

**THE PROTEIN NUTRITION OF ALBINO RATS FED SYNTHETIC DIETS,
INCORPORATING SINGLY AND IN COMBINATION, SOME
PROTEIN RICH PLANT AND ANIMAL FOODS.**

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TABLE OF CONTENTS.

	Page
LIST / TABLES	..
LIST / FIGURES	..
LIST / APPENDICES	..
I INTRODUCTION	1
II REVIEW OF LITERATURE	9
A. Issues in Nutrition	9
B. Protein Malnutrition	13
C. Nutritive Value of Vegetable and and Proteins	22
D. Experimental Relationship between Protein	31
E. Protein Protein Mixtures	35
F. Methods of Assessing the Nutritive of Proteins	51
III EXPERIMENTAL PROCEDURE	73
A. Planning the Survey	73
Selection of the Questionnaire	73
Selection of Families	74
Design of Survey	75
Results of the Survey	79
B. Logical Study	80
Food Selected for the Study	80
Selection of the Experimental Animals	82

	Page
3. selection of the Equipment ..	82
4. the Composition, Standardization and Preparation of Diets ..	85
5. feeding ..	89
6. length of Test Period ..	89
7. methods Studied ..	90
IV RESULTS AND DISCUSSION ..	95
A. Efficiency Ratio ..	95
B. K ₂ HPO ₄ Balance ..	104
C. Pal Appearance of the Animals ..	109
D. excretion of Haemoglobin and lood Corpuscles ..	115
E. Protein Regeneration ..	125
V SUMMARY AND CONCLUSION ..	129
BIBLIOGRAPHY ..	133
APPENDIX ..	

INDEX OF TABLES.

Table No.		Page No.
I	Survival function of germinants, <i>Brassica</i> and <i>Phaseolus</i> .	41
II	The distribution of <i>Brassica</i> and <i>Phaseolus</i> .	43
III	The distribution of <i>Brassica</i> and <i>Phaseolus</i> .	44
IV	The effect of protein: rich plant and animal foods consumed by urban families, with their frequency of consumption.	73
V	The effect of protein: rich plant and animal foods consumed by rural families with their frequency of consumption.	73
VI	The protein composition of the <i>Brassica</i> diet.	81
VII	Comparison of the <i>Brassica</i> diet.	82
VIII	The protein efficiency ratios of the proteins obtained in four periods of depletion.	83
IX	Statistical tests for significance between the protein efficiency ratios of the diets obtained in four periods of depletion.	87
X	Statistical tests for significance between the protein efficiency ratios of the diets obtained in four periods of depletion.	102
XI	The protein efficiency ratios and percentages of protein utilization of the proteins in diets of depletion.	103
XII	Comparison of the protein efficiency ratios of the proteins in the two periods of depletion.	106
XIII	Comparison of the nitrogen retention of the proteins in the two periods of depletion.	107

XIV	Results of the test for significance between weeks for nitrogen retention of five proteas.	108 a
XV	Competitive regeneration of haemoglobin by albino rats fed with the five proteins.	116
XVI	Competitive regeneration of red blood cells by the rats fed with these proteins.	122
XVII	Competitive regeneration of liver proteins by these proteins in albino rats.	126

LIST OF FIGURES

No.		Page No.
I	Rat Cross	83
II	Metabolism	83
III	Weighing Balance	83
IV	The Effect of Age On The Protein Efficiency Ratios Of The Five Proteins	93
V	Comparison Of The Average Protein Efficiency Ratios Of The Five Proteins	102 a
VI	Comparison Of Rats Fed On Test Proteins- Milk And Butten	111
VII	Comparison Of Rats Fed On Test Proteins- Milk And Red Gram Dhal	111
VIII	Comparison Of Rats Fed On Test Proteins- Milk And Rice-Pea Mixture	112
IX	Comparison Of Rats Fed On Test Proteins- Milk And Redgram Dhal - Milk Mixture	112
X	Comparative Regeneration Of Haemoglobin In Rats Fed On The Five Proteins	117
XI	Comparative Regeneration of Red Blood Corpuscles In Rats Fed On The Five Proteins	123
	Comparative Regeneration Of Liver Proteins In Rats Fed On The Five Proteins	127

I. INTRODUCTION

Among the many health problems which developing India needs to combat today, ~~mal-nutrition~~ and under nutrition - appear to be the most pressing ones. Diet surveys carried out in India from 1940 to 1958, by Aykroyd (1948)¹, Radhakrishna Rao (1953)², (1954)³, (1958)⁴, and reported by Pandit and Someswara Rao (1958)⁵ have revealed certain basic defects in Indian diets such as lack of protective foods like milk, meat, fruit and vegetables in Indian diets.

As early as 1839, Mulder stressed the importance of protein for growth and maintenance of life. They are the chief constituents of all animal and plant cells, that is, protoplasm in general. They build the body tissues and replace the wear and tear. Therefore, every system and tissue in the body is dependent on proteins for life.

In 1937, Rose⁶ showed that there were twentytwo amino acids present in proteins, but only eight of them were essential to man, namely, methionine, valine, isoleucine, leucine, tryptophan, phenylalanine, threonine and lysine. Therefore Brock (1960)⁷ considers proteins to be constellations of amino acids, rather than proteins as a whole. Block and Mitchell (1946)⁸ proved that the nutritive value of proteins was directly proportional to the number of essential amino acids in a protein. As shown by Elvehjem (1956)⁹, a proper balance of amino acids is also important for proper utilization of proteins.

As Henry and Ken (1958)¹⁰ and Kuppuswamy et al (1958)¹¹ point out, proteins have been classified as "good quality" and "poor quality" proteins. Hegsted et al (1955)¹² and Kuppuswamy (1958)⁶ ranked animal proteins as "high quality" and vegetable proteins as "poor quality" on the basis of their amino acid composition, because the essential amino acids present in animal foods, such as milk, eggs and meat as shown by Cuthbertson (1946)¹³, are adequate to support and maintain growth. On the other hand, several workers (Swaminathan, 1937)¹⁴ Kuppuswamy et al (1958)¹¹ and Evans (1962)¹⁵ have demonstrated that the essential amino acid make up of individual vegetable proteins is deficient in one or more of the essential amino acids. The only exception, as shown by Parsons (1943)¹⁶ is soya bean, which has a high nutritive value and has been found by Daniels and Nicols (1917)¹⁷ and Salmon (1943)¹⁸ to be almost equal to milk proteins, and thus, is the only vegetable protein which is almost complete. As early as 1914, Osborne and Mendel¹⁹ showed that casein was superior to gluten or seia in quality because of the difference in their amino acid composition. Other workers like Chow et al (1948)²⁰ and Rippon (1959)²¹ confirmed that finding. However all animal proteins are not complete, gelatin, an animal protein, is deficient in the essential amino acid, tryptophan as shown by McCollum et al (1917)²² and Cuthbertson (1946)¹³.

Comparing meat eaters with cereal eaters in India, McCarrison (1932)²³ found that there was an over all superiority in

health and physical development of those consuming the high quality animal protein diet. However, the superiority of animal proteins over vegetable proteins, has not always been consistent, as shown by Bricker *et al* (1945)²⁴ and Hegsted *et al* (1946)²⁵. The former found the protein requirement of a 70 kilogram man to be 42.1 grams per day, whereas Hegsted *et al* (1946)²⁵ showed that the protein requirement was reduced to 32.4 grams per day, when a mixed vegetable protein diet was given. Both Bricker *et al* (1945)²⁴ and Hegsted *et al* (1946)²⁵ showed that the requirement of protein for human beings was 27.6 and 27.1 grams per day when the protein levels in the diet were 47 and 33 per cent respectively. Since it has been shown that the smaller the amount required for growth and maintenance the greater the value of the protein, the findings of Bricker *et al* and Hegsted *et al* show that animal proteins are superior to vegetable proteins, but only by a small degree. This shows that the general belief that animal proteins are far superior to vegetable proteins, is yet to be confirmed.

When the diet does not supply the protein needs of the body in both quality and quantity, the protein reserves or the "labile" reserves are broken down to meet the more urgent needs of the body, namely, the supply of amino acids to the

*According to Allison (1960)²⁶ labile reserves are those proteins which can be depleted to contribute amino acids to the metabolic pool.

heart or brain tissue, in order to prevent them from degeneration. The chief labile reserves in the body are serum albumin and liver proteins. Wario et al (1953)²⁷ and Strickman and Patwardhan (1955)²⁸ have shown that some of the enzyme systems in the liver undergo inactivation and degeneration, when fed with protein deficient diets. When all the body reserves for protein are depleted, the body tries to break down some of the more resistant tissues such as nerve and heart tissues, and as a result the body becomes weaker, manifesting protein deficiency symptoms such as oedema and lassitude.

The deficiency of proteins in the diet manifests itself in various forms. The results of the deficiency are more severe in children than in adults, as the demand and need for protein is greater in the rapidly growing child than in the adult. Severe protein malnutrition, leads to the development of a syndrome called "Kwashiorkor" in children. This syndrome is characterized by cessation of growth, mental apathy, oedema of the dependent parts of the body such as hands and feet, changes in the hair, and dermatitis. "Kwashiorkor" is

*Trowell et al (1954)²⁹ explain the nomenclature of 'Kwashiorkor' as a name derived from the native African Ga dialect words meaning 'first' and 'second' which are applied to describe the disease as one which develops in the first child when he is displaced at the breast by the second.

a great problem in the so called underdeveloped, tropical countries, specially India and Africa, particularly in the poor socio-economic groups in rural areas. Besides kwashiorkor, the other effects of protein deficiency demonstrated on rats by Madden and Whipple in 1940, (1946)³⁰ are lowered resistance to disease and increased fatigue, greater susceptibility to the spread of disease and reduction in capacity for work.

■ A fast developing country such as India, cannot afford to sacrifice man power due to protein deficiency. At the same time, the poor people cannot afford the expensive protein rich foods such as eggs and milk in their dietaries. Diet surveys, carried out by Aykroyd (1948)¹ and Radhakrishna Rao (1958)⁴ have shown that the bulk of the food in Indian dietaries is from the cereals which contribute 80 per cent of the total energy, and also form the main source of protein. In a survey made by the investigator in the villages of Padupedere and Devampalayam in Coimbatore District, she observed that less than 15 per cent of the families surveyed consumed milk and the quantity of milk consumed by them was as low as 17 grams per day per person. The chief reasons given for its low consumption were the cost and non-availability of milk.

Thus nutritionists in India, are faced with the task of supplying good quality proteins at low cost to the poor people, so that the low income groups can afford to consume protein rich foods. A few successful attempts have been made in India and

Central America to combine vegetable proteins in certain proportions so as to arrive at a protein nearly equivalent to animal protein. In India, the Central Food Technological Research Institute, Mysore (Parpia et al 1957)³¹, (Subrahmanyan et al 1957)³² (Tasker et al 1960)³³, (Krishnamurthy et al 1960)³⁴ and the Nutrition Research Laboratories, Hyderabad (Venkatachalam et al 1956)³⁵, (Phansalkar et al 1957)³⁶ have developed successfully, combinations of vegetable proteins. At the Institute of Nutrition of Central America and Panama, Scrimshaw and his colleagues (1960)³⁷ have developed vegetable protein mixtures called "INCAP" vegetable mixtures 8 and 9".

There is yet another important justification for the efforts towards developing inexpensive combinations of vegetable proteins. Wright (1944)³⁸ has indicated that only 25 per cent of the food given to animals is converted into animal proteins. Thus the conversion is achieved at a high cost of energy and proteins. He reinforced this statement with the following example: Equal acreages of land devoted to the production of crops and live-stock, showed that an acre of land yielded as potatoes six million calories; as wheat about three million calories; as milk one million, and as meat only one-fiftieth of a million calories. In terms of crude protein, wheat and potatoes yielded 200 pounds per acre, while milk

* INCAP: Institute of Nutrition of Central America and Panama.

yielded about 100 pounds and meat less than 50 pounds. In India, where the average annual income is low, such a sacrifice of nutrients will be wasteful. Therefore the development of combinations of vegetable proteins has become a necessity, from the economic point as well.

The present study is an attempt at studying the effects of combinations of certain commonly used protein rich, vegetable foods, towards the improvement of the nutritive value of vegetarian diets. In this investigation, comparison of protein rich plant and animal foods has been made in terms of the nutritive value of their proteins using albino rats. The nutritive value of the proteins was assessed with the following criteria --growth, nitrogen balance, regeneration of red blood corpuscles, blood haemoglobin and total liver protein.

The diets containing protein rich plant and animal foods were identical in all respects but for the test protein. The basal diet was a synthetic, protein free diet, to which was added the test proteins at 10 per cent level forming the sole source of protein to the animals.

The five diets studied were:

1. Basal diet plus skim milk at eight per cent protein level.
2. Basal diet plus mutton at eight per cent protein level.
3. Basal diet plus red gram dhal at eight per cent protein level.

4. Basal diet plus red gram dal - Milk mixture in the ratio of 6 : 2 at eight per cent protein level.

5. Basal diet plus rice-pea mixture in the ratio of 4 : 4 at eight per cent protein level.

II. REVIEW OF LITERATURE

A. Proteins in Nutrition.

1. Definition of proteins:

Mulder (1952)³⁹, a Dutch chemist in 1939 suggested the name 'Protein' which according to West and Todd (1961)⁴⁰ is derived from the Greek work 'Proteios' meaning primary or holding first place.

2. Composition of Proteins:

Proteins include an enormous group of complex nitrogenous compounds which occur in all animal and plant protoplasm, and are chemically different and characteristic.

According to West and Todd (loc. cit) all proteins contain carbon, hydrogen, oxygen, nitrogen and generally sulphur. They yield amino acids on hydrolysis and it is these amino acids which are important in nutrition and not so much the proteins as a whole.

Rose (1952)⁴¹ states that interest in amino acids were initiated by two events which occurred more than sixty years ago, which were the publications of an approximately quantitative method for the separation of the basic amino acids by Kossel and Kutcher in 1900, and secondly the discussion of a procedure for isolation of the mono amino acids by the fractional distil-

lation of their ethyl esters by Fischer Rose (1952)⁴¹ states that as early as 1900, 12 out of 20 amino acids were recognized as constituents of proteins. The eight other amino acids discovered in the Twentieth Century were according to Rose (loc. cit) valine, proline, hydroxy-proline, tryptophan, isoleucine methionine, citrulline, and threonine. Rose (1952)⁴² in 1936 found that ten amino acids were necessary for the rats namely, valine, threonine, histidine, arginine, phenylalanine, methionine, lysine, tryptophan, leucine, and iso-leucine. In 1948, Rose (1952)⁴³ determined the essential amino acids necessary for human beings, which included all those necessary for the rats except histidine and arginine. He further found that the exclusion of any of these amino acids from the diet led to nutritive failure, loss in weight, diminished appetite and eventual death.

3. Importance of proteins

Proteins have been considered by Mulder (1952)³⁹ in 1839, as "unquestionably the most important of all known substances in the organic kingdom". Vernon (1952)⁴⁴ considered proteins even at the beginning of the present Century to be important, by stating that proteins stand at the centre of all organic life. Lewis (1952)⁴⁵ comments that even in the decade, they come first among all nutrients and form the fundamental building stones of protoplasm.

McGey (1952)⁴⁶ in 1932 showed that Bengalis were

inferior health than westerners, due to their low intake of protein. Rose (1946)⁴⁷, in 1938 showed that lysine deficiency resulted in nausea, dizziness and hyper sensitivity to metallic sounds. Madden and Whipple (1946)³⁰ demonstrated that the production of normal serum globulin was dependent upon the utilization of an adequate assortment of amino acids. Since globulins have been shown to be responsible for antibody synthesis, it can be inferred that proteins play a part in developing resistance to diseases. Cannon et al (1946)⁴⁸ showed that in protein deficiency the animal has a decreased capacity to fabricate antibodies. Cahill (1945)⁴⁹ asserts that proteins influence the process of growth and maintenance as well as reproduction and lactation. He further states that protoplasm without protein does not exist, and every tissue in the living system contains proteins including the hair, skin, nails etc. Muscles are made up chiefly of proteins. The nitrogenous building material for all the tissues in the body is derived from the proteins supplied in our food. Stewart (1946)⁵⁰ points out that proteins play an important role in the muscles; for lack of lysine or valine results in a curious sensitivity to touch and a lack of co-ordination in movement. Tai (1946)⁵¹ and Pellack (1947)⁵² gives clearly five body functions with which protein nutrition is intimately associated. They are a) maintenance of blood, b) wound healing, c) maintenance of tissue integrity, d) elaboration of antibodies and e) convalescence. Besides these functions there are other roles, such as the role in growth and liver which proteins play.

The role of protein in growth has been clearly demonstrated by Osborne and Mendel (1914)¹⁹, (1919)⁵³, Swaminathan (1937)¹⁴, Rippon (1959)²¹ and Mack *et al.* (1962)⁵⁴. They showed that tissue synthesis depends on the quality and quantity of protein in the diet, for a rapid increase in the weight of the animal is observed on a good quality protein as compared to that fed on a poor quality protein.

The role of protein in the liver, one of the vital organs of the body, has been studied by many workers who studied the effects of feeding a non-protein or low protein diet on the liver. (Miller (1950)⁵⁵, Wanie *et al.* (1953)²⁷, Tulpule (1958)⁵⁶, (1958)⁵⁷).

Proteins have been shown to be the basis of enzymes which emphasizes the importance of proteins, since the living system depends on these enzymes for the maintenance of life. Therefore, dietary proteins play a significant role in nutrition.

4. The Nature of Proteins - The Dynamic State of Body Proteins

Schoenheimer (1949)⁵⁸ stated that the body proteins are constantly in a state of dynamism and never static. They are constantly broken down and resynthesized in the animal body and thus a constant exchange of protein material in the "Metabolic Pool" takes place. Patwardhan (1960)⁵⁹ described a 'Metabolic Pool' as that made up of a number of substances derived from

the breakdown of body tissues and from those absorbed, from the intestines. Whipple (1956)⁶⁰ states that there is a ready interchange between the protein in ~~circulation~~ in extra cellular fluids, the cells and in the reserve stores within the body proteins. The dietary proteins become part of the "Metabolic Pool" as pointed out by Sannanheimer (1949)⁵⁸ and this pool has to be constantly replenished with fresh amino acids, for it is being constantly used up. When the diet lacks proteins either in quality or quantity, the reserves in the 'Metabolic Pool' are used up, and deficiency symptoms appear.

B. Protein Malnutrition.

1. Consequences of protein malnutrition:

When any living system is deprived of proteins which is the essence of life and the basis of protoplasm, serious metabolic, physiologic and mental changes take place. Almost all the vital organs and systems are affected during protein malnutrition.

a. Vitamin A metabolism in protein deficiency:

Krishnamurthy *et al* (1958)⁶¹ had shown that vitamin 'A' was linked with lipo proteins in the liver. Arroyave (1959)⁶² studied levels of vitamin A in the serum of children suffering from protein deficiency and observed a rise in the serum levels of vitamin A, when the sick children were fed with skim milk. Arroyave *et al* (1959)⁶² had confirmed earlier that the vitamin 'A'

tolerance in children fed on protein deficient diet was improved when fed with a high protein diet. These workers also observed a direct relation between vitamin A in the plasma and plasma albumin concentration. McLaren (1959)⁶³ stated that vitamin A and protein had a greater effect in curing corneal wound than vitamin A alone. Esh et al (1960)⁶⁴ observed that in protein deficient animals the animals stored a lower quantity of vitamin A in the liver during the repletion period when they were fed with high protein diet at both low and massive doses of Vitamin A feeding. They also observed greater weight gains when vitamin A was given in a high protein diet, thus showing that the vitamin A and protein absorption were related.

Bhattacharya and Esh (1961)⁶⁵ found that in vitamin A deficiency, the nitrogen efficiency and nitrogen retention decreased, and there was a decline in total plasma nitrogen with an increase in the non-protein nitrogen in the plasma and liver; but the above anomalies were corrected with a higher dosage of vitamin A. Esh and Bhattacharya (1961)⁶⁶ demonstrated the possible role of increased levels of vitamin A on the formation of enzyme proteins and on the improved utilisation of dietary proteins with a consequent increase in the weight gain under certain protein feeding conditions. Friend (1961)⁶⁷ observed a relationship between protein deficiency and transport of vitamin A in the blood of pigs, and found a significant correlation, between the two. They reported that in protein deficiency, the serum level of vitamin A decreased, which improved as the dietary intake of

protein was increased.

b. Liver changes in protein deficiency:

The role of protein in the liver has been studied in great detail by many investigators, by taking into consideration the liver changes during protein depletion. Williams and Elvehjem (1949)⁶⁸, Miller (1950)⁵⁵, Litwack (1952)⁶⁹, Wanio *et al* (1953)²⁷ have shown that the liver enzymes are decreased in activity during protein deficiency. Srinivasan and Patwardhan (1955)²⁸, have studied in detail, the effect of protein deficiency on some of the constituents of the liver, pancreas and blood of albino rats. They noted a decrease in enzymes like glutathione, succinic acid dehydrogenase and butyric acid dehydrogenase in protein depleted, adult rats, while in weanling animals, the picture of the liver was more serious, since the condition noted after 90 days in adult animals, was observed within 30 days in the weanlings. In conclusion they state that protein deficiency can severely change the enzyme pattern and structure of the liver. Tulpule (1958)⁵⁶ has studied the liver enzyme changes in protein deficiency. He found that in protein deficiency the D.P.N. (diphosphopyridine nucleotide) requiring enzymes of the liver such as the respiratory enzymes were adversely affected. Tulpule (1958)⁵⁷ further studied the break down and synthesis of D.P.N., in protein deficiency. He found that the D.P.N. content of the liver decreased in protein deficiency, which he explains to be due to the reduced capacity of the liver to synthesize D.P.N. under conditions of deficiency.

Eshwanger and Entenman (1958)⁷⁰ studied the effect of different levels of dietary protein on the total nitrogen, succinic acid dehydrogenase and xanthine oxidase, and also the sex differences in the response to protein deficiency, and found that at 10 per cent protein level, the enzyme activity was higher in the female than in the male. They further observed an adaptation mechanism which came into play after a certain period of protein deficiency, which helps to "tide over the effects of protein deficiency for some time".

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1. Keshwani: Besides these manifestations of protein deficiency, there is another severe condition called 'Keshwani' which is a protein malnutrition syndrome, and a manifestation of many other nutritional deficiencies. According to a F.A.O. report (1954)⁷¹, in South Africa it is known as "Infantile Pellagra", in Jamaica it is called "fatty liver disease or sugar baby," and in French as 'Dystrophie des farineux,' and in German as "Mehlschaden". In India, it is given the name of nutrition dystrophy or "Nutritional oedema syndrome". All these names mean the same sickness in children namely, protein malnutrition. The third Joint F.A.O./W.H.O. Expert Committee on Nutrition (1960)⁷ have defined protein malnutrition in general "as a state of ill health occurring where diets are habitually poor in protein, while they are more nearly adequate in calories. Clinically, protein malnutrition is most easily

~~summarized~~ when there is a relatively high intake of calories from starchy foods together with an insufficient intake of protein. The concept includes the effects of deficiency in the quantity of protein consumed, of imbalance of amino acids, and deficiency of factors, such as vitamin B 12, commonly found in foods in association with animal protein and concerned with protein ~~metabolism~~.⁶ Thus in these few lines, the causes of the syndrome have been explicitly shown.

Whenever, protein malnutrition is considered, the syndrome "~~Kwashiorkor~~" is taken up, since in this one condition all the ~~manifestations~~ of protein deficiency are evident. Therefore, in the following review, work done in connection with "~~Kwashiorkor~~" will be more discussed.

~~11. Summary:~~ Protein malnutrition is most evident in the post-war years, as shown by Gopalan and Ramalingaswamy, (1953)⁷², Autret and Behar (1954)⁷³, Rastamanthan (1954)⁷⁴, Rastamanthan (1956)⁷⁵, Brook (1960)⁷; and Scrimshaw and Bressani (1960)³⁷. Brook (1960)⁷ is of the opinion that protein deficiency may have effects on the constitutional susceptibility at all ages.

The clinical picture varies from region to region depending on the type of food given to children. Of all the complex ~~malnutrition~~ syndromes, the most widespread in its incidence, and the most damaging in its effects, is that known as Kwashiorkor, or protein malnutrition. This condition is also prevalent in

Central America, Latin America, India and in many parts of Asia. The effects of protein malnutrition ranges from a slackening in the rate of growth to a serious disease and finally death.

Sorinshaw (1959)⁷⁶ states that most frequently the protein deficiency begins when the mother's milk is not longer adequate in quantity and quality to supply the full needs of the growing child, and the lack of approximate supplementary sources of protein. He further states that the condition becomes more acute during and after weaning, because, the diets of children of their age group are frequently starch or cereals and have a very low content of protein. This fact has also been pointed out by Woods (1951)⁷⁷, Jelliffe (1953)⁷⁸, Venkatachalam *et al* (1954)⁷⁹, Autret and Behar (1954)⁷³, Trowell *et al* (1954)²⁹, Dean (1954)⁸⁰, Behar *et al* (1959)⁸¹ and Gopalan (1957)⁸². According to Gyorgy (1954)⁸³ and Cravioto (1958)⁸⁴ the disease is due to the lack of animal protein in the diet. Engel and his associates (1954)⁸⁵ developed severe generalized edema with hypalbuminemia, fatty liver and cirrhosis by employing a seven per cent of protein diet consisting of peanut protein with a little amount of casein. The main features of the multiple deficiency syndrome of 'Kwashiorkor' as pointed out by Venkatachalam *et al* (1954)⁷⁹ and Autret and Behar (1954)⁷³ are (1) the age at which they are most common, (which is the period of weaning in the areas studied (this is between one and three years of age), and (2) the dietary situation of the children. The children are mainly fed on starchy foods with little or no

protective foods. The other effects are discussed later.

iii. Effect on physiological functions: Venkatachalam et al (1954)⁷⁹ and Autret and Behar (1954)⁷³, have shown that a change in the general health, digestive system, liver, hair, and nails, are noticed in 'Kwashiorkor'. The other changes occur in the cardio-vascular, haemopoietic systems, and bones and muscles are also affected.

The main symptom of this syndrome is the loss of weight, and cessation of growth, write Autret and Behar (1954)⁷³, mild oedema may also be present at first. Furthermore, the other signs and symptoms of protein deficiency, as seen in the syndrome 'Kwashiorkor' are apathy, anorexia, vomiting, liver enlargement, fatty liver infiltration and muscular dystrophy. Autret and Behar (1954)⁷³ state that the mental apathy is so severe that a smile from the sick child is regarded as a sign of improvement.

iv. Metabolic changes: Venkatachalam et al (1954)⁷⁹ and Autret and Behar (1954)⁷³ have also pointed out the metabolic changes which occur as a manifestation of protein deficiency. They are, oedema, which is spread on the feet and legs at first and then travels upwards. Later, even the face and hands are affected. Sometimes, dehydration is also present causing folds and fissures in the skin. The blood protein level is also considerably lower in a protein deficient child than that in a normal child of his age, for in protein deficiency, the body tries to supply the proteins of the enzymes and important tissues by breaking down

the 'labile proteins' of the body. Allison (1960)²⁶ states that those proteins which can be depleted to contribute amino acids to the metabolic pool have been considered as reserves. He cites that plasma albumin is a reserve, as it is depleted during a period of protein starvation, but on the other hand, globulin tends to rise a little. Allison also states that the important tissues such as those of brain, heart and kidney are resistant to depletion, while liver comes under the category of reserves. Steatorrhoea is another manifestation of severe protein deficiency. Mehta *et al* (1956)⁶⁶ found this condition in 'Kwashiorkor' children, even on a fat free diet. They found that glucose aggravated the condition while groundnut & oil was better tolerated than other oils.

v. Changes in the Blood picture: Jayalakshmi *et al* (1957)⁸⁷ and Edozein (1960)⁸⁸ have observed the serum levels of protein and calcium in Kwashiorkor to be subnormal which increased when the children were placed on a high protein diet. Protein deficiency also results in elevated prothrombin level, as observed by Jayalakshmi and co-workers, which again come down to normal on a high protein diet.

vi. Changes in the Hair: Another manifestation of protein deficiency is that of hair change. Autret and Behar (1954)⁷³, Venkatachalam *et al* (1954)⁷⁸, Narasinga Rao and Gopalan (1957)⁸⁹ have reported of hair changes as a consequence of severe protein deficiency. Narasinga Rao and Gopalan (1957)⁸⁹ found the cystine

content of discoloured hair to be less than normal, and also found no difference in the cystine content of hair of Kwashiorkor children in India and Africa.

Vii. Changes in the liver: Ramalingaswamy et al (1954)⁹⁰ have shown a decreased activity of R.N.A (ribo nucleic acid) in Kwashiorkor while alkaline phosphatase of the liver increased. These workers further studied liver changes in Kwashiorkor. They found that the liver was seriously damaged in severe protein deficiency. But a follow up study of Kwashiorkor cases by Sri Kantia et al (1956)⁹¹ found that the persist even after treatment and right into adult-hood. They only observed an abnormal condition of the liver, which could be easily treated and brought back to normal with a good protein diet.

Thus from the above review, it is evident that a lack of protein in the diet, causes severe damages to important organs like the liver which in turn affects the normal function of the other organs, thereby, resulting in a very abnormal, unhealthy condition.

Can We Over Come then Protein Deficiency?

In order to overcome such a condition of gross malnutrition, the diets can be improved by the inclusion of protective foods such as milk, meat, eggs, pulses, and leafy green vegetables.

These foods are not only rich in protein, but also in vitamins and minerals and are therefore termed protective foods.

The diet should contain some vegetable or animal proteins, so as to prevent the consequences of protein deficiency; but animal proteins are expensive and not easily available. Thus cheaper sources of protein such as vegetable protein mixtures have been developed. The nutritive value of vegetable and animal protein will be discussed in the following paragraphs.

2. Nutritive Value of Vegetable and Animal Proteins.

1. Nutritive Value of Legume and Cereal Proteins:

a) Legumes: Mc Collum *et al* (1919)⁹² demonstrated that the protein of peas and navy beans have just half the dietary value of cereal protein for support of the growth in rats. These authors also found soya bean protein to be superior to that of the navy bean protein which holds good even today, from the results of analysis of soya beans by Kuppaswamy (1958)¹¹.

Among the pulses, red gram dhal has been proved by Saminathan (1937)¹⁴, Vijayaraghavan and Brinivasan (1953)⁹³ and Ramachandran and Phansalkar (1956)⁹⁴ to be the worst, since it cannot support nor maintain growth. Red gram has been shown to be deficient in tryptophan and theonine by Vijayaraghavan and Brinivasan (1953)⁹³ and Jaffee (1958)⁹⁵. Black gram has been shown to be the best by the same authors, while Baptist (1954)⁹⁶ has shown green gram to be the best.

Peas have been analysed for their amino acids by Kuppaswamy *et al* (1958)¹¹ and have been found to contain more lysine and

tryptophan in their albumin, than other legumes. Ramachandran and Phansalkar (1956)⁹⁴ have proved that pea protein contains as much as 11.4 per cent lysine. Legumes in general have been shown, by Baptist (1954)⁹⁶ and Kuppaswamy *et al* (1958)¹¹ to be rich in lysine. Hach *et al* (1947)^{97,98, 99} found that growing children fed legume diet to be inferior in health to those fed on meat diets, thus pointing out the inferior quality of legumes. But Venkatachalam *et al* (1956)³⁵ have shown that Bengal gram dhal was very effective in the treatment of nutritional oedema syndrome.

Esh and Som (1954)¹⁰⁰ showed that pulses were deficient in threonine when compared to casein and this was also proved by the results obtained on rats. Esh and Som (1955)¹⁰¹ studied the nutritive value of pulse proteins and found that when α -methionine was supplemented the nutritive value increased, but supplements of threonine, lysine and tryptophan did not have any effect except when given along with methionine. They found the PER of bengal gram to be 1.9 to 2.34 with methionine supplementation.

Esh and Som (1957)¹⁰² compared casein and a pulse diet and found that in weanling rats there was no difference in the liver xanthine oxidase activity between casein and pulse diet, but in the adult, pulse diet had a lower enzyme activity. When methionine was supplemented to the pulse diet, the activity of the liver enzymes was even superior to the casein diet.

Besides the growthpromoting value of legumes, Brinivasan (1957)¹⁰³ showed that tender field beans could reduce blood sugar levels, while mature field beans lacked this quality. Black gram dhal also had this quality. Sah, De and Basu (1960)¹⁰⁴ studied the nutritive value of two types of Bengal gram the high protein and low protein varieties. They found the biological value of high protein pulses to be less than that of low protein pulses, but the net protein value and PER of the former were higher.

Legumes and pulses thus form a very good source of vegetable protein. But there have been reports of their toxicity. Pal and Bose (1953)¹⁰⁵, in 1943 observed a severe toxic effect of a high pulse diet on the livers and kidneys of rats, resulting in death of the animals within six weeks, but De (1953)¹⁰⁶ studied the same diet once again to find out the cause of the early death of the animals. He found that this alleged toxicity was not correct and referred the death of the animals to lack of vitamins and minerals.

b. Cereals: Mc Collum et al (1917)²² showed that cereal proteins, when compared with milk proteins, which is the best protein for growth of rats, had only half to a third of the nutritive value of milk protein for the growth and maintenance in rats. Cereal proteins have been studied by later workers, (Swaminathan, 1937)¹⁴, Baptist (1954)⁹⁶, Kik (1956)¹⁰⁷, and Kuppaswamy et al (1958)¹¹ have shown that among the cereals, rice protein ranks first, while wheat comes second. Rice had a P.E.R of 2.17 with a Biological value of 80. Morgan (1931)¹⁰⁸ had earlier showed

that rice protein had a P.E.R. of only 1.4. Cereals proteins are deficient in lysine. (Swaminathan, (1937)¹⁴, Vijayaraghavan and Srinivasan(1953)⁹³, Kik (1956)¹⁰⁷, and Kuppaswamy, (1958)¹¹. Kik (1956)¹⁰⁷ showed that rice protein could be improved by the addition of threonine and lysine.

Hash and Baghchi (1952)¹⁰⁹ proved that whole wheat had a biological value between 60 and 70 while the digestibility proved to be between 85 and 92 per cent.

2. Nutritive Value of Soya Bean Protein:

Among the legumes, soya beans occupy an important place. Kuppaswamy et al (1958)¹¹ have analysed the soya beans for their amino acids and Shrivastekar et al (1961)¹¹⁰ have studied soya beans extensively. The soya bean occupies a place midway between legumes and oilseeds. They have a protein content of 40 to 45 per cent and fat content between 16 to 20 per cent. Soya beans according to Kuppaswamy et al (1958)¹¹ and Shrivastekar et al (1961)¹¹⁰ are deficient in methionine and cystine, and the latter authors find them to be otherwise good sources of essential amino acids. One limitation in the availability of protein from soya beans is the presence of trypsin inhibitors. The presence of these inhibitors have been observed by Osborne and Mendel in (1917)¹¹¹ and later by Leiner (1962)¹¹². Heat treatment destroys this factor and improves the nutritive value of soya beans. Salmon (1943)¹⁸ reports that heat treated soya beans are almost equal to milk, in their nutritive value.

3. Nutritive Value of Groundnut Proteins:

Groundnuts form one of the important sources of protein among the oil seeds. The nutritive value of their proteins have been studied extensively by Subrahmanyan *et al* (1957)¹¹³, Rama Rao *et al* (1957)¹¹⁴, found the P.E.R. of groundnut protein to be 1.45 at 10 per cent level. The low P.E.R. is due to deficiency of methionine as shown by Kappaswamy *et al* (1958)¹¹. Hawley *et al* (1948)¹¹⁵ studied the biological value of groundnut protein on human beings, and found it to be only 56, while the co-efficient of digestions was as high as 99. Lal (1952)¹¹⁶ studied the effect of groundnut protein on the nutritional status of children, and found it to have a good effect. So far the nutritive value of individual proteins have been discussed, and now the nutritive value of animal proteins in comparison with vegetable proteins will be considered.

4. Comparison of Animal and Vegetable Proteins

As early as 1914, it was realized that proteins differed in their ability to promote growth. Osborne and Mendel (1914)¹⁹ from their experiments on rats, concluded that animal proteins like casein could promote good growth in rats, while vegetable proteins like gliadin and zein could not. Gelatin has been shown by Daniels and Nicols (1917)¹⁷ to be deficient in tryptophan, and according to Cuthbertsen (1946)¹³, forms the only incomplete animal protein. Mitchell (1927)¹¹⁷ considers animal proteins to be "preeminent sources of protein", and this view

has been supported by many workers. Mitchell and Carmann (1924)¹¹⁸ found the biological value of eggs, pork and wheat proteins to be 93, 74 and 67 per cent respectively. Mitchell and Carmann (1926)¹¹⁹ found the biological value of whole egg, egg albumin, milk, veal and beef to be 94, 83, 85, 62 and 69 respectively. Pessen (1936)¹²⁰ showed that the animal proteins were superior to plant proteins and the best among the animal protein being milk and eggs which share the first place. Liver and kidney were found to be better than muscle proteins. Cereals were demonstrated to be inferior, but were better than legume proteins. Wilder and Keys (1942)¹²¹ placed milk proteins in the first place, but vegetable proteins last. They found the soya bean and peanut protein to be better than cereals. Mitchell (1942)¹²² found eggs and meat to be the most valuable protein foods while cereals and nuts came only next.

According to Beach et al (1943)¹²³ animal proteins as a class are superior in nutritive value to those of vegetable proteins, which is due to the amino acid composition of animal proteins. Marrack (1943)¹²⁴ showed that the needs of growing animals are better supplied by animals, than by vegetable proteins.

Murlin et al (1944)¹²⁵ state that the biological values of animal proteins like milk, and whole egg were superior to vegetable proteins like soya bean, cottonseed and pea nut proteins, with human beings. Wickers (1944)¹²⁶ emphasised that the animal proteins contained all amino acids essential for growth and main-

tenance in health and for reproduction. Turner (1945)¹²⁷ also states that animal proteins especially milk, egg, meat, fish and cheese are superior in nutritive value to most vegetable proteins.

Both Hegsted et al (1955)¹² and Evans (1962)¹⁵ have shown that vegetable diets lack lysine and methionine. In both their studies they found that supplementation of lysine and methionine improved the nutritive value of the vegetable proteins which was seen in the higher retention of nitrogen by the pigs in the study of Evans (1962)¹⁵ and by human beings in the study of Hegsted et al (1955)¹².

Kofranyi (1957)¹²⁸ found that by determining the lysine content of vegetable proteins, and relating the concentration of this essential amino acid to their nutritive value, the vegetable proteins were found to be inferior to animal proteins for two reasons: (1) as a group, vegetable protein apparently contain less of this essential amino acid and (2) they are less readily digested, as seen by pepsin digestion under ⁱⁿ vitro conditions.

Lintzel (1958)¹²⁹ had claimed that plant protein were more efficient than animal proteins in maintaining the nitrogen balance in sedentary fully grown human subjects, but later Kofranyi (1958)¹³⁰ who found many faults with the work of Lintzel (*loc. cit*) has proved that the results of Lintzel were wrong. Lintzel (*loc. cit*) had stated that animal proteins were necessary only

for growth, synthetic and regeneration processes, but the work of Kofranyi had shown that animal proteins are necessary for maintenance of the adult organism as well as for growth, regeneration and synthesis.

Gardener and Schultze (1955)¹³¹ have proved that certain all vegetable diets containing all known nutrients were inadequate for growth, but it was believed that an 'animal protein factor' existed which was lacking in vegetable foods. Earlier Schultze (1953)¹³² reports of the work of Picconi and co-workers who had also found such a factor which was water-soluble and heat stable. Esh (1955)¹³³ made a study on the nutritive value of plant proteins and demonstrated that vitamin B₁₂ could improve the growth promoting value and biological value of pulse protein and he found a greater increase in the nutritive value when methionine was also given in addition to vitamin B₁₂. Bhagavan (1958)¹³⁴ showed that vitamin B₁₂ could enhance the nutritive value of raw soya bean.

Wagle and Brindaman (1961)¹³⁵ have shown that a deficiency of folic acid and vitamin B₁₂ may be one of the reasons for the poor quality of vegetable proteins. They found that rats fed on a cereal legume, diet, showed good growth which was improved by the addition of folic acid and vitamin B₁₂ and the plasma protein levels also improved considerably. In order to study the effect of animal and vegetable proteins on human beings, studies on vegetarians and non-vegetarians have been made by many

Investigations.

Hirone (1954)¹³⁶ observed that prolonged consumption of a diet devoid of or low in, animal proteins produced no apparent deleterious effect on the health of the members of a community, which did not eat meat, but they found that the nutritive value of vegetable proteins could be improved by the addition of skim milk. Wokes *et al* (1955)¹³⁷ have shown that definite illnesses may appear in some "Vegans" of long standing. They also found that the deficiency symptoms in the "Vegans" could be alleviated by the administration of vitamin B₁₂.

Rajeshwar *et al* (1956)¹³⁸ compared five vegetarians with five non-vegetarians and found that the vegetarians had a lower serum albumin and higher globulin content than non-vegetarians. But when ~~amino acids~~ was given daily to the vegetarians, the levels of albumin and globulin reverted to the normal, thus showing that the poor amino acid composition of the vegetable proteins were not entirely responsible for their low nutritive value.

Hardinge and Stage (1954)¹³⁹ have reported that there existed no difference in the height or blood pressure in these groups. There was also no difference in the haemoglobin level, nor in the albumin and globulin levels, but they found the

* Vegans are the pure vegetarians who exclude from their diets all foods of animal origin.

'Vegans' 20 pounds less in weight than the others.

The serum cholesterol level has been shown to decrease in individuals fed solely on vegetable proteins by Walker *et al* (1961)¹⁴⁰. Thus from the findings of the workers reviewed above, Guthbertson (1946)¹³, Henry and Kon (1958)¹⁰, Kuyumjian *et al* (1958)¹¹ and others have concluded that animal proteins belonged to the first class while vegetable proteins come only next in quality. But the superiority of animal proteins is not only due to their amino acid make up, as seen from the above review, and therefore, according to Henry and Kon (1958)¹⁰ the terms first and second class do not hold good today. The vegetable proteins were designated as inferior, because only single, pure vegetable proteins had been studied. But, today the value of vegetable proteins in mixtures have been considered, and found to be of high nutritive value.

So far the nutritive values of individual proteins have been discussed, and now the nutritive values of protein mixtures will be considered.

Supplementary Relationship Between Proteins.

The supplementation of vegetable proteins has been thought of as early as 1916 by McCollum *et al*¹⁴¹. They demonstrated the supplementary value of ~~soybean~~ oat kernel. McCollum *et al* (1919)⁹² shows that the addition of even a small amount of a complete protein like casein could improve the

nutritive value of an incomplete protein like pea protein. Mitchell and Carman (1926)¹¹⁹ found that feeding two proteins together could enrich the nutritive value of the protein. According to them, "If the indispensable amino acid limiting the utilization of the nitrogen of meat, for example, is not the limiting factor for the nitrogen of white flour, it is evident that when the two foods are fed together, the nitrogenous compounds of meat will enrich the biological value of the nitrogenous compounds of white flour, and also the nitrogenous compounds of white flour will enrich the biological value of the nitrogenous compounds of meat".

Jones, (1946)¹⁴² proves that the proteins within the same seeds can be of good supplementary value. For example, the proteins of groundnut - arachin and conarachin when combined, resulted in a good protein, because the methionine and tryptophan in conarachin made up for the deficiency of methionine and tryptophan in arachin.

McCollum et al (1919)⁹² found gelatin to supplement wheat kernel, but wheat protein and zein did not promote growth, due to the lack of lysine in both the cereals wheat and maize. McCollum et al (1919)⁹² further proved that pea protein and zein were good supplements, due to the methionine content of zein in which peas are deficient, and the lysine content of peas in which zein is deficient. The analysis of these legumes and cereals for their amino acids was made only recently by Block

and Bolling (1951)¹⁴³ and Kappaswamy *et al* (1958)¹¹, and in the light of the work of Rose (1937)⁶, who distinguished between "essential" and "non-essential" amino acids, the supplementary value of legumes to cereals can be understood. Legumes, such as pulses, field peas, and navy beans have been proved to be deficient in methionine by Rameshram and Phansalkar (1956)⁹⁴ and Kappaswamy *et al* (1958)¹¹, while cereals have been shown to be deficient in lysine by the above workers. Thus the supplementary value of these two foods have been studied and have been found to supplement each other very well by workers like Srinivasan (1937)¹⁴⁴, Murray (1948)¹⁴⁵, Vijayaraghavan and Srinivasan (1953)⁹³, Phansalkar *et al* (1956)¹⁴⁶, and Kappaswamy *et al* (1958)¹¹.

Phansalkar *et al* (1957)³⁶ have shown that red gram dhal protein supported growth, when supplemented with wheat or jowar protein, but not with rice.

Legumes as cited earlier are deficient in methionine. When methionine is supplemented to legumes, their biological value is increased, as shown by Woods *et al* (1958)¹⁴⁷ who supplemented Alaska peas with methionine, and found the final product to be superior to casein in quality. Jaffe (1958)⁹⁵ proved that methionine supplementation of red gram, split peas or pigeon peas did not produce good growth in albino rats. When tryptophan and threonine were also added, the red gram dhal showed good results.

Bengal gram and Alaska peas have been shown to have a high biological value. Murray (1948)¹⁴⁵ reports that raw Alaska field peas were inferior to casein at 10 per cent level in the diet, but when supplemented by cereals to substitute half the peas, the biological value increased. This, he states, may be due to the methionine content of the cereals. Richardson (1948)¹⁴⁶ found that cooked legumes had a higher nutritive value than the raw ones. Only English peas were found that addition of methionine supplements to legumes improved their nutritive value especially when methionine was added after heating the legumes. Richardson (1948)¹⁴⁶ found that raw English peas improved markedly by supplementation with methionine and its nutritive value was superior to egg albumin, whereas the heated peas with the addition of the same amount of amino acid was not superior; but the addition of lysine and tryptophan resulted in a reasonable improvement.

Hirve and Magar (1953)¹⁴⁹ and Ganapathy et al (1958)¹⁵⁰ demonstrated that horse gram could be improved in its nutritive value by combining it with cereals and pulses. Ganapathy et al (1958)¹⁵⁰ combined, horse gram and cow pea with Italian millets and found a good supplementary value between the two. The lysine deficiency of the millets were made good by the richness of lysine in the legumes. Phansalkar et al (1957)¹⁵¹ have reported their study on the protein efficiency of a combination of cereals and pulses, and the effect of the addition of a leafy vegetable

like ~~ammaritus~~ to this combination, which resulted in a protein which ~~maintained~~ the haemoglobin and plasma protein levels effectively. The nutritive value of this combination was higher because of the protein of ~~ammaritus~~ which has been shown to be good by Deshpande and Rao (1954)¹⁵¹ and Kanath and Bohonie (1959)¹⁵² except for the deficiency of methionine.

Groundnut protein is another vegetable protein studied extensively for its supplementary value to other proteins. Johns and Finks (1920)¹⁵³ and Kappaswamy *et al* (1957)¹⁵⁴ demonstrated the supplementary value of groundnut protein to cereals, and found that wheat was more effective than rice. Sure (1948)¹⁵⁵ showed that groundnuts supplemented maize proteins. Moorjani and Subramanyam (1950)¹⁵⁶ had shown that groundnut milk and soya bean milk combined in the ratio of 3:1 proved to be as good as casein in the formation of blood proteins.

The above review points out the possibility of combining vegetable proteins in order to arrive at a complete protein of high nutritive value.

B. Vegetable Protein Mixtures.

1. Reasons for the Preparation of Vegetable Protein Mixtures:

Diet surveys carried out in India by Aykroyd (1948)¹ and Radhakrishnan Rao (1958)⁴ have shown that the amount of animal food included in the diets of Indian people is very little.

The reasons for not including ~~protective~~ foods in their diet as pointed out earlier are high cost and ~~non-availability~~ of milk and eggs; but in order to prevent the consequences of protein deficiency which has been discussed earlier, some protein food had to be made available to the poor people. As pointed out by Sai (1961)¹⁵⁷ foods like milk and eggs are not available in large quantities in countries like Africa, South and Central America, India and other parts of Asia, where the dietaries are deficient in protein. Thus many attempts have been made to develop vegetable protein mixtures which are inexpensive. The vegetable protein mixtures should be mixed in proper proportions so as to arrive at a good quality protein. Since the biological value of proteins depend on the essential amino acid composition, as pointed out by Block and Metcalf (1946)⁸ and Hart (1952)¹⁵⁸, the vegetable proteins can be mixed in a proportion which will supply all the essential amino acids in the right quantity, as set up by F.A.O. (1957)¹⁵⁹. The protein mixture has to be mixed correctly and its amino acid content studied before it is advocated for use, for Brock (1960)⁷ has pointed that proteins have to be considered as a 'constellation' of nutrients rather than as a single entity; and moreover as Chitre *et al* (1956)¹⁶⁰ state protein nutrition is actually amino acid nutrition.

Sai (1961)¹⁵⁸, states that certain factors have to be considered before the development of vegetable protein mixtures is undertaken, they being (1) the amino acid composition of the

proteins which should be adequate in quantity and balanced, (2) performance of Chemical and Biological tests, (3) the individual proteins for toxicity and (4) the ease of preparation.

2. a. Vegetable protein mixtures:

Joseph *et al* (1958)¹⁶¹ studied the supplementary value of bengal gram and sesame flour to that of low fat groundnut flour and found that this vegetable protein mixture was of high nutritive value.

This was due to the methionine content of sesame seeds which made up for methionine deficiency of groundnuts and bengal gram. The threonine content of bengal gram, supplements the protein of groundnuts, which lack threonine.

Shankar *et al* (1958)¹⁶² developed two vegetable protein mixtures consisting of wheat, red gram and amaranth, and another consisting of bajra, red gram and amaranth. They found that such mixtures were effective in the regeneration of haemoglobin and plasma proteins, but at a slower rate than skim milk powder and therefore they concluded that vegetable proteins do have the capacity of regenerating haemoglobin, but need more time than animal proteins. In another study, Shankar and Pathak (1956)¹⁴⁶ had showed that legumes and cereals could be effectively combined, for the egg replacement value of such a mixture was much higher than that of the cereal alone.

Krishnamurthy *et al* (1959)¹⁶³ showed that a blend of groundnut flour, soyabean, bengal gram and sesame has a high nutritive value. They found that the addition of soya bean to a mixture of groundnut, sesame and bengal gram already studied by Joseph *et al* (1958)¹⁶¹ could enhance the quality of the already nutritious mixture. Addition of sesame to a mixture of soya bean, groundnut and bengal gram, raised the P.E.R. from 1.7 to 2.1, which was almost equal to that of skim milk, which was 2.55. This study showed that addition of pure amino acids to vegetable diets can improve their nutritive value. Albanese *et al* (1955)¹⁶⁴ studied the effect of reinforcing infant diets with lysine and found a good result as seen by the increase in weight of the infants.

Tasker *et al* (1960)³³ studied a vegetable protein mixture containing sesame flour, groundnut flour and sunflower seeds (*Helianthus annuus*) and bengal gram. They observed a significant improvement in the mixture as compared to the nutritive value of individual proteins when fed at 10 per cent level in the diet.

Tasker and coworkers (1960)¹⁶⁵ developed a composite protein food with coconut meal, groundnut meal and bengal gram flour which formed a good supplement to the poor vegetarian diet. This is due to the supplementary value of coconut meal to the other proteins since the lysine and methionine deficiencies are made up. Subrahmanyam *et al* (1960)¹⁶⁶ combined whole wheat flour with tapioca flour and low fat groundnut flour and

called it "Paushtik Atta". They found that when "Paushtik Atta" replaced wheat in a diet, the nutritive value improved immensely, showing that the whole wheat proteins could supplement those of tapioca and groundnut flour. The P.E.R. of this mixture was found to be 1.2, while wheat had a P.E.R. of only 0.94, thus showing the increase in the growth promoting quality of "Paushtik Atta".

Joseph et al (1960)¹⁶⁷ studied the nutritive value of three blends of vegetable protein whose amino acid composition was similar to the composition of the F.A.O. reference protein pattern (F.A.O. 1957)¹⁵⁹ and in addition seven other blends having varied amounts of amino acids, and compared them to I.N.C.A.P (Institute of Nutrition of Central America and Panama) vegetable protein mixture 9 B. The results showed that the blends with amino acids similar to the F.A.O. reference protein had a nutritive value in the same order as that of skim milk. They found that groundnut protein improved in quality when lysine and methionine were added. INCAP 9 B had a lower nutritive value than the blend with the F.A.O. pattern. Thus it is possible to develop inexpensive nutritious vegetable protein mixtures with locally available protein rich foods. The Central Food Technological Research Institute has taken a major step in developing and manufacturing a cheap vegetable protein mixture called "Multipurpose Food".

b. Indian Multipurpose Food:

This composite food was termed the Indian Multipurpose

food (M.P.F) because it had to be differentiated from that of its American counterpart, "The American Multipurpose Food" which has been developed by Barcock (1951)¹⁶⁸ for the 'Meals for Million Foundation'.

Subrahmanyam *et al* (1957)³² developed the Indian M.P.F. on the same lines as that of the American M.P.F., but used only locally available, protein foods like *groundnuts*, bengal gram and sesame seeds.

The raw materials chosen were studied in detail at the Central Food Technological Institute and have been found to be of good nutritive value. *Groundnuts* has been used in many blends (Bains *et al* (1952)¹⁶⁹, Subrahmanyam *et al* (1954)¹⁷⁰, Gowri Sur *et al* (1954)¹⁷¹ and Subrahmanyam *et al* (1957)¹¹³. Bengal gram was chosen not only because it was nutritious, Narayana Rao *et al* (1959)¹⁷², inexpensive and available in large quantities, but also because it was already commonly consumed in India. Subrahmanyam *et al* (1957)³² considered the annual production of these proteins and decided to include them in M.P.F.

Table I gives the annual production of *groundnuts*, sesame seeds and bengal gram, as given by Subrahmanyam *et al* (1957)³².

TABLE I

ANNUAL PRODUCTION OF ~~GROUNDNUTS~~, BENGAL GRAM AND SESAME SEEDS.

Name of food	Production figures for 1954-55. (in 1000 tons)	
Groundnut in shell	3823	Approximate annual production of cake will amount to about one million tons.
Bengal Gram (whole)	5125	
Sesame seeds	592	

* Figures taken from All India Final Estimate issued by the Economic and Statistical Adviser in the Ministry of Food and Agriculture, Government of India.

In processing of Groundnut Cake, Sesame Cake and Bengal Gram
Cake.

The kernels of good quality ~~groundnuts~~ were cleaned, lightly roasted, and desolated ~~mechanically~~. The desolated kernels were crushed in an expeller press, till the cake contained only 8-10 per cent fat. The ~~groundnuts~~ were then powdered coarsely and roasted till an aroma was liberated. The nutty flavour of the groundnut was further enhanced by this roasting.

Sesame Cake Unit: The white variety of sesame was chosen, cleaned and crushed in an expeller press, till the oil content

came down to 10 per cent, after which the cake was ground to a coarse powder. [REDACTED]

Bengal Gram Grits: The gram obtained from the bazar was cleaned, roasted for 8 - 10 minutes at 120° - 130° C, and finally ~~a~~ ground.

Seasoning: To enhance the taste and flavour of the final product, seasonings such as coriander, pepper, cinnamon, turmeric, asafoetida and salt were added, after being roasted and powdered.

St. Studies Made to Test the Nutritive Value and Acceptability of the Mixture:

In order to find the best combination of these ingredients mentioned above, experiments were made by Subrahmanyan et al (1957)³². Four mixtures made with the following ingredients were tested:-

(1) Low fat groundnut. (2) Bengal gram. (3) Low fat sesame and (4) Black gram dhal. The composition of these four mixtures are given in Table II.

TABLE II

THE FOUR COMBINATIONS OF MULTI PURPOSE FOOD.

Constituents	Formula I %	Formula II %	Formula III %	Formula IV %
Groundnut meal	70	75	65	80
Bengal gram dhal	15	25	20	Nil
Black gram dhal	15	Nil	5	Nil
Sesame	Nil	Nil	10	20

Table II shows that sesame was added only to two combinations, formula II and IV, while black gram was added only to formula I.

The results showed that Formula II was the best among the four products, regarding the organoleptic tests. This was chosen to form the 'Indian Multipurpose Food'. It was fortified with vitamins and minerals, and was made almost equal to animal protein foods in quality. The chemical composition of both the Indian and American multipurpose food are given in Table III.

TABLE III

(**)
CHEMICAL COMPOSITION OF INDIAN AND AMERICAN MULTIPURPOSE FOOD.
 (VALUES PER 100 GM.)

Constituents	Indian M.P.F.	American M.P.F.
Moisture. (g)	6.8	6.7
Protein. (N x 6.49)	41.9	42.3
Fat. (g)	8.5	7.6
Ash. (g)	7.0	6.5
Carbohydrate (by difference) (g)	35.8	36.9
Calcium (g)	0.665	0.587
Iron. (mg)	5.1	7.0
P (g)	0.820	0.440
Vitamin B ₁ (mg)	1.3	0.7
Niacin. (mg)	14.0	7.0
Riboflavin (mg)	3.2	1.2
Vitamin A. (I.U)	3000	2940
Vitamin D. (I.U)	300	235
Calories	387	386

(**) As given by Subrahmanyam et al (1957)³².

The multipurpose food was prepared in three forms, namely seasoned, unseasoned and with 20 per cent of skim milk powder. Multipurpose food has been used in the preparation of many common dishes and a Brochure on dishes with multipurpose

food has been published. (1957)¹⁷³. [REDACTED]

Dishes made with Multipurpose food have been tested for shelf life (sterability) and it has been found that they could be stored for nine months at 37° C and for longer periods at lower temperatures.

The cost of one ounce of Multipurpose food is about 75 naya paise and according to Parpia *et al* (1957)³¹, the cost can be lowered if manufactured at a very large scale. Since an ounce contains ~ 12 grams of good quality protein, and costs only about 5 naya paise, this food will go a long way to alleviate the people of the lower socio-economic groups from protein malnutrition. Parpia *et al* (1957)³¹ state that the American multipurpose food has been found to be of immense value in Latin American countries and "some of the large industrial canteens have begun to realize that the general health and efficiency of their workers is directly dependent upon their diet, and that the multipurpose food can play an important role in this direction." He further pointed out that the American multipurpose food increased the efficiency of the workers by 10 per cent in Latin American countries. Since the Indian multipurpose food is almost similar to the American multipurpose food, this food can solve some of the major nutritional problems in India.

As Sai (1961)¹⁵⁷ had pointed out, a vegetable protein mixture has to be chemically and biologically tested, before it is recommended for widespread use. Such studies have been made with the multipurpose food.

iii. Studies made to Evaluate the Nutritive Value of Multipurpose Food:

Kuppuswamy (1957)¹⁷⁴ studied the nutritive value of multipurpose food and compared it to American multipurpose food and further studied the nutritive value of rice diets containing both these protein foods. They found that the biological value of multipurpose formula containing black gram, bengal gram and groundnut was 57.34 while the digestibility coefficient was 95.29. The multipurpose food of formula II (in Table II) had a biological value of 59.53 and digestibility coefficient of 95.68. The biological value and digestibility coefficient of formula III and IV were 60.62 and 60.43 and 95.8 and 96.26 respectively. The biological value and digestibility coefficient of American multipurpose food were 58.89 and 95.03. The results proved the formula III and IV to be the best, but the results were not statistically significant. There was no difference in the ability of American M.P.F. and Indian M.P.F. in supplementing the poor rice diet. The higher nutritive value of formula IV was due to the sesame content which contains methionine. Joseph et al (1957)¹⁷⁵ also studied the overall nutritive value of the multipurpose food. They found that rats grew better on American multipurpose food, but Indian multipurpose

food regenerated haemoglobin and plasma proteins better, otherwise there were no other differences. The multipurpose food also promoted normal reproduction in the rats, but the rate of growth in the first generation decreased,

Kuppuswamy *et al* (1957)¹⁷⁶ studied the supplementary value of M.P.F to vegetarian diets and found that addition of the M.P.F., increased the nutritive value of the poor vegetarian diet remarkably.

Joseph *et al* (1957)¹⁷⁷ also found a similar result when they supplemented poor vegetarian diet based on Italian millets.

Joseph *et al* (1959)¹⁷⁸ studied the nutritive value of 'Idli' fortified with multipurpose food. They found a significant weight increase with the Idli made with multipurpose food while organoleptic tests also favoured the same sample. Further studies on children had also been made. These studies have proved that multipurpose food can be regarded as an excellent source of protein, for the tropical countries, where protein malnutrition is widely prevalent. Joseph *et al* (1957)^{179, 180} and Subramaniam *et al* (1957)^{181, 182} had studied the nutritive value of multipurpose food on children by studying the effect of supplementing M.P.F to the children's orphanage diet and found that the children increased significantly in height and weight and also a higher haemoglobin content and also noted a

higher retention of nitrogen, calcium and phosphorus in those children consuming the M.P.F than in the control group.

3. Development of Vegetable Protein Mixtures in Other Countries:

a. American Multipurpose Food:

The American multipurpose food is an expeller produced product, reports Cooper (1951)¹⁸³ and distributed by the Meals for Millions Foundation of Los Angeles. It was enriched with vitamins and minerals. The based vegetable protein was that of soya grits. Cooper (1951)¹⁸³, values one ounce of the American multipurpose food to be equal to 1.4 cups of whole milk or two ounces of lean meat, and costs only two and a half cents per ounce, while milk costs 8 cents and provides the same amount of protein. The composition of the American M.P.F is given in Table III. - The Indian M.P.F was prepared on similar lines as the American M.P.F.

b. INCAP Vegetable Protein Mixtures:

The other protein mixture of importance, which is manufactured in a large scale like the Indian multipurpose food, is the vegetable protein mixture developed after 7 years of research work at the Institute of Nutrition of Central America and Panama.

Sorinshaw and Bressani (1960)³⁷ have reviewed the work done with the evolution of this protein mixture. They used only locally available vegetable protein sources such as maize masa, sesame, cottonseed and kikuyu grass leaf meal.

The first to be prepared was called vegetable mixture 8. It contained 28.1 per cent protein and provided 503 calories for every 100 gram. Sorimshaw and Bressani (1960)³⁷ reported that the most limiting amino acids in this vegetable protein mixture were those of lysine, tryptophan and methionine. The vitamin B complex were supplied by the Torula yeast added to the mixture. Biological studies by Squibb *et al* (1959)¹⁸⁴ were made to test the efficiency of the proteins of this vegetable mixture. The results showed that this food was well tolerated by animals and children and the nitrogen retention was equal to that of milk protein. Kwashiorkor was successfully treated with this protein mixture. Another vegetable protein mixture was developed replacing sesame with cottonseed flour known as INCAP vegetable mixture 9, and later marketed under the name 'INCAPARINA'. Bressani *et al* (1959)¹⁸⁵ proved that maize could be replaced by other cereals, in this mixture.

Thus another vegetable protein of high nutritive value was developed and according to Sorimshaw and Bressani (1960)³⁷ this product can make a significant contribution to the solution of the problem of protein malnutrition.

c. Other Vegetable Protein Mixtures

Prior and Derse (1957)¹⁸⁶ have studied the nutritive value of vegetable protein mixtures developed by them and ~~first~~ found that a mixture of wheat gram and defatted soya bean was

not only palatable but also had a high nutritive value. On the addition of vitamin B₁₂, this diet improved and weight gain of the animals fed with the vegetable mixture was 44 grams as against 41 grams with casein at 9.09 per cent level in the diet, but at 11.5 per cent level the casein was shown to be superior. They also compared this vegetable protein mixture with an animal protein diet and found them to be almost equal, in their nutritive value.

Many investigators had attempted to cure protein malnutrition using either animal proteins like skim milk powder or vegetable protein mixture mentioned above.

4. Attempts made to Cure Kwashiorkor with Vegetable Protein Mixture:

The nutritive value of vegetable protein mixtures have been studied in great detail and the investigators concerned are confident that such mixtures can be used in the treatment of 'Kwashiorkor' or nutritional oedema syndrome. Phansalkar *et al* (1957)⁵⁶ concluded from their experiments on vegetable protein mixtures that it is practical to obtain through a proper combination of vegetable proteins a protein mixture of high biological value which are comparable to that of skim milk protein. These protein mixtures can be used for the treatment of Kwashiorkor. Dean (1958)¹⁸⁷ treated Kwashiorkor in Uganda, by feeding soya bean and bananas, but later gave it up as soya beans could not be digested easily. In Uganda, Thompson (1955)¹⁸⁸

tried treating Kwashiorkor children by using milk and soya beans, but again found that soya beans took a longer time than milk to cure anorexia, but improved when vitamin B₁₂ was added along with methionine. Subramanyam *et al* (1955)¹⁸⁹ had treated Kwashiorkor by feeding casein fortified with calcium and B complex vitamin.

Venkatachalam *et al* (1956)³⁵ had treated nutritional oedema syndrome with a combination of skim milk protein and bengal gram powder very successfully. He also treated Kwashiorkor with bengal gram alone. Phansalkar and Patwardhan (1958)¹⁶² had showed that blood protein can be regenerated with vegetable protein mixtures.

Subramanyam *et al* (1954)¹⁹⁰ treated Kwashiorkor cases with M.P.F. with skim milk powder. Thus, this world wide problem of Kwashiorkor or protein malnutrition can be combated when the vegetable protein mixtures have been popularised in all the areas where such inexpensive, protein rich foods are necessary. It is also evident from the above review that animal proteins need no longer be considered as the only source of complete proteins, for vegetable sources can also be utilized just as well.

P. Methods of Assessing the Nutritive Value of Proteins:

In view of the important role of proteins in the diet, it is necessary to determine the quality of the protein, before it is consumed.

Rippon (1959)²¹ reports that ever since Rubner first demonstrated in 1879, a variation in the efficiency with which nitrogen from different proteins was retained in the body, many workers have attempted to evolve suitable methods for assessing the quality of proteins. Most of these methods have been based on the fact that proteins promote growth and maintenance of life. From their observations that different proteins promoted growth at different rates, Osborne et al (1919)⁵³ concluded that the greater the rate of growth produced by a protein, the better was its quality. In the second decade of the Twentieth Century, cites Rippon (1959)²¹, the other methods evolved include nitrogen balance in growth, maintenance, reproduction, fertility, lactation and longevity. In the fourth decade, the regeneration of blood proteins started by Madden and Whipple was included. In the present decade, Rippon cites, the recent trends are those of analysis of the entire carcass of animals, estimation of amino acid content of foods to evolve a chemical score, estimation of the ratio of essential nitrogen to total urinary nitrogen, determination of the changes in ¹⁵N nitrogen and liver enzymes, and relating these to protein quality. Each method has its own advantages and limitations.

~~These methods~~ ~~are~~ ~~can be~~ ~~classified~~
into ~~biological~~ ~~chemical~~ ~~methods~~ ~~and~~ ~~microbiological~~
methods. The various methods of assay are:

1. Biological Methods:

- a. Rat growth method or determination of "Protein efficiency Ratio" (PER).
- b. Nitrogen balance studies.
- c. Rat depletion-repletion studies.
- d. Carcass nitrogen method.
- e. (i) Liver protein regeneration, and (ii) enzyme changes in the liver.
- f. Hemoglobin and plasma protein regeneration.
- g. Ratio of creatinine nitrogen to total urinary nitrogen.
- h. Short term Nitrogen retention test.
- i. Effect of protein quality on bone development.

2. Chemical Methods:

- a. Chemical score.

3. Microbiological methods.

1. Biological Methods:

- a. ~~The rat growth method or determination of PER~~
~~Protein Efficiency Ratio: Since the classical ex-~~
~~perimentation of Osborne et al (1914), that dif-~~

-ferent proteins supported growth and maintenance differently, evaluating the quality of proteins through rat growth methods has been popular. They fed different groups of rats with comparable diets, each containing a different protein, and the average gain or loss in weight over a period of 30 days, served as an index of comparison of the nutritive value of different proteins. The results showed that casein produced the greatest gain, while gliadin permitted only maintenance of the initial

weight and zein or gelatin produced a loss in weight in the animals. Osborne and Mendel (1919)⁵³ introduced the concept of PER as a refinement of the simple growth method. They compared the nutritive value of different proteins by taking the ratio of gain in body weight of the young rats to the amount of protein consumed.

$$\text{PER} = \frac{\text{Gain in weight in grams}}{\text{Amount of protein consumed in grams}}$$

Osborne and Mendel (1919)⁵³ found that a definite level of each individual protein ⁱⁿ the diet, produced the greatest gain per gram of that ingested protein. This concept later received the support of other workers (Swaminathan, 1937¹⁴, Bender, 1958¹⁹¹). From the results of the numerous experiments conducted on rats, first by Osborne and Mendel (1914)¹⁹ and followed by Mitchell (1924)¹⁹¹, Chow et al (1948)²⁰, Bender (1958)¹⁹¹, Albanese (1959)¹⁹³ and Allison (1959)²⁶ and Hippon (1959)²¹ it can be seen that better the quality of protein, lower is the level required in the diet to produce the highest PER. This emphasizes the importance of proper balance of all amine acids in order to produce optimum metabolic efficiency. The recent trend, according to Bender (1958)¹⁹¹ is to determine the PER at 10 per cent level in the diet. According to Frost (1959)¹⁹⁰ casein and lactalbumin have been shown to have their optimum growth at 12 and 7 per cent respectively.

The factors affecting the PER of proteins are: 1. Age

since the PER obtained with younger rats are higher than that obtained with older ones. This has been shown by Swaminathan (1937)⁴, Subrahmanyam *et al* (1957)³², Joseph *et al* (1957)¹⁷⁵ and others who state that the PER in four weeks is higher than that in eight weeks. 2. Sex, as shown by Swaminathan (1937)¹⁴ and Cahill (1945)⁵⁰. 3. The period of experiment also affects the PER, as shown by Fixsen (1936)¹⁹⁵. For a higher value is obtained with a shorter period than with a longer period. Fixsen considered that at least 60 days are necessary for accurate results. 4. The percentage of protein fed in the diet can affect the PER, as shown by the experiment of Rippon (1959)²¹ who found that all the animals should be fed with the same amount of protein, in order to obtain a correct value for PER. 5. The amount of water intake can affect the PER, as shown recently by Keane (1963)¹⁹⁶ who demonstrated that the larger the amount of water, the higher the PER.

Frost (1959)¹⁹⁴ stated that PER is considered as a critical index of protein value, and is most critical when fed at a near minimum level of 1-1.5 per cent nitrogen added to a protein free diet.

This method was criticised by Mitchell, according to Rippon (1959)²¹ as an ~~inadequate~~ method for assessing the nutritive value of proteins. Moreover, the growth method classified proteins according to their adequacy to meet the needs for tissue synthesis. In spite of Mitchell's criticism, the rat growth

method is popular, for its simplicity of technique, states Rippon, and is often recommended for routine work. Among the advantages of this method, the one which stands out is that values of PER can be obtained simultaneously with other experiments conducted with the same rats. Rippon (1959)²¹ studied the PER of casein at various levels in the diet. He also studied the effect of the following conditions on the PER:

1. Ad libitum feeding.
2. Pair feeding.
3. Litter mate animals.
4. Animals grouped at random.
5. Altering the previous supplement in depletion-repletion tests.
6. Albino rats.
7. Hooded rats.

He demonstrated that varying the level of intake of protein from 0.87 grams to 1.74 gram per day does not significantly affect the PER value. The result was the same in depletion-repletion tests as well. Secondly ad libitum feeding at 10 per cent gave a higher PER than when pair fed. Thirdly, he found no difference between litter mates and random grouped animals. Fourthly, he found that the PER obtained in depletion-repletion tests differed according to the diet given before the commencement of the experiment and finally he found no difference in the PER obtained with hooded and albino rats. Rippon's final conclusion was that PER is a good method to

assess the nutritive value of a protein, if the depletion-repletion technique is followed.

b. Nitrogen Balance Studies: Frost (1959)²⁰⁷ denotes the Nitrogen Balance technique as the second oldest method of protein assay. Nitrogen Balance study was first conceived by Thomas in 1909¹⁸, (1959)¹⁹⁷ who reported that the percentage of digestible nitrogen retained by the adult human from a test food was different for different proteins. Mitchell (1924)¹⁹² developed this concept further and Mitchell and Larmann (1926)¹¹⁸ announced the technique. Applying the principle of Thomas, Mitchell obtained on growing rats the requirement of nitrogen for both growth and maintenance. Mitchell et al (1959)¹⁹⁸ in 1945 succeeded in evolving the method to a high degree of accuracy and precision, using paired feeding technique. Allison (1959)²⁶ surmises the principle of Nitrogen balance method as "the difference between nitrogen intake and the nitrogen excreted. If nitrogen balance is positive, the living system is gaining in protein, either through the growth of new tissues or the repletion of depleted 'stores'. Negative nitrogen balance means a loss in tissue nitrogen through excess catabolism, the dietary protein intake being insufficient to meet the needs for maintenance of the body proteins." Allison (1959)²⁶ has defined the "dynamic catabolic anabolic interrelationship" and has integrated it into an equation.

$B = I - (U \text{ plus } F)$, where B is the nitrogen balance, I is the nitrogen intake, U is the excretion of urinary nitrogen, and F excretion of faecal nitrogen. According to Allison (1959)²⁶, "when the intake I just equals excretion (U plus F) the system is in equilibrium and is said to be maintaining the status quo, catabolic activity being nicely balanced with anabolism." This in short, is nitrogen balance.

The balance sheet method of Mitchell is used to assess the quality of proteins, by determining the biological value (BV) of proteins. The determination of BV includes the determination of the normal nitrogen turnover of the body, determination of nitrogen in the faeces and urine, feeding a known quantity of the test proteins as the sole source of nitrogen, and thus becomes a lengthy procedure. Niyogi *et al* (1930)¹⁹⁹ (1931)²⁰⁰, Swaminathan (1937)²⁰¹, Mitchell and Beadles (1949)²⁰², and Rippon (1959)²¹ and many other workers have determined, biological values of proteins, and have emphasised the use of metabolic cages, for the collection of urine and faeces.

The nitrogen retained on ingestion of the test protein is expressed as a proportion of the nitrogen ingested or absorbed, making an allowance for the nitrogen lost during a period of nitrogen starvation. This has been expressed in an equation by Albanese (1959)¹⁹³.

$$B.V. = \frac{\text{Retained food nitrogen}}{\text{Absorbed food nitrogen}} \times 100, \text{ where}$$

Absorbed food nitrogen = Food nitrogen — faecal nitrogen.

Faecal nitrogen = Faecal nitrogen — metabolic nitrogen of faeces.

Retained food nitrogen = Absorbed food nitrogen — excreted food nitrogen

Excreted food nitrogen = (Urine nitrogen — endogenous nitrogen of urine) + faecal nitrogen.

As pointed out by Rippon (1959)²¹ it is not sufficient to subtract faecal nitrogen from dietary nitrogen in order to determine the proportion absorbed, as there is always a faecal nitrogen excretion even on a non-protein diet, which is the 'metabolic' or 'endogenous' nitrogen. Similarly, in order to measure the nitrogen retention, the urinary nitrogen has to be corrected for endogenous losses. Rippon (1959)²¹ proved that the endogenous nitrogen differed from animal to animal, and from time to time in the same animal, and that the endogenous nitrogen varies in litter mates as well and also in those chosen at random and therefore, he emphasises that the endogenous nitrogen should be determined for every nitrogen balance experiment. The determination of 'BV' of proteins further requires an adequate diet in all aspects, except that of the protein to be tested. It should be adequate in calories, so that the carbohydrate and fat present spare the test protein from being used up for energy. Rippon concludes that in order to compare several proteins for their biological value, the test food should be fed at the same level since the greater the amount of nitrogen ingested, the less was the biological value of the protein. Albanese (1959)¹⁹³ who has given

the biological values of certain proteins, has pointed out that the values are different when fed at different levels. For example, the BV of milk at five and ten per cent level are 93 and 85, per cent respectively. Earlier Morgan (1931)¹⁰⁷ and Henry and Kon (1957)²⁰³ reported a similar finding. Henry and Kon (1957)²⁰³ demonstrated the effect of age of test animals also on the biological value of proteins. They found that the sulphur amino acid requirement of rats decreased with age, which affects the B.V. They also suggested that 8 per cent levels bring better results regarding relative nutritive value of proteins, than at lower levels. The determination of the nutritive value of proteins, using Mitchell's balance sheet technique, is quite popular, inspite of the tediousness of procedure. This is because the results obtained by this method according to Hippen (1959)²¹ are 'reliable' and the procedure is a 'straight forward' one and more or less uniform with little difficulties, once the metabolic cages have been set up. Moreover, the digestibility of the protein is also determined in this method.

Allison and Anderson (1959)²⁰⁴ introduced a Nitrogen Balance Index in 1945 which obviates errors in measuring endogenous nitrogen excretion. They standardized dogs till the endogenous excretion was fairly constant, then plotted the absorbed nitrogen against nitrogen balance to obtain a straight line.

Comparing the PER method with the BV method, the BV

is found to be superior to PER method. Bender (1958)¹⁹¹ showed that in the case of PER method, there is an assumption that weight increase is an index of protein synthesis which need not be necessarily so. Secondly the results vary with the difference in levels of protein intake. The results depend on the adequacy of calories and other nutrients, finally only those protein which can promote growth can be evaluated at all. In order to modify the PER method, to eliminate errors such as the variation in results due to the different levels of protein intake, and the inability to assess the nutritive value of those proteins which do not promote growth, Miller and Bender (1955)²⁰⁵, Bender and Loell (1957)¹⁹⁸ have introduced a new technique. They measured the protein retention efficiency (PRE) of a protein, by subtracting the final weight of a group of rats fed on a non-protein diet for a week, from the final weight of the experimental group fed a test protein. They found that PRE correlated highly with net protein utilization determined by the carcass analysis method.

c. Rat depletion-repletion studies: The rat depletion-repletion method involves, as its name suggests, a period of depletion of the tissue and labile protein reserve of the body, and ^{then} feeding the test proteins at definite levels, Bender (1958)¹⁹¹ cites that in this method, the weight gained is that of only tissue synthesis in rats. Hippen (1959)²¹ reported that the nutritive value of proteins can be assessed quite accurately by this method. Frost and Sandy (1949)²⁰⁸ conducted a series of depletion and repletion tests using the same animals over and over again to study the

quality of proteins. This method was used by Frier and Berse (1957)¹⁸⁶, and later by Rippon (1959)²¹. The animals were placed on a protein-free diet for seven days, followed by repletion of 5 days on the test protein, and an equilibration period of 2 days, on a stockdiet. The weight gained during repletion is taken for the calculation of PER, and Rippon has found this method to be very accurate, for the determination of PER.

Miller (1950)⁵⁵, Sanio (1953)²⁷, Rippon (1959)²¹ had conducted studies on depletion and repletion, but they did not include the two days on stock diets, as Frost and Sandy did.

The depletion-repletion technique is advantageous because the recovery of the weights of the animals parallels, changes in the carcass protein and moreover, the same animals can be used over again.

d. Carcass nitrogen method: Rippon (1959)²¹ refers to ixsen attributing the origin of the method of carcass analysis to Mc Collum and Shuckers many years ago. It is a short method, involving estimation of the total nitrogen content of the defatted carcass of test animals, and the percentage retention of nitrogen is measured from the carcass analysis, instead of the difference between intake and output. Joseph et al (1957)¹⁷⁵ Rippon (1959)²¹ and Korala (1959)²⁰⁸ used this method to assess

the nutritive value of proteins. In order to compare the carcass analysis method with other methods to measure the nutritive value of proteins, Rippon (1959)²¹ studied the advantages and disadvantages of this method. One group of animals were depleted of protein, for a week and were then sacrificed, and the total nitrogen of the depleted animal was obtained. Two other groups were ^weighed, and then fed with the test protein at ten per cent level. After a week on the test protein, the animals were sacrificed, and analysed for nitrogen. The net protein retention was calculated by subtracting the initial nitrogen value (that is of the depleted animal) from the final value, which is that of experimental group. Rippon found the results to be almost as good as those obtained with BV or P.E.R method, and therefore, recommends the latter method as it is not unpleasant. Moreover, the BV and P.E.R methods, allow the use of the same animals for other experiments, but in the case of carcass analysis, the animals can no longer be used. A new technique has been developed by Henry and Toothill (1962)²⁰⁹. The procedure is as follows:

The body nitrogen is determined by a formula, using the body water content since Bender and Miller have shown according to Dreyer (1957)²¹⁰ that the $N:H_2O$ ratio is of great constancy and that it could be used for calculating carcass nitrogen from a knowledge of the water content and age of the rat. Miller and Bender (1956)²⁰⁵ and Henry and Toothill (1962)²⁰⁹ have used this method in evaluating proteins.

The value obtained by this method have been found to be quite accurate, and in line with those obtained by BV or P.B.R methods. Thus, the carcass analysis method has no special advantages, and can be replaced by other techniques of protein assay.

c. (1) Liver Protein Regeneration and Storage changes: Another important method, used often to assess the nutritive value of proteins, is that of liver tissue regeneration.

The body has certain protein reserves called the "labile reserves" which are used up in emergency to supply proteins to the body when the diet lacks proteins in quality or quantity. Addis et al (1936)²¹¹ showed that the liver lost the maximum protein, when rats were put on a protein deficient diet for two days. Miller (1950)⁵⁵ showed that the protein of liver could be depleted after a week of protein deficient diet. Allison (1960)²⁶ states that the most labile proteins are those of albumin in the blood. Liver proteins come next in order. Kosterlitz (1959)²¹² was the first to suggest the sensitivity with which the nitrogen content of the liver responds to different dietary proteins. Chow et al (1948)²¹ describes the procedure they adopted to estimate liver protein regeneration. The animals were depleted of their liver proteins by feeding nothing but water for 48 hours, and with in this period the liver tissues are depleted of the labile reserves. The animals were then killed by a blow on the head, and the livers are removed and analysed for nitrogen. This group represented the control group. Another

set of animals were fed with the test protein for a definite period of time and the livers were analysed for nitrogen, at the end of the period. The difference between the two gives the value for the regenerated tissue proteins. The regenerated liver protein is directly proportional to the quality of the protein. Henry (1959)²¹³, Joseph *et al* (1957)¹⁶⁴, Korula (1957)¹⁹⁴ and Rippon (1959)²¹ have also used this method, to assess the nutritive value of proteins. In the liver nitrogen method, an assumption has to be made, since the initial value is obtained only from a control group.

11. Liver enzyme changes: Studies have shown that besides liver nitrogen changes, liver enzymes are also changed during protein depletion. Williams and Elvehjem (1949)⁶⁸, Litwack *et al* (1952)⁶⁹, Wario (1953)²⁷, Allison (1960)²¹⁴ had pointed out that RNA (Ribonucleic acid) activity is increased in protein deficiency, and this finding that the greater the deficiency of protein, the higher the activity of RNA in the cells has been used as an index of protein quality. Thus, the quality of proteins can be assessed, by measuring the degree of activity of the cell constituents, and by the degree of inactivity of the liver enzymes.

12. Hemoglobin and Plasma Protein Regeneration: The labile reserves present in the blood are mainly three in number—albumin, globulin and haemoglobin. As pointed out by Williams (1959)⁶⁸ and Allison (1960)²¹⁴ these reserves are depleted and then regenerated with the test protein. The degree of regeneration of these proteins has been related to the quality of proteins. Cox and Muller (1959)²¹⁵ used this finding, in assessing the nutritive value of problems.

Chow et al (1948)²⁰, (1949)²¹⁶ have shown that not only various proteins do differ in their ability to regenerate plasma proteins, but some favour the production in the plasma of globulin than albumin. Chow et al (1948)²⁰ depleted the blood proteins of dogs by three methods, and studied the effect of whole proteins and hydrolysates in regenerating plasma proteins. The three methods of depletion were - 1) Plasmapheresis, which was done by placing the dogs on a protein free diet, for a week, followed by a removal of one-fourth of the blood, calculated on the basis of body weight. The blood was citrated, centrifuged and the plasma was removed. The red cells were washed once with sterile 0.85 per cent saline solution, and then suspended in a normal saline solution equal in volume to that of the plasma removed. The suspension was returned to the animal intravenously. This process was repeated till the plasma protein level came to 4.5 to 5.2 grams per 100 ml., 2) Protein free diet. The animals were placed on protein deficient diet, till the plasma protein concentration came to the desired level, which was usually after six to eight weeks on the diet., 3) By feeding a diet inadequate in calories, but otherwise nutritionally adequate with enough protein for the maintenance of nitrogen balance, the plasma concentration was brought down by 35 to 40 per cent of the original content in all the three methods.

The results obtained by Chow et al (1948)²⁰ showed that the total circulating proteins increased, but very little

difference was seen between the different proteins fed. Maybee and Morgan (1951)²¹⁷ also used this technique. They found a correlation between P.E.R and serum protein levels. Albanese (1959)¹⁹³ had shown that this method could be used as a method to assess the nutritive value of protein even though the method has its drawbacks. In conclusion, Albanese (1959)¹⁹³ regards this method to be a dependable criterion for the assessment of the nutritive value of proteins.

G. Ratio of creatinine nitrogen to total urinary nitrogen: In 1905, Folin (1959)²¹⁸ showed that the output of creatinine by an individual was almost constant. Albanese (1959)¹⁹³ states that creatinine excretion is influenced by the body weight and muscular tissue, and it is independent of a diet devoid of creatinine. Bender (1958)¹⁹¹, in his review of the methods of protein assay explains the principle of this method. The urinary nitrogen increases as the quality of protein decreases, and since creatinine excretion is constant, a ratio is obtained from which the quality of the protein can be assessed. Barrows and Chow (1959)²¹⁹ have stated that Malin and coworkers found a direct relationship between biological values and urinary creatinine of animals fed on a range of proteins. Arroyave (1960)²²⁰ has also stated that this method can be used to assess the nutritive value of proteins. Rippon (1959)²¹ found no correlation between BV proteins and the creatinine excretion, but he states that an idea about the quality of the test proteins can be obtained, from the use of this method.

~~1. Effect of protein quality on nitrogen balance:~~ Since the balance sheet and protein efficiency ratio methods take long time to evaluate the quality of a protein, shorter methods have been devised. Bender (1958)¹⁹¹ reports of a modified method developed by Silber and Porter, which takes just one day. After a week of protein depletion, rats were given the test protein in a single meal, and the total amount of alpha-amino acids excreted in urine was measured and the retention of nitrogen was calculated from this method. Bender remarks that this method is useful in the testing of hydrolyzates.

~~1. Effect of protein quality on bone development:~~ Yet another method for the measurement of protein quality is that of the study of proteins and bone development. Batresera et al (1961)²²¹ fed mature rats with equal amounts of essentially 180 ~~calorie~~ diets containing 4.5, 9, 13.5 and 18 per cent protein as ~~protein~~ albumin, for 30 days, and the effect on the rat was studied. The animals of 4.5 per cent diet, did not increase in weight, while the other three increased proportionately. The results also showed that the bones were affected by the quantity of the protein in the diet, and not by quality, thereby showing that this method cannot be relied upon, to assess the nutritive value of proteins. But Mack et al (1962)⁵⁴ have shown that even quality of proteins affect the bone development for they found that ~~children~~ on lysine supplemented diet had denser bones than those fed with a lysine deficient diet.

2. Chemical methods:

a. Chemical score: Eversine² Rose in (1937)⁶ showed that proteins were made up of essential and non-essential amino acids, the nutritive value of proteins has been ascertained from its amino acid composition.

Mitchell and Block (1946)²²² and Oser (1951)²²³ developed a new method of evaluating the quality of proteins by a chemical method. They suggested the essential amino acid content of proteins as a measure of protein quality. The 'chemical score' method of evaluating a protein requires an accurate analysis of the test protein for essential amino acids in order to determine the most 'limiting' or the most deficient amino acids in the test protein, in relation to the essential amino acids of whole egg protein, which is used as a standard, for Mitchell and Garman (1926)¹¹⁸. Hawley *et al* (1948)¹¹⁴ have shown egg proteins to be 100 per cent utilized. Thus, in the chemical score method as pointed out by Davidson *et al* (1960)²²⁴, the amino acids are of importance and not the protein as a whole. Mitchell and Block (1946)²²² evaluated a protein as follows: The percentage deficiency of the most deficient amino acid relative to whole egg protein, is subtracted from 100, to give a value called the 'chemical score.' Mitchell and Block (*loc. cit*) plotted the BV calculated from nitrogen balance values, against the percentage deficiency of the most deficient protein foods and from the relationship a regression equation

derived.

$$Y = 102 - 0.634 x.$$

where, Y = Biological value and x = percentage deficiency of the most limiting amino acids.

The chemical score method has been used by many investigators (Vijayalingam and Srinivasan, 1953⁹²; ~~Amasakur~~ and Phansalkar 1956⁹³) to evaluate the quality of a protein, and have found it to be an objective method, for the individual differences of animals, as in the biological studies, do not effect the result. But, there are many drawbacks, pointed out by some of the workers who have used this method. Davidson et al (1960)²²⁴ point out that though a great deal is now known about the composition of individual proteins in terms of their constituent amino acids, attempts to give them a 'chemical score' on the basis of their composition, and to assess their nutritive value from this score is not very successful. This is because the digestion of proteins and the release of amino acids are rarely complete, as pointed out by ~~Putnam~~, (1960)²²⁵ that some proteins are so firmly held in the cell walls of the plant, that they are not completely absorbed. Secondly, Davidson et al (loc. cit) point out, that some animal proteins have been found to be even more nutritious than what is concluded from their chemical analysis, which is due to the presence of an animal protein factor, which is vitamin B₁₂ and is found only in animal foods. Thirdly, Davidson et al point out that the chemical score is not very practical, for individuals differ in their ability to digest and utilize a protein. Fourthly,

Rippon (1959)²¹ points

out that some vegetable proteins improve on germination as shown by Everson and coworkers, but the amino acid content of the germinated seeds are the same as in the ungerminated state. Fifthly, the most deficient essential amino acid should not be considered as a guide to evaluate a protein, for Harper (1959)²²⁶ and Elvehjem (1956)⁹ have shown that an excess of one amino acid increases the requirement of another.

In order to correct some of the drawbacks of the chemical score method Sheffner et al (1956)^{227,228} calculated the essential amino acids released during enzymatic digestion of proteins, thereby overcoming one of the errors pointed out earlier. This was called 'the Pepsin Digest Residue Index'. The chemical score method has its advantages too, for it has been used by many workers. The work of Mitchell (1916)²²⁹, Hemmington (1937)¹⁴, Hart (1952)¹⁵⁷, Mack et al (1962)⁵⁴, Evans (1962)¹⁵ and many others show that the amino acid content of foods do determine their nutritive value.

Thus, from the above review, it is evident that every assay method has its advantages and disadvantages, and moreover, if the nutritive value of a protein has to be studied in detail, more than one method should be made use of, as each method evaluates a protein from different angles.

2. Microbiological methods:

The third type of assay used to evaluate a protein, is that of the microbiological assay. Rockland and Dunn (1959)²³⁰

first introduced this method in 1949. They found that the protozoan, *Tetrahymena pyriformis*, has an amino acid requirement similar to that of higher animals. Moreover, the organism was able to digest whole proteins. Fernell and Rosen (1956)²³¹ developed this technique further and evaluated a protein in terms of the ammonia production over a four day growth period. Horn *et al* (1952)²³² showed by a microbiological method, that cotton seed proteins had their nutritive value altered during processing as the amino acids availability was altered. These workers found a high correlation between the rat feeding methods and microbiological method. Anderson *et al* (1951)²³³ have achieved in determining the availability of amino acids from proteins by the use of microorganism, which is also very necessary to evaluate a protein. Ford (1960)²³⁴ has succeeded in evaluating the nutritive value of proteins using "*Streptococcus zymogenes* NCDO 592", which needed the same amino acids as the growing rats. They also obtained a high correlation between the results of microbiological assay and biological methods. Rosen and Ferbekk (1956)²³⁵ also found a high correlation between the *Tetrahymena* method, and rat assays.

The advantages of this method, as set forth by Bender (1958)¹⁹¹ are:

1. It is a quick method and therefore of value, and
2. Large numbers of proteins can be tested simultaneously, and thereby easy to compare the effects of processing on proteins.

III. EXPERIMENTAL PROCEDURE.

The purpose of the study was to compare some plant and animal protein foods, with special reference to their protein quality. The protein foods selected were from those commonly consumed by the people, as indicated by a survey conducted by the investigator, in a rural and urban area of Coimbatore District. Accordingly the experimental procedure was carried out as follows:-

A. Description of the Survey

1. Evolution of Survey Schedule:

An interview schedule as shown in Appendix I was evolved to elicit information regarding the consumption of protein rich plant and animal foods.

The survey was conducted in a village as well as in an urban area and the schedule forms were different for the two areas.

a. Urban area survey: In order to find out the common protein foods, a detailed list of all the commonly available protein foods were listed. The list included green leafy vegetables too, since lately, the importance of leaf proteins has been gaining ground. Pre-prepared foods like "Papad" were included since papad has been known to be a common side dish in most

South Indian homes. The quantity of the foods consumed and the frequency of their use were also included in the questionnaire, since the data give information regarding the most commonly and popularly used protein foods by the families.

In order to find out if the protein foods differed according to the caste, religion, native place or even the income level of the family, relevant questions to elicit these details were included in the questionnaire.

b. Rural areas: The data regarding the consumption of protein rich foods in a rural area, were obtained by the use of an interview schedule given in Appendix II, which was designed for a project made by the investigator.

2. Selection of Families:

a. Urban area: The survey was conducted on fifty families as this size of sample was considered statistically adequate. In order to find out the common plant and animal protein rich foods consumed, twenty five vegetarian and non-vegetarian families respectively, were chosen for the study. The families belonged to the middle class according to their annual income and were chosen from the location situated close to the college premises.

b. Rural area: The rural area selected for the study belonged to the villages of Devianspalayan and Pudupodur in Coimbatore District. The number of families selected for the survey totalled upto 153 families, belonging mainly to the lower income

group. Out of the 153 families, only four families were pure vegetarians. The interview schedule was developed to include questions on both protein rich plant and animal foods.

3. Method of Survey:

The interview method was adopted in both the urban and rural areas, to elicit the information required. The lady of the house was interviewed, and when she was not available the eldest daughter or the head of the family was interviewed.

4. Results of the Survey:

The results of the urban family survey are given in Table IV.

TABLE IV.

THE COMMON PROTEIN RICH PLANT AND ANIMAL FOODS CONSUMED
BY URBAN FAMILIES WITH THEIR FREQUENCY OF CONSUMPTION

Food	No. of families.	Frequency of use
<u>Quantities over 100 gm. per day:</u>		
1. Red gram dhal	45	Daily
2.	2	Thrice a week
2. Milk	16	Daily
<u>Less than 100 gm. per day:</u>		
3. Black gram dhal	0	Daily
	30	Twice a week
	3	Once a week
4. Amaranth	5	Daily
	30	Once in three days
	5	Once in a week
5. Agathi	0	Daily
	16	Twice a week
	2	Once a week
6. Peas	20	Once a week
7. Coconut	22	Once a day
8. Papad	16	Daily
9. Cucumber	20	Once a week
10. Bengal gram	5	Once a week
11. Green gram	10	Once a month
12. Eggs	0	Daily
13. Mutton	12	Twice a week
	1	Twice a week
	8	Fortnightly
14. Fish	2	Fortnightly
15. Chicken	1	Once a month
16. Field beans	6	Rare
17. Mungani	2	Rare
18. Whole bengal gram	3	Rare

The above Table gives a list of the protein foods consumed by urban families. They are classified according to the quantities consumed, and according to the frequency of consumption.

The results of the urban survey showed that 90 per cent of the families consumed red gram dhal daily and in appreciable amounts. Milk was consumed daily by only 32 per cent of the families, and only in small amounts. Next in order, come black gram dhal which was used twice a week by 64 per cent of the families, and by six per cent weekly once, but the quantities consumed per day was small. Green leafy vegetables were consumed by over sixty per cent of the families twice a week. 'Amaranth' was preferred to 'agathi'. The quantities consumed, though little, add to the nutritive value of their diets, since greens are considered as a store house of nutrients.

Peas were consumed by 40 per cent of the families who used it once a week. Coconut, though consumed daily formed only a small quantity. Papad - a ready made, black gram dhal dish was consumed daily. Among the pulses bengal gram dhal and whole bengal gram were not very popular. They were used only for seasoning, and sometimes prepared for tea. Green gram was taken only by 20 per cent of the families and only once a month. Field beans and Karamani were seldom used. Among the non-vegetarian foods, mutton ranked, first. Out of the 25 non-vegetarian families surveyed, 48 per cent consumed

mutton twice a week, while 3 per cent consumed it fortnightly. The rest were found to be just called ~~non-vegetarians~~ because they did not mind including meat in their diets, which they seldom did.

Fish and chicken were not popular, because they were not available easily, and involved much labour in cleaning.

The families surveyed used more of red gram dhal than other pulses, since it has been a tradition to eat only red gram dhal. It is now eaten without questioning its value as a food. Amaranth was the most popular among leafy vegetables, since it was easily available.

Table V shows the consumption of common plant and ~~non-animal~~ protein foods in a rural area.

TABLE V.

THE PROTEIN RICH PLANT AND ANIMAL FOODS CONSUMED BY RURAL FAMILIES WITH THEIR FREQUENCY OF CONSUMPTION.

Food	No. of families	Frequency.
A. Above 100 sq. m. per day:		
Red gram dhal	106	Thrice a week.
	47	Four times a week
Horse gram	91	Twice a week
	15	Once a week
Mutton	80	Thrice a week
Beef	68	Once a week
B. Less than 100 sq. m. per day:		
Milk	3	Daily
Black gram	8	Once a month

Table V shows that the rural families consumed a limited number of foods, when compared to the urban families. Their diets were simpler, lacked variety and involved less labour. The chief protein rich plant food - red gram dhal, was consumed by all the 153 families. 69 per cent of the families consumed it thrice a week, while the rest consumed it four times a week. Two days during the week, horse gram was consumed. 59 per cent of the families consumed horse gram twice a week, while about 10 per cent consumed it only once a week.

The villagers consumed pulse on all the days during the week, except when they had some non-vegetarian food, which was invariably mutton for the caste Hindus, and beef for the Harijan community. Mutton was consumed twice a week by 52 per cent of the families. Beef was consumed once a week usually on Sundays, by 44 per cent of the families surveyed. Milk was consumed daily in negligible amounts by about 15 per cent of the families. The average milk consumption came up to 17 gram per day per person. —

Thus from the survey conducted in both the urban and rural areas, it can be concluded that the vegetarians derive their main protein from red gram dhal and cereals in urban and rural areas. The sources of protein for non-vegetarians were animal and plant foods in both the areas.

A. Nutritional Study:

1. Foods selected for the Study:

From the surveys conducted, the foods to be included in the biological study for the comparison of protein rich plant and animal foods with special reference to the nutritive value of their proteins, were decided as (a) milk, (b) mutton, (c) red gram dhal, (d) ~~rice and red gram dhal~~ and (e) rice and peas.

a) Milk: ~~was selected as a standard protein~~ standard protein, with which the plant and animal proteins could be compared. Milk proteins have been shown to be excellent

proteins, by Pixsen (1935)¹²⁰, Wilder and Keys (1942)¹²¹, Mitchell (1942)¹²² and Hart (1952)¹⁵⁷. Hart (1952)¹⁵⁸ cited that milk proteins were used as standard proteins in many experiments due to its high nutritive value. Thus in this study, skim milk formed the reference protein.

b) Mutton: Mutton was selected to represent the protein rich animal food in the diet to be studied. From the results of the surveys conducted, it was clear that mutton formed an important animal protein food for the non-vegetarians, and was the most common among all the animal foods available.

c) Red gram dhal: The plant protein food chosen for the study was red gram dhal as the results of the surveys had showed that it was consumed widely.

d) Red gram dhal - milk mixture: The fifth protein was that of a mixture of milk and red gram dhal. Red gram dhal protein has been shown to be a very poor protein, deficient in methionine and tryptophan. It was postulated that the addition of a small amount of a complete protein like milk would improve its quality. Skim milk and red gram dhal were combined in the ratio of 6 : 2.

e) Rice and pulses: The plant proteins chosen for this diet were rice and pulses. These foods were chosen because, it was postulated that by combining rice protein, which forms the bulk of the proteins in a vegetarian diet as

pointed out by Aykroyd (1948)¹, with a legume protein, the nutritive value of the diets of vegetarians could be enhanced.

Since it has been shown earlier, that rice proteins though of good quality lack in lysine, it was combined with peas which have a high content of lysine and therefore can supplement rice. They were combined in the ratio of 4:4.

2. Selection of Experimental Animals:

The study was made on albino rats, which are 'the most widely used laboratory animals', due to their low cost, small space requirement, omnivorous diet, short time span of generations, large litters and the fact that the rats can be standardized easily. (Parris and Griffith, 1949)²³⁶. Moreover, the results obtained from this study can be compared with those of earlier workers who had also used albino rats.

Twenty weaned male rats, 28 days old, belonging to the 'Wistar' strain were used for the study. The animals were divided into five groups, four in each group, and the animals were so grouped that the average weight of all the groups was nearly the same, ranging between 48 and 50 gm.

3. Selection of Instruments:

The rat cages were made of aluminium with raised screen bottom which made the collection of food waste easier. They were rectangular shaped as shown in Figure I with the

FIGURE II METABOLIC CAGE

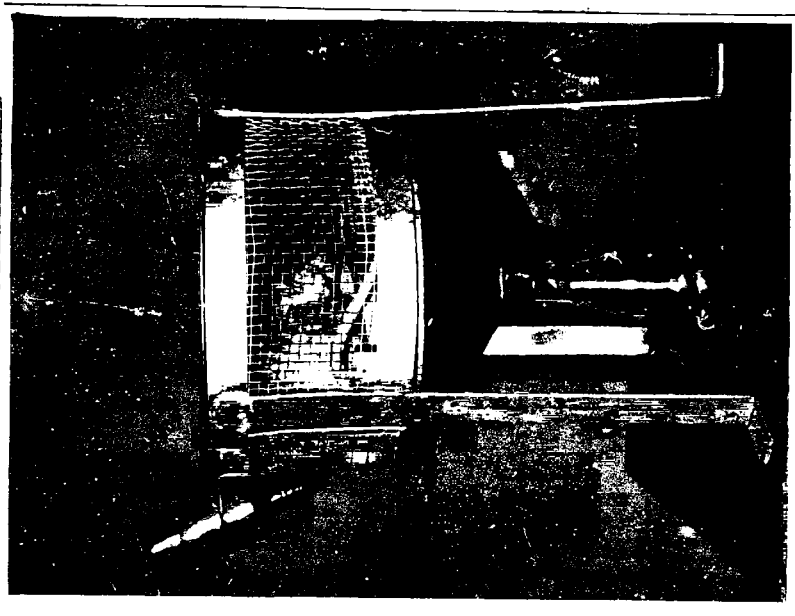


FIGURE III WEIGHING BALANCE.

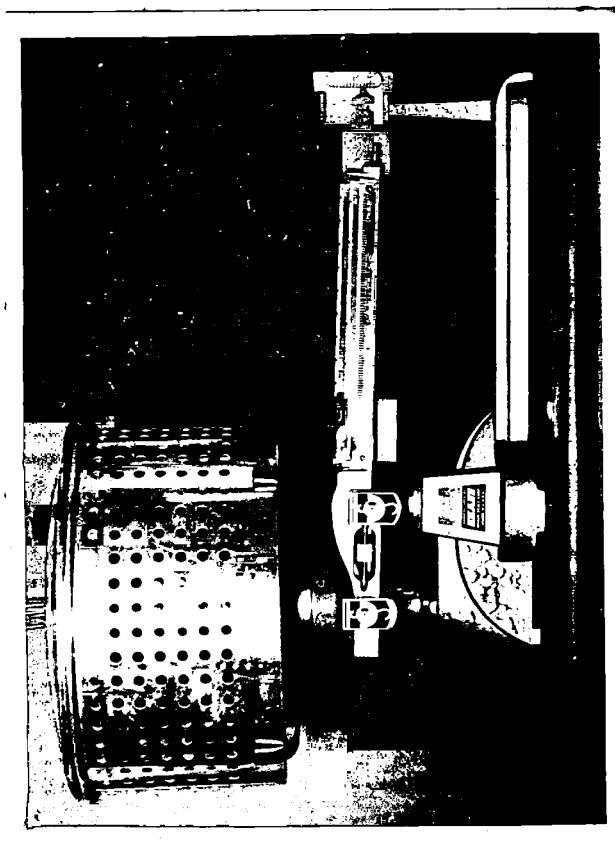


FIGURE I RAT CAGE



following measurement: Length - 12 inches; breadth - 8 inches and height - 9 inches. The cages were provided with cards with details of date of experiment, number of experiment, weight of animals, food consumed and a column for remarks, where the physical changes of rats could be noted.

Food was given in enamel plates, which reduced spilling and scattering. Water was provided from a bottle, fixed to one of the sides of the cage. The bottle was fitted with a tight fitting cork, through which a small bored metal tube passes, and water from the bottle which is in an inverted position when fixed on the wall, flows down when the rat sucks at the open end end of the tube.

The cages were cleaned once in two days with phenyl water and soap. The bottom tray was removed daily, and cleaned well, after the left over food had been collected.

The metabolic cages were specially designed to facilitate proper collection of urine and faeces. They are cylindrical in shape as seen from Figure II with a funnel which is elongated and fits into the neck of a bottle placed beneath the funnel to collect the urine. The animal is placed on the wire screen and is thus separated from the urine.

The food was given in a plate. Any spilling of food was caught up at the neck of funnel, where a filter paper was placed.

In order to weigh the animals and the food, an accurate balance of the 'Ohaus' type with the animal box, (Figure III) graduated upto the first decimal, was used.

The other equipment used were those connected with the nitrogen estimation of faeces and urine. The Macro Kjeldhal method as described by Hawk et al (1954)²³⁷ was adopted. Appendix XI gives the detailed procedure followed in this study.

The Macro Kjeldhal method required the digestion chamber, distillation apparatus and kjeldhal flasks. In order to estimate the nitrogen content of liver, the Micro Kjeldhal method was adopted. Appendix, XI gives the detailed procedure. The moisture content of food and liver were determined, by drying them in a hot air oven.

4. The Composition, Standardization and Preparation of Diets

There were five types of diets to be prepared. The basis of all these diets were the same. That is the basal, protein free diet, and only the protein foods differed. Table VI gives the percentage composition of the Basal diet. (Hippon, 1959)²¹.

TABLE VI.
COMPOSITION OF THE BASAL DIET.

Ingredients	Amount (%)
Starch	73
Vitamin Mixture *	5
Salt Mixture**	4
Codliver oil	1
Sucrose	7
Butter	10

* ~~Vitamin Mixture~~ (mg) (In 100 gm. of Sucrose) (Rippon, 1959)²¹.

Thiamine hydrochloride	- 10	~~Thiamine~~	- 190
Pyridoxine	- 10	Inositol	- 400
Pantothenic acid	- 50	Choline chloride	- 4
Riboflavin (added last) - 10			

** ~~Salt Mixture~~ (gm) (Rippon, 1959)²¹.

Sodium chloride	- 50	Potassium chloride	- 50
Calcium Phosphate	- 400	Sodium acid phosphate (anhydrous)	- 105
Ferric nitrate	- 35	Potassium chloride	- 1.0
Magnesium sulphate	- 80	Manganese sulphate	- 0.2
Sodium Fluoride - 0.04			

The above table, shows that the nitrogen content of the diet is very low, and as pointed out ^{by} Rippon, this diet contains only 0.09 per cent nitrogen.

The ~~composition~~ of the five diets, with the protein free diet as basal, is given in Table VII.

TABLE VII.
COMPOSITION OF THE FIVE DIETS.

Diets	Test food pro- tein. gm.	Starch gm.	Vita- min mix- ture gm.	Salt mixture gm.	Sucrose gm.	Butter gm.	Cod liver oil. gm.
1. Basal diet plus skim milk	21.0	53	5	4	7	10	1
2. Basal diet plus mutton	43.2	30.8	5	4	7	10	1
3. Basal diet plus red gram dhal	35.8	38.2	5	4	7	10	1
4. Basal diet plus red gram dhal - milk mixture.	R.G.dhal 26.8 Skim milk 5.2	42	5	4	7	10	1
5. Basal diet plus rice - pea mix- ture.	Rice 58.0 Pea 22.0	0	5	4	7	10	1

a. Basal diet plus skim milk:

The diet containing skim milk was prepared thus:
The weighed amount of skim milk was taken in its dry state and
mixed with the synthetic diet and fed to rats.

b. Basal diet plus mutton:

Any fat or bones present in the minced meat was
removed. It was then steamed in an 'Idli' vessel till it was

cooked, and mixed with starch. The vitamins and other nutrients were then added. The whole diet was mixed well. Mutton was obtained once in three days and the protein content was obtained from the values given in the Health Bulletin No. 23 (1962)²³⁸.

c. Basal diet plus red gram dhal:

The red gram dhal was obtained in a bulk locally and powdered. In order to cook it, the weighed amount of dhal was mixed with just enough water to make it a stiff paste, and then steamed in an 'Idli' vessel for five minutes. The nitrogen content of the dhal was calculated from the figures given in the Health Bulletin No. 23 (1962)²³⁸.

d. Basal diet plus red gram dhal and skim milk:

The fourth diet was that of red gram dhal and skim milk in the ratio of 6:2. Red gram dhal was cooked as described above, and mixed thoroughly with the skim milk powder. This was then mixed with the synthetic diet.

e. Basal diet plus rice and peas:

The fifth diet was that containing a mixture of rice and peas in ratio of 4:4. The rice was bought in bulk locally and pulverised into a coarse powder. The requisite amount was taken and mixed with just enough water to make a smooth paste. This dough was steamed for 5 minutes, in an 'idli' steamer. The peas were also bought locally, in bulk. It was pulverised into a coarse powder, and cooked in water. These two were

mixed together thoroughly and then added to the synthetic diet.

5. Feeding:

The diets were prepared in bulk containing eight per cent of the test protein, which also formed the sole source of protein in the diet, since the basal diet contains only 0.09 per cent of nitrogen, according to Rippon. The diets were given ad. lib. to the animals. In order to ensure that the animals did receive ad. lib. feeding, an adequate amount was given so that at least ten grams of food, is left over on the next day. Every morning the food left over was weighed, and the amount of food consumed was calculated.

6. Length of the Test Period:

The total length of the experimental period lasted for 64 days. These 64 days were actually made of 4 periods of 16 days each, and the criteria used were studied during these four periods. This method of study was adopted from that of Frost and Sandy (1949)¹⁹⁴ and later used by Frier and Burns (1957)¹⁸⁶ and Rippon (1959)²¹, who in a depletion - repletion modification of the growth method, used initial depletion for seven days, then a five day test period followed by two days on stock diet, and then 7 days on a ~~protein deficient diet~~, before another 5 day test period and a ~~repletion of the system~~. In this method the same animals were used repeatedly, according to the scheme outlined above. In this study a slight modification was made by the investigator who extended the 5 day test

protein period, as it enabled the nitrogen balance study to be conducted, for a week.

7. Methods Employed:

a. P.E.R.: The P.E.R. was first used by Osborne and Mendel (1919)¹⁵ to evaluate the nutritive value of proteins, based on the ability of the protein to promote growth. This was later modified, and improved by many scientists, such as Summation (1937)¹⁴. The P.E.R. was calculated for a week, and obtained four times throughout the whole study. The rats were first depleted for a week by feeding them with the protein deficient, synthetic diet and water only. The repletion was then started by feeding the animals the test diet ad. lib. which lasted for a week. The weights of the animals were taken at the beginning and end of the week of repletion period. Thus the increase in weight during the week of repletion, is due to the proteins given, since the basal diet contains no proteins. This is followed by two days on stock diet which brings back the animal to equilibrium, according to Rippon. Thus the animal is subjected to depletion once again and the cycle is repeated.

The P.E.R. was calculated as follows:-

Weight increase of experimental animals (gm) over a suitable period of time.

Weight of protein consumed (gm).

b. Nitrogen Balance: The nitrogen balance studies were carried out according to the method of Mitchell, modified by Swaminathan (1937)²⁰¹. During the periods when nitrogen balances were determined, the animals were placed in metabolism cages with a mesh bottom supported by aluminium funnels. Under the funnels glass conical flasks containing ~~sufficient~~ sulphuric acid to acidify the urine samples were placed. The fine mesh screen over the top of the funnel retained the faeces, which were collected daily and stored. Sulphuric acid was not added, for Womack (1953)²³⁹ found that there was no loss in nitrogen when the faeces were stored without sulphuric acid. Several times during the period, the cages and funnels were washed down with a fine stream of hot dilute sulphuric acid (0.1N) solution. This procedure was again employed at the end of the period when the animals were transferred to clean cages. The used cages were washed in a similar manner, and all the washings were added to the urine collection. At the end of the period, the combined urine samples and washings for each animal was filtered. The filter paper was washed thoroughly, and the filtrate and washings diluted to a constant volume that is 250 ml.

The faeces for each animal for each four day period were dried separately, powdered and mixed well. Aliquots were weighed and the nitrogen content estimated.

The nitrogen was estimated in the food, faeces and

urine by the Macro Kjeldhal method (Appendix X).

The nitrogen balance study was carried out twice during the whole experimental period, once when the animals were 40 days old and the second when they were 72 days old.

The collection of faeces and urine was made, during the last four days of the repletion period, allowing the animal to get acclimatized to the diet during the first three days. From the results of the analysis for nitrogen of faeces, urine and food, the following were calculated. (Bender, 1958)¹⁹¹.

$$\begin{aligned}
 1. \text{ True digestibility} &= \frac{\text{Absorbed Nitrogen}}{\text{Food Nitrogen}} \times 100 \\
 &= \frac{\text{Food Nitrogen} - \text{Faecal Nitrogen}}{\text{Food Nitrogen}} \times 100 \\
 2. \text{ Net protein utilization} &= \frac{\text{Retained Nitrogen}}{\text{Food Nitrogen}} \times 100 \\
 &= \frac{\text{Food Nitrogen} - (\text{Faecal Nitrogen} + \text{Urine Nitrogen})}{\text{Food Nitrogen}} \times 100
 \end{aligned}$$

The fourth criteria by which the proteins were evaluated, was by studying the regenerative ability of the proteins to regenerate haemoglobin and red blood corpuscles in the rats.

The animals were depleted of proteins for a week, and then repleted for a week with the test proteins. The

haemoglobin content and red blood corpuscles count were determined at the end only, so that the ~~comparative~~ ability of the various proteins to regenerate haemoglobin and red blood corpuscles could be studied.

The blood was drawn from the tail of the rats, as in the method described ~~Griffith et al~~ (1949)²⁴⁰.

Procedure: -

The rat was held securely by an assistant, wrapped up in a towel without squeezing its chest, leaving only the tail protruding. The tip of the tail was dipped in warm water and rubbed with a saturated solution of Sodium citrate, in order to increase the flow of blood to the tail, and prevent clotting. The tip was amputated quickly, and the blood was allowed to flow freely. The blood was taken for the ~~enumeration~~ of red blood cells, by the ~~Wintrobe's~~ ~~Technique~~, and for the haemoglobin estimation by the method as described by Hepler (1958)²⁴¹. (Appendix X)

~~Preparation of total liver extract:~~

The liver nitrogen estimation was adopted from that followed at the Central Food Technological Research Institute (Joseph, (1957)¹⁷⁵, Korula (1959)²⁰⁸).

The animals were anaesthetised with chloroform, at the end of the fourth repletion period. They were bled through

the abdominal aorta to ensure a minimum and relatively uniform amount of residual blood in the liver. The whole liver was quickly excised, washed with normal saline, wiped between folds of filter paper and weighed accurately to the fourth decimal. The moisture was determined by drying at $90^{\circ} - 95^{\circ} \text{C}$ in a hot air oven, to constant weight. The dried liver was powdered and aliquots were taken for total nitrogen estimation. The total nitrogen was determined by the micro kjeldhal method. (Appendix XI.)

IV. RESULTS AND DISCUSSION.

The nutritive value of the proteins of plant and animal foods were studied. The foods studied were, milk, mutton, red gram dhal, rice-pea and red gram dhal-soya mixtures.

The results of the various methods adopted, to study their nutritive value are given below:-

Protein Efficiency Ratio

The protein efficiency ratio, was calculated from the protein intake and gain in weight. Table VIII gives the value of protein efficiency ratios obtained over four periods of repletion, along with an average for the whole period of study. The protein intake and increase in weight are also given in Table VIII. Table IX gives the statistical treatment of this data for significance.

TABLE VIII.

THE PROTEIN EFFICIENCY RATIOS OF FIVE PROTEINS OBTAINED
IN FOUR PERIODS OF REPLETION.

Proteins	No. of rats.	1st repletion			2nd repletion			3rd repletion			4th repletion			Average PER. for four weeks.
		weight in-crease	Pro-tein in-jected	P.E.R.	weight in-crease	Pro-tein in-jected.	P.E.R.	weight in-crease.	Pro-tein in-jected.	P.E.R.	weight in-crease	Protein in-jected	P.E.R.	
Skin milk	4	14.80	4.72	3.08	24.60	9.12	2.89	17.5	7.2	2.43	13.6	6.31	2.12	2.60
Hutton	4	8.90	4.55	1.97	12.90	6.59	1.86	14.5	8.15	1.78	16.6	8.94	1.68	1.82
Red gram dal	4	4.80	6.87	0.71	3.20 ^f	5.49 ^f	0.62 ^f	5.8 ^f	8.86 ^f	0.98 ^f	7.5 ^f	13.4 ^f	0.43 ^f	0.58
Red gram dal plus milk.	4	8.66	4.98	1.74	14.50	8.41	1.63	16.2	10.3	1.58	14.0	9.43	1.45	1.60
Rice plus peas	4	14.20	5.89	2.42	16.20	7.97	2.04	21.2	12.10	1.82	13.80	8.60	1.59	1.98

N.B: ^f Average for 3 rats, as one animal lost weight in the second period and third period and died during the fourth repletion period.

TABLE IX.

RESULTS OF TESTS FOR SIGNIFICANCE BETWEEN DIETS FOR THE PROTEIN EFFICIENCY RATIOS OF FIVE PROTEINS, OBTAINED IN FOUR WEEKS OF REPLETION

Comparison of diets	Mean differences in four periods of repletion.				Average
	1	2	3	4	
Milk ~ Mutton	1.11**	1.03**	0.65**	0.44**	0.78**
Milk ~ Red gram dhal	2.37**	2.27**	1.85**	1.69**	2.02**
Milk ~ Red gram dhal - milk mixture.	1.34**	1.26**	0.85**	0.67**	1.00**
Milk ~ Rice - pea mixture.	0.66**	0.85**	0.61**	0.57**	0.62**
Mutton ~ Red gram dhal	1.26**	1.27**	1.20**	1.25**	1.24**
Mutton ~ Red gram dhal - milk mixture.	0.23**	0.23**	0.20**	0.23**	0.22**
Mutton Rice pea Rice pea mixture ~ mutton.	0.45**	0.18**	0.04 (N.S)	0.09 (N.S)	0.16*
Red gram dhal - Milk mixture ~ Red gram dhal.	1.03**	1.01**	1.00**	1.02**	1.02**
Rice - Pea mixture ~ Red gram.	1.71**	1.42**	1.24**	1.16**	1.40**
Rice - Pea mixture ~ Red gram dhal - milk mixture.	0.68**	0.41**	0.24**	0.14*	0.38**

* Means that the difference between the diets is significant beyond 5 per cent level.

** Means that the difference between the diets is highly significant, beyond 1 per cent level.

N.S: Means that the difference is not significant.

THE EFFECT OF AGE ON PROTEIN EFFICIENCY

RATIO OF THE FIVE PROTEINS

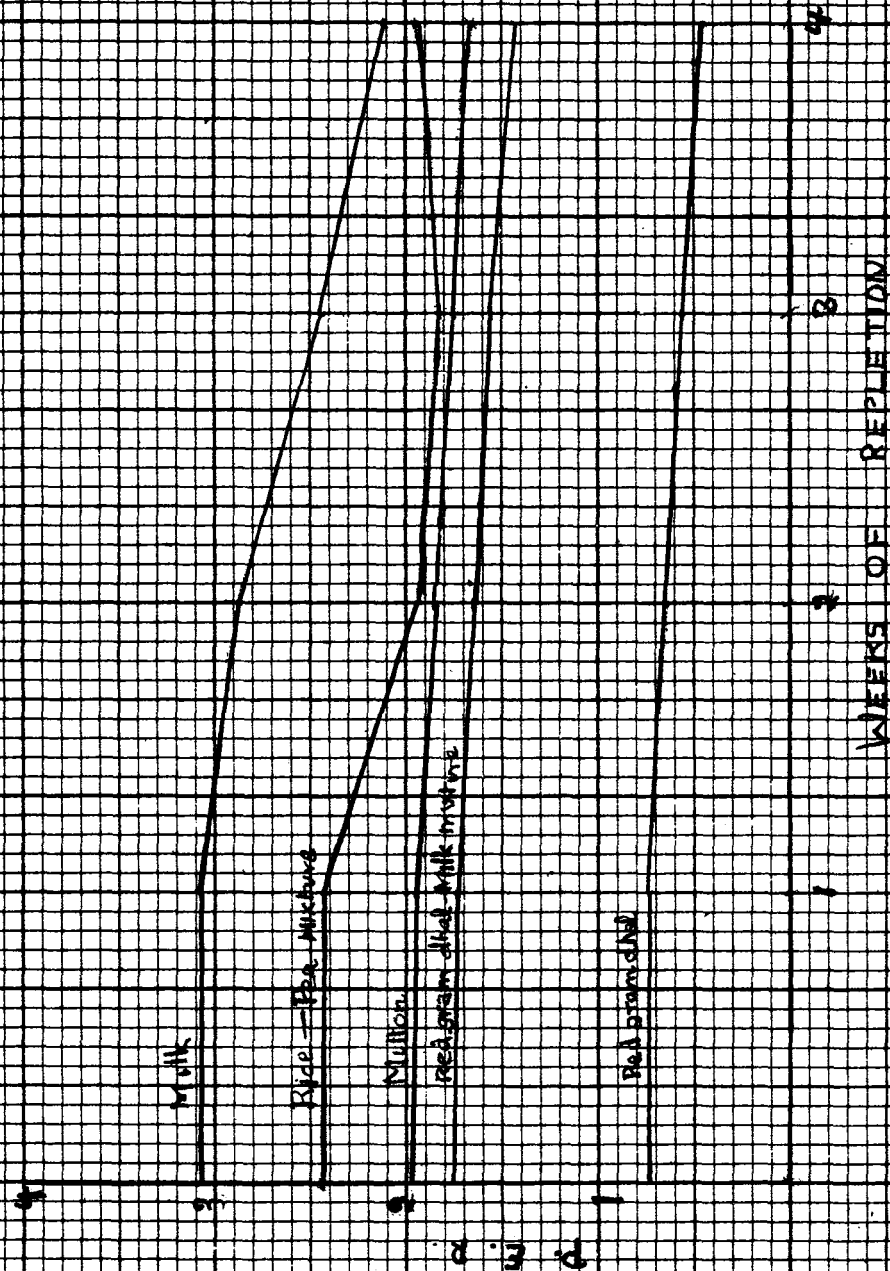


FIGURE IV

The results show that during the first period of repletion (that is when the animals were 35 days old,) the Protein Efficiency ratio obtained for the five proteins were higher than those obtained during subsequent trials Figure IV. This is because, younger animals grow at a faster rate, and therefore, the increase in weight in that period is comparative, greater, for the amount of protein ingested, than in other periods. The statistical treatment of the differences in P.E.R. are given below in Appendix V.

1. Skim Milk: The protein efficiency ratio of milk proteins is the highest in all periods of repletion, thereby showing that milk protein is the best among the five proteins studied for growth and maintenance as seen from the average P.E.R. shown in the last column of Table VIII and Table IX. The P.E.R. of milk proteins is significantly better than the other proteins throughout the period. Earlier workers, cited in the review, such as Mc Collum ¹⁴¹ et al (1919) Swaminathan (1937) ²⁰¹, Hart (1951) ¹⁵⁸ and others have shown milk proteins to be the best for the growth and maintenance. They have used milk proteins as a standard protein for comparison with other proteins, due to its high nutritive value. The Protein Efficiency Ratio of skim milk in the first period of repletion is 3.08, and it decreases to 2.12 in the fourth repletion period as shown in Figure IV. The average protein efficiency ratio for the four trials is 2.63 which compares favourably with those obtained by other workers.

~~2. Repletion~~ The P.E.R. of mutton during the first period of repletion was 1.97 and ~~decreased~~ to 1.68 in the fourth repletion and the ~~difference~~ was highly ~~significant~~ when the ability ~~of~~ to utilize food is less as it is older. The average P.E.R. of the four periods was 1.82. Meats are always considered a good source of protein, as indicated by Mitchell (1927)¹¹⁷ and the study of Mack et al (1947)^{97,98,99.} But in this study, they were ~~significantly~~ inferior to skin milk ^{and} to the vegetable protein mixture of rice and peas, but was very superior to red gram dhal protein and red gram- milk mixture, throughout the period of study except during the third and fourth weeks, when milk was almost level with rice-pea mixture.

~~3. Red gram dhal~~ The red gram dhal protein is of the a very poor quality as it was ~~significantly~~ inferior to all other proteins during the entire period of study, as seen from the values of P.E.R. in Table VIII. The growth of the rats was very poor, and moreover one animal lost weight during the second and third repletion period and died during the fourth repletion period. Thus, from these values, it can be stated that red gram dhal protein is not very ~~efficient~~ in promoting growth in albino rats. ~~Swaminathan~~ (1937)²⁰¹ had also met with similar results, for in his study all the three albino rats died when fed on red gram dhal. ⁹³ ~~Vijayalakshmi and~~ Srinivasan (1953)¹⁰⁰, Esh and Som (1954)¹¹ and ~~Engelmann~~ (1958) have shown that red gram dhal lacks

~~methionine~~ and ~~tryptophan~~. Bah has shown that valine is deficient. All these facts substantiate the reasons for the failure in growth on such deficient diet. The amount of proteins consumed by the animals is not very small, for the average intake for the first repletion period was 6.8 gram and in the fourth week it was as much as 13.8 grams. But in spite of this high intake the growth was poor, thus pointing out the inefficiency of the protein in promoting growth due to its poor amino acid composition.

4. ~~Red gram dhal - milk mixture~~: In order to improve the low nutritive value of the red gram dhal, which is consumed commonly as an important source of protein, the investigator substituted 3 grams of the ~~10~~ grams of red gram dhal, with skim milk in 100 grams of the diet. Thus the animals received six per cent of red gram protein, and two per cent of skim milk protein. The P.E.R. of this mixture of proteins was very significantly superior to that of red gram alone, in all the four periods of repletion. The protein efficiency ratio was raised from 0.71 (that of red gram dhal in the first period.) to 1.74 (that of red gram dhal - milk mixture in the first period.) Milk as it has been pointed out earlier, is a complete protein and has an ideal amino acid composition. When milk is added in a very small quantity to the red gram dhal, the resulting nutritive value was far superior to that of the red gram dhal alone. This superiority was a obvious throughout the entire period of study.

~~5. Rice-pea mixture~~ The second best protein is that of the combination of rice and peas. Rice and peas were combined in the ratio of 4:4, to provide 18 per cent protein diet. This proportion has proved to be of good nutritive value as seen from the value of Protein Efficiency Ratio, but it is very significantly inferior to that of milk.

²⁰¹
~~Summation~~ (1937) had showed that the P.E.R. of rice fed at 5 per cent level for eight and four weeks were 1.70 and 2.17 respectively. In this study it has been shown that the protein Efficiency Ratio, of the rice and pea combination is 1.98, over four repletion periods, thereby pointing out the superior quality of the combination. Rice protein is deficient in lysine, and rich in methionine as pointed out earlier while peas in the review contain lysine which enables it to supplement the rice protein. Thus a mutual supplementation between the proteins of peas and rice have resulted in a good quality protein.

The Protein Efficiency Ratio of rice-pea protein was found to be very significantly superior to mutton protein during the first and second periods of repletion, while there was no significant difference in the subsequent periods. But considering the average for the whole period, the difference was significant, thus showing that the rice-pea protein was superior to an animal protein. The combination of rice-pea protein and red gram dhal was highly significant statistically in all the

COMPARISON OF THE AVERAGE PROTEIN EFFICIENCY RATIOS OF THE FIVE PROTEINS

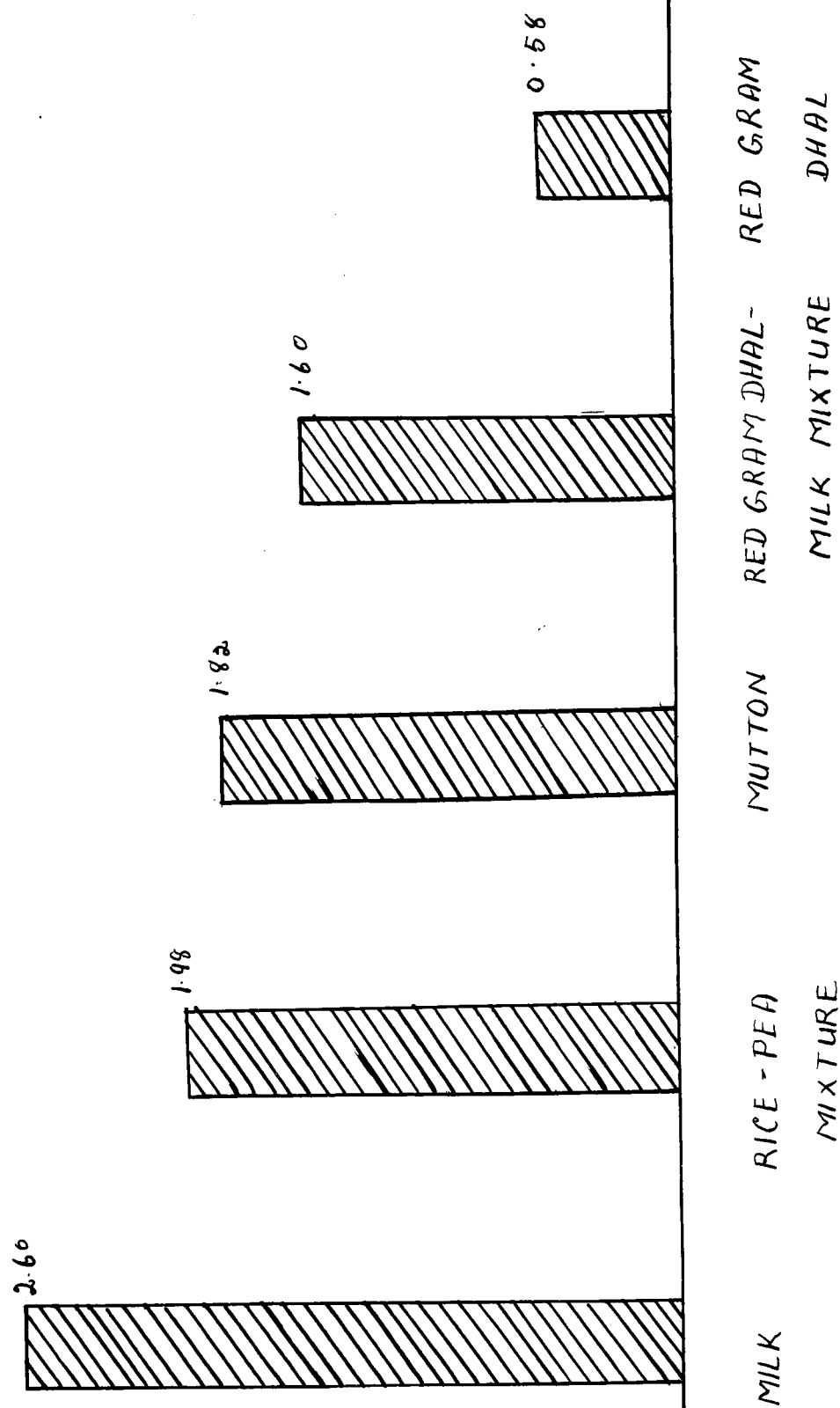


FIGURE V

TABLE X.

RESULTS OF TEST FOR SIGNIFICANCE BETWEEN WEEKS,
FOR THE PROTEIN EFFICIENCY RATIO OF FIVE PROTEINS,
OBTAINED IN FOUR PERIODS OF REFECTION.

Comparison of periods of reple- tion.	Milk	Mutton	Red gram dhal	Red gram dhal - milk mix- ture	Rice - Pea mixture
1st ~ 2nd	0.19**	0.11 N.S	0.09 N.S	0.09 N.S	0.38**
1st ~ 3rd	0.65**	0.19**	0.13*	0.16*	0.60**
1st ~ 4th	0.96**	0.29**	0.28**	0.29**	0.83**
2nd ~ 3rd	0.46**	0.06 N.S	0.04 N.S	0.05 N.S	0.22**
2nd ~ 4th	0.77**	0.16*	0.19**	0.18**	0.45**
3rd ~ 4th	0.31**	0.10 N.S	0.15*	0.13*	0.23**

** Mean that the difference between the weeks is highly significant beyond 1 per cent level.

* Means that the difference is significant beyond 5 per cent level.

N.S: Means that the difference is not significant.

TABLE X.

RESULTS OF TEST FOR SIGNIFICANT DIFFERENCES
FOR THE PROTEIN NUTRITION RATIO OF FIVE PROTEINS
OBTAINED IN FOUR PERIODS OF REPRODUCTION.

Comparison of periods of reprod- uction	Milk	Wheat	Barley	Red clover - hay	Alfalfa - hay
1st - 2nd	0.19**	0.11 N.S.	0.09 N.S.	0.09 N.S.	0.35**
1st - 3rd	0.09**	0.19**	0.17*	0.16*	0.60**
1st - 4th	0.09**	0.29**	0.28**	0.29**	0.87**
2nd - 3rd	0.46**	0.06 N.S.	0.04 N.S.	0.05 N.S.	0.35**
2nd - 4th	0.77**	0.16*	0.19**	0.18**	0.46**
3rd - 4th	0.71**	0.10 N.S.	0.15*	0.17*	0.52**

** Means that the difference between the means is highly significant beyond 1 per cent level.

* Means that the difference is significant beyond 5 per cent level.

N.S.: Means that the difference is not significant.

four repletion periods. Rice-pea protein was also very significantly superior to red gram ~~and~~-milk protein combination in the first three periods while the ~~difference~~ was significant in the fourth period.

Thus, by combining rice and peas, a high quality protein is obtained, which though inferior to milk, is significantly superior to other proteins studied.

Figure V shows the ~~difference~~ in growth promoting quality of the five proteins as measured by the P.E.R of the proteins, and as shown above, milk ranks first.

The ~~difference~~ between the P.E.R. between weeks was also studied. Table X points out that the maximum utilization of all proteins was during the first repletion period, when the animals were very young. The P.E.R of milk proteins in the first repletion was significantly higher than in the subsequent weeks. Rice and Peas mixture was also utilized at a maximum in the first repletion period, and it is significantly higher than in later periods. In the case of mutton, red gram and red gram plus milk, the ~~difference~~ between the first and second weeks was not significant, but the ~~difference~~ between the first and other weeks was significant. This again points out the fact that younger animals retain more nitrogen, and ~~utilize the protein better than older animals.~~

2. Nitrogen Balance.

1. Digestibility Coefficient:

Table XI gives the digestibility coefficient of five proteins. Table XII gives the result of statistical analysis.

EXPERIMENTAL RESULTS

1. Effect of Temperature

Table I gives the effect of temperature on the rate of reaction. The rate increases with increasing temperature. Table II gives the effect of concentration on the rate of reaction. The rate increases with increasing concentration. Table III gives the effect of catalyst on the rate of reaction. The rate increases with increasing catalyst.

TABLE XI.

THE DIGESTIBILITY, EFFICIENCY AND PERCENTAGE OF NITROGEN UTILIZATION OF FIVE PROTEINS IN TWO PERIODS OF REFINITION.

Proteins	No. of rats	Average initial weight of rats	Average final weight of rats	Average amount of nitrogen diet consumed.	Average amount of nitrogen excreted in urine	Average amount of nitrogen in faeces	Percentage of nitrogen utilisation	Digestibility co-efficient.
Milk	1st Period 4	43.4	58.2	0.171	0.0056	0.0025	94.40	98.54
	2nd Period 4	49.4	74.0	0.233	0.0148	0.0072	90.80	96.90
Mutton	1st Period 4	41.4	50.3	0.125	0.0099	0.0060	89.10	95.19
	2nd Period 4	43.0	57.5	0.199	0.0168	0.0125	85.80	93.71
Red gram dhal	1st Period 4	45.6	50.4	0.176	0.0137	0.0328	74.2	83.77
	2nd Period 4	48.1	53.9	0.278	0.0101	0.0301	80.70	89.19
Red gram dhal - 1st Period 4	41.4	50.1	0.144	0.0142	0.0067		85.06	95.35
Red gram dhal - 2nd Period 4	45.4	61.6	0.265	0.0189	0.0143		84.80	94.61
Rice - 1st Period 4	44.3	58.5	0.159	0.0050	0.0050		75.60	84.28
Rice - 2nd Period 4	48.1	69.3	0.310	0.0120	0.0192		89.90	93.80

TABLE XII.

MEAN DIFFERENCES IN DEMONSTRATED CO-EFFICIENT OF
THE FIVE PROTEINS OVER THE TWO PERIODS
OF RESEARCH.

Comparison of diet	1st Period	2nd Period
Milk Mutton	3.4 N.S	3.2 N.S
Milk Red gram dhal	14.8**	7.8**
Milk Red gram dhal - milk mixture	3.2 N.S	2.3 N.S
Milk Rice - pea mixture	14.3**	3.1 N.S
Mutton Red gram dhal	11.4**	4.6*
Mutton Red gram dhal - milk mixture	0.2 N.S	1.2 N.S
Mutton Rice - pea mixture	10.0**	0.1 N.S
Red gram dhal - Milk mixture Red gram dhal	11.6**	5.5*
Rice - Pea mixture Red gram dhal	0.5 N.S	4.7*
Rice - Pea mixture Red gram dhal - Milk mixture	11.1**	0.8 N.S

** Means that the difference between the diets is highly significant beyond 1 % level.

* Means that the difference is significant beyond 5 % level.

N.S: Means that the difference is not significant.

Milk was found to be significantly better digested than red gram dhal, or rice-pea mixture in the younger animals, (first balance) while there was no significant difference in the digestibility when compared to both mutton or red gram dhal-milk mixture. This shows that the addition of a small amount of milk can enhance the nutritive value of a poor protein like that of red gram dhal. In older animals, the digestibility of rice-pea mixture improved significantly and when compared to milk, there was no significant difference between the two proteins in the older animals. Red gram had a poorer digestibility coefficient than milk, ^{and} mutton and red gram dhal-milk mixture, but there was no significant difference between this mixture and rice-pea mixture (in the first balance). There was no significant difference between mutton and red gram dhal-milk mixture while the difference between mutton and red gram dhal alone is highly significant. This finding once again points out the enhancement of a protein of inferior quality by the addition of a small amount of milk.

Regarding the rice-pea mixture, the digestibility coefficient was found to be as poor as that of red gram dhal and there was no significant difference between the two proteins in the first balance, while the vegetable protein mixture improved significantly in the second balance study.

The digestibility of a protein depends on its fibre content as pointed out by Kamath and Schonitz (1959)¹⁵² and

TABLE XIII.

MEAN DIFFERENCE IN NITROGEN RETENTION OF FIVE PROTEINS
DURING THE TWO PERIODS OF EXPERIMENT.

Comparison of diets	1st period mean difference	2nd period mean difference
Milk - Mutton	5.3*	5.0*
Milk Red gram dhal	20.2**	10.1**
Milk Red gram dhal - milk mixture	9.4**	6.0**
Milk Rice - pea mixture	18.8**	0.9 N.S
Mutton Red gram dhal	14.9**	5.1*
Mutton Red gram dhal - milk mixture	4.1*	1.0 N.S
Mutton Rice - pea mixture	13.5**	4.1*
Red gram dhal - milk mixture Red gram dhal	10.8**	4.1*
Rice - Pea mixture Red gram dhal	3.4 N.S	9.2**
Red gram dhal - Milk mixture Rice pea mixture	9.4**	5.1*

** Means that the difference between the diets is highly significant beyond 1% level.

* Means that the difference is significant beyond 5% level.

N.S. Means that the difference is not significant.

therefore the lower digestibility coefficient of red gram dhal and rice and peas mixture can be accounted for, for both being plant protein foods, contain a higher amount of protein than animal protein foods.

The ~~digestibility~~ coefficient of the five proteins was calculated from ^{the} data presented (Appendices VI and VII).

2. Nitrogen Utilization:

Table XI gives the percentages of nitrogen utilization in the five proteins by albino rats. The nitrogen utilization from milk was found to be very significantly superior to the red gram dhal proteins and red gram -milk mixture in both the periods of study. When compared to mutton, milk is very significantly superior in the first balance study while it decreases slightly in the second period. But when compared to rice - peas mixture, it is significantly superior only in the younger animals, while the plant protein mixture has improved significantly (Table XII) in the second period so as to almost equal the nutritive value of milk.

Mutton which is advocated often as a good quality protein has been shown in this study also to have a high percentage of utilisable nitrogen. In the second balance study, rice-peas mixture was found to be utilized significantly better than mutton thereby showing that vegetable protein mixture could also be of high nutritive value.

TABLE XIV.

RESULT OF THE TEST FOR SIGNIFICANCE
BETWEEN WEEKS, FOR NITROGEN RETENTION OF
FIVE PRISONERS.

Diets	Mean difference
Milk	3.6 N.S
Mutton	3.3 N.S
Red gram dhal	6.5**
Red gram dhal - milk mixture	0.2 N.S
Rice - pea	14.2**

** Mean that the difference is highly significant beyond 1 % level.

N.S: Means that the difference is not significant.

Red gram dhal has proved to be inferior to milk and mutton, but when a little skim milk was added to the red gram dhal, its nutritive value was improved very significantly. Rice - pea mixture proved to be rather inferior to the other proteins, but in older animals, the utilization was significantly better.

The difference between the utilization of the nitrogen during two different periods was studied and the results are given in Table XIV. The results show that in the case of milk, mutton and red gram dhal -milk mixture, there is no significant difference between the two weeks, while both red gram dhal and rice - pea mixture have been significantly better utilized in the second week. Thus both the plant proteins were utilized appreciably in the older animals, while both the animal proteins have been less well utilized by the older animals. In both the digestibility coefficient and nitrogen utilization, the statistical analysis, as given in Appendices VIII and IX, was performed by the method outlined by Snedecor (1956)²⁴¹.

5. Nutritional Assessment of the Animals

Proteins can be judged for their nutritive value by many methods, and one of them is the changes which take place in the physical conditions of the experimental animals.

Albino rats are used as experimental animals, because

within a short period, all the changes which take place in a growing animal can be observed. Thus, any deficiency in the diet, fed to the albino rats can be observed on them easily. In the present study, the albino rats were observed weekly, for any changes in the coat, eyes or tail, for these areas are the parts most easily affected by any deficiency in the diet.

Figures VI - IX show the differences between the rats fed on milk protein (which had been used as the standard protein, due to its excellent amino acid make up) and the rats fed on the other proteins namely, mutton, red gram dhal, rice - peas mixture and red gram dhal - milk mixture. The weekly observations made on the animals are shown in Appendix X.

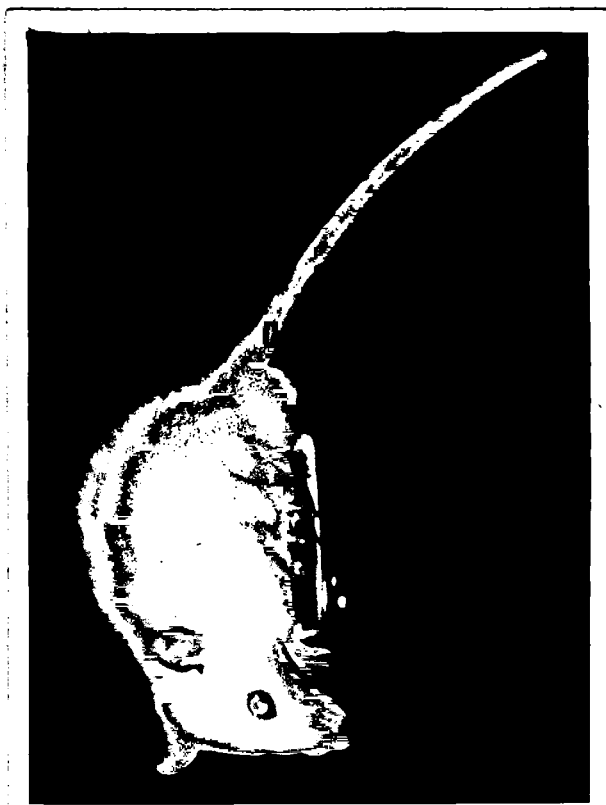
The photographs were taken at the end of the fourth repletion period.

Size of the Animals

The animal fed on milk is definitely the healthiest among all the five animals shown.

On comparing each animal with the animal fed on milk protein, it can be seen that the greatest difference is between milk protein and red gram dhal protein. Red gram dhal being very poor in quality could not promote good growth, in the animals, and therefore the rat in Figure VII looks very small. The rat fed on rice - peas protein, looks almost as

COMPARISON OF RATS FED ON TEST PROTEINS.



MILK



FIGURE VI MUTTON



FIGURE VIII RED GRAM DHAL
MILK MIXTURE



FIGURE VII RED GRAM DHAL.



FIGURE IX RICE - PEA
MIXTURE.

big as the animal fed on milk protein, but the other animals appear smaller.

Coat: The coats of the animals were examined carefully every week, for any changes in texture, colour or for signs of dropping hair.

The rat fed on milk diet had a white soft coat, with no signs of bald spots, while, the rat fed on the other proteins had a coat which showed some signs of ~~malnutrition~~. The animal fed on meat, had a white coat, but the hair started dropping during the fourth repletion period but no bald spots were noticeable. The rat fed on red gram dha, had a slightly yellowish coat, and the hair around the neck started thinning down, during the third repletion period. The texture of the hair also changed, as the softness decreased.

The rat fed with red gram dhal milk mixture, even though not very ~~malnourished~~, did show some changes in the coat. The hair under the chin, was thin and slightly ~~as~~ yellowish.

Tail: The ~~condition~~ of the tail in the rat fed on milk was taken to be the best. It was smooth and shining, with no signs of ~~neurosis or depigmentation~~. Comparing the condition of the tail of the milk protein rat with the other albino rats, there was not much of a ~~difference~~. The tails of the rats fed on red gram dha, red gram dhal-milk mixture and the mutton showed a few hyperpigmented spots.

The tips of the tails of all the rats in the pictures are cut, because these pictures were taken after the blood of these rats were drawn from the tail for ~~hemoglobin~~ and red blood corpuscle estimations.

Eyes: The eye is another part of the animal which is affected by any severe deficiencies in the diet. Vitamin A in the diets, fed to the animals, were adequate to prevent any Vitamin A deficiencies in the rats. The eye changes in these animals therefore, are due to the low nutritive value of the proteins, rather than vitamin A deficiency. Earlier, in the review of the literature, it has been cited that vitamin A metabolism is related to the protein in the diet. Protein is necessary for the proper utilization of vitamin A and therefore when the protein ingested is not of a high quality the Vitamin A in the diet is not utilized, and as a result, vitamin deficiency symptoms appear.

Figure VII shows the eye of the rat on red gram dhal to be slightly inflamed, and changed in shape. The corner of the eye was inflamed and red and the eyes watered constantly. The eyes of the other animals in the pictures do not show any signs of inflammation but are just as sparkling as that fed on milk diet. The symptoms once again point out the low nutritive value of the red gram dhal.

Other symptoms: It was also observed that the animals fed on red gram dhal became very timid and very frightened when it

the slightest noise. They were irritable and nervous. Towards the end of the third repletion period, they started dragging their hind feet, and exhibited signs of extreme weakness. These signs became worse in the subsequent weeks. The red gran was not tolerated very well, for the faecal material was loose and watery.

B. Haemoglobin and Red Blood Corpuscle Regeneration.

Tables XV and XVI give the results of the haemoglobin content and red blood cell count of the blood of albino rats respectively, fed with the five test proteins.

Appendices XIII and XIV show the method adopted for the determination of 'P' value. These values were calculated from the data given in Appendix XII.

TABLE XV.

COMPARATIVE NUTRITION OF ~~HEAVYWEIGHT~~ BY ALBINO RATS FED WITH THE FIVE PROTEINS.

Proteins	No. of animals	Haemoglobin gram/100 ml.
Milk	4	10.9
Mutton	4	10.5
Red gram dhal	3	5.9
Red gram dhal - milk mixture	4	8.6
Rice pea mixture	4	9.3

Comparison of diets	Mean difference
Milk Mutton	0.4 N.S
Milk Red gram dhal	5.4*
Milk Red gram dhal - milk mixture	2.3 N.S
Milk Rice pea mixture	1.6 N.S
Mutton Red gram dhal	4.6*
Mutton Red gram dhal - milk mixture	1.9 N.S
Mutton Rice pea mixture	0.8 N.S
Red gram dhal - Milk mixture Red gram dhal	2.7 N.S
Rice pea mixture Red gram dhal	3.4 N.S
Rice pea mixture Red gram dhal - milk mixture	0.7 N.S

* Means that the difference between diets is significant beyond 5 per cent level.

N.S: Means that the difference is not significant.

COMPARITIVE REGENERATION OF HAEMOGLOBIN IN RATS FED ON THE FIVE PROTEINS

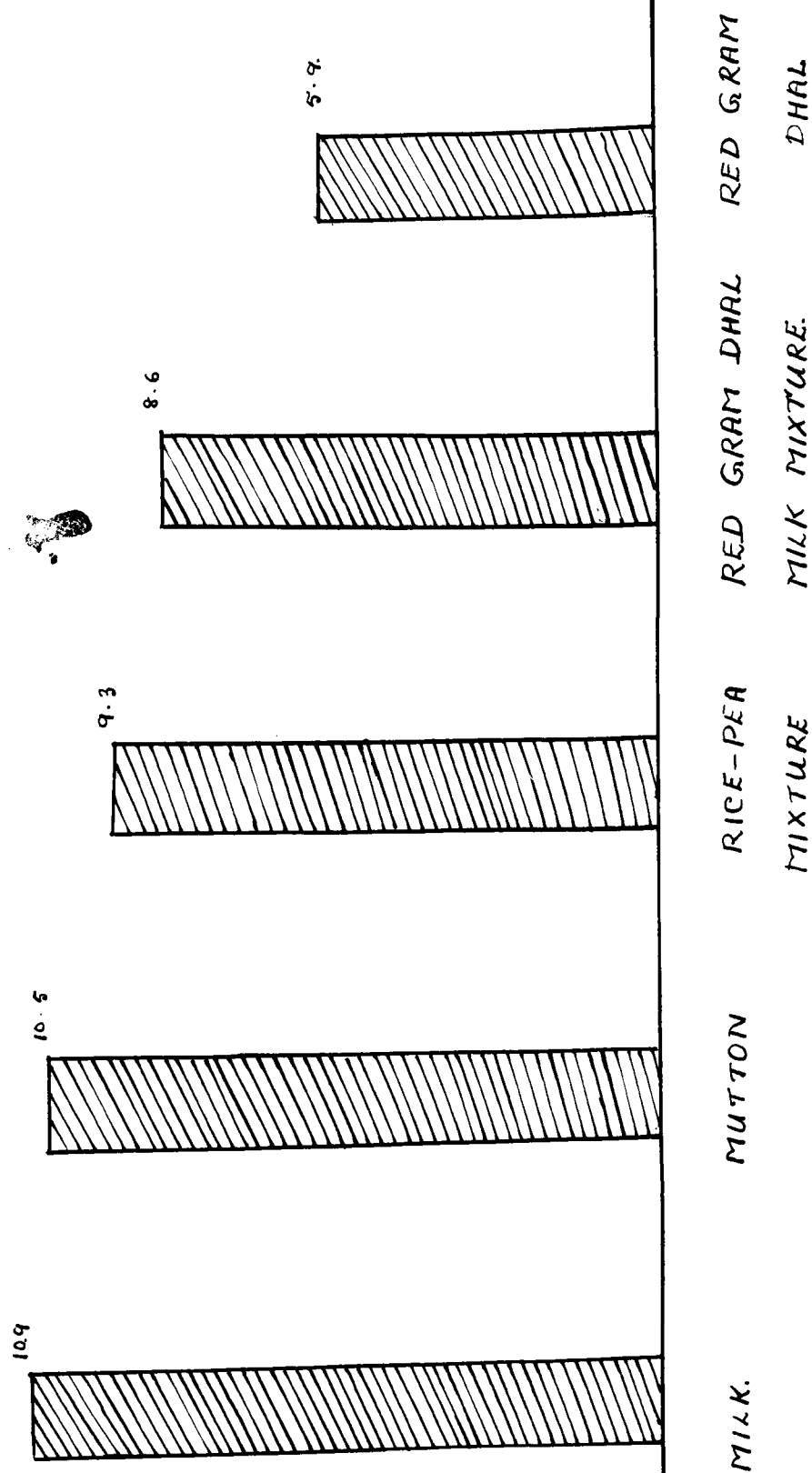


FIGURE X

Table XV shows that the ~~hemoglobin~~ of rats ~~on~~ fed on milk protein, is ~~regenerated~~ to the maximum, when compared with the other proteins as also shown in Figure X. The hemoglobin content of a normal, healthy rat ~~amounting~~ to Creakoff ~~et al~~ (1949)²⁴⁰ is 15.4 grams per ml. of blood. But Table XV shows that the ~~hemoglobin~~ content of the milk rat, is only 10.9 grams. The probable ~~explanation~~ of this low value ~~observed~~ could be the ~~inadequacy~~ of the length of each ~~repletion~~ period to bring back the depleted blood protein level to normal and therefore at the end of the fourth ~~repletion~~ period the ~~hemoglobin~~ level is lower than that of a normal, healthy albino rat. As all the animals were given adequate quantities of all other nutrients but the protein, and ~~more-~~ over treated under similar ~~conditions~~, the results given in Table XV point out the ~~difference~~ in the capacity of various proteins to ~~regenerate~~ hemoglobin.

Next in order, considering ~~the hematopoietic~~ capacity of mutton, as the ~~hemoglobin~~ contains 10.5 grams, but the difference between milk protein and mutton protein is not significant as seen in Table XV. Rice-pea mixture was also comparable to the animal proteins, since the ~~hemoglobin~~ content which was 9.3 grams, was not found to be ~~significantly~~ different from ~~milk~~ proteins, nor to mutton proteins.

Redgram dhal, which was chosen as a plant protein, was found to have a very poor capacity to ~~regenerate~~ haemoglobin. Table XV shows the ~~haemoglobin~~ content of rats fed on red gram dhal to be ~~significantly~~ lower than milk and mutton. But, when the milk was added to the red gram dhal, there was an ~~increase~~ in ~~haemoglobin~~ content but this ~~difference~~ was not found to be significant. Therefore, the ~~nutritive~~ value of red gram can be enhanced only ~~very~~ slightly by the addition of a complete protein like milk.

As shown by Pearson *et al* (1937)²⁴², Orten and Orten, (1943)²⁴³, (1946)²⁴⁴, Cresskoff *et al* (1949)²⁴⁰ and later by ~~Aschkenasy~~ (1960)²⁴⁵ dietary deficiencies can easily influence the blood picture in rats. The investigator found this to be true, as seen from the results of the blood ~~examination~~ given in table XV. ~~Aschkenasy~~ (1960)²⁴⁵ has pointed out that either ~~incomplete~~ deprivation of protein, such as a four per cent protein diet, or an inferior quality protein diet, results in a lowered ^{re} albumin content of the serum, and later the concentration of ~~haemoglobin~~ and red blood corpuscles are lowered. ~~Aschkenasy~~ has also pointed out that a deficient protein diet lowers the ~~haemoglobin~~ concentration rather than the red blood cell ~~concentration~~. Further ~~he states that a "protein"~~ diet, if ~~inadequate~~ does not increase the ~~concentration~~ to normal. This was found to be very true in this study, for after ~~x~~ every depletion, the concentration of ~~haemoglobin~~ could not be brought to the normal, level, within the short repletion period of

one week, with the test proteins, and therefore, at the end of the fourth repletion period, the haemoglobin values were subnormal.

Aschkenasy (1960)²⁴⁵ states that muscles' proteins, are highly haematopoietic. This was found to be true in this study, for compared to the vegetable proteins, the haemoglobin content of mutton protein was significantly superior.

Aschkenasy (1960)²⁴⁵ has also stated that in protein depleted rats, eggs, meat and soya beans have been found to regenerate haemoglobin very effectively, and were found to be superior to casein in this respect. Aschkenasy stated that isoleucine was found to be indispensable for haemoglobin synthesis, while lysine was also of importance for haematopoiesis. But Chitre and Vallary (1956)²⁴⁶ who made a systematic comparison between cereals and pulses, with reference to their ability to regenerate blood and plasma proteins, found that wheat had a high haemoglobin synthesising capacity, equal to casein. Rice was found to be slightly inferior to casein, but all the same of good quality. They found red gram to be a very poor protein, since, even after ten weeks of feeding the rats with red gram dhal at 10 per cent level, the haemoglobin value came up to only 9.87 grams as compared to 14.87 of casein. But when red gram was autoclaved, it improved a little. This inability of red gram to regenerate haemoglobin is due to the lack of tryptophan, since Ramachandran and Phansalkar (1956)⁹⁴ have shown red gram to be deficient in tryptophan. Thus, from these studies it is evident that tryptophan is a necessary

amino acid for the synthesis of haemoglobin. The other amino acids necessary for haemoglobin synthesis, as stated by Aschkenasy (1960)²⁴⁵ are methionine, lysine and phenylalanine.

2. Red Blood Corpuscle Enumeration: (R.B.C)

The results of the R.B.C. count is given in Table XVI and illustrated in Figure XI.

TABLE XVI.

COMPARATIVE REGENERATION OF RED BLOOD CELLS BY ALBINO
RATS FED WITH FIVE PROTEINS.

Proteins	No. of animals	Red Blood Cells millions/cmm.
Milk	4	7.76
Mutton	4	5.62
Red gram dhal	3	3.89
Red gram dhal - milk mixture	4	6.33
Rice - pea mixture	4	6.44

Comparison of diets	Mean difference.
Milk ~ Mutton	2.14 N.S.
Milk ~ Red gram dhal	2.87*
Milk ~ Red gram dhal - milk mixture	1.43 N.S.
Milk ~ Rice - pea mixture	1.53 N.S.
Mutton	
Red gram dhal ~ Mutton	1.73 N.S.
Red gram dhal - Milk mixture ~ Mutton	0.71 N.S.
Rice - Pea mixture ~ Mutton	0.81 N.S.
Red gram dhal - Milk mixture ~ Red gram dhal	2.43 N.S.
Rice - Pea mixture ~ Red gram dhal	2.55*
Red gram dhal - Milk mixture ~ Rice - pea mixture	0.10 N.S.

* Means that the difference between the diets is significant beyond 5 per cent level.

N.S: Means that the difference is not significant.

COMPARATIVE REGENERATION OF RED BLOOD CORPUSCLES IN RATS FED ON THE FIVE PROTEINS

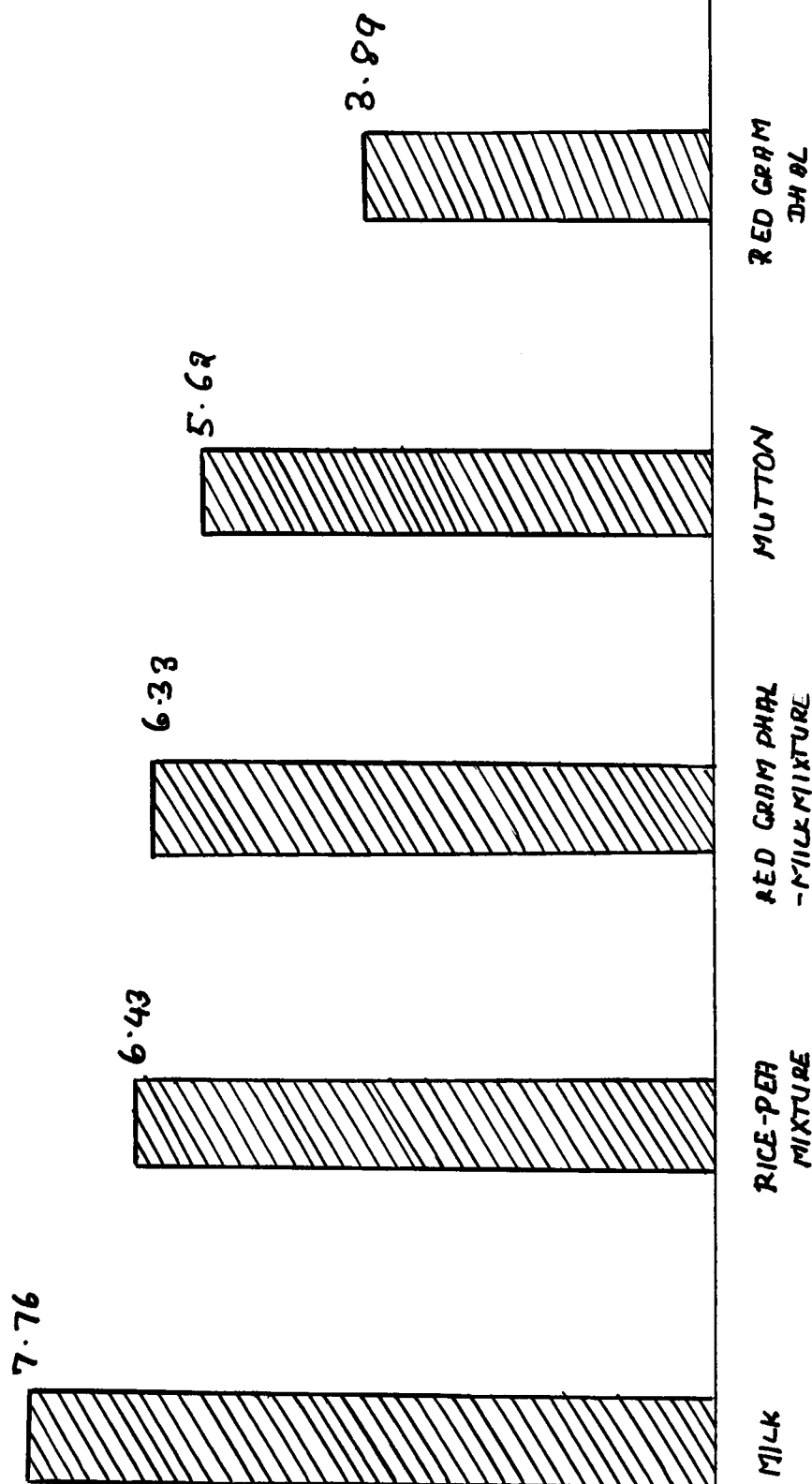


FIGURE XI

The R.B.C. concentration of a normal, healthy male adult rat, according to Creskoff et al (1949)²⁴⁰ is 9.27 millions per cubic millimeter. The values shown in Table XVI indicate the superiority of milk proteins in regenerating red blood corpuscles. Milk was found to regenerate R.B.C. better than other proteins studied, but it was significantly superior only to red gram dhal and the difference between milk and other proteins was not significant. Mutton was superior to red gram dhal but there was no significant difference between the two proteins. Beach et al (1943)¹²³ have shown that the amino acids present in m muscle meat are good for R.B.C. Red gram dhal, when fed alone proved to be of poor quality, but when milk was added to the red gram dhal the hemoglobin regenerating capacity was improved considerably to almost that of milk but the difference was not significant. Rice pea mixture also proved to be very effective, in this respect, as there was no significant difference between milk and this mixture, and it was also significantly better than red gram dhal.

The R.B.C. values, as seen from Table XVI have not been drastically reduced, for the normal value for an adult male rat is only 9.7 millions. This is because as Aschkenasy (loc. cit.) states the red blood corpuscles are not as drastically reduced, as hemoglobin or serum albumin, on a protein deficient diet.

Aschkenasy (loc. cit.) ~~concluded~~ that the capacity of

animals and plant proteins to regenerate blood proteins, depended on their amino acids content and the amount in the diet. He further stated that whenever a protein was adequate for growth and weight maintenance, either because of their balanced amino acids composition, or the amount of the diet, they were also effective on haematopoiesis. Factors which affect the biological value of proteins such as digestion, time of ingestion and cooking and methods of processing also affect the haematopoietic capacity of the proteins. ~~Aschkenasy~~ (1960)²⁴⁵ also pointed out that when proteins are fed simultaneously, as a mixture, the rate of haematopoiesis was improved, as the two proteins supplement each other. These conclusions of ~~Aschkenasy~~, were shown to be true in this study as shown in the above discussion.

~~Liver Protein Regeneration~~

Table XVII and Figure XII give the data regarding the efficiency of the five proteins to regenerate liver proteins.

TABLE XVII.

**DIETARY REGENERATION OF LIVER PROTEIN BY FIVE
PROTEINS IN ALBINO RATS**

Dietary	Liver proteins (On dry wt. basis) % protein	Moisture
1. Milk	81.5	65.4
2. Mutton	79.3	73.4
3. Red gram dhal	56.5	70.5
4. Red gram dhal - milk mixture.	59.7	74.5
5. Rice pea mixture	80.4	75.4

Comparison of diets	Mean difference
Milk ~ Mutton	2.2 N.S
Milk ~ Red gram dhal	25.0**
Milk ~ Red gram dhal - Milk mixture	21.8**
Milk ~ Rice - pea mixture	1.1 N.S
Mutton ~ Red gram dhal	22.8**
Mutton ~ Red gram dhal - milk mixture	19.6**
Mutton Rice pea mixture ~ Mutton	1.1 N.S
~~Red gram dhal~~ Red gram dhal - milk mixture ~ Red gram dhal	3.2 N.S
Rice pea mixture ~ Red gram dhal	23.9**
Rice pea mixture ~ Red gram dhal - milk mixture	20.7**

** Means that the difference between the diets is highly significant, beyond 1 per cent level.

N.S: Means that the difference is not significant.

COMPARATIVE REGENERATION OF LIVER PROTEINS IN RATS FED ON THE FIVE PROTEINS

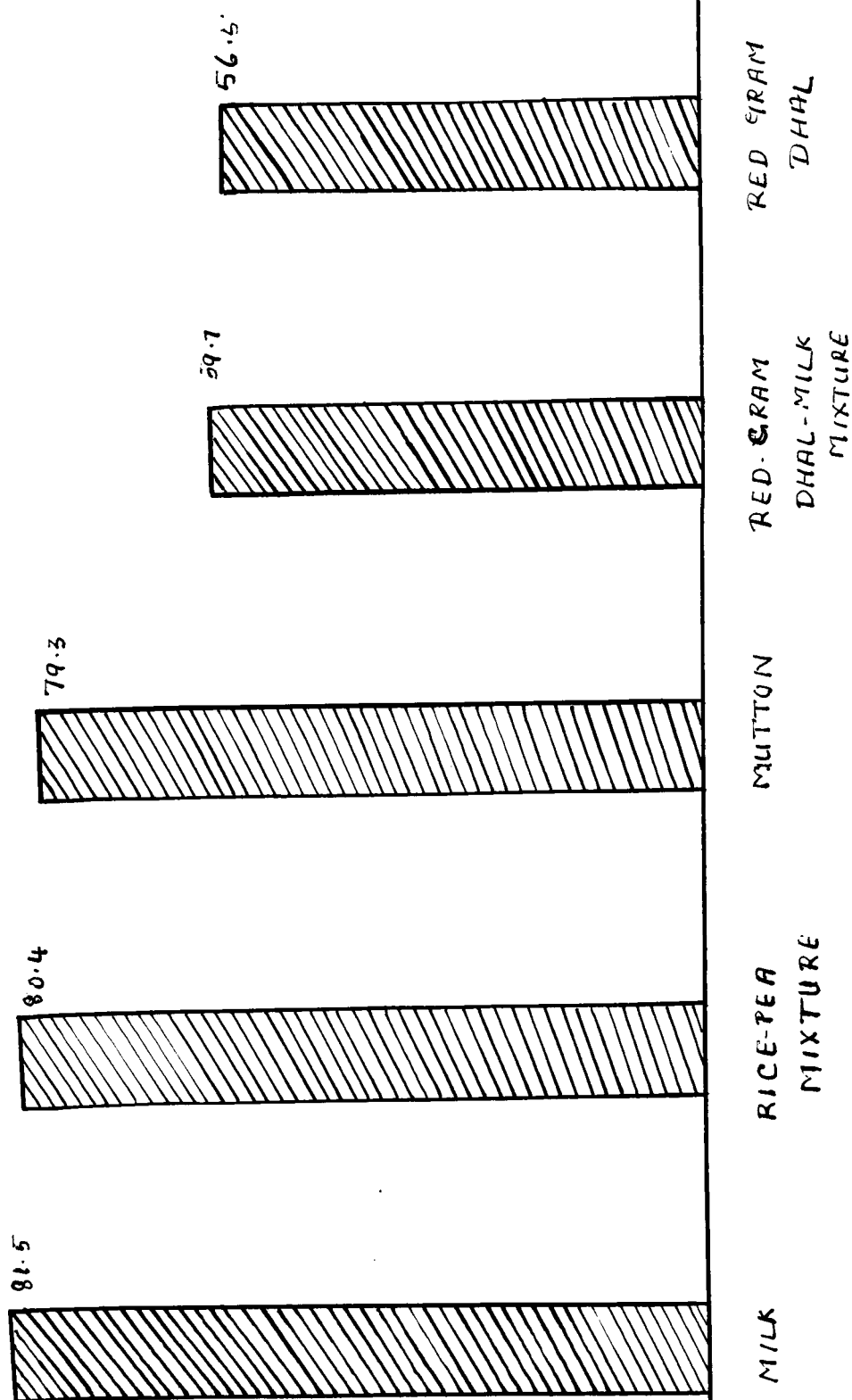


FIGURE XII

The protein values of the liver, shown in Table XVII, point out that the five proteins can be divided into categories, namely, those which can appreciably regenerate liver protein, and those which lack this capacity. Milk, mutton and rice-pea mixture come under the former category while red gram dhal and red gram dhal milk mixture fall under the latter category. Table XVII also points out that the differences between milk and mutton, milk and rice-pea mixture are not statistically significant, while the difference between any protein of the group and red gram dhal alone or in combination with milk is highly significant. In short, red gram dhal and red gram dhal milk-mixture are very significantly inferior to milk, mutton and rice-pea proteins. Red gram dhal is very poor in regenerating liver proteins, and it cannot be improved appreciably by the addition of an excellent protein like milk. The statistical analysis were performed with the data given in Appendix XIV, by the method outlined in Appendix XVI.

V. SUMMARY AND CONCLUSION.

1. A survey, conducted in a rural and an urban area of a representative, sample, showed the common sources of plant and animal protein foods to be: i) Rice, ii) Red gram dhal and iii) mutton. These foods were chosen to study the nutritive value of their proteins. In addition to these, certain protein mixtures like rice and peas, and red gram dhal and milk were also studied.

2. In order to study the nutritive value of the five proteins the following criteria were used:-

- (a) Protein Efficiency Ratio; (b) Nitrogen balance;
- (c) Haemoglobin and red blood cell regeneration and (d) liver protein regeneration.

3. The Protein Efficiency Ratio(P.E.R) of milk proved to be significantly higher than the other proteins, namely mutton, red gram dhal, red gram dhal-milk mixture and rice-pea mixture in all the four repletion periods. The P.E.R of rice-pea mixture proved to be the second best having a P.E.R of 2.42 in the first week as compared to 1.97 of mutton, 1.74 of redgram dhal-milk mixture and 0.71 of red gram dhal.

4. The digestibility coefficient of the five proteins in the order of their digestibility, are as follows:-
Milk, red gram - milk mixture, mutton, rice - peas mixture and red gram dhal.

5. The percentage of nitrogen retention of the five proteins showed that milk protein had the maximum retention and the rest in order of quality were: Mutton, red gram dhal - milk mixture, rice - peas mixture and red gram dhal.

6. The order of proteins with regard to their capacity to regenerate haemoglobin were:

Milk, mutton, rice - pea mixture, red gram dhal - milk mixture, mutton and red gram dhal. But of all the mean differences only that between milk and red gram dhal and between mutton and red gram dhal are significant.

7. The R.B.C. regenerative capacity of the five proteins in order were:

Milk, rice - peas mixture, red gram dhal - milk mixture, mutton and red gram dhal. Milk and rice - pea mixture are significantly superior to red gram, no other difference is significant.

8. The liver protein synthesis were studied with the five proteins and in the order of their ability they were as follows:

Milk, mutton, rice - peas mixture, red gram dhal - milk mixture, mutton and red gram dhal. The first three constitute one group and the other inferior in this respect. The difference between each superior protein and each inferior protein is statistically significant. Within each group there is no significant difference.

It can be concluded, that on the basis of the protein nutrition of albino rats, the five proteins, belonging to animal and plant sources, can be classified as those of high and low quality proteins. Milk is decidedly the best protein for support, and maintenance of growth in the rat, and also for proper regeneration of blood proteins and liver proteins. The vegetable protein mixture such as rice-pea mixture has also been found to be of good quality, since not only did it compare well with milk, but it was found to be well tolerated by the animals. Red gram dhal, though found to be a poor source of protein, can be enhanced and utilised better, with the addition of a complete protein like milk. Mutton an animal protein and usually known to be an excellent protein, has been found to be of high quality in all the aspects studied except that of regeneration of R.B.C.

Therefore, it can be concluded that vegetable protein mixtures can take the place of animal foods, for this study has shown that the nutritive value of the vegetable protein mixtures were comparable to animal proteins. Moreover, they are cheaper and more easily available. This study further points out the fact that proteins of lower nutritive value can be made more nutritious by adding only a small amount of a complete protein. It is evident that good quality proteins can be obtained for animal use, by the use of locally available and commonly used protein foods.

It can be suggested that, more studies based on combinations of commonly used vegetable protein foods can be conducted, so as to evolve diets for the lower socio-economic groups, whose diets have been shown to lack good proteins. The above investigation can be further studied, by varying the proportions of rice to peas and milk to dhal, and also by substituting the rice and dhal with other cereals and pulses.

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247. Hepler

APPENDIX.

APPENDIX I.

INTERVIEW SCHEDULE TO FIND OUT THE MOST COMMONLY CONSUMED PROTEIN RICH VEGETABLE AND ANIMAL FOODS.

(UNDER SURV.)

Name of the head of the family : Serial No:
Caste: Religion: Mother tongue:
Native Place: Address: Income per month:
Number of members in the family : Age Above 21:
Below 21:

1. Vegetarian or Non-vegetarian:

Name of member.	Vegetarian.	Non-vegetarian.	Frequency.
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Go

2. The most commonly consumed vegetarian and non-vegetarian protein rich foods in order of frequency:

Vegetarian food	Quantity per day	Frequency	Non-vegetarian foods	Quantity per day	Frequency
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1. Dhal:

- a.
- b.
- c.
- d.

2. Legumes:

- a.
- b.
- c.
- d.

3. Nuts:

- a.
- b.

4. Greens:

- a.
- b.

5. Paped:

1. Meat:

- a.
- b.
- c.
- d.

2. Fish:

- a.
- b.
- c.
- d.

3. Egg:

- a.
- b.

4. Milk:

- a.
- b.

APPENDIX II.

INTERVIEW SCHEDULE TO FIND OUT THE MOST COMMONLY CONSUMED PROTEIN RICH VEGETABLE AND ANIMAL FOODS. (RURAL AREA)

Serial Number of the
Family surveyed:

1. Name of head of family:
2. Caste:
3. Religion:
4. Address:
5. Income:
6. Vegetarian or Non-vegetarian:

Name of member	Vegetarian	Non-vegetarian and type.	Frequency
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7. The most commonly consumed vegetarian and Non-vegetarian protein rich foods:

Food	Quantity/day	Frequency
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APPENDIX III.

The protein efficiency ratios of five proteins obtained
in four periods of repetition.

Protein	First period	Second period	Third period	Fourth period
1. Milk	2.90	2.70	2.49	2.10
	2.86	2.69	2.50	2.30
	2.94	2.74	2.30	2.10
	2.84	2.60	2.40	2.00
2. Mutton.	1.94	1.86	1.86	1.72
	2.00	1.90	1.74	1.70
	1.94	1.81	1.73	1.60
	2.06	1.89	1.80	1.69
3. Red gram dhal	0.86	0.54	0.60	0.51
	0.40	(Loss of weight)	dead	dead
	0.79	0.61	0.54	0.30
	0.80	0.70	0.61	0.49
4. Red gram dhal milk mixture	1.7	1.64	1.50	1.42
	1.69	1.60	1.52	1.42
	1.76	1.69	1.60	1.41
	1.80	1.60	1.70	1.54
5. Rice pea mixture	2.62	2.06	1.72	1.54
	2.36	2.10	1.81	1.64
	2.40	2.00	1.74	1.59
	2.45	1.91	1.70	1.58

APPENDIX IV.

THE RECORD OF WEIGHT GAIN OF ALBINO RATS FED ON FIVE PROTEINS
DURING FOUR PERIODS OF DEPLETION.

		1st period		2nd period		3rd period		4th period	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final
		g	g	g	g	g	g	g	g
Milk	1.	45.2	60.0	44.8	68.3	56.9	74.8	64.6	78.6
	2.	42.8	50.4	42.1	56.3	42.8	64.5	63.5	78.5
	3.	44.1	58.1	41.9	72.1	53.5	70.9	62.2	76.6
	4.	43.2	58.5	46.1	69.2	59.9	77.5	68.0	80.0
Mutton	1.	51.2	60.4	58.5	70.3	60.2	73.7	72.2	97.0
	2.	46.8	59.0	54.1	65.6	51.1	65.9	61.5	78.9
	3.	48.4	54.7	55.7	67.0	53.3	64.6	55.7	72.5
	4.	41.5	49.3	47.5	62.4	48.2	66.7	55.1	68.3
Red graml. dhal	1.	47.5	53.3	48.1	52.0	47.0	55.5	44.8	53.4
	2.	48.5	51.6	48.8	46.0	dead	dead	dead	dead
	3.	43.8	50.0	46.8	50.1	43.2	50.0	42.0	51.7
	4.	42.7	46.0	46.0	49.5	43.7	46.2	43.7	49.8
Red graml. plus dhal milk mixture	1.	45.9	55.0	50.8	65.0	43.6	63.5	64.2	79.0
	2.	50.0	58.7	54.8	67.9	54.6	74.7	60.3	75.0
	3.	49.0	57.7	52.5	67.1	51.6	67.0	56.0	68.0
	4.	44.6	53.4	49.1	65.1	50.0	69.5	55.6	68.2
Rice — plus pea mixture	1.	43.8	58.7	52.8	70.3	54.6	76.7	62.6	76.4
	2.	49.0	62.7	54.2	70.1	54.7	76.2	69.2	83.9
	3.	49.2	62.3	53.3	70.2	56.2	76.4	59.6	74.6
	4.	47.4	62.3	53.3	67.1	58.5	79.5	67.3	79.5

APPENDIX V.

ANALYSIS OF VARIANCE FOR THE PROTEIN EFFICIENCY RATIO OF FIVE PROTEINS OBTAINED IN FOUR PERIODS OF REPLETION.

Source of variation	Sum of squares	d.f.	Mean squares	F
Total	28.71	75	0.3828	
Between diets	22.6	4	5.65	156.5**
Week by diets	3.98	12	.331	9.187**
Error	2.13	59	.036	

Standard error (within variance) $\sqrt{\frac{\text{Error within rats}}{df}}$ $\times \frac{2}{19}$

$$= 0.0616$$

Minimum difference at 1 per cent level = $3 E_w \times t_{01} = 0.0616 \times 2.678 = 0.17$

Minimum difference at 5 per cent level = $3 E_w \times t_{05} = 0.0616 \times 2.001 = 0.1232$

2 Four df. were lost in data, missed through death of a rat.

** Means that the difference between the sets is highly significant, that is beyond 1 per cent level.

APPENDIX VI.

THE VALUES OF FAECAL AND URINARY NITROGEN, AND THE PERCENTAGE OF NITROGEN RETENTION AND DIGESTIBILITY COEFFICIENT OF FIVE PROTEINS OBTAINED DURING THE FIRST NITROGEN BALANCE STUDY.

	Protein intake gm	Faecal nitrogen gm	Urinary nitrogen gm	Nitrogen retention %	Digestibi- lity coef- ficient %
Milk	1.0.19200	0.00230	0.00330	94.5	98.8
	2.0.14300	0.00320	0.00400	93.1	97.8
	3.0.19900	0.00470	0.00300	96.3	97.8
	4.0.15000	0.00320	0.00620	93.7	97.9
Mutton	1.0.13200	0.00461	0.01074	88.3	76.6
	2.0.17300	0.00793	0.01003	89.4	95.4
	3.0.08900	0.00182	0.00591	91.3	86.7
	4.0.10500	0.0059	0.01250	87.5	99.4
Red gram dhal	1.0.18900	0.02815	0.00800	73.8	85.0
	2.0.19800	0.02997	0.00240	83.2	90.7
	3.0.20500	0.01800	0.00230	64.2	91.2
	4.0.112500	0.03200	0.00210	85.1	66.0
Red gram milk mixture	1.0.14800	0.00771	0.00885	89.3	87.9
	2.0.14300	0.00700	0.00895	89.2	95.3
	3.0.14700	0.00555	0.01260	83.0	97.5
	4.0.13400	0.00860	0.02670	73.7	93.6
Rice-pea mixture	1.0.17300	0.01230	0.00330	72.3	87.1
	2.0.22000	0.01750	0.00330	90.6	92.0
	3.0.11000	0.2370	0.00860	64.7	73.0
	4.0.13500	0.02970	0.00404	75.02	79.9

APPENDIX VII.

THE VALUES OF FAECAL AND URINARY NITROGEN AND
THE PERCENTAGE OF NITROGEN RETENTION AND
DIGESTIBILITY COEFFICIENT OF FIVE PROTEINS
OBTAINED DURING THE SECOND NITROGEN BALANCE STUDY.

	Protein intake gm	Faecal ni- trogen gm	Urinary nitrogen gm	Nitrogen retention %	Digestibility coefficient %
Milk	0.226	0.00390	0.01312	92.45	98.3
	0.211	0.01208	0.01750	85.96	94.3
	0.263	0.00952	0.00918	92.62	96.3
	0.233	0.00320	0.01881	91.66	98.6
Mutton	0.180	0.02020	0.3901	78.29	88.8
	0.220	0.00872	0.03916	88.57	96.0
	0.174	0.01143	0.02509	85.61	93.4
	0.220	0.00990	0.02046	90.77	95.5
Red gram dhal	0.331	0.03710	0.00624	86.9	70.7
	0.260	0.04100	0.01356	79.0	84.2
	0.232	0.03120	0.00741	83.3	89.0
	0.133	0.01122	0.420	73.36	84.0
Red gram-milk mixture	0.26900	0.01980	0.02318	84.37	92.7
	0.16900	0.101470	0.01137	75.36	91.3
	0.32500	0.01008	0.02450	89.35	96.9
	0.30000	0.01264	0.01663	90.24	95.8
Rice-pea mixture	0.32000	0.01789	0.01056	91.09	94.4
	0.31000	0.02262	0.01443	88.02	92.7
	0.30000	0.01932	0.00744	91.07	93.5
	30000	0.01694	0.00487	89.58	92.8

APPENDIX VIII.

ANALYSIS OF VARIANCE FOR THE DISSEMINATION COEFFICIENT OF FIVE PROTEINS OBTAINED DURING TWO PERIODS OF REPLETION.

Source of variation	Sum of squares	d.f.	Mean	F
Total	2519.5	39	64.6	
Between diets	1028.3	4	257	5.942**
Week by diets	151.0	4	37.7	.8734
Error	1340.20	31	43.2	N.S.

$$\text{Standard error} = \sqrt{\frac{\text{Error within sums}}{\text{d.f.}}} \times \frac{2}{20}$$

$$= 2.08$$

$$\text{Minimum difference at 1 per cent level} = S.E. \times t_{.01} =$$

$$2.08 \times 2.75$$

$$= 4.63$$

$$\text{Minimum difference at 5 per cent level} = S.E. \times t_{.05} =$$

$$= 2.08 \times 2.04$$

$$= 4.24$$

** Means that the difference between the diets is highly significant beyond 1 per cent level.

N.S. ~~The difference is not significant.~~

APPENDIX IX.

ANALYSIS OF VARIANCE FOR THE NITROGEN RETENTION OF FIVE PROTEINS BY ALBINO RATS, OBTAINED DURING TWO PERIODS OF REPLETION.

Sources of variation	Sum of squares.	d.f.	Mean squares	F
Total	2508.55	39	36.2	
Between diets	329.71	4	93.8	5.481**
Week by diets	505.86	4	58.8	5.295**
Error	1172.98	31	25.8	

Standard error =

$$\sqrt{\frac{\text{Mean square error}}{d.f.}} = \sqrt{\frac{25.8}{31}}$$

$$= 1.945$$

$$\text{Minimum difference at 1 per cent level} = S.E. \times t_{.01}$$

$$= 1.945 \times 2.75$$

$$= 5.348$$

$$\text{Minimum difference at 5 per cent level} = S.E. \times t_{.05}$$

$$= 1.945 \times 2.04$$

$$= 3.968$$

** Means that the difference is highly significant beyond 1 per cent level.

APPENDIX X.

THE OBSERVATIONS MADE ON THE ALBINO RATS FED WITH FIVE TYPES OF PROTEINS, FOR CHANGE IN PHYSICAL APPEARANCE AND FOR SIGNS OF MALNUTRITION.

Period	Observation.
1st repletion period	No change in physical appearance. The animals were active.
2nd repletion period	4B (rat fed on red gram dhal) showed signs of extreme weakness, and lost weight during this period.
3rd repletion period	4B lost weight. The other three rats fed on red gram dhal, showed extreme weakness dragged their feet, and became very timid, and nervous. The coat was unchanged. The eyes of one of the animals showed slight inflammation. Regarding animals fed on mutton, the hair dropped heavily.
4th repletion period	4B died at the end of the depletion week. ~~The rest~~ of the animals fed on red gram dhal showed a slight change in colour to yellow. For under the chin became scanty. The eyes of one of the rats fed on red gram dhal was inflamed, and was red. It also wattered constantly. The coat of the other animals were not as soft as those fed on red gram dhal, showed spots of hyper pigmentation.

APPENDIX XI.

DESCRIPTION OF THE VARIOUS TECHNIQUES INVOLVED IN THE EXPERIMENTAL PROCEDURE.

The various techniques involved in the experimental procedure, are described in detail below:-

1. Kjeldhal method:- For the estimation of total nitrogen in the ^afeces and urine.

Principle: The principle of this method is the conversion of the various nitrogenous compounds of the urine into ammonium sulphate by boiling with concentrated sulphuric acid, the subsequent decomposition of the ammonium sulphate by means of a fixed alkali (NaOH) and the collection of the liberated ammonia in an acid of known strength. Finally, this partly neutralized acid solution is titrated with an alkali of known strength and the nitrogen content of the urine under examination computed.

Procedure: Placed 20ml. of urine in a 500 ml. long necked pyrex-glass, Kjeldhal flask, added 20 ml. of concentrated sulphuric acid, about 0.2 grams of copper sulphate and three grams of potassium sulphate, and boiled the mixture till it became pale green in colour, with no black specks. The whole digestion process was conducted in a fume cupboard, in which six electric furnaces were fixed, thus enabling the digestion of six samples simultaneously.

When the oxidation of the sample was completed, allowed the flask to cool and diluted the contents with about 200 ml. of ammonia free water. Added a little more of a concentrated solution of NaOH (40%) than is necessary to neutralize the sulphuric acid, down the side of the flask in such a way that the alkali does not mix with the solution in the flask but forms a layer beneath it, and introduced into the flask a few porcelain bits, to prevent bumping. This flask is at once connected to a condenser and delivery tube, whose end dips into a conical flask containing 25 ml. of 4% boric acid to which 3 drops of ~~Methylorange~~ indicator has been added. The ammonia is distilled over when the digested mixture is heated after being well mixed into the boric acid,

The end of the distillation is indicated by testing the tip of the delivery tube for ammonia, with a wet red litmus paper. The Boric acid is titrated against 0.1N H_2SO_4 , the end point being the formation of a pink colour. The calculation for total nitrogen is as follows:

Taking the number of millilitres of 0.1N H_2SO_4 taken to neutralize the half neutralized boric acid to be X, the nitrogen content of the urine sample is:

1 cc of 0.1N H_2SO_4	= 0.0014 gram of nitrogen.
∴ X cc of 0.1N H_2SO_4	= 0.0014 X -do-
∴ 20 ml. of urine	= 0.0014 X X gm. of N
∴ 250 ml. of urine contains	
(The total of 4 days collection)	$\frac{0.0014 \times X \times 250}{20}$

Thus the total urinary nitrogen for the four days, was calculated. The Nitrogen was determined in triplicates in both ²feces and urine samples.

2. The microkjeldhal, as adopted for the determination of total nitrogen of liver.

Principle - The same as given under Macro-Kjeldhal method.

Procedure: A sample of liver weighing about 100 grams was digested in a 50 ml. Kjeldhal flask with 5 ml. of concentrated H_2SO_4 and a few grains of selenium dioxide, over a Nicoburner, till the mixture was clear and pale green in colour, with a no black specks. Porous bits to prevent bumping were also added. The digested sample was cooled, and made up to 50 ml. Aliquots of this diluted, digested sample were used for the determination of nitrogen.

10 ml. of the digested sample was pipetted into the distillation flask, and 15 ml. of 40% NaOH was added to the digested sample. Water was boiled in the mean time, and steam was allowed to pass through the sample. The tip of the delivery tube was dipped into a conical flask containing 5 ml. of boric acid containing 2-3 drops of Murexide indicator. The blue colour of the indicator changes to green when ammonia distills over. Distillation is now continued for five more minutes. The tip of the delivery tube is tested for the absence of ammonia and then down into the conical flask. The contents of the

conical flask are titrated against N/100 HCl.. The nitrogen is estimated as follows:-

- 1 cc of 0.01 N HCl = 14 mg. of nitrogen
- . X cc N HCl = 14 x X -do-
- . 10 ml. of sample contains 14 x X mg. of N
- . 50 ml. of sample contains $14 \times X \times \frac{50}{10}$ mg.
- i.e. Y gram of liver sample contain $14 \times X \times \frac{50}{10}$ mg.
- . 100 grams of liver sample contain $14 \times X \times \frac{50}{10 \times Y}$ milligrams of nitrogen.

Thus the nitrogen in liver was determined.

Hastings' estimation by the Sahli - Hollingworth's

1. A Sahli- Hollingworth's colorimeter was used.
2. Place Hcl upto the mark 10 in the graduated tube.
3. Obtained a large drop of blood, and pipetted upto the 20 mark and blew it into the Hcl, rinsed the pipette well by drawing up the acid in the tube several times.
4. Allowed it to stand for 4 minutes for the haemoglobin to change to haematin.
5. Diluted with distilled water, drop by drop, mixing after each addition, until it matched the colour of the standard comparison tube.
6. The reading on the graduation was read directly for grams per 100 ml. as well as percentage.

4. Red blood cell count

1. Used a pipette marked 101 above the bulb.
2. Pipetted blood upto 0.5 mark. Wiped the tip for any adhering blood and then drew in diluting fluid upto the 101 mark ^{and} mixed thoroughly.

3. Adjusted the microscope. Placed the counting chamber and focussed the ruled area.
4. Blew out a part of the contents of the pipette.
5. Placed a small drop of this fluid on the counting chamber, placed the cover glass and allowed some time for the cells to settle.
6. Adjusted the microscope, to see clearly the rulings of the counting chamber.
7. Counted 5 big squares - the four corners and the centre squares so as to get as nearly an average as possible. Added four ~~systems~~ to this value, to get the number of millions per cubic millimeter.

APPENDIX XII.

AND

THE VALUES FOR HAEMOGLOBIN, RED BLOOD CELL REG-ENATION,
BY THE FIVE PROTEINS.

Proteins.	Haemoglobin g/100 ml.	Red Blood cell millions/c.mm.
1. Milk.	8.0	8.94
	7.2	7.77
	14.0	7.31
	14.2	7.02
2. Mutton.	9.0	4.44
	12.0	8.66
	9.0	8.04
	12.0	6.35
3. Red gram dhal.	6.0	3.68
	4.8	5.32
	7.0	8.61
4. Red gram dhal- milk mixture.	7.00	5.53
	9.5	6.37
	7.6	4.90
	10.5	8.52
5. Rice-poa mixture	10.5	643
	11.0	615
	8.0	769
	7.6	546

APPENDIX XIII.

ANALYSIS OF VARIANCE FOR THE REGENERATION OF HEMOGLOBIN IN ALBINO RATS BY FIVE PROTEINS.

Source of variation	Sum of squares	d.f.	Mean squares	F
Total	216.6	19	11.4	
Between diets	101.3	4	25.3	3.232
Error (within diets)	115.3	15	7.7	

$$\text{Standard error} = \sqrt{\frac{\text{Mean square error}}{\text{d.f.}}} = \sqrt{\frac{7.7}{15}} = 1.96$$

$$\begin{aligned} \text{Minimum difference at 1 per cent level} &= 3.2 \times 1.96 \\ &= 1.96 \times 2.94 \\ &= 5.3 \end{aligned}$$

$$\begin{aligned} \text{Minimum difference at 5 per cent level} &= 3.2 \times 1.96 \\ &= 1.96 \times 2.13 \\ &= 4.2 \end{aligned}$$

* Means that the difference between the diets is significant beyond 5 per cent level.

APPENDIX XIV.

THE WEIGHT, MOISTURE AND PROTEIN CONTENT OF LIVERS OF
ALBINO RATS FED WITH FIVE PROTEINS.

Proteins.	Weight of Liver (dry) gms.	Moisture content gms. %	Protein gms. %
Milk	1. 1.61 2. 1.644 3. 1.35 4. 1.08	53.37 56.24 54.30 67.56	89.40 84.30 83.80 81.40
Mutton	1. 0.340 2. 1.1056 3. 0.8183 4. 1.09	73.02 71.31 75.00 73.47	72.51 70.63 99.02 77.09
Red gra dhal	1. 0.56 2. — 3. 0.40 4. 0.58	68.73 — 74.94 67.90	50.31 — 57.94 60.56
Red gra dhal plus ilk	1. 0.70 2. 0.92 3. 0.64 4. 0.45	68.76 74.30 71.48 82.77	52.63 56.04 70.33 55.79
Highline peas mixture	1. 0.67 2. 0.61 3. 0.60 4. 0.64	73.30 77.38 76.88 7.51	69.74 89.00 97.70 68.31

APPENDIX V.

ANALYSIS OF VARIANCE FOR THE ESTIMATION OF RED BLOOD CELLS, IN ALBINO RATS BY FIVE PROTEINS.

Sources of variation	Sum of squares	d.f.	Mean squares	F
Total	78.93	19	4.13	
Between diets	38.1	4	9.5	3.529*
Error (within)	40.48	15	2.69	

$$\text{Standard error} = \sqrt{\frac{\text{Error sum of squares}}{\text{d.f.}}} = 1$$

$$\text{Minimum difference at per cent level} = S.E. \times t_{0.01}$$

$$= 1.25 \times 2.94$$

$$= 3.675$$

$$\text{Minimum difference at per cent level} = S.E. \times t_{0.05}$$

$$= 1.25 \times 2.04$$

$$= 2.55$$

* Means that the difference between the diets is significant beyond 1 per cent level.