The author disagrees with the FAO/WHO Expert Group's (1963) approach of determining protein requirements. Amongst other, the protein that replaces the net nitrogen loss from the body is considered to be equal to the basal protein requirements of adults. The most important nitrogen loss from the body is considered to be the basal urinary nitrogen output. This is determined experimentally on protein free diets but according to the author urinary nitrogen output does not reach a constant level on a protein free diet, but falls continuously as the body becomes depleted of protein. It is not so easy to determine the so-called sharp inflection point of the curve of the urinary nitrogen output. BMR also decreases gradually as the protein depletion proceeds. It is important to know at what stage of depletion the end point of normal adaptation is reached and at what stage abnormality sets in. To get clarification on this point the author decided to investigate the physiological changes that occur with protein deficiencies.

Low protein diets were investigated on healthy young medical students. The subjects consumed a standard habitual Japanese diet for a few weeks (1.18-1.23 g/Kg/day with a protein score of 79-70 consisting of cereals, meat, or fish) and were then transferred to the experimental diet for three weeks, consisting of:

a. Low protein content and of low protein quality (0.56 g/Kg/day, protein score 63, consisting of cereals and vegetables);
b. medium protein content and quality (0.77 g/Kg/day, chemical score 67-73 consisting of cereal, pulse and vegetables); and
c. low protein content but high quality protein (0.57 g/Kg/day, chemical score 95, consisting of meat and egg), (Table 1).

Using the figure of 0.35 g/Kg/day of FAO/WHO as a requirement of the reference protein, it is evident from table 1 that the protein intakes on diets (b) and (c) were higher than that prescribed by FAO/WHO. On multiplying the protein intake/Kg with the protein score, expressed as "effective protein value" (EPV), the same figure, 52-58 was obtained for diets (b) and (c), which is close to the practical allowance of FAO/WHO (0.35 × 100 × 1.5 = 52). The EPV for the standard diet was 83-93. Diets (b) and (c) are therefore deficient in comparison to the standard diet, but not in terms of the FAO/WHO requirements. The low protein content and low quality diet (a) has a low EPV of 35, thus being deficient in protein.

Various criteria were used to determine the effects of the different diets body weight, body metabolism, total body water, ECF, total Hb, total serum protein content, A/G ratio, PBI, ADS in serum, daily urinary excretion of 17 Ks and 17 OHCS, etc. Control values were obtained on the standard diet and experimental values again periodically on the other diets.

With all the experimental diets (a, b and c), nitrogen balance was well maintained for a few weeks under the experimental conditions. The total Hb content and the total serum protein content, however, decreased on all three experimental diets,
while the BMR (and PBI) decreased significantly on diet (a). The extra-cellular fluid volume increased significantly; the ADS in the serum as measured by Birnie’s method also increased indicating an increased water reabsorption in the kidney. Daily urinary excretion of 17 Ks and 17 OHCS decreased considerably in the protein deficient state.

Investigations on diets (b) and (c) were prolonged on a pair of subjects for 12 weeks in order to study whether changes were temporary and would disappear with adaptation (fig. 4). The physiological changes tended to be restored after 6-7 weeks, but the restoration was not complete and the reduction in blood protein content and the urinary excretion of 17 Ks and 17 OHCS persisted after 12 weeks (fig. 5). The ADS content in the serum was also still higher than the control value after 12 weeks, an increased activity considered to be due to a decreased function of the liver (Morita). The effects therefore, were not temporary. It was shown in rat experiments that the reduction in 17 Ks and 17 OHCS could be due to a reduced activity of the pituitary. The pituitary and the liver, therefore, seem to be sensitive to protein deficiency.

It may be mentioned that the protein "deficient" diets used in the long term experiments, contained sufficient protein to meet the requirements of FAO/WHO. On these proteins the nitrogen balance was well maintained and tended to become rather positive whereas the physiological functions were impaired. The conclusion drawn is that physiological functions of important organs in the body cannot be maintained by providing protein which is sufficient to cover the loss of nitrogen in the urine. It therefore, is doubtful whether calculations of total nitrogen loss from the body is useful to determine the protein that is needed to maintain normal physiological functions of the important organs in the body.

An hypothesis of the existence of reserve protein seems useful to explain the effects of protein deficiency. Whipple believes that protein reserves stored in the body are utilized to maintain the protein metabolism of important organs, either when the protein consumption is reduced or when the requirements of organs are increased beyond the level of exogenous protein supply. Albumin is believed to be the representative reserve protein to be utilized for this purpose.

Rittenberg believes in the existence of a protein pool consisting of metabolic nitrogen originating from the food consumed and from the protein catabolized in the body. This pool keeps the protein metabolism of the organs in the body in a state of dynamic equilibrium. Reserve protein which has a high turn-over rate can provide nitrogen to the metabolic pool; reserve protein is therefore, also lost rapidly in protein depletion.

The protein content of the various organs of rats fed on a standard diet (14 cl.% of protein), were compared to those on a protein deficient diet (2.3 Cal.% of protein) killed after 7 and 20 days respectively on the experimental diet (Table III). The total loss of the protein after 20 days on the protein deficient diet was found to be 8.09 g/200 g. rat - about 25% of the total body protein. Organs that lost more than 25% of their protein were the liver, spleen, kidneys, blood, gut, heart and testes. Isotope studies also indicated that the half life of organ protein is relatively short in the liver, serum, spleen, heart, kidney, intestine and other internal organs (Sprinson and Rittenberg), possibly because these organs contain a higher percentage of labile protein than the average.

It was further indicated that labile protein may consist largely of flavoprotein.
The author also cannot agree with Waterlow that not much labile protein is lost with protein depletion; according to Yamamoto about 14% of the total body protein is lost during a period of 14 days on a low protein diet.

**Mechanism of Adaptation to Protein Deficiency**

In regard to the protein losses with protein depletion the author believes that more than 10% of the total body protein can be lost in a period of 2 weeks, which is in contrast to the 1% quoted by Waterlow. The loss of such a large amount of protein can affect the activity of the hypophysis, adrenal cortex and the thyroid. It has already been shown that the adrenal cortex is intimately involved in protein metabolism. Changes in this organ could be responsible for some of the changes brought about by protein depletion.

It was furthermore found that the reduction of the activity of the enzyme cathepain II in the liver and kidney of rats on a protein deficient diet was closely related to the loss of protein in both organs. With the reduced activity of such catabolic enzymes the catabolic rate of labile protein is also reduced, which helps the body to adapt to protein deficiency. The reduction of the catabolic rate of the body protein with protein deficiency may therefore be attributed either to a hormonal mechanism, to a direct effect on catabolic enzymes, or both. Reduced excretion of metabolities may be another contributing factor.

Reduced serum albumin with protein deficiency is understandable, but the cause of the Hb reduction is difficult to explain, for the Hb contained in erythrocytis is not easily utilizable. The anaemia with protein deficiency was therefore also studied in the 4 subjects on the low protein diet (0.6 - 0.8 g/Kg/day). It was found that the anaemia was hypochromic - microcytic in the early period of protein deficiency but became normocytic later. Some degenerative processes developed towards the end to facilitate the destruction of total Hb content in 3 weeks of protein deficiency. Retioueocyte counts increased showing that blood formation was not necessarily reduced. The enzyme systems in the red cells were also adversely affected. It therefore appears that the reduction of metabolic activity associated with protein deficiency may initiate abnormal physiological functions beyond the scope of normal adaptation.

The exact extent of the protein deficiency which causes the pathological disorders is not known, but it can be stated emphatically that a pathological state may be initiated even though nitrogen balance is well maintained.

**An approach to the evaluation of protein utilization**

Summarising, it is concluded that the activities of various endocrine organs as well as the activities of protein catabolic enzyme systems may be impaired on low protein diet in which nitrogen balance is maintained by adaptation. Indices, especially such as serum protein but also Hb content, along with 17 Ks and 17 OHCS seem sensitive to measure quantitatively the effect of protein deficiency. More sensitive methods however, are needed to determine the hormones. The water content of the blood may also affect the Hb and serum protein values. More work is needed on reliable criteria to estimate the effects of protein deficiency.
An approach to estimate protein requirements

Yamamoto in the author's laboratory using criteria such as Hb and serum protein, but not of water content, recommended a protein requirement for use of 1 g/Kg/day for the adult. This finding was checked by Yoshioka who also measured total blood volume in conjunction with the Hb and serum protein concentration, in order to obtain the total Hb and serum protein contents. Normal values were obtained on protein intakes of 0.9 - 1.3 g/Kg/day consisting mainly of cereals and pulses (Protein score 62-63). In previous studies a protein intake of 0.8 g/Kg/day of medium quality protein (Protein score of 70) was found to be inadequate to maintain normal physiological functions. A consumption of 1 g/Kg/day was therefore, recommended for adults when the dietary protein consists commonly of vegetable protein. In follow-up studies Yoshimura and co-workers reduced the protein intake of subjects to 50% after being for weeks on an average Japanese intake of 2,500 calories and a protein intake of 1.25 g/Kg/day, consisting mainly of rice combined with 30% of animal protein, i.e. a reduction to 0.5 g/Kg/day. Amino acids were added stepwise to this basic diet, taking into consideration the provisional pattern of FAO. Serum protein, Hb and the excretion of 17 Ks and 17 OHCS were measured. Non-essential amino acid were added in the form of glycine and glutamic acid in the ratio of 2 to 1 in order to increase the amount of nitrogen to the equivalent of 1 g of protein/Kg/day.

With the addition of the non-essential amino acids distinct anaemia as well as a hypoproteinemia developed, but the basal metabolism was normal. In summarizing the results of the above experiments it was concluded that more than double the amount of amino acids as recommended by FAO in 1957 will be needed to maintain serum protein and the urinary excretion of the corticosteroids normal in adults. Further investigations however, are needed to compare the recommendations of the FAO/WHO expert group of 1965 with that of the 1957 recommendations. Several criteria should be used, essentially also to distinguish between responses of abnormal adaptation and those of normal adaptation; also to determine the end point of normal adaptation and the commencement of the abnormal state. Other factors also to be taken into consideration are the calorie contents, the amount and composition of the non-essential amino acids, the pattern of the essential amino acids, etc., before any final recommendation can be made on protein requirements. It will not be possible to recommend to the Japanese people a protein requirement of less than 1 g/kg/day, which has proved to be the minimum for maintaining the blood properties normal on a habitual vegetable diet. Nutritional status surveys of people accustomed to particular diets are needed to estimate practical protein allowances.
<table>
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<tr>
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<th>Standard Diet</th>
<th>Low qualified protein deficient diet (1)</th>
<th>Middle qualified protein deficient diet (2)</th>
<th>High qualified protein deficient diet (3)</th>
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<td>2 (3W)</td>
<td>2 (12W)</td>
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