GENERAL INTRODUCTION

"A brief review of and an introduction to the literature on protein malnutrition with special reference to the experimentation on animals has been attempted."
Proteins are the universal and one of the principal constituents of living cells. Next to water, proteins quantitatively constitute the largest single component of protoplasm. Proteins cannot be replaced by any other biological material. It has been recognized that they play an important role in almost all activities of living matter, more especially in growth, maintenance and reproduction. The importance of proteins was recognized as early as in 1844 by Mulder. Whereas plants can synthesize proteins from inorganic nitrogen or very simple organic nitrogenous compounds, animals have to depend on the extraneous sources of preformed proteins for their protein requirements, which are necessary for building up new tissue, for the elaboration of enzymes, hormones, antibodies, etc., so vital for life.

Proteins which are composed of amino acids are broken down to smaller peptides and amino acids by the action of various proteolytic enzymes in the digestive tract. These hydrolytic products are absorbed and carried away by the portal blood and lymph to various tissues and organs where they are resynthesized into specific proteins with the help of synthetic mechanisms involving various enzymes. Protein synthesis in the body will, therefore, depend upon the adequate supply of various amino acids which in turn will depend mostly upon the quality and quantity of dietary protein.

The pioneer work of Wilcock and Hopkins (1906), of Osborne
and Mendel (1914, 1916) established the nutritive indispensibility of certain individual amino acids. For the last two decades, Rose has intensively worked on this aspect, particularly its quantitative aspect. The amino acids which are the building stones of proteins can, as a result of the work of Rose, be divided into two classes — (1) Indispensable or essential and (2) Dispensable or non-essential. Those amino acids which are not synthesized in the body with the materials ordinarily available to it at a rate commensurate with the demand for any particular requirement such as growth are called as indispensable. The dispensable amino acids are synthesized at the required rate (Rose 1938). Hence an adequate amount of indispensable amino acids must be incorporated in the diet. The presence of dispensable amino acids in the diet saves the body from the strain of synthesizing them from indispensable ones.

Those proteins which contain all the indispensable amino acids in adequate proportions are called as "complete" proteins. They are mostly of animal origin, viz., proteins of egg, milk, meat, etc. They have high biological value. In contrast are "incomplete" proteins which are deficient in one or more of the indispensable amino acids. Examples are the proteins of cereals, pulses, etc. These vegetable proteins are of comparatively low biological value. Thus it may be seen that not only an adequate amount of any protein but particularly of complete or good quality protein is necessary to meet the requirements of the body. This requirement of the body could be
met by the common dietary proteins of animal origin or by a mixture of foodstuffs of vegetable origin supplying the essential amino acids in requisite quantities.

The requirements of protein for different phases of life have not yet been worked out fully. There is wide diversity in the reported values. As early as in 1902, Voit recommended an inclusion of 110 - 120 g protein per day in the diet. Chittenden, who emphasized the physiological economy of protein intake, demonstrated that strength and vigour can be maintained for several months on a daily protein intake of 36 - 40 g. Nitrogen equilibrium (when nitrogen output equals nitrogen intake) could be maintained on a daily intake of 0.6 - 0.8 g protein per kg body weight. The Nutrition Advisory Committee (1944) of the Indian Council of Medical Research (formerly Indian Research Fund Association) has recommended an intake of 1.5 g protein per kg body weight per day for adult man and woman. The recommended values for the latter are increased during pregnancy and lactation. For children and adolescents the values vary between 3.5 g per kg to 2.0 g per kg body weight. The requirements which are highest during infancy gradually decrease as age advances. Phansalkar and Patwardhan (1956) and (1957) have calculated the minimum protein requirement for maintenance in the Indian adult which is 0.4 - 0.5 g per kg per day. These values do not appreciably differ from those reported by other workers outside India (Hegsted et al 1946; Bricker et al 1945). In maintenance,
protein is used to replace the daily wear and tear of tissue proteins. Growth involves the formation of new proteins in addition to maintenance. Hence the requirement of protein is increased in growth, so also in pregnancy, lactation and convalescence. An adequate amount of protein in the diet ensures increased resistance to infection (Cannon 1944) and helps in wound healing.

Protein requirements have been expressed in terms of essential amino acids since it is the latter which decides the biological value or efficiency of protein. The minimum essential amino acid requirements of rat and man, for different phases of life, have been determined by various workers (Rose 1938; 1949 and 1950; Mitchell 1950; Albanese 1950, 1952; Leverton 1954).

A survey of literature concerning consumption of proteins in various parts of the world would reveal that there is wide variation not only in the quantity but also in quality. Eskimos live on a high protein diet, the protein being of animal origin. To the other end are the people in the tropical regions where they subsist, for one reason or other, on a predominantly vegetarian diet in which the protein content is often inadequate either in quality or quantity or sometimes both. In the diet surveys carried out in different regions of India between 1935 to 1943, it was observed that the average total protein intake was 63 g per adult unit per day, a figure which is somewhat lower than that observed in the temperate regions. Proteins from cereals, pulses and other
vegetable sources constituted a major portion, while animal protein formed only 1⁄3 of the total protein intake (Patwardhan 1952, 1956).

Individual vegetable proteins have lower growth promoting efficiency than that shown by the animal proteins. Hence the predominantly vegetable protein diets of Indians are likely to be unsuitable for the young growing child. Because of the poverty of the people and shortage of cow milk the Indian infant is given during the weaning periods supplementary feeds of starchy pastes and cereal gruels which are very poor in proteins. Gopalan (1955 a) and Someswara Rao et al. (1956) have examined the intake of protein in the infants and children in South India and have observed gross deficiency of proteins and almost of every essential amino acid as compared to the figures given by Albanese (1950) on ideal weight basis. It is during this period, i.e., when the infant is in need of extra nourishment due to increased demands for growth, that it is likely to be vulnerable to protein malnutrition, owing to inadequacy of the protein. Indeed one is struck with the absence of protein malnutrition in the early infancy and "peak" incidence observed between the first and third years (Gopalan & Ramalingaswami 1955). It is important to note that in India, prevalence of protein malnutrition among the children beyond the third year is markedly higher as compared to that in other tropical countries (Trowell 1951). Frequently adults from very poor class who have to subsist
for a prolonged period on a fairly inadequate diet also fall victims to the effects of protein malnutrition. For these reasons protein malnutrition has become a problem of growing concern, more especially in infants and children. This problem is not restricted to India alone but is equally important for the tropical countries in Africa, West Indies, Central and Latin America and some parts of South-East Asia, as will be seen from the reports of Gambia (1952) and Jamaica (1953) Conferences on Protein Malnutrition. Thus this is more or less the problem of underdeveloped countries. The clinical manifestation of protein malnutrition has been known by several names like "kwashiorkor", "nutritional oedema syndrome", etc. (Trowell 1952). Since the term "kwashiorkor" has been universally accepted, it will be used in this thesis to denote the protein malnutrition syndrome.

The changes observed in protein malnutrition can be broadly divided into three parts: (1) Clinical, (2) Histopathological, and (3) Biochemical.

The clinical features are: (1) Failure of growth which sets in very early stages, (2) Oedema, (3) Skin changes - pavement dermatosis, (4) Dyspigmentation of hair, (5) Fatty liver, and (6) Alimentary disorders.

The histopathological changes include structural and histochemical changes mainly seen in livers of the patients.

Biochemical changes, which will be described in detail, are
classified as (i) Changes in various organs and tissues, and (ii) Changes in enzyme Activities. It is obvious that these biochemical changes or "lesions" are due to altered metabolism brought about by protein deficiency. Indeed, Gillman and Gillman (1951) have referred to them as "altered metabolic regulations". According to these authors whenever there is a minimum supply, quantitative or qualitative, of raw materials (in this case protein), new metabolic regulations are brought into force which are manifested in the maintenance of metabolism at a minimum level compatible with survival. Jolliffe (1950) is of the view that biochemical changes precede morphological modifications, which view is also shared by Gillman and Gillman (loc.cit). At least the former changes will accompany, if not precede, the latter. The study of these biochemical changes will be of great help in the proper understanding of protein malnutrition.

It must be mentioned at the outset, that protein malnutrition is not due to a mere deficiency or inadequacy of protein alone but it involves many other nutrients such as deficiencies of calories, one or more members of the vitamin B-complex and minerals. Other environmental factors such as alimentary disorders resulting from infections and infestations which interfere with the digestion, absorption or utilization of protein contribute to precipitate protein malnutrition. A study of the relative importance of several factors would, therefore, be of great help in the elucidation of the intricate problem of protein malnutrition.
I. Protein Deficiency

Simple protein deficiency may occur due to (1) inadequate intake of protein or amino acids and particularly of essential amino acids, (2) increased loss of blood and tissue proteins as in surgical operations and haemorrhage, and (3) poor absorption and faulty synthesis of proteins. In kwashiorkor, the first one is the most important, with the possibility of defective absorption and utilization playing a role as well.

(A) Changes in various organs and tissues of the body. Protein deficiency will lead to depletion of the proteins of many organs of the body. This depletion will, however, depend upon the previous nutritional status of the body and the degree of protein deficiency. Time is an important factor in bringing about the changes. Infants and children will be affected earlier and more severely than adults. Though protein deficiency affects most of the organs at some time or other, it should be pointed out that all the organs do not respond equally to such a deficiency. It will be evident from the discussion in the following pages that some organs lose more protein, some less and still others lose insignificant amounts. Some organs part with their protein rapidly while others resist the depletion for a longer period. The classical studies of Schoenheimer and Rittenberg (1940) would explain these different responses. They have demonstrated that the protein turnover varies with the
different organs or in other words the various protein compartments of the body lose or gain at different rates. Proteins of serum, liver, intestine and pancreas have very high turnover (Tarver 1954). Priority of the proteins also varies from organ to organ and will also be responsible for the preferential loss or retention of proteins of some of the organs. High priority proteins will be maintained at the cost of low priority ones. This would be obvious from the competition between the various organs to retain as much protein as is possible. One may as well consider the degree of such retention as an index of economic importance of the organ or tissue concerned from the metabolic point of view. This is borne out by the findings of Addis et al (1940). These considerations would seem to indicate that the effects of protein depletion are most felt in the organs with the highest protein turnover.

The body does not store protein unlike fat and carbohydrates. Some of the organs part with varying proportions of their total protein in cases of emergency. This is sometimes referred to as labile proteins.

The earlier work of Lusk (1936) and Addis et al (1936) had shown that during protein deficiency or inanition liver of rat loses rapidly its protein as compared to kidney, muscle and alimentary tract.

Kosterlitz (1944) correlated this loss of liver protein with the diminution in the stainable cytoplasm and vacuolization. There
was no change in the number of liver cells. It is interesting to note that the female rats lost more liver cytoplasm than the males.

This lability of liver proteins has inspired several workers to focus their attention on liver during protein depletion. Low protein diets deficient in sulphur amino acids induce liver necrosis (Himsworth and Glyn 1945; MacLean and Beveridge 1952). Vijayaraghavan and Patwardhan (1952) found fatty livers in the rats fed low protein poor rice diet. It could be corrected by methionine and choline supplementation.

Glutathione, an important tripeptide concerned with the oxidation reduction reactions, is found to be reduced in liver in protein deficiency (Leaf and Neuberger 1947). Edwards and Westerfield (1952) correlated the changes in glutathione and xanthine oxidase, a labile enzyme, on protein-free diet. They found that in liver the loss of glutathione was a little earlier than that of xanthine oxidase.

Muntwyler et al (1950) studied the effect of protein depletion (protein-free diet for 21 days) on various intracellular components of liver and found that nitrogen was lost from all the fractions except the nuclear one. They found that desoxyribonucleic acid (DNA) concentration increased. Of all the fractions (nuclear, mitochondrial, microsomal and residual cytoplasmic) microsomal fraction was the worst affected.

The nitrogen integrity of essential tissues is maintained by
cellular and plasma proteins, which are in dynamic equilibrium with each other. Reduction of the dietary nitrogen which replenishes these proteins will naturally affect the equilibrium. Hence in protein depletion both cellular and plasma proteins are reduced.

Considerable work has been done on the changes in the plasma proteins in protein deficiency (Elman and Heifetz 1941; Allison et al 1946). Plasma albumin is reduced markedly and β-globulins gradually. There is a wide diversity in the reports of the changes in α-globulins. Some reports state that they are unchanged while others claim a rise in their concentration. Chow (1950) found that α-globulin is lowered on continued protein depletion and that this lowering indicates irreversible and irreparable damage.

Madden and Whipple (1940) in their classical work on plasma proteins in which they have used plasmapheresis technique showed that a normal healthy dog forms, on fasting, plasma proteins from tissue proteins. The quality of plasma proteins thus formed may exceed the normal total mass of circulating proteins. They evolved the concept of "dynamic equilibrium" from their work, which concept was also originated from the isotopic studies of Shoenheimer. Weech et al (1935) have studied the absolute and relative losses of various fractions of serum proteins during hypoproteinemia.

Electrophoretic studies of the blood proteins of the
depleted animals have revealed the same changes as above (Zeldis et al 1945). However, the changes in blood proteins do not always show the true picture of depletion of proteins of the various organs. Blood proteins have priority over other tissue proteins, as a result of which they are not reduced as early as the other proteins. It may happen that a well defined reduction in proteins may take place in many organs and still the blood proteins may not show any change. This would be apparent from the studies of Allison (1950) and Elman (1947) who have shown that 30 times as much body nitrogen is lost from the soft tissues as from the circulating proteins in animals fed diets inadequate in protein.

Efforts have been made to correlate the changes in blood with those in liver. Elman and Heifetz (loc. cit) found parallel changes in serum and liver. The serum protein concentration falls as liver loses its cytoplasm and shows dysfunction as revealed by bromosulphthalein retention (Wang et al 1949).

Muscle is by far the largest individual organ in the body and it contributes the largest amount of protein during depletion as far as absolute amounts are concerned. Roche, A. (1935) and Roche, J. (1942, 1947) have shown that during protein deficiency there is a wastage of muscle proteins. These two authors contended that there was a preferential loss of myosin as revealed by the decreased content of diamino acids. There was a rise in the content of mono-
amino acids. It is surprising to find that complete starvation did not show any changes.

Kidney damage (Dicker et al. 1946) and bone atrophy (Armstrong et al. 1947; Estremera and Armstrong 1948) have been observed in protein deficiency.

Ingestion of low protein diet induces temporary rise in the excretion of creatinine and creatine (Tidwel 1946) and increased glutamine levels in most of the tissues (Tigerman and MacVicar 1949).

Zamcheck and coworkers (1953) have shown that prolonged protein deficiency leads to ulceration of stomach and small intestine of rat.

Protein depletion drags the animal towards negative nitrogen balance progressively till it attains a somewhat constant level. In general, nitrogen metabolism is reduced considerably.

(B) Changes in enzyme activities: Since most of the enzymes which are protein in nature are synthesized from the dietary proteins in the body itself, it is evident that protein deficiency should affect the activities of various enzymes. High priority enzymes are maintained at the effective strength while the partially dispensable enzymes will be sacrificed. These latter enzymes participate, according to Miller (1960), in a nonspecific manner in the metabolism of proteins.

As has been mentioned above, protein malnutrition brings about biochemical changes which are mostly due to changes in enzyme
activities. Since enzymes are responsible for the dynamic state of cellular activity, the study of enzyme systems in protein deficiency would give a clue to the solution of the complex problem of protein malnutrition. It appears from the evidence, both in humans and experimental animals, that the organs which suffer heavy and rapid loss of proteins also lose enzymes as well. Much work has been done on the changes in enzyme activities during protein deficiency in experimental animals.

Lightbody and Kleinman (1939), it seems, were the first to show the relationship between protein deficiency and enzyme changes.

The liver occupies a key position in metabolism, participating in a multitude of physiological processes. Liver enzymes, which account for the varied functions of liver, have been studied in detail. Perhaps xanthine oxidase has been studied in greatest detail of all the liver enzymes. This is the most labile enzyme in the liver. Many workers have reported varied losses of this enzyme in protein deficiency (Westerfield and Richert 1949; Litwack et al 1950).

Succinic oxidase and succinic acid dehydrogenase, the important enzymes in the Kreb's tricarboxylic acid cycle have been found to decrease in rat liver during protein deficiency (Potter and Klug 1947; Elson 1947; Srinivasan and Patwardhan 1955).

Cytochrome-c-oxidase, one of the most important oxidative and respiratory enzymes, was found to be unaffected in protein
depletion both in liver and heart of rats (Hillman 1951; Wainio et al 1953). The resistance of this enzyme to protein depletion is to be expected in view of its important nature.

Arginase, which participates in the urea cycle, is affected in protein deficiency (Lightbody and Kleinman, loc. cit.). This result can explain the lowered urea excretion in malnourished children (Platt 1952) and possibly also the low urea concentration in blood (Dean and Schwartz 1953; Ramanathan 1955).

Adenosine triphosphatase which brings about the breakdown of adenosine triphosphate to adenosine diphosphate yielding energy rich phosphate group, has been found to be reduced in liver but not in skeletal muscle (Hillman, loc. cit.). Lowered activity of this enzyme in brain, kidney and lung was also reported by Bargoni (1952).

Great interest has been evinced in the study of alkaline phosphatase in liver because of its abnormal behaviour in protein depletion. It is interesting to note the increased activity of this enzyme in liver (Ely and Ross 1951; Srinivasan & Patwardhan, loc. cit) under the above conditions.

Other enzymes which have been found to be affected in liver are D-amino acid oxidase, catalase, uricase, cathepsin II, esterase, transaminase, choline oxidase and rhodanese (Awapara 1953; Hoch-ligeti 1953; Miller 1948 and Rosenthal et al 1949).

Pancreatic lipase activity was found to be lowered in experimental animals. The changes were reflected in plasma
Pancreatic tryptic activity was affected, as was shown by Platt (loc. cit.). Changes of enzyme activity on deficiencies of single amino acids have been studied by the Wisconsin group of workers (Williams et al. 1949, Williams and Elvehjem 1950; Williams and Bothwell 1951).

Because of the possible diagnostic value, the study of the changes in enzyme activities in protein malnutrition has been receiving great attention during the past few years. But because of the association of factors such as calorie deficiency, and inadequacy of vitamin B-complex with the protein deficiency, the changes observed in clinical protein malnutrition may not be entirely due to protein deficiency. Rather they should be regarded as the result of the combined effect of multiple deficiencies.

Carvalho et al. (1947) and Veghelyi (1948) independently reported the losses of lipase and amylase activity in the duodenal contents of children suffering from protein malnutrition. Similar studies have been made by Thompson and Trowell (1952). McCance et al. and Widdowson (1948) have found low plasma-pseudo choline esterase in undernourished German prisoners.

Waterlow (1952) and Waterlow and Patrick (1954) studied enzyme activities in African and Jamaican children. They found reduced pseudo-choline esterase in liver.

Srinivasan and Patwardhan (1952) have reported low plasma lipase and esterase in malnourished children and adults. Dean and
Schwartz (loc.cit) found reduced serum enzyme activities in African children.

It would be evident from these reports that a variety of enzymes is reduced in protein deficiency. The study of enzymes in gastrointestinal tract, liver and blood, has come into prominence in protein malnutrition. The interpretation is, however, complicated by the fact that clinical protein deficiency is, as mentioned above, usually accompanied by calorie and vitamin B-complex deficiencies.

II. Calorie Deficiency

Calorie malnutrition or undernutrition is historically the most important problem of nutrition.

As early as in 1903, Landergreen had pointed out that the characteristic of undernutrition is a prolonged loss of body nitrogen when the calorie content of the food is insufficient to cover the needs of the individual.

Little is known about the role of calories in protein malnutrition.

Calories have great influence on protein utilization. Higher the calories better is the utilization of protein and vice versa (Benditt et al 1948). In man too, calorie starvation impairs protein utilization. These authors have suggested that there is some similarity between the requirement for growth and that for repletion.
Allison (1951) has shown that nitrogen balance decreases as calorie intake decreases. Elman et al. (1945) have emphasized the inclusion of more protein in low calorie diet. It is known that during calorie starvation protein serves as a source of energy rather than enter the protein metabolism of the body.

Keys and coworkers (1950) have studied the effects of semi-starvation on human beings. They observed that the basal metabolism was reduced during semi-starvation.

Addis et al. (1940) and Miller (1948) have reported losses of rat liver protein and enzymes respectively on a seven-day fast.

Richert and Westerfield (1955) have demonstrated that low protein diets could affect liver xanthine oxidase only in the presence of adequate calories.

The relation between protein deficiency and calorie restriction is illustrated by the study of Rose (1938). It was shown that lack of protein in the diet lowers the food intake. This indicates that on prolonged protein deficiency the animal would be led to calorie starvation because of the voluntary reduction in food intake. The possible role of calories in protein malnutrition, though not known, seems to be interesting in view of the fact that a proper balance between protein and calories is necessary to ensure prompt utilization of proteins. A disturbed total calorie/protein calorie ratio in association with deficiency of certain B-vitamins may be more harmful in that it leads to fatty
infiltration of liver. In fact, Trowell (1952) suggested such a disturbance in the balance as one of the causes of kwashiorkor. However, in India, the balance is not very much disturbed for Indian cases of kwashiorkor are known to suffer from both calorie and protein undernutrition. In spite of that kwashiorkor is prevalent. The role of calories, therefore, appears to be complex and deserves further investigation.

III. Vitamin B-complex deficiency.

Protein malnutrition also involves a deficiency of substances involved in protein metabolism and synthesis such as vitamins B₁, B₂, B₃, and B₁₂ (Waterlow 1953).

There is a close physiological relationship between proteins and members of vitamin B-complex as some of them are known to take an active part in enzymic reactions concerned in metabolism.

Low protein diets increase the intestinal synthesis of thiamine (Balkrishnan and De 1951). This is because such diets are usually rich in carbohydrates, which form a suitable medium for bacterial growth. High protein diet has sparing effect on vitamin B₁ whereas high carbohydrate (low protein) diet has harmful effect especially in the absence of vitamin B₁ as carbohydrate metabolism is disturbed. It would seem then that increased intestinal synthesis of vitamin B₁ has no beneficial effect during protein deficiency.
Inadequate protein intake causes poor utilization of riboflavin (Rama Sastri et al 1950; Rama Sastri 1953). Axelrod and Elvejhem (1941) have shown that riboflavin deficiency reduces liver xanthine oxidase activity by 25% of normal. D-Amino acid oxidase is also affected by riboflavin deficiency. Both enzymes require flavin adenine nucleotide for their action.

Vitamin B₆ is closely concerned with transamination, being the cofactor. Transaminase and cysteine desulphhydrase activities of liver are affected in vitamin B₆ deficiency (Mister et al 1953). There is a derangement of tryptophan metabolism under these circumstances. Thio amino acids hasten B₆ deficiency. On low protein, B₆ deficient diet the blood urea level was decreased because of reduced transaminase activity (Thompson et al 1953).

The deficiency of pantothenic acid is accentuated by protein depletion. Pantothenic acid deficiency is characterized by reduced coenzyme A activity (Nelson et al 1947).

The importance of lipotropic action of choline has been repeatedly demonstrated. Choline supplementation corrects only the fatty changes and has no effect on liver damage on combined deficiency of choline and protein (Koch-Wesser et al 1953). Marked fatty infiltration was seen in the combined deficiency in addition to liver damage. The combined deficiency also hastens the formation of oedema (Alexander and Engel 1952). It has an adverse effect on serum proteins.
György and Rose (1950) reported hepatic necrosis or cirrhosis on low protein diets deficient in vitamin B₁₂. Like protein and riboflavin deficiencies, B₁₂ deficiency also lowers xanthine oxidase activity of rat liver. Betaine-homo-cysteine-transmethylase of the rat liver is markedly reduced in B₁₂ deficiency.

It will be seen from the above account that the influence of protein and vitamin B-complex deficiencies are best manifested in the changes in enzymes, some of which have to depend on both proteins and members of the vitamin B-complex for their activities.

PRESENT INVESTIGATION

The object of the present investigation was to study certain aspects of experimental protein deficiency in order to elucidate the influence of associated factors which play an important role in clinical protein malnutrition. Needless to say, the subject is very vast. Hence it was necessary to confine the field of investigation to certain aspects usually associated with protein malnutrition. As has been mentioned above, calorie starvation and deficiency of one or more members of vitamin B-complex are some of the factors which are responsible, along with the protein deficiency, for the clinical protein malnutrition as seen in kwashiorkor. So it was felt that a comparison of the effects
of protein and calorie deficiencies, alone and combined, on the organs of the highest protein turnover, viz., liver, small intestine and pancreas would serve a useful purpose in furthering our understanding of their relative roles in kwashiorkor. Since the enzymes represent the dynamic aspects of the living cell and its metabolic activity, it was felt worthwhile, to concentrate on the study of the changes in enzyme activities of the above organs in these investigations.

To study the effect of vitamin B-complex deficiency super-added on protein deficiency, it was considered desirable to concentrate on one member from this group and continue the study to that vitamin only in the first instance. This is because there are many members of the vitamin B-complex and it would not be possible to study all of them. Vitamin B₆ is known to be a cofactor for the transaminase enzyme which is closely connected with the metabolism of protein. Vitamin B₆ also has influence on the protein synthesis. In view of these facts, it was felt that the deficiency of vitamin B₆ and protein may be studied with special reference to transaminase. In order to find out the changes in this enzyme due to these deficiencies over a long period on the same animal, changes in transaminase in blood were studied. An attempt was also made to find out the effects of realimentation with the missing nutrient. These two deficiencies were further studied on superimposed calorie restriction and subsequent rehabili-
On surveying the literature on the effects of protein deficiency it was not clear when and at what level of protein intake, a slightest and earliest deleterious effect of protein inadequacy can be seen, or in other words what is the critical level of protein at which deficiency could be considered to set in, involving the nitrogen integrity of the various tissues. To achieve this, it was necessary to select the most labile enzyme which would be very sensitive in its response to changes in dietary proteins. Liver xanthine oxidase appeared to be the most suitable for this purpose. The adaptation phenomenon which was exhibited by this enzyme was studied using different proteins in an attempt to assess the critical level of protein in terms of varying nutritive value of dietary protein.