REVIEW OF LITERATURE
2.1 Nutrition is a subject having relevance almost everywhere whilst not fitting in anywhere in particular. The subject is getting more and more attention since the last second world war. No matter whether it is the foetus or the fully grown up individual, a politician or a scientist everyone wants food in proper form, proper quality, proper proportion for one's physical, mental and also for social well being.

The Agent - Host-Environment interaction as suggested by Greenberg (1953) in the epidemiology of disease is also equally applicable to nutrition. McLaren (1972) suggested the Agent-Host-Environment interaction in nutrition as represent in Fig. 1.

![Diagram](image)

Fig. 1: Representing the Agent-Host-Environment interaction as suggested by McLaren.
This interaction has been modified and in the Host i.e., in the body physiological processes like digestion, absorption, transport, storage, excretion and many other intracellular chemical reactions are the factors to influence one's nutrition.

The agent i.e., the nutrients are the macro and micronutrients namely carbohydrate, fats, proteins and vitamins and some elements and presence or absence of the agent maintains or disturbs the nutrition of the individual. The agent lies within the environment.

The foodstuffs processing, storage, preparation, hygienic condition all constitute the environment. Bad handling, bad and unscientific processing, bad sanitary and hygienic habit reflect an utilization of food by the body and thus on the nutrition proper. Disturbances in the Agent-Host-environment reaction may thus cause impairment in nutrition leading to malnutrition.

Malnutrition may thus cause due either to primary or endogenous and secondary or exogenous causes and it may be excess, toxicity, overnutrition, deficiency and/or under-nutrition.

An overall picture of nutritional disorders all over the world has been presented by McLaren as follows (TABLE I).
Malnutrition is not a problem of medical man alone, but its causes are dysfunctions in economic, demographic, cultural and ecological processes. Pregnant and lactating woman and young children are especially vulnerable to nutritional stress and deficiencies. Deficiencies of minerals and vitamins especially vit. A and vit. C in mothers reduce the foetal stores of these nutrients. The period between the start of weaning and the fifth birthday is the most vulnerable nutritionally, because of rapid growth, loss of passive immunity with undeveloped active immunity.

Surveys conducted by the Indian Medical Council in 1956 and 1972 revealed that in the age range of 1-5 years children 92% suffered from some sorts of malnutrition and nearly half of a million of children between the age group of 1-6 years had protein calorie malnutrition.

The nutrition in the man as stated earlier is the interaction of the Agent-Host-environment including the different physical, biological and cultural factors.

William (1962) summarised the causes of undernutrition and malnutrition and can be well presented diagramatically as shown in Fig.2.

It has been observed throughout the world that undernutrition is very commonly found in the low income groups and is more prominent in the developing countries. An interaction between the nutritional status of the individual and his
Disasters, accidents
Sickness
of nature—Drought
Flood
man-made-Vers.
Civil disorders

Food habits
Traditions

Food intake defective
Quality, balance, timing
Quantity, physical, biochemical

Presentation

Food malnutrition

Other diseases
Infective—tuberculosis
Scabies
Endocrine—metabolic
Peptic ulcerative—allergic

Malabsorption

Gastrointestinal disorders of doubtful origin
Steatorrhea, sprue
Crohn's disease
Ulcerative colitis

Increased needs
Individual variations
Growth, pregnancy
Lactation
Injury, illness
Physical work

Congenital defects
Prematurity
Metabolic errors
Nutritional defects
Anatomical defects
Developmental defects
Infective

Malnutrition

Enzyme changes
Bacterial changes
Intestinal atony
Intestinal atrophy

Lack of habituation
Emotional
Somatic

Lack of sanitation
Lack of education

Environmental contamination

Contamination

Purgatives
Enema
Slimming

Maternal deprivation
Psychological disorders

Lack of habituation
Emotional
Somatic

Fig. 2. Causes of malnutrition
## Table I

Nutritional disorders all over the world

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Related Nutritional disorders</th>
<th>Precipitating factors</th>
<th>Major clinical features</th>
<th>Geographical distribution</th>
<th>Number approximate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Protein Energy malnutrition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Developing countries</td>
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<tr>
<td>a) early stages</td>
<td>mild or moderate deficiency of all</td>
<td>Early weaning infection</td>
<td>Stunting</td>
<td></td>
<td>200 m</td>
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<tr>
<td>b) Marasmus</td>
<td>Severe deficiency mainly energy</td>
<td>Gastroenteritis, urbanization</td>
<td>Gross wasting</td>
<td>Slum (Asia, Latin America)</td>
<td>15 m</td>
</tr>
<tr>
<td>c) Kwashiorkor</td>
<td>Severe deficiency mainly protein</td>
<td>Measles and gastroenteritis</td>
<td>Dermatosis, Rural oedema, fatty liver (Africa)</td>
<td></td>
<td>5 m</td>
</tr>
<tr>
<td>2. Xerophthalmia</td>
<td>carotene vit. A</td>
<td>Rice staple, measles, early weaning</td>
<td>Night blindness, East Asia xerosis of conjunctiva and cornea, keratomelacia</td>
<td></td>
<td>100,000</td>
</tr>
<tr>
<td>3. Rickets, osteomalacia</td>
<td>calciferol (vit. D)</td>
<td>Unfortified milk, sunlight lack</td>
<td>Bone Asians softening, in U.K., hypocalca-Urban emia tropics</td>
<td></td>
<td>Several thousands</td>
</tr>
<tr>
<td>4. Beriberi</td>
<td>Thiamine (vit. B)</td>
<td>Nonparboiled rice, alcoholism</td>
<td>Heart failure, Asia, neuropathy of cities of thy, anca-philopathy</td>
<td></td>
<td>Several thousands</td>
</tr>
<tr>
<td>Disorder</td>
<td>Related Nutritional disorders</td>
<td>Precipitating factors</td>
<td>Major clinical features</td>
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<tr>
<td>5. Pellagra</td>
<td>Nicotinic acid</td>
<td>Maize (as parridge), alcoholism, jowar,</td>
<td>Diarrhoea dermatosis</td>
<td>Parts of Africa, middle east, Central India</td>
<td>Occasional</td>
</tr>
<tr>
<td>6. Scurvy</td>
<td>Ascorbic acid</td>
<td>Lack of fruit, excess cooking</td>
<td>Haemorrhages impaired wound healing</td>
<td>Desert</td>
<td>Common</td>
</tr>
<tr>
<td>7. Folic acid</td>
<td>Folic acid</td>
<td>Excess cooking, pregnancy, drugs</td>
<td>Megaloblastic anaemia</td>
<td>Common</td>
<td></td>
</tr>
<tr>
<td>8. Vitamin B₁₂</td>
<td>cobalamin</td>
<td>Veganism, genetic</td>
<td>Megaloblastic anaemia, subacute combination degeneration of the cord</td>
<td>Occasional</td>
<td></td>
</tr>
<tr>
<td>9. Iron deficiency</td>
<td>Iron</td>
<td>Prematurity, pregnancy, milk diet</td>
<td>Microcytic hypochromic anaemia</td>
<td>World wide, many millions</td>
<td></td>
</tr>
<tr>
<td>10. Goitre</td>
<td>Iodine</td>
<td>Mountains goitrogenes</td>
<td>Thyroid enlargement, deaf mutism, deafness</td>
<td>Hill areas, many millions America, Middle East, Asia</td>
<td></td>
</tr>
<tr>
<td>11. Flourine deficiency</td>
<td>Flourine content</td>
<td>Low water content</td>
<td>Dental caries</td>
<td>Parts of many countries, many millions</td>
<td></td>
</tr>
</tbody>
</table>
Disorder | Related Nutritional disorders | Precipitating factors | Major clinical features | Geographical distribution | Number approximate

12. Obesity | Excess energy sources lack of exercise, overeating | increased adiposity, poverty risk | Disease of civilization and groups seen among affluent communities | upto 50% affected

13. Athero-sclerosis | Excess smoking, lipid, hypertension, saturated salt, diabetes, cholesterol, bates, rol, sucrose-mellitus, lack of stress minerals, fibres | coronary heart disease, stroke | many million

food intake, physical health, psychological make up, family function and social group factors has been suggested by Cobos and Gnevara (1970).

The staple food of a good number of people of the vast areas of different countries specially of Africa, India, Indonesia, Central America, Chile, Greece, Italy and Spain is the starchy diets and thus the people of these areas are left without adequate intake of animal proteins for quite a long period of time.

Czerny (1906) reported the cessation of growth and development of oedema in Weilnigs fed almost exclusively on cereal flours cooked in water with or without added butter or salt, and termed the disease as "Mehlnahrschaden" which means "nutritional damage by cereal flour". The same condition was known as "starchy
food dystrrophy" in Italy and "Malodie de la famine" in France.

Different diseases impair the protein metabolism, on the other hand, improvement of protein intake is manifested with disappearance of different deficiency signs and symptoms in the different organs of the body more particularly in vital organs like liver, brain etc.

2.2. Effect of protein-calorie malnutrition on liver:

The livers of the protein calorie deficient children or pigs are either yellow brown or bright yellow in colour, have well marked lobules and are extremely friable. Presence of fat globules is common findings, accumulation of glycogen in the liver may be quite high (Mukherjee and Nath, 1957; Heard et al., 1958; Wang et al., 1949; Baltour, 1954; Halder, 1962). Histologically the cells of the liver become angular in appearance, enlarged, distended. Fibrosis of the liver in the protein depleted animals is not the constant findings. Wannemacher Jr. et al. (1963) reported a decrease in liver protein and RNA in the protein depletion in dogs and was found to be correlated with the body nitrogen losses. The drop in the liver protein concentration may be the result of a decreased synthesis and concentration of soluble
cytoplasmic and nuclear proteins. Analysis of blood serum suggested that as the dog lost body nitrogen, there was a decrease in the concentration and synthesis of serum albumin and Beta globulin. In the protein depleted dog there was evidence also for an increase in the relative half life of all serum protein fractions which did not appear to be correlated with the rates of synthesis of the various fractions. By using the current concept of protein synthesis, it is possible to explain some of these changes in serum and liver protein in the protein depleted dogs through an amino acid RNA-PNA are interaction.

Evidence has been put forwarded for a progression from the fatty liver of Kwashiorkor (Trowell et al., 1954) to the development of cirrhosis (Davies, 1948; Ramalingaswami, 1964). There is considerable weight of opinion against such events in man (Gillman and Gillman, 1951; Haggison et al., 1957; Waterlow and Bras, 1957; Brook, 1966). This is supported by an investigation with liver histology (Suckling and Campbell, 1957).

The fatty liver in Kwashiorkor develops a starlike pattern round the portal tract and originally described as incipient cirrhosis, many investigators considered Kwashiorkor a precirrhotic condition.
The liver in malnutrition shows its alteration in colour, histological changes, like distended cells, disappearance of cytoplasm with accumulation of glycogen and fat (Platt et al., 1964; Gillman and Gillman, 1951; Waterlow, 1948; Davies, 1931; Bahar et al., 1956; Chand, 1938). These changes are associated with biochemical variations as reflected in the blood by low serum protein, low blood urea, nonprotein nitrogen, low cholesterol, low serum amylase, lipase and esterase, low values for pancreatic enzymes like trypsin, amylase, lipase.

Hepatic disfunction is observed along with these biochemical changes, however, limited studies have been done as regards to liver enzymes and plasma hormones in malnutrition.

Much experimental evidence has been reported relating levels of dietary protein to tissue enzymes contents. There are reports showing that, the activities of various enzymes in animals tissues are affected by nutrition alteration. A number of research workers have investigated the effect of protein free and low protein diet upon the activities of liver enzymes in animals as compared to that of diet containing an adequate amount of protein. In general liver enzymes are labile and different enzymes respond to protein depletion in different ways.
2.3. Effect of Protein Calorie Malnutrition on Hb and Plasma protein

Recently a great deal of research has been carried out to develop new methods to evaluate protein utilization.

The earliest surveys of nutritional status were the recording of body weight co-related with height, age and sex.

Anthropometry has provided many co-relation with racial or geographical or economic background. Anthropometric standard of reference are particularly useful in the objective interpretation of nutritional survey data from economically underdeveloped areas.

Although measurement of body weight, height, as related to sex and age are generally considered to offer most objective and probably the most sensitive direct measure of nutritional status of an individual, the validity of these criteria however depends on the fact of assumption that the body composition remains constant. However, review of literature reveals that appreciable shifts in the tissue compartments, water, fat and protein frequently accompany changes in the dietary, nutritional status and age of the experimental animals.

The laboratory methods most widely used in appraising nutritional status are the determination of plasma proteins.
and haemoglobin content of the blood.

The simplest and oldest method used to evaluate the nutritional properties of proteins in an animal is to determine the nitrogen intake and nitrogen excreted. This difference, called nitrogen balance, shows whether the animal gains or loses body nitrogen. The measurement of nitrogen balance alone is not a sound and infallible criterion of protein nutrition.

Addie et al. (1936), Kosterlitz (1947) and Harrison and Long (1945) observed in a series of animal experiments that animals either starved or fed with a protein free diet, suffered from very outstanding loss of liver protein. The effects of various dietary protein preparations on the reflection of liver proteins have also been calculated out.

It has been noted that if the dietary protein intake is restricted, the continual catabolic activity of the living system results in a reduction in amino acids supplied to the metabolic pool and thereby decreasing the protein stores.

Melnich et al. (1936) and Cox and Mueller (1944) suggested that various food proteins differ qualitatively in their ability to promote synthesis of plasma protein.

Madden et al. (1938) demonstrated that some dietary proteins may favour the production of plasma albumin and other may favour the production of plasma globulin.
The experiments of Madden and Whipple (1940) suggested that their observation on divergence of haemoglobin content and body weight may constitute and be an expression of haemoconcentration effect of severe malnutrition.

Peters (1942) has pointed out considerable justification of the fact that three dimensional functions cannot be evaluated by two dimensional measurement. Hence, variations in total circulating protein and plasma volume cannot be correctly estimated from percentage of protein concentration alone.

Despite of these obvious defects, it is still widely thought and believed that hypoproteinemia generally with a reduced ratio of albumin and globulin, is the hallmark of starvation.

Youmans et al. (1943) have failed to demonstrate hypoproteinemia in low income group in U.S.A. and Canada.

In a group of well-fed dog the effect of protein depletion in diet on the concentration of plasma protein was studied by Chow et al. (1945). The animals were subjected to malnourishment by offering a protein free diet, which contain sufficient amount of calories and vitamins to meet the daily requirements. After 6 to 8 weeks of feeding it was observed that, the plasma protein concentration dropped down from a value
of 6.8 to 5.0 g%. Further studies with electrophoretic analysis of dog plasma during pre and post feeding revealed that, the circulating plasma albumin decreased remarkably in comparison to the total circulating globulin.

Hegsted et al. (1946) reported that in men the total protein and also plasma albumin and globulin showed tendency to decrease, when they were fed with low protein, all vegetable diet which was quite low enough to produce negative nitrogen balance. However, Keys et al. (1946) observed that there was slight decrease in plasma protein in a group of 34 men maintained on a European type famine diet for a period of 6 months.

Kark et al. (1947), in studies on nutrition of troops in tropical areas, found that there was reasonable correlation between the average protein intake and its concentration in the serum. Indian troops, with lower protein intake than American and Canadian had lower serum protein level.

Alper et al. (1950) studied the utilization of an enzymatic digest of casein by dogs depleted of protein either by prolonged protein free feeding alone or in conjunction with plasmapheresis. Nitrogen retention and plasma protein regeneration were determined, during both phase of experiments.
It was concluded in hypoproteinemia effected by restricted plasmapheresis, the "reserve protein stores" of experimental animals were not permanently depleted although plasma protein and plasma volume were markedly reduced. On the other hand, "reserve protein stores" of the animals fed with protein free diet over a long period were reduced first followed by depletion of plasma protein. The higher retention of orally administered nitrogen indicates the importance of the pattern of amino acids and peptides available to the animals for the synthesis of its body tissues.

Studies of Scrimshaw et al. (1951) have shown that the problem of interpretation which is posed by the serum protein data is not only one of failure of low protein intake to be reflected in serum protein levels, but also the occurrence of a distinct elevation of serum protein above normal levels under certain conditions in relatively undernourished individuals in Central America. Values as high as 7.39 - 7.61 gm per cent were found in group who were well hydrated but distinctly undernourished. However in animals, Albanese (1952) observed that the total serum protein became low when the animals were kept in poor protein diet or with single amino acid. Many studies were conducted during 1st and 2nd World War in famine areas where the majority of human population has suffered from serum hypoproteinemia.
Tissue enzymes change due to nutritional condition in the initial studied were restricted to specific vitamin and metal deficiency. Axelrod et al. (1939–1940; 1941 and 1942) found that Riboflavin deficiency causes reduction of liver and kidney. d-amino oxidase, liver xanthine oxidase and succinic oxidase, the coenzyme of these enzyme being riboflavin. Hove et al. (1940) has worked out that the Zno deficiency causes a reduction in carbonic anhydrase which is activated by zinc ion. The concentration of riboflavin in the liver depends on the level of protein diet as studied by Farett and Perlzweig (1943).

It is well known that when an animal changes from a high to low protein diet or vice versa; it loses or gains proteins. These proteins are considered to be labile and are called "reserves".

Dietary protein is not only the substrate for certain tissue enzymes, but also structural material of all tissue enzymes. Therefore, the phenomenon are self regulatory and complex, so that many more studies are needed to clarify exact physiological meaning of enzyme changes caused by alteration in dietary protein.

Addie et al. (1936) showed that the rate of loss and gains of protein varies in different tissues, liver
protein being the most labile. By fractionation of tissue protein using a salting out procedure, Luck (1936) was not able to demonstrate the existence of a special reserve protein in the liver which differs chemically from others. It was concluded that no single protein can be regarded as reserve material and all liver proteins participates in the function of storage. The fact that enzymes are protein and tissue proteins contains enzymes suggested the possibility that the enzymes in tissue also decrease with the protein depletion.

Lightbody and Kleinman (1939) reported that liver arginase activity of rat fed a 6% milk protein diet was about one half that of rats fed 25% milk protein diet.

Potter and Klug (1947) had observed that liver octanoate and succinate oxidase were depressed in rats fed only 6 to 10% protein diets.

Miller (1948) confirmed that inanition in rats caused a loss of liver catalase, alkaline phosphatase, xanthine dehydrogenase and cathepsin which paralleled or exceeded the loss of liver protein. Various investigators have extended this study to include the effect of protein depletion on many kinds of enzymes and enzyme system. As a result it was found that different enzymes respond to protein in different way. Some decrease, some remain unchanged and others
increase in their unit activities, the physiological meaning of the changes of each enzyme content in different level of dietary protein remain unexplained in many cases.

Seifter et al. (1948) made an interesting observation that both liver arginase and D-amino acid oxidase are lost more rapidly than liver nitrogen in rats fed a non-protein diet.

Williams et al. (1949) observed that liver xanthine oxidase in rats especially sensitive to subtle changes in dietary protein. The liver of animal fed a 14.6% casein diet with 0.25% dietary methionine has got xanthine oxidase level twice than those animals, which did not receive the methionine. Supplement with lysine deficiency a 50% decrease in liver xanthine oxidase activity was associated with marked decrease in succinic and liver choline oxidases.

Litwack et al. (1950) observed that the rapid loss of xanthine oxidase activity induced by a non-protein regime, proceeds at a greater rate, than the loss of flavin adenine dinucleotide co-enzymes or other liver proteins.

Litwack and Williams (1952) studied the relationship of liver xanthine oxidase to quality of dietary protein. In their study of this relationship between the liver xanthine oxidase activity and quality of protein, casein, lactalbumin
gliadin and meat has been made, along with method of evaluating the value of dietary protein in terms of liver xanthine oxidase activity of the animals fed the protein. Using this method for evaluating various proteins, the following relative values were obtained, assuming gliadin equals to 1.0 and meat protein 11.7 casein 9.7 lactalbumin 8.3.

Dean and Ruth Schwartz (1953) studied the serum chemistry in uncomplicated kwashiorkor. They observed that, in actual stage of the disease, total serum protein was reduced, albumin being mostly the low component. Enzyme activity was also observed to be very low. Treatment with large amount of milk protein rapidly caused alteration in the blood, which coincided with alterations in contents of the duodenal juice with the improvement in clinical condition of children. In their study, they noticed that total protein, blood urea, cholesterol, alkaline phosphatase, pseudocholinesterase, amylase, esterase and chlorides showed a lower level than the normal before the treatment; and after 28 days of treatment with milk protein, the values of all became near the normal levels.

In general, protein depletion decreases the total activity of various liver enzymes, because the average weight of the liver in the depleted animals is significantly lowered.
than that of ad-libitum fed control. Jainio et al. (1953) studied a group of enzymes, namely cytochrome, oxidase, succinoxidase, succinic dehydrogenase, d-amino acid oxidase, DPN-cytochrome C-reductase, uricase and xanthine oxidase in liver tissue of protein depleted rat. It was observed that most of these enzymes decreased in case of animal with protein depletion as compared to the animals which are fed ad-libitum diet. Since rats on a protein free diet reduce their food intake, in another set of experiment, Jainio et al. (1953) employed pair-fed and ad-libitum fed control in order to compare the effect of protein and partial food restriction. The use of pair fed control can exclude the effect of consuming more energy in ad-libitum control than in depleted animal. It has been observed that the unit activity of succinoxidase, succinic dehydrogenase, d-amino acid oxidase, DPN-cytochrome C-reductase and uricase decreased in protein depletion as compared with ad-libitum fed controls, whereas the cytochrome oxidase level increases slightly and, xanthine oxidase was found to be unchanged in protein depletion.

Margaret et al. (1954) studied a type of malnutrition in African adults due to deficiency of dietary protein. In their study, adult men suffering from protein deficiency were treated with a high calorie high protein diet. During treatment their red cell count, serum proteins, serum pseudocholinesterase and protein bound lipid were studied.
They observed low level of serum albumin and raised level of serum globulin at the beginning of the treatment in all the patients, with or without clinical evidence of liver damage. During treatment with high calorie, high protein diet the serum albumin and red cell count rose. The changes in globulin were not consistent. Serum pseudocholinesterase was low in such patients and elevated with increase of the serum albumin. Low lipid and the lipo-protein in the person with malnutrition was also observed.

The susceptibility of enzyme response to protein depletion differs in different tissue. Wainio et al. (1959) studied the enzyme cytochrome oxidase, succinate-cytochrome C-reductase and DPNH-cytochrome C-reductase activity of brain, kidney, skeletal muscle and spleen tissues were assayed after rats had been fed a protein free diet for a period of 49 days. The effects of sub-acute food restriction were interrupted with aid of pair fed animals, which were given a diet containing 18% casein. It was observed that the food restriction alone had little or no effect on the unit activities of the enzymes. Some unit activities rose slightly others fell. Food restriction did, however, slightly decrease the total enzyme activities, since the organs of the pair-fed animals except brain were somewhat found to be smaller that the organ of the animal fed ad-libitum. Further they have observed that the protein depletion with
its attendant food restrictions had no effect on the unit activities of the brain enzyme, but it lower the activities of the enzymes of kidney, skeletal muscle and spleen by about 10-20%.

Waterlow and Patrick (1954) noted an elevated level of transaminase in liver tissue in malnutrition, however, their findings were not constant and uniform, and it was not supported by the subsequent findings of Burch et al. (1957). It has also been suggested that protein anabolic potentiality is not lost in malnutrition (Robinson et al., 1957). Recently it has been observed that gamma-amylase is responsible for amylosis process involved in glycogenolysis and variation of amylase was observed after addition of dietary item (Sarkar and Goswami, 1975, Sarma et al., 1975) but the effect of malnutrition on this particular enzymes has not been studied.

As mentioned above there are many reports in change in enzyme response following alteration in dietary protein level. However, the physiological mechanism of these changes are not clear.

Kunkell et al. (1956) studied the cytochrome oxidase activity and body weight in rats and in three other species of large animals. The cytochrome oxidase activity of skeletal muscles (specially gracilis) in rat, sheep, swine and cattle was found to be roughly an inverse function of the body weight. A logarithmic regression of 0.239 was calculated. Although
the cytochrome oxidase activity per unit weight of liver in sheep, swine and cattle series does not reflect the variation in body mass, the regression of logarithm of total liver cytochrome oxidase activity on the logarithm of body weight was calculated to be 0.647. The results support that the total measurable cytochrome oxidase activity is proportional to body to 3/4 power and hence to the basal metabolic rate.

Burch et al. (1957) studied tissue enzymes in human malnutrition. The enzymes selected were four flavin containing enzymes i.e., glycolic acid oxidase D. P. N. H. dehydrogenase, xanthine oxidase and D-amino acid oxidase. The first two enzymes remained unchanged, but xanthine oxidase and D-amino acid oxidase activity decreases significantly in the liver of malnourished humans. Their results are in accord with those obtained in earlier experiment in group of animals fed with protein deficient diet.

Ross and Bitt (1957) have studied the relationship between activity of hepatic enzymes and diet and the age of the rat, and between liver enzymes. They observed that when rats are maintained on diets varying in amount of casein and dextrose, the level of activity differed with age. The older the rat, the lower the liver alkaline phosphatase and the cathepsin activity and higher the liver histidase and D-amino acid oxidase activity. In all age groups these four
enzymes were found to vary also with casein content of diet. Therefore, the level of these activities appear to be dependent upon the diet and the age and the growth of the rat. This would indicate the existence of diet-age pattern for liver enzymes. The level of enzymes can be altered at any given age by a change in the level of diet. A diet containing small amount of casein alters the levels of activity of enzymes in older animals, whereas, a diet containing only a large amount of casein can change the level of enzymes in young animals. Further they have postulated that biological ageing may be a manifestation of naturally occurring enzymatic changes and alteration of the enzymological activities through diet might possibly influence the ageing.

Barrows Jr. (1958) studied the effect of dietary proteins on the level of plasma cholinesterase of rats. They observed that, the concentration of plasma cholinesterase of female animals differs according to protein preparation in diet, this was however not observed in male animals. Animals fed wheat gluten had low plasma cholinesterase levels. Addition of lysine and methionine to this diet causes an increase in the level of enzymatic activity. Although the growth rates of the female rats fed with protein preparations of high nutritive quality were indistinguishable, the plasma cholinesterase activities of the animals fed with diet
containing beef muscle or whole desiccated liver were markedly higher than those of animals fed other protein preparations. This high concentration of plasma cholinesterase activities is believed to be due to the presence of some factors in beef muscles and whole desiccated liver rather than to difference in the amino acid contents of dietary proteins. By fractionation of whole liver yielded a fraction, which when added at a level of 4% to a 24% casein diet caused a distinct elevation of plasma cholinesterase activity.

Wainio et al. (1959) studied the effects of threonine deficiency on changes in enzyme activity and liver fat deposition with time and observed an alteration in the activities of xanthine oxidase and malic dehydrogenase with time in the groups of albino rats fed with low protein diet (9% casein) deficient in threonine in comparison to the control group of animals fed with same low protein diet but supplemented with 0.36% of DL-threonine. The maximum decrease in activities of these enzyme systems in the threonine deficiency group occurred on the 19th day of experiment. This phase of decreasing activity was followed by a period of recovery.

Waterlow (1959) thoroughly reviewed the effect of malnutrition on enzyme activity. Cholinesterase, alkaline phosphatase, transaminase and other enzymes were measured.
Malnutrition did not affect the cholinesterase activity of the red cells but the activity in the serum was greatly reduced. Waterlow concluded that the measurement of serum cholinesterase is of practical value. His group also studied six oxidative enzymes, malic, lactic, glutamic dehydrogenase, (NADH) cytochrome C-reductase, cytochrome oxidase and glutamic oxaloacetic transaminase in human malnourished liver and observed that, these enzymes were not reduced in human and were quite different from the findings in the rat. Of these enzymes, transaminase and malic dehydrogenase had higher activity per unit weight of tissue during the stage of malnutrition than after recovery.

Weber et al. (1961) found that various enzymes namely 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, fructose 1-6-diphosphatase, phosphoglucomutase and phosphohexoisomerase involved in the utilization of glucose are decreased in chicken fasted for 7 days. After one day of refeeding the activities returned to near normal levels except for glucose-6-phosphate dehydrogenase which increased by three times by the control values. The result indicated that those enzymes involved in glucose-6-phosphate metabolism showed widely different behaviour during nutritional depletion and restoration process. However, Allard et al. (1957), Landau et al. (1958) and Vaughan et al. (1960) have observed that the enzymic studies of Weber et al. (1961) has not only affected by changing in the protein and
nitrogen pool of the liver, but were also involved in the control of blood glucose level, and any alteration in their activity due to change in the diet was considered a metabolic adoption of tissue enzyme for the maintenance of homeostasis.

Muramatsu and Ashida (1962) studied activities of ten liver enzymes of rats fed with diets containing various levels of casein after a period of starvation. According to response curve obtained, the enzymes studied could be categorized into four groups. Xanthine oxidase, succinic dehydrogenase, glutamic acid dehydrogenase were found to decrease on protein free diets. With increase of protein intake these enzymes reached a maximum activities and was not effected by a further increase in dietary protein. Arginase, glutamic pyruvate transaminase, glutamic oxaloacetic transaminase and uricase increased with the increase in the dietary protein level, suggesting a specific role of these enzymes in nitrogen metabolism. Cathepsin was essentially unchanged and alkaline phosphatase increased in the rats fed with protein free diets. The enzymes in the first instance have patterns of response similar to those of liver nitrogen and the growth rate of the animals.

Muramatsu and Ashida (1963) studied the effect with varying levels of eggalbumin, fishmeal, soybean protein, wheat gluten and casein supplemented with or without methionine for two weeks in the diet of the growing rats. They
observed marked influence with the variation of dietary protein on growth rate, liver weight, liver nitrogen, the activity of liver xanthine oxidase and the liver succinic dehydrogenase. It was found that, when rats received different dietary proteins, except soybean protein, the response curve of the liver xanthine oxidase or succinic dehydrogenase plotted against the protein level in the diet was similar to those of growth rates, liver weight and liver nitrogen plotted in a similar way. The correlation of enzyme activities with the growth rate was higher in xanthine oxidase than in succinic dehydrogenase. They also observed that when rats were fed with casein diets supplemented with methionine, the minimal protein level for maximal growth and the maximal activity of the liver xanthine oxidase was shifted from the 20% casein level to the 12% one. Liver xanthine oxidase concentration could be utilized as a criterion for the quantity of dietary protein required for maximal growth of young rats. However they felt some limitations in this procedure because besides protein level factor like protein adequacy could also influence the enzymes activity, as in case of soybean protein.

Waldorf et al. (1963) observed a variation in glutamic oxaloacetate and glutamic pyruvate transaminase activities in liver of rats, subjected to minimum food intake or diets with high in casein, or fats. An increase in the
activity of the enzymes occurs on a high casein diet and response is greater in young than old rats. But by feeding a low carbohydrate, high fat diet and adequate in protein caused no significant change in the activities of either enzymes.

The patterns of decreases in total liver enzyme activities, body weight and some selected liver constituents in young and old rats were studied during progressive starvation by Freedland (1967) and observed that in the young rats, total liver protein liver glycogen, phosphoglucomutase, fructose diphosphatase, aldolase, succinic dehydrogenase and fumarase decreased in activity after one day of starvation and remained relatively constant during the next 3 days of starvation. A decrease in soluble liver protein, phosphohexoseisomerase, glyceraldehyde 3 phosphate dehydrogenase, lactate dehydrogenase, alpha glyceralphosphate dehydrogenase, malic enzyme (TPN), isocitric dehydrogenase (TPN) and malic dehydrogenase was noted on 1, 2 and 4 days of starvation. A decrease in the activities of phosphofructokinase, glucose 6-phosphate dehydrogenase 6-phosphogluconate dehydrogenase and the pentose phosphate metabolizing enzymes was also observed on days 1, 2 and 4 of starvation. Glucose-6-phosphatase and uridine diphosphate glucose dehydrogenase activities remained relatively constant during 3 days of starvation and decreased on day 4. The patterns of decreases
in total liver enzyme activities in old rats were similar to those for young rats with a few notable exceptions. The most striking of these was the sharp decrease in the activity of uridine diphosphate glucose dehydrogenase on day 1 of starvation in old rats. The activities of glucose 6-phosphatase and lactate dehydrogenase were maintained to a greater extent in young rats than in old rats. But the activities of aldolase, alpha glycerol phosphate dehydrogenase, isocitrate dehydrogenase (TPN) and glucose 6-phosphate dehydrogenase appeared to be more stable during starvation in old rats. In general, the activities of enzymes involved in gluconeogenesis, the citric acid cycle and the enzymes of the pentose phosphate metabolic process were equally stable to starvation. The activities of enzymes of the Embden-Meyerhof Pathway (phosphoglucomutase) aldolase, glyceraldehyde 3-phosphate dehydrogenase and lactate dehydrogenase were somewhat less stable. The activities of those enzymes which are thought to be associated with lipogenesis (glucose 6-phosphate dehydrogenase, malic enzyme and alpha glycerol phosphate dehydrogenase) were the least stable.

The effects of some nutritional and hormonal factors on the level of the activity of methionine adenosyltransferase in rat liver were studied by Toopav and Tarver (1967) with the following results. (1) Thyroideotomy and cor...
treatment increased the activity of this enzyme whereas adrenalectomy and administration of bovine growth hormone or triiodothyronine lowered its activity in the liver (2) The activity also changed with dietary protein content. Intact animal fed a high protein diet had high activity but the adrenalectomised (or hypophysectomized) animals had low activities irrespective of the protein content of their diet. (3) Excess methionine added to a 27% casein diet caused little change in the activity of adenosyltransferase. However, rats fed a protein free diet supplemented with methionine or its 2-hydroxy analogue (but not glycine) retained a higher level of activity than those on the protein free diet (4) starvation for 2 days brought about a decrease in the activity which returned to the normal level in the subsequent 2 days.

Bella Szepesi and Freendland (1967) studied on alterations in the activities of several rat liver enzymes at various times after initiation of high protein regimen. They have investigated the time course and increase of several rat liver enzymes following the feeding of a high protein diet (90% casein and carbohydrate free). They have used the male Sprague-Dawley rats of 6-8 weeks of age weight ranging 150-200 gms. To these animals 90% glucose protein free diet was fed for 4 days in order to obtain maximal responses in activity after dietary change. The animals were killed and liver enzyme activity was determined at 0, 1, 2, 3, 4 and 7 days after rats were offered the high protein diet. All the enzymes studied,
with the possible exception of Phosphorlyase, were increased by the high protein diet. Two patterns of increase in enzyme activity were observed. A number of enzymes including fructose 6-phosphatase, alpha glycerophosphate dehydrogenase, pyruvate kinase, malic enzyme and tyrosine after keto glutarate transaminase reached maximum activity within 48 hours or earlier after the change in the diet or either remained nearly constant or declined with time. The activity of another group of enzymes including fructose 1-6 diphosphatase, glucose 6 phosphate dehydrogenase, GOT, GPT and serine dehydrase rose sharply in the second and third day after the dietary change.

Belaeepei and Freedland (1968) studied the effects of dietary changes and starvation in meal fed, male rats of Sprague Dawley strain following a training period of 2 weeks, and a protein deprivation period of 4 days. They observed that 90% casein diet increased the activities of the enzymes glucose-6-phosphatase, glucose 6 phosphate dehydrogenase, serine dehydrase, tyrosine-alpha-keto-glutarate transaminase, glutamic pyruvic transaminase and glutamic oxaloacetic transaminase, 24 hours post meal. Serine dehydrase and glutamic oxaloacetic transaminase activities were further increased in animals killed 48 hours after the protein meal. They also observed an increase in the activity of serine dehydrase in rats killed 48 hours after their last protein
free meal. The feeding of diets containing both carbohydrate either glucose or fructose and casein increased glucose-6-phosphate dehydrogenase and malic enzyme activities to a greater extent than the 90% casein diet but the increase in transaminase activities were less than those obtained by feeding the 90% casein diet. Serine dehydrase activity was not increased by a 65% carbohydrate diet and was increased only slightly by diets containing 57.5% casein plus 32.5% carbohydrate. Tyrosine-alpha-ketoglutarate transaminase activity increased by this latter diet almost similar to the 90% casein diet. The inducing effect of carbohydrates on enzymes activities was observed even if the test diets contained considerable amounts of protein, whereas the inducing effect of protein was considerably decreased by even a moderate amount of carbohydrate.

Belasezepsi and Freedland (1968) studied the effect of high carbohydrate (protein free) diet on the activities of several liver enzymes in rats which were previously adapted to a 90% casein, carbohydrate free diet. Both, the 90% glucose and 90% fructose diet decreased all the liver constituents and enzyme activities studied with the exception of pyruvate kinase, the activity of which was increased temporarily by both the carbohydrate diets. They have suggested the possibility of a control system regulating the synthesis
of this enzyme at the translation level. Phosphorvlase, glucose-6-phosphatase, fructose 1,6, diphosphatase, L-alpha-glycerophosphate dehydrogenase, glucose-6-phosphate dehydrogenase and malic enzyme activities were decreased to variable extent. In some cases glucose-6-phosphate dehydrogenase and malic enzyme activities were increased. Tyrosin-alpha-ketoglutarate transaminase activity was minimal after 1 day of carbohydrate feeding. Glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and serine dehydrate activities declined logarithmically. In the glucose fed animals glutamic oxaloacetic transaminase declined with a half life of 4 days, glutamic pyruvic transaminase with a half life of 2 days and serine dehydrate with a half life of 1.7 days. Fructose feeding increased the half life of both glutamic oxaloacetic transaminase and glutamic pyruvate transaminase but not the half life of serine dehydrate. Pair feeding experiments had shown that the tendency of the fructose diet to increase liver size and the half life of the 2 transaminase (glutamic oxaloacetic transaminase and glutamic pyruvate transaminase) was not due to an increased consumption of the fructose diet.

Bela Szepesi and Freedland (1968) also studied the effects of feeding a high protein diet to rats previously adapted to a high carbohydrate diet containing adequate protein. The studies were carried out on male rats of Sprague-Dowley strain. The high carbohydrate diet was found to stimulate
growth which was not augmented noticeably by feeding the high protein diet. Liver protein reached maximal values one day after the dietary change, whereas relative liver size values remained relatively constant. The glycogen content of liver was minimum one day after the dietary change which was followed by relatively higher and constant values. The enzymes studied were divided into two groups based on the pattern of change in enzyme activity after the dietary shift. In one group which included fructose 1,6 diphosphatase, glucose 6 phosphatase, glutamic oxaloacetic transaminase, glutamic pyruvate transaminase, serine dehydrase and tyrosine alpha ketoglutaric transaminase, enzyme activity increased with time. Furthermore, the activity of these enzymes increased without a lag, with the exception of fructose 1,6-diphosphatase which increased in activity only after a one day delay. In the second group which included L-alpha-glycerophosphate dehydrogenase, glucose 6 phosphodehydrogenase, pyruvate kinase and malic enzyme activity decreased by feeding the high protein diet, except that the activities of pyruvate kinase and malic enzyme temporarily increased one day after the dietary change. They have discussed the physiological significance of a lag in enzyme induction, when such occurs and a possible mechanism for the transitory induction of pyruvate kinase and malic enzyme by the high protein diet.
Bergner et al. (1968) studied the relations between protein nutrition, thyroid secretion rate and some enzyme activities. They used 2-5 growing pigs for each of 9 diets with different proteins and a tenth group had protein free diet. The biological values of the proteins ranged from 4-6 for gelatin, to 89.9 for dried skimmed milk. Next, the thyroid secretion rate of each pig was estimated and then increasing injections of thyroxine were given and serum was taken for the estimation of glutamic pyruvic and glutamic oxaloacetic transaminases, arginase and leucine aminopeptidase. Apart from gelatin, the biological values of the proteins showed a significant positive correlation with thyroid secretion rate and also negative correlation with each of the enzymes, the closest being with arginase ($r=0.98$); correlations of thyroid secretion rate with the enzymes were very low and not significant.

Chang et al. (1968) studied the effect of diet, dietary regimens and strain differences on some enzyme activities in rat tissues of Wistar R BHE rats which were on a stock diet containing 25% whole egg powder. Rats of both strains had greater weights of liver and perirenal fat pads and more liver protein on the egg diet. In the liver glucose-6-phosphate dehydrogenase activity was higher on the stock diet but glucose-6-phosphatase and aldolase were higher on the egg
diet. BHE rats had higher glucose-6-phosphate dehydrogenase and Beta glucuronidase activities in liver but less alkaline phosphatase in serum and liver than Wistar rats on the same diet. In both strains fasting for 16 hours showed less aldolase in liver and less alkaline phosphatase in liver, serum and kidney. Wistar rats on the egg diet oxidised the C-1 of glucose more rapidly than the C-6. They had the lowest glucose-6-phosphate dehydrogenase activity in liver and appeared to use the hexose-6-monophosphate shunt more than other groups. Total 14CO₂ output from glucose U₁⁴C was similar in all groups.

Sassoan et al. (1968) studied the dependence of rat liver glucose 6 phosphate dehydrogenase levels on diet. They have noted that the level of glucose-6-phosphate dehydrogenase in liver is altered strikingly by starvation and also by refeeding, but the steps by which increased synthesis of this enzyme is induced have not been satisfactorily identified.

Recent work suggested that hormones may have a general, rather than a specific role. When starved rats were reeled with diets varying either in amount or in carbohydrate content, their liver glucose 6-phosphate dehydrogenase increased directly with their carbohydrate intakes. Insulin and other hormones which were tested had no effect on liver glucose 6-phosphate dehydrogenase levels when given with adequate carbohydrate, but with adequate protein.
Hemon and Berby (1968) studied the changes of enzyme activities with diet and thyroxine during postnatal development of the rat. They have described the evolution of alpha-glycerophosphate oxidase, oxidoreductase and malate dehydrogenase activities in the brown adipose tissue of young rats both normal and hyperthyroid. Both enzyme activities were greater in adipose tissue than in liver, and less sensitive to thyroxine when the rats were fed from weaning with semi-synthetic diet high in fat or in carbohydrate the alpha-glycerophosphate oxidase activity was the same as with the stock diet. The malate dehydrogenase activity increased during the weaning period with the high carbohydrate diet, but stayed at the suckling level with the high fat diet. In the liver the alpha-glycerophosphate oxidase activity failed to decrease after weaning with either semisynthetic diet. Liver malate dehydrogenase increased more and was found to be more sensitive to thyroxine with the high carbohydrate diet than with the high fatty diet.

Toulou et al. (1974) studied the activities of liver aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) in chickens, fed on diets of ground maize and fish meal, soyabean oilmeal or ground nut oil meal to provide 40% protein. Both enzyme activities showed a significant rise in response to increase of dietary protein with all the protein types. The rise in GOT activity as a
function of protein content of the diet was about linear in chickens on fish meal or ground nut oil meal but was progressively smaller in chickens on sovameal. Unlike the activity of GOT, that of GPT was affected significantly by the type of dietary protein.

Peret (1975) studied the effect of quantity and quality of dietary proteins on variations in enzymic activity. In their experiments they had taken male dietary CF rats aged 8 weeks and weighing 180g to 200g. They were given a diet with no protein or with casein or wheat gluten providing 10-70% protein which displaced a portion of starch and sucrose. Feeding was for 8 days for the protein free group and 23 days for the groups getting casein and gluten. At autopsy the liver was taken for the estimation of the activity of pyruvate kinase (PK), glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme, glutamic pyruvate transaminase (GPT), glucose-6-phosphatase (G6Pase) and phosphoenol pyruvate carboxykinase (PEPCK). Gain in body weight was highest in the groups on 30 to 40% casein and on 40% to 50% wheat gluten. Activity of glucose-6-phosphatase was not affected by the quantity or quality of protein in the diet, that of G6PDH depended mainly on the quality of the protein, being highest with casein. Activity of GPT rose with the quantity of protein in the diet, that of PEPCK and PK fall.
It was concluded that maximum growth rate on diet containing any given amount of protein depended on the balance between glycolysis and gluconeogenesis.

Trocher and Collins (1977) studied the effect of protein intake on the activities of liver specific enzymes in the plasma of dairy cows. Activities of glutamic dehydrogenase (GLDH), sorbitol dehydrogenase (SDH) and ornithine carboxyl transferase (OCT) were estimated in tissues of cattle after slaughter. The liver had most of all 3 enzymes and the kidney cortex also had high activities of GLDH and SDH, OCT was negligible in tissues other than liver. GLDH and OCT were estimated in plasma of 2 groups of cows given different amounts of protein before calving and upto 14 weeks after calving and then the same feed for all. Increase of both the enzymes after calving was noted. In both the groups, reaching 3 to 7 times more than the values before calving and between 7 and 17 weeks after calving and declining thereafter. In the group given protein at the recommendation quantity of the ARC (NAR 36,5513) the value of both the enzymes are higher than that of in the group given 25% less protein. The conventional interpretation of this is usually attributed to the hepatic pathology associated with larger intake of protein though other explanations are not ruled out.
Roger et al. (1977) studied the lack of hepatic enzymatic adaptation to low and high levels of dietary protein in the adult cat. The activities of the three enzymes of urea cycle, and also several catabolic gluconeogenic and lipogenic enzymes, were measured in the liver of adult cats. By giving a commercial kibble, a purified diet with 17.5% or 70.5% protein or starved for 5 days, it was noted that except an increase in thyroxine aminotransferase after the high protein diet there was no change in the activities of the hepatic enzymes with the variation of the dietary protein content. Starvation had little effect on the activities of the enzymes compared with that found in similar experiments in rats. These results indicate that the cat may have only minimal capabilities for enzyme adaptation, compared with many herbivores and omnivores and may provide an explanation as to why cats have unusually high protein requirements compared with many other animals.

2.5. Biochemistry of Protein metabolism

General aspects of protein metabolism: The complexity of protein metabolism and the central fundamental role of protein which is vital for the existence of all forms of life has stimulated a tremendous amount of research. Many
investigators have contributed greatly to understand the biochemistry of protein and have given adequate information to formulate a basic understanding of protein metabolism. However, many of our concepts are still theoretical and elementary in nature. New and creative research will be required to broaden our understanding and to solidify our concepts relating to protein metabolism.

A. Digestion and absorption:

It has been established by Christensen (1949), Dent and Schilling (1949), Parshin and Kubel (1951), Denton and Ilvheim (1954) and Stein and Moore (1954), that under normal conditions ingested proteins are broken down into free amino acids and are absorbed as such into the blood. These blood amino acids are an integral part of the metabolic amino acid pool. However, the mechanism by which free amino acids are absorbed from the gastrointestinal tract into the blood stream is yet controversial to date. It had been assumed that the transfer of amino acids takes place by simple diffusion, but Christensen et al. (1954, 1958, 1959), Korelitz and Frank (1959) and Fridhandlor and Quastel (1955) indicate the existence of an active and selective mechanism for amino acid absorption. Though the present methods for detection of the absorption of low concentrations of short chain peptides are inadequate, the amount of absorption occurring in this manner is however appears to be insignificant.
B. Anabolism and Catabolism—Dynamic Equilibrium:

After absorption, the amino acids of the blood, an integral part of the metabolic pool are translocated throughout the body via the circulatory system. At this stage the amino acids can enter into one of the two metabolic pathways i.e., anabolism or catabolism. Anabolism is the pathway of synthesis of cellular protein pathways 4 and 5 (Fig. 3). Catabolism is the reverse process involving the breakdown of body protein to amino acids pathway 5 (Fig. 3) followed by the breakdown of amino acids with the excretion of urea and other nitrogen compounds such as creatinine pathway 7 (Fig. 3). The two pathways viz. anabolism and catabolism are in dynamic equilibrium since the metabolic pool is supplied with amino acids from the breakdown of tissue proteins which at this stage are indistinguishable from those contributed by dietary protein sources. According to Polin (1905) the protein metabolism associated with creatinine production is called "endogenous protein metabolism" and the protein metabolism which controlled urea excretion and dependent upon dietary protein is called "exogenous protein metabolism." However, the two metabolic pathways are not independent and interdependent. The body fluids serve to transfer amino acids for the synthesis of body proteins and other nitrogen containing substances. Free amino acids in the body fluid are derived both from
exogenous and endogenous sources thus creating a common bond between the two metabolic routes. Supply and utilization of the amino acids have been termed as the metabolic pool and it serves to establish a "dynamic equilibrium" between dietary and body proteins and also between tissue proteins. The rapid interchange of dietary and cellular amino acids have been demonstrated by Schonheimer et al. (1939) by using isotopically labeled amino acids.

C. Factors affecting protein metabolism:

The great importance of protein to the living organism is clearly manifested by the complexity of protein metabolism and the great number of physiological functions performed by proteins. Protein metabolism is influenced by many factors; the major factors being (i) amino acid composition of the dietary protein (ii) dietary calorie intake (iii) dietary protein intake and (iv) the nutritional and physiological status of the individual when other necessary constituents of diet are provided in adequate amount.

I. Amino acid composition of dietary protein:

Approximately twenty two amino acids are known to be needed for growth and maintenance of cellular tissue and also for other metabolic functions. Under physiological conditions, eight of these twenty two amino acids cannot be synthesized by the adult human being, and must be present in the diet to permit physiological functions to proceed.
These amino acids are called 'essential' or 'indispensable'. Under normal conditions the remaining amino acids can be synthesized by the body and thus are not required in the diet. These are referred to as 'nonessential' or 'dispensable' amino acids. Willcock and Hopkins (1906) and Osborne and Mendel (1914) first realized the fact that the utilization of a dietary protein for anabolic purposes depended upon the pattern of the essential amino acids provided by the protein. The more closely the essential amino acid pattern of the dietary protein meets the animals' needs, the greater is the utilization of the protein. It has been demonstrated that efficient synthesis of tissue proteins occurs only when all the essential amino acids are supplied simultaneously and in proper proportions. For example, if a dietary protein has a 50% deficiency of one essential amino acid, approximately 50% of the dietary amino acid supplied to the 'metabolic' pool will be used by the body for the synthesis and maintenance of tissue pathways 4 and 6 (Fig. 3). The rest will be metabolized via pathway 6 and 7 (Fig. 3).

Thus only 50% of the protein is utilized for synthesizing or replenishing body proteins. These values are however only approximate, since in vivo amino acid studies suggest that an amino acid deficiency of a dietary protein may be alleviated partially by a temporary supply of the
Fig. 3. Schematic outline showing the general pathways of protein metabolism.
deficient amino acid available from the blood plasma. Therefore, in this hypothetical example, it is quite possible that more than 50% of the dietary protein could be utilized to meet the protein needs of the body. When the percentage of one (or more) of the amino acids in a diet is very low, amino acid imbalances occur. In amino acid imbalance not only does the efficiency of protein utilization fall, but some additional adverse effect, such as a drop in food consumption, a depression in growth or an increase in the need for one or more amino acids become evident.

Amino acid imbalances can be distinguished from amino acid toxicity in which adverse effects are caused by excesses of amino acids. Excessive intakes of certain individual amino acids such as methionine or tyrosine appear to cause definite toxic reactions which are highly specific and give rise to symptoms which cannot be prevented by adding a small quantity of the most limiting amino acids to the diet.

The importance of the amino acid composition of the dietary protein to the utilization of this protein thus can be well understood. The relative degree of the amino acid disproportion will determine whether the efficiency of protein utilization will be affected solely by, or in association with disturbances in physiological functions usually attributed to amino acid toxicity.
II. Dietary Calorie Intake

Rosenthal and Allison (1951) and Leverton et al. (1951) have reported the effect, the calorie content of a diet upon protein utilization. If the calorie content of a diet supplied by fat and carbohydrate is insufficient to meet the calorie needs of the body, amino acids from the "metabolic pool" will be oxidized via pathway 6 (Fig.3). As a result, dietary protein will be used for energy production, thus decreasing the anabolic activity (pathway 4 and 5; Fig.3) and resulting in less efficient utilization of the dietary protein for the synthesis of body proteins.

On the other hand, if adequate calories are supplied by fat and carbohydrate, anabolic activity will be maximal, permitting efficient utilization of dietary protein for the synthesis of body proteins. The different aspects of dietary calorie intake have been reviewed excellently by Swanson (1959) and Allison (1958).

III. Dietary Protein Intake

Protein cannot be stored by the body if fed in excess of its requirement. For this reason the efficiency of utilization of dietary protein is dependent upon the protein content of the diet. With excess dietary protein, anabolism and catabolism will occur. Anabolic pathway 4 and 5 (Fig.3)
will function to satisfy the body protein needs, while the
protein in the diet in excess of this need will enter the
catabolic pathways 6 and 7 (Fig.3). When the protein intake
is at or below the body requirements, anabolic functions
pathway 4 and 5 (Fig.3) will predominate and promote
maximum protein utilization to meet the protein needs of the
body. If the dietary protein supply remains below the level
required by the organism to maintain a satisfactory nutritional
and a physiological status, body protein will be catabolized
in an attempt to help supply certain necessary protein
constituents to the body.

IV. Nutritional status:

The various protein compartments of the body such
as plasma proteins, tissue proteins etc. whose protein levels
vary directly with the dietary protein intake, have been
referred to as the labile protein reserves or the dispensable
protein stores. With a high protein intake the "protein stores"
are full, and have a rapid turnover, resulting in a high
urinary nitrogen excretion. When dietary protein is restricted
for a substantial time, the "protein stores" are used by
catabolic processes in an attempt to supply the body with the
necessary amino acids normally provided in the diet. As these
"protein stores" are being catabolized, urinary nitrogen
diminishes until it reaches a low and constant value when the
'protein stores' are completely or nearly depleted. When protein is provided again in the diet, retention of the dietary protein will be high in order to replenish the body 'protein stores'. Allison (1949) has pointed out that during regeneration nitrogen stores that are depleted last are probably refilled first. This may indicate that the store depleted are the most essential.

2.6 Endocrine changes:

Complex endocrine changes take place in protein calorie malnutrition (P.C.M.). Recent work shows that pattern of response in malnutrition in adult differs from that of child. Changes of plasma hormone during malnutrition result from an alteration in rate of synthesis or destruction. Less appreciated changes occur as a result from an alteration in plasma constituents hormone binding protein. Synthesis of plasma protein is reduced in P.C.M. as a sequel of over all reduction in anabolism. This has a specific significance in case of steroid and thyroid hormone binding globulin. The total plasma concentration of a particular hormone may be reduced but if this is competed with a greater reduction in appropriate binding globulin, the net result may be an increase in the concentration of the free plasma hormone.
Physiology of Thyroid gland:

Historical Background: Seven years following the discovery of iodine by Courtois in 1813, it was recognized that iodine was the likely active ingredient in the ashes of seaweed and sponges which had been in use as a remedy for goiter for several centuries. Subsequently, it was appreciated that iodine is not only effective in the treatment of goitrous patients, but that a deficiency of this substance might be the causative factor in the development of goiter. The intimate association of thyroid with iodine was further documented in 1896 when Bauman demonstrated high concentrations of this element in the thyroid gland. Gley and Bauroost (in 1900) identified protein bound iodine in serum. Kendall in 1915 isolated thyroxine a physiologically active, iodinated amino acid from the thyroid gland.

Iodine and the Thyroid:

Diet: The principal source of iodine is the diet. The amount of iodine ingested varies with dietary composition and geographic location relative to iodine concentration in the soil and water supply until supplementation in recent times, the daily intake in certain iodine deficient areas such as Great lakes areas of the United States, Middle Europe, and the Andes region of South America was below 10 mg. The typical American diet includes 200 to 300 mgm of iodine daily. But this also varies depending upon the use of foods high in iodine content, such as iodized table salt, white bread.
with iodinated preservatives and increasing quantities of sea food. Health and food fed enthusiasts using seaweed, sea salt and vitamin-mineral supplement may ingest several milligrams of iodine daily. In addition, medications, food dyes and X-ray contrast media provide sources of organically bound iodine. The variation in dietary iodine has an impact on the use of iodine kinetics for thyroid diagnosis, since the variations in dietary iodine will be reflected in differences in the handling of the tracer iodine.

**Distribution of Iodine**

Iodine as free iodine or organically bound iodine, is usually reduced to the iodide ion during digestion and absorption from the gastrointestinal tract. It is rapidly distributed throughout the extracellular space and penetrates the red blood cell membrane in equilibrium with the plasma concentration. A small fraction is excreted by salivary glands and gastric mucosa. The iodine in saliva and gastric secretion is reabsorbed from the gastro-intestinal lumen.

Plasma inorganic iodine is removed almost entirely by the kidneys and the thyroid gland. Except in severe renal insufficiency, the kidneys will clear approximately 35 ml of plasma/min. The thyroid clearance in the euthyroid state varied between 10 and 35 ml/min. If no other factors are involved, we can see that a 17 ml/min. thyroid clearance and
34 ml/min. renal clearance would partition an administered dose of tracer radioactive iodine, resulting in 33 per cent of the dose being extracted by the thyroid. Increased thyroidal clearance, up to as high as 250 ml./min. is observed in glandular insufficiency. Impaired renal clearance is observed in renal insufficiency, leading to retention of dietary iodine and enlarged body and thyroidal iodine pools.

Inorganic iodine is cleared from the plasma by the thyroid acinar cell, apparently by an active process, since a gradient can be maintained in which the thyroidal inorganic iodine concentration may be 10 to 20 times plasma concentration. Nevertheless, this iodine is still freely exchangeable with the plasma iodide. In hyperthyroidism, or iodine deficiency, or with elevated levels of thyroid stimulating hormone (TSH), this gradient may increase from 150 to 200 times the plasma iodide concentration. Despite the fact that the iodide is exchangeable, this process called the "iodine trap" is the first step toward synthesis of the thyroid hormones, thyroxine and triiodothyronine. Other ions, such as perchlorate, pertechnetate and thiocyanate, with similar ionic charge and size in biological systems, may also be cleared at this step. If the concentration of these ions in plasma greatly exceeds the iodine concentration, the mass action effect will block iodine trapping. The competitive clearance via the "iodine trap" represents the basis for the use of these ions as diagnostic or therapeutic agents.
Thyroid hormogenesis and homeostasis (Fig. 4):

Approximately 80 mg of thyroxine and 28 mg of triodothyronine are synthesized daily by the gland. This synthesis is accomplished by series of steps: (Intrathyroidal hormogenesis). The thyroid follicular cells actively clear iodide from the plasma (thyroid clearance rate 20 ml/min) and can be concentrated up to 40 times the blood level at the rate of 2 micro gram/hour thyroid iodide trap. This trapping of iodide salts is influenced by TSH and other thyroid stimulators and by anions such as thiocyanate and perchlorate which competes the iodide for the transport system. This aspect of glandular function can be studied by use of early (10-20 minutes) thyroid uptake following intravenous administration of radio-active iodine or pertechnetate. Organic binding or organification of trapped iodide salts occurs very rapidly. In certain situation, however, this organic binding does not take place (for example, congenital biosynthetic defects, autoimmune thyroiditis, therapy with thiourylene drugs). Organification iodine requires the oxidation of iodine by a peroxidase enzyme system and this allows for the spontaneous iodination of tyrosyl groups on the thyroglobulin molecules.

Thyroglobulin, a 19S glycoprotein (M.W. 650,000) is synthesized by follicular cells, initially in 8-S subunit, 4 of which aggregate to form the complete molecule.
which contain 115 tyrosine residues. Iodination takes place at or near the apical surface of the follicular cells during and following aggregation and leads to the formation of mono and diiodotyrosine ($\text{MIT}$ & $\text{DIT}$) in approximately equal quantities. In iodine deficiencies, there is preferential formation of MIT and rise in $\text{MIT}/\text{DIT}$ ratio leading to a greater production of $\text{T}_3$ to $\text{T}_4$. Coupling of MIT and DIT results loss of an alanine side chain from one of the molecular forming $\text{T}_3 (\text{MIT} + \text{DIT})$ and $\text{T}_4 (\text{DIT} + \text{DIT})$. This reaction appears to occur most readily between molecules which are in close physical proximity of the thyroglobulin framework. The release of thyroid hormones takes place after coupling of iodothyroninase, in this process colloid is taken up from the follicle lumen by pinocytosis and the droplets formed coalesce with lysosomes in the cytoplasm. Proteolysis of thyroglobulin follows resulting in the liberation of $\text{T}_4$, $\text{T}_3$, DIT and MIT. The iodothyronines are released from the base of the cell into the bloodstream, together with tiny amount of thyroglobulin and iodothyrosinase, yielding bulk of the later are deiodinated within the cell by a dehydrogenase. The iodide so released is eventually reutilised for further formation of thyroid hormone. Under normal circumstances about 35% of the thyroxine secreted by the thyroid gland is converted to triiodothyronine in non-thyroidal tissue, this may be referred as extra thyroid hormone synthesis. In such case 24 of the 28 microgram of $\text{T}_3$ produced daily is derived from $\text{T}_4$. Only 15% (about 4 microgram) of the $\text{T}_3$ produced daily arises from thyroidal secretion.
Fig. 4. Showing out line of thyroside bio synthesis.
In the homeostasis of thyroidal hormones the hypothalamus and the anterior pituitary play a key role by secreting of thyrotropin releasing hormone (TRH) and thyroid stimulating hormone (TSH). The stimulation by TSH of thyroid gland metabolism, its growth and synthesis is a part of homeostatic feedback system in which the synthesis and release of TSH by the pituitary is controlled by the concentration of thyroid hormones. The hypothalamus responds to the T3 concentration causing stimulation/suppression of thyrotropin releasing hormone secretion (Fig.5). Thyroid stimulating hormone (TSH) is a glycoprotein (Mw 25,000) consisting of 2 nonidentical subunit (alpha and beta) and formed by the specific thyrotroph cells in the anterior pituitary cells under the influence of TRH. The TRH, is a tripeptide (L-pyroglutamyl-L-Histidyl-L-protein amide) is made by neurosecretory cells within the hypothalamus and is stored in the neuronal axon within the median eminence. It is then transported to the anterior pituitary through the portal vessel and stimulate the synthesis and release of TSH via an axon on adenyl cyclase. The thyroid hormone inhibit the action of TRH on the thyrotroph cells and this suppressive function is mediated through a step which involves synthesis of protein (Inhibitory feedback effect). The action of thyroid hormone is principally the action of T3. It appears likely that metabolic activity of T4 is accounted for peripheral deiodination to T3. Nevertheless, T4 has some direct effect.
Fig. 5. Normal control of thyroid hormone secretion.
Thyroid hormone transport and degradation:

About 99.98% of circulating $T_4$ and 99.9% of $T_3$ are bound to serum proteins. Thyroid binding globulin (TBG) has specific binding sites for thyroid hormones; normally it is one third saturated with thyroid hormones (Fig. 6). The remaining bound hormone is carried by thyroid binding prealbumin and albumin. The slower turnover rate and longer half life of $T_4$ partly results from its tighter binding to proteins than that of $T_3$. Large numbers of evidence indicate that only the free hormone (Unbound portion) exerts metabolic effects on tissues and is susceptible to degradation. The bound hormone serves as a metabolically inert reservoir. Thus, free thyroid hormone levels provide a better index of thyroid hormone levels provide a better index of thyroid activity than the total (bound and unbound) level of hormone. The latter may change markedly with the concentration of thyroid binding proteins, which vary in response to multiple nonthyroidal factors.

A major route of $T_4$ degradation is extrathyroidal deiodination to $T_3$. Approximately 20% of the released $T_4$ is excreted in the faeces as glucuronyl and sulfated conjugates. Other means of metabolizing thyroid hormone include deamination and oxidative decarboxylation to yield acetic pyruvic and lactic acid derivatives. None of the metabolites is more potent than either $T_3$ or $T_4$. 
Representing
Fig. 6. Normal ratios of $T_4$ and TBG
Biological function and pathway of hormone metabolism

The three most important aspects of thyroid hormone activity are effect on oxidative metabolism, growth, and differentiation of tissue. Other metabolic processes influenced by thyroid hormone action are metabolism of carbohydrates, protein, lipids, water, and electrolytes. Thyroid function also plays an important role in reproduction and can be determinant of reproductive potential. The function of thyroid gland is controlled by the hypothalamus and pituitary hormones. The release and distribution of the stored thyroid hormones occur, after stimulation of gland by TSH. Under this influence the thyroglobulin reenters the thyroid epithelium by pinocytosis. As the pinocytic vacuoles fused with lysosome, enzymatic activity releases $T_4$ and $T_3$ from thyroglobulin, and both hormones enter the circulation through capillary adjacent to the follicular epithelium. Once in circulation, $T_4$ and $T_3$ are protein bound, except for a small fraction of each which remain free as the physiologically active moiety. $T_3$ has greater physiologic activity than its congener $T_4$ in fact, some 6-8 times the activity. Interestingly, about 60% of the circulated $T_3$ arises from the peripheral monodeiodination of $T_4$. This finding has suggested that $T_3$ may be the active hormone and $T_4$ the prohormone. In the blood, essentially all $T_4$ and $T_3$ are transported bound to proteins, this render them metabolically inactive. The three proteins which bind the
thyroid hormone are referred collectively as thyroxine binding protein (TBP). Components of TBP are thyroxine binding prealbumin (TBPA) and albumin. TBG is an alpha globulin in which migrates electrophoretically between the alpha 1 and alpha 2 globulins and binds about 60-70% of circulating T4 and T3. TBPA a specific binding protein for T4 binds approximately 30% of the circulating T4 but has no avidity for T3. The remaining T4 and T3 are bound to albumin, which has the lowest association constant of the three binding proteins but the largest capacity.

Pathway of hormone metabolism:

Recent studies of T4 and T3 demonstrated that the major source of T3 in the peripheral tissues of man is not a direct secretion from the thyroid but it is generated from the peripheral deiodination of T4. Long standing speculation as to whether T4 gives rise to T3 during its peripheral metabolism was laid to rest by the demonstration that athyreotic patients given a mixture of highly purified stable and radiiodine labeled T4 as replacement therapy displayed in their serum substantial quantities of stable and labeled T3 (Braverman et al., 1970). Subsequent studies by more complex techniques which permit calculation of the fractional rate of conversion of T4 to T3 and the absolute quantity of T3 derived there from have fully confirmed this finding, and have
indicated that minimum of about 40% of $T_4$ is metabolized via monodeiodination to $T_3$ (Surks et al., 1973). The tissues in which $T_4$ to $T_3$ conversion takes place in vivo are unknown; however, generation of $T_3$ from $T_4$ in vitro has been demonstrated in human fibroblasts, in liver and kidney cells in culture, and in freshly isolated human leukocytes.

Several important concepts have emerged from these findings. First, when allowance is made for the daily disposal of $T_4$, its fractional conversion to $T_3$, and the smaller molecular weight of $T_3$ relative to $T_4$, it can be calculated that approximately 27 microgram of $T_3$ are produced daily from $T_4$. This value is only slightly less than the total daily production and disposal rate for $T_3$ described above. This indicates that only a small portion at most of $T_3$ is normally secreted by the thyroid. The implication that a high $T_4: T_3$ ratio is the normal pattern of thyroid secretion is in accord with recently refined analysis of normal thyroid tissue which reveal an unexpectedly high $T_4: T_3$ ratio (Chopra et al., 1973).

Second, the large proportion of $T_4$ metabolized to $T_3$, together with a 2-3 times greater biological potency of $T_3$, has led to the suggestion that virtually all of the metabolic action of $T_4$ is derived from the $T_3$ generated therefrom. Viewed in this light, $T_4$ need have no intrinsic biological activity but would merely serve as a stable
prohormone for the evanescent, physiologically active metabolite, $T_3$. Consonant with this concept are observations that agents, such as the thiouracils, which partly inhibit the deiodination of $T_4$ and its conversion to $T_3$, also diminish the metabolic potency of $T_4$. However, they reveal only that $T_4$ gains potency through its conversion to $T_3$ and do not indicate whether $T_4$ has intrinsic, though lesser, biologic activity.

Thirdly, from the observation it is noted that virtually all the $T_3$ normally arises in the periphery from $T_4$ and it is concluded that, if a hypothyroid patient is given adequate maintenance therapy, he does not require either administration of both hormones or provision of $T_4$ in quantities substantially greater than those normally secreted.

The experiments that have demonstrated the peripheral conversion of $T_4$ to $T_3$ have also revealed that tetrac is a normal metabolite of $T_4$ in man. Similar findings have been noted in animals.

Studies in man using $T_4$ labeled either with radiiodine in the inner ring or with $^{14}C$ have revealed rates of disappearances from plasma that are similar to those of $T_4$ containing radiiodine in the outer or phenalio ring. Such findings suggests, first that neither ring is preferential deiodinated and second, that significant quantities of long
lived products are not generated. After complete deiodination of $T_4$, the resulting excretory products retain the ether link of the original thyronine nucleus intact (Surks and Oppenheimer, 1971).

Studies of the products of the deiodination of $T_3$ in man are much more difficult to perform in view of its much faster rate of metabolism relative to $T_4$. The major product that has been identified is an iodoprotein that is manufactured peripherally and that appears in the plasma. The iodoprotein, resembles to serum albumin on electrophoretic analysis. The turnover rate of the iodoprotein is much slower than that of $T_3$, with the result that after a single injection of labeled $T_3$ the iodoprotein becomes an increasingly prominent component of the plasma radioactivity with the passage of time. Nevertheless, the proportion of the administered radiiodine that appears in the plasma as iodoprotein is exceedingly small. The iodoprotein is insoluble in organic solvents, and hence, the iodine contained therein is referred to as nonextractable iodine or NFI.

The foregoing discussion has been concerned with the fate of the approximately 80% of $T_4$ and $T_3$ that is metabolized via deiodinative pathways. The remaining 20% of both hormones ultimately appears in the stool as either free hormone or as conjugates with glucuronate or sulfate. Sulfoconjugation is a particularly prominent mechanism in the case of $T_3$. 
Studies of thyroid hormone metabolism in animal or in vitro have complemented the findings obtained in man. In the rat as in man, T₃ is generated peripherally from T₄, and an iodoprotein appears in plasma as a product of deiodination. In addition, studies in the living rat suggest that removal of iodine atoms from T₄ occurs in random order. On the other hand, in rat and dog, liver iodines in the outer ring of T₄ are apparently removed more readily than those in the inner ring. Organ related differences may occur, however, since, in the intact dog, iodines in the inner ring are apparently removed more rapidly than those in the outer ring. These observations suggest that deiodination may not be the result of a truly random process. The question is one of extreme importance with regard to the mechanism of T₃ generation from T₄. If deiodination is truly random, then the proportion of all T₄ undergoing deiodination that is metabolized via T₃ must be fixed at 50%, regardless of the operation of factors that change the overall rate of deiodination. If, however, deiodination is not random, than the possibility emerges that the generation of T₃ from T₄ is a specific process that undergoes a specific change in rate during various physiological states.

Studies of the metabolism of T₄ and T₃ in the rat have revealed the formation of tissue iodoproteins whose rate of formation is proportional to the rate of hormone deiodination. In liver, they are found predominantly in the
microsomal fraction, which is the site of greatest deiodinative activity. Hydrolysis of iodoproteins yields iodide, iodothyronine, and most interestingly, the original iodothyronine. Generally similar iodoproteins are formed in vitro during deiodination of T₄ by all tissues studied.

The capacity to deiodinate T₄ and probably T₃ is a property of all tissues studied in vitro to date. In most, the greatest deiodinative activity is found in the microsomal fraction. However, no specific deiodinase for T₄ or T₃ has been isolated. Indeed, tissue preparations in vitro in which deiodination of thyroid hormones has been studied have exhibited peculiar properties, including activation by short periods of boiling. This may indicate that deiodination in such systems is a mixture of enzymic and direct chemical deiodination, the later perhaps representing a technical artifact. There is considerable evidence that deiodination is an oxidative process, possibly mediated by tissue peroxidases and inhibited by catalase. Ideologically, this is an attractive hypothesis, since the degradation of thyroid hormones would thereby be linked to oxidative processes in the cell. The possibility is intriguing that enzymes similar to those which mediate ioddinations in the thyroid also catalyze hormonal deiodination in the periphery.

2.7. Thyroid status in malnutrition:

Protein calorie malnutrition (PCM) is known to induce variety of metabolic disturbances some of which may be
mediated through the dysfunction of endocrine glands. The first effect of PCM is an growth than slowing, caesatran or loss of weight and finally delayed bone maturation. The pituitary and thyroid functions have been studied by various workers in order to explain the mechanism of growth and retardation. Thyroid status has been studied by different thyroid function tests like thyroidal uptake of radio active iodine, PBI, Butanol extractable Iodine (BII), perchlorate discharge test, thyroxine and thyrotrophine levels in serum and BMR.

L.S.H. is within normal limit, T₃ uptake is also normal in maximum cases. As serum total protein and albumin are low, thyroid binding globulin (TBG) is also low.

These observations have been supported by Graham et al. (1973), Parra et al. (1973) and Gholmv et al. (1967).

Besar et al. (1966) reported slow uptake of radioactive iodine and postulated hypothyroidism in malnutrition. Monckeberg (1968) showed thyroid hypofunction by diminished BMR and decreased radiiodine uptake and BII.

Many workers have suggested thyroid hypofunction in malnutrition. But with nutritional rehabilitation there is a dramatic catch up of growth suggesting normal thyroid function.
With deprivation of food serum PBI level falls. The decrease in thyroid uptake and clearance of I^{131} and low level of serum PBI during deprivation of food suggesting that there is a decrease in thyroid activity; Cochran et al. (1964) also noted a decrease in serum PBI during fasting. It is not known how these effects are produced.

Suppression of thyroidal uptake of iodine by increased cortisol secretion is unlikely since normal or low level of urinary steroid excretion have been found during fasting while cortisol results in clearance of iodide as opposed to the decreased clearance found in patients. Deprivation of food have been found to result in increased secretion of GH, by the pituitary and it may be that accompanying decrease in secretion of thyroid stimulating hormone occurs, resulting in decreased protein catabolism by thyroid hormone.

It is well known that calorie restriction causes morphological changes in various endocrine organs. The regressive changes found in thyroid and gonade of starved animals stimulated these in hypophysectomy and can be reversed with pituitary substance (Julinos and Pomerantz, 1941), Stephens (1940), Stephens and Allen (1941). D. Angelo et al. (1942). It has been hypothesized that, in calorie restriction there is shift in the quality of trophic hormone activity in adenohypophysis with augmenting adrenocorticotrophine (ACTH)
secretion probably occurring at the expense of less cortical trophic hormones (D. Angelo et al., 1948; Routwell et al., 1948). In undergrowth animals reduced activity of thyroid appears to parallel the body weight loss. The thyrotrophic hormones level in blood of adult male rats and mice are significantly reduced by acute starvation (D'Angelo, 1942).

Increase of plasma growth hormone (GH) in PCM has been observed. It should be noted, however, that GH has other actions which may be appropriate to the adoption to malnutrition such as stimulation of hypophysis. Stimulation of somatic cells in growth also depend upon an effect of GH on the liver to produce a group of smaller peptide hormones named somatomedins.

The pituitary thyroid axis gives a good example of complexity of endocrine adoption to malnutrition. The BMR of malnourished is decreased when expressed as a function of lean body mass; early measurements of thyroid function show general agreement that both plasma T.S.H. and thyroidal hormone levels are reduced.

However, it is indicated that, plasma thyroid stimulation hormone measured by FIA is normal and that there is a normal response in plasma TSH to stimulation by thyroid releasing hormone (TRH).
In adult suffering from PCM the total serum thyroxine is normal but the free fraction is elevated due to reduction of binding globulin. At the same time both total and free $T_3$ levels are low possibly due to impairment of extrathyroidal conversion of $T_4$ to $T_3$.

Forsheim et al. (1970) studied thyroid function in protein-depleted rats and observed that renal iodide clearance in rats was reduced rapidly when the animals were fed a protein depletion diet. If the iodide content of the protein depletion diet is high it results in an increase in the serum iodide levels to concentrations in excess of 150 µg/100 ml. Protein bound iodine was elevated due to the accumulation of iodinated serum albumin. Thyroid hormonal iodoaminoacid content was transiently depressed, presumably by the mechanism described by Soloff and Charoff (1945).

Thyroxine metabolism was not affected except for a change in the partition of thyroxine between liver and serum. There was no evidence for pituitary involvement in the effect of protein depletion on thyroid function.

Stelzig and Ribeire (1974) estimated the thyroxine level in the serum of highly inbred Wistar rats after giving a basal diet of 12 g daily and after 3 days a diet containing the basal diet alone or with tricin 2 mg/12 g. Concentration of thyroxine was $3.89 \pm 0.56$ and $4.95 \pm 0.88$ µg/100 ml.
without and with tricin. This result showed that the stimulation of basal metabolic rate resulting when flavonoids were given to rats is caused by increased serum thyroxine.

Eales (1979) made a comparison of thyroxine and 3,5,3-tri-iodo-L-thyroxine kinetics in fed and starved rainbow trout (Salmo gairdneri). After 3 days of starvation L-thyroxine (T\textsubscript{4}) in plasma and rate of degradation of T\textsubscript{4} (DR) in rainbow trout 1 year old kept at 11°C-12°C were decreased but 3,5,3 tri-iso-L-thyroxine (T\textsubscript{3}) and T\textsubscript{3} DR were hardly affected. The decrease of T\textsubscript{4} DR was accompanied by decreasing deiodination of T\textsubscript{4} and decreased in conversion of T\textsubscript{4} to T\textsubscript{3}. But there was a little change in biliary excretion of T\textsubscript{4}. T\textsubscript{3} kinetics differed from T\textsubscript{4} kinetics and suggested a more rapid fractional exchange of T\textsubscript{3} between plasma and tissue compartments. In contrast of T\textsubscript{4} and T\textsubscript{3} underwent negligible deiodination.

Christopherson et al. (1979) studied the effect of temperature and feed intake on plasma concentration of thyroid hormone in beef cattle housed either indoors in a heated barn or in outdoor pens. In their experiments a comparison was also made of the effect of four feed intake level ranging from one to two times maintenance. They observed that the outdoor steers had higher plasma concentration of T\textsubscript{4} (P/0.01) and T\textsubscript{3} (P/0.05) and higher FTI values (P/0.05) than the indoor steers. Higher plasma concentration
of $T_4$ (P<0.01) and FTI values (P<0.05) were recorded in February and March when mean outdoor temperature was $-15^\circ C$. Under these circumstances increasing the level of feed intake to twice the maintenance reduced plasma $T_4$ concentration and FTI values. Level of feed intake had no effect on either plasma $T_4$ or FTI values in steers kept in a heated barn. Reducing food intake from twice the maintenance to maintenance levels resulted in a decrease in plasma $T_3$ concentration in steers kept outdoors but not in those kept indoors in a heated barn. These results indicate that thyroid hormone responses of cattle to cold environment are influenced by the level of feed intake.

Moss and Balnave (1979) studied the influence of elevated environmental temperature and nutrient intake on thyroid status and hepatic enzyme activities in immature male chicks. On 25th day of age chickens which had been given feed and water to appetite had environmental temperature maintained at $22^\circ C$ or changed to $30^\circ C$. Chickens at $22^\circ C$ had feed and water to appetite or had the same amount of feed as that taken by those at $30^\circ C$ and of water as those at $22^\circ C$. They were killed at intervals for up to 28 days later. Serum thyroxine and liver ATP citrate lyase increased in chicken which restricted in feed intake at $22^\circ C$ or were exposed to $30^\circ C$. Liver phospho-fructokinase activity decreased at $30^\circ C$. Thyroid function and enzyme activities became adapted to the controlled diet during the 28 days but not in higher temperature.
Balnak et al. (1981) observed seasonal and nutritional influences on growth hormone and thyroid activity. Blood samples were drawn monthly for 21 months from 4 penned adult female white-tailed deer (Odocoileus virginianus) on high energy diet, 6 does on low energy diet and for 6 months from 3 does semistarved in winter and spring. Somatotropin showed no difference with diets, but was increased in late spring and summer in all groups. Thyroxine $T_4$ and Triiodothyronine ($T_3$) increased significantly in late spring, presumably in conjunction with advance pregnancy. Concentration of $T_4$ and $T_3$ between winter and summer were not different for deer on high or low energy diet. Does on high energy diet exhibited greater amount of $T_4$ and $T_3$ during winters and early spring compared to those on low energy diet. The $T_4$ and $T_3$ values dropped sharply throughout winter in the semistarved does. The findings suggest that decreased circulating thyroid hormones may serve as a mechanism for reducing energy requirements, when deers are confronted with acute winter malnutrition. Measurement of serum $T_3$ appears to provide a particularly valuable indicator of nutritional stress during winter and probably other periods.

Okamura and Dis Tefane (1981), observed that rats receiving a nutritionally deficient diet displayed markedly elevated serum free $T_3$ levels but showed no increase in oxygen consumption. This was associated with greatly reduced
ratio of hepatic cellular and nuclear 125 I-T<sub>3</sub> to serum 125 I-T<sub>3</sub>. Kinetic data supported the conclusion that cellular uptake of T<sub>3</sub> was decreased in the nutritionally deficient rats. The lack of metabolic effect despite the elevated serum T<sub>3</sub> levels is attributable to reduced availability of serum T<sub>3</sub> to tissue nuclear receptor.

Okamura, Taurog et al. (1981) in course of their experiment on iodine deficiency observed that the Remington diets supplied by the ICN Nutritional laboratories though very deficient in iodine (≈20 μg/kg), did not lead to the rapid loss of thyroid iodine, and the rapid decrease in serum T<sub>4</sub> as expected on the basis of previous studies with a similarly iodine deficient Remington diet from another source. It was noted that the ICN Remington diet was nutritionally much more deficient than Remington diet from other suppliers. It was also observed that when rats were placed on an iodine supplemented ICN Remington diet, there was a marked increase in serum T<sub>3</sub> and T<sub>4</sub>. In one experiment rats receiving the ICN Remington diet plus KI in the drinking water for 16 days showed a serum T<sub>3</sub> level of 109 ± 16 ng/dl and a serum T<sub>4</sub> level of 6.6 μg/dl compared to 52 ± 7.8 and 4.4 ± 0.8 respectively in control rats on a stock diet. These elevations were not simply the result of increased binding of serum proteins. Serum protein binding studies by the method of equilibrium dialysis showed a very slight decrease in the percent dializable fraction for T<sub>3</sub> and T<sub>4</sub>. However calculated free T<sub>3</sub>
levels were significantly elevated ($P < 0.001$) and increases in free $T_4$, though less striking were also significant. The elevation were not accompanied by evidence of hyperthyroidism as judged by measurements of $O_2$ consumption or serum TSH.

Although the specific nutritional factors and mechanisms have not yet been defined the studies demonstrate that nutritional deficiencies in a Remington diet may act to oppose the effect of iodine deficiency itself. Their observation that the iodide supplemented ICN Remington diet has a marked serum $T_3$ and $T_4$ elevating effect offers a possible explanation for the blunted thyroidal responses of rats to the same diet taking added iodide. Their studies also suggest that alteration of peripheral $T_4$ to $T_3$ conversion may not be the only mechanism by which nutritional factors affect serum $T_3$ and $T_4$ levels.

Attempts have been made to explain the thyroid function in the light of protein deficiency in deficiency disease by groups of workers. Chopra (1981) found that in protein-calorie deficiency there is a decrease in serum total-$T_4$ level but the free $T_4$ level is not much affected, maintaining a physiologically euthyroid state in malnutrition. He remarked that in protein-calorie deficiency there is low TBG in serum and this causes a marked decrease in bound and hence total $T_4$ level in serum.
Larsen (1981) discussed that the lower results obtained in patients with reduced TBG concentration due to any cause are associated with an elevation in the fraction of $T_4$ and $T_3$ that are free, and such patients do not show any manifestations of hypothyroidism.

Dauncey et al. (1982) studied the increase in plasma concentration of thyroid hormones in piglets after a meal. In their experiments, out of 12 piglets kept at 25°C and given a meal of standard feed once daily 6 were given a low energy intake and 6 were given a high intake (H) equivalent to 2L, from 2 to 8 weeks old. When the piglets were 7 weeks old a catheter was placed into the jugular vein. At 8 weeks the intake were 300 and 600g feed and 10 blood samples were taken via the catheter starting from 3 hours before the meal to 2.5 hours afterwards. The concentration of $T_3$ increased within 15 min of eating and reached a peak after 60-90 min. Before the meal basal $T_3$ was 1.0 n mol/litre for both L and H group. Peak values of $T_3$ were 1.4 n mol/litre for the L meal and 2.3 n mol/litre for the H meal. The increase in $T_3$ after the H meal of 300g was significantly greater than after 300g feed. The plasma concentration of $T_4$ increased slowly during the 2.5 hours after the meal and the rise was significant for the H intake only. In another study 4 piglets 6 weeks old were housed singly at 26°C and were given a meal of 400g feed once daily for one week. Test
meals containing a high proportion of glucose, sucrose, fat as protein were then given on separate days with at least 2 days between the test. The increase in T\textsubscript{3} in blood after the meal was significant for carbohydrate and fat but not for protein. The increase in T\textsubscript{4} was variable and reached a significance for sucrose and fat only.

The level of different diets seems to influence the synthesis and secretion rate of thyroid hormones.