues as well as in serum and urine. It has been suggested that human serum ribonuclease (RNase) originates in the pancreas, from where

it enters the blood and is then excreted in the urine (Reddi, 1978); however, not all authors are in agreement (Houck and Berman, 1958). There are believed to be several ribonucleases, some acidic and some alkaline, present in human urine (Reddi, 1977). The present discussion will deal with serum and urine alkaline RNase.

Estimations of normal levels of RNase vary according to the method of assay. Values calculated using serum or plasma of normal subjects include 0.38 $\pm$ 0.04 µg/ml (Houck and Berman, 1958), 0.209 $\pm$ 0.023 µg/ml, with a range of 0.180 - 0.240 µg/ml (Levy and Rottino, 1960) and 0.079 $\pm$ 0.018 µg/ml (Shenkin et al., 1976). No significant difference has been observed between serum and plasma RNase activity (Houck and Berman, 1958; Albanese et al., 1971). Levy and Rottino (1960) in five normal subjects found urine RNase levels ranging from 0.532 to 0.770 µg/ml. Other authors have expressed RNase as arbitrary units of activity (Sigulem et al., 1973; Sheid et al., 1977; Warshaw et al., 1980).

Serum RNase levels in normal subjects have been reported by some authors to increase significantly with age (Sved et al., 1967; Albanese et al., 1971), although more recent studies (Sheid et al., 1977; Maor et al., 1978) found no significant difference. Maor et al. (1978) also reported no significant difference according to sex. Serum levels appear to be decreased in pregnancy (Sved et al., 1967).

Sigulem et al. (1973) found that malnourished young children showed elevated plasma alkaline RNase levels, while

the severely marasmic children also had low ratios of urine RNase to plasma RNase. The low ratios were due mainly to a decreased concentration of urine RNase. In subjects with less severe degrees of malnutrition, this ratio was in the normal range. In severely marasmic children, following two weeks of dietary treatment the plasma RNase levels fell to near normal values. Prabhavathi et al. (1977), however, concluded that plasma RNase activity is elevated only in severe forms of protein-calorie malnutrition, and is not of use in detecting mild or moderate malnutrition.

Shenkin et al. (1976) suggest that the assessment of nutritional status is "one of the most interesting potential applications for plasma RNase assays". However, they caution that plasma RNase levels are dependent upon renal function, making it difficult to interpret elevated levels in patients with renal impairment.

A further difficulty is that several disease states besides malnutrition have been associated with altered RNase activity. Serum and plasma RNase levels are elevated in renal insufficiency (Shenkin et al., 1976; Karpetsky et al., 1977; Reddi, 1978). In fact, RNase has been suggested as an indicator in the assessment of renal function (Reddi, 1978). Elevated serum or plasma levels have also been reported in a variety of malignant diseases, including ovarian cancer (Sheid et al., 1977), lung cancer (Maor et al., 1978), pancreatic cancer (Warshaw et al., 1980), Hodgkin's disease and leukemia (Kottel et al., 1978). In addition, increased serum levels

have been seen in cirrhosis, fractures (Levy and Rottino, 1960), acute necrotizing pancreatitis (Warshaw et al., 1980) and sickle cell anemia (Prasad et al., 1975). On the other hand, Houck and Berman (1958) obtained serum RNase levels within normal limits for patients with carcinoma and pancreatic diseases, but reported elevated levels in congestive heart failure and myocardial infarction. Above-normal serum RNase levels have also been seen in long-term smokers compared to healthy non-smokers (Maor et al., 1978).

Very few measurements of alkaline RNase in human urine are available, so it is not known at present what disease conditions might affect RNase excretion.

A problem which has limited the wide-spread investigation of RNase as an indicator in disease states is the "poor analytical precision" of the assay (Shenkin et al., 1976). The range of linearity is limited (Kottel et al., 1978), and RNase is hypersensitive to environmental conditions such as minor temperature fluctuations (Sheid et al., 1977). Activity is strongly influenced by pH and substrate concentration (Sved et al., 1967). Levy and Rottino (1960) warn that trace amounts of detergent can inactivate serum RNase. Problems have also been reported due to contaminants present in the substrate or in plasma (Sheid et al., 1977).

Most researchers have used assay methods modified from Roth (1967). Recently, newer methods have been introduced which may improve the sensitivity and accuracy of the RNase

assay (Reddi, 1978; Kottel et al., 1978). However, the present study was performed using a modification of the earlier method.

7. 3-Methylhistidine

3-methylhistidine (N^{τ}-methylhistidine; 3-MEH) is an amino acid produced by the methylation of histidine residues in the peptide chains of actin and myosin, the contractile proteins of skeletal muscle (Asatoor and Armstrong, 1967; Johnson et al., 1967; Young and Munro, 1978). During muscle catabolism, 3-MEH is released, but unlike other amino acids is not reutilized for protein synthesis (Young et al., 1972). Instead, it is quantitatively excreted in the urine (Young et al., 1972; Long et al., 1975). Therefore, it appears that the urinary excretion of 3-MEH may serve as a measure of muscle protein catabolism (Long et al., 1975; Young and Munro, 1978).

Long et al. (1975), using (^{14}C) 3-methylhistidine in three patients, found that 75% of the administered dose was excreted in 24 hours and 95% in 48 hours.

In normal males, 24-hour urinary excretion of 3-MEH has been reported as 203 $\pm$ 10 μmoles/day (mean $\pm$ SEM) (Wannemacher et al., 1975), 211 $\pm$ 18 μmoles/day (mean $\pm$ SE) (Bilmazes et al., 1978) and 225 $\pm$ 8 μmoles/day (mean $\pm$ SE) (Lukaski and Mendez, 1980). Neuhäuser and Fürst (1979) reported mean 24-hour urinary excretion of 299.4 $\pm$ 23.8 μmoles in females and 545.5 $\pm$ 35.2 μmoles in males. Munro and Young (1978) observed that daily 3-MEH excretion was greater in the young than in the elderly, both as total excretion and as excretion per

kilogram of body weight. The decrease is apparently due to the loss of muscle mass with age.

Young et al. (1973) found that in three obese subjects undergoing prolonged starvation, urinary excretion of 3-MEH decreased progressively during the starvation period to about 60% of the reference value by the twentieth day. Children with protein-calorie malnutrition were found to have below-normal 24-hour urine 3-MEH excretion, which increased toward normal values following treatment (Rao and Nagabhushan, 1973). Urinary excretion of 3-MEH did not appear promising for the early detection of protein-energy malnutrition, but the ratio of 3-MEH to urinary creatinine might serve "as an indicator of the state of muscle protein turnover" (Nagabhushan and Rao, 1978).

In six patients receiving parenteral nutrition, excretion of 3-MEH increased gradually over the course of treatment, almost doubling the mean initial value by the third week although remaining below normal levels (Kim et al., 1979). On the other hand, Long et al. (1977) reported a continuing decrease in 3-MEH excretion in a severely debilitated patient who was undergoing nutritional replenishment. The authors suggested that an adaptive decrease in muscle protein breakdown occurs during severe malnutrition.

The excretion of 3-MEH is markedly increased by consumption of meat (Bilmazes et al., 1978). This is not generally a problem in patients receiving parenteral nutrition, since very few of them are able to ingest meat products. Valid urinary

levels of endogenous 3-MEH can be obtained by the third day after stopping meat intake (Tomas et al., 1979).

An increase in 24-hour urine 3-MEH excretion has been observed in several stress situations including surgery (Gross, Holbrook and Irving, 1978; Neuhäuser et al., 1980), fever (Long et al., 1977; Wannemacher et al., 1975), some but not all episodes of infection (Milewski et al., 1980) and some cases of long bone fractures (Williamson et al., 1977). Acute starvation, unlike chronic starvation, is associated with an increase in protein catabolism (Seashore et al., 1980), and it seems likely that 3-MEH excretion would also increase in this condition.

The urinary excretion of 3-MEH appears to be a promising indicator of nutritional status and should be further investigated. Unfortunately, results are not available from this study since a practical assay method was not developed. The usual method of assay is by an amino acid analyzer, which was not available. Several other methods, based on reports in the literature, were attempted. These were unsatisfactory due either to lack of sensitivity or to lack of the necessary equipment.

8. Nitrogen Balance

Nitrogen balance can be defined as a patient's daily nitrogen intake minus total daily nitrogen output. Nitrogen balance will be monitored in the present study to determine whether a patient is in a state of anabolism or catabolism

during the course of TPN.

In an already well-nourished patient who is to be maintained in his or her present state, a daily nitrogen balance of +1.0 to -1.0 grams is appropriate. However, a depleted patient should receive sufficient nourishment to produce a nitrogen balance of +4.0 to +6.0 grams per day (Kaminski and Ruggiero, 1979).

Nitrogen balance measures nitrogen utilization, not the actual nutritional state. A patient in consistent nitrogen equilibrium may be either well-nourished and not in need of further nitrogen retention, or depleted and receiving or utilizing insufficient nutrients to achieve a positive balance.

About 90% of daily nitrogen loss is through the urine. Of this, about 70% is excreted as urea nitrogen (Kaminski, 1976). An approximation of daily nitrogen loss can be obtained from the urinary urea nitrogen (UUN) plus a constant of 2 grams to represent non-UUN losses (Kaminski, 1976). A comparison of this method with the traditional macro-Kjeldahl technique showed that the former method provides a reasonable estimate, although it tends to slightly overestimate the nitrogen loss (Kaminski, 1976).

In view of the gastrointestinal complications seen in many TPN patients, the balances obtained should be considered as approximations of the actual state.

9. Skin Surface Lipids

The skin has been seen to exhibit changes during malnutrition. Protein depletion has been reflected in progressive

changes of the epidermal layer in a group of fasting obese subjects (Alvarez et al., 1975). Downing et al. (1972) collected lipids from the surface of the forehead in adults. From this area, about 95% of the lipids present are derived from the sebaceous glands. The composition of this sebum shows little change in normal subjects. However, the proportion of squalene present was seen to increase markedly in obese subjects undergoing prolonged fasting (Pochi et al., 1970) and also in normal nonobese fasting subjects (Downing et al., 1972). In malnourished children, however, the proportion of squalene was lower than in normal controls, possibly due to the fact that secretion of skin surface lipids in children differs from that of adults (Strauss et al., 1978).

Green et al. (1970) found that sebum samples could conveniently be collected from the forehead, which yields 150 - 300 μg of sebum per cm^2. The collection method is simply to wipe a polyurethane sponge over a measured area of the skin surface. Such a method is non-invasive, easy to perform and causes no discomfort to the patient.

A nutritional marker based on the above procedure would be very welcome in the clinical setting, especially for use with severely ill or debilitated patients. Unfortunately, the advantage of the simple sample collection is negated by the difficulty of assay. A variety of methods have been proposed for the analysis of sebum lipids, but most of these would not provide the squalene/total lipids ratio. The method of Downing (1968) does provide this ratio, but although this

method could readily be carried out in a research setting, it is unlikely that it could be attempted routinely in a hospital environment. Due to the lack of a suitable method for hospital use, the procedure was removed from the study.

10. Ethanol Dilution

The calculation of total body water (TBW) is useful for evaluating observed changes in body weight. However, the methods commonly employed for this measurement - such as the use of deuterium oxide, tritiated water or antipyrine - are not always practical in a clinical setting. Pawan and Hoult (1963) recommended the use of blood ethanol levels to calculate TBW. More recently, Loeppky et al. (1977) found that TBW could be determined by using ethanol levels in expired air to provide an estimate of blood ethanol levels. The method involved serial exhalations of air into an analyzer every 15-30 minutes following ingestion of a given dose of ethanol per kilogram of body weight. The readings obtained were used to estimate the initial ethanol concentration (assuming instantaneous equilibration). This concentration was used to calculate the TBW, using the formula:

$$TBW = \frac{\text{quantity of ethanol ingested (in gm.) x 0.8}}{\text{ethanol concentration in whole blood (in gm/litre)}}$$

The TBW can be used to estimate the lean body mass.

For the present study, it was intended to measure ethanol dilution by means of a breathalyzer of the type used by local

law-enforcement agencies. However, several complications arose. The major limitation was that very few TPN patients would be capable of taking part in the test, which would involve the ingestion of ethanol and the provision of repeated, completely exhaled breath samples. In addition, the required measurement period of two to five hours would be inconvenient and impractical for the patient as well as the researcher. Furthermore, there was some doubt concerning the use of the TBW to calculate the patient's lean body mass. Although this calculation can be performed for healthy, euhydrated subjects, it is doubtful whether the same ratios would apply to the TPN patients, particularly those who were dehydrated or edematous (Loeppky et al., 1977).

Due to the many difficulties involved in the procedure, it was withdrawn from the study.

D. Immune Competence

In patients with protein-calorie malnutrition, an increased incidence of infectious diseases has been observed. It has been suggested that there is an increased susceptibility to infection, occurring due to some defect in the production of a normal immune response against the invading organism (Munson et al., 1974; Jagadeesan and Reddy, 1979; Kielmann and Curcio, 1979).

There are two basic types of immunological response: cell-mediated immunity (CMI) and humoral (antibody-mediated) immunity. CMI is an important defence mechanism against both

microorganisms and endogenous neoplastic growth (Waldman and Ganguly, 1979). In addition, CMI mediates delayed cutaneous hypersensitivity (DCH) reactions, and has a role in allograft rejection and in regulation of the autoimmune system (Waldman and Ganguly, 1979). The CMI response is mediated by macrophages and by T lymphocytes, which originate in the thymus (Bowry, 1977).

Humoral immunity is mediated by the B lymphocytes. Stimulation of B lymphocytes by an antigen results in formation of plasma cells which secrete antibodies (Bowry, 1977). Humoral immunity is involved in prophylaxis of certain viral diseases and of infections involving production of toxins (Bowry, 1977). The humoral immune response involves the immunoglobulins (antibodies) and the complement system. Figure 1 outlines the main steps involved in cell-mediated and humoral immunity.

There has been controversy over whether malnutrition has a direct effect on immune function and, if so, what part of the system is affected. Most investigators agree that CMI is depressed in protein-calorie malnutrition; however, it has not been definitely established whether or not humoral immunocompetence is affected (Munson et al., 1974). One possible explanation for the conflicting results is that the response of the immune system may be affected by the degree of malnutrition as well as by the presence of an underlying disease state (Munson et al., 1974).

Immune competence can be assessed either by measuring

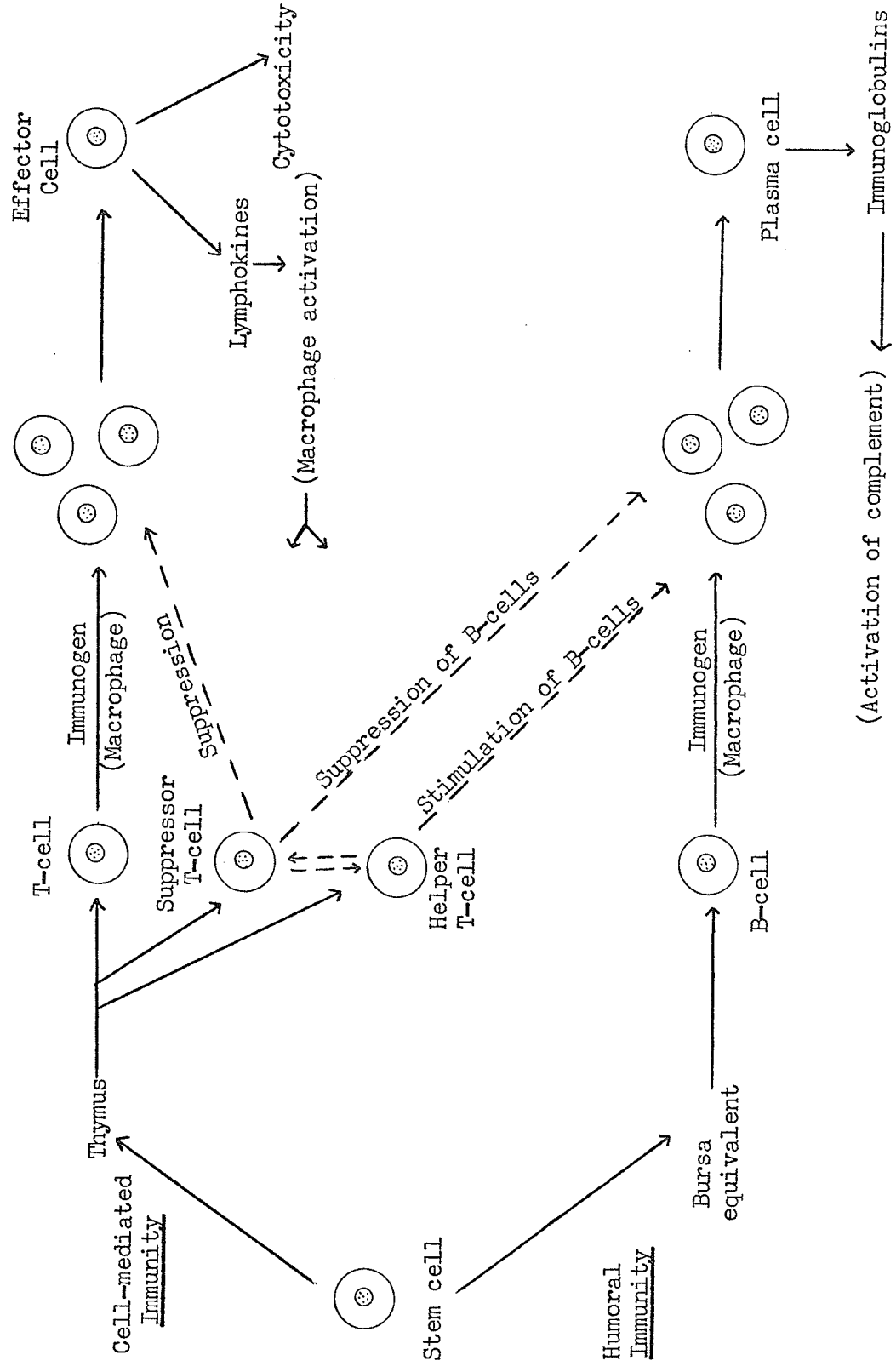

Figure 1. Cell-mediated and Humoral Immunity

individual components of the immune system or by observing the final immune response. The latter generally involves skin testing for DCH. Individual components which can be measured include immunoglobulin levels, total lymphocyte count, and percentage of T cells compared to percentage of B cells. DCH provides the most reliable evaluation of overall immune function but does not identify the site of dysfunction.

One aim of the present study was to determine whether malnourished patients are immunologically competent, and, if not, whether nutritional repletion would improve immune function.

1. Immunoglobulins

The immunoglobulins - IgG, IgM, IgA, IgD and IgE - are components of the humoral immune system. They are secreted by plasma cells in response to the presence of an antigen (Stiehm, 1977; Waldmann et al., 1976). They have a wide range of antibody functions. Immunoglobulin production can be augmented or suppressed by the influence of T-lymphocytes (Stiehm, 1977).

The most common form is IgG, which comprises about 75% of the serum immunoglobulins, and is important in defence against diffusing toxins and micro-organisms (Bowry, 1977). Both IgG and IgM are capable of activating the complement system (Bowry, 1977). IgA, whose function in serum is uncertain, is also included in routine determinations of serum immunoglobulins. Normal serum concentrations, in mg%, as determined

by the Immunology Department, St. Boniface General Hospital are: for IgG, 800-1800 (mean 1253); for IgA, 113-563 (mean 263); and for IgM, 54-222 (mean 112) in males and 62-250 (mean 143) in females.

In most studies of malnourished children, serum immunoglobulin levels are either normal or elevated (McFarlane et al., 1970; Neumann et al., 1975; Kielmann et al., 1976; Rafii et al., 1977; Suskind et al., 1977; Chandra, 1979). The increased levels are not believed to be directly related to malnutrition. Instead, they have been generally attributed to repeated exposure to infection, a common complication in malnourished children (Suskind et al., 1977; Chandra, 1979). Other hypotheses have also been proposed (Chandra, 1979).

Reports in malnourished adults are conflicting. In one group, serum levels of IgG, IgM and IgA did not differ significantly from controls, either before or after treatment (Law et al., 1973). Another study showed abnormally high IgG levels both before and after treatment together with slightly above-normal levels of IgA and IgM (Munson et al., 1974).

Although elevated immunoglobulin levels do not appear indicative of malnutrition, they may signify the presence of infection. Since infection may affect some of the nutritional parameters employed in this study, it was decided to measure serum IgG, IgM and IgA levels to permit more reliable interpretation of these nutritional parameters.

2. Complement

The complement system is an essential part of the normal humoral immune response. A key component of the system is complement C3, which may be activated by either the classical or the alternative pathway (Whicher, 1978; Johnston, 1977). Activation of the pathway provokes an inflammatory response and leads to phagocytosis and lysis of invading pathogens such as bacteria, fungi and viruses (Bowry, 1977).

Since malnutrition is often associated with increased susceptibility to infection, the state of the complement system has been investigated in malnourished children. The majority of authors have reported abnormally low levels of serum complement (Sirisinha et al., 1977; Jagadeesan and Reddy, 1979; Kielman et al., 1976) and of component C3 (Sirisinha et al., 1973; Haller et al., 1978; Kielmann and Curcio, 1979; Jagadeesan and Reddy, 1979). It is suggested that the decreased levels are due to both a decreased synthesis and an increased catabolism of C3 (Sirisinha et al., 1977; Haller et al., 1978). Furthermore, levels of complement components in malnourished children have been observed to increase to or above control levels following dietary treatment (Sirisinha et al., 1977; Haller et al., 1978).

Complement C4, a precursor of C3 in the classical pathway, does not appear to be decreased in malnourished subjects (Sirisinha et al., 1973; Haller et al., 1978; Neumann et al., 1977).

Normal serum levels of C3 in adults have been reported as

90-190 mg% (Immunology Laboratory, St. Boniface General Hospital), 122.9 ± 22.9 mg% (mean ± S.D.) (Young and Hill, 1978) and 146.2 ± 18.5 mg% (Heidland and Kult, 1975). Reports of normal serum values for C4 include 20-50 mg% (Immunology Laboratory, St. Boniface General Hospital) and 36.2 ± 8.0 mg% (Heidland and Kult, 1975). A study of preoperative surgical patients found very few abnormal C3 levels compared to anthropometric and visceral protein abnormalities (Mullen et al., 1979).

There are two major factors which may limit the use of complement components in the assessment of malnutrition. In the first place, several researchers have observed that complement levels appear to be markedly altered only in severe cases of protein-energy malnutrition (Neumann et al., 1977; Miller, 1978; Jagadeesan and Reddy, 1979), and particularly in kwashiorkor (Sirisinha, 1977; Neumann et al., 1977). Although Kielmann et al. (1976) reported significant reductions in C3 levels in young children with borderline malnutrition, other possible contributing factors were not definitely ruled out.

A second limitation is the effect of episodes of infection on serum complement levels. According to Chandra and Scrimshaw (1980), serum C3 levels are increased during infection in well-nourished subjects, but in malnutrition, infection further lowers the already decreased level of C3. Sirisinha et al. (1977) observed that complement levels were generally lower in malnourished subjects with severe infections

than in those with mild infections.

Significantly increased levels of plasma C3 have been observed post-operatively (Young and Hill, 1978) and in many inflammatory conditions including mildly active rheumatoid arthritis (Whicher, 1978). Decreased levels of C3 have been seen in anemia (Jagadeesan and Reddy, 1979), certain infectious diseases, glomerulonephritis and in immune-complex diseases such as systemic lupus erythematosus and severe forms of rheumatoid arthritis (Whicher, 1978).

3. T and B Lymphocytes

Counts of total lymphocytes in the peripheral blood can be routinely obtained. However, these counts can be depressed by several factors, as cited in Miller (1978). Enumeration of T and B lymphocytes may provide a better indication of immune competence, although absolute numbers do not indicate activity or function.

The percentages of T and B cells in healthy, white adults have been reported for T cells as 71.3 $\pm$ 6.8% (mean $\pm$ SD) and for B cells as 7.8 $\pm$ 2.2% of total lymphocytes (Glassman and Bennett, 1980). A decrease in the number and/or proportion of rosette-forming T lymphocytes (see Method) in the peripheral blood during malnutrition has been reported in children (Chandra, 1974; Reddy et al., 1976; Neumann et al., 1977a; Rafii et al., 1977; Kulapongs et al., 1977; Salimonu and Osunkoya, 1980). These values returned to normal levels during nutritional repletion (Chandra, 1974; Neuman et al.,

1977a; Kulapongs, 1977). Rafii et al. (1977) found no significant change in the percentage of B cells in malnourished children.

A limitation of the absolute T lymphocyte count is that it does not distinguish between helper T and suppressor T cells, which have opposing effects on antibody formation. Consequently, a T cell count could be misleading in some patients (Miller, 1978).

The numbers or percentages of T and B lymphocytes may be useful in evaluating any abnormalities found in other immune function tests, such as delayed cutaneous hypersensitivity.

4. Lymphocyte Transformation

Lymphocyte transformation is an in vitro test to assess immunological competence by evaluating the ability of the lymphocytes to divide when stimulated with a mitogen. Commonly employed mitogens include phytohemagglutinin (PHA) and Concanavalin A (Con A), which primarily stimulate T lymphocytes (Oppenheim and Schecter, 1976). The response is assessed by measuring incorporation of radioisotope precursors such as tritiated thymidine by the mitogen-stimulated cells. This test has the advantage of being able to detect some dysfunctions in cellular activity. However, although depressed proliferation of cells indicates an immune dysfunction, a normal mitogen response does not necessarily indicate normal immunocompetence (Miller, 1978).

Although normal responsiveness to PHA has been reported

using lymphocytes from marasmic children (Schlesinger et al., 1977), other authors have found responsiveness to be significantly lowered and/or in the low normal range in malnourished children (Chandra, 1974; Reddy et al., 1976; Neumann et al., 1977a; Kulapongs, 1977). Law et al. (1973) reported a significant improvement in response in malnourished adults treated with parenteral nutrition.

Many of the conflicting results reported may be due to difficulties with the assay, as discussed by Miller (1978). An additional problem with this procedure is the high cost of materials. The lymphocyte transformation tests could not be performed in the present study due to lack of funds.

5. Delayed Cutaneous Hypersensitivity

Delayed cutaneous hypersensitivity (DCH) is generally considered a reflection of the state of cell-mediated immunity. A test for DCH measures the end result of a complex series of interactions (Miller, 1978).

Skin testing for DCH may measure either a primary response to an antigen to which the patient has not previously been exposed, or a secondary recall response to an antigen previously encountered. The present study will be concerned only with testing for the secondary response. Such testing involves use of a battery of antigens which the subject might reasonably be expected to have encountered (Ruggiero et al., n.d.). A definite positive reaction to any of these antigens indicates immune competence, while a subject who fails to

respond to any of them is considered anergic.

An impaired response to DCH testing has been observed in malnourished children, particularly those with more severe conditions (Neumann, 1977a; Kielmann et al., 1976), and improved nutritional status was associated with an increased proportion of positive responses (Kielmann et al., 1976). A somewhat impaired response has also been reported in malnourished adults (Law et al., 1973; Bistrian et al., 1975b). Spanier et al. (1976) found a good correlation between anergy and malnutrition in patients receiving TPN.

Due to the complexity of the immune response, a wide range of factors can interfere with production of a positive skin test. Besides malnutrition, a negative response has been associated with trauma, shock, infection, sepsis and old age (greater than 80 years) (Pietsch et al., 1977; Johnson et al., 1979) as well as with malignancy, anemia, leukocytosis or immunosuppressive medications (Palmer and Reed, 1974). The latter observed that age (greater than 70 years) was the variable most frequently associated with loss of reactivity. Surgery is associated with a high incidence of anergy (Slade et al., 1975; Pietsch et al., 1977). The former investigators reported an impaired skin response postoperatively, with a return to the preoperative response after 10-12 days for SK/SD while the response to candida was still impaired after two weeks. However, Pietsch et al. (1977a) suggested that impaired DCH postoperatively is due to "abnormalities in host defense and not a normal response to surgery", and that

healthy individuals undergoing surgery need not develop anergy.

Irrespective of the cause of anergy, patients who remained normal (immune competent) or who converted from anergic to normal during their hospital stay, were found to have a substantially lower incidence of sepsis and mortality than those who remained anergic or who converted from normal to anergic (Pietsch et al., 1977). Johnson et al. (1979) reported greater postoperative morbidity and mortality in anergic than in immune competent patients. Restoration of body cell mass by the use of TPN has been associated with return of a normal DCH response. However, treatment of malnutrition with TPN "does not guarantee improvement in body cell mass or restoration of cutaneous responses" (Pietsch et al., 1977).

It must be emphasized that some apparently healthy subjects will not react to the typical recall antigens, sometimes due to lack of prior exposure to those antigens. In addition, suppression of the inflammatory response can partially or completely interfere with the local cutaneous reaction (Ruggiero et al., n.d.). Therefore, a negative response to skin testing does not necessarily indicate a true state of anergy.

In a large-scale study of hospitalized patients, Palmer and Reed (1975) reported the following percentages of positive reactions: mumps 66.8%, candida 62.9%, tricophyton 62.2% and PPD 33.3%. Using a battery of the four antigens, 91% of patients reacted to at least one antigen.

Another possible source of misleading negative results is

improper technique of preparation, administration or reading. Care must be taken to follow a standard protocol.

E. Summary

The articles referred to in the preceding sections describe a wide range of methods which have shown some promise in the assessment of nutritional status. An evaluation of the available information suggests, however, that some methods will be more useful than others in the clinical setting.

The anthropometric measurements are expected to be particularly useful since they are relatively simple to perform and cause little inconvenience or discomfort to the patient. Although these measurements are less sensitive than many of the other parameters, they do appear to be dependable as a measure of change, and therefore could have a valuable role in the assessment of TPN patients.

Serum albumin is not expected to be a useful nutritional marker, except in cases of severe, long-term malnutrition. Serum prealbumin and serum transferrin, which have shorter half-lives than albumin, appear to be more readily responsive to changes in nutritional state. However, most of the literature regarding the use of prealbumin and transferrin in nutritional assessment has been performed in children with long-term malnutrition. The present study will evaluate these parameters in adults with varying degrees of malnutrition.

Daily creatinine excretion, and the creatinine-height index, have been shown to be decreased substantially in chronic

malnutrition. In addition, Schiller et al. (1979) reported increased creatinine excretion in the early stages of muscle wasting. It appears that creatinine excretion can be useful in indicating changes in nutritional status. However, individual day-to-day variability could be a problem in evaluating some patients.

Abnormally low concentrations of serum or plasma zinc have been reported in malnourished children; however, more recent work suggests that urine zinc excretion may be more useful than serum or plasma concentrations as an indicator of metabolic status. A potential difficulty in using zinc levels in nutritional assessment is the many other factors which can produce altered levels.

Recent studies suggest that serum RNase, which is elevated in malnourished children, is a promising indicator of nutritional status. Less work has been done with regard to urine RNase excretion, but this parameter deserves further attention. Little information is available in adults regarding RNase in nutritional assessment.

The urinary excretion of 3-MEH shows promise as an indicator of nutritional status. It is unfortunate that results could not be obtained for this parameter. The other procedures for which results are not available - measurement of skin surface lipids and ethanol dilution - would probably have had only limited clinical application due to the complexity of the methods and, for the latter, the problems involved in performing the test in a clinical setting.

A variety of immunological parameters are to be measured. Delayed cutaneous hypersensitivity can be a useful indicator of the state of cell-mediated immunity. However, many factors other than malnutrition can affect the response to the test antigens. Enumeration of T and B lymphocytes may be useful, in conjunction with other tests such as delayed hypersensitivity, in assessing immune function. Some potential problems with this test are the relatively large quantity of blood required, the time required and the difficulties inherent in the assay. The lymphocyte transformation, which was not performed due to lack of funds, suffers from similar limitations.

The measurement of serum immunoglobulins is not expected to provide information on nutritional status, but is included primarily to alert the researcher to the presence of infection. Measurement of serum complement may be useful in cases of severe malnutrition.

Of the tests to be performed, those most promising for nutritional assessment in a clinical setting appear to be the anthropometric measurements, prealbumin, transferrin, creatinine-height index and possibly ribonuclease.

METHODS

A. Anthropometric Measurements

1. Weight

Each patient was weighed on admission to the study and then twice weekly, or more often if ordered by the attending physician. Except as stated below, all weights included in the study were performed by the same investigator, at approximately the same time of day, and using the same scale (Healthometer, Continental Scale Corp., Bridgeview, Illinois) for all the weights on one patient. In most cases, each patient was weighed barefoot and wearing only a standard hospital smock; every patient in whom this was not practical wore the same clothing on each occasion. Each patient was asked to empty his or her urinary bladder before weighing. This was not always possible in one patient (#9). If the patient was unable to stand, a chair scale was used (Acme Chair Scale, Acme Scale Co., Oakland, California); these weights were taken by ward personnel. Weight was recorded to the nearest 0.1 kg. All measurements were entered on a data flow-sheet (Appendix IIa and IIb). The intervals at which the various measurements were taken is shown in Appendix III.

2. Height

Each patient's height was measured at the time of the first weighing, using the apparatus built onto the scale. If the patient was unable to stand, a tape measure was used. Height was recorded to the nearest centimetre.

3. Triceps Skinfold

Triceps skinfold thickness was measured using the Lange skinfold calipers (Cambridge Scientific Industries Inc., Cambridge, Maryland). These calipers meet the requirements of Jelliffe (1966) that the instrument should have a standard contact surface area of 20-40 mm^2, should read to 0.1 mm accuracy and should exert a constant pressure of 10 g/mm^2 throughout the range of skinfold thicknesses at all distances of separation of the jaws.

The skinfolds measured consist of a double layer of skin and subcutaneous fat. Since the thickness of the fat of the upper arm is not uniform, the same site must be used for all measurements (Jelliffe, 1966; Ruiz et al., 1971). The site used was midway between the tip of the acromial process of the scapula and the olecranon process of the ulna (Fig. 2).

The method of measurement was adapted from Jelliffe (1966) and Ruiz et al. (1971). The midpoint distance was read to the nearest 0.1 cm and an indelible mark was made at the time of initial measurement so that subsequent readings would be as close as possible to the original site. All readings were taken by the same investigator to minimize variations due to technique. Whenever possible, measurements were taken with the arm hanging freely at the side; however, this was not possible with totally bedridden patients. A lengthwise skinfold approximately one centimetre above the marked midpoint was grasped between thumb and forefinger and pulled outward to separate the skinfold from the underlying muscle (Fig. 3).

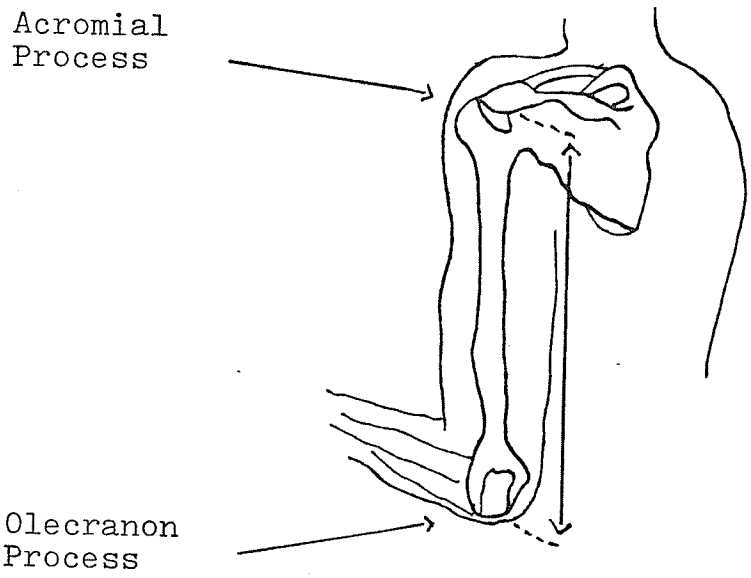

Figure 2. Assessing midpoint of the upper arm.
From: Jelliffe, D. B. The Assessment of the Nutritional Status of the Community, W.H.O., Geneva, 1966.

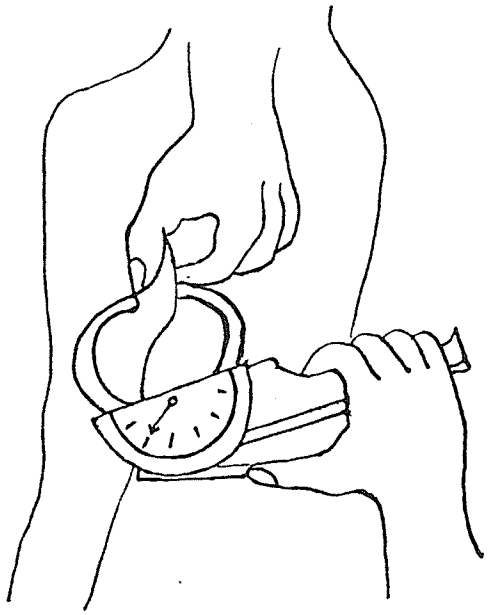

Figure 3. Measurement of triceps skinfold.
From: Grant, A. Nutritional Assessment Guidelines, Berkeley, 1979.

The jaws of the calipers were placed over the skinfold at the midpoint, the spring released and the dial read when the indicator needle had stopped moving. In patients with excessively thick skinfolds, the jaws tended to slide slowly for some seconds after the rapid initial movement. In these cases, the reading was taken when the needle had completed the initial rapid swing. Readings were recorded to the nearest 0.5 millimetre. Three readings were taken for each measurement and the results were averaged.

It has been recommended (Jelliffe and Jelliffe, 1979) that measurements should be performed on the non-dominant arm, and this was done initially unless the use of that arm was impractical. It was discovered that, since the handle of the Lange calipers bends to the right, its use is not practical on the left arm of bed-ridden patients. It was, therefore, decided midway through the study that all measurements should be performed on the right arm. Womersley and Durnin (1973, cited in Durnin and Womersley, 1974) found no significant difference between measurements on either side of the body.

4. Mid-upper Arm Circumference

The mid-upper arm circumference was measured using a cloth tape measure at the midpoint obtained for the triceps skinfold measurement. All readings were taken by the same investigator. Whenever possible, measurements were taken with the arm hanging freely at the side. The tape was placed firmly but gently around the site to avoid compression of the soft tissue

(Jelliffe, 1966). Readings were recorded to the nearest 0.1 centimetre. Three readings were taken for each measurement and the results were averaged.

5. Mid-upper Arm Muscle Circumference

The mid-upper arm muscle circumference was calculated from the triceps skinfold thickness and the mid-upper arm circumference, using the formula $Cm = Ca - \pi S$ (Jelliffe, 1966), where Cm = muscle circumference in cm, Ca = arm circumference in cm and S = triceps skinfold in cm. This value was expressed to the nearest 0.1 centimetre. For derivation of the formula, see Appendix IV.

B. Biochemical Methods

All blood samples were obtained by staff of the Biochemistry Laboratory, St. Boniface General Hospital. All tests were performed on serum, which was separated by centrifuge shortly after collection. For non-routine procedures, ie. prealbumin, zinc and ribonuclease, the serum samples were frozen until assay. The intervals at which each test was performed are shown in Appendix III.

All containers for urine collections were acid-washed with an aqueous solution of approximately 11% hydrochloric acid. The container was allowed to stand for a minimum of 24 hours and then rinsed out with distilled water. This procedure served to remove any trace contamination with zinc. Twenty-four hour urine collections were performed by ward personnel

and delivered to the Biochemistry Laboratory for measurement and routine tests. For non-routine procedures, ie. zinc and ribonuclease, 10 ml aliquots were taken and kept frozen until assay.

1. Albumin

Serum albumin was measured by the St. Boniface General Hospital Biochemistry Laboratory by an automated colorimetric technique using bromocresol green. This method was adapted from a method of Doumas, described by Annino and Giese (1976). It is rapid, sensitive and fairly specific for albumin. The absorbance of the albumin-bromocresol green complex was measured at 620 nm. on a Technicon SMA (Simultaneous Multiple Analyzer) 12/60. The normal range for serum albumin as obtained by this method is 3.5-5.0 gm%.

2. Prealbumin

Serum prealbumin was measured by radial immunodiffusion, a method which has been found to be simple, accurate and reproducible (Ingenbleek, 1972). The procedure involves allowing an antigen to diffuse from a well into agar containing antibody, and measuring the ring of precipitation, which marks the antigen-antibody interaction, around the well (Markowitz and Jiang, 1976).

M-Partigen immunodiffusion plates were obtained through Behring Diagnostics Division, Hoechst Canada. These plates contain specific antiprealbumin antiserum in an agar-gel layer. Control Plasma for Partigen (lyophilized) and

Protein Standard Serum B (prediluted) were obtained from the same source.

The wells of the plates were filled with 5 µl of serum sample, control or standard. The standard was applied undiluted and in dilutions of 1:2 and 1:4. These solutions were allowed to stand at room temperature for 42 hours. The diameters of the precipitin rings formed were then measured to within 0.1 mm, using a calibration viewer (Transidyne General Corporation, distributed by Kallestad Laboratories, Inc., Chaska, Mn.). The squares of the diameters of the precipitin rings (in mm^2) obtained with the standard dilutions were plotted against the antigen concentration (in mg/100 ml). This resulted in an almost linear plot. The "best fit" straight line was obtained using the principle of least squares. The concentrations of the serum samples were then obtained directly from this straight line.

For the study patients, serum prealbumin levels were obtained when TPN was initiated, after 2 weeks and when TPN was stopped. Although this schedule provided a good evaluation of prealbumin levels in relation to a patient's initial and final clinical status, it did not reveal the rate of change in prealbumin levels in relation to changes in albumin. To investigate this aspect, serum samples were obtained from three follow-up patients two or three times weekly.

3. Transferrin

Serum transferrin levels were estimated from the total iron-binding capacity (TIBC) using the following formula:

serum transferrin (mg%) = [0.8 x TIBC (μg%)] - 43

(Blackburn et al., 1977).

The TIBC is the sum of the serum iron plus the unsaturated iron-binding capacity (UIBC).

UIBC was determined by a method modified from White and Flashka (1973) and Yee and Goodwin (1974). This method involves saturation of the serum transferrin by addition of an excess of ferrous iron, followed by complexing of unbound iron with ferrozine. The decrease in free iron in the test sample is a measure of its UIBC. The iron-ferrozine complex produced was measured at 547 nm on an American Monitor KDA (Kinetic Data Acquisition).

Serum iron was determined by a similar method, involving complexation of ferrozine with ferrous ion. The resulting chromogen was measured at 547 nm on the KDA.

4. Creatinine

Serum creatinine determination was based on the Jaffe reaction using alkaline picric acid, as described by Henry (1974) and modified for use with an SMA 12/60 (Technicon). Creatinine reacts with alkaline picric acid to form an amber yellow creatinine picrate complex which is measured at 510 nm. By this procedure, the normal range is 0.5-1.2 mg/100 ml.

Urine creatinine was measured on the SMA 12/60 by the same method as for serum creatinine, but using one-tenth the amount of sample. Twenty-four hour urine creatinine excretion was obtained by multiplying the creatinine concentration per

millilitre by the 24-hour urine volume.

The creatinine-height index was calculated by dividing the 24-hour urine creatinine excretion (in mg) of that patient by the 24-hour urine creatinine excretion of a normal subject of the same height and sex. Normal values were adapted from a table of Blackburn et al. (1977) which in turn was based on the creatinine coefficient and on ideal weight for height. The creatinine coefficient is assumed to be 23 mg/kg ideal body weight for men and 18 mg/kg ideal body weight for women (Blackburn et al., 1977).

5. Zinc

Serum and urine samples were compared to standard zinc solutions using a Perkin Elmer 403 Atomic Absorption Spectrophotometer (Perkin-Elmer Corp., Norwalk, Connecticut), which is designed for measuring the concentration of metallic elements in solution. The instrument uses a hollow cathode single element lamp for zinc at 214 UV, slit 4(7Å), and uses air-acetylene providing an oxidizing (lean blue) flame.

Determination of zinc levels by atomic absorption spectrophotometry has the advantage of being simple, sensitive and precise (Halsted et al., 1974).

A stock standard solution was prepared, containing zinc 20 µg/ml, calcium 100 µg/ml, magnesium 10 µg/ml, cadmium 40 µg/ml, chromium 100 µg/ml and copper 100 µg/ml. This solution was prepared from separate stock standards (Fisher Scientific Company). The working solution was made fresh

daily by diluting the stock standard solution to 1:100, 2:100, 3:100 and 4:100 with triple deionized water, providing zinc concentrations of 0.2 µg/ml to 0.8 µg/ml. These solutions were used to obtain a standard curve, which was linear up to 1 µg/ml. Calibration was checked using a reference standard, Cation-Cal™ (Dade Division, American Hospital Supply Corp., Miami).

Approximately 1.5 ml of serum or urine sample was aspirated directly onto the burner. Samples were not diluted unless the zinc concentration exceeded the linear range, in which case the sample was diluted with triple deionized water.

Zinc is frequently found as a contaminant of many substances including glass, rubber, water and many chemicals (Davies et al., 1968). Consequently, zinc samples can be readily contaminated in the hospital and laboratory environment unless proper precautions are taken. A major source of contamination is collection tubes with rubber stoppers or with clot activator (Ralstin et al., 1979). In the present study, serum samples were collected in sterile, glass Vacutainers (Becton Dickinson). The study protocol called for urine samples to be collected and stored in acid-washed containers. In some patients, the collections were made in a clean plastic container and transferred immediately to the acid-washed container. This procedure was standardized for each patient, and had no apparent effect on the changes observed in urinary zinc levels.

6. Ribonuclease

The method of assay was modified from Roth (1967) and from Sigulem et al. (1973). The basic method, as noted by Roth (1967), is relatively simple, "adaptable to a large number of samples, and does not require special purification of the substrate or the use of expensive samples of RNA". It does, however, have limitations which will be discussed later. The method measures the absorbance of acid-soluble substances produced by the action of RNase on RNA substrate. Serum and urine samples were compared to a standard solution of bovine pancreatic RNase, using a Unicam SP 1800 Ultraviolet Spectrophotometer.

a. Preparation of reagents. Most reagents were prepared according to or slightly modified from Roth (1967) as described briefly below.

1) RNA substrate. Ribonucleic acid (Type II-S, Sigma Chemical Co.) was used to prepare a 1% aqueous solution. This was adjusted to pH 7.0 by the addition of 0.1 N NaOH, made up to volume and stored frozen in 10 ml aliquots.

2) Buffer. The buffer used was veronal acetate 0.07 M, with EDTA 0.05 M. The stock solution was prepared from 2.943 gm of barbital sodium (Fisher Scientific Co.) and 1.943 gm of sodium acetate trihydrate (Fisher) dissolved in 100 ml of distilled water, and stored under refrigeration. To 10 ml of this stock solution was added about 25 ml distilled water and 0.05 M trisodium edetate

(ethylenediamine tetraacetic acid) prepared either from the powder (Sigma) or using Sodium Versenate 1 gm/5 cc ampoule (Riker Laboratories). Dilute (10%) HCl was further diluted 1:5 and used to adjust the pH to 7.8. The solution was made up to 50 ml with distilled water and stored frozen in 10 ml aliquots.

3) Precipitating reagent. This solution was prepared from 12 ml of concentrated HCl, 8 ml of distilled water and 0.5 gm of lanthanum chloride (Sigma) made up to 100 ml with 95% ethanol. This preparation was kept under refrigeration, although it may be stored at room temperature indefinitely (Roth, 1967).

4) Standard. The standard solution was prepared from bovine pancreatic ribonuclease A (Type I-A, Sigma). A stock solution of 5 mg in 100 ml of 0.1% gelatin was stored frozen in aliquots and used within three weeks. At the time of assay, 1 ml of this solution was diluted 1:50 with 0.1% gelatin and kept on ice until use. Gelatin solutions were prepared with distilled water heated to 40° C, and were stored under refrigeration for up to one week.

b. Preparation of samples. RNase has been found to be stable in the frozen state in serum for at least three months (Houck and Berman, 1958) and in plasma at -20° C for at least one year (Sved et al., 1967). Frozen serum samples and frozen 10 ml aliquots from 24-hour urine collections were thawed and centrifuged at 1000 rpm for twenty minutes. From each sample,

0.2 ml of the clear supernatant was transferred to a clean glass test tube. To urine and to serum tubes were added 0.6 ml and 0.2 ml respectively of cold veronal acetate buffer.

c. Method of assay. This assay involves incubation of samples with buffer and ribonucleic acid (RNA). Addition of the precipitating agent precipitates unhydrolyzed RNA and protein, which are removed by centrifugation. The amount of remaining acid-soluble products is determined by measuring the absorbance against a blank determination. The use of a blank is essential because measurable quantities of acid-soluble material can be produced by the action of the precipitating agent on RNA. In addition, most commercial RNA preparations contain appreciable amounts of various degradation products (Roth, 1967). The assay was performed on ice to minimize enzyme degradation and extraneous substrate hydrolysis.

From the freshly-prepared standard RNase solution (1 μg/ml), dilutions of 0.02 to 0.40 μg/ml were prepared in distilled water. Veronal acetate buffer 0.3 ml was transferred to the appropriate number of clean test tubes. To these tubes was added 0.1 ml of either water (for the blank) or the standard dilutions just described or the sample/buffer mixtures previously prepared. Then 0.2 ml of RNA substrate was added serially to each tube. The tubes were agitated, and incubated in a water bath at 37°C for exactly 30 minutes. After 30 minutes, the reaction was stopped by the serial addition of 0.6 ml of cold acid-lanthanum-alcohol solution. The tubes were agitated and allowed to stand on ice for ten minutes,

then centrifuged at 2000 rpm for ten minutes, after which 0.5 ml of the clear supernatant was drawn off and diluted 1:10 with distilled water. The absorbance of the standard and sample solutions was measured against the blank at 260 mμ. The values from the standard dilutions were used to prepare a standard curve, against which the absorbance values of the samples were plotted. The results were expressed as RNase concentration in μg/ml. However, it must be remembered that these values are only a means of expressing the activity of the RNase in the sample in relation to the activity of the known amounts of bovine pancreatic RNase (in μg/ml) used in preparing the standard curve.

7. Nitrogen Balance

Nitrogen balance was estimated using the formula:

$$\text{nitrogen balance(gm)} = \text{N intake}_{(\text{parenteral + oral})} - (\text{UUN} + 2)$$

where UUN is urinary urea nitrogen (in grams) excreted in 24 hours. Nitrogen intake is the sum of the nitrogen content of the TPN solution used (4.2 gm/litre x volume infused in 24 hours) plus the nitrogen content of any oral intake. The volume of TPN solution actually infused was determined from daily fluid balance sheets kept on each patient by the nursing staff. Oral protein intake was estimated from records kept by the Department of Dietetics, and divided by 6.25 to give the nitrogen content. The determination of oral intake by this method is, however, less accurate than the calculation of intravenous intake.

C. Immune Function

1. Immunoglobulins

Serum levels of IgA, IgG and IgM were determined by the Immunology Laboratory, St. Boniface General Hospital, using a radial immunodiffusion technique similar to that described for prealbumin, but using an incubation time of 16.5 hours.

2. Complement

Serum levels of complement components C_3 and C_4 were determined by the Immunology Laboratory, St. Boniface General Hospital, using a radial immunodiffusion technique similar to that described for prealbumin, but using an incubation time of 16.5 hours.

3. T and B Lymphocyte Detection

Human T lymphocytes are capable of adhering to sheep erythrocytes, forming rosettes. This property is now widely used to permit the enumeration of T cells in peripheral blood. B lymphocytes, unlike T cells, have readily detectable immunoglobulins on their surfaces (Claman, 1975), so can be detected by a fluorescent antibody technique.

About 20 ml of peripheral blood was collected in heparin and mixed with phosphate buffered saline (pH 7.2). The mononuclear cells were separated by centrifugation in Ficoll-Isopaque as performed by the Immunology Department, St. Boniface General Hospital.

For determination of T cells, a lymphocyte concentration of 6×10^6 cells/ml in azide-free phosphate buffered saline

was used. Sheep red blood cells (SRBC) were washed with phosphate buffered saline and incubated with diluted papain solution at 37°C for 10 minutes to expose additional binding sites on the cell. The SRBC's were then washed, counted and the concentration adjusted to 120 x 10^6 cells/ml. To 0.1 ml of lymphocyte suspension was added 0.1 ml of SRBC suspension (cell ratio 1:20). This mixture was incubated at 37°C for 5 minutes, then centrifuged at 600 rpm for 5 minutes and allowed to stand at room temperature for one hour. The cells were then resuspended and the rosettes were counted with a phase contrast microscope, and expressed as a percentage of total lymphocytes. Three or more SRBC surrounding a lymphocyte constitutes a rosette.

For determination of B cells, the concentration used was 20 x 10^6 cells/ml in phosphate buffered saline with sodium azide. To 0.1 ml of this suspension was added 0.1 ml of a fluorescent conjugate of antibodies (to human IgG, IgA and IgM) used as a 1:20 dilution. The mixture was incubated at 4°C for one hour, then washed with phosphate buffered saline with sodium azide at 750 rpm. The cells were resuspended in residual liquid and immediately counted on a "Bellco" slide using a fluorescent microscope. The percentage of immunoglobulin-positive (fluorescent) cells was determined. Cells from a normal control donor were also counted to confirm the proper activity of the conjugate.

4. Delayed Cutaneous Hypersensitivity

The following tests were administered intracutaneously on the forearm or upper arm:

candida (Monilia) 1:100 (Beecham) 0.02 ml;

tricophyton 1:100 (Beecham) 0.02 ml;

tuberculin P.P.D. (Mantoux) 1 T.U. (Connaught) 0.1 ml;

streptokinase-streptodornase (Varidase—Lederle) 6.25 u/0.1 ml.

The doses used were those routinely employed for delayed hypersensitivity testing at St. Boniface General Hospital, except for the substitution of PPD 1 T.U. for the usual 5 T.U. The low doses were preferred in order to minimize the risk of severe local reactions, which are infrequent but could present difficulties if occurring in the study patients. Patients were not skin tested if there was a history of cutaneous manifestations of allergy, or of severe local response to previous skin testing. During the latter part of the study, Varidase was in short supply, so patients #10 to #16 received only the 3 other tests.

The streptokinase-streptodornase (SK-SD) was diluted in normal saline to obtain a final concentration of 62.5 u/ml. The other antigens were used undiluted. Administration was by means of a 1-ml tuberculin syringe with a 26-gauge, 3/8 inch needle. Syringes were prepared shortly before use.

The skin area to be used was cleansed with isopropyl alcohol and allowed to dry. The testing sites were marked

with an indelible pen, and the antigens were injected in the same order each time. Results were read at 24 hours and 48 hours after injection. The diameter of erythema and of induration was read in two directions, and recorded in millimetres. A positive response consisted of 5 mm or more of induration at the test site at either reading. Testing was repeated in two weeks, and when TPN was discontinued.

RESULTS

Seventeen subjects entered the study. One (#3) died soon after starting TPN, and was excluded due to insufficient data. Of the remaining 16 subjects, 15 received parenteral nutrition for periods ranging from 8 to 85 days. Subject #17 received enteral feeding due to an infection of her subclavian catheter site which precluded the use of TPN, and was included as a non-improved, malnourished control. Subjects #17 and #8 were the same person studied at different times.

The patients ranged in age from 28 to 93 years, with a mean age of 57.1 years. The age, sex and diagnosis for each patient are shown in Table 4. Reasons for receiving parenteral nutrition are shown in Table 5. The study population consisted of nine women and seven men. The former received TPN for a mean of 30 days compared to 16 days for the men.

A majority of the patients had normal renal function on entering the study. However, one patient (#7) had poor renal function with a creatinine clearance of less than 20 ml/min, and five other patients (#2, #4, #8, #9, #10) had initial values for creatinine clearance of less than 50 ml/min. In the latter group of patients, especially patient #7, most urinary parameters had relatively low values and narrower ranges in comparison to the other patients. However, there was no apparent relationship between the creatinine clearance and the direction of change in these parameters during the course of TPN.

Table 4. Patient Characteristics

Patient Number	Age	Sex	Diagnosis	Duration of TPN (Days)	Initial Weight (kg)
1	74	M	Ca stomach (metastatic)	13	58.3
2	56	F	Ca colon (metastatic)	11	65.1
4	69	M	duodenal obstruction	11	77.3
5	52	M	Ca bladder	8	93.0
6	36	F	Crohn's disease	32	67.1
7	71	M	congestive heart failure	8	-
8	59	F	malnutrition, malabsorption, scleroderma	18	29.0
9	93	F	malabsorption syndrome; atherosclerosis, peripheral circulatory problems; mild diabetes	36	40.9
10	60	F	intestinal fistula	85	70.5
11	48	F	gastric outlet obstruction	21	67.7
12	43	M	ulcerative colitis	27	70.0
13	52	M	post-cholecystectomy	26	75.9
14	28	M	bowel resection	22	94.4
15	55	F	Crohn's disease with short-gut syndrome	28	55.5
16	59	F	suspected cholecystitis; pancreatitis	15	80.0
17	59	F	malnutrition, malabsorption, infection, scleroderma	18	29.9

Table 5. Changes in Patients' Conditions during TPN

Patient Number	Nutritional Status	Clinical Condition	Reason for TPN
1	improved	non-improved	pre-operatively
2	improved	non-improved	post-operatively
4	improved	non-improved	pre- and post-operatively
5	improved	improved	wound healing (post-op.)
6	non-improved	non-improved	bowel rest
7	non-improved	non-improved	postprandial abdominal angina
8	improved	improved	malnutrition
9	improved	non-improved	malnutrition
10	non-improved	non-improved	wound healing (long-term post-op. complications)
11	improved	non-improved	post-op. complications; pre- and post-operatively
12	improved	improved	weight loss; poor response to treatment
13	non-improved	non-improved	gastric outlet obstruction; biliary fistula; weight loss
14	improved	improved	post-operatively
15	improved	improved	dehydration; malnutrition
16	improved	improved	abdominal mass and pain
17	non-improved	non-improved	malnutrition

The 16 patients were evaluated with regard to changes in nutritional status and clinical condition during the period of TPN. These evaluations were based partly on laboratory data traditionally used for the purpose, and partly on impressions and observations by the researcher, the medical and nursing staff and the patient. The results of this assessment are shown in Table 5. Eleven patients showed an improvement in nutritional status while five patients were classed as non-improved. Of the latter, one patient died, two were transferred to a larger centre for further investigation and one patient (#6) was nutritionally healthy when TPN was begun and maintained this state. The fifth patient (#17) was unable to receive TPN due to infection at the catheter site. The nutritionally improved patients remained in the study until the attending physician decided that TPN was no longer required.

Clinically, six patients improved, six patients showed no apparent change and one patient (#7) died a few days after TPN was discontinued. Two patients (#4, #11) had surgery during the course of TPN and one patient (#6) showed no apparent change until a sudden deterioration requiring surgery. Patient #10 required surgery near the end of a long course of TPN, and most parameters were evaluated only up to the time of surgery (day 75).

The parameters studied were evaluated separately for each patient, for all patients and for nutritionally improved patients. Results were expressed as the actual values and

also in relation to each initial value. Those parameters which appeared useful in predicting or assessing nutritional changes were further evaluated. Initial and final values of these parameters were compared using paired t-tests, usually one-tailed. Each variable was also plotted against time. The plots were examined by means of the line of best fit through the data, to determine whether the variable had undergone a net change during the period of TPN. Unless otherwise indicated, mean values are expressed as mean $\pm$ one standard deviation. Most of the statistical data were obtained using an S.P.S.S. programme on a Cyber 171 computer.

A. Anthropometric Measurements

1. Weight

Weights were available on 15 patients. The mean of initial weights was 64.97 $\pm$ 19.74 kg, while that for final weights was 66.47 $\pm$ 17.94 kg. Initial and final weights were not significantly different, using the paired t-test.

The weights are plotted in Fig. 4a and 4b. In all plots of variables against time, values for patient #10 (on TPN for 85 days with no net improvement) are shown only up to day 39.

Of the eleven patients who were classed as nutritionally improved by subjective assessment, seven had a net increase in weight and four showed a net decrease. Final weights of all improved patients grouped together were not significantly different from their initial weights, due to the effect of

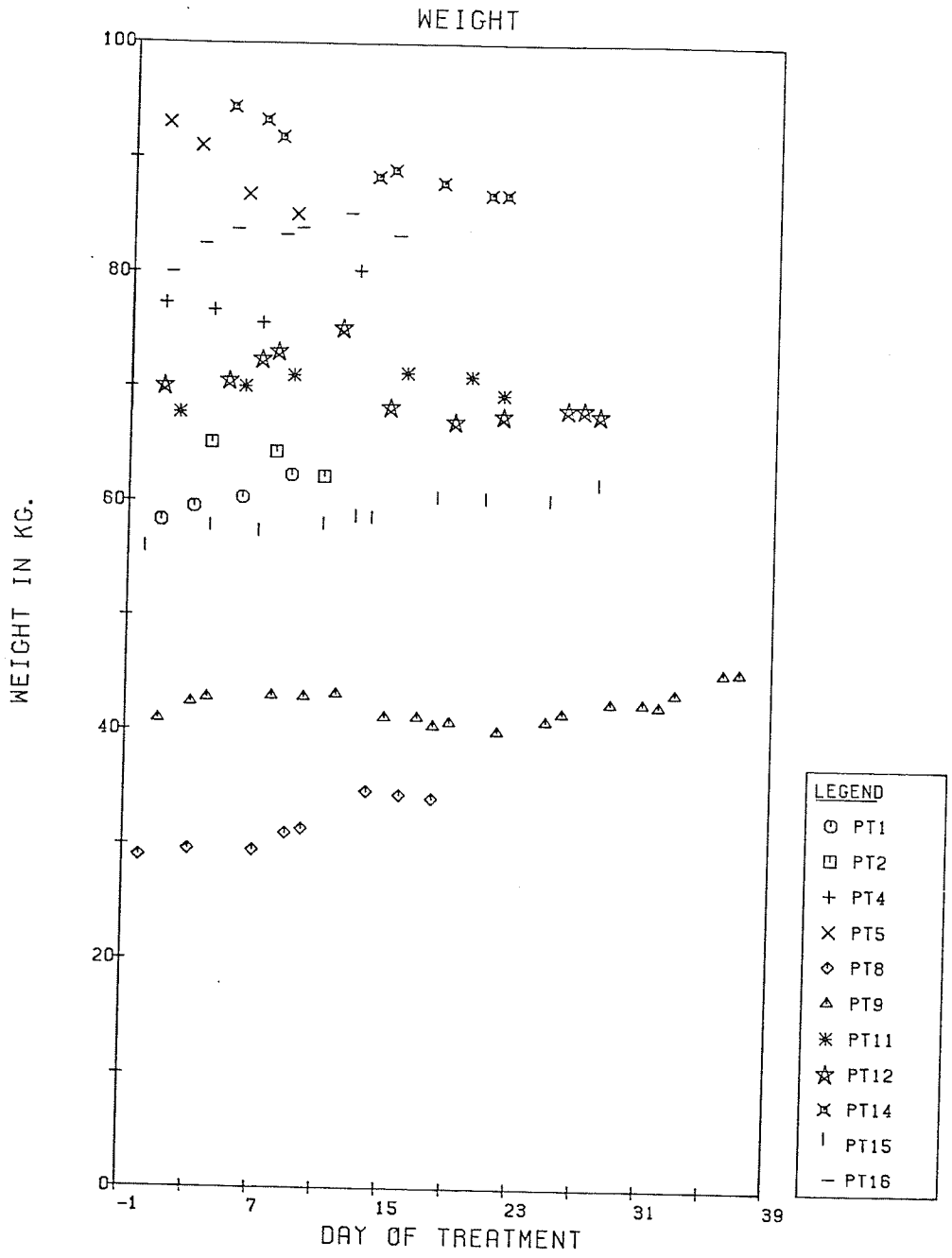

Figure 4a. Weight in improved patients.

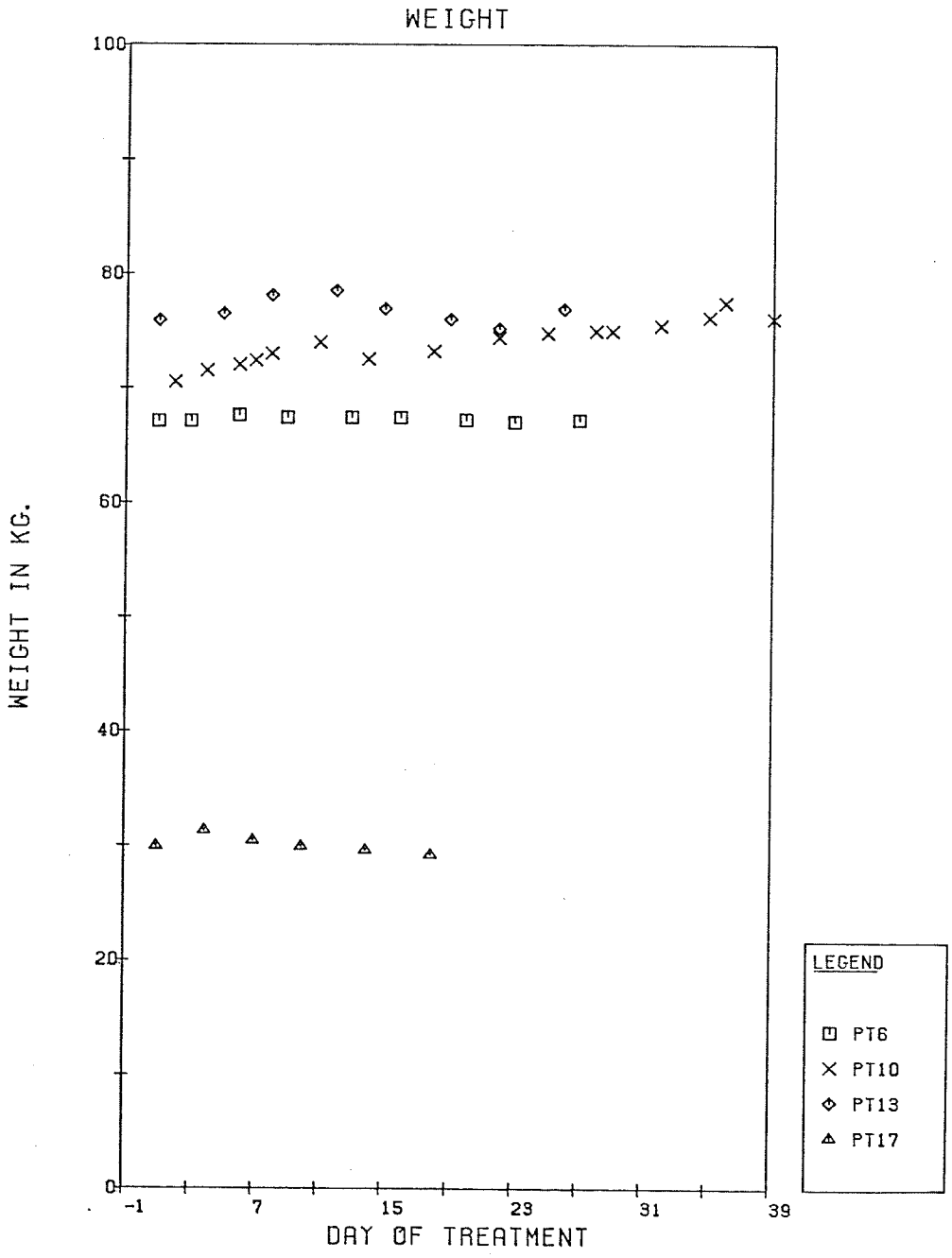

Figure 4b. Weight in non-improved patients.

the four patients whose weights decreased during TPN.

Of the four patients who did not improve according to subjective assessment, one had an increase in weight and three showed no net change.

2. Triceps Skinfold

Measurements of triceps skinfold thickness were available on 15 patients. Skinfold values for normal subjects according to sex and age group, as determined by Durnin and Womersley (1974), are reproduced in Table 6. Values obtained in the present study are plotted against time in Fig. 5a and 5b.

Table 6. Normal Triceps Skinfolds (mm)

Sex	Age (years)	Mean	S.D.	Range
Men	17-19	11	7.0	4-35
	20-29	9.8	5.8	3-39
	30-39	11	5.2	9-33
	40-49	11	5.6	5-29
	50-72	11	5.9	4-27
Women	16-19	16	8.0	5-41
	20-29	21	10	7-50
	30-39	25	12	7-54
	40-49	23	10	10-55
	50-68	25	9.7	13-49

From: Durnin, J. V. & Womersley, J. Br. J. Nutr. 32: 77-97, 1974.

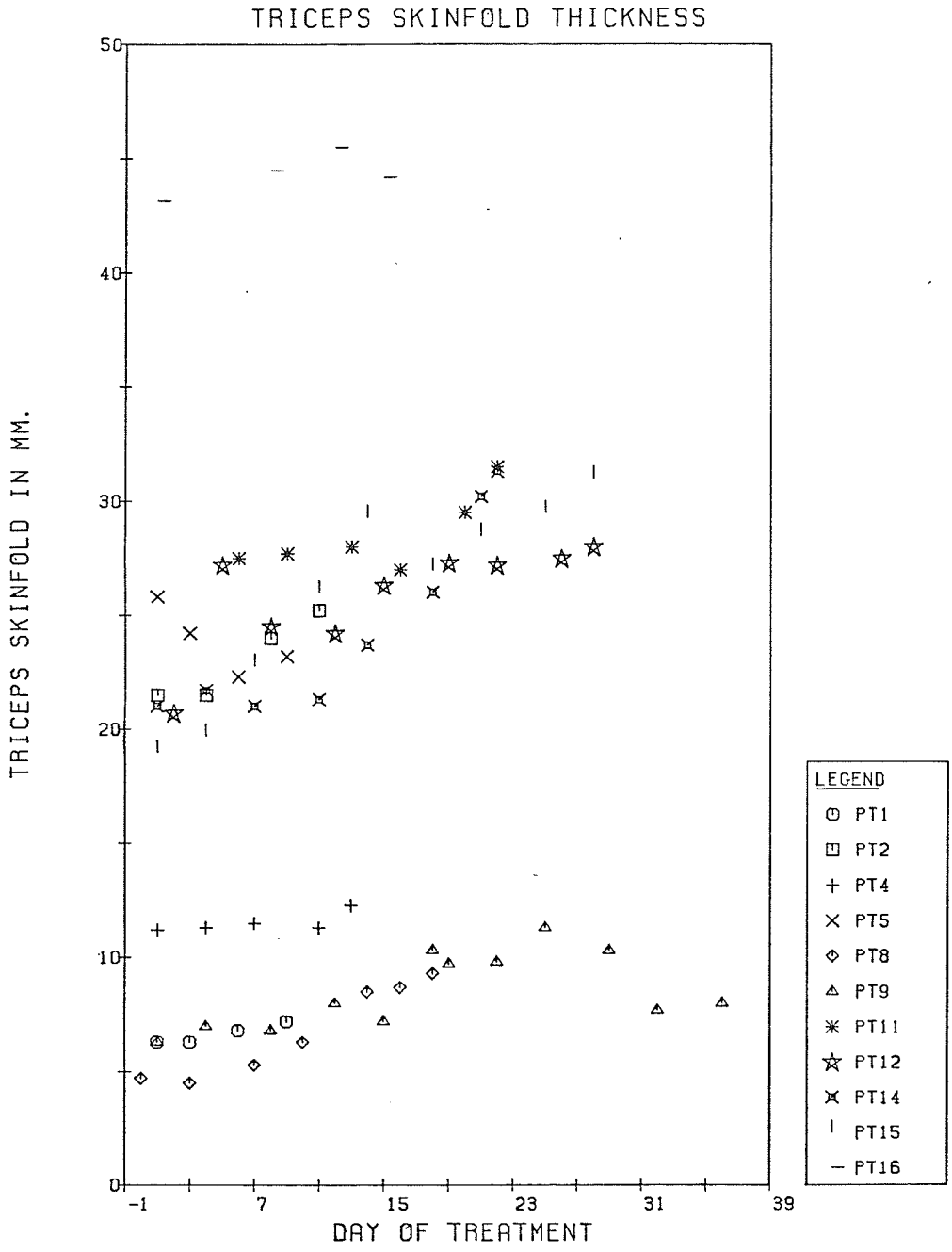

Figure 5a. Triceps skinfold in improved patients.

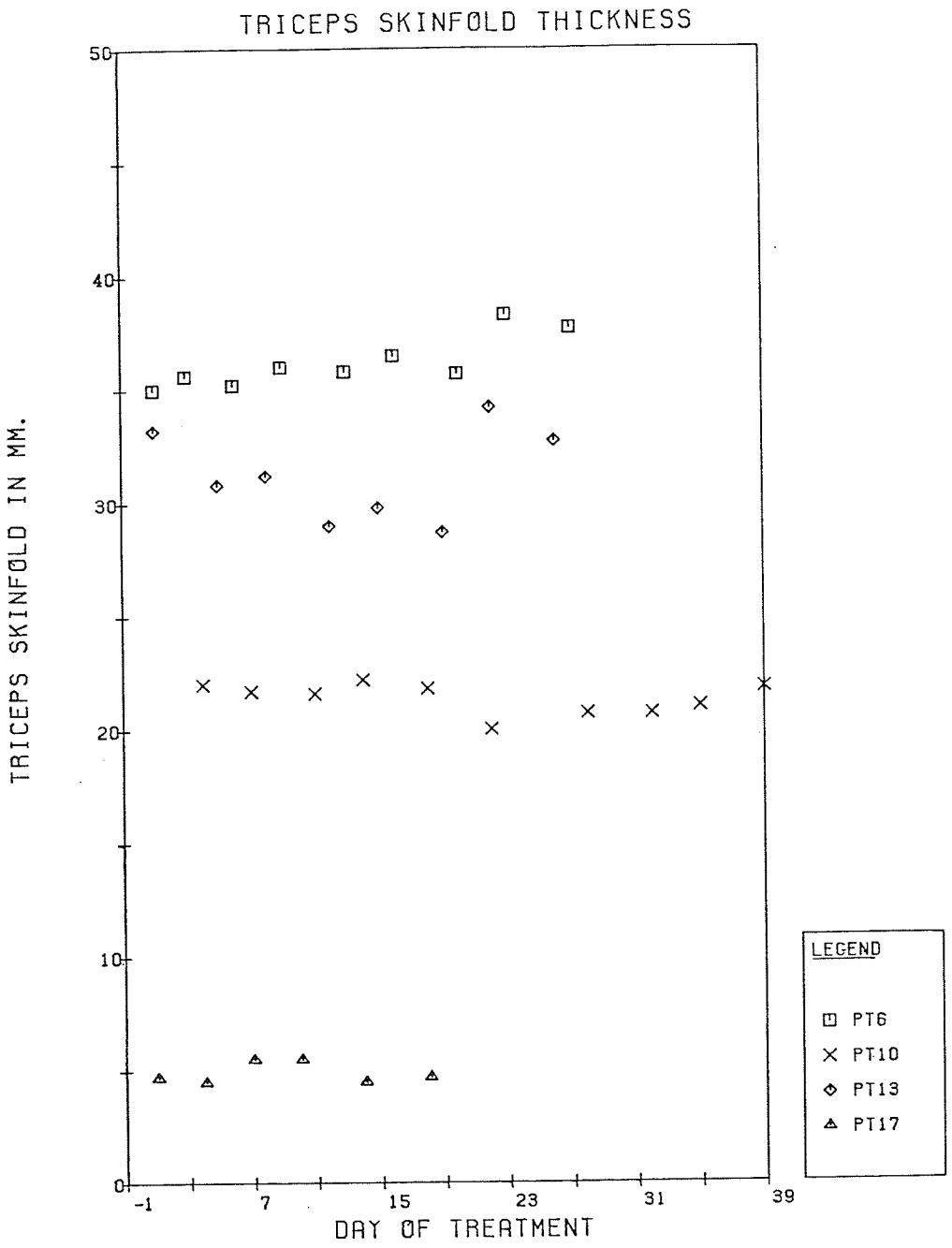

Figure 5b. Triceps skinfold in non-improved patients.

The mean of initial values was 20.46 ± 11.94 mm, compared to a mean of 23.67 ± 12.34 mm for the final values. The final values were significantly greater than the initial measurements ($P<0.005$). Of the eleven nutritionally improved patients, ten exhibited a net increase in triceps skinfold thickness. Of the four "non-improved" patients, one showed an increase, two showed a decrease and one had no net change in skinfold thickness. The final measurements of the improved patients were significantly greater than the initial values ($P<0.01$); those of the non-improved patients were not.

3. Mid-upper Arm Circumference

Measurements of mid-arm circumference were available for 16 patients, and are plotted in Fig. 6a and 6b. The initial mean of 27.80 ± 6.53 cm was not significantly different from the final mean of 28.06 ± 6.07 cm. These values were within the normal range (Frisancho, 1974).

No definite relationship was found between changes in arm circumference and changes in nutritional state, although the five patients with a marked increase in arm circumference also had a substantial increase in triceps skinfold.

4. Mid-upper Arm Muscle Circumference

The mid-arm muscle circumference was calculated for 15 patients. Results are plotted in Fig. 7a and 7b. Both the initial mean of 21.97 ± 4.17 cm and the final mean of 21.08 ± 3.47 cm were within the normal range (Frisancho, 1974). The decrease was statistically significant ($P<0.05$).

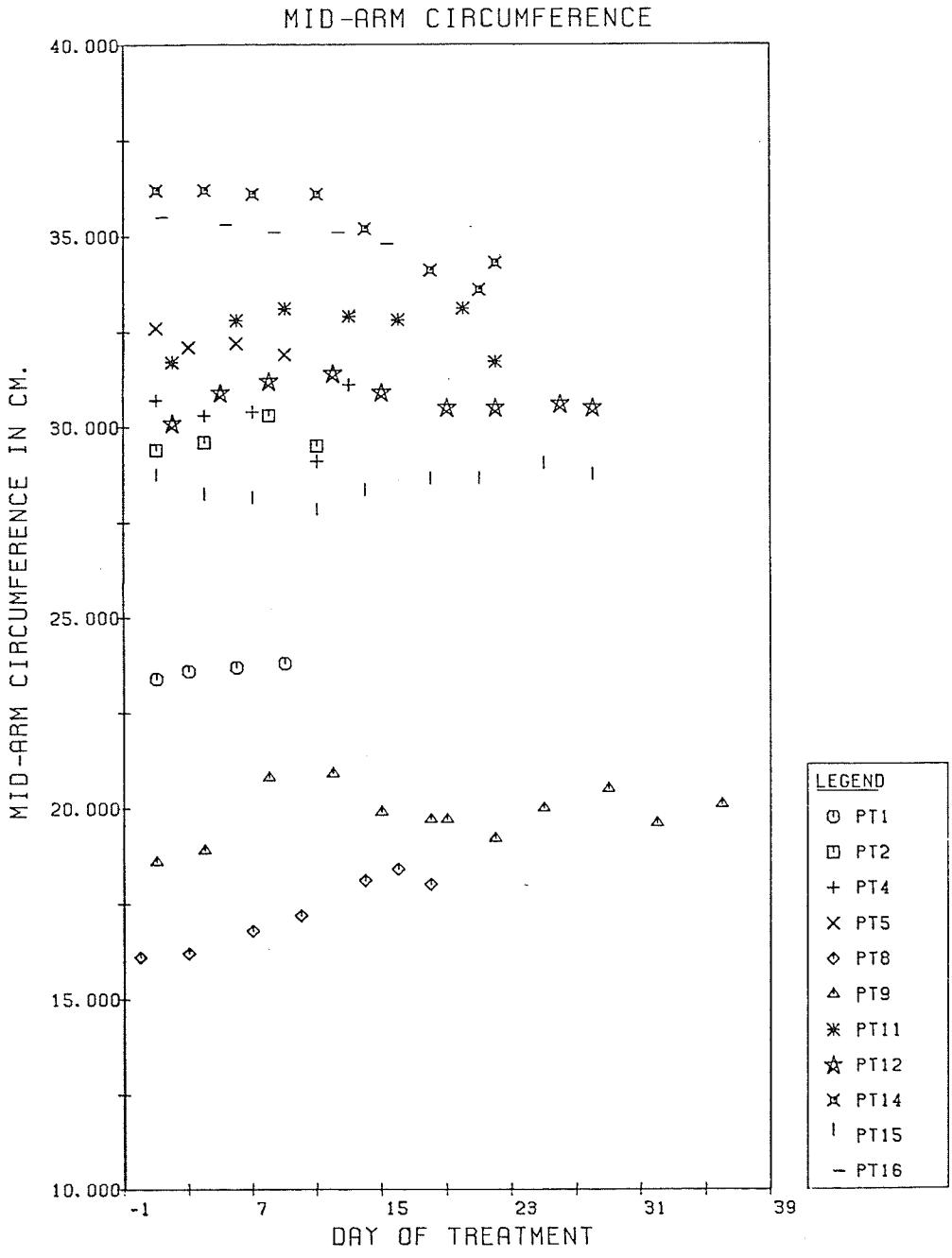

Figure 6a. Mid-arm circumference in improved patients.

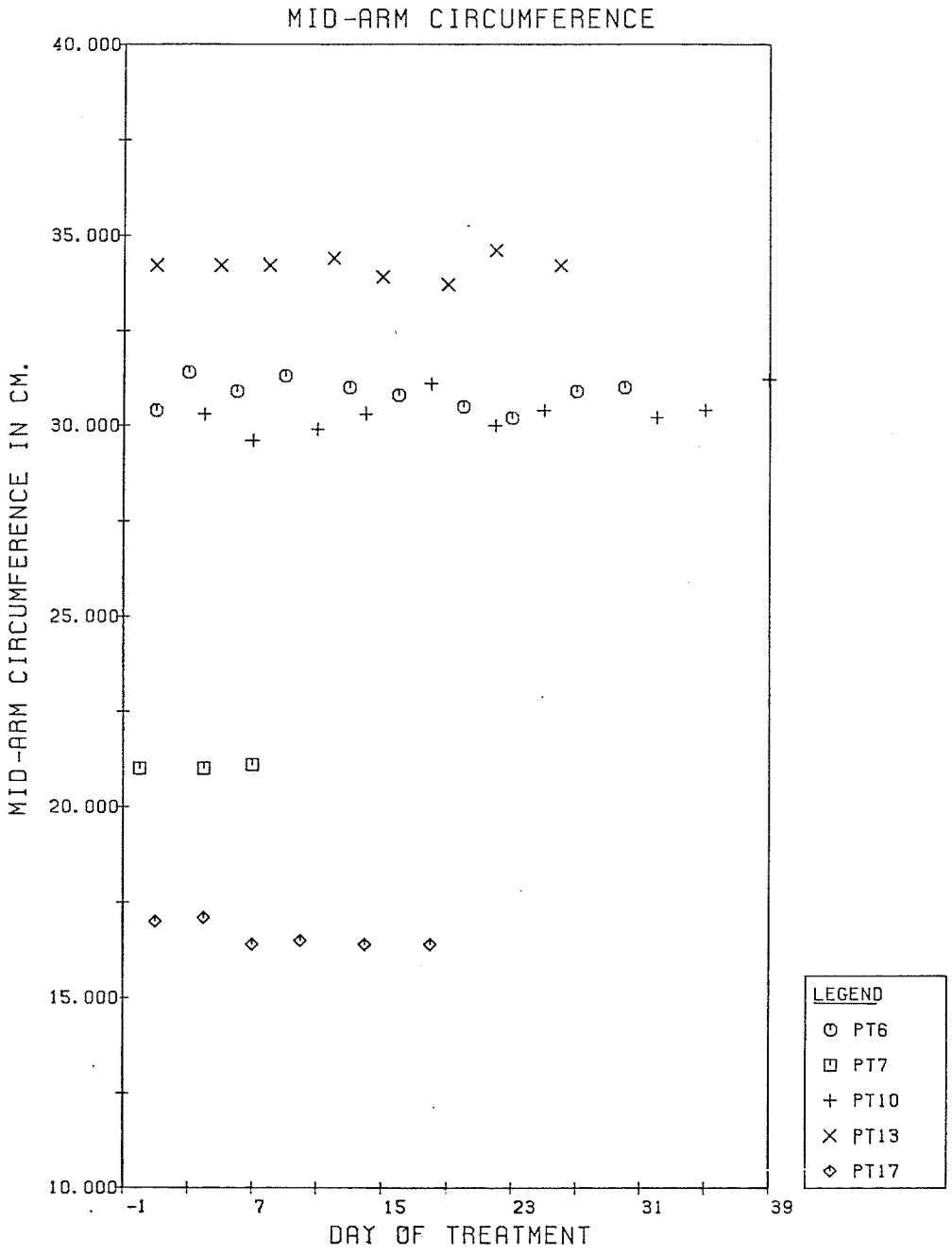

Figure 6b. Mid-arm circumference in non-improved patients.

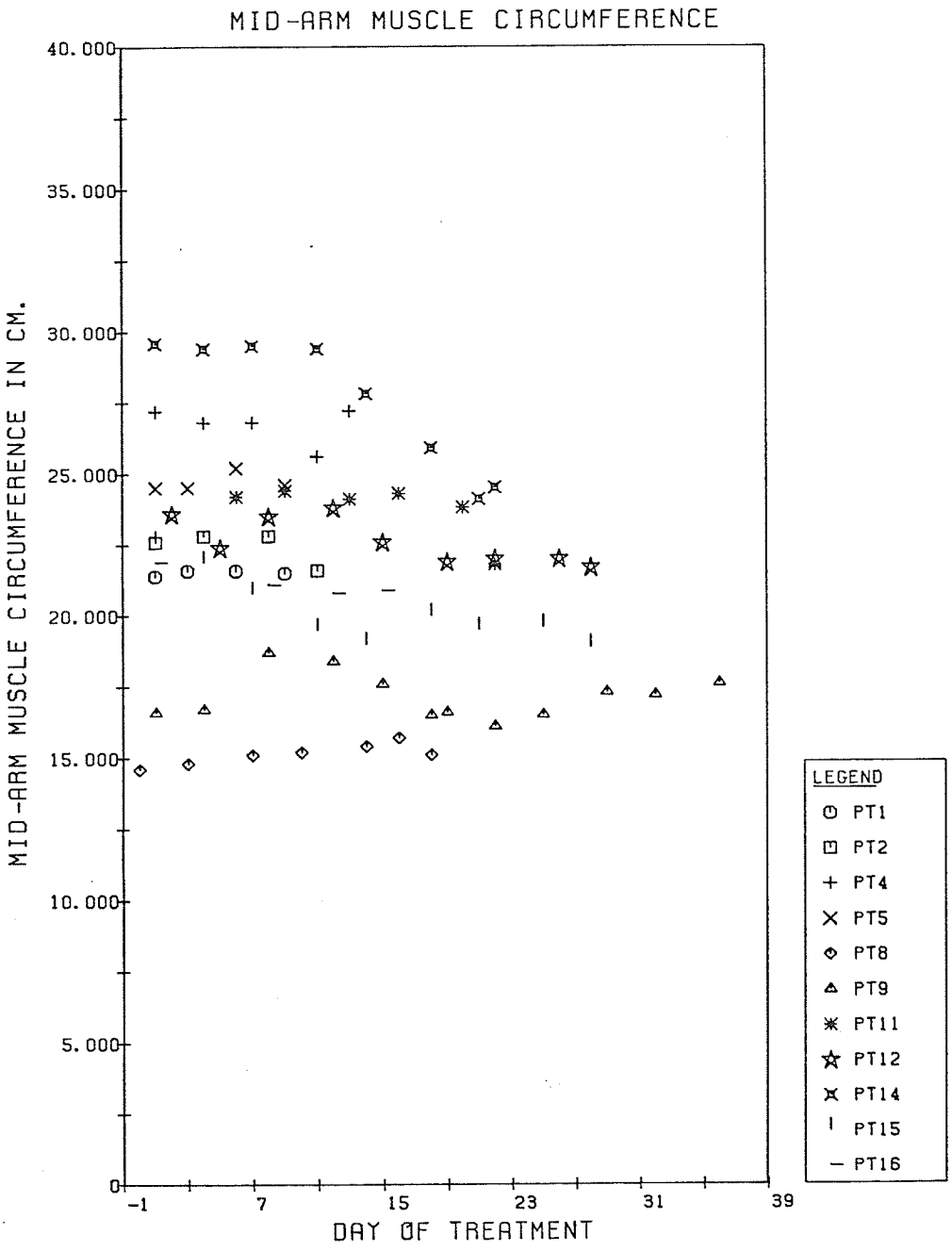

Figure 7a. Mid-arm muscle circumference in improved patients.

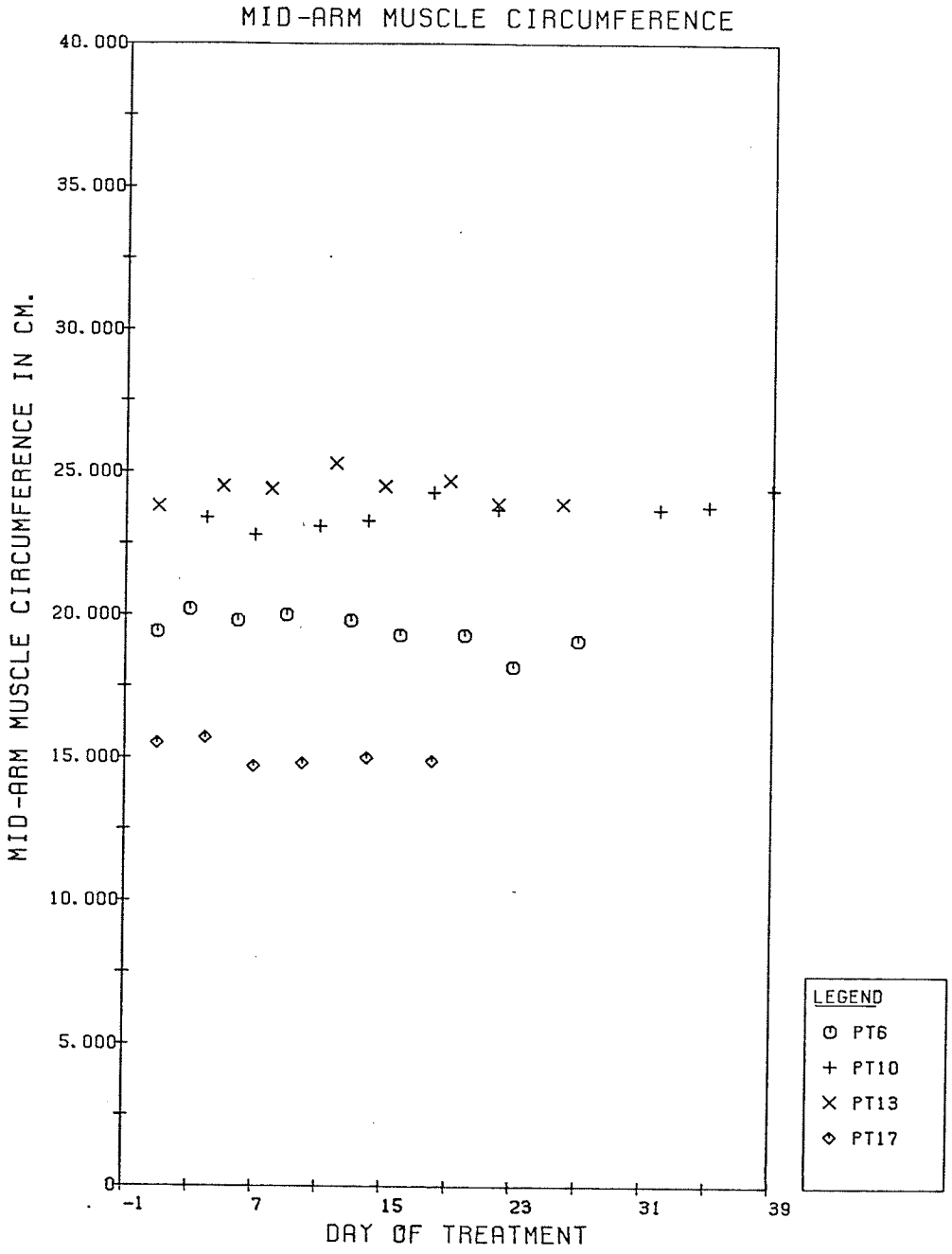

Figure 7b. Mid-arm muscle circumference in non-improved patients.

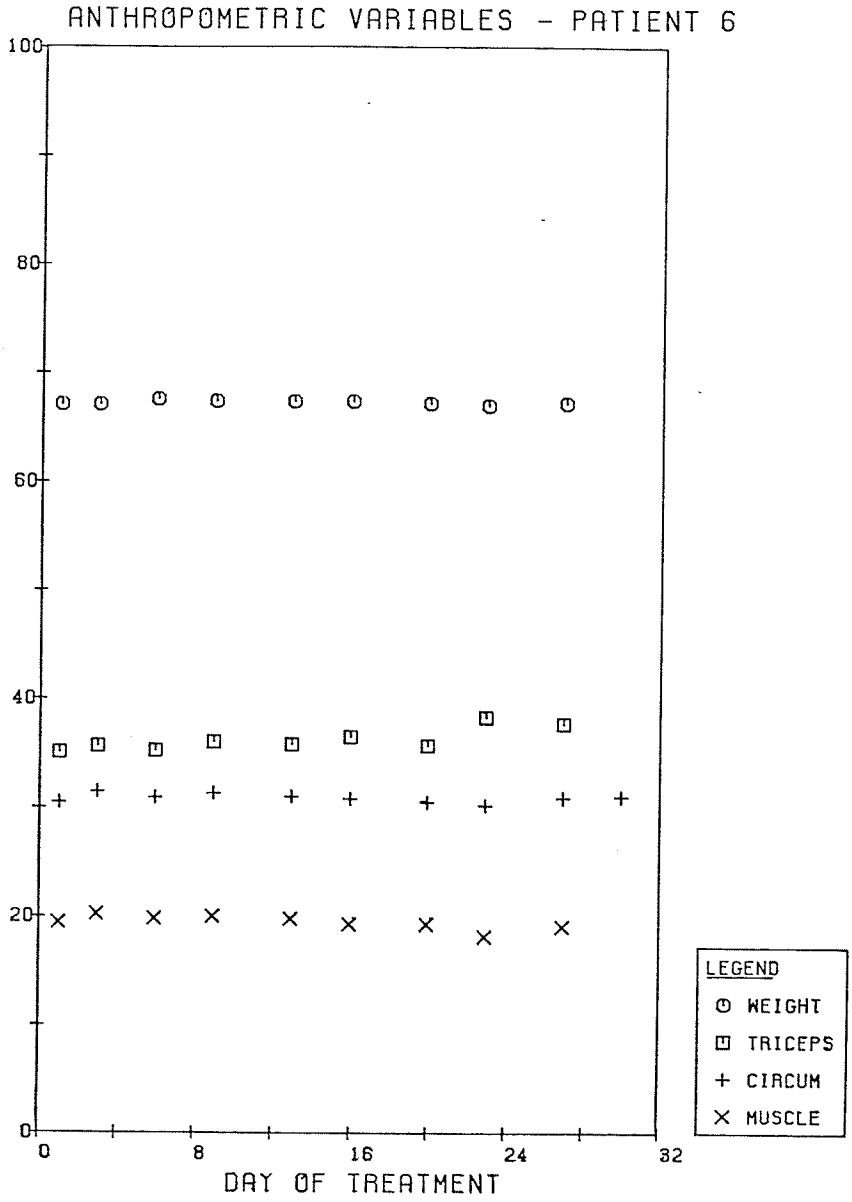

Figure 8a. Anthropometric measurements - Patient 6.

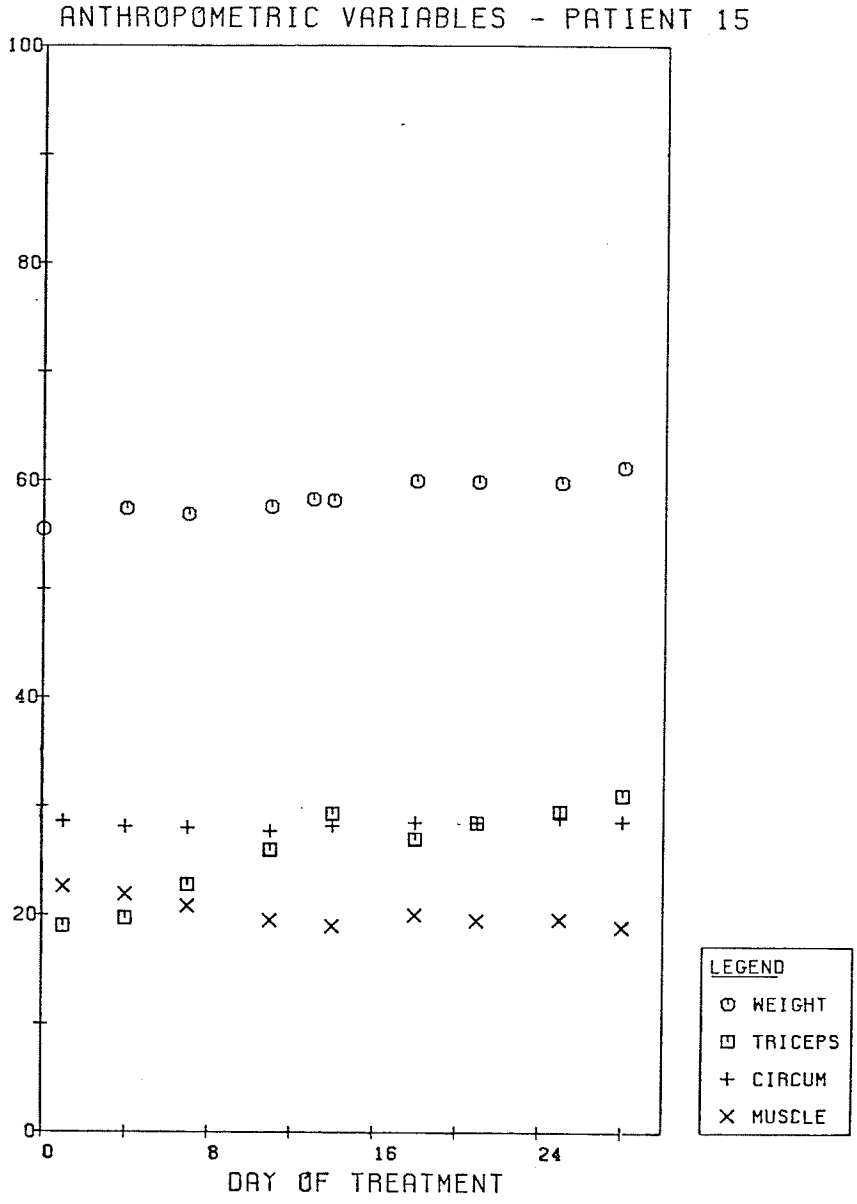

Figure 8b. Anthropometric measurements - Patient 15.

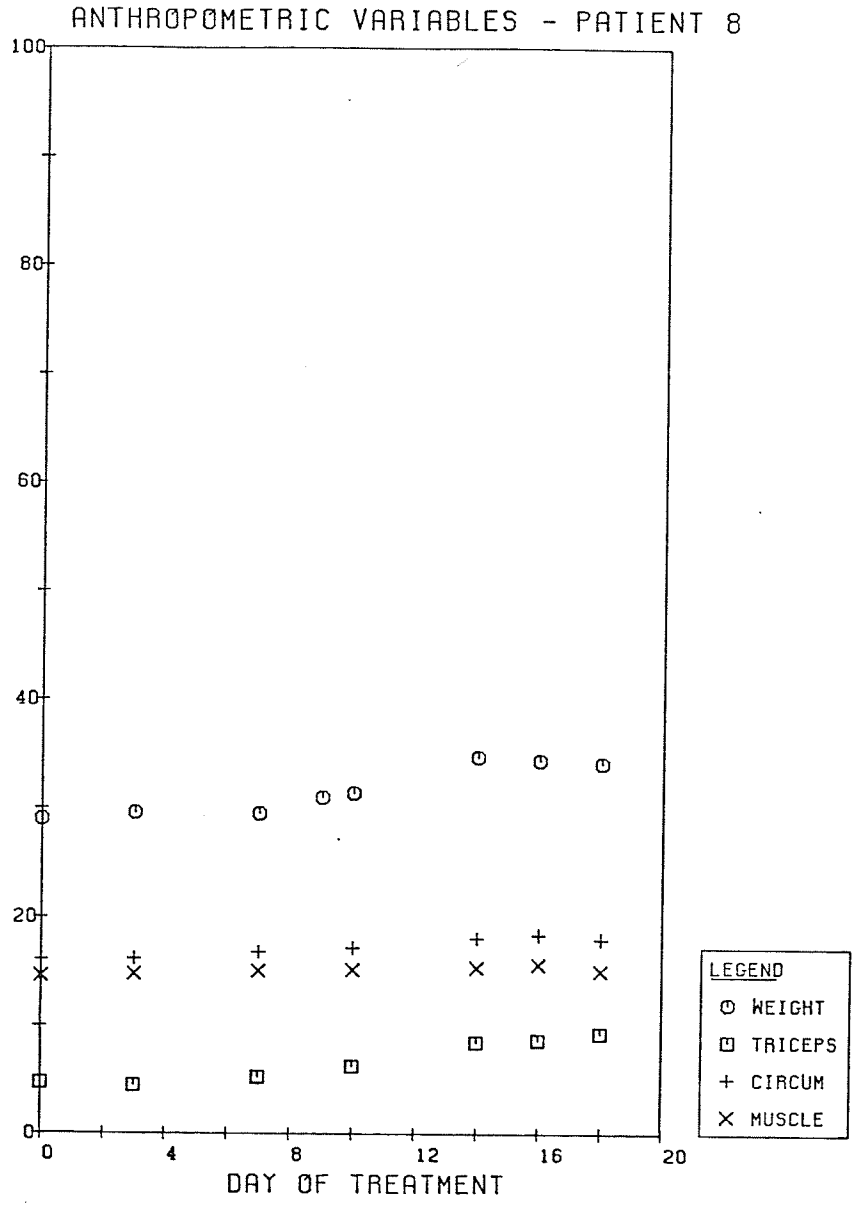

Figure 8c. Anthropometric measurements - Patient 8.

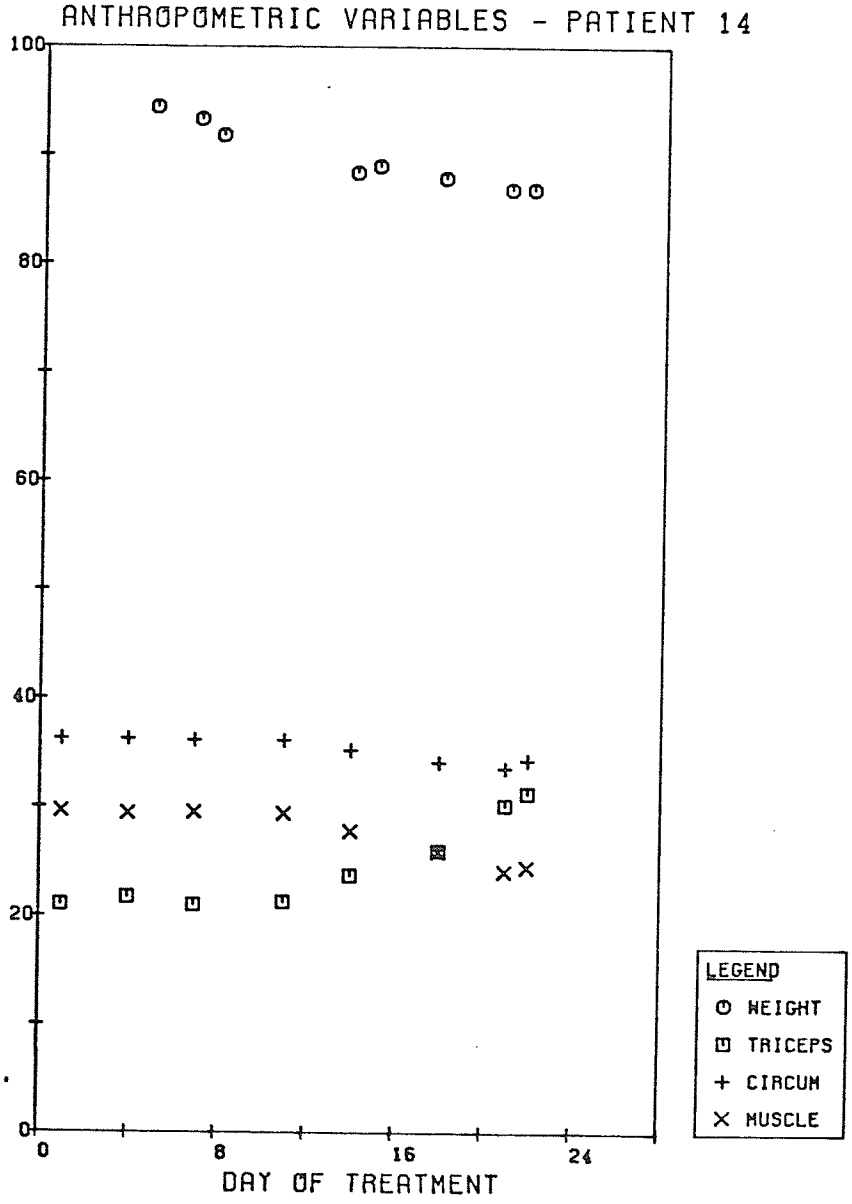

Figure 8d. Anthropometric measurements - Patient 14.

5. Interrelationships

Examples of the relationships among the various anthropometric variables are shown in Figs. 8a to 8d. Figure 8a is a good example of the stability of these parameters in a well-nourished patient who underwent minimal change during TPN. Typical improved patients are represented in Fig. 8b and 8c. The latter shows the most emaciated subject in the study, whose skinfold thickness was less than 20% of the reference standard. Her other anthropometric measurements were approximately 60% of the standard. Patient #14 (Fig. 8d) was one of the few patients whose change in triceps skinfold thickness did not correspond to the change in weight.

B. Biochemical Measurements

1. Albumin

The initial and final mean serum albumin concentrations for the 16 patients were not significantly different. For the improved patients, these values were, respectively, 3.07 ± 0.46 gm% and 3.18 ± 0.59 gm%. There was considerable individual day-to-day variability in some patients. In patients #5, #8, #9 and #13, most values were below 3.0 mg%. An increase in concentration of ≥ 0.7 gm% occurred in three improved patients - #15, #14 and #12.

2. Prealbumin

Serum prealbumin concentrations were available for ten patients, and are plotted in Figure 9. All ten patients

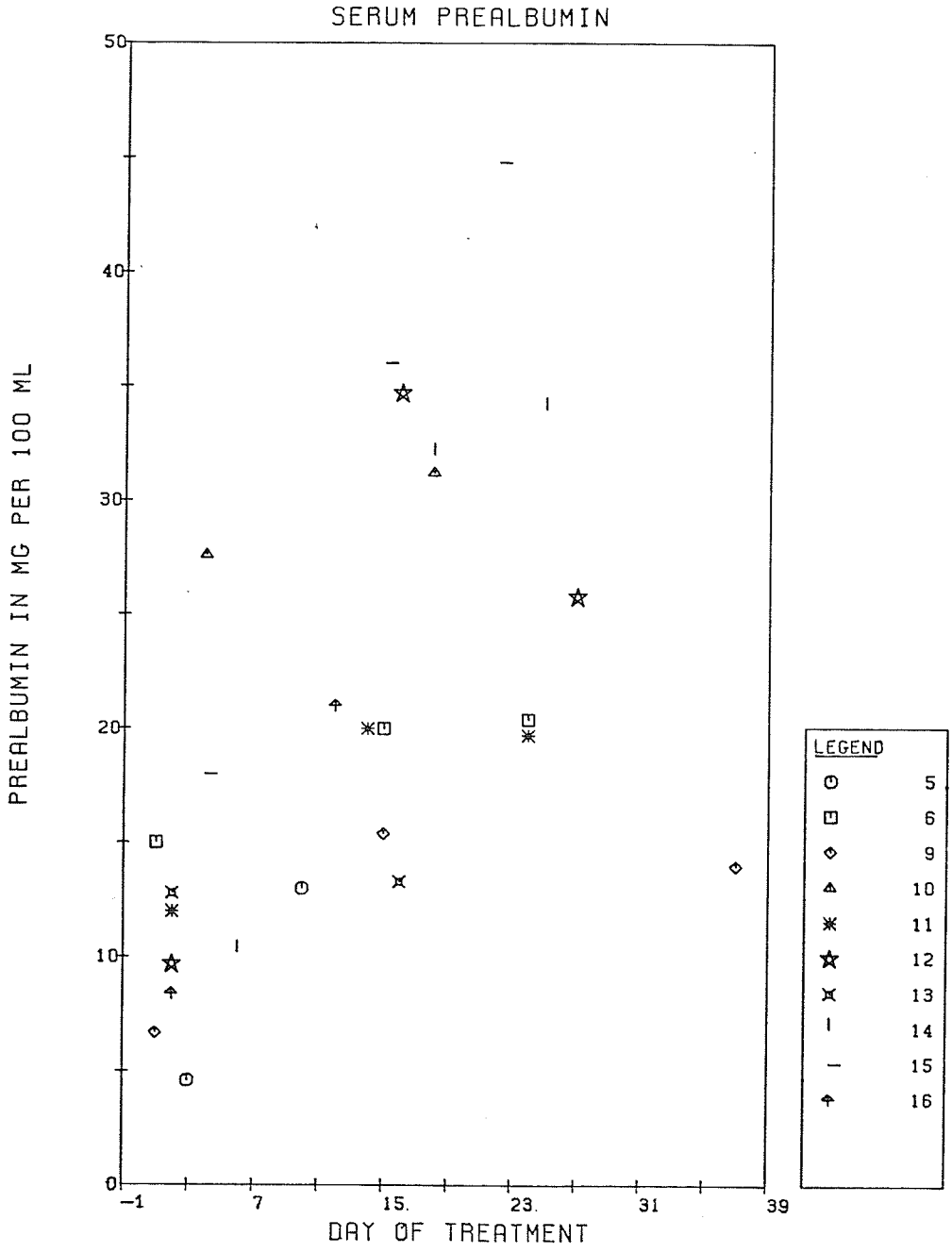

Figure 9. Serum prealbumin.

showed an increase from their initial values. The seven improved patients all had an increase to at least 1.5 times their initial concentrations, and in six of these, the final concentration was more than twice the initial concentration. The three non-improved patients had an increase in serum albumin of less than 1.5 times their initial levels. For two of these patients, the increase was less than 15% of the initial levels. The actual increase from the first to the second values in these three patients ranged from 0.5 to 5.0 mg%, compared to 8.0 to 25.0 mg% for the improved patients.

Serum prealbumin samples were obtained when the patient started TPN, after two weeks and/or when TPN was stopped. Of the six patients for whom the third prealbumin determination was available, four showed a change of equal to or less than 2.0 mg% from the previous determination. One patient exhibited a marked increase and one patient a marked decrease.

Initial values ranged from 4.6 to 27.6 mg%, the second values ranged from 13.0 to 36.0 mg%, and the third values from 14.0 to 44.8 mg%. All but one patient (#10) had initial values below 20 mg%, the lower limit of normal. Seven patients had subsequent concentrations at or above 20 mg%. Initial concentrations (mean 12.50 ± 6.59 mg%) were significantly lower than the concentrations after two weeks (mean 23.66 ± 8.97 mg%), $P<0.005$.

Figure 10 emphasizes the magnitude of the change in prealbumin concentration compared to that of serum albumin and total protein in a patient who underwent substantial

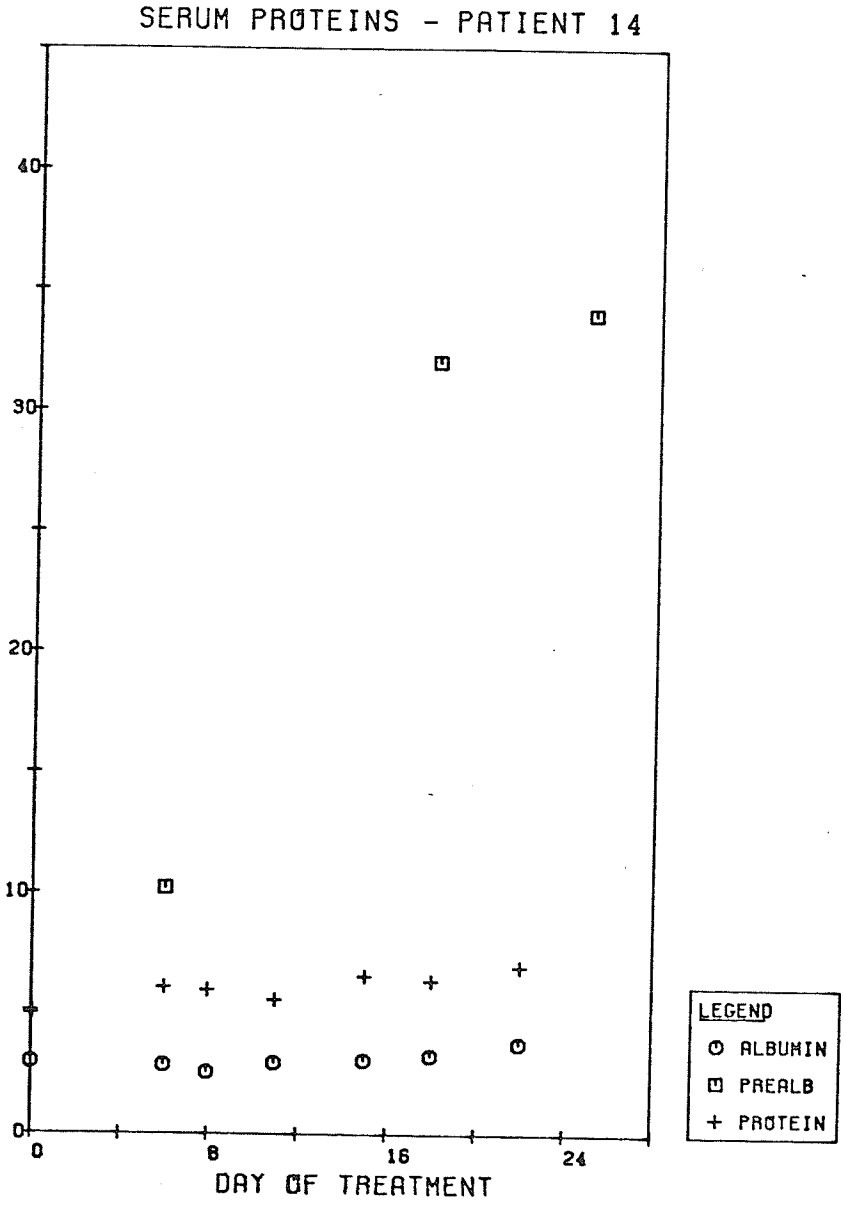

Figure 10. Serum albumin, prealbumin and total protein - Patient 14.

nutritional and clinical improvement.

Prealbumin levels were also investigated two to three times weekly in three follow-up patients to observe the time required for changes in prealbumin levels to occur. Patient A, whose initial concentration was below normal, exhibited a concentration in the normal range by the fourth day of TPN. Patient B, whose levels were initially normal, showed little change until the third week, when a substantial increase occurred. This patient later underwent surgery. The prealbumin level on the third post-operative day was about 50% of the value prior to surgery, but increased steadily thereafter. Patient C, whose prealbumin levels were abnormally low, showed a gradual decrease in concentration during TPN.

3. Transferrin

Serum transferrin levels were obtained for 13 patients. These values are plotted in Fig. 11, with the exception of patient #15 who received an iron dextran infusion during the course of TPN. This patient's transferrin levels, except those within 48 hours of the infusion, were included in the computations although not in Fig. 11.

The initial mean was 110.97 ± 40.78 mg%, which was not significantly different from the final mean of 136.69 ± 72.17 mg%. Only three patients had initial values and four patients had final values above 155 mg%, while almost one-half of the initial values were below 100 mg%. Normal concentrations in this laboratory range from 155 to 340 mg%.

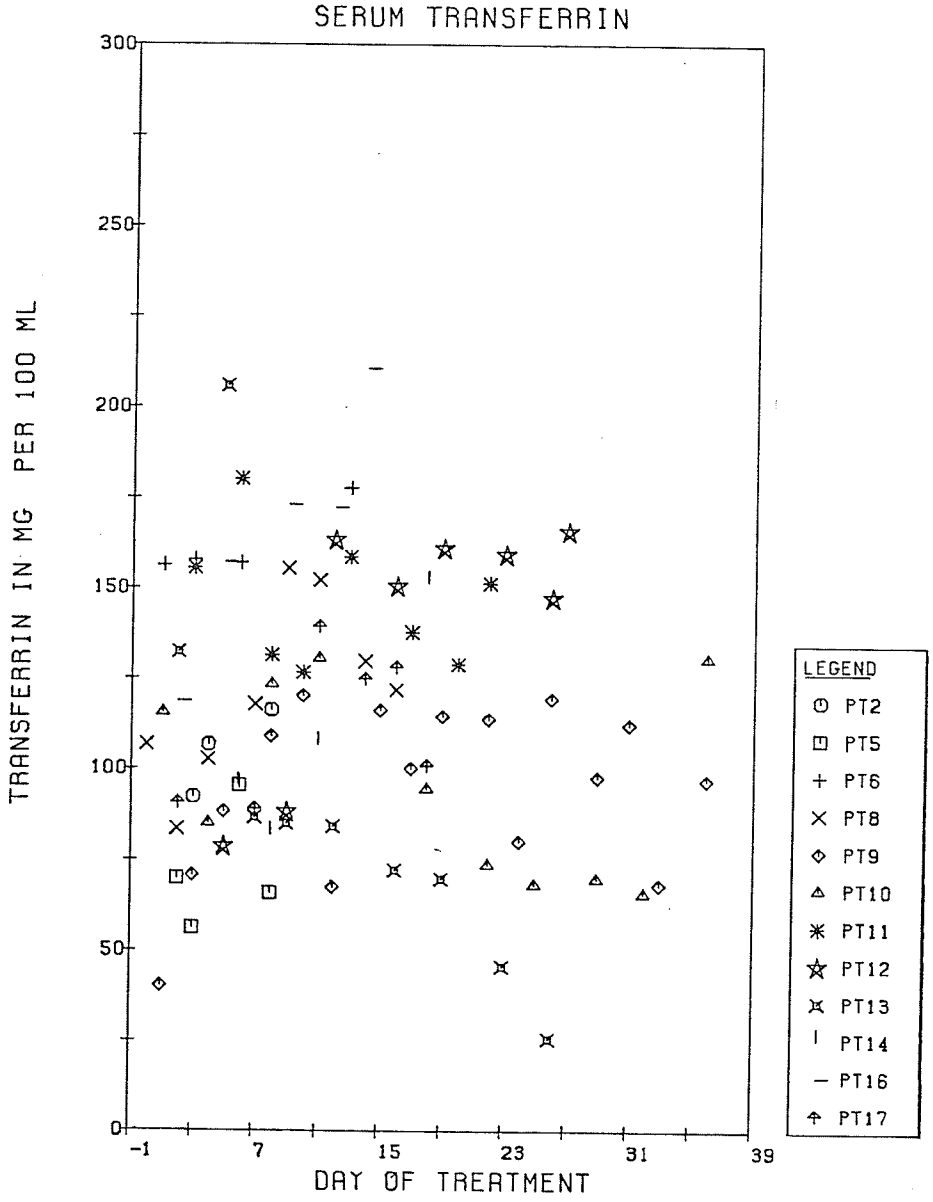

Figure 11. Serum transferrin.

Of the nine nutritionally improved patients, seven showed a net increase in transferrin concentration, one patient had a decrease and one patient showed no net change. The initial mean of the improved patients was 105.36 $\pm$ 45.74 mg%, which was significantly lower ($P<0.01$) than the final mean of 152.20 $\pm$ 75.01 mg%. Of the four non-improved patients, two had an increase and two a net decrease in serum transferrin.

4. Creatinine and Creatinine-height Index

Serum creatinine was normal in all but three patients. The range was 0.4 to 3.8 mg%. The highest values occurred in patient #7, with congestive heart failure. The normal range is 0.5 - 1.2 mg%.

Measurements of 24-hour urine creatinine excretion were available in 14 patients, and ranged from 120 to 2800 mg. Initial and final values were not significantly different.

Of the ten nutritionally improved patients, four had a net increase in 24-hour creatinine excretion and two had a net decrease. Four showed little net change although two of these had wide day-to-day variability. Of the four non-improved patients, one had a net increase in excretion and two had little net change but wide day-to-day variability. In the fourth patient (#7), creatinine excretion decreased as renal function deteriorated.

The creatinine-height index was determined in 14 patients. These values, which are plotted in Fig. 12a and 12b, ranged from 0.110 to 1.890.

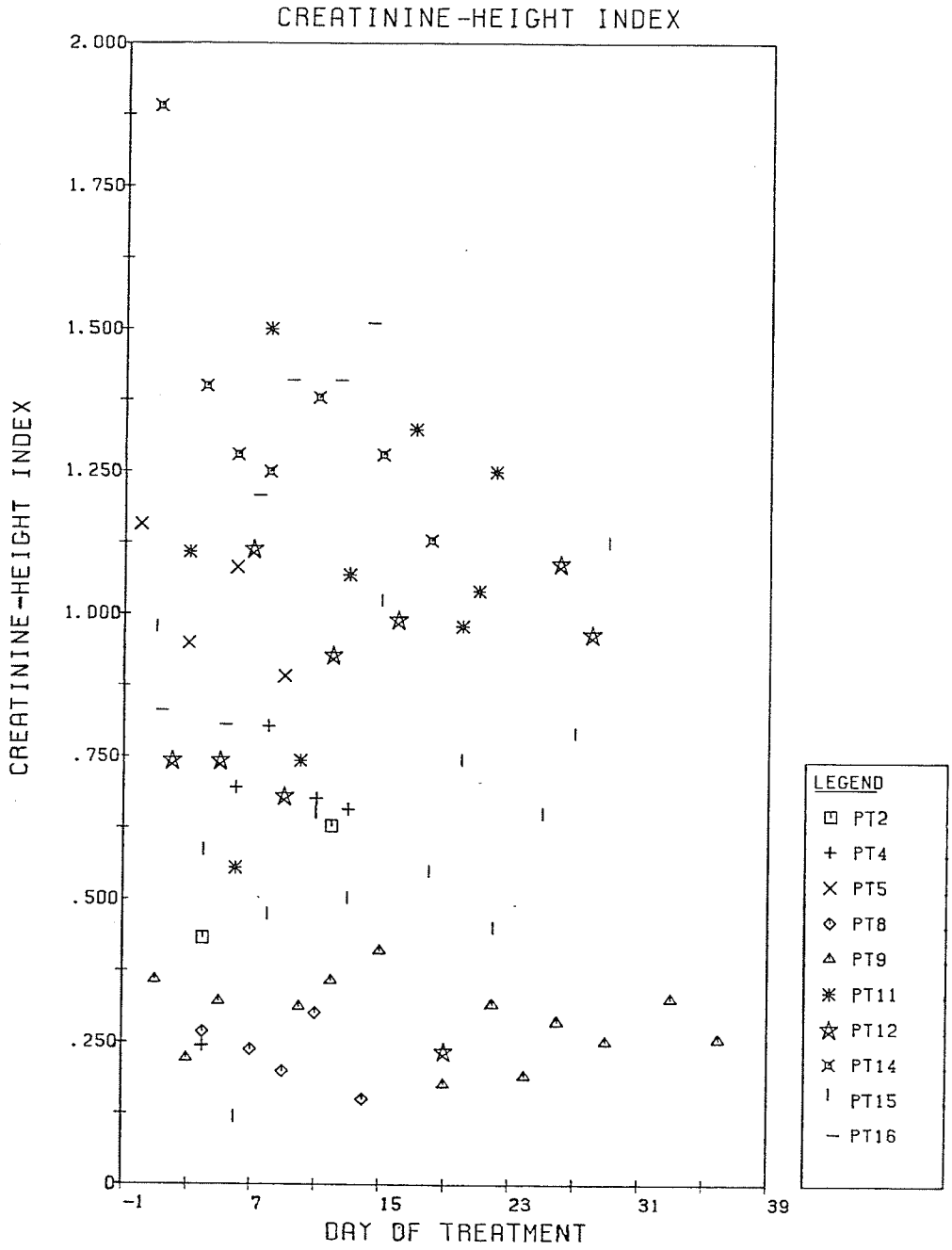

Figure 12a. Creatinine-height index in improved patients.

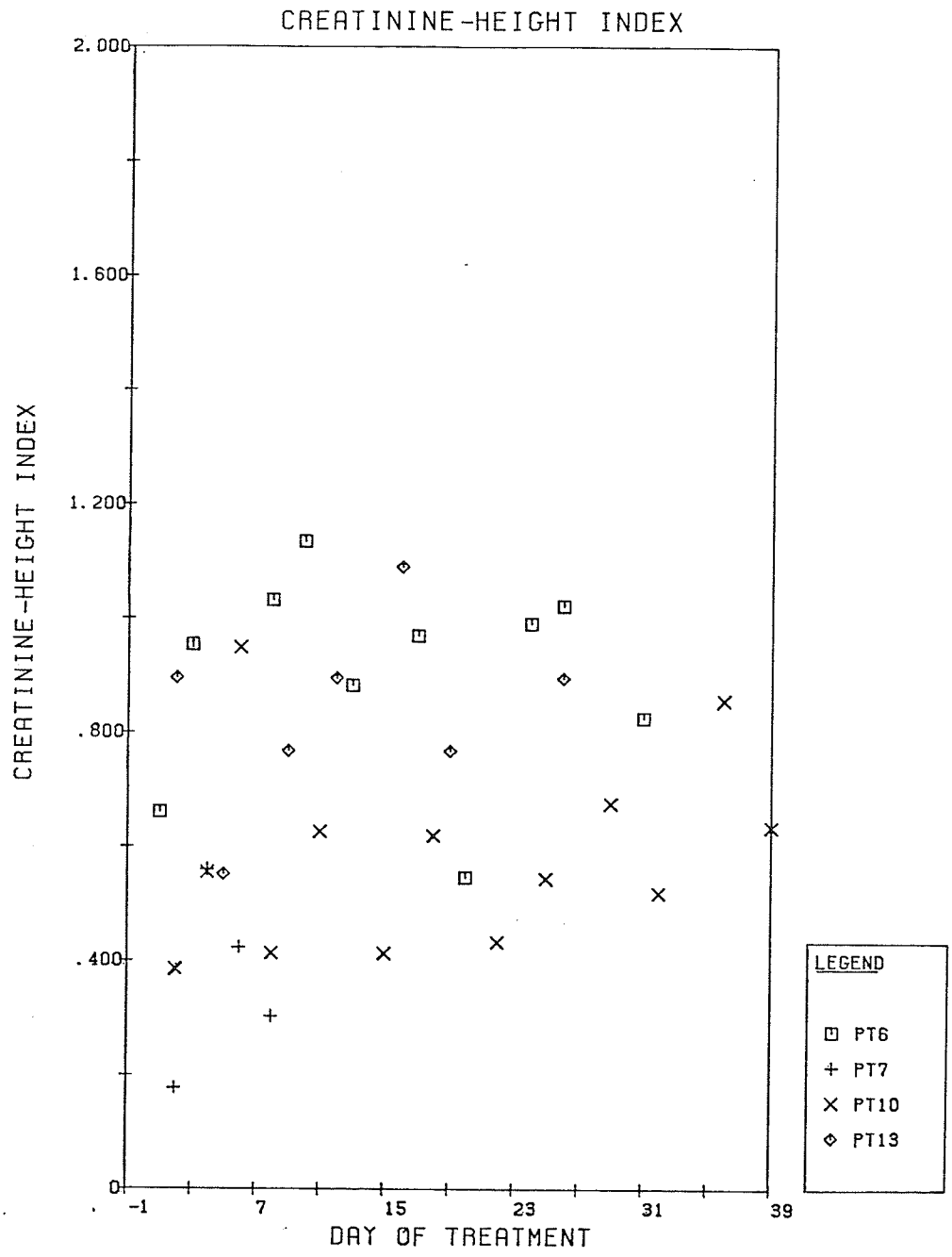

Figure 12b. Creatinine-height index in non-improved patients.

In nutritionally improved patients, the mean CHI was 0.800 ± 0.512 for initial measurements and 0.856 ± 0.433 for final measurements. This increase was not statistically significant. Four improved patients exhibited a net increase in CHI, two patients showed a decrease and four patients had little net change. Two of the latter had wide day-to-day variability. The non-improved patients, except for patient #7, also exhibited fairly wide day-to-day variability. Two of these patients had a net increase and one showed little net change.

The final value obtained for the CHI was within the normal range (0.8-1.2) for five improved and two non-improved patients. In two other improved patients, the CHI was increasing towards normal.

The ratio of urine creatinine to serum creatinine concentrations was also examined in 16 patients, and is plotted in Fig. 13a and 13b. The ratio decreased in nine of eleven improved patients and in one of five non-improved patients, and increased in one improved and two non-improved patients. However, three of the improved patients had isolated values near the end of treatment which in each case exceeded the initial value. Urine creatinine concentration itself gave similar results. Initial and final ratios were not significantly different either for improved patients or for all patients grouped together.

The ratio of urine urea nitrogen to urine creatinine,

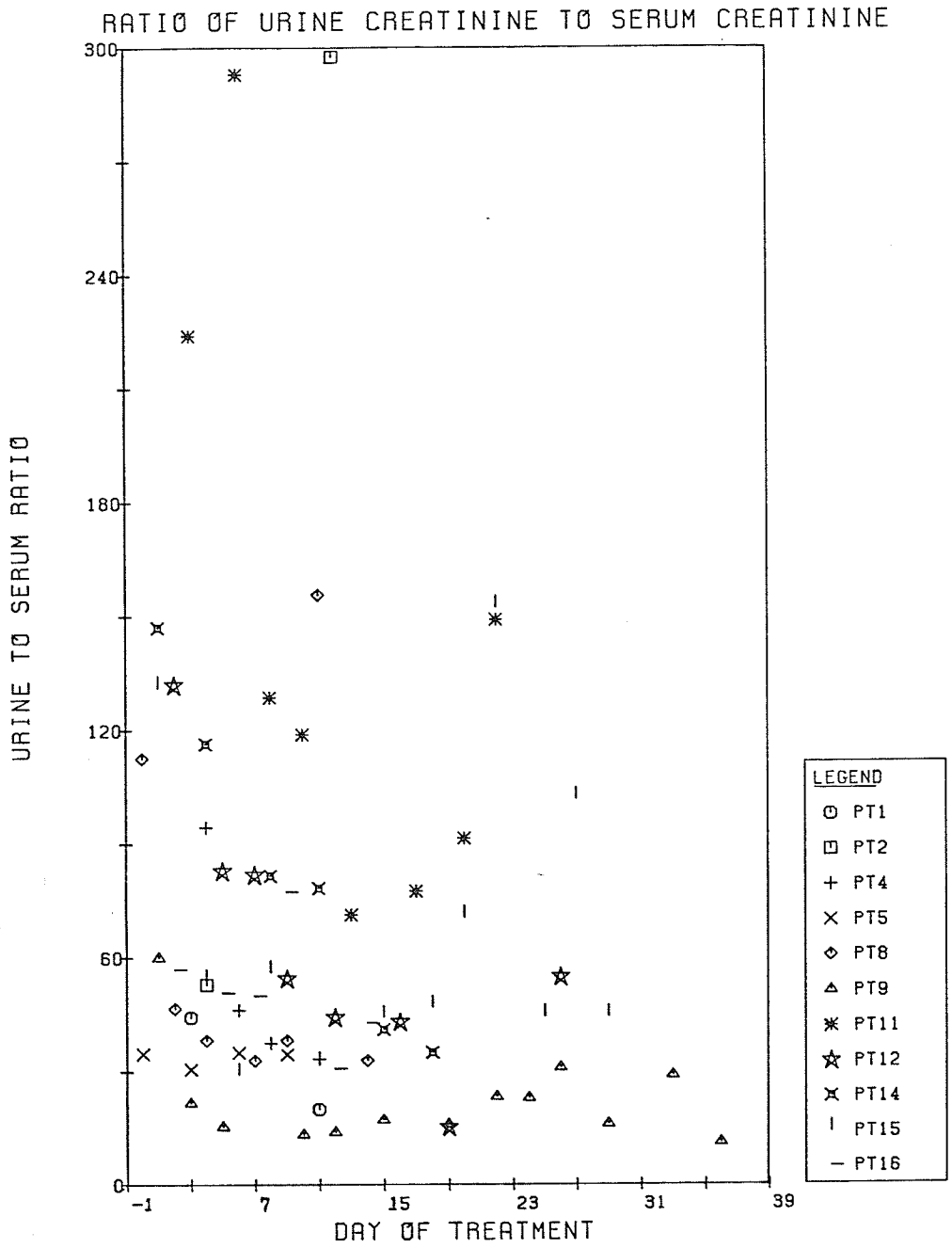

Figure 13a. Ratio of urine to serum creatinine in improved patients.

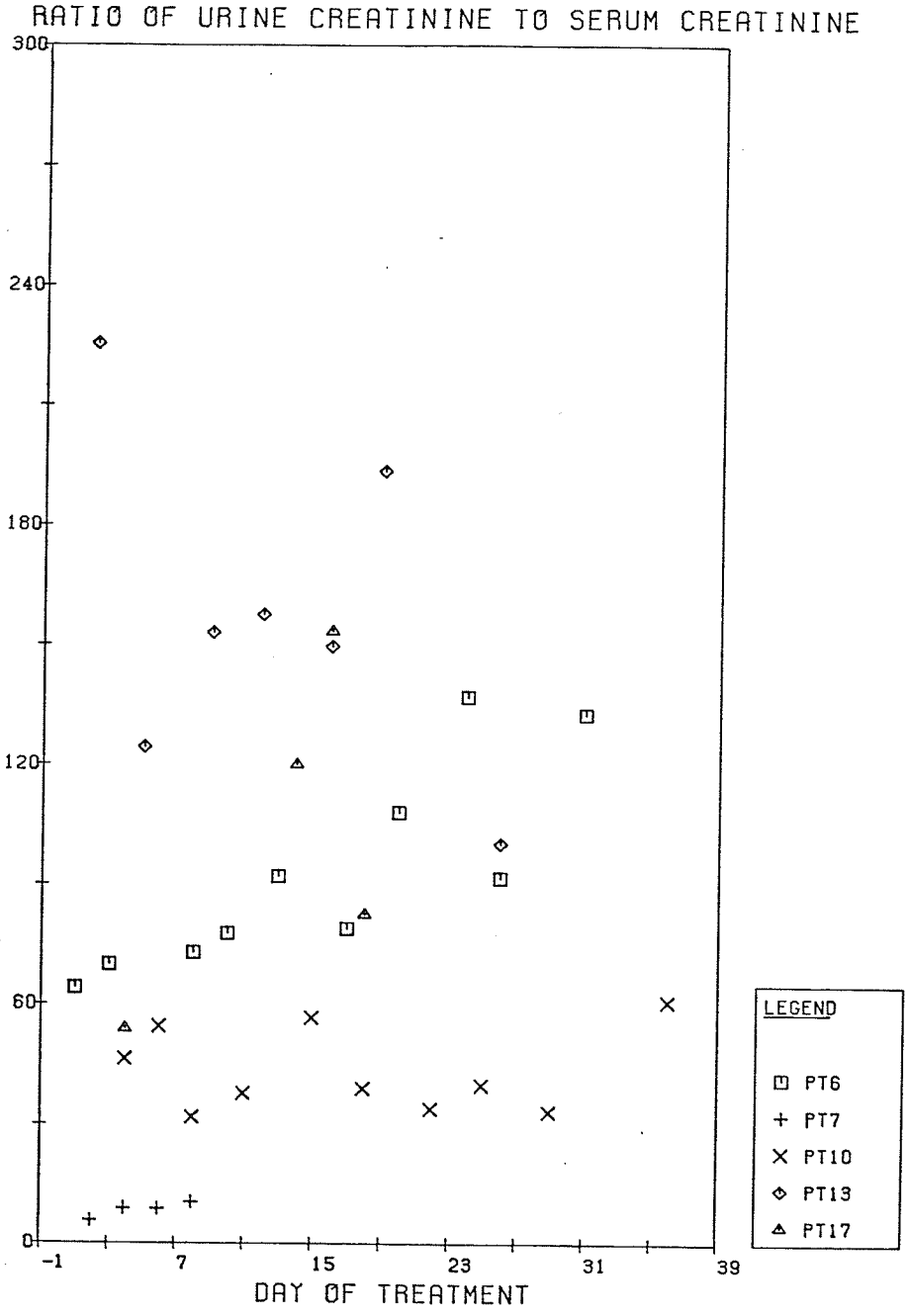

Figure 13b. Ratio of urine to serum creatinine in non-improved patients.

which has been investigated in malnourished children (Dugdale and Edkins, 1964; Simmons, 1972) gave no useful information in the present study.

5. Zinc

Serum zinc concentrations were available in 13 patients, and are plotted in Fig. 14a and 14b. Values ranged from 0.204 to 1.990 µg/ml. Initial and final mean concentrations were not significantly different. The normal range using the method employed in this hospital is 0.440 - 0.970 µg/ml. Abnormally high values occurred in patient #16 and to a lesser extent in patient #15. Several other patients had considerable fluctuation in serum concentrations, but these remained within the normal range. Only three patients had isolated values below 0.400 µg/ml.

Urine zinc concentrations were available in 15 patients, and ranged from 0.053 - 13.800 µg/ml. The broad range of values adversely affects interpretation of the results. A range of normal values for this institution was not available. Initial and final concentrations are shown in Table 7.

Extremely high urine zinc concentrations occurred in patient #13. Abnormally high concentrations were also found in patients #2 and #4 and on one occasion in patient #11. The concentrations of patients other than #2, #4 and #13 are plotted in Fig. 15. [One value for patient #11 (3.84 µg/ml on day 6) lies outside the limits of this plot.]

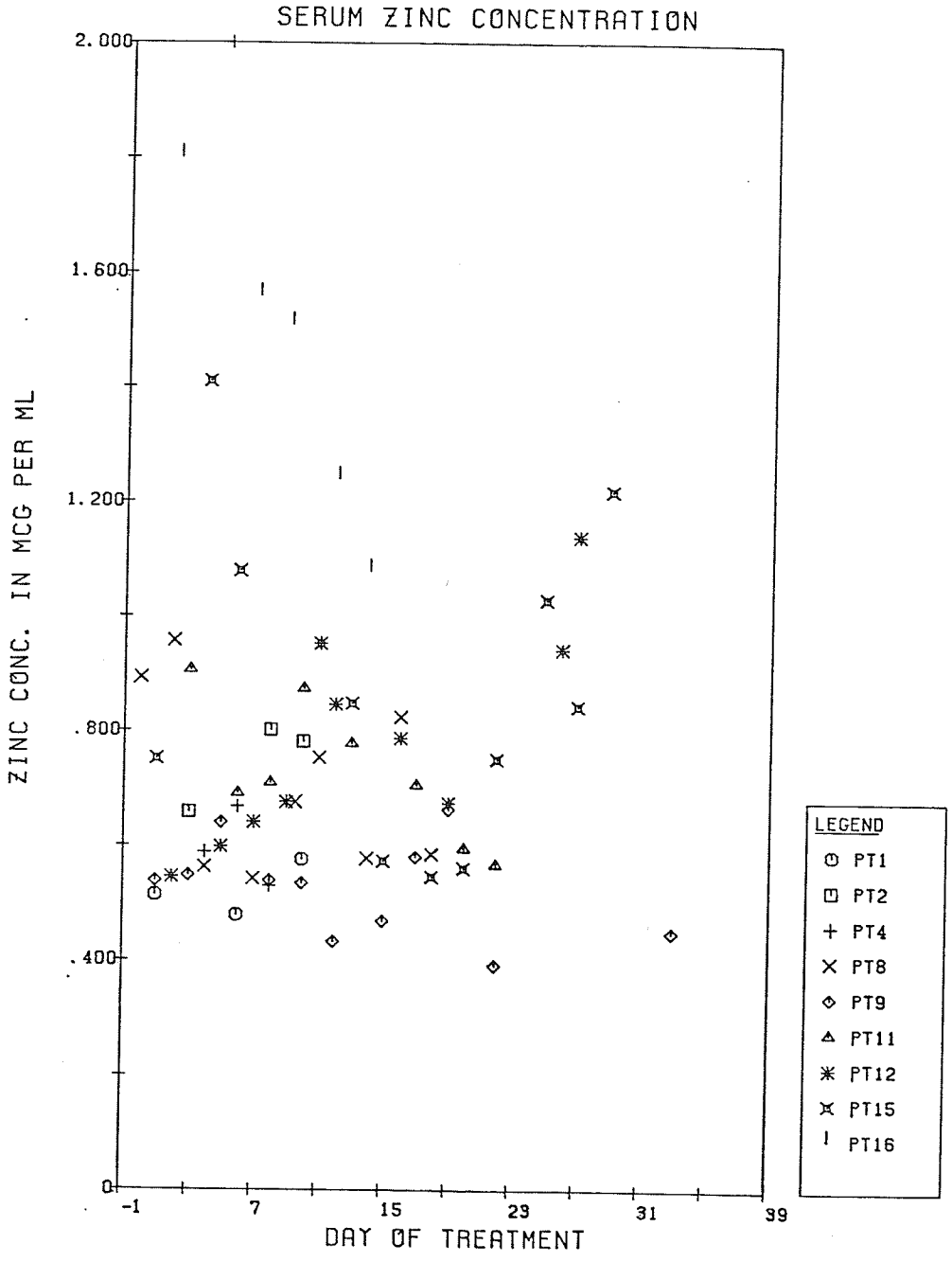

Figure 14a. Serum zinc in improved patients.

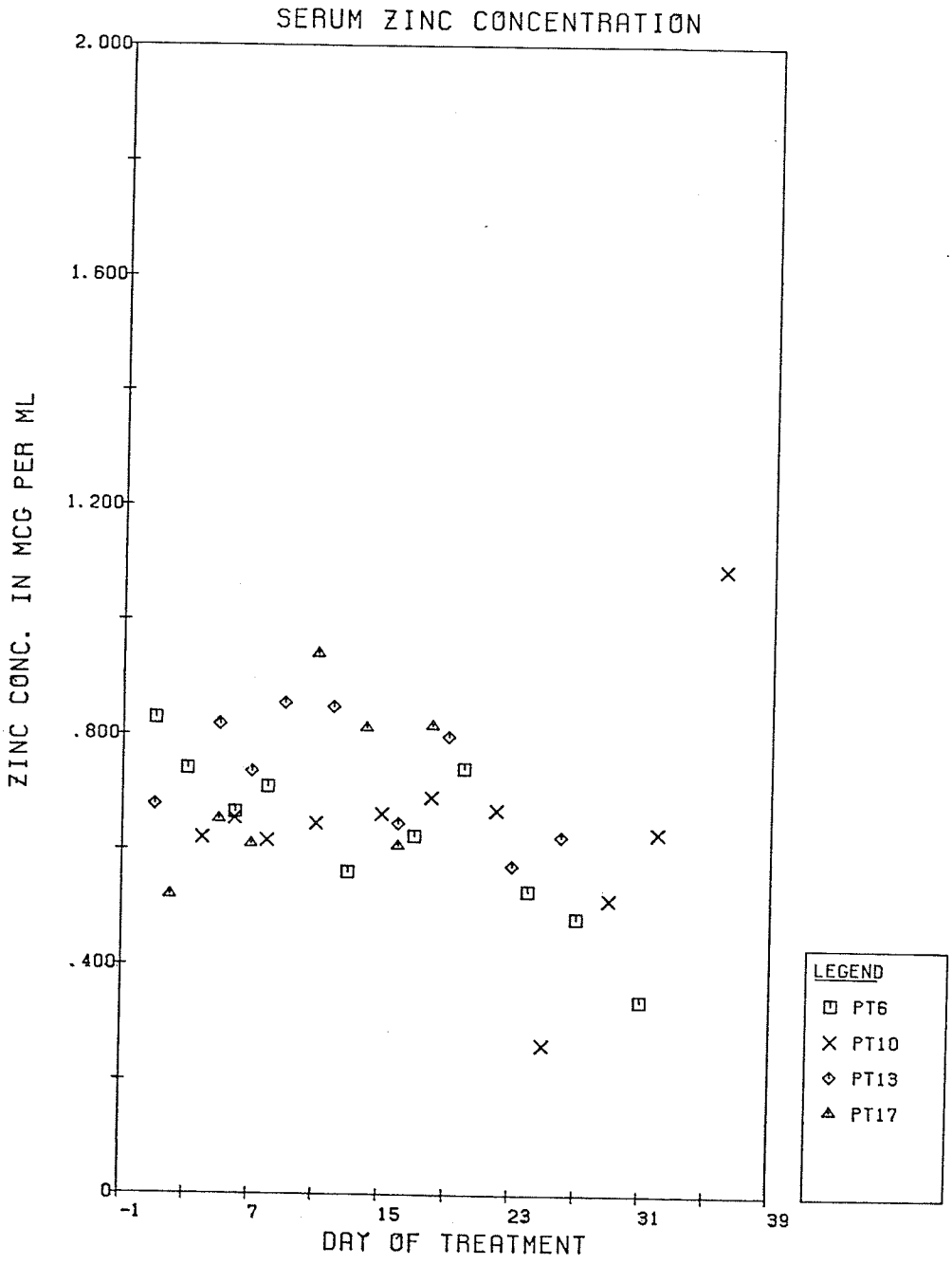

Figure 14b. Serum zinc in non-improved patients.

Table 7. Urinary Zinc Concentrations - Initial and Final Values

Classification	Patient #	Initial Concentration (μg/ml)	Final Concentration (μg/ml)
Improved patients	2	1.51	6.89
	4	3.28	.478
	5	.578	.438
	8	.297	.244
	9	.657	.061
	11	1.53	.478
	12	1.34	.978
	14	1.80	1.23
	15	.284	.181
	16	.755	.941
Non-improved patients	6	.082	.053
	7	.410	.413
	10	.316	.206
	13	12.20	2.42
	17	.461	.287

Table 8. 24-hour Urine Zinc Excretion - Initial and Final Values

Classification	Patient #	Initial Excretion (μg)	Final Excretion (μg)
Improved patients	4	6429	817
	5	1792	1511
	8	134	138
	9	322	140
	11	2173	550
	12	1350	2150
	14	245	196
	15	204	326
	16	1095	2992
Non-improved patients	6	103	57
	7	582	458
	10	316	430
	13	8420	3750

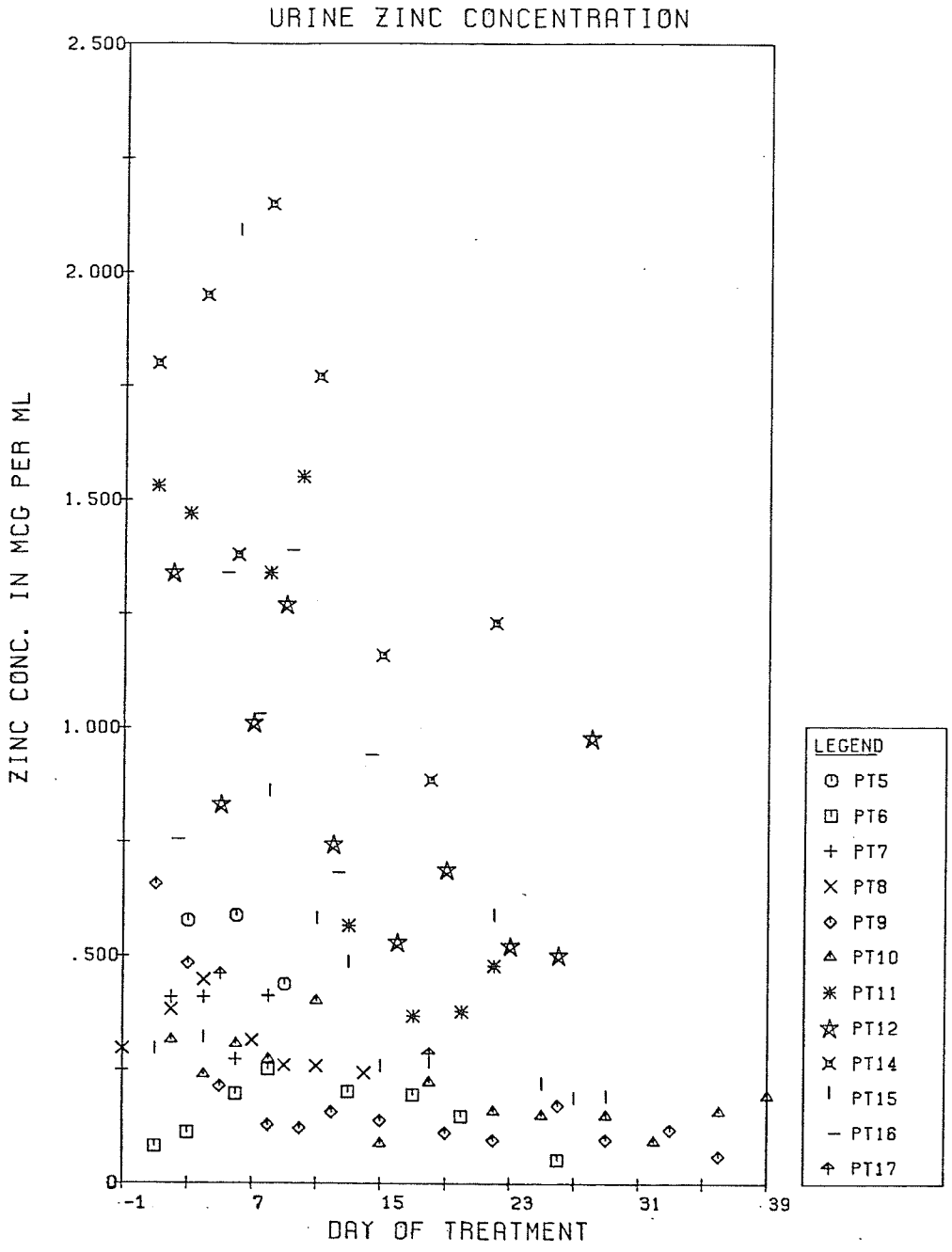

Figure 15. Urine zinc concentration.

Of the 10 improved patients, six showed a decrease and two an increase in concentration. Concentration decreased in three non-improved patients (two of whom had very high initial values) and showed little change in another. In three patients (#6, #8 and #15), there was an early increase in concentration (several-fold in patient #15) but little difference between initial and final values. There was no significant difference between the mean values for initial and final concentrations, either in the improved patients or when all patients were grouped together.

Twenty-four hour urine zinc excretion was obtained in 13 patients. Values ranged from 57 to 13,272 μg. Dawson and Walker (1969) have reported normal means of 414 μg for women and 585 μg for men. Initial and final values are shown in Table 8. Extremely high values were seen for patients #4 and #13. The 24-hour urine zinc excretions of the remaining patients are plotted in Fig. 16a and 16b. Initial and final values were not significantly different, either in improved patients or when all patients were grouped together.

The ratio of urinary zinc concentration to serum zinc concentration was also examined in relation to nutritional status. Extremely high ratios were observed in two patients (#4, #13). Of the 9 other patients for whom ratios were available, five patients (3 improved and 2 non-improved) showed a net decrease, one patient (#16) had an increase and three patients had little net change. There was no significant difference between the initial and final ratios in

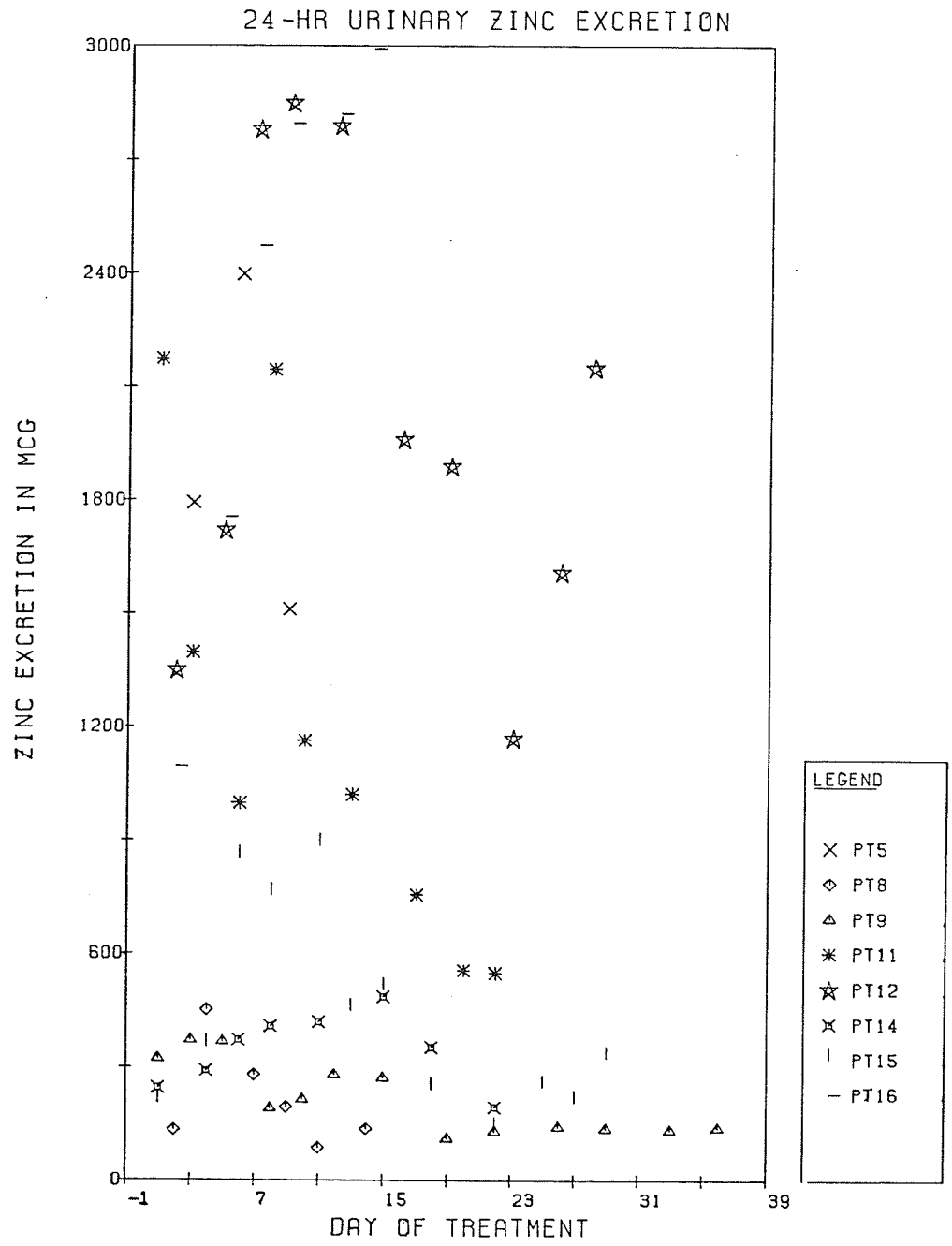

Figure 16a. 24-hour urine zinc excretion in improved patients.

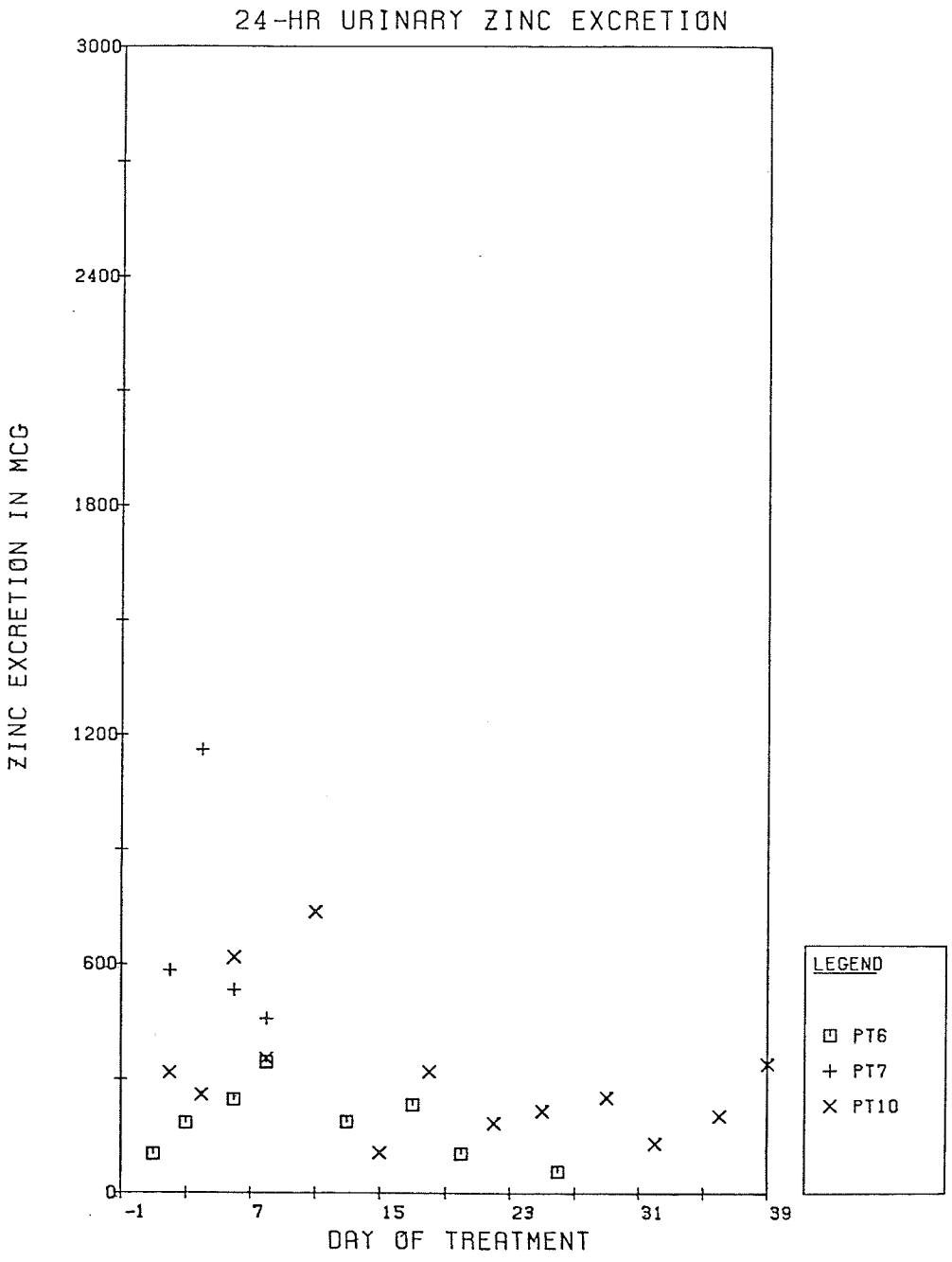

Figure 16b. 24-hour urine zinc excretion in non-improved patients.

improved patients or when all patients were grouped together.

6. Ribonuclease

Serum RNase concentrations, which were obtained in 15 patients, are plotted in Fig. 17a and 17b. Values ranged from 0.110 to 0.864 μg/ml. Houck and Berman (1958) reported a mean of 0.38 ± 0.04 μg/ml for normal subjects. A normal range of RNase values for this institution was not obtained.

A majority of patients, both improved and non-improved, exhibited an increase in serum RNase concentration during TPN. In improved patients, the initial and final concentrations, with means of 0.274 ± 0.037 μg/ml and 0.351 ± 0.187 μg/ml respectively, were not significantly different. However, when all patients were grouped together, the mean of the initial concentrations, 0.281 ± 0.086 μg/ml, was significantly lower than the mean of the final concentrations, 0.384 ± 0.191 μg/ml ($P<0.025$). Similarly, in non-improved patients, the mean of the initial concentrations, 0.295 ± 0.150 μg/ml, was significantly lower than the mean of the final concentrations, 0.450 ± 0.202 μg/ml ($P<0.01$).

Several patients showed a wide range of serum concentrations. Patient #9 had high concentrations and a wide range of RNase values, in contrast to most other variables in this patient.

Urine RNase concentrations were available in 14 patients, and are plotted in Fig. 18a and 18b. Values ranged from 0.052 to 1.528 μg/ml. A range of normal values for this institution was not obtained.

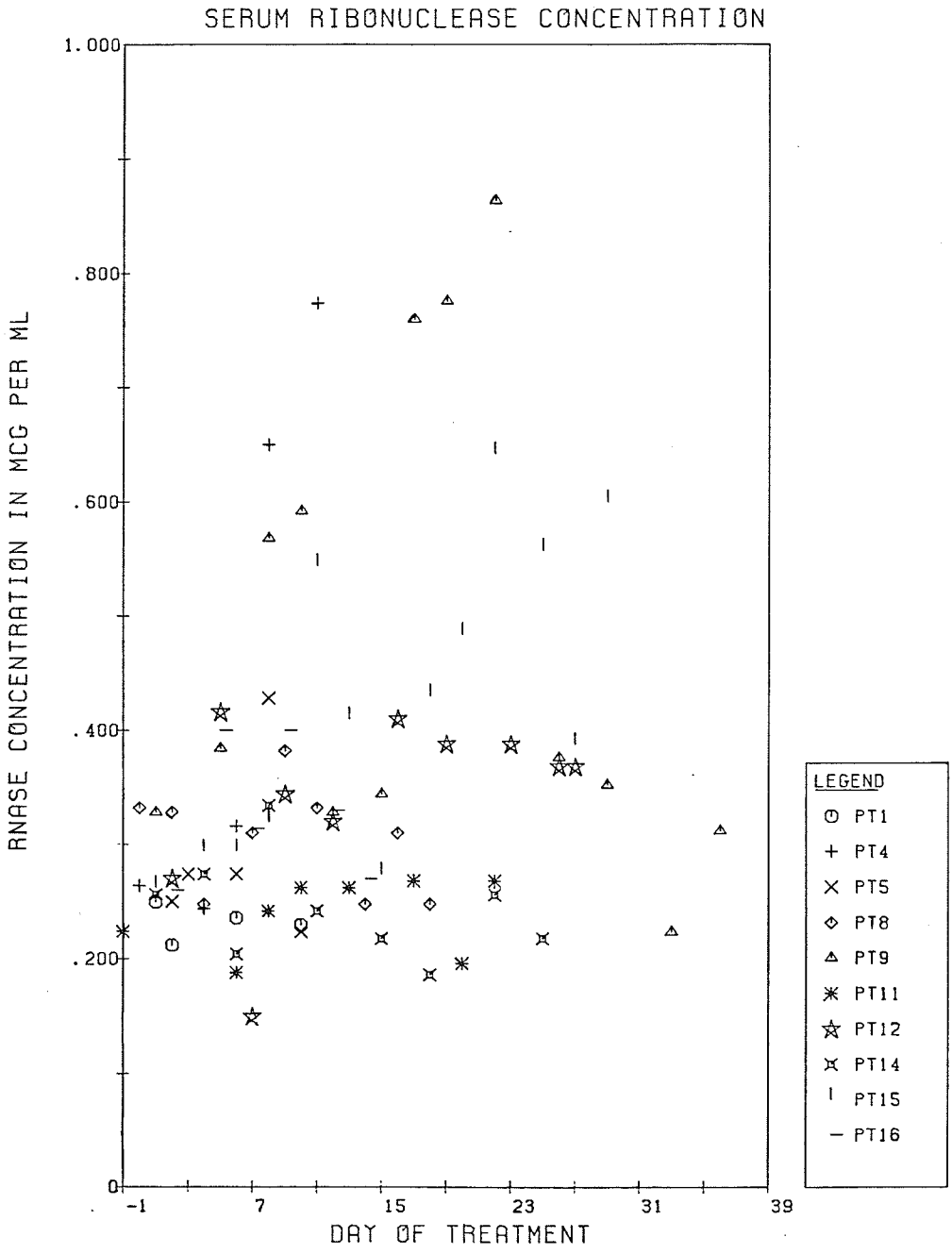

Figure 17a. Serum ribonuclease in improved patients.

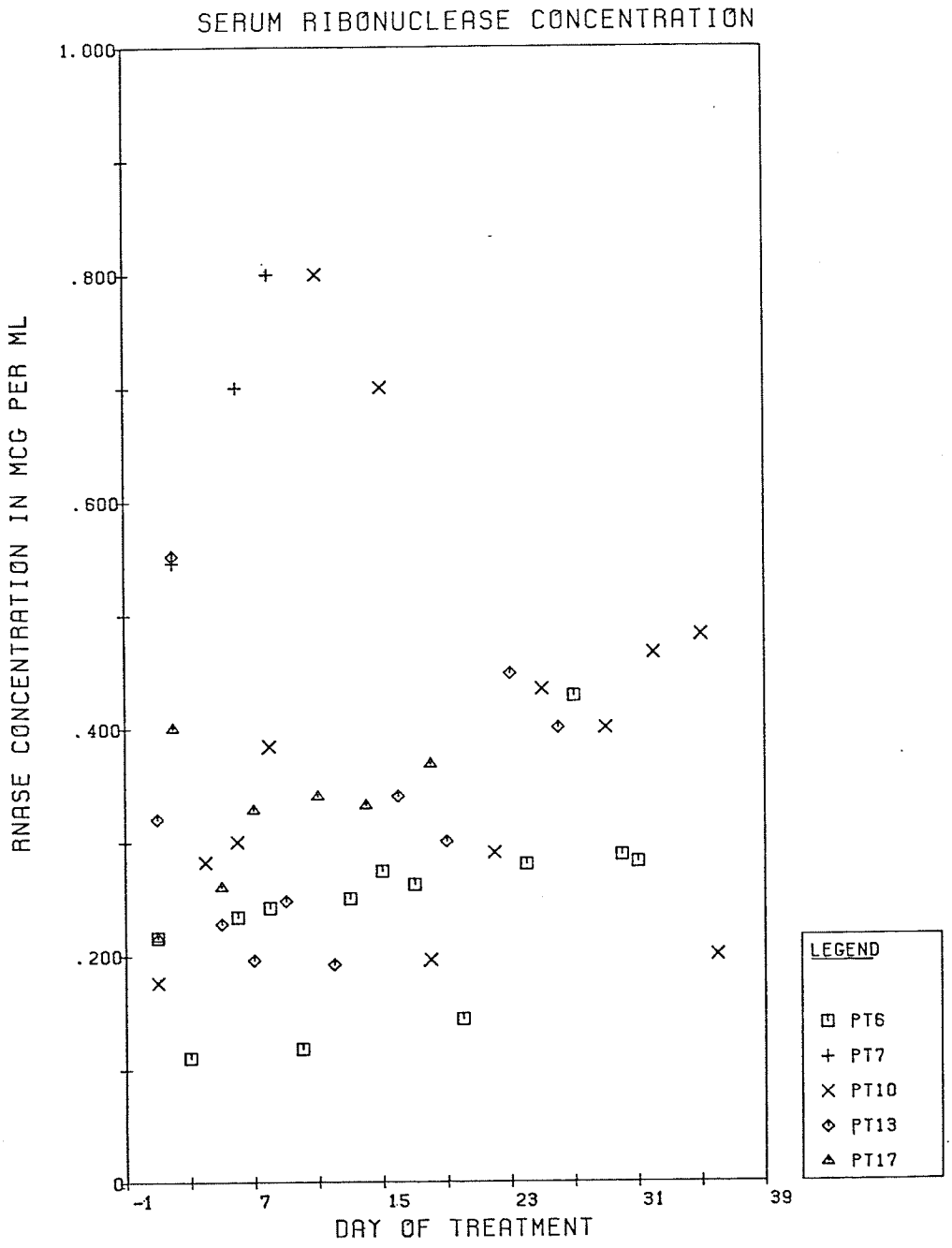

Figure 17b. Serum ribonuclease in non-improved patients.

Of the ten nutritionally improved patients, eight exhibited a net decrease in urine RNase concentration. Of the four non-improved patients, two patients showed a marked increase, one patient had a decrease and one patient had fairly wide fluctuations but little net change. The initial values of the improved patients, with a mean of 0.773 ± 0.425 µg/ml, were significantly higher than the final values, with a mean of 0.425 ± 0.134 ($P<0.025$). When all patients were grouped together, the initial and final means, 0.690 ± 0.410 µg/ml and 0.505 ± 0.374 µg/ml respectively, were not significantly different.

Patient #6, with Crohn's disease, showed a fairly steady, marked increase in urine RNase concentration which was not associated with any visible stress. To investigate this observation, RNase levels were determined for 2 other patients with Crohn's disease, who were not part of the original study. Both these patients received TPN for bowel rest, for periods of about 26 days. One patient (A) was then discharged, while the other (patient B) underwent surgery and a further 10 days of TPN. Serum concentrations of both patients were in the high normal range. Urine concentrations were unusually low, as were the initial concentrations in patient #6. In patient A, the urine RNase concentration decreased gradually but not substantially. In patient B, the concentration decreased quite steadily until the time of surgery, after which it promptly increased to well above the initial level. There was no increase prior to surgery

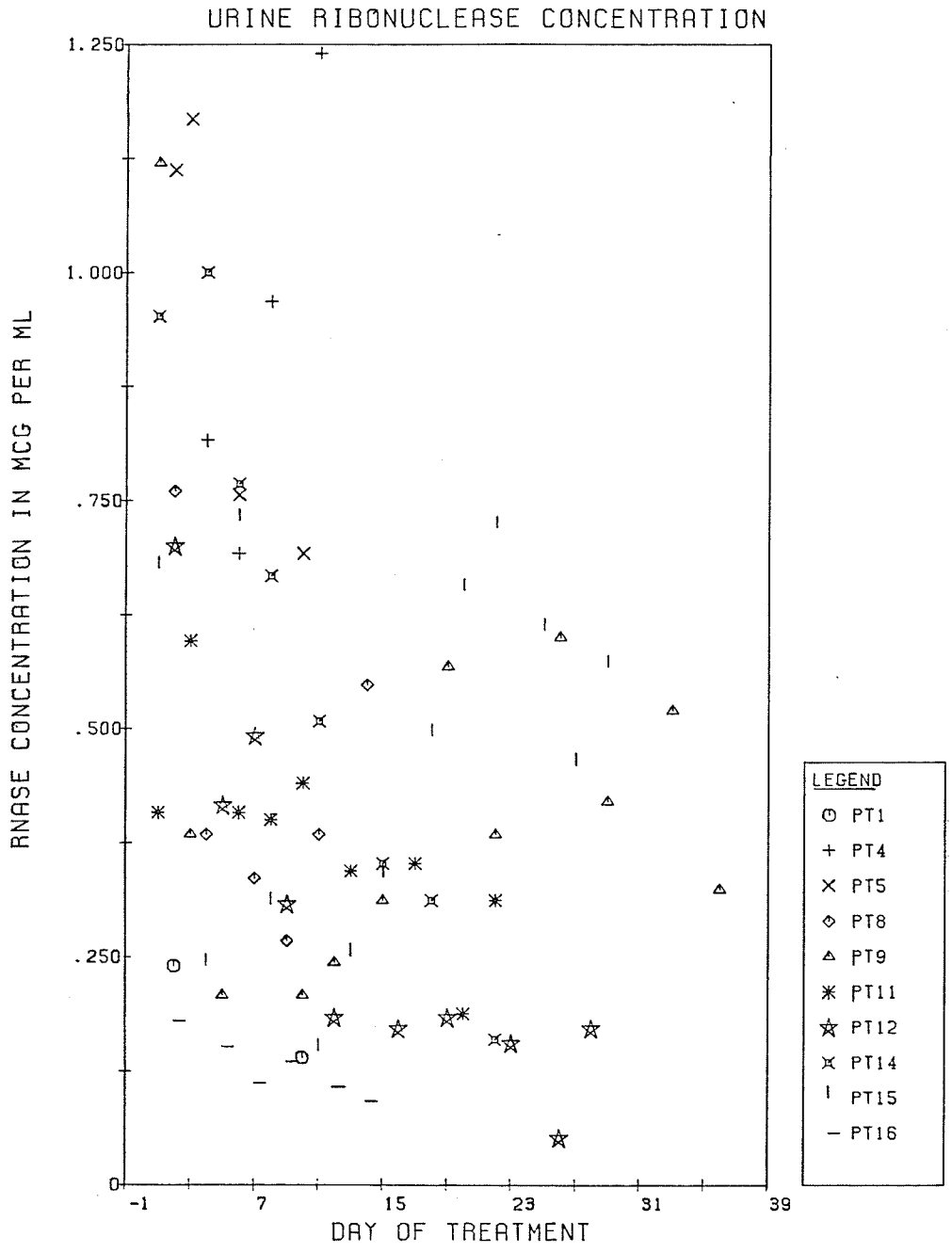

Figure 18a. Urine ribonuclease in improved patients.

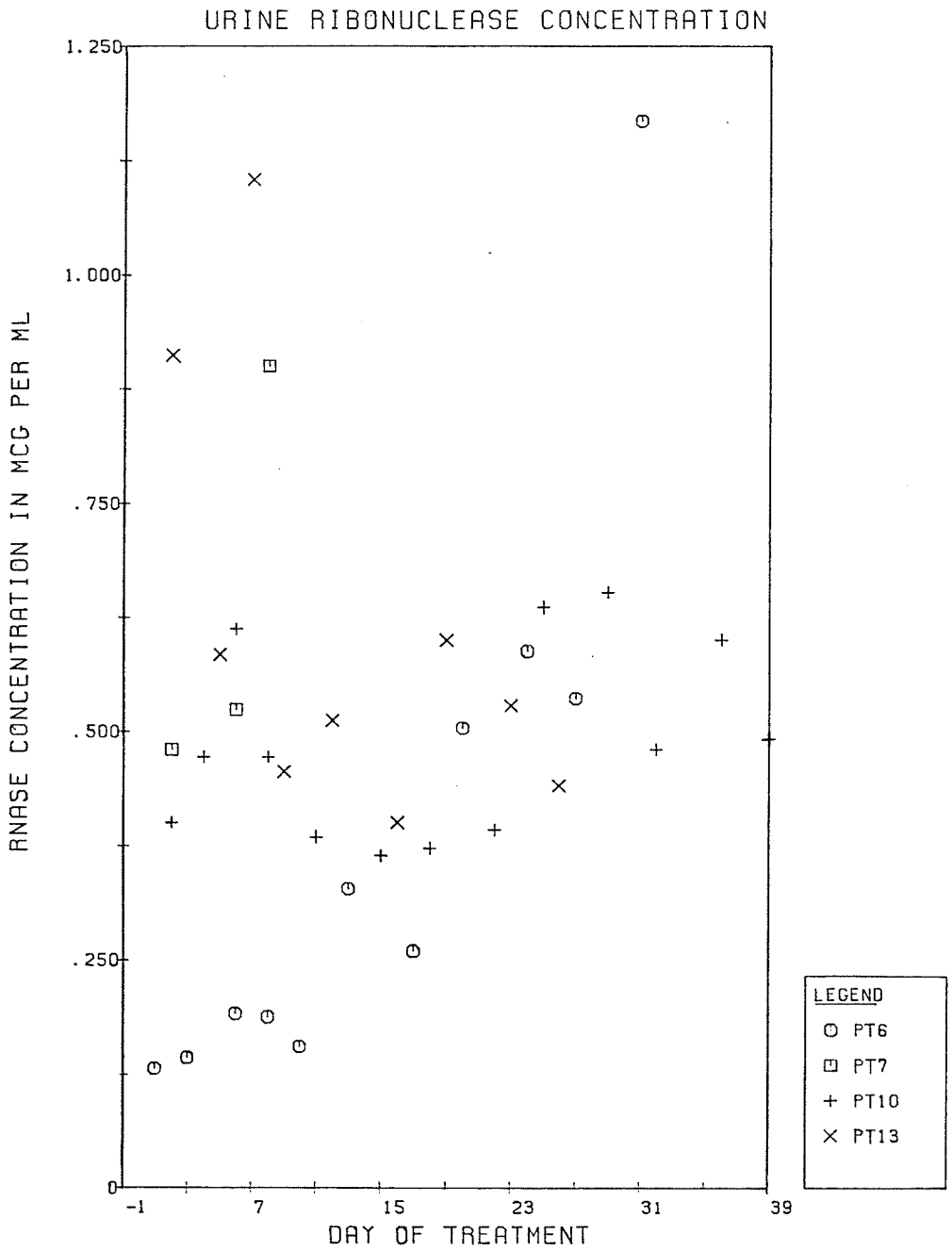

Figure 18b. Urine ribonuclease in non-improved patients.

as occurred in patient #6.

The 24-hour urinary excretion of RNase was determined in 13 patients. Values ranged from 106.1 to 2604.0 μg in women and from 166.4 to 3620.8 μg in men. The difference between initial and final values was not significant either for improved patients or for all patients grouped together.

In the four non-improved patients, 24-hour urinary RNase excretion either increased or showed little change. Changes in the improved patients often did not correspond to changes in the urinary concentration. Only three of the improved patients showed a net decrease in 24-hour RNase excretion, while five patients exhibited little net change.

The ratio of urine RNase concentration to serum RNase concentration was also examined in relation to nutritional status. Results were available in 14 patients, and are plotted in Fig. 19a and 19b.

All of the 10 nutritionally improved patients showed a net decrease in urine/serum RNase concentration. Of the 4 non-improved patients, the ratio increased markedly in one, showed little net change in another, and showed wide fluctuations in the remaining patients (#10, #13).

The mean of the initial values of the improved patients, 2.873 ± 1.302, was very significantly higher than the mean of the final values, 1.177 ± 0.905 ($P<0.0005$). When all the patients were grouped together, the initial mean, 2.396 ± 1.361, was still significantly higher than the mean of the

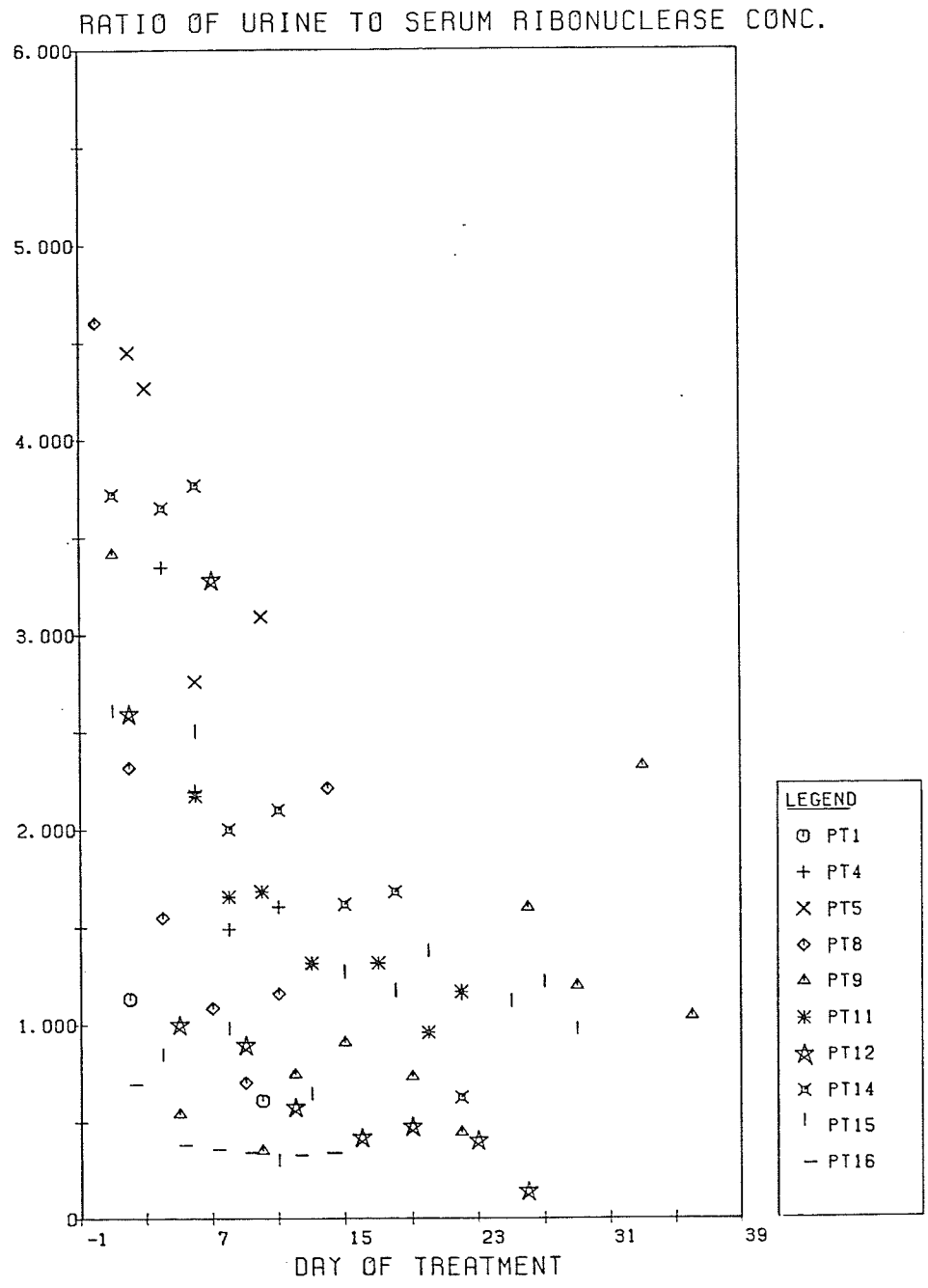

Figure 19a. Ratio of urine to serum ribonuclease in improved patients.

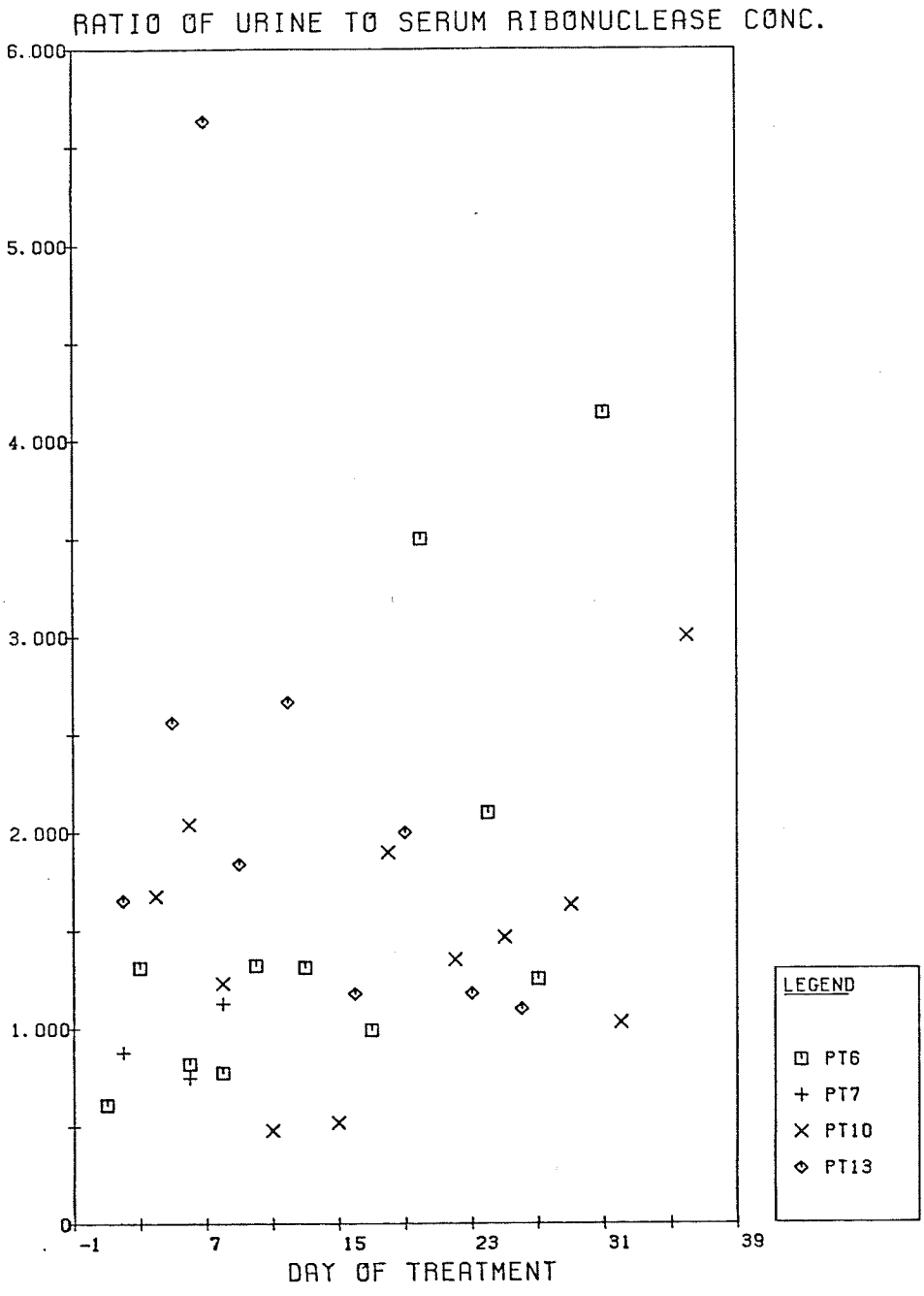

Figure 19b. Ratio of urine to serum ribonuclease in non-improved patients.

final values, 1.359 ± 1.102 ($P<0.025$), but an examination of Fig. 19b shows that this latter result is clearly due to the values of the improved patients.

Relationships between the various RNase measurements are shown for specific patients in Figures 20a, 20b and 20c. In these plots, values are expressed as a fraction of the initial value. The symbols in the legend represent, in descending order, the urine RNase concentration, the 24-hour urinary excretion and the ratio of urine to serum concentration.

7. Nitrogen Balance

Nitrogen balances, which were obtained for twelve patients, ranged from -19.1 to +15.0 grams. The results are shown in Fig. 21a and 21b.

The lowest values occurred in the two post-surgical patients (#5, #14) and the highest values in patient #15. Only two patients (#9, #15) had consistent nitrogen balances greater than 4.0 grams. Two other patients had predominantly positive balances and three patients had predominantly negative balances. In three patients the values fluctuated between -4.0 and +4.0 grams, and in one patient the balance remained at approximately zero.

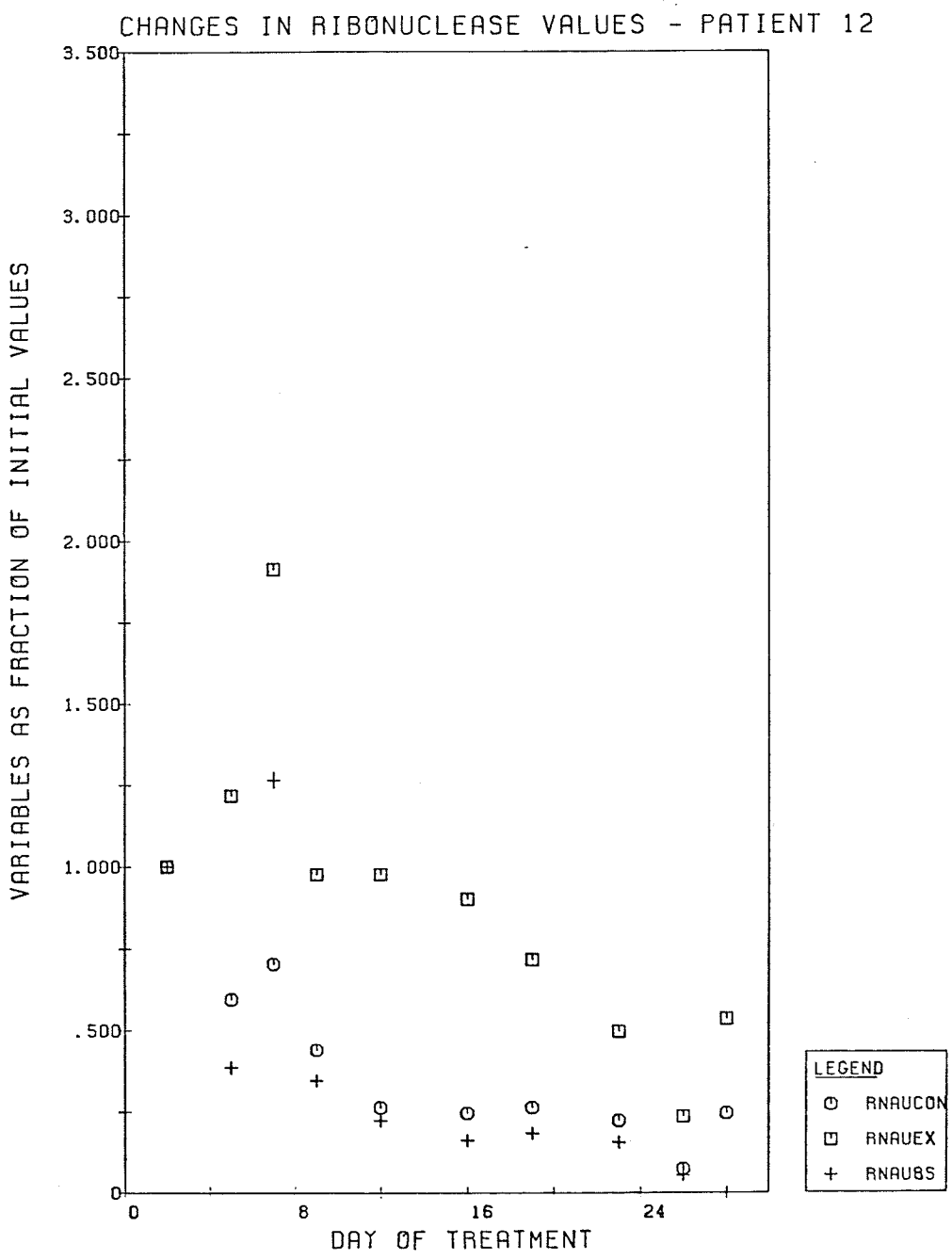

Figure 20a. Ribonuclease values - Patient 12.

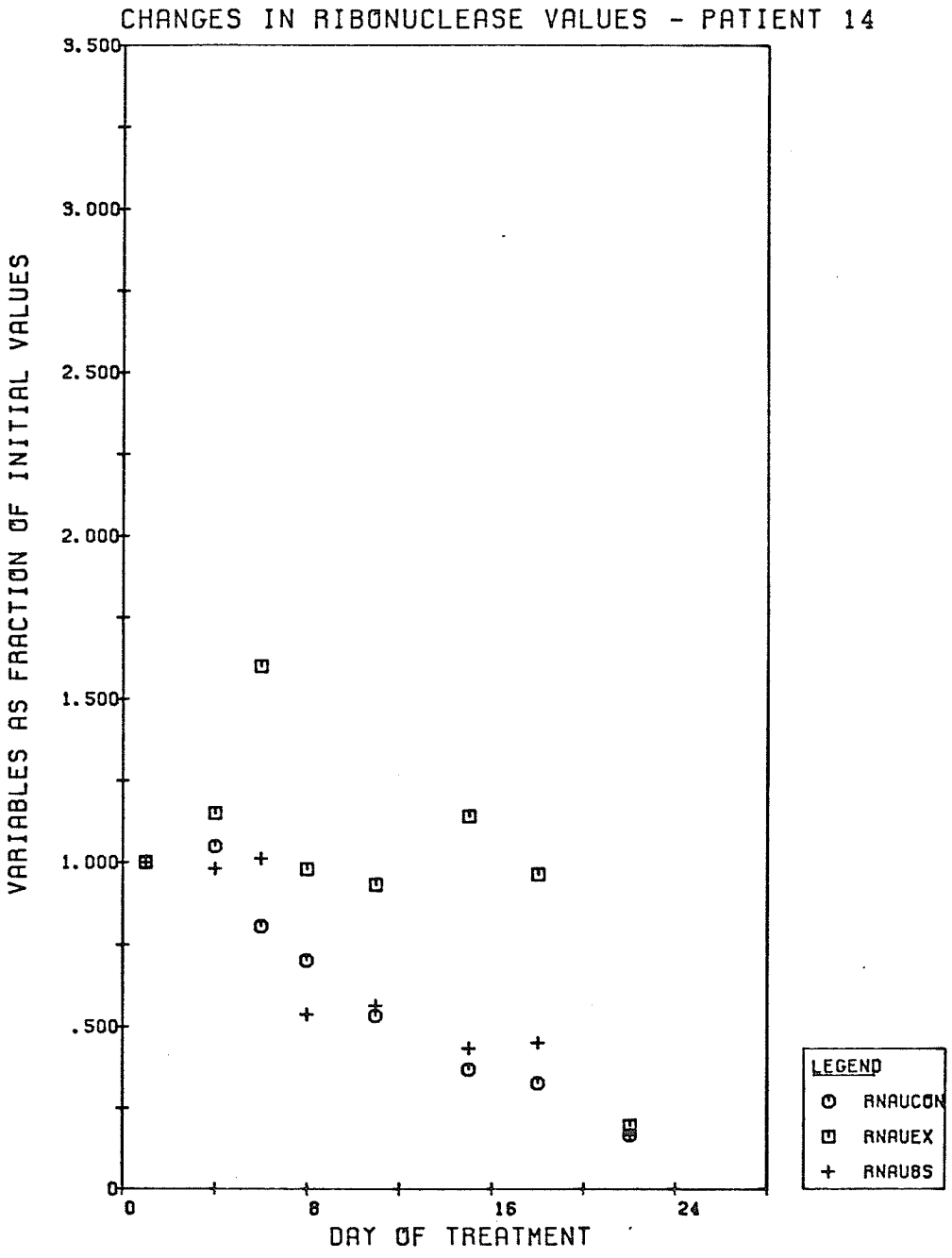

Figure 20b. Ribonuclease values - Patient 14.

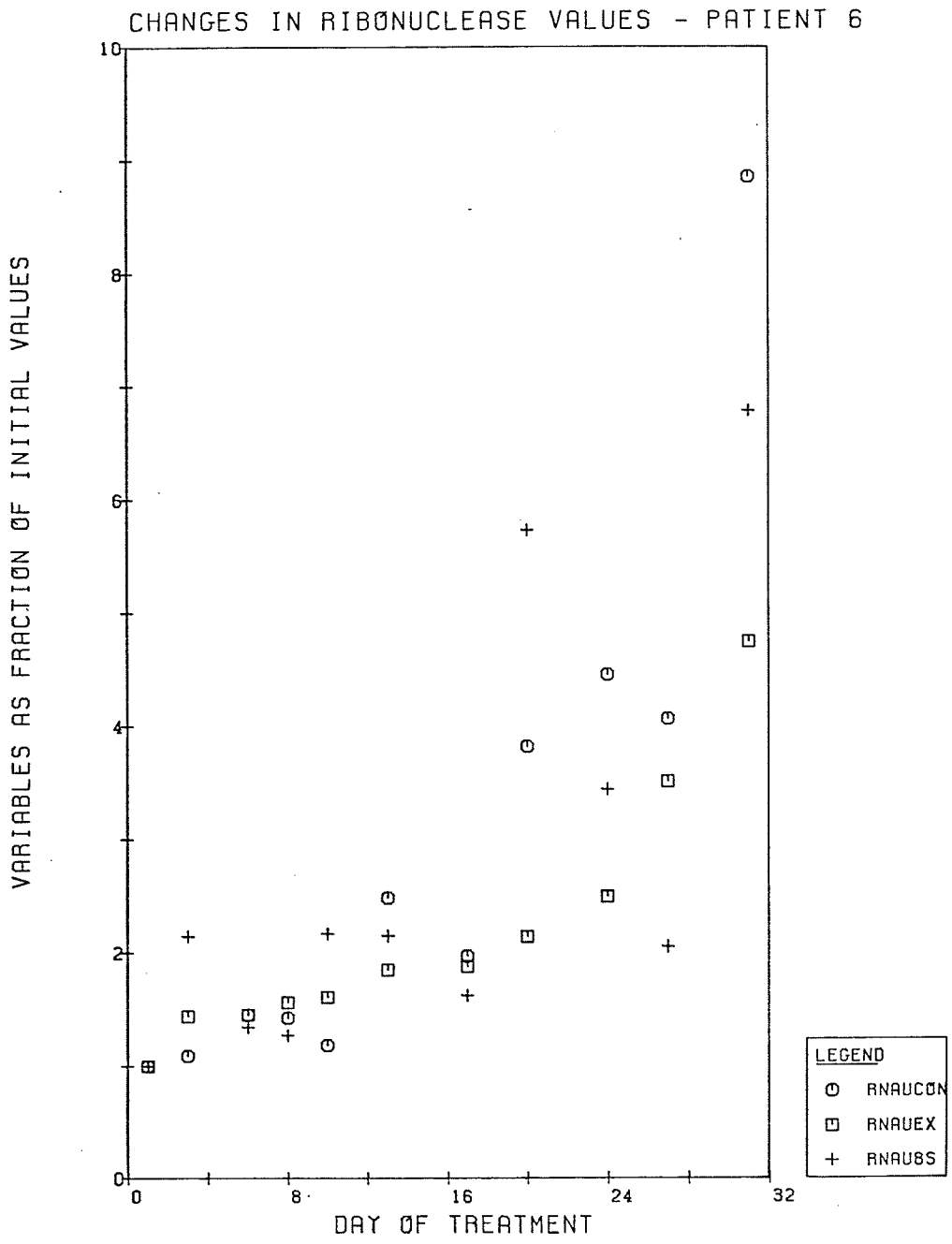

Figure 20c. Ribonuclease values - Patient 6.

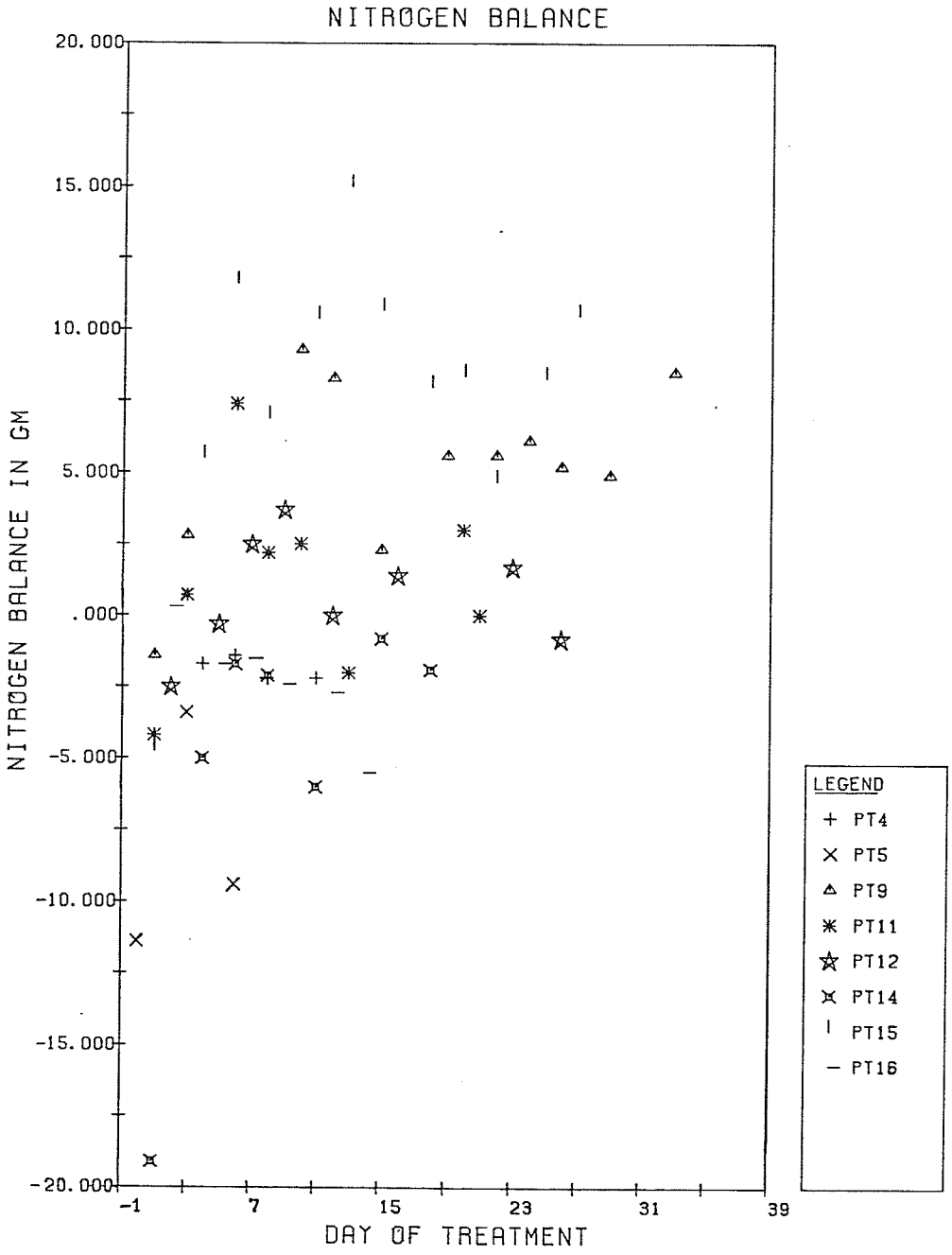

Figure 21a. Nitrogen balance in improved patients.

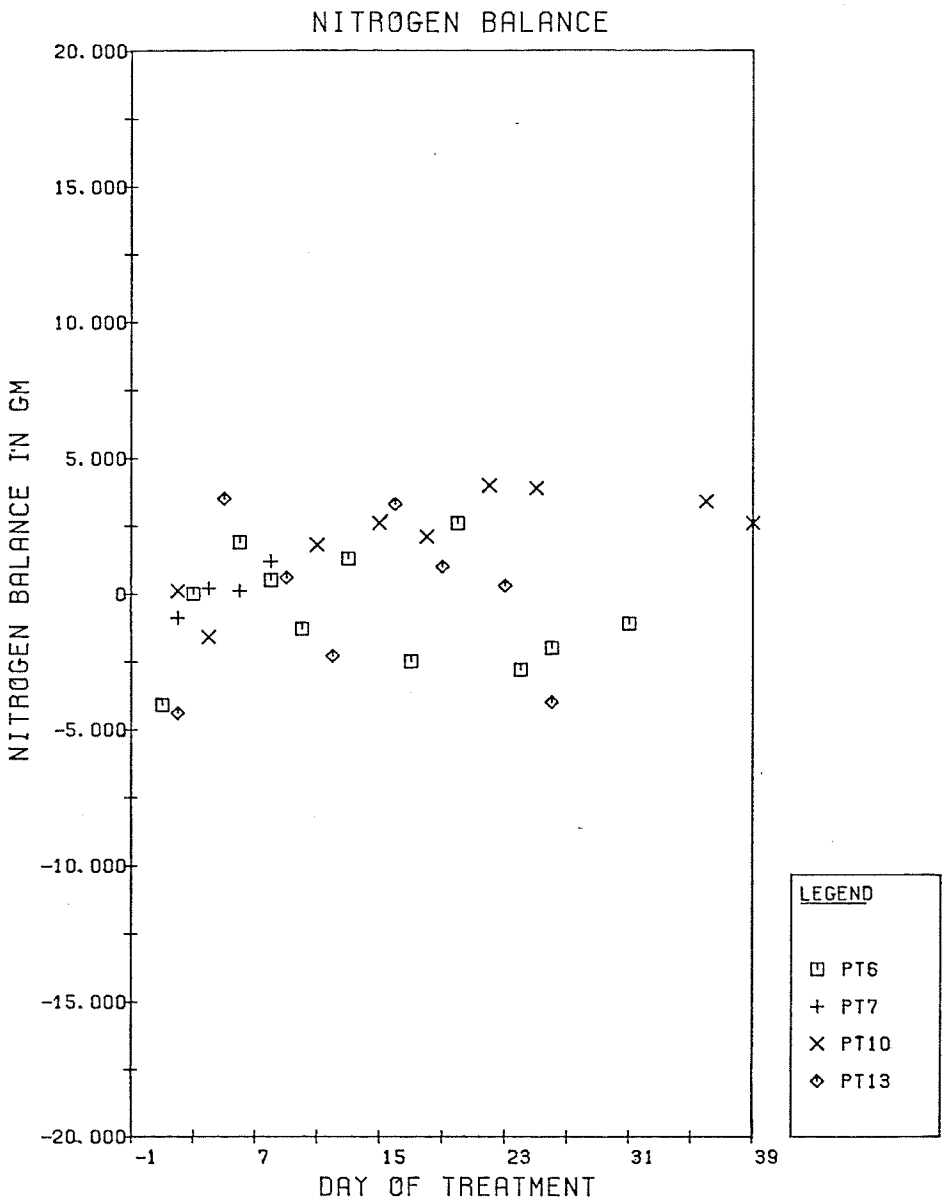

Figure 21b. Nitrogen balance in non-improved patients.

C. Immune Competence

1. Immunoglobulins

Serum concentrations of immunoglobulins IgG, IgM and IgA were available for 11 patients, and are shown in Table 9. Most concentrations were within the normal range.

For IgG, no patients had concentrations above the limits of normal, while two patients had values below the normal limits. Seven patients exhibited a moderate increase in concentration during TPN, while two patients showed little change and two patients had a slight decrease in concentration.

For IgM, two patients had concentrations above the normal limits. Three patients had initial concentrations below the lower limits of normal. During TPN, six patients showed an increase in IgM concentration, two patients showed a decrease and three patients exhibited little change.

For IgA, two patients had concentrations above the normal limits and one patient had concentrations below normal. Seven patients exhibited an increase in concentration during TPN, one patient (#9) had little change and three patients showed slight decreases in concentration.

2. Complement

Serum concentrations of complement, which were available for 11 patients, are shown in Table 9. Most concentrations were within the normal limits.

Table 9. Immunoglobulin and Complement Levels (in mg%)

Patient #	Day of Therapy	IgG	IgM	IgA	C3	C4
4	2	1310	298	286	86	27
	16	1525	333	320	106	30
5	3	835	94	410	124	23
	10	1100	114	493	178	29
6	1	625	50	176	133	38
	15	800	127	234	137	41
	24	840	128	217	144	37
9	1	1300	58	585	66	66
	15	1290	67	520	84	51
	37	1330	105	581	88	50
10	4	1325	46	755	157	47
	18	1250	57	715	154	43
11	2	695	143	236	121	39
	14	720	148	259	107	35
	24	860	157	285	170	51
12	2	925	107	89	105	37
	16	880	108	101	125	40
	27	975	128	115	162	43
13	2	1335	159	535	189	49
	16	1200	144	502	177	40
14	6	990	337	358	116	26
	18	1110	223	327	184	33
15	4	925	98	264	165	46
	15	920	135	280	200	43
	22	970	130	300	180	43
16	5	940	133	260	187	32
	16	910	117	300	155	38

For C3, only one patient (#9) had serum levels below the normal range, and none had levels above the normal range. Eight patients exhibited an increase in concentration during TPN, two non-improved patients (#10,#13) showed little change, and one patient (#16) had a decrease in concentration. The mean of the final values, 154.4 $\pm$ 31.2 mg%, was significantly higher ($P<0.05$) than the mean of the initial values, 131.7 $\pm$ 39.5 mg%.

For C4, only one patient (#9) had serum levels above the normal range, and none had levels below the normal range. Five patients had a moderate increase in concentration during TPN, two patients (#9,#13) had a moderate decrease and four patients showed little change. There was no significant difference between the initial concentrations (mean of 39.1 $\pm$ 12.5 mg%) and the final concentrations (mean of 39.7 $\pm$ 7.3 mg%).

3. T and B Lymphocytes

Initial and final counts of T-lymphocytes were obtained in only six patients, although a single count was done on most patients. Results are shown in Table 10. All six patients had below-normal percentages of T-lymphocytes, although one patient (#16) had a final value within the normal range. Extremely low values were observed in patient #10. Values increased in three of the four improved patients, but initial and final percentages were not significantly different.

Table 10. T-lymphocytes: Initial and Final Values

Patient #	Day of Therapy	Percent of T-lymphocytes
6	3	53
	24	46
10	5	10
	87	23
11	2	44
	24	57
12	5	47
	28	47
15	4	17
	22	50
16	5	54
	16	70

The B cell count was expressed in terms of the fluorescent, mature, non-secretory immunoglobulins adhering to the B lymphocytes. The total immunoglobulin count was subdivided according to whether IgG, IgM or IgA adhered to the lymphocyte. Each of these was expressed as a percentage of the total immunoglobulins.

Initial and final percentages of immunoglobulins were available in only five patients. The results are shown in Table 11. There was little change in the total percentage of immunoglobulin (Ig) cells during the study period. The percentage of IgG cells increased markedly in three patients but decreased markedly in patient #15. The initial and final

Table 11. Percentage of Immunoglobulins

Patient #	Day of Therapy	Total Ig cells (%)	IgG cells (%)	IgM cells (%)	IgA cells (%)
6	3	10	10	2	-
	24	14	15	6	-
11	2	20	10	5	3
	24	24	11	9	4
12	5	32	13	5	3
	28	30	22	6	0
15	4	27	21	3	0
	22	27	9	6	1
16	5	25	13	7	2
	16	25	23	7	0
Mean $\pm$ SD (%)		23.4 $\pm$ 6.9	14.7 $\pm$ 5.4	5.6 $\pm$ 2.0	-

values were not significantly different. The percentage of IgM cells increased in four patients, but there was no significant difference between the initial and final values. In some cases, IgA cells could not be detected.

4. Delayed Cutaneous Hypersensitivity

Results of initial and final skin testing for delayed hypersensitivity were available for only five patients, although most patients received the initial set of skin tests. The initial tests were negative in all patients. During the final set of tests, only one of the five patients exhibited a definite positive reaction (with induration). This patient (#16) reacted positively only to tricophyton. Two other

patients (#11, #14) showed definite redness but no induration.

D. Statistical Relationships

1. Correlation Coefficients

A Pearson product-moment correlation coefficient, which describes the relationship between two mutually dependent variables, was computed for any two study parameters between which a relationship might be expected. All values for all patients were included in the computations for each parameter.

Good correlations were found in only seven instances. Anthropometric correlations occurred between weight and height ($r = .7578$, $P<0.01$), between weight and mid-arm circumference ($r = .9554$, $P = 0.001$), between weight and triceps skinfold thickness ($r = .7220$, $P = 0.00001$) and between triceps skinfold thickness and arm muscle circumference ($r = .8216$, $P = 0.001$). The remaining three correlations were between related variables. These were between urine zinc concentration and 24-hour urine zinc excretion ($r = .8843$, $P = 0.001$), between urine RNase concentration and the ratio of urine to serum RNase ($r = .7920$, $P = 0.00001$) and between urine zinc concentration and the ratio of urine to serum zinc ($r = .9804$, $P = 0.001$).

2. Discriminant Analysis

Discriminant analysis is a statistical procedure which evaluates a number of variables according to their ability to distinguish between two or more groups of cases. In the

present example, discriminant analysis was performed to determine which variables were best able to distinguish between the improved and the non-improved groups of patients. The variables included in the analysis were those which appeared most useful, based on the data already presented. These variables were weight, triceps skinfold, serum prealbumin, serum transferrin, urine ribonuclease concentration and the ratio of urine to serum ribonuclease concentration.

For weight, triceps, transferrin and urine RNase concentration, all values of each variable for each patient were plotted against the day of therapy, and the regression lines were calculated. For the remaining two variables (prealbumin and the urine/serum RNase ratio), for which fewer data points were available, the value used for each patient was the slope of the line obtained by the following formula:

$$\text{slope} = \frac{\text{final value - initial value}}{\text{number of days between obtaining initial and final samples}}$$

Patients #1, #2, #4, #7 and #17 were excluded from the analysis due to an insufficient number of data points.

The stepwise discriminant procedure employed (Klecka, 1975) first chooses the single variable that best divides the two patient groups. This variable is then paired with each remaining variable, one at a time, to determine which variable best improves the initial selection. These two variables are then coupled with each remaining variable and evaluated as to further improvement in grouping. This process continues until additional variables no longer provide a sufficiently large

improvement in discrimination of the patient groups. As variables are selected, some of the variables previously selected may become redundant, and will be removed, because their contribution to the selection process is provided by some combination of the other included variables. When the discriminant functions have been chosen, a discriminant score can be obtained for each patient by multiplying each discriminating variable by its computed discriminant function coefficient, then adding the products plus a constant. This score can be tested by using it to classify the original set of data.

The discriminant analysis of these six variables selected the following variables in descending order of importance: urine/serum RNase ratio, urine RNase concentration, serum transferrin and triceps skinfold thickness. Weight was never considered for inclusion, while prealbumin was always somewhat less useful than another variable.

The equation for the discriminant score was as follows:

Score = (2.786778) (slope of triceps) + (-.3140220) (slope of transferrin) + (-133.6017) (slope of urinary RNase concentration) + (61.97656) (slope of RNase ratio) + 1.914488

When the patients were re-classified according to this score, each patient was allotted to the proper group (8 improved, 3 non-improved). A histogram of patients plotted according to discriminant scores showed a good division between groups.

Because the inclusion of both urine RNase concentration

and urine/serum RNase ratio appeared redundant, the former was omitted and the discriminant analysis repeated with the 5 remaining variables. The following variables were selected in descending order of importance: urine/serum RNase ratio, serum transferrin and serum prealbumin. Weight was never considered for inclusion, and triceps skinfold was classified as less useful than prealbumin.

By this analysis, the equation for the discriminant score was:

Score = (-.6619754) (slope of transferrin) + (2.479456) (slope of prealbumin) + (24.99924) (slope of RNase ratio) + 0.8795456

When the patients were re-classified according to this score, each patient was allotted to the proper group. The division between groups as shown by histogram was good although the distance separating the groups was less than that obtained using the other set of variables. It appeared that an acceptable classification of patients could be obtained using either combination of variables.

DISCUSSION

In order to gain maximum benefit from the use of parenteral nutrition, it is essential to develop an effective method of evaluating a patient's progress during the course of therapy. It has been established that no single parameter will serve this purpose in all patients. However, various methods have been developed which can contribute to the overall assessment of nutritional status. The present study investigated a wide range of parameters which have been recommended for this purpose. Emphasis was placed on determining which variables would most reliably identify changes in nutritional status during therapy.

A. Anthropometric Measurements

The use of anthropometry in the nutritional evaluation of hospitalized patients has been widely recommended in recent years. Anthropometric measurements have the advantage of being quick and easy to perform (by experienced personnel) while causing minimal discomfort to the patient. Furthermore, they are the only tests of which the results are immediately available. However, to derive maximal benefit from the test results, close attention must be paid to the method of measurement and to factors which may alter the readings.

1. Weight

When the procedure is performed consistently, weight is

relatively free from measurement error. However, it is clear from the results of this study, in which only seven of eleven nutritionally improved patients had a net increase in weight, that several factors besides nutritional changes can lead to substantial weight change.

Of the four improved patients who exhibited a net decrease in weight, two had undergone surgery just prior to initiation of TPN, one was twelve days post-surgery and suffering from post-operative complications, and one (#12) stopped receiving ACTH infusions during the course of TPN. Weight loss in the latter appeared to be due mostly to fluid loss. The two patients already receiving TPN prior to surgery did not show marked post-surgical weight loss during the period of TPN.

The most common factor associated with rapid weight gain was fluid retention, which often occurred during the first several days of TPN and was followed by a slight weight loss.

Excluding the four patients discussed above, and allowing for episodes of edema, most improved patients showed a gradual but fairly steady increase in weight. Although failure to empty the bladder before weighing can noticeably affect weight in some patients, this was not a problem in patient #9. All but one of the non-improved patients had minimal weight change. The exception, patient #10, had a marked increase in weight which was not accompanied by other indications of improvement. This patient was already overweight and almost bedridden, so that a weight increase was not desirable. This

patient was receiving TPN in an unsuccessful attempt to repair a fistula.

As a nutritional "marker", weight appeared to be useful in some patients, particularly for intermediate and long-term assessment since many of the day-to-day fluctuations were probably due to changes in fluid balance. The main factors associated with a weight change were change in fluid balance, nutritional change and surgery.

2. Triceps Skinfold

To obtain reliable, consistent values for triceps skinfold thickness requires careful attention to the method of measurement. The major factors leading to variability in results are interobserver error (the effect of measurements being performed by different individuals) and change in site (Burkinshaw et al., 1973; Ruiz et al., 1971). These problems were largely avoided in the present study since all measurements were performed by the same individual on a site marked at the time of the first measurement. However, some measurement error did occur even under these conditions, particularly in obese subjects in whom it was difficult to distinguish between muscle tissue and adipose tissue.

Another factor which could affect consistency of results is the patient's position during measurement. Jensen et al. (1979) reported no significant difference between triceps skinfold measurements taken with patients either supine or

upright. However, it was observed in the present study that the supine position could affect the results if the patient's position was such that it interfered with access to the exact site used during previous measurements. This problem was particularly noticeable in overweight, uncooperative patients, and when attempting to measure the left arm of a bedridden patient using right-handed calipers.

In spite of some problems with measurement, very good results were obtained for triceps skinfold thickness in most patients. Ten of the eleven nutritionally improved patients exhibited a net increase in triceps skinfold. The exception, patient #5, who showed a marked decrease in skinfold thickness, was an overweight postsurgical patient who lost weight during parenteral feeding. A similar patient (#14) showed a substantial increase in triceps skinfold, so there is no clear explanation for the decrease in patient #5. However, patient #14 did have a substantially greater intake of TPN solution. It is possible that the efficient wound healing in patient #5 occurred at the expense of the somatic compartment. Nitrogen balance was negative in both patients, but especially in patient #5.

Of the four patients judged "non-improved" by the largely subjective criteria mentioned earlier, one patient showed a marked increase in triceps skinfold, although not in weight. This increase might be related to the reduced physical activity in this patient. The extremely wide fluctuations

in another patient (#13) were probably due partly to measurement error and partly to edema.

Final values of skinfold measurements were significantly higher than initial values. This was especially noticeable in those patients who showed the greatest overall improvement both nutritionally and clinically.

Triceps skinfold measurements appear to be useful in the on-going assessment of TPN patients. Day-to-day variability is narrow in most patients in comparison to the variability of many of the non-anthropometric measurements, although not in comparison to weight. Skinfold measurements appear to be less affected than is weight by edema and stress situations such as surgery, although some response to these factors was noted. On the other hand, weight is less subject to measurement error.

The problem of measurement error, which occurred mainly in obese and/or bedridden patients, could probably be minimized by very close attention to the exact measurement site. Ruiz et al. (1971) showed that results are affected by horizontal as well as vertical deviation from the original site. In the present study, some degree of horizontal deviation occurred occasionally in at least three patients. Another problem in obese patients is reading the calipers at exactly the right point. The method used here was to take the reading when the needle had completed its initial rapid swing. Another method

suggested for all patients by Grant (1979) is to take the reading three seconds after release of the caliper spring. This method was not employed in the present study, but might be useful in obese patients to obtain more consistent measurements.

As mentioned previously, the triceps skinfold was chosen for investigation due to its ready accessibility in almost all patients. However, the work of others (Durnin and Womersley, 1974; Bradfield et al., 1979) has shown that greater sensitivity is obtainable with the use of multiple skinfolds, particularly including the subscapular skinfold. In view of these reports, it seems reasonable to consider measurement of the subscapular skinfold, in addition to the triceps, in those patients in whom the site is accessible.

3. Mid-upper Arm Circumference

Measurement of mid-arm circumference was found to be simple and readily reproducible in most patients, although accurate measurement was more difficult in bedridden patients. Changes in arm circumference were small in relation to the actual circumference. Unfortunately, there was no apparent relationship between changes in mid-arm circumference and changes in nutritional status.

The relatively wide variability in patient #9 was probably due partly to edema but mainly to measurement error resulting from the patient's supine position and poor skin resiliency.

The close correlation between mid-arm circumference and weight suggests that arm circumference might serve as an alternative measurement in long-term assessment of bedridden patients. However, the relationship appeared less reliable in the non-improved patients, a factor which would severely limit its usefulness.

4. Mid-upper Arm Muscle Circumference

Since the mid-arm muscle circumference is derived from the measurements of triceps skinfold thickness and mid-arm circumference, any discrepancy in either measurement will affect the calculation of muscle circumference. This calculation is an approximation only, since it does not allow for the presence of the bone and other non-muscle tissue, and since it assumes the arm is exactly cylindrical (Jelliffe and Jelliffe, 1979). Since triceps skinfold is susceptible to measurement error, especially in overweight patients, readings of muscle circumference are also subject to variability. Furthermore, readings of a substantial increase in triceps skinfold in conjunction with a small increase in arm circumference can lead to a calculated decrease in muscle circumference which probably does not correspond to an actual change. Calculations of mid-arm muscle area (Gurney and Jelliffe, 1973) and mid-arm muscle volume (Jelliffe and Jelliffe, 1979) have also been recommended, but these were not evaluated in the present study.

No relationship was observed between changes in mid-arm muscle circumference and changes in nutritional status. There is a possibility that the decreased muscle circumference readings in four patients who exhibited substantial nutritional improvement was due to the problems inherent in the calculation. However, an actual decrease in muscle circumference is also definitely possible. Such a decrease could have occurred in at least two patients due to lack of exercise and in a third patient due to muscle catabolism following surgery. In fact, a decrease in muscle circumference might be expected in most hospitalized patients receiving long-term TPN, since such patients almost always experience a marked reduction from their normal degree of physical activity. An appropriate exercise programme for these patients could be useful in maintaining or improving muscle function.

5. Summary

The newer anthropometric measurements appear to be a promising addition to the nutritional assessment of TPN patients. Anthropometric measurement is non-invasive, and not subject to the many problems which can be associated with collection of blood and urine samples. Weight change is one of the most valuable single tests of nutritional status. However, there are some patients in whom weight is either unobtainable or unreliable, and measurement of the triceps skinfold thickness can be especially useful in these cases.

For maximum reliability, anthropometric measurements should be performed by trained personnel using a standard procedure. This is particularly important when determining skinfold thickness. Whenever possible, all measurements on the same patient should be performed by a single individual.

B. Biochemical Measurements

1. Albumin

Serum albumin is one of the traditional parameters of nutritional assessment. Its concentration is substantially depressed in severe prolonged malnutrition, and in this condition it is undoubtedly of diagnostic value (Shetty et al., 1979). In the present study, albumin levels were depressed in the two patients (#8, #9) who exhibited severe, long-standing malnutrition. The levels in these patients continued to fall for several days after initiation of TPN.

Few patients in the present study exhibited severe depression of serum albumin, although most values were below the normal range. The majority of the study patients, like most hospitalized patients who are candidates for TPN, were not suffering from severe malnutrition but from some acute condition which resulted in malnutrition of fairly recent onset. Serum albumin concentration appears to be less useful in such cases. This is in agreement with the observation of Shetty et al. (1979) that albumin shows poor responsiveness

to short-term changes in protein and energy intake.

An abrupt decrease in albumin concentration was observed in most patients following surgery, and a marked but gradual decrease in one patient (#13) who suffered a clinical deterioration. The largest increases occurred in the three most improved patients (#15, #14, #12), although several improved patients did not have an increase in albumin concentration. In some cases, the reasons for changes in concentration could not be identified.

Serum albumin appears to have only limited value as a measure of nutritional status in most hospitalized TPN patients, although it can be useful in long-term assessment.

2. Prealbumin

Serum prealbumin was investigated as a short-term indicator of changes in nutritional status. Since many patients receive TPN for periods of two weeks or less, it is important to be able to identify promptly any change in status.

It was observed in the present study that all ten patients showed some degree of increase in prealbumin concentration during the first two weeks of TPN. In all but one of the seven improved patients, the second value was at least twice the initial concentration. The remaining patient (#11) underwent surgery the day before the second prealbumin sample was obtained. The three non-improved patients had much smaller increases in concentration.

Patients who remained on TPN for more than two weeks had a third serum prealbumin determination performed when the TPN was stopped. Most of these patients exhibited little change from the second determination. The reason for the marked decrease in one improved patient (#12) is unknown, but it should be noted that the final concentration was still more than twice the initial concentration. Other investigators have observed that most of the increase in concentration occurs, in most patients, within the first two weeks of treatment. Attainment of peak levels may be followed by a levelling off or a slight decrease in concentration (Ingenbleek et al., 1972; Large et al., 1980).

Changes in prealbumin levels correlated well with changes in patients' conditions. The more marked increases in prealbumin were observed to occur in those patients who improved both nutritionally and clinically.

Factors reported to decrease prealbumin levels include surgery, malignancy, ulcerative colitis and Crohn's disease. The two patients who started TPN shortly after surgery (#5, #14) had initial prealbumin concentrations equal to or less than 10 mg%. Patient #5, with the lowest initial value, also suffered from cancer. Patient #11, who received surgery during the course of TPN, has already been mentioned. Patient #12, with ulcerative colitis, had an initial value below 10 mg%, but showed a substantial increase after two weeks. The two patients with Crohn's disease had initial values slightly below

the normal range, with a substantial increase in the improved patient (#15) and a lesser increase, to low normal levels, in patient #6.

In the three follow-up patients, the frequent prealbumin determinations confirmed that marked changes can occur over a few days. A steady increase was seen in Patient A (diagnosis: abdominal abcess) over one week. Initial values were, unexpectedly, well within the normal range in Patient B, a Crohn's disease patient undergoing bowel rest. The substantial increase in prealbumin concentration during the third week of TPN could not be explained. The decrease following surgery was prompt and readily identifiable, as was the subsequent increase in concentration. The magnitude of the decrease was about 50%, similar to that reported by Surks and Oppenheimer (1964). The somewhat more rapid recovery here than that observed by these authors might be attributed to the use of TPN in the present study. Patient C, who exhibited a gradual decrease in concentration, was 16 days post-surgery, with cancer of the duodenum. It is possible that these two factors largely accounted for his abnormally low levels, although the reason for the continuing decrease is not clear. Two of the study patients with cancer had undergone surgery prior to starting TPN (patients #2, #5). Patient #5 had an extremely low initial prealbumin concentration (4.6 mg%) and a low final concentration although a substantial increase did occur. Patient #2 had only one prealbumin sample obtained, so her results were

not included in any evaluation of data. This single initial level was 4.5 mg%, almost identical to the initial level of patient #5. Surgery and/or malignancy appear to be associated with particularly low prealbumin levels.

When the above factors are taken into consideration, changes in prealbumin concentration show definite promise as an indicator of changes in nutritional status. Long-term results were not available in the present study. However, Smith et al. (1973) in a study of children undergoing oral nutritional replenishment, found that prealbumin levels in most patients peaked between the 20th and 40th day, then decreased substantially by the 60th to 90th day. In view of this report, and until more information is available regarding long-term changes, it appears reasonable at this time to recommend the use of prealbumin concentration primarily in initial, short-term assessment.

3. Transferrin

The serum half-life of transferrin is intermediate between that of prealbumin and albumin. Consequently, transferrin has been recommended in preference to albumin as an indicator of changes in nutritional status. Promising results have been obtained in malnourished children, but few studies have been done on hospitalized adults receiving TPN. The present study illustrates some of the problems associated with this measurement in such patients.

Most improved patients exhibited a substantial increase in serum transferrin levels, and initial and final values were significantly different in the improved patients. However, few of these patients achieved normal values. In many of the study patients, transferrin levels were below the lower limits of normal throughout the study period. Only one patient (#15) had consistent serum levels well within the normal range. Several of the patients had conditions - such as malignancy, chronic inflammatory conditions, and old age - which have been previously associated with lowered transferrin levels (Woo et al., 1979). In some of the remaining patients, prolonged malnutrition is a reasonable explanation. However, there are a few patients in whom the abnormally low levels cannot be readily explained.

Several patients exhibited substantial day-to-day variability which interfered with interpretation of the results. Some patients had isolated extreme values which could not be readily explained. This observation agrees with that of Ingenbleek et al. (1975) that plots of transferrin levels in patients undergoing dietary treatment are characterized by "sometimes unpredictable...isolated values". In addition, there was wider inter-subject variation compared to that observed with serum albumin; this is in agreement with the observations of Young and Hill (1978).

There was no clear relationship between surgery and changes in serum transferrin, although post-surgical patients did have

depressed transferrin levels. The pronounced decrease in serum transferrin in patient #13 occurred in conjunction with a deterioration in clinical condition.

In view of the variable results obtained, it may be necessary to re-evaluate the use of an indirect method of measurement. It is possible that direct measurement by the use of radial immunodiffusion, which is less susceptible to analytical problems, would have produced more consistent results. The latter method is recommended by some authors (Von der Heul et al., 1972; Dudrick et al., 1979; Rowlands et al., 1979) although others, as discussed previously, maintain that reliable results can be obtained using the TIBC.

In spite of the problems discussed above, transferrin was found to be much more dependable than albumin as an indicator of changes in nutritional status. Serum albumin levels are doubtless useful as long-term nutritional markers, but for short-term monitoring, levels of transferrin and prealbumin provide more reliable information.

4. Creatinine and Creatinine-height Index

Daily creatinine excretion has been observed to increase during the early stages of muscle wasting and decrease in the later stages (Schiller et al., 1979). In the present study, the lowest values, which were maintained throughout, occurred in the two patients (#8, #9) with severe chronic malnutrition which was only partially corrected during the course of TPN.

The highest values occurred in a previously healthy young patient who had just undergone major surgery for treatment of an acute condition.

In most cases, changes in daily creatinine excretion did not occur in conjunction with changes in nutritional status. The initial and final values were not significantly different, although substantial changes occurred in a few patients. Six patients had day-to-day variability sufficient to interfere with interpretation of their results. This is in agreement with the observation of Chattaway et al. (1969) that day-to-day creatinine excretion, while reasonably constant in some patients, varies considerably in others. It has been suggested that some of this variability may be avoided by averaging the results of three subsequent 24-hour urine collections (Gross, Holbrook and Thornton, 1978). However, this method is not always practical and was not attempted in the present study.

Daily creatinine excretion alone does not appear useful in nutritional assessment except in cases of long-term malnutrition. A number of researchers have recommended use of the CHI as a more reliable indicator of nutritional status (Viteri and Alvarado, 1970; Bistrian et al., 1975a; Dudrick et al., 1979). Unfortunately, the CHI is also susceptible to problems such as day-to-day variability (Rich et al., 1977). This was confirmed in the present study, in which six patients showed wide day-to-day variation, often but not always

associated with changes in 24-hour urine volume. Chattaway et al. (1969) found this correlation between creatinine excretion and urine volume in some subjects, particularly in those with fairly wide daily variations in excretion.

Creatinine excretion exhibits day-to-day variability even in healthy individuals engaged in normal activity (Greenblatt et al., 1976). The authors also observed considerable inter-subject variability, which likewise occurred in the present study. In addition, Scott and Hurley (1968) observed that the excretion pattern of a single individual may be altered from time to time.

The effect of surgery on the CHI was not uniform. The two post-surgical patients did show a fairly consistent decrease in CHI, and two surgical patients exhibited a transient increase in CHI following surgery. However, two other surgical patients (#6, #10) showed no such increase.

Gross, Holbrook and Thornton (1978) found marked day-to-day variability in creatinine excretion in patients with intestinal fistulae. This was also observed in the present study in patient #10, and in patient #13 who had a biliary fistula.

One factor which could affect creatinine excretion is ingestion of meat products (Chattaway et al., 1969). In the present study, only one patient (#8) was served meat products during the course of TPN. It was not known to what extent food could be absorbed from her gastrointestinal tract.

Another factor which can alter creatinine excretion is exercise. However, most of the study patients received minimal but fairly consistent amounts of exercise. In fact, wide day-to-day creatinine fluctuations were seen in some bedridden patients.

As is pointed out by Gray and Gray (1980), the standards for the CHI are based on a relatively small number of individuals. Large-scale surveys are still required to determine the range of values for the normal population. In particular, more data is required regarding the CHI in women and in subjects of unusual height. Proper evaluation of a patient also requires that the CHI be adjusted for age. This has been calculated by Driver and McAlevy (1980) based on creatinine excretion data from Rowe et al. (1976). However, equivalent data is not available in women, and more large-scale surveys in the elderly would be helpful. In addition, more information is required regarding the effect on the CHI of various disease states.

In the present study, there was a trend in some but not all patients for the CHI values to approach normal limits during the course of TPN. However, initial and final values were not significantly different. The two chronically malnourished patients (#8, #9) maintained a very low CHI (less than 0.400) throughout. This is in agreement with the observations of others (McLaren et al., 1970; Viteri and Alvarado, 1970) that daily creatinine excretion and the CHI

are significantly depressed in severe long-term malnutrition.

Although the results indicate that the CHI may be of use in some patients, no clear relationship to nutritional status was seen except in cases of severe, long-term malnutrition. This is not in agreement with the results of Schiller et al. (1979) who reported that urine creatinine excretion gradually increased as the nutritional state improved. No definite reason for the lack of significant change in the present study could be identified, although it is possible that many of the patients who were included suffered from other conditions affecting the CHI. Schiller et al. (1979) did observe that patients with serious illnesses had variable levels of urine creatinine excretion.

An unexpected observation was the decrease in the ratio of urine creatinine to serum creatinine in all but two improved patients. There was no significant difference between initial and final values when all patients were included, but the final value for patient #2 was extremely high. When this patient was excluded, initial values for the improved patients, with a mean of 103.7 ± 53.2, were very significantly higher than final values, with a mean of 45.8 ± 38.3 ($P<0.0005$). Unfortunately, the values for this ratio, like the 24-hour urine creatinine excretion, showed wide day-to-day intra-subject variation which in four cases was sufficient to interfere with interpretation of the results. The use of such a ratio has the advantage of not requiring a 24-hour urine collection.

However, this measurement has not been reported in nutritional assessment, and its validity for the purpose has not been established.

5. Zinc

In recent years, there has been increased interest in the effect of stress on the body's zinc stores, but as yet, there is limited data available regarding such changes in response to different forms of stress, including malnutrition. Abnormally low serum and plasma zinc concentrations have been seen in malnutrition, but changes in urinary zinc excretion during malnutrition are less well documented. However, the observations of Kay et al. (1976) indicate that zinc excretion appears to be a more reliable index of metabolic status than is plasma zinc.

In the present study, most serum zinc concentrations were within the normal range, although some patients showed considerable variation within these limits. Only three patients had isolated values below the lower limit of normal, although abnormally low serum concentrations have been previously reported in numerous clinical conditions including malignancy, infection, pancreatitis and Crohn's disease in addition to malnutrition. Neither the abnormally high results observed in patient #16 nor the wide range of values in patient #15 could be readily explained.

Halsted and Smith (1970) found no effect of fasting or

diurnal variation on plasma zinc levels. On the other hand, some authors have reported higher plasma zinc levels from fasting samples than from samples taken following a meal or following administration of glucose (Dawson and Walker, 1969; Davies et al., 1968). Few of the study patients had substantial oral intake. However, most received enteral feeding and/or a light diet at some time during the course of TPN, and patient #15 received regular oral supplementation. It is possible that some day-to-day variability in zinc levels was due to postprandial sample collection. Although most blood collections were performed at 0800 hours, on occasion they took place at 1400 hours, following a meal.

Falchuk (1977) found that an ACTH infusion produced a marked decrease in serum zinc concentration in patients with normal zinc levels but not in those with initially low zinc levels. In patient #12, serum zinc was seen to increase following discontinuation of the ACTH infusion, but no definite relationship was established.

Serum zinc levels did not appear to correlate well with changes in patients' clinical conditions or nutritional status. Studies in young children (Golden and Golden, 1979) showed low plasma zinc levels to be associated especially with kwashiorkor, which is characterized by depression of visceral proteins such as albumin. In the present study, few patients exhibited severe depression of serum albumin, although most patients had values below the normal range.

No clear conclusions could be reached regarding disease states which are capable of altering serum zinc concentrations. During TPN, serum zinc levels decreased to below normal limits in a patient with Crohn's disease. However, abnormally low concentrations were not observed in the other patients with conditions reportedly associated with low serum zinc levels, although in some cases values were in the low normal range.

Urine zinc concentrations were difficult to evaluate due to the broad range of values and due to the very high concentrations observed in a few patients.

Concentrations remained within a fairly narrow range for seven patients. In the remainder, some of the sudden changes occurred in conjunction with known stress situations such as surgery. One patient (#14) started TPN post-operatively and two patients (#4, #11) received surgery during the course of TPN. In addition, patient #12 was undergoing treatment for ulcerative colitis, which has been associated with increased urinary zinc excretion (Mills and Fell, 1979). Other changes could not be readily explained. The extremely high concentrations in patient #13 could not be accounted for, although the stress due to post-operative complications was probably partly responsible. Two other patients (#15, #16) had inexplicable wide variability. These two patients also had abnormally high serum zinc concentrations.

When all patients were included, there was no significant difference between initial and final concentrations. However,

when the patients with extremely high values (#2, #4, #13) were excluded from the computations, the initial values, with a mean of 0.905 $\pm$ 0.577 µg/ml, were significantly higher than the final values, which had a mean of 0.569 $\pm$ 0.428 µg/ml ($P<0.025$).

It was observed that the lowest concentrations and the narrowest intra-subject ranges of zinc excretion occurred in patients with chronic, long-term conditions, including the three patients with long-term malnutrition as well as patient #10 (long-term post-operative complications) and patient #6 (Crohn's disease). High values were associated with more acute conditions. It may be that patients with certain chronic conditions, including malnutrition, have low urine zinc concentrations due to an adaptive decrease in the rate of skeletal muscle breakdown. Catabolism of skeletal muscle is believed to be a major source of urinary zinc (Fell et al., 1973).

The total 24-hour urine zinc excretion is more likely to provide useful information than is the urine concentration alone. However, in many subjects, the wide day-to-day variability made interpretation of results difficult. Following initiation of TPN, most of the patients exhibited an initial abrupt increase in 24-hour excretion, followed by a fairly rapid decrease to a level at or below the initial level. This increase appears to be partly but not entirely associated with

the initial increase in urine volume that occurs in many patients receiving TPN. Only one patient (#16) had a steady increase in excretion which was not followed by a decrease. The mean initial and final values of 24-hour urine zinc excretion were not significantly different.

The 24-hour excretion, like urine concentration, was lowest in the patients with long-term malnutrition and low in patients #6 and #10 who had chronic conditions. However, excretion was also low in an acutely ill patient (#14) in spite of a high urine zinc concentration.

A marked increase in urine zinc excretion was expected following surgery, according to the work of Fell et al. (1973). Such an increase was observed in patient #4, but not in patient #11. However, patient #11 exhibited abnormally high initial levels of zinc excretion which could have been related to her previous surgery or its related complications. Of the two patients who started TPN following surgery (#5, #14), a marked increase occurred several days after surgery in the former but not in the latter. In fact, urine zinc excretion in patient #14 remained in the low normal range in spite of his having undergone major surgery. No single relevant factor was identified which distinguished the surgical patients who did exhibit an increase in zinc excretion from those who did not.

Another condition which is reportedly associated with increased urine zinc excretion is active ulcerative colitis (Mills and Fell, 1979). Patient #12, who was being treated

for ulcerative colitis, did excrete very high quantities of urinary zinc. Also in agreement with Mills and Fell (1979), patient #6, with active Crohn's disease, did not exhibit excessive zinc excretion.

Extremely high 24-hour urine zinc excretion was observed in patient #13. Kay et al. (1976) reported similar results in severely catabolic patients. Patient #13 was not believed to be sufficiently catabolic during the study period to account for such excessive zinc excretion. However, this patient had been admitted with acute pancreatitis and cholecystitis one month prior to initiation of TPN, and suffered complications following surgery. The high zinc excretion might reflect severe catabolism occurring prior to the study period.

Four patients received zinc supplementation, which was added to the TPN solution, throughout the course of TPN. Two other patients, #9 and #13, received supplementation beginning on day 10 and day 19, respectively, of TPN. There was no apparent effect in these two patients on either urine zinc concentration or 24-hour excretion. This is in agreement with Wolman et al. (1979) who concluded that zinc losses "were independent of the amount infused and dependent upon the volume of gastrointestinal fluids lost and the presence or absence of high urinary nitrogen losses". An earlier study involving administration of oral zinc (Henzel et al., 1970) found that patients with normal biologic zinc stores demonstrated an increase in urine zinc excretion following

zinc ingestion. On the other hand, patients who were initially zinc deficient exhibited a lag period before the increase in urinary zinc occurred. The initial state of the zinc stores in the patients of Wolman et al. (1979) was not specified. In the same study, Wolman et al. (1979) observed an increase in plasma zinc concentration which was correlated with the amount of zinc infused. No such relationship was seen in the two applicable patients in the present study; however, the quantities of zinc infused were less than those used by Wolman et al.

As was also observed by Wolman et al. (1979), abnormal zinc excretion can occur during diarrhea and by means of fistula drainage. One or both of these complications is believed to have significantly interfered, on more than one occasion, with the urine zinc results in three patients.

Urine zinc excretion can also be affected by certain diuretics. Wester (1980) found that both mean urine zinc concentration and mean 24-hour urine zinc excretion, particularly the latter, increased during treatment with thiazide diuretics. During treatment with furosemide and triamterene, there was some increase in mean 24-hour excretion but a slight decrease in mean zinc concentration. In the present study, one patient (#7) received furosemide throughout the course of TPN. Two other patients (#8, #9) received furosemide during the study, on days 2-18 and days 9-25, respectively. There was no clear effect on urine zinc

concentration, nor on 24-hour excretion in patient #9. Patient #8 exhibited a relatively marked, transient increase in 24-hour excretion following initiation of furosemide therapy. No patients received thiazide diuretics during the study period. Although no definite effect could be identified from these two patients, the use of diuretics should be taken into account when evaluating measurements of urine zinc.

The urine zinc concentration alone provides limited information, and results are sometimes difficult to interpret due to the occurrence of isolated extreme values. It was hoped that adjusting for the corresponding serum level might minimize the effect of these isolated values. In some instances, using the ratio of urine zinc concentration to serum zinc concentration did produce a narrower range of values. However, the ratio did not provide any more useful information regarding nutritional status.

Neither serum nor urine zinc measurements appear to be useful in the short-term assessment of nutritional status. Urine zinc measurements may serve as an indicator of muscle breakdown, but there appears to be a "lag" period between the occurrence of a stressful situation and the increase in urine zinc excretion. Similarly, it appears that the increased excretion continues for some time after initiation of TPN. There may be a role for zinc measurements in long-term metabolic assessment, but such a role would require further investigation.

6. Ribonuclease

Although RNase levels have recently been investigated in the diagnosis of certain diseases, particularly cancer (Sheid et al., 1977; Warshaw et al., 1980), little work has been done to evaluate the use of RNase in the assessment of malnutrition. Information is particularly scarce regarding the effect of malnutrition on urinary RNase.

In the present study, serum RNase concentration increased in several patients during TPN. This was unexpected since the work of Sigulem et al. (1973) indicated that plasma RNase levels were elevated in malnourished children and fell following nutritional improvement. However, Prabhavathi et al. (1977) found elevated plasma RNase levels only in severe forms of protein-calorie malnutrition, and most patients in the present study were not severely malnourished. The increase observed in patient #7 can be attributed to deteriorating renal function, but the marked increases in some of the other patients are difficult to explain. Final concentrations were significantly higher than initial concentrations in the non-improved but not in the improved patients, suggesting that the increased levels may be related to stress or disease.

Albanese et al. (1972) reported an inverse relationship between plasma RNase and nitrogen balance, but no such relationship was seen in the present study. No relationship was observed between serum RNase and surgery. Elevated RNase levels were not observed in the cancer patients. The latter

observation is in agreement with Hisada (1968) who reported a relatively low incidence of elevated serum RNase in carcinoma of the stomach and colon.

The urine RNase concentration decreased in all but two of the improved patients, and both of these patients exhibited initial decreases. In patient #4, the initial decrease was followed by a very marked increase which began the day after surgery. In patient #15, the fairly abrupt marked increase coincided with the onset of severe diarrhea. Of those improved patients who exhibited a net decrease, all but two had a reasonably steady fall in urine RNase concentration. The remaining two patients (#8, #9) showed a steady decrease for the first ten days, then both exhibited a marked increase which did not, however, approach the initial concentration. This increase could not be explained. Only two of the improved patients exhibited wide day-to-day variability. Results in the non-improved patients, as with most variables, were less consistent.

Urine RNase concentration increased substantially following surgery in patient #4 and in patient #6 (whose surgery was performed the day prior to discontinuation of TPN), but not in patient #11. The most extensive decrease (from 0.952 to 0.160 μg/ml) occurred in patient #14 who began TPN following major surgery and made a rapid uneventful recovery. A substantial decrease (1.112 to 0.692 μg/ml) was also observed in the other post-surgical patient (#5). [Although patients #10,

#11 and #13 were suffering from post-operative complications, they were not classified here as post-surgical patients because their surgery occurred at least two weeks prior to initiation of TPN, as opposed to patients #5 and #14, who started TPN within a few days after surgery.]

The wide fluctuations which developed in patient #9 after two weeks of TPN could not be explained. For most parameters, this patient displayed relatively low concentrations and a narrow range of values. This patient also had a wide range of serum RNase levels. The wide fluctuations seen in patient #10 may be partly attributed to periodic difficulties in obtaining reliable urine collections in this patient. Much of the increased urine RNase concentration in patient #7 can be attributed to deteriorating renal function.

The results suggest that urine RNase concentration could serve as a useful parameter in nutritional assessment. Concentrations decreased quite consistently in the improved patients who were not subjected to acute stress during TPN. Furthermore, day-to-day variability was narrow in most patients. These results were unexpected since urine concentrations, in general, are relatively poor indicators due to their dependence on the volume of excretion. Further work is required to determine whether these factors might account for some of the day-to-day variability in urine RNase concentration.

Patient #6 demonstrated a fairly steady increase, which was not associated with visible stress, in urine RNase concentration. This patient was well-nourished initially and her

nutritional state did not change during the course of TPN. Her clinical condition did not appear to change until shortly before surgery; in retrospect, however, the deterioration may have been taking place for some time. The unexpected marked increase in RNase concentration was not accompanied by increases in any other urinary variable except creatinine ($r=0.767$, $P=0.01$). It was hoped that RNase might prove to be useful as an indicator of whether healing was occurring during bowel rest. However, results with the two follow-up patients were inconclusive, since only one patient underwent surgery and that patient did not experience complications prior to surgery. Patient #6, on the other hand, required emergency surgery due to intense pain following an attempt at oral intake.

In general, changes in the urinary concentration of RNase appeared to correlate well with changes in nutritional status. The most substantial decreases occurred in patients who improved both nutritionally and clinically.

The 24-hour urinary excretion of RNase, unlike the urine concentration, did not appear promising as a nutritional marker. Changes in the total daily excretion frequently did not correspond to changes in urine RNase concentration. As expected, measurement of the total daily excretion served to narrow the range of values in most patients. The majority of the patients exhibited fairly consistent RNase excretion or gradual changes with few extreme values. However, these results showed no real relationship to nutritional change.

Plots of the urine RNase concentration show occasional isolated values which can interfere with interpretation of the results. In an attempt to minimize the effect of these isolated values, the urine concentration was divided by the corresponding serum RNase concentration. The urine/serum RNase concentration showed a net decrease in all of the nutritionally improved patients and none of the non-improved patients. These results are not in agreement with those of Sigulem et al. (1973), who found a low urine/plasma ratio in severely marasmic infants. It is possible that the age of the subject and the degree and duration of malnutrition affect the urine RNase concentration.

A potential disadvantage with use of a ratio is that an abnormal value for either or both parameters may result in a grossly abnormal ratio. This might account for the wide variability in a few of the patients. In other patients, however, the ratio did serve to minimize the effect of wide variations in the urinary concentration. In patient #15, use of this ratio diminished the effect of the broad fluctuations seen in both the urine and serum concentrations, although the increase at approximately day 15 did still occur. The post-surgical patients (#5, #14) had initially high ratios followed by a marked decrease, as occurred with the urinary RNase concentration. However, the abrupt increase in urine concentration following surgery in patient #4 was not observed with the ratio, and its occurrence in patient #6 was difficult

to interpret due to a widely fluctuating ratio prior to surgery.

Both the urine RNase concentration and the urine/serum RNase concentration correlated well with changes in nutritional status. The ratio appears to be somewhat more reliable and less affected by stress situations such as surgery. Where assays can be performed readily, it seems preferable to perform both urine and serum determinations. However, the urine RNase concentration alone could be used in settings where the determination of both urine and serum levels is not feasible.

In general, very promising results were obtained using the present assay method. However, the method as performed in the present study involved assay of all of one patient's samples at one time, to avoid between-batch variability which could interfere with interpretation of results. Consequently, some minor adjustments must be made in order to use this procedure for on-going assessment. In addition, a normal range of values using this method must be determined in healthy subjects. Automation of the method is also desirable, since the present method is time-consuming and would not be practical if performed frequently with only a few samples at one time.

Another consideration is that some researchers, including Scott (1979), have recently developed assay methods for RNase which they claim are superior to adaptations of the method of Roth (1967). The use of these methods to evaluate nutritional status has not been reported, but may warrant investigation.

These methods, however, were developed for use specifically with serum or plasma samples. As yet, no method specifically intended for urine samples has been reported which appears preferable to the adaptation from Roth (1967) and Sigulem et al. (1973) which was used in the present study.

7. Nitrogen Balance

Nitrogen balance determinations were performed to identify to the investigator any patients who were in a catabolic state. It was expected that most patients would achieve positive balance, preferably by approximately 5.0 grams daily. However, the mean nitrogen balance was found to be only slightly positive, with most patients failing to achieve the balance of +4.0 to +6.0 grams which is recommended for a nutritionally depleted patient.

The initial low results are to be expected due to the necessity for gradual increases in the rate of infusion of the TPN solution. A minority of patients are unable to tolerate the recommended quantity of solution, and maintain an inadequate intake. However, the more frequent reason for failure to achieve a sufficiently positive nitrogen balance was technical difficulties with the infusion. In at least one-half of the patients, actual intake was less than the intended intake on several occasions. Most patients did not receive their TPN solutions via infusion pumps, which might have prevented much of the day-to-day variability in intake.

In a few patients, problems with the infusion line occurred quite regularly, resulting in a substantial deficiency in intake.

The patient with the highest nitrogen balance exhibited the most regular and impressive improvement in nutritional status (until diarrhea developed after about two weeks of TPN). It should be mentioned that the two patients with nitrogen balances consistently greater than 4.0 grams received regular oral supplementation. Of the three patients whose nitrogen balance fluctuated between -4.0 and +4.0 grams, only one was well-nourished and probably did not require additional intake.

A problem observed in some patients was an abrupt decrease in nitrogen balance at the time parenteral nutrition was stopped, suggesting that oral intake was not yet adequate to maintain the existing state.

To permit maximal improvement, it is necessary for the patient to receive an adequate quantity of nutrients. Two steps which could be taken to help achieve this aim are to ensure more regular delivery of TPN infusions, and whenever possible, to continue parenteral feeding until the patient's oral intake is adequate to establish or maintain an anabolic state.

C. Immune Competence

The ability to generate a positive immune response against an invading organism depends on the proper functioning and

interaction of a variety of defence mechanisms. Any of these mechanisms can be inhibited by a wide range of disease conditions.

A problem in evaluating the tests of immune function in the present study was the limited number of patients in whom data was available. Furthermore, only two or three measurements were obtained on each patient.

1. Immunoglobulins

Although a majority of patients exhibited some increase in immunoglobulin concentrations during TPN, there was no significant difference between the initial and final values. No definite relationship could be found between changes in immunoglobulin levels and changes in nutritional status.

Most concentrations of IgG were within the normal range. The reason for the low levels in patients #6 and #11 is not clear. According to Jensen and Jarnum (1976), serum IgG concentration is usually normal in Crohn's disease (patient #6). Abnormally high levels of IgM were seen in two patients (#4, #14) following surgery. However, patient #5, who was also post-surgical, had IgM levels well within the normal range. Very high values of IgA occurred in patient #10, although her levels of IgG and IgM were high normal and low normal respectively. Patient #12 showed abnormally low concentrations of IgA, but normal levels of IgG and IgM. Due to the wide range of values and the variety of disease

conditions observed in the study patients, it was not possible to identify specific factors which might have altered immunoglobulin levels. No consistent relationship was observed between immunoglobulin levels and infection, although high concentrations of one or more immunoglobulins did occur concurrently with a few episodes of fever. The small number of samples obtained per patient precludes more definite observations. The high incidence of elevated immunoglobulin levels in some studies of malnourished children have usually been attributed to repeated exposure to infection (Suskind et al., 1977; Chandra, 1979). It may be that such exposure has not occurred to the same degree in the study patients.

2. Complement

Serum levels of complement C3 and C4 were investigated partly as nutritional markers but also as possible indicators of the presence of infection.

Levels of C3 were within normal limits for all but one patient, in agreement with the observation that C3 concentration is substantially altered only in severe cases of malnutrition. The exception was patient #9, who was suffering from long-term malnutrition. During treatment with TPN, her C3 level increased almost to the lower limits of normal. The other patient with long-term malnutrition, patient #8, had only one set of complement measurements so was not included in the computations. However, her initial C3 concentration

was very similar to that of patient #9.

There was a significant increase in the final values of C3 compared to initial values. The post-surgical patients, #5 and #14, exhibited marked increases in C3 concentration at the second measurement, but not at the first measurement which was obtained within a few days after surgery. These results are similar to those of Young and Hill (1978) who found that an increase occurred more than one week after surgery.

An increase in C3 occurred in all but one improved patient, and in only one of the three non-improved patients. It appears that, contrary to expectations, C3 might be a useful indicator of changes in nutritional status even in moderate malnutrition. No consistent relationship was observed between C3 levels and episodes of infection.

Levels of complement C4 were within normal limits for all but one patient (#9). It is not known why this patient's values of C4 were above normal. There was no significant difference between initial and final concentrations, and no apparent relationship between changes in C4 concentration and changes in nutritional status. This is in agreement with the work of others (Sirisinha et al., 1973; Neumann et al., 1977; Haller et al., 1978).

3. T and B Lymphocytes

There were two major difficulties in evaluating the results of the T and B lymphocyte counts. The first problem

was the small number of patients on whom results were available. This problem occurred partly due to the quantity of blood required, but mainly because the tests must be performed with fresh blood. Since the tests require several hours, it was not always possible to have the counts performed on the appropriate day. On a few occasions, the counts could not be performed at all.

The second difficulty was that the assay method, even with careful attention to procedure, is susceptible to interference, so that precise counts are difficult to obtain.

With one exception, all T lymphocyte results were well below the normal range. The low initial values were to be expected in malnourished subjects, but final results (except for patient #16) were also below normal. A very marked increase occurred in one patient (#15), who underwent substantial nutritional improvement during that period, while no increase was seen in patient #12 who was also substantially improved. No definite conclusions regarding T lymphocyte counts can be reached from the available data. However, the use of this test in routine nutritional assessment is not recommended, since the test is not always practical to perform, and the results do not justify the time required.

Similarly, no definite conclusions can be drawn from the data on percentages of immunoglobulins. Although the percentage of IgG and IgM cells increased in a majority of the five patients, there was no significant difference between

initial and final values. The percentage of total Ig cells was markedly elevated in all patients except patient #6, whose values were within the normal range of 5-15% of total immunoglobulins (Waller and MacLennan, 1977).

4. Delayed Cutaneous Hypersensitivity

DCH can be considered a reflection of the state of the overall cell-mediated immune system, since a positive response requires the input of all components in the system. Consequently, numerous factors can influence the cutaneous response to the test antigens.

Even with a battery of three or four recall antigens, as were employed in the present study, a positive response does not occur in all apparently healthy subjects. However, the number of negative responses which occurred in the present study was unusually high in view of the fact that none of the five subjects were severely malnourished. This lack of response may be partly due to use of inadequate doses of test substances. Most reported anergy testing has involved larger doses of antigens than those employed in the present study. On the other hand, Palmer and Reed (1974) recommended use of the low doses, since they had observed "excessive reactions" during a preliminary study with the usual test doses.

Of the four patients with a negative response, one patient (#14) was post-surgical and one patient (#11) received surgery during the period of TPN. Surgery has been associated with a high incidence of anergy (Slade et al., 1975; Pietsch et al., 1977) although the latter suggest that such anergy is due to abnormalities in host defence rather than being an inevitable response to surgery. Slade et al. (1975) found that the response to candida antigen was still impaired after two weeks. In patient #14, the final tests were given three weeks after surgery. Neither patient was tested with SK-SD, for which a normal response has been seen 10-12 days following surgery (Slade et al., 1975).

A lack of immune response was not unexpected in patient #10, who suffered from a long-standing, non-healing fistula and had periodic bouts of infection. On the other hand, a positive response had been expected in patient #15, who underwent marked nutritional improvement prior to the second skin testing. The complete lack of response in this patient cannot be readily explained. However, this patient was anemic at the time, and anemia has been reported to be associated with some impairment of response to skin testing (Palmer and Reid, 1974). The same authors reported an incidence of "25% fewer positive or large reactions" than expected in patients with a WBC greater than $10,000/mm^3$. Patients #14 and #10, and to a lesser extent patient #15, frequently had WBC results above this level. It may be that the presence of a sufficient number of such

separate contributing factors will result in anergy.

The present study involved too few patients to draw any firm conclusions regarding the use of skin tests in nutritional assessment, particularly in view of the unexpectedly high incidence of negative responses. A further trial with higher test doses is recommended.

Ruggiero et al. (n.d.) commented that DCH, although important, "is only a single parameter of a battery of tests which comprises the nutritional assessment". Furthermore, an extensive literature review of skin testing in nutritional assessment of adults (Twomey et al., 1979) concluded that, in view of the scarcity of suitable trials, the usefulness of DCH in nutritional assessment has not yet been proven. This statement remains valid at the present time.

5. Summary

Serum levels of C3, somewhat surprisingly, appeared to offer promise as an indicator of nutritional improvement, and warrant further investigation.

Testing for DCH was not useful by the method employed in this study. The results of the lymphocyte counts were inconclusive, and the immunoglobulins, as expected, were not useful as nutritional markers. Although definite conclusions cannot be drawn due to the small number of patients involved, these tests of immune competence do not appear useful or practical for short-term nutritional assessment.

D. Relationships Among Variables

1. Correlation Coefficients

Good correlations were found between very few of the variables studied. The only separate parameters for which reasonably good coefficients were obtained were weight with arm circumference, height and triceps skinfold thickness; and the triceps skinfold thickness with arm muscle circumference. Furthermore, these relationships are based on all patients together and do not necessarily hold true for each individual patient. For example, several patients have a correlation between weight and triceps of ≥ 0.800, but in a few patients the correlation was very poor. It appears that the relationship observed between these two variables is valid only under certain conditions, probably because the two variables can change at different rates. The worst correlations between weight and triceps occurred in two patients who had a sudden decrease in weight. The best anthropometric correlation was between weight and arm circumference. A close relationship was seen between these two variables in almost all of the improved patients, but was less apparent in the non-improved patients.

The correlation between urine zinc concentration and 24-hour excretion suggests that a 24-hour urine collection is not required for zinc determinations. However, there were also some individual patients who exhibited poor correlations

between the two variables. Furthermore, the present study did not find any clear relationship between nutritional status and urinary zinc.

High correlations were expected between urinary concentration and urine/serum ratios for zinc and RNase because the value of each ratio depends largely on the urinary concentration. Consequently, the value of r=0.7920 for the RNase variables seems relatively low, and suggests that the use of the urine/serum ratio is preferable to use of the urine concentration alone. This is in agreement with the observations upon classifying the patients according to net increase or decrease. Using the urinary concentration alone, eight of ten nutritionally improved patients showed a net decrease compared to ten of ten patients using the urine/serum ratio.

2. Discriminant Analysis

The discriminant analysis selected four variables - urine/serum RNase ratio, urine RNase concentration, serum transferrin and triceps skinfold thickness - as being the most useful in correctly classifying the study patients as nutritionally improved or non-improved. When urine RNase concentration was excluded, the variables chosen were the urine/serum RNase ratio, serum transferrin and serum prealbumin. With regard to these selections, some comments must be made, based on an overall view of the information derived from the study.

In the first place, the discriminant analysis was retrospective. Although use of a retrospective analysis is valid as an investigative tool, in practice a nutritional evaluation must be continuous and able to provide information during the period of therapy. Secondly, even the most useful parameters showed considerable day-to-day variability in some patients, although to a lesser extent than several of the parameters which were not selected for the analysis. Substantial variability was noted with urine RNase concentration and serum transferrin, and to a lesser extent with the urine/serum RNase ratio. In addition, five of the sixteen patients, including four short-term patients, were excluded from the analysis due to insufficient data.

Consequently, the results of the discriminant analysis must be viewed in perspective. It is highly unlikely that all patients could be classified according to changes in nutritional status using only three or four variables. Many patients can be expected to have complicating factors which will invalidate one or more of the parameters. However, in this study, the original evaluation of net change in nutritional status - which involved a broad range of factors, many of which were subjective - was successfully substantiated in each patient included in the analysis, using only three and four objective criteria. To obtain such consistent results with a group of patients suffering from a variety of disease conditions shows definite promise for these parameters as dependable, objective "markers" in nutritional assessment.

E. Summary

Of the numerous parameters evaluated in this study, several were found to show promise in assessing changes in nutritional status. Of the anthropometric measurements, the triceps skinfold thickness was found to be the most reliable. The proper procedure for obtaining this measurement has been discussed. Although excluded by the discriminant analysis, weight is useful as a nutritional marker in most patients provided that allowance is made for other factors which can produce changes in weight. In addition, weight helps to monitor changes in fluid balance. Measurements of both triceps skinfold and weight should be obtained for all patients.

Serum albumin appears useful chiefly as a long-term marker in patients with severe, chronic malnutrition, and therefore is of limited value in most hospitalized TPN patients.

The increase in serum prealbumin was found to correlate well with changes in nutritional status. This parameter appears particularly promising as a short-term marker, for use during the first three weeks of TPN. Further information is required regarding use of frequent determinations, such as two or three times weekly.

Changes in serum transferrin were also found to correlate fairly well with changes in nutritional status, although some patients had isolated extreme values or repeated fluctuations.

Neither daily creatinine excretion nor the creatinine-height index appeared consistently useful in nutritional assessment, except in cases of severe, long-term malnutrition.

Better results were obtained with the ratio of urine to serum creatinine. However, all parameters involving urinary creatinine exhibited wide day-to-day variability.

Serum zinc levels were not correlated with changes in nutritional status. All tests involving urinary excretion were subject to wide day-to-day variation, and this problem was especially noticeable with zinc excretion. In addition, urine zinc was affected by many non-nutritional factors. Urine zinc concentration and 24-hour excretion were low in those patients with severe, long-term malnutrition, but in other cases, the wide fluctuations appeared to preclude its use as a nutritional marker.

In this study, neither serum RNase levels nor 24-hour RNase excretion were useful as nutritional markers. However, both urine RNase concentration and the urine/serum RNase ratio, particularly the latter, correlated well with changes in nutritional status. These two parameters appeared especially promising for nutritional assessment.

With the possible exception of complement C3, the tests of immune function did not appear to be useful in short-term nutritional assessment, probably due to the complexity of the immune system.

CONCLUSIONS

This study has investigated ways of assessing nutritional changes in patients receiving TPN. Most of the tests evaluated are useful as nutritional markers in certain cases, although a number of them were not appropriate for this group of patients.

Based on the results of this study, those parameters which are recommended for nutritional assessment of hospitalized TPN patients are: the ratio of urine to serum RNase, the urine RNase concentration, triceps skinfold thickness, serum prealbumin, serum transferrin and weight. Prealbumin appeared to be the best short-term marker, although triceps skinfold and urine RNase can also show substantial change over a short period. The RNase values, triceps, transferrin and weight can also serve as intermediate markers.

This study did not attempt to evaluate long-term nutritional markers; however, a few observations were made. Weight and serum albumin are well established as reliable long-term markers in patients with severe malnutrition. Data in the present study suggested that the creatinine-height index and urinary zinc might also be useful for this purpose. Long-term studies would be required to investigate this possibility, and also to determine whether these parameters would have any advantages over the more readily obtainable serum albumin levels.

Using discriminant analysis, it was possible to correctly

classify the study patients as nutritionally improved or non-improved, using only three or four objective parameters. In practice, however, it is more logical to perform additional tests so as to minimize the effects of factors which could interfere with the determinations.

Several factors, as discussed below, will affect the intervals at which tests of nutritional status should be performed. From the results of this study, the following is recommended as a basic schedule, with alterations to depend on the condition of the patient:

1. weight - daily. For nutritional assessment, twice weekly would be adequate, but daily weights help to assess fluid balance.
2. skinfold thickness - twice weekly. Once weekly would be adequate for nutritional assessment, but skinfold thicknesses are also useful for assessing weight changes.
3. urine and serum RNase - once weekly. With the present assay method, more frequent determinations are not practical in a clinical setting.
4. serum transferrin - once weekly;
5. serum prealbumin - twice weekly for the first two weeks, then once weekly;
6. nitrogen balance - twice weekly;
7. serum albumin - every two weeks;
8. complement C3 - every two weeks.

In a patient whose condition is unstable, more frequent determinations would be advisable. On the other hand, some long-term, stabilized patients could be adequately monitored by less frequent determinations. During and following situations of acute stress, such as surgery, monitoring should be more frequent. Regular monitoring of electrolytes, trace metals and blood components is also essential for TPN patients.

Those parameters which were not useful as nutritional markers in the present study were: mid-arm circumference and mid-arm muscle circumference, creatinine excretion and creatinine-height index, urine and serum zinc levels, immunoglobulins, T and B lymphocytes, and delayed cutaneous hypersensitivity.

In spite of the poor results with the above parameters, some of them appear to warrant further study. The following areas are recommended for investigation:

1. the effects on muscle circumference of different degrees of physical activity in long-term TPN patients, and
2. testing for delayed cutaneous hypersensitivity, using higher test doses.

In addition, more information is required on some of the parameters which have shown promise in this study as nutritional markers. The following investigations are recommended:

1. determination of serum transferrin levels using radial immunodiffusion, to determine whether this method would result in less variability;
2. long-term prealbumin measurements to determine the role of this parameter in on-going assessment, and further study of factors which could alter prealbumin values;
3. improvement of the method for RNase assay, and further study of factors which could affect RNase levels, especially urine values, and
4. further study of C3 concentrations in TPN patients.

Any patient receiving TPN requires constant assessment of his or her nutritional status. This study has identified tests which can substantially improve such assessment. In addition, it has indicated some established tests and some of the newer tests which do not appear to be useful in many of the hospitalized patients who receive TPN.

GLOSSARY

anergy - the absence of reaction to a specific antigen or allergen or to a battery of skin test antigens.

kwashiorkor - a state of protein deficiency occurring in the absence of a significant caloric deficiency. This state results from a protein-deficient diet which includes an excessive supply of calories, and which may be related to severe catabolic stress. The condition is characterized by depletion of the visceral protein stores, while the somatic reserves remain normal.

marasmic kwashiorkor - a life-threatening condition characterized by depletion of both somatic reserves and visceral proteins. It usually arises from mobilization and exhaustion of the subject's reserves of fat and lean body mass, followed by rapid depletion of visceral proteins in an attempt to handle catabolic stress.

marasmus (adult) - a prolonged, gradual wasting of muscle mass and subcutaneous fat due to an inadequate intake of both protein and calories. Anthropometric measurements will be decreased, but levels of visceral proteins often remain normal.

mitogen - a substance that stimulates mitosis and lymphocyte transformation.

somatic - pertaining to the framework of the body, including the skeletal muscle, but not to the viscera.

visceral proteins - all non-muscle proteins, such as albumin and transferrin.

The definitions of kwashiorkor, marasmic kwashiorkor and marasmus were adapted from: Jeejeebhoy, K.M. and Kaminski, M.V. 1978. Nutritional Assessment and Intravenous Support. Indications for Parenteral Nutrition (Physician's Monograph). Medical Directions, Inc., Chicago, Illinois.

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Appendix I

Standard Solutions

Central Standard Solution

This solution is composed of 25% glucose and 2.5% amino acids and provides the following per 1000 ml:

Nitrogen	- 4.2 g (26.25 g protein)
Kcal (non-protein)	- 850
Na	- 50 mM
K	- 30 mM
Mg	- 3 mM
Ca	- 3.5 mM
P	- 7.5 mM

Peripheral Standard Solution

This solution is composed of 5% glucose and 4% amino acids and provides the following per 1000 ml:

Nitrogen	- 6.72 g (42 g protein)
Kcal (non-protein)	- 170
Na	- 100 mM
K	- 60 mM
Mg	- 6 mM
Ca	- 7.2 mM
P	- 15 mM

Appendix IIa.

TPN Assessment Study--Pharmacy Data Sheet

Name: _____ Height(cm.) _____

Medical Records Number _____ Ideal Weight (kg.) _____

Actual Weight (prior to starting TPN)(kg.) _____ Midpoint of upper arm (cm.) _____

Date															
Day of therapy															
Anthropometric:															
(a) weight (kg.)															
(b) skinfold thickness (mm):															
(i)															
(ii)															
(iii)															
Average															
(c) arm circumference (cm.):															
(i)															
(ii)															
(iii)															
Average															
(d) arm muscle circumference (cm.)															
(e) creatinine-height index															
Immune Competence (mm. erythema) (time of test; readings at 24 & 48 hr. after test)															
(a) Candida															
(b) Tricophyton															
(c) Tuberculin(PPD)															
(d) Varidase (SK-SD)															
Total mm. induration (at 48 hr.)															

Appendix IIb.

TPN Assessment Flow Sheet

Name _____ Height (cm.) _____

Medical Records Number _____ Ideal Weight (kg.) _____

Actual Weight (prior to starting TPN)(kg.) _____ Age _____

Date											
Day of therapy											
Anthropometric:											
(a) weight (kg.)											
(b) skinfold thickness (mm.)											
(c) arm circumference (cm.)											
Biochemical (urine):											
(a) creatinine (mg %; mg/vol.)											
(b) alk. ribonuclease (mcg/ml)											
(c) zinc (mcg/ml)											
(d) 3-methylhistidine (mmoles/ml)											
(e) creatinine-height index											
(f) urine volume (ml/24 hr)											
Biochemical (serum):											
(a) albumin (gm %)											
(b) creatinine (mg %)											
(c) TIBC (mcg %)											
(d) pre-albumin (mg %)											
(e) alk. ribonuclease (mcg/ml)											
(f) immunoglobulins: IgA (mg %)											
IgG (mg %)											
IgM (mg %)											
(g) complement: C_3 (mg %)											
C_4 (mg %)											
Immune Competence:											
(a) skin testing: Candida											
Trichophyton											
PPD											
SK-SD											
(b) T-B lymphocyte detection:											
(i) B cells (%)											
(ii) T cells											

Appendix III

Orders for TPN Assessment Study

First Week

1. 24-hour urine for Na, K, creatinine and urea nitrogen -- Mon., Wed., Fri.
2. Serum creatinine, BUN, albumin, total protein, glucose, T.I.B.C., Zinc, Na, K, Ca, phosphate. } Mon., Wed., Fri.
3. Serum Mg and trace metals weekly on Mon.
4. CBC weekly on Mon.; differential (once only)
5. Serum pre-albumin; IgG, IgA, IgM; C_3, C_4; T-B cells } once only

Second Week (after at least 1 full week of TPN)

1. may decrease 24-hour urine to each Mon. & Fri.
2. may decrease the Mon., Wed., Fri. bloodwork to each Mon. & Fri. (physicians may wish to continue electrolytes Mon., Wed., Fri.)

After 2 Weeks (on 14th day of TPN)

serum pre-albumin; IgG, IgA, IgM; C_3, C_4

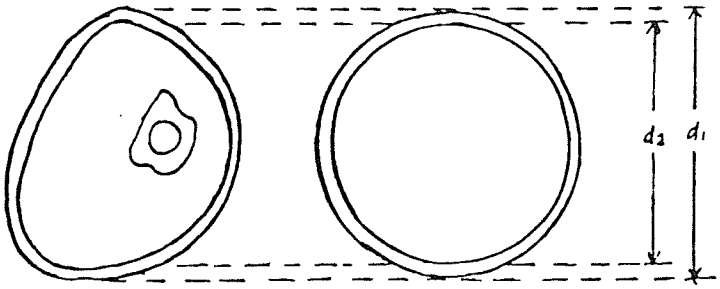
When Stopping TPN

1. 24-hour urine for Na, K, creatinine and urea nitrogen
2. Serum creatinine, zinc, T.I.B.C.
3. Serum pre-albumin; IgG, IgA, IgM; T-B cells.

Appendix IV

Formula for Mid-Upper Arm Muscle Circumference

The mid-upper arm muscle circumference is calculated using the relationship between triceps skinfold thickness and mid-upper arm circumference, as described below.



d_1= arm diameter

d_2= muscle diameter

Skinfold S = 2 x subcutaneous fat = $d_1 - d_2$

Arm circumference $C_a = 2\pi r_1 = \pi d_1$

Muscle circumference $C_m = \pi d_2 = \pi[d_1 - (d_1 - d_2)]$
$= \pi d_1 - \pi(d_1 - d_2)$

Substituting, $C_m = C_a - \pi S$

From: Jelliffe, D.B. The Assessment of the Nutritional Status of the Community, WHO, Geneva, 1966.