Nutritional Changes in some Nigerian foods during dietary preparation and their effects on the mammalian body.

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By

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ABSTRACT

Starting with a comprehensive review of the research already done in the related field of nutrition, the chemical composition of 20 Nigerian food materials which are used in the preparation of six common local diets has been investigated. The diets were prepared using accepted recipes and commonly used methods of preparation. Simultaneously, the same amounts of food materials, as used for cooked diets, were mixed raw to correspond to each cooked diet. The changes in the nutritional value of each of these diets, which were brought about by cooking, have been determined from the analyses of the raw mixed and cooked diets. The effects of feeding these diets to rats have been studied by measurement of the growth of these animals and of various protein constituents of the blood. The digestibility, nitrogen balances, and biological values of the food proteins have been determined using a specially designed battery of wire net cages in which it is possible to collect faeces and urine of the rats separately.

A method has also been described for the quantitative estimation of various amino acids in food proteins using an automatic amino acid analyser. The amino acid patterns of these diets have been deduced and compared with the known requirements of various amino acids for rat growth. A comparison of the amino acid patterns of these diets and the

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FAO Provisional pattern of amino acid requirements has also been made. The results of the chemical analysis are examined with reference to growth of rats when these diets are fed to them.

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INTRODUCTION

Food is essential for human life. Different materials are used in various countries depending upon the agricultural production, traditions, social and economic pattern, and, to some extent, religious belief. It also varies (in narrow limits) according to individual tastes. To survive, man must not only eat, but he must eat a nutritionally balanced diet. During the last fifty years or so the dietary requirements have been determined in terms of proteins, carbohydrates, fats, vitamins and minerals. Further more, some 55 different nutrients which are necessary to maintain a man in good health have so far been recognized (King 1963). At the same time much progress has been made in acquiring new knowledge concerning factors affecting the nutritional status of man, and diet has been recognized as the most important single factor in promoting health or causing disease. This wider acceptance of the importance of the knoeledge of nutrition has given a great impetus to detailed nutritional studies in order to find out firstly, what people eat, secondly, the nutritive value of the diet consumed and thirdly the extent to which it supplies known physiological needs.

A recent FAO study shows that between 300 and 500 million people suffer from actual lack of food; and between one third and one half of the world population of 3,000 million suffer from varying degrees of malnutrition. (FAO, 1960).

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In Nigeria, such studies are in progress or have been completed on different groups of the population. Basir (1953) has reviewed most of them carried out during 1933-53. Scattered through the literature are other reports all pointing to the various nutritional deficiencies and their impact on health in this country.

One fundamental requirement in such studies is the knowledge of the chemical composition of food material consumed. Generally for this purpose a reference is made to the food composition tables as published by the FAO or by certain other agencies such as M.R.C. "Tables of representative values of food commonly used in tropical countries (1960)". In all these tables, it is the composition of the uncooked food materials that is given.

In recent years, attention has been drawn to changes which occur during cooking and preparation of food (Bender 1966). Loss of certain water soluble substances during cooking, leaching of nutrients in boiling water, dehydration during frying, oxidation in open pan cooking and the interaction during stewing bring about highly complex reactions which have a direct bearing on the nutritional value of food before it is ready to be served. For instance, it has been reported that the major portion of the thermolabile vitamins is lost during cooking, while certain classes of foodstuffs, especially some

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of the legumes, can improve in protein value as a result of heat processing, either through the inactivation of trypsin inhibitors or through an increase in the availability of certain amino acids, particularly the sulphur-containing ones (Kuppuswami 1958). Conversely, cooking also adversely affects the nutritive value of proteins present in certain other classes of foodstuffs, either by destroying or altering certain amino acids, or by altering certain proteins in such a way that they become resistant to the action of digestive enzymes (Fraxon 1960). Such changes will not only affect the quantitative availability of various nutrients but are also likely to affect the digestibility and nutritional value of the diet as a whole.

Some studies of the apparent losses under controlled conditions of cooking and processing of individual foods have been reported in more advanced countries. In Nigeria, where the methods of cooking are different from the ones employed in those countries, this type of information is still required.

The present study has been undertaken to investigate the nutrient changes in some Nigerian foods during dietary preparations and their effects on the mammalian body. In the following pages an account of six of the most common Nigerian diets, when fed raw and cooked is presented.

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Development of the Concept of Nutrition :-

People everywhere must have been conscious that certain types of food were more desirable than others because of taste and odour and the sense of satisfaction which they gave when eaten. Individual experiences taught observant people the folly of over-eating. The notorious Roman banqueters were as uncomfortable as are the over-indulgent in food and drink of today. Socrates advice to his pupils to eat only when hungry and to drink only when thirsty and a Scots proverb saying: "feed sparingly and defy the physician" are good examples. Illnesses following over-eating and the intake of spoiled food must have been frequent from the earliest times. Herodotus related to the early Egyptians that all diseases to which mankind was subject proceeded from food (Komroff 1936). Athenaeus 200 A.D. wrote the oldest cookery book "The Sophists at Dinner" (Gulick 1927).

Thirteen hundred years later Cornaro (1558) wrote an essay entitled "The Sure and Certain Method of Attaining a Long and Healthful Life" in which emphasis was laid on abstemiousness in eating. He restricted his daily allowance to twelve ounces of solid food and fourteen ounces of wine. Later he experimented with further reduction of his food intake and found he could sustain himself on one egg a day. He lived for ninety three years.

The earliest record of a nutritional experiment with human

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subjects, in which two contrasting diets were compared, is recorded in the book of Daniel (Bible).

Evolution of the idea of Chemical analysis and its development.

The identification of the essential constituents of an adequate diet, their chemical detection, and their quantitative determination are problems which have confronted chemists since the beginning of the effective study of nutritional problems. The provision of oxygen for respiration and the nitrogenous (albuminous) material for construction and repair of the muscles and organs of the animal body were already thought to be of fundamental importance by the chemists of the early nineteenth century (Mccollum, 1957). Methods were therefore evolved for their estimation. Following the basic work of Black, Priestley and Cavendish , Lavoisier (1794) explained the phenomena of combustion. He proved that respiration in animals results in the oxidation of carbon and hydrogen of food. The problem of the determination of the content of carbon and hydrogen in organic substances was solved by Gay-Lussac and Thenard (1811)

Prout (1827) used this method for the analysis of foodstuffs. Dumas (1835) improved upon this method, and Varrentrapp and Willy (1841) both his students, described a method for the estimation of nitrogen. It was based on the observation that when any organic substance was heated strongly with an excess of potassium hydroxide, its nitrogen was converted into ammonia which could be accurately measured. This method was employed

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by the nutritionists for the next forty-two years until Kjeldahl (1883) described his method which depended upon the easy and complete conversion of the nitrogen of most organic substances into ammonium sulphate by boiling with strong sulphuric acid in presence of a catalyst, followed by a subsequent release of ammonia by the action of sodium hydroxide. This method with certain modifications is used even today for the estimation of the content of total nitrogen.

Henneberg and Stohmann (1860) by combining the chemical methods of previous workers, devised a system of food analysis which became known as Weende method. The essential features of this method were the determinations in the sample, of moisture, fat, nitrogen, ash, crude fibre and the estimation (by difference) of a fraction which was designated as the nitrogen free extract. They carried out studies with mature cattle kept under well defined conditions and fed on known amounts of feed of known composition. Urine and faeces were collected and analyzed. These experiments were designed to answer the following two main questions. (1) What are actually the nutritive ingredients in different feeding stuffs, and in what proportions do they occur? (2) In what proportions must their nutritive ingredients be fed in order to produce from a minimum of food, a maximum of flesh (lean), or fat, or both?

Henneberg soon found that his chemical methods did not give the desired results. He also followed the work of Pettenkofer (1862) on quantitative respiration and calorimetric investigations

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with animals; but no new insight into the nature and number of essential nutrients was made.

The beginning of the analysis of food (and of metabolic end products) for the energy contents was made by Frankland (1866) who used a bomb calorimeter and with it provided analytical data in terms of calories. Stohmann (1893) following the lead, provided the caloric value for one gram of most of the important known derivatives produced during the metabolism of proteins in the organism. These included several amino acids, fatty acids, fats, nitrogenous constituents of urine, starch and several sugars.

Schulze (1879) was the notable investigator who described an elaborate method for distinction of different classes of nitrogenous compounds, true proteins, peptones, peptides, nitrate and ammonia. He assumed that by this differentiation between true protein and other nitrogenous substances a more accurate appraisal of the nutritive value of the food could be made.

"Official methods of food analysis" were published for the first time in (1889). Since then the American Association of Official Agricultural Chemists (A.O.A.C.) has under-taken to publish and revise every five years the known methods of food analysis.

Proteins, carbohydrates, fats and minerals as essentials of nutrition :-

The science of nutrition has undergone many changes ever since Albert Thear (1957) put forward the concept of hay equivalents.

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Boussingault (1844) was the first to show, by chemical analysis the effects on animals of foods inadequate in quality. He found that when potatoes or beet roots, (both of which are deficient in proteins) were fed ad libitum as the sole food, they were incapable of preventing loss of weight in cows. In his writings Boussingault (1850) on nutrition considered also common salt which he demonstrated by experiments to be indispensable for the well being of farm animals. He also included potassium, calcium and phosphate as necessary nutrients, thus showing the importance of inorganic minerals in the rations.

Liebig (1803-73) and Henneberg (1825-1890) calculated nutrients for growth and energy and conducted the first digestibility trial. Pettenkofer (1862) analysed the urine and faeces and Kellner investigated energy balances.

Lawes and Gilbert (1866) started their pioneer work in the field of animal nutrition and agronomy at Rothamsted in 1843. They analysed the entire bodies of the farm animals and provided results in the form of percentage water, protein, fat and mineral matter. They also did some work on crop analysis.

Discovery of vitamins and their importance in nutrition :-

In the begining of present century Pekelharing (1905) fed mice a diet composed of a baked mixture of casein, eggs albumen, rice flour, lard and salt, which he called bread, and which he believed to be of physiological importance. Fed together with

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eater, this bread was however unable to support mice and all the animals died within a period of four weeks. When he tried a small addendum of whey with his diet of bread the animals remained healthy. He stated "My intention is to point out that there is still an unknown substance in milk which even in very small quantities is of paramount importance to nourishment. If this substance is absent, the organism looses its power to properly assimilate the well known principal parts of food, the appetite is lost and the animals die of want. Undoubtedly this substance not only occurs in milk but in all sorts of food stuff, both of vegetable and animal origin".

Rohman (1908) reported highly instructive results by feeding small animals on purified diets with only small additions of natural foods. The purified diets appeared to lack nutritive value, while some success was achieved on addition of the natural foods. Stepp (1909) tried to show that fats were essential in diets. His alcohol and ether extracted bread diets failed to support rats even for thirty days. He later added lecithin, cholesterol, and cephalin to the purified diet, but none proved useful. He also prepared a cold alcohol extract from egg yolk and divided it into two portions, one was heated and the other was not heated. These extracts were fed to rats together with the purified diet. All the rats which received the cold extract survived while those to which the heated extract was given died.

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He then concluded "what ever it was that dissolved out of bread with alcohol is of physiological importance to mice, and can be easily destroyed by heating."

McCollum and Davis (1913) while trying their basal diet composed of milk sugar (which was mixed with whey) and the ether soluble fraction of butter observed that the animals failed rapidly when lard or olive oil was substituted for fat. Thus it became evident that hitherto unsuspected nutrient existed and that it was carried by some fats. This constituent was first called 'fat soluble 'A' and later renamed as 'vitamin A'.

In 1922 McCollum and his co-workers demonstrated the existence of anti-rickettic vitamin D and noted that rats when kept indoors, away from direct sunlight, and deprived of this vitamin, developed abnormal bones.

Moore (1929) provided proof that carotene is provitamin A and is converted into vitamin A in the body. He fed highly purified carotene to vitamin A depleted young rats and demonstrated that their livers became rich in vitamin A.

Eijkman and Grijns(1896) described the properties of the dietary factor which cured pigeons of the neuritis produced by feeding them only polished rice. This factor was soluble in water and in dilute alcohol and could diffuse through a semi-permeable membrane.

Many studies followed to isolate this antineuritic vitamin William et al (1934) perfected a method in which the vitamin was

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absorbed on Fullers earth and eluted with a solution of quinine. They further established its structure.

Funk (1914) observed that polyneuritis in pigeons occurred sooner when the carbohydrate content of the diet was high, and Collazo (1923) noted an apparent toxicity from introducing carbohydrates into the crops of pigeons depleted of antineuritic vitamin.

Lohmann and Schuster (1937) made the important discovery that in yeast, a diphosphate ester of vitamin B_1 acts as the co-enzyme, co-carboxylase, for the enzyme carboxylase. Carboxylase functions in the conversion of pyruvic acid into carbon dioxide and acetaldehyde. Co-carboxylase is present in thiamine pyrophosphate chloride, and it is the key substance in biochemical de-carboxylation. It catalyses the de-carboxylation of many α-keto acids. Peter and his associates (1939) presented evidence that vitamin B_1, in the form of its pyrophosphate is indispensable for the removal of pyruvic acid, and indirectly of lactic acid in the normal metabolic scheme.

Morrison and Sarett (1959) gave weanling rats a diet, otherwise adequate, but containing low, adequate or high levels of one B vitamin, in combination with low, adequate or high levels of a second B vitamin. A deficiency of thiamine, pyridoxine or pantothenate significantly reduced weight gain and efficiency of food utilization. Those animals deficient in thiamine and

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another vitamin grew at rates similar to those deficient in thiamine alone. High levels of other vitamins had no influence on the weight gain or efficiency of food utilization by rats deficient in pyridoxine, pantothenate or riboflavin. Similarly, no effects of high levels of pyridoxine, pantothenate or riboflavin were observed on the severity of thiamine deficiency. A study was also made of the effects of administering excess thiamine, riboflavin, pyridoxine or pantothenate to animals deficient in the other three B vitamins. No adverse effect of thiamine, pyridoxine or pantothenate was noted, but excess riboflavin depressed weight gain and food conversion efficiency and increased the mortality of rats which were deficient in other vitamins. In a second series of experiments, the same author (1959) reported that excess thiamine or pyridoxine or both had no effect on the weight gain or the reproductive performance of the animals after parturition and lactation.

Holst and Frohlich (1907) ushered a new era in the study of scurvy. They conducted extensive investigations into the effect of a diet in inducing or curing scurvy. Their guinea pigs remained healthy on a diet of cereals and cabbage; when restricted however to only grain, they developed scurvy and died after 20 to 40 days. They found that supplements of fruits, fresh vegetables or their juices, protected the animals against this disease. They found that 30 g of fresh raw cabbage,

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another vitamin grew at rates similar to those deficient in thiamine alone. High levels of other vitamins had no influence on the weight gain or efficiency of food utilization by rats deficient in pyridoxine, pantothenate or riboflavin. Similarly, no effects of high levels of pyridoxine, pantothenate or riboflavin were observed on the severity of thiamine deficiency. A study was also made of the effects of administering excess thiamine, riboflavin, pyridoxine or pantothenate to animals deficient in the other three B vitamins. No adverse effect of thiamine, pyridoxine or pantothenate was noted, but excess riboflavin depressed weight gain and food conversion efficiency and increased the mortality of rats which were deficient in other vitamins. In a second series of experiments, the same author (1959) reported that excess thiamine or pyridoxine or both had no effect on the weight gain or the reproductive performance of the animals after parturition and lactation.

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cranberries, dandelion leaves, sorrel or carrots prevented the disease. These antiscorbutic foods lost their effectiveness when heated at 100°C. Cohen and Mendel (1918) had produced scurvy in guinea pigs on diets which were complete from a nutritional stand point except that the antiscorbutic vitamin was absent.

The fact that fresh, raw, vegetable food, raw milk and numerous other substances contain an antiscorbutic substance liable to heat, drying and exposure to oxidation, excited great interest among biochemists. Drummond (1920) proposed to name the antiscorbutic substance vitamin C, a name which was widely adopted until 1933, when Szent-Gyorgyi and Haworth suggested that it be renamed ascorbic acid. That vitamin C is very easily destroyed by oxidizing agents and by air was shown by Delf (1920).

Tillmen and associates (1932) discovered a striking correlation between the reducing values of food and their vitamin C content, the reagent 2-6-dichlorophenolindophenol proved to be fairly accurate for the quantitative estimation of vitamin C, in foods except when some other strong reducing substances are present.

Many methods of vitamin assay have since then been described by various authors. The Association of Vitamin Chemists published the methods of vitamin assay in 1947 and revised it in 1957. The methods given in the 2nd edition have been followed in this study.

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Nitrogen balance and Biological value

Trial and experience were the means by which the art of feeding animals was originally developed. Today many of the problems of nutrition are being studied on small animals, such as the rat. The process of growth, reproduction and lactation can be effectively investigated and the value of various feeds for these various functions determined. An important feature of feeding trials which has been developed along with the use of laboratory animals is the employment of purified diets (Maynard, 1951).

Magendie (1816) fed diets of pure sugar and of pure fat to dogs to ascertain whether protein was required in the food. Boussingault, McCollum, Osborne and Mendel (1911) and many others reported the use of purified diets on small animals which led to the discovery of many unknown nutritional requirements.

Nitrogen balance.

In order to determine the quantitative protein requirements of various species and the changes which proteins undergo in living organisms, the time - honoured concept that nitrogen entering the body of mammals as food, is ultimately stored in the form of body protein or eliminated chiefly through urine and faeces has been followed. The Nitrogen balance (NB) is therefore determined as :- NB = NI - (UN + FN) where NI is the Nitrogen intake, UN is Urinary nitrogen and FN is faecal nitrogen.

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Although the basic principle of the method remains unchanged there have been many refinements. The relationship of the nitrogen intake to the nitrogen balance was analysed by Martin and Robinson (1922). They used the term protein utilisation to indicate the percentage of the ingested food nitrogen actually assimilated. Thus the approximate percentage nitrogen

$$\text{utilisation} = \frac{(\text{food N} - \text{faeces N}) \times 100}{\text{food N}}$$

As all the faecal nitrogen does not originate entirely from the food, a correction is necessary for the metabolic or the endogenous nitrogen. To overcome this Mitchel (1923) expressed his results as :-

Biological value =

$$\frac{[\text{food N} - (\text{faecal N} - \text{metabolic N}) - (\text{urinary N} - \text{endogenous N})] \times 100}{\text{food N} - (\text{faecal N} - \text{metabolic N})}$$

values for "metabolic nitrogen" of faeces and "endogenous nitrogen" of the urine were obtained by the Kjeldahl analysis of the excreta collected during non-nitrogenous but isocaloric dietary periods.

In addition to its usefulness in determining the qualitative and quantitative nitrogen needs, the nitrogen balance method has also been employed with success to explore metabolic relationships of various foodstuffs. The capacity of non-nitrogenous nutrients, carbohydrates and fat to diminish the extent of protein catabolism has long been known. Animal and human experiments on

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this subject by the early investigators starting with Voit (1869) were summarised and reviewed by Lusk (1928).

Miller and Payne (1964) have given an integrated view of the interrelationship between nutrients as they affect the nitrogen balance. In quantitative terms.

Nitrogen balance, B = I (E) - M

where, I = Nitrogen Intake

(E) = The efficiency of nitrogen utilization.

M = Nitrogen used for maintenance

The efficiency of utilisation (E) is the proportion of the intake of nitrogen that is retained i.e. net protein utilisation. It may be measured by the balance technique or by body analysis. The various dietary factors influencing (E) have been described as (a) amino acid composition of the protein (b) the protein concentration (c) the caloric intake (d) the level of minerals in the diet (e) the level of vitamins in the diet.

Miller and Payne (1964) have further pointed out that in those countries with staples such as cassava, plantain or sago, positive balance may not be reached, because the protein intake is low. A good deal of emphasis has been placed on the need to consume proteins of high biological value, but the first important factor is the consumption of enough protein.

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Dietary requirements of amino acids.

The nutritive value of a dietary protein is determined by its amino-acid content. Not all amino-acids present in a protein source are available to the animal body especially when the protein is either from a vegetable source or has had some of the amino-acids destroyed or rendered unavailable due to processing. These losses could be substantial. Melnick (1946) showed that 49% of the methionine present in soyabean meal fed to rats appeared in the faeces and therefore was not available to the animal. Kuiken (1952) reported variations in availability of lysine and methionine in a cotton seed meal depending upon the conditions of processing. Albanese (1959) has published data showing that wheat gluten is 35% lower in lysine than whole wheat.

The problem of heat injury to dietary proteins has been reviewed by the Food and Nutrition Board of the National Research Council (1950). This review has emphasized, especially the tremendous loss of lysine that occurs in the toasting of ready-to-eat cereals. This loss may be partly, but not wholly compensated for, when these foods are eaten with milk. Friedman and Kline (1950) showed a nutritive loss of lysine when protein hydrolyzates were autoclaved in 5% glucose. The loss of lysine in heat processed milk has been studied by Mauron and Hodson (1956) the sterilization of commercial evaporated milk and raw milk was found to result respectively in an 11% and 10% loss of lysine.

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Approximately twenty-two amino acids are known to be needed for growth and maintenance of cellular tissue and also for other metabolic functions. Under physiological conditions, eight of these twenty-two amino acids cannot be synthesized by the adult human being and must be present in the diet to permit physiological functions to proceed satisfactorily. These amino-acids are called "essential" or "indispensable". Under normal conditions the remaining amino-acids can be synthesized by the body and thus are not required in the diet. These amino-acids are referred to as "non-essential" or "dispensable" Rose (1952).

The utilization of a dietary protein for anabolic purposes is greatly dependent upon the pattern of the essential amino-acids provided by the protein. This fact was first realized by Willcock and Hopkins (1906) and Osborne and Mendel (1914) who also demonstrated that the more closely the essential amino acid pattern of the dietary protein meets the animal needs the greater is the utilization of the protein. The efficient synthesis of the tissue proteins occurs only when all the essential amino-acids are supplied simultaneously and in proper proportions.

Amino-acid imbalance has been defined by Harper and Kumte (1959) and by Elvehjem (1956) and Salmon (1958). "Amino-acid imbalances occur when the percentage of one (or more) of the amino-acids in a diet is so low that, not only does the efficiency of protein utilization fall, but some additional adverse effect

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such as a drop in food consumption, a depression in growth or an increase in the need for one or more amino-acids becomes evident." To point out the difference between amino-acid imbalance and amino-acid toxicity Salmon (1958) continued, "It should be made clear that amino acid imbalance can be distinguished from certain other cases in which adverse effects are caused by excess of amino-acids. Excessive intakes of certain individual amino-acids such as methionine or tyrosine appear to cause definite toxic reactions. These seem to be highly specific and give rise to symptoms which cannot be prevented by adding a small quantity of the most limiting amino-acid."

Harper and Roger (1965) studied the effects of amino-acid imbalance in diets of rats and concluded that imbalancing the amino-acid mixture, results in an abnormal pattern of amino acids in certain body fluid compartments, by causing a quantitative shift in the normal pathways of amino-acid metabolism, probably by stimulating incorporation of amino-acids into tissues that are actively synthesising protein. In such conditions food intake is deprsssed and hence growth is retarted.

Bender (1965) investigated the correlation between the nutritive value (biologically determined) of proteins and amino-acid mixtures of known composition and their amino-acid make-up. He used egg protein as the standard and evaluated its biological value to be 97% with a surplus of about 20% of all the essential

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amino acids. From the biological values of mixtures of known chemical composition he constructed a curve relating these two measurements. Within the limits of experimental error, the biological values agreed numerically with the corresponding chemical score at biological values greater than 50. Below this value, the curve depended upon the limiting amino-acid. Only when valine or the sulphur amino acids were limiting did the curve follow the theoretical relation to zero. The reliability of forecasting Biological value from the chemical score was tested by the analyses of a number of proteins for their amino-acid content and by comparing the predicted biological value with the value determined by biological assay. The results on UNICEF reference milk, Nigerian dried fish, coconut protein and cotton seed flour showed a high degree of correlation.

It is convenient when comparing food proteins to use a standardized procedure using one fixed level of protein in each test diet. Such experiments can answer some questions, but not how much of one particular type of protein food must be used in order to obtain the desired rate of growth. Where protein A has given a lower value than the protein B, we may still reasonably consider using A rather than B, provided that the total cost of a still satisfactory diet based on A is less than that of a similarly satisfactory diet based on B. The combination of foods available is very great and it is usually very defficult to attempt to predict the quality of a particular diet from its

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chemical composition.

Carpenter and Kuoleasvere (1965) reviewed the problem of high protein diets of unbalanced amino-acid composition in relation to various criteria of predicting quality of protein and have stated that "under certain conditions higher levels of poor protein will result in nearly as good growth as can be obtained with practical diets containing good quality protein. This good growth is obtained notwithstanding an increased requirement for the limiting amino acid with increased protein levels. The possibility of predicting the performance to be obtained with a particular protein depends firstly on the accuracy of chemical score assigned to it, which is in turn governed by 3 factors namely the requirement for the essential amino acids by various species, amino acid composition of the protein and biological availability of these amino acids. Our knowledge of these factors is still not complete and as such great variations are encountered." They further conclude that although amino acid requirements can increase with higher total protein levels the Miller and Payne (1961) equation for predicting this effect may need modification for higher protein diets.

Bassir (1964) has studied the problem of protein nutrition in Nigeria. Using gari and soya, the staple foods of this country he tried various diets containing different proportions

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of these material. None of these were able to stimulate growth in rats to the extent of casein standard diet. He further supplemented these diets with casein and found that 2 : 1 : 1, Gari : soya : casein gave the best results even better than those of the control. He concludes that in 1 : 2 : 2 gari, soya, casein diet there is a marked imbalance of amino acids and gross deficiencies of methionine, lysine, isoleucine and valine while in the 2 : 1 : 1 diet this is corrected to a great extent although it may not attain the provisional pattern (FAO 1957) of the essential amino acids.

In his next set of experiments he has used 1 : 1 gari, soya, as the basal diet and then supplemented it with varying quantities of different amino-acids and casein. He thus concludes that supplementation with both lysine and methionine is required in order to obtain a maximum growth with this 1 : 1 gari soya diet. The amino acid imbalance with respect to tryptophan, valine, isoleucine does not appear to have an effect on the growth of rats. Rose (1948) fed rats on diets containing highly purified amino acids instead of proteins and by successive removal of each amino acid from such diets, established the role of each amino acid. The results demonstrated that for growing rats ten amino acids are essential dietary components. They are valine, leucine, isoleucine, threonine, methionine, phenylalanine, tryptophane, lysine, histidine and arginine. The exclusion

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from the food of any one of these other than arginine, led to profound nutritive failure, loss in weight and death. Arginine deprivation merely decreased the rate of growth.

Rose (1949) determined the minimum daily requirements of amino acids and recommended daily intakes of essential amino acids for man as shown below. It is assumed that the diet furnishes sufficient nitrogen for the synthesis of non-essential amino acids.

Amino Acid	Minimum daily requirement (g)	Recommended daily intake (g)
L-Tryptophan	0.25	0.5
L-Phenylalanine	1.10	2.2
L-Lysine	0.80	1.6
L-Threonine	0.50	1.0
L-Valine	0.80	1.6
L-Methionine	1.10	2.2
L-Leucine	1.10	2.2
L-Isoleucine	0.70	1.4

Interrelationship of nutrients

When the basic principles of nutrition were being established, it was clearly necessary to consider nutrients independently, in order to identify their significance, and to determine quantitative needs. This approach has tended to overshadow the obvious fact that individual nutrients are not consumed in isolation,

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but as a part of diet providing a large number of variable components. For example, the nutritive values of proteins are usually determined under conditions ensuring their maximal utilisation; but such studies do not adequately represent the fate of protein in natural diets, in which not only are the absorbed amino acids derived from several foods but the nutritive value of the mixture is affected by the amounts of other dietary constituents, such as energy yielding nutrients, the minerals and vitamins (Munro, 1964).

When purified, semi-synthetic diets were available, attention changed to a study of the functions of individual vitamins and interest in interrelationships lagged. However during the middle of the present century, attention has been given to the study of this important interrelationship. (Campbell, 1964) has given a comprehensive review of recent findings.

Proteins and Vitamins:- The literature contains many conflicting reports on the relation of protein deficiency and the absorption and utilisation of vitamin A and carotene. Kah, Bhattacharya and Som (1960) for instance reported that when there was insufficient protein or poor quality protein in the diet, the storage of vitamin A was reduced. On the other hand Murray (1961) showed that rats were able to store ample amounts of vitamin A on diets of poor-quality protein or even on a protein-free diet. This finding has also been confirmed by Mathews & Beaton (1963) who

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used diets containing 0, 4, and 20% casein protein. Rechcigl, Berger, Loosli and Williams (1962) assayed the livers and kidneys of rats fed on protein free diet. They found that with increasing proportion of dietary protein there was a progressive decrease in the amount of vitamin A in the liver and an increase of it in the kidney. In rats ingesting an 18% casein diet, less vitamin A was found in the liver, and more in the kidney than in the slower - growing animals given 18% gluten or zein. The efficiency of vitamin A utilization was decreased by protein of inferior quality, but was not affected by the level of dietary protein. Differences observed in total vitamin A per liver were not as great as in the amount per unit weight of liver. Results reported by Mathews and Beaton (1963) suggest that proteins are closely concerned with vitamin A transport.

Friend, Heard, Platt, Stewart and Turner (1961) showed that pigs on low-protein diets had lower contents of vitamin A in their livers than animals on diets adequate in protein. This was considered to be due, both to impaired conversion of carotenoids and to reduced absorption of vitamin A in the protein deficient diets. The concentration of vitamin in the serum was also reduced in animals fed on protein deficient diets. Berger, Rechcigl, Loosli and Williams (1962) reported that although a nitrogen free diet decreased the conversion of carotene and its storage as vitamin A, it did not prevent it. Mathew and Beaton (1963) found that the liver and blood of rats which were given

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carotene contained less vitamin A when the diets were low in protein. In general therefore the data suggest an interference at low protein level with the absorption or conversion of carotene.

Protein - riboflavine Cenoskea and Guggenheim (1946) fed rats on diets in which casein supplied 11, 20 and 39% of the calories and which contained either graded amounts of riboflavin or none at all. On the low protein diet the vitamin content of the organs was low, whatever the amount given and its excretion was relatively high. Apparently the rats could not retain and make use of riboflavin. On the high protein diet more riboflavin was required to maintain the normal level of riboflavin, than was necessary on normal 20% protein diet. The amount of riboflavin required was influenced in the same way by any fat in the diet and the authors suggested that the effect of both protein and fat was to reduce intestinal synthesis of riboflavin. This theory was supported by other work with rats in which the riboflavin requirement was shown not to increase with protein intake when sucrose was the source of carbohydrate. It has been established that animals and man on low protein intake, excrete relatively large amounts of riboflavin, (Bro-Rasmussen, 1958).

Carbohydrates and vitamins :-

Morgan and Yudkin (1957) found that when D-sorbitol was added to a diet deficient in thiamine normal growth occured in rats. Okudu, Hasu and Chen (1960) demonstrated that when

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D-sorbitol was added to a diet deficient in vitamin B_6, it increased the urinary excretion and the concentration in the liver of vitamin B_6; in adult rats, and improved growth rate of weanling rats. Peppler, Muller and Cramer (1960) were unable to confirm the thiamine sparing action of sorbitol in man. Morgan and Yudkin (1962) reviewed this whole field and concluded that there appears to be two types of sparing action involved. The first is the limited sparing action on vitamin B_{12} and folic acid; whilst the second can be demonstrated by experiments on rats and mice, in which they can be made entirely independent of dietary sources of a variety of B vitamins. The data as a whole demonstrate that results may vary markedly with experimental conditions.

Minerals and Vitamins :-

Gershoff and Faragalla (1959) established that the endogenous excretion of oxalate in man, cats, and rats is inversely related to the amount of pyridoxine in the diet. This is important because of the formation of urinary calculi which are largely composed of calcium oxalate. Gershoff and Andrus (1961), while studying the problems of over-nutrition, pointed out the defects of high calcium diets. They were of the opinion that under circumstances favourable to the production of calcium oxalate, the intake of low calcium diets may be advisable.

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Protein - Calorie :-

Vera Cabak and Dickerson (1963) investigated the response of young rats when deprived of proteins or of calories they also studied the plasma protein patterns of these rats. It was concluded that malnutrition whether brought about by a low protein diet (cassava and banana) or a restricted high protein diet affected the concentration of plasma protein and urea, Both decreased B globulins but did not appreciably change the proportion of albumins. Striking similarity between the plasma protein patterns in such malnourished rats was shown by paper electrophoresis.

Dietary preparation of foodstuffs :-

The effects of cooking or processing of food vary with the type of food, the duration and severity of the process, and the size and condition of the portion of food. The most sensitive of all the nutrients are the vitamins. Several, but not all, may suffer considerable loss on processing. Most proteins do not suffer damage unless they are severely heated or stored for long periods. Losses of carbohydrates and mineral salts occur in wet processing by leaching but usually can be ignored. (Bender, 1966).

Ezell and Wilcox (1962) found that as much as 75% carotene in Kale and Collard is lost in 4 days when stored at 70 °F under conditions of rapid wilting. At 50 °F there are 20% loss on slow wilting and 30% loss on rapid wilting. When wilting was prevented and Kale stored at 32 °F there was only 2% loss of

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carotene in 4 weeks.

Both vitamin A and carotene are stable to mild cooking and processing, but are destroyed at high temperature, in presence of oxygen. Maqsood, Haque and Khan (1963) reported the loss of vitamin A in enriched ghee and vanaspati. Frying at 200 °C caused a 40% loss in 5 min.; 60% in 10 min. and 70% in 15 min. Boiling in water caused 16% loss in 30 min. 40% in 1 hr. and 70% in 2 hr.

Adam, Mercer and Stanworth (1942) have pointed out that greatest losses during processing are caused by leaching out of water soluble vitamins. The smaller the state of sub-division of the food the more is leached out. The amount lost also depends upon the temperature and time of water treatment. Steam blanching causes smaller losses than hot water treatment. Vitamin B_1 is stable to acid even at boiling point and up to 120 °C; but unstable at neutral or alkaline pH. Roy and Rao (1963) reported that cooking in tap water caused 8-10% loss whilst cooking in well water reduced the loss by 36%. Again when large volumes of water are used for cooking (10-15)volumes as in the case of rice gruel) the loss of vitamin B_1 can be as great as 80%. This was due to the alkalinity and not leaching as the same preparation in distilled water lost only 5% B_1.

Zacaringer and Personius (1949) showed that toasting bread for 30-70 seconds results in loss of 10-30% of thiamine. Cuendet et al (1954) reported that cereals when stored as whole grain can suffer a loss of thiamine depending upon the moisture content.

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In one series of observations there was a 30% loss during 3 months storage at 17% moisture; 12% loss at 12% moisture; and no loss after one year, when the moisture was reduced to 6%. Fish was found to lose up to 50% of its thiamine on boiling and 75% on canning, and eggs lose some of it on cooking. One report gives the loss in eggs as 9% when scrambled and 29% when they were boiled (Lane, Johnson and Williams, 1942).

Vitamin B_2 is stable to oxygen and to acid conditions, but unstable to light and to alkali. Heat alone is not harmful. Its sensitivity to light leads to the destruction of both vitamin B_2 and vitamin C in milk. About 50% vitamin B_2 in milk can be destroyed in 2 hrs. by exposure to bright sunlight and 20% on a dull day. The vitamin B_2 is coverted into lumiflavin and this destroys vitamin C. (Harris and von Loesecke 1960).

There is evidence of a deficiency of pyridoxine (vitamin B_6) in pregnancy as indicated by the inability of some individuals to metabolise completely a test load of tryptophan until extra pyridoxine has been administered (Hunt 1957). This may be due to the lack of B_6 in the food. Bunting (1965) examined the stability of vitamin B_6 when added to food. Maize meal when stored for 1 year at 100 °F and at 100% relative humidity, retained 90-95% of the added vitamin. When maize was baked into bread, the recovery was 100%. Macaroni retained 100% of the added vitamin on storage but lost 50% in water when macaroni was cooked.

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Vitamin C is perhaps the most readily destroyed of all the vitamins and its retention is often used as an index of the severity of processing and storage conditions. It is oxidised in air under alkaline conditions and this reaction is catalysed by traces of copper. The greatest losses are due to leaching into processing water. The amount lost depends, on the amount of water rather than on the time of treatment. Vegetables when fully covered with water may loses 80% of the ascorbic acid, when half covered 60%, and when quarter covered 40%. The amount destroyed may be quite small compared with leaching losses. A sample of cabbage cooked in 10 volumes of water lost 10% by destruction whilst 80% was lost into cooking water, only 10% was retained. (Bender, 1960).

In an examination of hospital diets, Platt, Eddy and Pellett (1963) showed that when peeled potatoes were soaked in water overnight, they lost 45-60% of their vitamin C content. The authors pointed out that this loss was due to mechanical peeling as leaching does not occur if tissues are undamaged. Hand peeling followed by 14 hrs. soaking in water caused an average loss of 9% of vitamin C.

Oke (1966) has reported losses of ascorbic acid in some Nigerian foods during cooking. Yam when cooked lost 51%, cassava 67%, and sweet potato 63%. He observed negligible losses when yam flour or cassava flour were cooked. In plantain, he observed 64% loss of ascorbic acid.

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Mineral salts.

The loss of minerals into the processing water is not a matter of nutritional importance. The reverse process in which the foods absorb minerals from the water may be of greater significance in certain instances. It can be a serious problem in the preparation of low-sodium foods for dietetic purposes. In one series of observations, the sodium content of peas which was 1.7 mg/100 g. was increased to 12 mg/100 g. For this reason water containing more than 10 mg/100 ml. sodium is considered unsuitable for cooking, (Bender, 1966).

Calcium can also be absorbed by foods from hard water (Horner 1936-37) but the amounts involved are not great enough to be nutritionally significant.

Proteins

Damage to proteins may be of two main types (a) destruction of amino acids and (b) combination of part of one or more of the amino acids in a linkage that is not hydrolysed during digestion. There can also be small losses of soluble proteins leached into the cooking water; but this mostly affects vegetables.

Lee (1958) and Ellis (1959) reported combination between the amino group of amino acids and reducing groups present in the foodstuff, and also between the amino groups and carbonyl compounds formed by the oxidation of fats. These links cannot be hydrolysed by digestive enzymes, hence the amino acids

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become unavailable. As these compounds can however be hydrolysed by the acid treatment, the chemical estimation may give misleading results. Bender (1958) has therefore emphasized the need of biological assay methods.

Harris and Von Loesecke (1960) reported that lysine can react with reducing substances, via its amino group projecting from the protein molecule. This loss of available lysine can occur with relatively small amounts of reducing substances. Dried egg containing 8% protein and 3% glucose will deteriorate both in nutritional value and flavour during prolonged storage, If by treatment with yeast or glucose oxidase this small amount of glucose is removed, it can be stored without harm.

Iriarte and Barnes (1966), and Ford (1962) have shown that not only lysine but other amino acids in some instances are also affected in a similar manner sometimes even more than lysine.

Bressani et al (1963) determined the effect of cooking and of amino-acid supplementation on the nutritive value of black bean (Phaseolus vulgaris). Diets varying in protein content from 14.1 to 17.2%, cooked for a time varying from 0 to 40 minutes and supplemented with methionine, leucine, tryptophan, lysine, valine, phenyl alanine, isoleucine and threonine, in different combinations, were fed to rats. It was concluded that pressure cooking from 10 to 30 minutes did not decrease the nutritive value of beans but that cooking for more that 30 minutes did decrease the nutritive value. Home cooking in an open kettle did not decrease the nutritive value. No apparent

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change in the methionine, lysine or valine content of bean flour resulted from any method of cooking. The content of the free amino group of lysine however decreased in pressure cooked samples, except for the 40 minutes cooked sample. This did not occur in those cooked in an open kettle. 0.1% supplementation with DL-methionine in the diet produced significant increase both in protein efficiency ratio and in weight gain of the rats when compared with the group on unsupplemented diet. Further additions of methionine (up to 0.3%) produced proportionately smaller increase. When various combinations of amino-acids were added to the diet, none of them improved the weight gains beyond the value obtained by the addition of methionine alone. Methionine together with tyrosine and methionine together with lysine however improved the protein efficiency ratio beyond the values obtained on methionine alone. When other amino-acids were added in the presence of methionine it did not increase the value any further.

Mary (1949) studied the effect of cooking on meat. It was found that during cooking of meat, ascorbic acid was totally lost, aneurin largely lost and riboflavin was only slightly affected. Nicotinic acid survived well in most of the cooking methods.

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CHAPTER 2

Materials

(a) Sources and nature of foodstuffs

Investigations were carried out on the following six diets, commonly used in Nigeria.

1. Eko and akara.
2. Pounded yam, efo, and snails.
3. Elubo, egusi soup and ponmo.
4. Fried plantain and beef stew.
5. Gari, okro soup and dry fish.
6. Moinmoin with shrimps.
7. For the purpose of comparison a semi-synthetic diet consisting of Amylum (maize starch), casein, a mineral mixture and a vitamin mixture, known to contain the optimum requirements for the rats, was also studied.

All the food materials were bought in the local market and quickly brought to the laboratory for analysis and preparation of the diets. Ogi (wet maize starch) was bought for the preparation of eko and beans (*Vigna unguiculata*) were bought for the preparation of akara and moinmoin. Pounded yam was prepared in a local restaurant so as to be able to use a wooden mortar and pestle for pounding. All the other foods were cooked in the laboratory.

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The generally accepted recipes and methods of preparation which also closely agree with the various cooking books (William 1962) were followed. The methods of preparation of these diets are described below. The actual quantities of various ingredients used for the preparation of each diet are shown in table No. 1 to 7 on page 43 to 49.

DIET NO.1. Eko and Akara

Eko:-

Ingredients: Ogi (Wet maize starch)

Method:- To a smooth paste of ogi, boiling water was added until it thickened, stirring all the time to stop lump formation. The material was then heated for 15 minutes in order to cook the starch.

Akara:-

Ingredients: White beans, onion, red pepper, palm oil and salt.

Method:- The beans were soaked in water till the skin became soft. The skin was removed by gentle rubbing in the hands and the remaining portion was ground until it was smooth, then beaten with fingers until light. Ground onion, pepper and salt were added and fried in small portions in palm oil until brown.

DIET NO.2. Pounded Yam, Efo and Snails

Pounded Yam:-

Ingredients: Yam tubers.

Method: The yam was peeled, cut into pieces and boiled in

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water until soft. Water was strained off, the yam put in a wooden mortar, and pounded until smooth and elastic to touch.

Efo and Snails.

Ingredients : Efo (*Crotalaria* spp.), snails (*Vivapera quadrata*), pepper, salt, tomato, palm oil, onion.

Method: Efo leaves were removed from the thick stems and cut into about one inch pieces. They were then boiled and the water strained off. Snails were removed from the shells and then washed with alum to remove all saliva, then cut into pieces and allowed to cook in their own water with some salt until soft. Palm oil was added and the snails were fried. Ground tomato, salt, onions and pepper were then added, and frying continued for another five minutes. Boiled efo was then added and cooked for about 15 minutes, stirring occasionally.

DIET NO. 3. Elubo, Egusi soup and Panla (dried fish).

Elubo.

Method: Yam flour was sprinkled in some hot water and stirred with a wooden spoon. The yam flour was added a little at a time and stirred vigorously to avoid lumps. Some more hot water was added and the mixture was allowed to cook until it was smooth and of a soft consistency.

Egusi soup and Panla.

Ingredients: Panla, egusi (melon seed), palm oil, tomato, onions pepper and salt.

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Method: Panla was cut in small pieces and boiled in a little water with some salt until soft. Ground tomato, onion, pepper and salt were fried in palm oil. Then ground egusi and boiled pieces of panla were added and allowed to cook for about ½ hour more.

DIET NO.4. Fried Plantain and Beef Stew

Fried Plantain

Ingredients: Plantain, palm oil.

Method:- Plantains were peeled and cut in to about 3" long pieces. There were fried in hot palm oil until brown.

Beef stew

Ingredients: Beef, palm oil, tomato, onion, salt and pepper.

Method: Small pieces of beef were boiled with a little salt and water. Ground tomato, onion, pepper and salt were fried in palm oil and pieces of meat were then added. The mixture was fried for sometime after which the water left from the meat was added and the mixture was allowed to cook until the meat was quite soft.

DIET NO. 5 Gari, Okro soup and Dry fish

Gari:

Ingredients: Gari (dried grated cassava tuber).

Method: Gari was added to boiling water and continuously stirred to form a smooth paste.

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Okro soup and dry fish

Ingredients: Okro, dry fish, palm oil, pepper and salt.

Method: Okro was sliced into small pieces about ¼" long and put in the boiling water. The dry fish was cut into small pieces, and pepper and palm oil were added. The mixture was allowed to cook and then salt was added.

DIET NO. 6. Moinmoin with Shrimps

Ingredients: Beans, shrimps, eggs, palm oil, tomato, onions, pepper and salt.

Method: Beans were soaked in water and the skins rubbed off. They were then ground to a paste. Tomatoes, onions, pepper and salt were ground separately. Beaten eggs, shrimps and all the ingredients were mixed with warm palm oil and rolled in leaves in small portions. They were then steamed in a big pan by placing a perforated bottom in between the boiling water and the leaf rolls. Keeping the top of the pan loosely covered, the steaming was done for about one hour in order to cook the starch properly.

DIET NO. 7. Standard

Ingredients: Amylum (maize starch); casein, butter fat, mineral mixture and vitamin mixture, as shown in table 7.

Method: The ingredients were mixed properly by adding a little water to bring it in the form of a paste.

Formulation of the diets

All the diets from No. 1-5 essentially consist of two dishes.

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One portion is generally some form of starch while the other is a stew, containing meat, fish or some other form of protein. To ascertain as to what quantity of one dish will be consumed with the other a survey was carried out. Eleven persons were invited and asked to take the quantities of each dish they would like to consume at one time. These quantities were weighed and median values were taken to be the amount of one dish which will be consumed together with the other in case of each diet. In the case of each diet, the two dishes were mixed in this fixed ratio. Care was taken to homogenise each dish before and after mixing so as to get a uniform distribution. The process was repeated for all the diets from 1-5. Diet No. 6 was a single dish. The results of this survey are presented in Table Nos. 1 to 6 on pages 43 to 48 along with the actual recipes.

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TABLE I

(a) Recipe for diet No. 1 Eko and Akara.

EKO		AKARA	
Name of ingredient	Wt. Used in g.	Name of ingredient	Wt. Used g.
Ogi (Wet maize starch)	990	Beans	2620
		Onions	455
		Pepper	103
		Salt	59
		Palm oil	575
Total Wt. raw mixed	990	Total Wt. raw mixed	3812
Total Wt. prepared	6608	Total Wt. prepared	3290

(b) Relative weights of Eko/Akara per meal as chosen by different individuals

Subjects	Quantity of Eko in g.	Quantity of Akara in g.
1	250	23.2
2	250	35.0
3	250	110.0
4	250	33.8
5	250	84.5
6	250	60.0
7	250	122.3
8	250	78.9
9	250	47.6
10	250	88.0
11	250	86.4

Median 250 : 78.9
Mixing ratio for cooked foods = 250 g. Eko with 78.9 g. Akara
Corresponding amount of raw mixed foods = 37.4 g. ogi with 91.3 g. of raw mixed ingredients of akara.

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(b) Relative weights of Eko/Akara per meal as chosen by different individuals

Subjects	Quantity of Eko in g.	Quantity of Akara in g.
1	250	23.2
2	250	35.0
3	250	110.0
4	250	33.8
5	250	84.5
6	250	60.0
7	250	122.3
8	250	78.9
9	250	47.6
10	250	88.0
11	250	86.4

Median 250 : 78.9
Mixing ratio for cooked foods = 250 g. Eko with 78.9 g. Akara
Corresponding amount of raw mixed foods = 37.4 g. ogi with 91.3 g. of raw mixed ingredients of akara.

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TABLE II

(a) Recipe for diet No. 2. i.e. Pounded yam, efo, and snails

Pounded Yam		Efo and Snails	
Name of ingredient	Wt. Used in g.	Name of ingredient	Wt. Used in g.
Yam	5900	Snails	1000
		Efo	1000
		Onion	350
		Tomato	120
		Pepper	50
		Palm oil	500
		Salt	30
Total Wt. raw mixed	5900	Total Wt. raw mixed	3050
Total Wt. prepared (cooked)	7264	Total Wt. prepared (cooked)	2334

(b) Relative weights of pounded yam/efo and snails soup per meal as chosen by different individuals.

Subjects	Wt. of Pounded Yam in g.	Wt. of Efo and Snail soup in g.
1	349	65
2	340	93
3	339	133
4	262	112
5	206	74
6	407	58
7	534	148
8	509	264
9	249	186
10	205	49
11	421	138

Median 340 : 74

Mixing ratio for cooked food - 340 g. of pounded yam with 74 g. of stew

Corresponding amount of raw mixed food = 276.1 g. of raw yam with 95.8 g. of raw mixed wt. of stew.

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TABLE III

(a) Recipe for diet No.3 i.e. Elubo, Egusi,soup and Panla

Elubo		Egusi soup with panla	
Name of ingredient	Wt. Used in g.	Name of ingredient	Wt. Used in g.
Elubo	1447	Egusi	250
		Panla	250
		Onions	225
		Pepper	30
		Tomato	150
		Salt	52
		Palm oil	275
Total Wt. raw mixed	1447	Total Wt. raw mixed	1232
Total Wt. prepared (cooked)	4512	Total Wt. prepared (cooked)	2326

(b) Relative Wt. of Elubo/Egusi soup and panla per meal as chosen by different individuals

Subjects	Wt. of Amala in g.	Wt. of Egusi soup and panla in g.
1	576.0	136.2
2	574.0	176.2
3	515.5	86.5
4	389.01	86.0
5	182.0	61.0
6	620.0	59.5
7	517.0	86.0
8	670.0	77.5
9	697.5	135.5
10	320.7	89.5
11	232.0	40.5

Median = 517 : 86
Mixing ratio for cooked foods = 517 g. of elubo with 86 g. of soup
Corresponding amount of raw mixed foods = 165 g. elubo with 45.5 g. of raw mixed soup.

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TABLE IV

(a) Recipe for diet No. 4 i.e. Fried plantain and Beef stew

Fried Plantain		Beef Stew	
Name of ingredient	Wt. Used in g.	Name of ingredient	Wt. Used in g.
Plantain (peeled)	3000	Beef	600
Palm oil	241	Onion	142
		Tomato	125
		Palm oil	225
		Salt	30
		Pepper	20
Total wt. raw mixed	3241	Total Wt. raw mixed	1142
Total Wt. prepared (cooked)	2210	Total Wt. prepared (cooked)	994

(b) Relative Wt. of Fried plantain/Beef stew per meal as chosen by different individuals

Subjects	Fried plantain in g.	Beef stew in g.
1	122.0	76.0
2	222.0	106.0
3	297.0	117.0
4	219.0	59.0
5	206.0	107.0
6	167.0	25.0
7	128.0	61.0
8	235.0	124.0
9	232.0	75.0
10	257.0	92.0
11	246.0	45.0

Median = 222 : 76

Mixing ratio for cooked foods = 222 g. fried plantain with 76 g. stew

Corresponding Wts. of raw mixed foods = 325.5 g. of raw mixed plantain with 87.3 g. of raw mixed stew.

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TABLE V

(a) Recipe for diet No. 5 i.e. Gari, Okro and dry fish.soup

Gari		Okro and dry fish soup.	
Name of ingredient	Wt. Used in g.	Name of ingredient	Wt. Used in g.
Gari	1300	Dry fish	170
		Okro	240
		Palm oil	250
		Salt	25
		Pepper	17
Total Wt. raw mixed	1300	Total Wt. raw mixed	702
Total Wt. prepared (cooked)	4280	Total Wt. prepared (cooked)	1445

(b) Relative wt. of Gari/Okro soup and dry fish per meal chosen by different individuals

Subjects	Gari (Eba) g.	Okro soup and dry fish g.
1	414.0	167.0
2	316.0	126.0
3	432.0	126.0
4	411.0	151.0
5	414.0	141.0
6	273.0	123.0
7	481.0	89.0
8	272.0	123.0
9	440.0	148.0
10	262.0	162.0
11	220.0	172.0

Median = 411 : 141
Mixing ratio for cooked food = 411 g. gari : 141 g. soup
Corresponding Wt. of raw foods mixed = 124.8 g. of gari with 68.4 g. of raw mixed soup.

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TABLE VI

(a) Recipe for diet No. 6 Moimmoin

Name of the ingredient	Wt. Used in g.
Beans	1500
Onion	622
Palm oil	513
Shrimps (dry)	55
Eggs (Wt. without shells) 6 in No.	272
Tomato	170
Pepper	60
Salt	80
Total Wt. raw mixed	3272
Total Wt. prepared (cooked)	5802

(b) Being a single dish, no mixing was required.

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TABLE VII

Composition of Control diet

Name of the ingredient	Wt. Used in g.
Amylum (Maize starch)	700
Casein	250
Butter (pure cream butter, "Danish butter")	50
Vitamin mixture	20
Mineral mixture	20
Water to make it into a paste	

The mineral mixture consisted of the following as described by Hubbell, Mendel and [illegible] (1937)

$CaCO_3$	54.300 g.
$Mg\ CO_3$	2.500 "
$Mg\ SO_4$	1.600 "
$NaCl$	6.900 "
KCl	11.200 "
KH_2PO_4	21.200 "
$Fe\ PO_4.4H_2O$	2.050 "
KI	0.008 "
$Mn\ SO_4$	0.035 "
NaF	0.100 "
$Al_2\ (SO_4)_3.K_2\ SO_4$	0.017 "
$CaSO_4$	0.090 "
	100.000

The vitamin mixture consisted of the following:-

Vitamin A	=	5000	i.u	per ml
Vitamin B_1	=	2 mg.		"
Vitamin B_2	=	1 mg.		"
Vitamin B_6	=	10 mg.		"
Vitamin C	=	50 mg.		"
Vitamin D	=	1000 i.u.		"
Vitamin E	=	3 mg.		"

To this mixture 2 mg. each of pyridoxine and choline along with 50 µg. of B_{12} and 1 mg. of Vitamin K were added so as to bring it up to the optimum standard of vitamin requirements of rats as described by Cuthbertson (1957).

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CHAPTER 3

METHODS

(a) Dry matter (Association of Official Agricultural Chemists) official method.

10 g. of a representative sample of food was dried to a constant weight, in a tared moisture dish by placing it in a hot air oven at 100 °C ± 2, and subsequent cooling in a desiccator.

(b) Total Proteins

Total nitrogen was estimated by the Kjeldahl's method as recommended by the Association of Official Agricultural Chemists (A.O.A.C. methods of analysis 1960) for food samples.

The samples were digested with conc. H_2SO_4 and potassium sulphate using mercuric oxide as catalyst so as to convert all the organic nitrogen into ammonium sulphate. A measured volume of this digested material when treated with excess alkali liberated ammonia which was quantitatively collected in 4% boric acid and titrated with standard acid.

The quickfit micro-Kjeldahl's steam distillation apparatus (Gallenkamp No. RR-50) was used for the distillation of the ammonia. The protein was estimated by multiplying the amount of nitrogen with the factor 6.25.

Reagents.

Sulphuric acid. Analar Sp. gr. 1.84 (nitrogen free).

Mercuric oxide (reagent grade).

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Potassium Sulphate, (nitrogen free).

Boric Acid A 4% solution was prepared.

Sodium Hydroxide-Sodium Thiosulphate solution 500 g. of NaOH and 40 g. $Na_2S_2O_3.5H_2O$ were dissolved in water and made to 1000 ml.

Indicator. Methyl red-bromocresol green solution. 1 part of 0.2% alcoholic methyl red solution was mixed with 5 parts of 0.2% alcoholic bromocresol green solution.

Method.

1 g. of a chosen sample was transferred into a kjeldahl digestion flask and to it was added 0.7 g. mercuric oxide and 15 g. powdered potassium sulphate followed by 25 ml. concentrated sulphuric acid. The flask was placed inclined on the digestion rack and heated gently until frothing ceased. The contents of the flask were then boiled briskly until it cleared and then for another 30 minutes longer. The flask was cooled and the digestate transferred with distilled water into a 200 ml. flask (volumetric) and made to mark. 10 ml. aliquots from the volumetric flask were taken in the microkjeldahl distillation flask followed by the sodium hydroxide - sodium thiosulphate solution until the mixture was alkaline; about 10 ml. of the alkali was sufficient. The contents were then steam distilled into a

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receiver containing 10 ml. 4% boric acid solution and 2 drops of the indicator solution. About 40 ml. of the distillate were collected and titrated against 0.01 N. HCl until the first appearance of violet colour.

(c) Amino acids

On the report of Spackman, Stein and Moore (1958), Hennig (1959) described an apparatus for the quantitative estimation of individual amino acids in protein hydrolysates. This apparatus as manufactured by Messrs Bender and Hobein, Munchen, West Germany was used for the determination of amino acids in food hydrolysates.

The method consists of using ion-exchange resin columns of suitable length and diameter packed with synthetic sulphonated cation exchange resins of very fine particle size. A small quantity of (0.2 - 0.4 ml. corresponding to 1-2 mg. protein) of hydrolysate is applied and individual amino-acids are eluted by the citrate buffers at a gradient pH. 3-7. This process takes place under highly standardised conditions of flow rate, temperature and pressure on the column. The eluate is mixed at a constant flow rate with a specially prepared ninhydrin reagent and is passed through a capillary tube 20 metres long, coiled and dipped in a boiling water bath. The blue color of the ninhydrin complex develops in 20 minutes. It is then passed through an

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automatic recording colorimeter (modified Elphor Integraph) by which the transmissions are recorded on a graph sheet every 20 seconds at two different wave lengths (436 $m\mu$ and 578 $m\mu$) giving two Gaussian curves. The areas under the individual peaks as related to the concentration of each individual amino acid, can be calculated by reference to standard curves.

<u>Description of the apparatus</u>

A labelled photograph (plate 1.) showing various parts of the amino acid analyser is given on page 54. The different parts are the following :-

P = Switch panel for operating the assembly.

G_1 = Syringe filled with ninhydrin reagent

G_2 = Syringe filled with buffer pH 3.12

G_3 = Syringe filled with buffer pH 5.12

G_4 = Syringe filled with buffer pH 7.0

M = Mixing vessel mounted on a magnetic stirrer

Tr_1 and Tr_2 = Large column 130 cms. long for the elution of acidic and neutral amino acids, filled with Dowex 50 resin enclosed in a hot water jacket at 50 °C.

Tr_3 = Short column 50 cms. long for the elution of basic amino-acids filled sith Amberlite (RC-50 enclosed in a hot water jacket at 40 °C.

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Plate I

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AMINO ACID ANALYSER
(Bender & Hobein 1959).

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Th = Thermostat with pumping device to circulate hot water in the outer jackets of the columns.

U = Contact thermometer

H = Heating bath

K = Water bath containing 20 metre of coiled plastic tube submerged in boiling water; a condenser is fitted at the top.

Ex = Elphor integraph

R = Recorded graph sheet as it comes out of the integraph.

Modifications.

Shortly after assembling the apparatus as described it was realised that the separation of the amino-acids on the short column was not sharp; this was probably due to the quality of resin. The manufacturers when contacted were unable to give a satisfactory explanation. However they suggested the use of Aminex A-4 resin manufactured by M/S Bio-RAD Laboratories Richmond Calif : U.S.A. on both the column. Consequently, the following modifications were made.

1. Change of column Instead of the 130 cm. long column and the 30 cm. short column; only a 50 cm. long column and a 12 cms. short column were fixed.

2. Change of buffers Sodium citrate buffers of pH 3.2 and pH 4.2 were used on the long column instead of 3.1 and pH 5.1. Similarly a buffer pH 5.28 was used on the short

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column instead of pH 7.0. The rate of elution on the short column was also changed from 30 ml./hr. to 60 ml./hr.

3. <u>Temperature</u> The temperature of both columns was maintained at 50 °C during elution.

4. <u>Elution of ammonia and arginine</u> After the elution of histedine 40 ml. of N/40 NaoH were passed through the short column so as to hasten the elution of ammonia and arginine. The passing of alkali also served to regenerate the column.

An assembly of the apparatus is shown in the plate 2 on page 57.

These modifications reduced the elution time on the large column from 16 hours to 12 hours and on the short column the run could now be completed in 3½ hours instead of 4½ hours.

<u>Reagents.</u>

Buffers of the various pH were prepared using the quantities of various chemicals as given in table No. 8 on page 58. All the buffers were adjusted to the correct pH using a pH meter and then boiled to exclude all air. Finally 1 ml. of caprylic acid was added to each to prevent the growth of fungi. They were then covered with a layer of liquid paraffin and stored in an air-conditioned room in plastic bottles.

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AMINO ACID ANALYSER MODIFIED

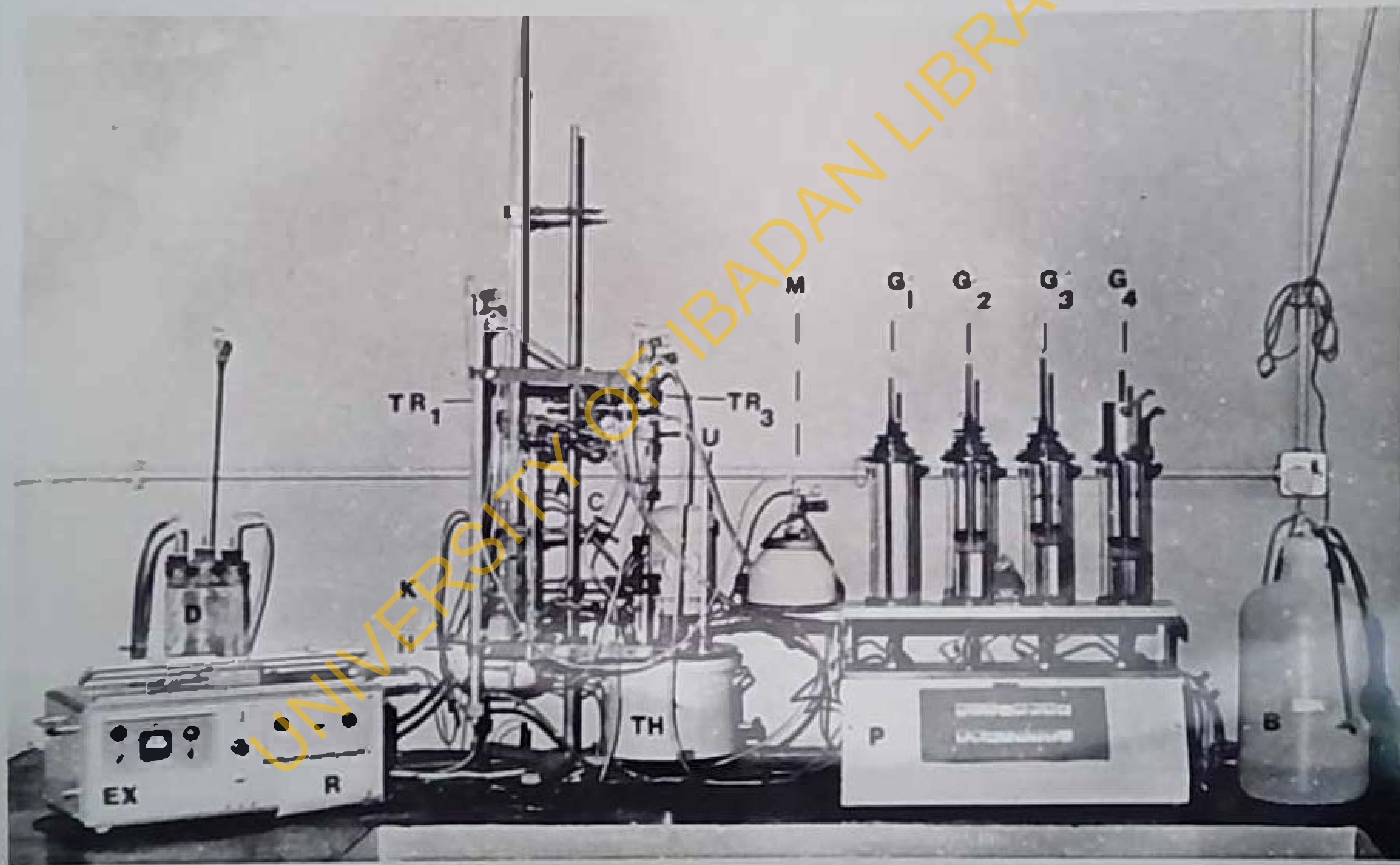

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TABLE 8

Quantities of various chemicals used for the preparation of buffers.

Buffer	pH	Citric acid-monohydrate g.	Sodium Hydroxide g.	Sodium citrate g.	Sodium acetate g.	Hydrochloric acid 32% ml.	Acetic acid glacial ml.	Thiodiglycol ml.	Polyethylene-glycol monolauryl ether ml.	Volume made up Lit.
1	2.2 ± 0.05	105	42	-	-	60.0	-	-	-	5
2	3.1 ± 0.02	210	83	-	-	135.0	-	50	50	10
3	3.22	-	-	196	-	123.5	-	50	20	10
4	4.25	-	-	196	-	83.7	-	50	20	10
5	5.1 ± 0.02	105	47	-	136	-	20	50	50	10
6	5.28	-	-	345.5		65.0			20	10
7	5.5	-	-	-	360	-	250	-	-	2.5
8	7.0 ± 0.02	210	120							5

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Preparation of ninhydrin reagent

Methyl cellosolve was distilled using 1% mixture of ferrous sulphate in H_2SO_4 so as to remove any peroxides; until it gives no yellow colour with KI.

750 ml. of methyl cellosolve were then mixed with 250 ml. of buffer pH 5.5. To displace air nitrogen was passed (after passing through H_2SO_4) for 15 minutes. Then 20 g. of ninhydrin were dissolved in the mixture 0.4 g. of $SnCl_2 \cdot 2H_2O$ were then added, and immediately a layer of paraffin was put over. The reagent was stored in a plastic bottle in an air-conditioned room.

Preparation of hydrolysates.

0.5 g. of the dry food sample was taken in a pyrex tube 100 ml. capacity and 75 ml. of 6N HCl were added. Oxygen free nitrogen was then passed through the solution for half an hour, and tubes were immediately sealed. They were then placed in a hot air oven at 105 °C for a period of 24 hours. The tubes were removed, cooled, and the sealed end cut open. The solution was filtered through a Whatman No.1 filter paper and a calculated quantity corresponding to 1-2 mg of the protein was evaporated to dryness at low temperature. This was done in a desiccator containing a mixture of sodium hydroxide and calcium chloride and by applying mild suction by means of a water pump. The dry hydrolysates were then taken up in a known volume of buffer pH 2.2.

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Elution on the acidic column.

0.5 ml. of the sample (hydrolysate in buffer pH 2.2) was applied at the top of the column Nr. 2 (plate 1 page 54) and the sample was allowed to soak in completely for about 45 minutes. 0.4 ml. of the buffer pH 2.2 was then again applied to the top of the column so as to wash in the sample completely and soak it in the column. A 2nd washing with the same buffer, was given and when this had also soaked in, the column was filled up to the top with the same buffer and connection made with the pump G_2 containing buffer pH. 3.1 via mixing vessel "M".

In the meantime the thermostat TH., had already been set on and the contact thermometer set to 50 °C. The hot water circulation was also set on, and the column was now at 50 °C. The ninhydrin reagent and buffer pH. 3.1, and pH 5.1 were filled in the syringes G_1, G_2 and G3, respectively, taking care that no air bubble got in during the operation. The connection was made with the mixing vessel M, and the buffer pH 3.1 was allowed to run in to the column via the mixing vessel M, at the rate of 30 ml. per hour, by pressing the appropriate controls on the panel P. The eluate leaving the column was connected to the mixing tube M_2. Simultaneously the water bath, integraph registration and ninhydrin were switched on. After 20 minutes the adjustment of zero was

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carried out on the integraph, on which two curves of wave lengths of 436 m/u and 578 m/u were recorded simultaneously.

If cysteic acid was present it appeared first from the eluate, the amino acid aspartic acid appeared after about two hours and then the others as shown in the diagram on page 63A Cystine when present appeared between alanine and valine.

Elution on the short column.

The basic amino acids were separated on the 12 cm. column, filled with Aminex A-4 resin. 0.5 ml. of the sample (hydrolysate in buffer pH 2.2) was applied at the top of column Nr. 3 (plate 2 page 57) and the sample was allowed to soak in as described under elution on the long column. Appropriate controls on the panel P were operated and the elution was started with buffer pH 5.28 at 60 ml./hr. Zero adjustment was made on the integraph 20 minutes after starting the elution. First of all a peak was obtained from the acidic and neutral amino acids which appeared together. The first basic amino-acid lysine appeared after one hour and then came histidine, ammonia and arginine. After the appearance of the histidine peak the pH of the eluting buffer is raised, by passing 40 ml. of the N/40 sodium hydroxide through the column. This is done by closing the pinch cork C and allowing the buffer to push out N/40 sodium hydroxide from the alkali tube A. This is then passed over the column. By doing so

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the ammonia and arginine came out quickly. After passing the alkali the pinch cork C is opened again and the buffer is allowed to pass through the column for one more hour. A typical elution pattern of the various amino acids is shown in the figure 63.1 on page 63A

Regeneration of the columns. No special regeneration of the short column is necessary because the N/40 alkali which is passed during the elution also regenerates the column. On the long column immediately after a run is over, about 100 ml. of the 0.2 N sodium hydroxide are passed through under pressure from the bottle D, followed by 100 ml. of buffer pH 3.28.

Standardisation of the instrument

(i) The positions of the peaks of the various amino acids were marked as they appeared on the graph sheet, and the time taken to eluate them from the column was noted by running various amino acids first singly and then all of them in a mixture.

(ii) To check the linearity of the recovery of the amino acids solutions in three different concentrations were prepared and run on the column. The areas under each peak were calculated and plotted against the concentration. The results for 4 different amino acids are presented in the figure 63.3. It will be seen that in the case of each individual amino acid the curve is a straight line. Hence the areas are proportional to the concentration and the

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recoveries are linear under the conditions of the experiment. It was noticed, however, that although equimolar amounts of different amino acids were taken, the areas for the same concentration of different amino acids varied slightly. This is due to the colour factor, error in weighing, and the purity of the amino acids used. A standard mixture of the amino acids provided by the Messrs Bender and Hobein Ltd. Munchen, W. Germany was therefore employed as the reference standard and all calculations were based on this mixture. The results are reported in Table 9 on page 64.

(d) Ether extract

The ether soluble material was extracted for six hours in a soxhlet apparatus using petroleum-ether (60-80) as the solvent. At the end of this period the solvent was removed from the extract on a water bath. The flasks were dried in a desiccator and weighed.

$$\text{Ether extract percentage} = \frac{\text{Wt. of residue}}{\text{Wt. of sample}} \times 100$$

(e) Carbohydrates.

The amount of total carbohydrates was estimated by difference. The sum total of moisture, crude protein, ether extract and mineral matter was substracted from 100.

(f) Total mineral matter (Ash) (A.O.A.C. Method)

A weighed amount of sample was charred over a flame in a crucible. It was then put in a muffle furnace at 550 °C, until a white ash was obtained. The time of ashing

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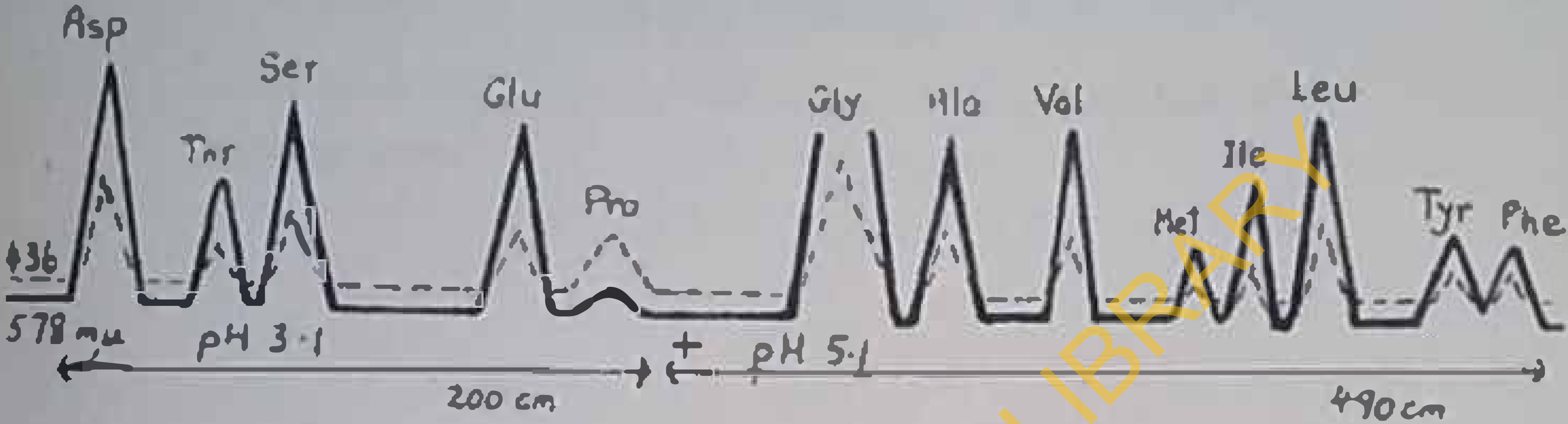
SEPARATION OF AMINO ACIDS

Non-Basic amino acids, Order of elution on 130 cm. column



Basic amino acids, order of elution on 12 cm. column

Linearity of area of gauss curves with increasing concentration of amino acids.

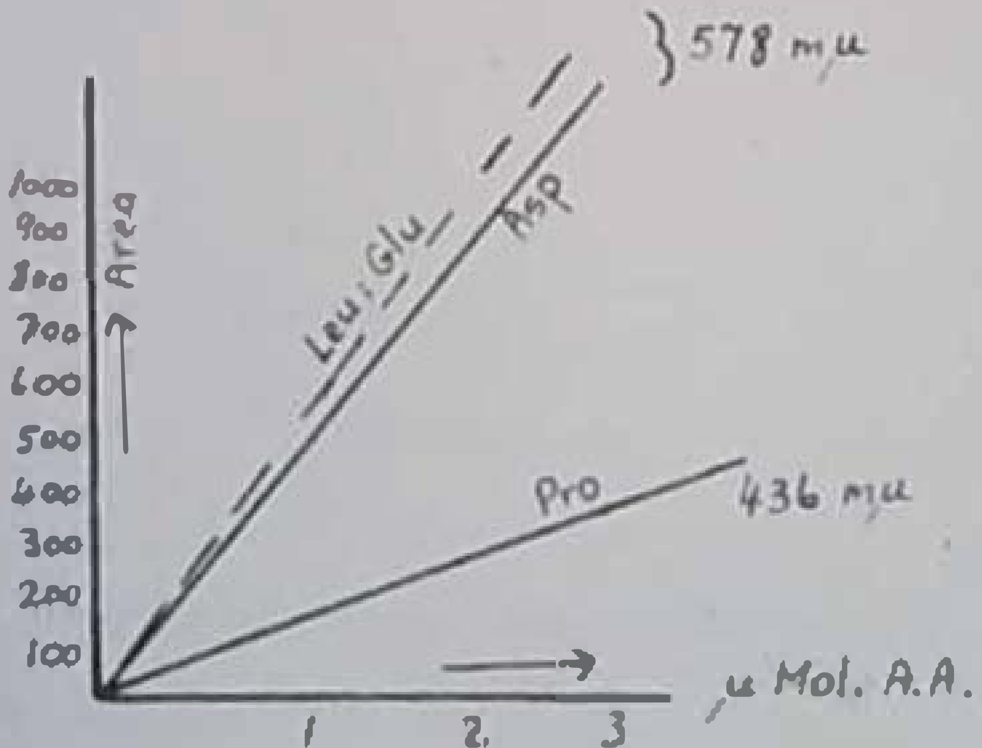


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TABLE IX

Areas per mg. for various amino-acids as recorded by the integraph

Amino-acid	Area (square cm.)
Aspartic acid	30.30
Threonine	33.78
Serine	38.57
Glutamic acid	28.83
Proline	9.21*
Glycine	54.13
Alanine	46.26
Cystine	39.50
Valine	25.21
Methionine	28.18
Isoleucine	32.51
Leucine	32.51
Tyrosine	23.42
Phenylalanine	25.69
Lysine	29.49
Histidine	27.48
Arginine	24.36

* Proline read at 440 mμ.

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varied for different materials. The crucible was then cooled and weighed.

$$\text{Mineral matter \%} = \frac{\text{Wt. of the ash} \times 100}{\text{Wt. of the sample}}$$

(g) Minerals

A wet digestion method as described by A.O.A.C. (1960) was followed so as to get all the mineral elements into solution. The method consists of digesting the sample first with nitric acid and subsequently with 60% perchloric acid until the solution becomes nearly colourless; (heating to dryness is highly dangerous because perchloric acid might cause an explosion). 50 ml. of water are then added and the solution is boiled to drive out any nitrous oxide fumes. It is then cooled and made up to volume.

Calcium. (A.O.A.C. method)

Calcium was precipitated as calcium oxalate at a pH of 4.4 - 4.6. This precipitate was dissolved in hot dilute sulphuric acid (1 : 4) and titrated against standard $KMnO_4$ at a temperature of 70-75 °C. The end point was a light pink persisting for one minute.

Reagents.

Nitric acid Conc.

Sulphuric acid (analar). A dilute solution was prepared by adding 200 ml. of conc acid in 800 ml. of water.

Perchloric acid 60%

Ammonium oxalate (reagent grade). A saturated solution

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was prepared.

Ammonium hydroxide. 2% conc. ammonia solution.

Potassium permagnate. An N/10 solution was prepared and standardised against an N/10 standard solution of sodium oxalate.

Method

A 25 ml. aliquot of the mineral solution was taken into a beaker, diluted to 100 ml. and 2 drops of methyl red added. Dilute ammonium hydroxide was added dropwise to a pH 4.4 - 4.6. The solution was further diluted to about 150 ml. and brought to boil with the addition of 10 ml. hot saturated solution of ammonium oxalate. The contents of the beaker were left to stand overnight for the precipitate to settle. The supernatant was filtered through Whatman No. 42 paper and precipitate washed thoroughly with 2% conc. ammonia solution. The filter paper with the precipitate was put back in the original beaker and to it was added a mixture of 125 ml. water and 25 ml. conc. H_2SO_4. The contents were heated to about 70 °C. and titrated hot with 0.05 N. potassium permanganate solution to a first slight pink colour. A correction for the reagent blank was made.

Calcium was calculated as:-

1.c.c. 0.1N$KMnO_4$ = 0.002 g. Ca.

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(h) Phosphorus (Gomori, 1942)

Phophorus in the form of phosphate reacts with ammonium molybdate to form a complex phosphomolybdate. This complex is reduced to form a molybdenum blue. The blue colour is read at 680 m μ on a SP 600 spectrophotometer.

Reagents.

Ammonium molybdate solution was prepared to contain 7.5 g. salt in about 200 ml. water, then 100 ml. of 10 N. sulphuric acid were added and made up to 400 ml. with water.

10% Trichloracetic acid.

Metho (p-dimethyl amino phenol sulphate) 2 g. in 200 ml. of 3% $NaHSO_3$ solution.

Standard phosphorus solution: this was prepared by dissolving 2.22 g. of KH_2PO_4 in water and made up to a litre, adding a few drops of chloroform. 1000 ml. of solution contained 5 g. phosphorus.

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Method

The solutions were mixed according to the following plan:

	Test	Standard	Blank
Solution of food sample (mineral solution)	1 ml.	-	-
Standard phosphorus solution	-	0.5 ml.	-
10% tricholoracetic acid solution	4 ml.	4.5 ml.	5 ml.
Ammonium molybdate solution	1 ml.	1 ml.	1 ml.
Metol solution	1 ml.	1 ml.	1 ml.

The solutions were mixed in each test tube and allowed to stand for 30 minutes before being read for their optical densities in 2 mm. cuvettes at 680 m/u in a SP 600 spectrophotometer (Unicam Instrument Ltd., U.K.)

Phosphorus was calculated by reference to the reading for the standard.

(i) Iron.

The method of Ramsay (1954) was used. It involved the reduction of iron to the ferrous form, which gives a pink colour with α-α dipyridyl. This colour was read in a S.P. 600 spectrophotometer at 510 m/u. The concentration of the test solution was read from a graph which was prepared by using known quantities of iron.

Reagents.

Standard iron solution was prepared by dissolving 0.7 g.

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$Fe(NH_4)_2(SO_4)_2.6H_2O$ in a mixture of 20 ml. conc. HCl and 50 ml. water, and then diluted to a litre. 100 ml. of the solution were transferred to a litre volumetric flask and made to mark with water. Each ml. of this solution contained 0.01 g. iron.

Acetate buffer solution. This was prepared by dissolving 8.3 g. of anhdrous sodium acetate in water and then adding 12 ml. of acetic acid, and diluting to 100 ml.

α-α dipyridyl solution was prepared by dissolving 0.1 g. of the reagent in water and diluting to 100 ml.

Hydroxylamine hydrochloride. A 10% solution was prepared.

Method

10 ml. of the mineral solution prepared by the wet oxidation method, were transferred to a 25 ml. volumetric flask. In a similar manner, 2 ml., 5 ml., 10 ml. and 15 ml. of the standard iron solution were transferred to a number of 25 ml. volumetric flasks respectively. To each flask was added 1 ml. of 10% hydroxylamine hydrochloride solution and after 5 minutes, 5 ml. of buffer solution and 2 ml. of α-α dipyridyl solution and then made to mark with water. An aliquot was transferred from each solution to a 2 mm. cuvette and read for optical density at 510 mμ in an SP 600 spectrophotometer. The values for the unknown samples were read from a graph (concentration of iron against optical density) plotted on

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the results of the standard solutions.

(j) Vitamin A. (Carr- price blue colour method, 1938).

The method is based on the measurement of the unstable blue colour formed by the interaction of vitamin A and antimony trichloride. The absorbancy of this blue solution at 620 m/u is a function of the concentration of vitamin A.

The ether extract of the sample is saponified with alcoholic KOH and the solution is then neutralised with HCl. The unsaponifiable matter is then extracted with ether. Ether is then evaporated on a water bath and the sample is taken up in chloroform. The colour is then developed by the addition of antimony-trichloride and immediately optical density is determined at 620 m/u on an SP 600 spectrophotometer. The reading is converted to the concentration of vitamin A by using a graph, where standard concentrations of vitamin A are plotted against absorbancy.

Reagents.

Potassium hydroxide solution. 50 g. of KOH pellets in 50 ml. of distilled water

Diethyl ether (peroxide free).

Ethanol. 96%

Anhydrous sodium sulphate. Checked not to retain any vitamin A.

Phenolphthalein 1 g. in 100 ml. alcohol 98%.

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Chloroform. Moisture free.

Antimony trichloride reagent. 25 g. in 100 ml. of moisture free chloroform.

Vitamin A reference solution.

Method

10 g. of each sample was extracted in a continuous soxhlet apparatus with ether for 6 hours. The solvent was evaporated. 30 ml. of ethanol and 3 ml. of KOH, were then added to the flask containing oil. It was then connected with a reflux condenser and heated until saponification was complete (about 25 minutes). The flask was cooled, the contents diluted with about 100 ml. of water and transferred to a separating funnel. The saponification flask was washed with another 50 ml. of ether and similarly transferred to the separating funnel. The total contents were shaken carefully to avoid the formation of emulsions and allowed to seperate into layers. The aqueous layer was drawn off into another separating funnel, whilst another 50 ml. of ether which had been used to wash the saponification flask the second time, was also used to extract the aqueous layer. The operation was repeated twice, before the ether extracts were bulked in the first separating funnel and again washed by pouring through 50 ml. of water without shaking. The aqueous layer was drawn off and discarded. The ether extract was again washed with 50 ml. of 0.5 N NaOH solution,

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shaking gently, then allowed to separate and the aqueous layer drawn off and discarded.

The ether extract was then washed with water repeatedly until the aqueous layer drawn off was alkali-free on testing with phenolphthalein. The extract was filtered through anhydrous Na_2SO_4 placed on filter paper in a funnel into a 250 ml. flask. The separating funnel was rinsed twice with two - 25 ml. portions of ether and poured into 250 ml. flask. Some glass beads were added to the ethereal solution and evaporated to dryness on a water bath. The residue was taken up with 10 ml. chloroform. The following table was followed in developing the colour with antimony tricholoride reagent:-

	Test tube 1	Test tube 2	Test tube 3
Pure chloroform	2 ml.	1 ml.	1 ml.
Antimony tricholoride reagent	9 ml.	9 ml.	9 ml.
Unknown sample extract	-	1 ml.	-
Standard vitamin A solution	-	-	1 ml.

Other test tubes were made to hold 0.5 ml. and 1.5 ml. standard vitamin A solution respectively with 9 ml. antimony trichloride reagent and corresponding volumes of pure chloroform to make to 11 ml. each. The optical density of the mixed solutions in each test tube was quickly read at 620 mμ in an

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SP 600 spectrophotometer and the values of the unknown samples deduced from a graph plotted on the results of the standard solutions of vitamin A.

Carotene was estimated separately and the values obtained were expressed in terms of international units of vitamin A.

Enzymatic digestion and release of B vitamins: 10 g. of each sample was diluted with 60 ml. of 0.2 N. Sodium acetate buffer having a pH 4.5 - 4.7. Then 10 ml. of a freshly prepared enzyme suspension containing 200 mg. of papain and 200 mg. of takadiastase were added. The enzyme suspension was prepared by mixing 200 mg. of papain with 10 drops of glycerine, then adding 200 mg. of takadiastase, and making to 10 ml. with water. The enzymes were mixed with the samples, a few drops of toluene added to each, covered loosely, and incubated for 24 hours at 37 °C. At the end of this period, the samples were autoclaved for 10 minutes at 10 lbs pressure. They were then shaken and filtered through Buchner funnel containing Whatman No.1 paper into volumetric flasks (100 ml. capacity). The residues were washed with more water and the washings collected with the filtrate in the volumetric flasks which were then made up to volume. The extracts were transferred into amber bottles, a few drops of toluene added and the mixtures stored in a refrigerator until wanted for use.

(k) Vitamin B_1

Vitamin B_1 was estimated by the method of Conner and

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Straub (1941). It involved the oxidation of thiamine by mild oxidation with alkaline ferricyanide to thiochrome which fluoresces in ultraviolet light, under standard conditions when other fluorescing substances are absent. The fluorescence is proportional to the thiochrome present and hence to the thiamine in the original solution.

The standard thiamine samples and test solutions are estimated simultaneously.

Reagents.

Sodium hydroxide. 15% solution in water

Sodium acetate buffer. 2.5 M. 205 g. of anhydrous $NaC_2H_3O_2$ were dissolved in water and made up to 1 litre.

Alkaline potassium ferricyanide solution - 3 ml. of 1%. $K_3Fe(CN)_6$ were diluted to 100 ml. with cool 15% NaOH solution and kept in a brown bottle.

Standard thiamine solution - 100 mg. of dry thiamine hydrochloride were dissolved in 25% ethanol and diluted to one litre with the same reagent. 5 ml. of this solution were then diluted to 100 ml. with water. The final concentration was 0.2 µg. of thiamine/ml.

Working Quinine sulphate solution - 100 mg. of quinine sulphate were dissolved in 0.1 N H_2SO_4 and dilute solution were further diluted to 1 litre with

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0.1 N. H_2SO_4 to give concentration of 0.3 mg/litre.

Method.

5 ml. of the enzyme digested sample were pipetted into each of two reaction vessels the first was added 3 ml. of alkaline ferricyanide, 15 ml. of isobutyl alcohol. To vessel No. 2 was added 3 ml. of 15% NaOH solution followed by 15 ml. of isobutyl alcohol. It was also shaken vigorously for 90 seconds. Similar vessels were prepared using 5 ml. each of the standard Thiamine solution. The reaction vessels were all centrifuged for 3 minutes to separate them into two layers. The aqueous layers (lower) were run out using separatory funnels. About 2 g. of anhydrous Na_2SO_4 were added to each of the alcohol solutions before shaking for 30 seconds. They were allowed to stand until clear. At least 10 ml. of the clear, colourless isobutyl alcohol solutions from each vessel were decanted into matched cuvettes. The fluorescence of the isobutyl alcohol solutions was determined in terms of galvanometer deflections, operating the Coleman photofluorimeter according to the manufacturer's directions. The photofluorimeter was checked at start, in between readings and at the end with the working quinine solution.

Thiamine content of the sample in µg. per g.

$$= \frac{U - UB}{S - SB} \times \frac{1}{5} \times \frac{100}{\text{wt. of sample}}$$

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Where U = deflections of unknown.

UB = deflections of unknown blank.

S = deflections of standard.

SB = deflections of standard blank.

(1) Vitamin B_2

The microbiological method described by Snell and Strong (1939) was used.

Lactobacillus casei a bacterium known to require riboflavin for its growth was used for the assay. *Lactobacillus casei*, a homofermentative bacterium, will produce lactic acid when it ferments a carbohydrate substrate. This growth and acid production of certain lactobacilli are, within definite limits, proportional to the amount of riboflavin available to the culture.

A riboflavin-free basal medium was prepared which was complete with respect to other required nutrients, so that the bacterial response was limited only by riboflavin. This medium was distributed among a series of test tubes; graded amounts of sample extract or standard riboflavin solution were added and the tubes were sterilised. They were then inoculated with the test organism and incubated. A standard curve was prepared by plotting the volume of 0.1 N. NaOH used against the amount of riboflavin present. The riboflavin content of the test solution was then calculated by interpolation.

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Reagents.

Salt solution A. K_2HPO_4, 5 g., KH_2PO_4, 5 g., water 50 ml.

Salt solution B. $MgSO_4 \cdot 7H_2O$, 10 g., NaCl, 0.5 g., $FeSO_4 \cdot 7H_2O$, 0.5 g., $MnSO_4 \cdot 4H_2O$, 0.337 g., water 250 ml.

A complete medium for Lactobacillus casei was prepared as follows:- Casein hydrolysate 10 g., sodium acetate 12 g., Glucose 20 g., Asparagine 500 mg., Tryptophan 200 mg., Cystine 200 mg., Salt solution A 10 ml., Salt solution B 10 ml., Xanthine 10 mg., Uracil 10 mg., Thiamine hydrochloride 200 μg., Biotin 10 μg., Folic acid 20 μg., Riboflavin 200 μg., Calcium pantothenate 400 μg., Nicotinic acid 400 μg., Pyridoxine hydrochloride 800 μg., distilled water 1000 ml. and pH adjusted to 6.8.

Method

1 ml., 3 ml., and 5 ml. of each sample extract were transferred into 3 test tubes respectively. Each tube was made to 5 ml. with distilled water. 5 ml. of the complete medium prepared above were added to each tube to make a total volume of 10 ml. Similar preparations were made using standard vitamins in place of the sample extract. The standard

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solutions covered a range from 0.05 µg. to 0.5 µg. The tubes were plugged with cotton and sterilised at 10 lbs/psi pressure for 15 minutes. After cooling, the tubes were inoculated with a suspension of *L. casei* and incubated. The amount of growth was determined by titration of the acid produced using 0.1 N. NaOH with bromothymol blue as the indicator. Values obtained from the dilutions of the vitamin standard were used to construct a standard curve from which the vitamin content of any dilution of the sample was calculated. Only values falling within the linear portion of curves were accepted from assay values of the samples.

(m) Vitamin C.

The method described by Harris and Oliver (1942) was used. The ability of ascorbic acid to reduce 2 : 6 dichlorophenol indophenol dye was used as a measure of ascorbic acid concentration in the sample extracts. The amount of standard ascorbic acid required to reduce a definite volume of dye solution was first determined. Then, from the volume of the unknown solution required to reduce the same volume of dye, the percentage of ascorbic acid was calculated in the unknown.

Reagents.

Metaphosphoric acid, 6%.

Metaphosphoric acid, 3%.

Standard ascorbic acid 100 mg. of ascorbic acid (U.S.P. reference standard) were dissolved in 500 ml. of 3% HPO_3.

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2, 6-Dichlorophenol indophenol. 50 mg. of the dye were dissolved in approximately 150 ml. of hot water containing 42 mg. $NaHCO_3$, cooled and diluted with water to 200 ml.

Standardisation of dye solution. A 5 ml. aliquot of the standard ascorbic acid solution (containing 1 mg. ascorbic acid) was diluted with 5 ml. of 3% Metaphosphoric acid. This was titrated with the dye solution to a pink colour which persisted for about 15 seconds since this volume of dye represented 1 mg. of ascorbic acid, the ascorbic acid equivalent (T) of 1 ml. of dye solution is equal to 1 divided by the volume in ml. of the dye solution used in the titration.

Method

40 g. of each of the powdered samples were blended with an equal weight of 8% acetic acid and mixed to give a homogeneous slurry. 20 g. of this slurry were weighed into a beaker and transferred to a 200 ml. flask and made up to the volume with water. The solution was mixed thoroughly and centrifuged. The supernatant was decanted and filtered through Whatman No. 1 filter paper. 25 ml. aliquot of the filtrate was pipetted into an Erlenmeyer flask and titrated immediately with the standardised solution of 2, 6-dichlorophenol indophenol to a faint pink end point which persisted for 15 seconds.

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Calculation:-

$$\frac{V \times T}{W} \times 100 = \text{mg. ascorbic acid per 100 g. sample.}$$

V = ml. dye used for titration of aliquot of diluted sample.

T = Ascorbic acid equivalent of dye solution expressed as mg./ml. of dye.

W = g. of sample in aliquot titrated.

(a) Total serum proteins.

Biuret method as reported by King (1964) was followed.

Reagents.

Solution A. 45 g. of sodium potassium tartrate (Rochelle salt) were dissolved in 400 ml. of 0.2 N. NaOH in a beaker. 15 g. of copper sulphate was then added and dissolved completely. To this 5 g. of potassium iodide was added and mixture made up to 1 litre with 0.2 N. NaOH.

Solution B. 0.5% potassium iodide was made in 0.2 N. NaOH.

Working biuret reagent. 50 ml. of solution A were diluted to 250 ml. with solution B.

Calibration curve.

Versatol A was used as standard protein and calculated quantities of protein solution (1 g. to 10 g./100 ml.) were

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taken in test tubes. The volume was made up to 3 ml. in each case. Then 3 ml. of working biuret reagent was added to each tube. All the tubes were placed in a waterbath at 37 °C for 10 minutes. The optical density was read in a SP 600 spectrophotometer at 540 mu, setting the instrument to zero with a distilled water and reagent blank. The optical density was then plotted against the known protein concentration and the standard line obtained.

Method.

0.1 ml. of serum from test samples was taken in test tubes the volume was made up to 3 ml. with distilled water in each case, 3 ml. of working biuret reagent were added and the optical density determined in same manner as for the standards. The protein concentrations were read from the calibration curve.

(e) Paper electrophoresis

The method as described by Smith in his book "Chromatographic and Electrophoretic Techniques," Volume II (1960) was adopted for the separation of various proteins in the serum of animals fed on experimental diets.

In a vertical tank (as supplied by M/S Ltd. Shandon Ltd. London) the separations were carried out using the barbitone buffer pH 8.6. A current of 100 volts was passed for 16 hours. The proteins exist as anions and so migrate to the anode. However, due to the electro-endosmotic flow the

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globulin remained stationary at the origin.

The strips were dried immediately after the running period and then stained with amido black 10B dye.

Apparatus and reagents.

(1) The vertical electrophoresis tank specifications as supplied by M/S Shandon

(2) Stabilised D.C. power supply. "Volkam" power pack

(3) Buffer pH. 8.6 barbitone buffer was prepared by dissolving 10.3 g. of sodium barbitone in about 900 ml. of water, 1.83 g. of barbituric acid was then dissolved by slowly shaking. The volume was made up to 1 litre.

(4) Whatman No. 1 paper strip of 3 mm. width.

(5) Dye solution 1% solution of amido black 10B in 50 ml. methanol and 50 ml. water.

(6) Washing solution: 75 ml. acetic acid and 50 ml. methyl alcohol made up to 250 ml.

Method.

The buffer solution of pH 8.6 is added in the outer two compartments on each side of the tank so as to dip the electrodes completely. The paper wicks connecting the two adjacent compartments serve to maintain a uniform level of buffer in all the compartments. Whatman No. 1 paper strips 3 mm. wide and 56 mm. long are folded in the middle and hung on the nylon thread. The lower ends of the paper strips remain dipping in the buffer solution. The strips were allowed to soak in buffer solution until they were completely

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soaked (for about 45 minutes). The samples were then applied (0.07 ml. by means of a micro pipette) near the nylon thread in a uniform streak. The lid of the tank was replaced and the electric terminals connected to the power supply. The current of 100 volts was passed for a period of 18 hours. At the end of this period the current was stopped and the paper strips removed gently by mean of a glass rod. They were then dried and stained. Staining was done by dipping the paper strips in the solution of dye for 30 minutes. The excess dye was then poured off and paper strips, washed in methanol for 15 minutes. The washing of excess dye was then done with 75 ml. acetic acid in 250 ml. of water, until a clear back ground was obtained. The strips were then dried and scanned in a scanner.

(p) Starch gel electrophoresis.

The method of Smithies (1955) was used for the separation of serum proteins. Hydrolysed starch made into the gel by heating 72 g. with 600 ml. boric acid-sodium hydroxide solution was allowed to set for six hours. The samples were applied in incisions across the depth of the gel block and the block was fixed in a vertical position. Current was passed through at 25 m. A for 18 hours. At the end of this period the gel blocks were removed and sliced across the thickness. They were then stained using amido black dye. The excess dye was drained off in successive washings with

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methanol acetic acid and gel plates photographed proteins.

Apparatus and reagents.

Trays 25 x 11 cm. and 0.5 cm. deep with thick ends, made of plastic

Buffer container, 6" x 6" and 4" deep with a partition 2" high and an electric terminal

Power supply

Staining trays

Hydrolysed starch

Boric acid - sodium hydroxide sol:- This was prepared by dissolving 8.39 g. of boric acid and 2 g. of sodium hydroxide in 5 litre of distilled water

Buffer pH8 boric acid 92.76 g. and sodium hydroxide 12 g. were dissolved in 1000 ml. of water.

Preparation of gel:- 72 g. of hydrolysed starch were dissolved in 600 ml. of boric acid - sodium hydroxide solution and gently warmed. The material was poured into 25 x 11 x 0.5 cm. trays covered with a plastic sheet, avoiding air bubbles. This material was allowed to set for 6 hours. At the end of this period the cover sheet is removed and at a distance of 3.0 cm. from the end three uniform incisions were made in the gel at equal distances. 0.07 ml. of the serum sample was applied and the gel was again covered with a piece of clean polythene sheet where upon the trays were placed in slanting positions in the buffer containers. The buffer from the outer

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compartment of the containers was connected with inner by means of filter paper wicks. The terminals were connected to the power pack and current passed through for 18 hours.

At the end of this time the gel was then gently removed from the tray and sliced. The upper slice was stained in the staining trays with amido black 10 B. dye 1% solution. The excess dye was then washed and the back ground made as clear as possible by washing with successive dilutions. The washed gel plates were dried with filter paper and photographed.

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(q) Animal feeding experiments.

Albino rats from our own colony were used for the weight gain studies, determination of digestibility of protein and biological value of proteins of raw and cooked diets.

Selection of animals:- Ten female rats known to produce more than eight offsprings at a time were selected and mated. When the litters came out seven groups out of these which had more than eight litter-mates were retained and others discarded. The young rats were allowed to remain with their respective mothers for a period of 21 days and were fed on a stock diet. After this period they were numbered, weaned, and put into individual cages.

Housing of rats:- All wire netting cages of 8" x 8" x 10" were specially designed to house individual rats. Each cage was clamped on an angle iron and a perspex sheet bent upwards at two opposite sides for the easy flow of urine, was introduced under each cage. The top of the perspex sheet was covered with a wire mesh so as to hold back the faeces. (Plate 3.)

Each cage was fitted with 3 wire loops one for holding the food beaker inside the cage, one for holding the urine collecting beaker infront of the cage, and one

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PLATE 3

PLATE 4

PLATE 3 A single wire net cage 8"x8"x10" for an individual rat, with arrangements for faeces and urine collection.

PLATE 4 A battery showing rats in their individual cages during experiment.

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for holding the water bottle at the back of the cage. The cages were arranged in the form of a battery in groups of 8 cages in each line (plate 4).

Management and feeding:- Every eight litter mates in each group were put on the same diet, half of them on the raw mixed and the other half on the cooked diet. They were fed ad lib, the amount of diet which each rat would consume in 24 hours was approximately ascertained during the first 3 days of non-experimental period. Every day a known weighed amount of diet containing more than what the rat had eaten during the previous day, was given to each rat at 9 o'clock in the morning. Any remains of the food left over were removed from the food beaker before providing fresh food and weighed next morning.

In this manner it was possible to provide ad lib feeding and the amount of food spilled from the beaker was very low.

Collection of urine and faeces:- 5 ml. of diluted H_2SO_4 (20.90) were added to each urine collection beaker every morning and the amount of urine for 24 hours was collected. The perspex sheet was washed with distilled water into the beaker before the urine was collected. This procedure prevented any losses due to evaporation of urine. Faeces were collected daily at the same time as the urine from the wire gauze on top of the perspex sheet. They were transferred into weighed petri dishes and immediately dried in a hot

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air oven at 100 °C ± 1 for 8 hours and cooled and weighed.

The daily collections of urine and faeces were pooled for every week, and analysed for total nitrogen.

<u>Growth</u>. Growth in rats was measured by determining body weight on every alternate day.

The experiment was continued for 3 weeks and at the end of this period the rats were killed by a single stroke and their blood was pooled in each group separately. From these pools the serum was obtained.

The experiment was repeated using a fresh batch of rats.

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CHAPTER 4

Experiments and Results

1. Nutrient values of various foodstuffs

Procedure.

The foodstuffs collected as described on page 37 were quickly brought to the laboratory and analyses carried out on them for Dry matter, Proteins, Ether extract, Carbohydrates, Minerals (Calcium, Phosphorus and Iron) and Vitamins (A, B_1, B_2 and C).

All experiments were carried out in duplicate on the edible portion only, except in the case of fish where the bones were included. The methods followed for the analyses are described on pages 65 to 8[illegible].

Results.

The results of the chemical analyses of various foods, used in the preparation of diets are presented in Table 10 and 11. In Table 10, the composition of the major nutrients in the food materials is given. It can be seen from these results that ogi (wet maize starch), yam and yam flour, plantains and gari (grated cassava flour) are rich in carbohydrates; while beans, egusi (melon seeds), snails, panla (stock fish) beef, dry fish, eggs and shrimps are the protein-rich materials. Vegetables such as efo, okro, tomato, onions etc. do not contain much of the major nutrients but are rather a source of vitamins.

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The composition of minerals and vitamins in these foodstuffs has been presented in Table 11. It can be seen that three of the staple foods i.e. yam, elubo (yam flour) and plantain have low levels of calcium and ~~vitamins.~~ A low level of vitamin A is also evident in ogi, beans, gari, and stockfish while palm oil contains a high concentration of beta carotene.

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TABLE 10

The Composition of the Major nutrients in the Food Materials

No.	Name: Commonly used	Name: Other names/Botanical	g./100 g. of edible portion: Moisture	Total Protein Nx6.25	Ether-extract	Carbohydrates
1	Ogi	Wet maize starch; (Zea mays.)	49.5	4.3	Tr.	43.0
2	Beans	Cowpeas; (Vigna unguiculata)	7.7	23.1	5.0	60.5
3	Yam	(Dioscorea rotundata)	59.0	1.7	-	38.6
4	Efo	Tete; (crotalaria spp.)	90.9	3.7	0.3	3.6
5	Snails	(Vivapara quadrata)	76.0	14.0	2.0	3.5
6	Elubo	Yam flour; (Dioscorea spp.)	13.0	4.0	2.0	78.6
7	Egusi	Melon seeds; (Citrullus vulgaris)	8.0	24.3	21.2	41.3
8	Panla	Stockfish;	35.7	56.0	2.1	Tr.
9	Plantain	(Musa paradisiaca)	61.5	0.9		35.5
10	Beef	Beef lean	73.5	22.9	2.4	Tr.
11	Gari	Cassava flour; (Manihot esculenta)	13.6	1.3	0.4	82.2
12	Dry fish	Smoked fish;	52.0	42.0	2.0	
13	Okro	(Hibiscus esculentus)	82.8	2.4	0.3	13.2
14	Eggs	Hen eggs	76.1	10.5	11.4	1.0
15	Shrimps	Palaemonetes varians	69.0	22.0	2.5	-
16	Onion	(Allium cepa)	91.8	1.3	-	6.2
17	Pepper	(Piper nigrum)	7.3	13.0	2.7	70.0
18	Tomato	(Lycopersicum esculentum)	92.0	3.0	Tr.	4.0
19	Palm oil	(Elaeis guineensis)	Tr.	-	100.0	-
20	Butter		16.5	0.5	82.5	Tr.

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TABLE 11

The Composition of Minerals and Vitamins in the Food Materials

No	N A M E		Total mineral matter g./100g.	mg./100 g. of the food						Vitamin A I.U./100 g.
	Commonly used	Other names/Botanical		Calcium	Phosphorus	Iron	Thiamine B_1	Riboflavin B_2	Ascorbic C	
1	Ogi	Wet maize starch (Zea mays.)	1.2	40	116	7.0	0.03	0.03	-	-
2	Bean	Cowpeas; (Vigna unguiculata)	3.7	86	315	4.0	0.70	0.20	-	Tr.
3	Yam	(Dioscorea rotundata)	0.7	15	60	Tr.	0.10	0.03	10	-
4	Efo	Tete; (Crotolaria spp.)	1.5	180	28	4.0	0.10	0.50	100	2500
5	Snails	(Vivapera quadrata)	4.5	1200	148	8.0	Tr.	0.05	-	Tr.
6	Elubo	Yam flour; (Dioscorea spp.)	2.4	30	132	5.0	-	-	20	-
7	Egusi	Melon seeds; (Citrullus valgaris)	4.5	50	60	8.0	0.01	0.15	10	-
8	Panla	Stockfish;	6.2	300	160	4.0	0.15	0.18	-	-
9	Plantain	(Musa paradisiaca)	2.1	Tr.	30	0.5	0.02	0.05	15	-
10	Beef	Beef lean	1.1	10	250	3.0	0.08	0.15	Tr.	Tr.
11	Gari	Cassava flour; Manihot (esculenta)	2.3	130	50	Tr.	0.05	0.07	8	-
12	Dry fish	Smoked fish;	3.2	2000	160	2.0	0.10	0.20	-	-
13	Okro	(Hibiscus esculentus)	1.3	80	68	Tr.	0.10	0.10	25	200
14	Eggs	Hen eggs	1.0	54	210	3.0	0.12	0.35	Tr.	1000
15	Shrimps	Palaemonetes varians	6.5	120	80	0.8	Tr.	0.05	-	1200
16	Onion	(Allium cepa)	0.7	30	30	Tr.	0.05	0.10	50	300
17	Pepper	(Piper nigrum)	7.0	150	260	8.0	0.48	0.69	1200	-
18	Tomato	(Lycopersicum esculentum)	1.0	15	21	Tr.	0.06	-	16	650
19	Palm oil	(Elaeis guineensis)	Tr.	-	-	-	-	-	-	β [illegible]000
20	Butter		Tr.	15	20	0.2	-	-	-	2000

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2. The effect of cooking on the Chemical Composition of diets.

Procedure.

Representative samples of all the six diets both in raw mixed and cooked forms were analysed for the following:-

(a) Dry matter, total protein, ether extract, Carbohydrates and total mineral matter

(b) Minerals:- calcium, phosphorus and iron

(c) Vitamins:- A, B_1, B_2, and C.

The analytical methods followed are described on pages 50 to 52 and 63 to 79. The methods of preparation and mixing of the diets are described on pages 38 to 49.

Results.

The results for (a) (b) and (c) are presented in Tables 12, 13 and 14, respectively. From the Tables 12 and 13 it will be seen that there were only negligible losses during cooking in the cases of total protein, ether extract, carbohydrates and minerals. On the other hand, a slight increase during cooking (0.8%) of total proteins is noticeable in diet No. 1 and diet No. 6. This is explained by the fact that both these diets contain large amount of beans (see pages 42 and 49). During the preparations for cooking, the beans are soaked and their testas are rubbed off. It is known that the testa contains very little protein about 3% as compared to rest of the bean seeds which contain about 25% protein.

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Vitamins losses.

The results of the vitamin estimations are presented in Table 14. It can be seen that about 25% to 50% of vitamin A is lost during the process of cooking; but since palm oil is the main source of fat in all the diets, which is very rich in carotenes this loss is of little nutritional significance.

The loss of the B vitamins Thiamine and Riboflavin varies considerably from diet to diet. It was noticed that it varied closely with the method of preparation of food. There were for instance no losses in the case of diet No. 6 where the cooking was accomplished in steam under pressure, while losses were severe when prolonged heating, in open pan along with spices which can produce acid or alkaline conditions, was involved.

The loss of vitamin C was high in all the diets. It varied from total destruction to 50%, again it was noticed that diet No. 6 (steam cooked) retained about 55% vitamin, while the losses were much heavier in the other cases.

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TABLE 12

The Composition of raw mixed and cooked diets

No.	Diet	Dry Matter	Per 100 g. of the diet dry wt.		
			Total Protein	Ether-extract	Carbohydrates
1	Eko and Akara				
	Raw	59.6	18.5	20.1	57.1
	Cooked	26.3	19.2	19.8	56.9
2	Pounded yam, efo and snails				
	Raw	47.0	7.5	10.7	78.1
	Cooked	34.0	7.0	10.4	79.1
3	Elubo, egusi soup and ponmo				
	Raw	53.2	9.5	9.7	76.4
	Cooked	27.0	9.5	9.6	76.5
4	Fried plantain and beef stew				
	Raw	38.0	7.8	24.9	61.9
	Cooked	57.5	7.8	23.7	63.1
5	Gari-Okro and dry-fish				
	Raw	54.5	6.4	16.9	72.6
	Cooked	27.0	6.4	16.8	72.7
6	Moinmoin				
	Raw	59.3	18.2	24.6	53.0
	Cooked	38.0	19.6	25.7	50.6

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TABLE 13

The mineral composition of raw mixed and cooked diets

Diet	(Per 100 g. of the diet)			
	Mineral matter (g.)	Calcium (mg.)	Phosphorus (mg.)	Iron (mg.)
Eko and akara				
Raw	4.2	87	289	6.1
Cooked	4.1	75	278	6.1
Pounded yam, efo and snails				
Raw	3.7	408	162	10.6
Cooked	3.5	307	150	10.0
Elubo, egusi soup and Panla				
Raw	4.4	212	163	11.7
Cooked	4.3	215	157	11.2
Fried plantain and beef stew				
Raw	5.4	16	123	1.7
Cooked	5.4	16	118	1.7
Gari, okro and dry fish				
Raw	4.1	344	72	6.4
Cooked	4.1	318	70	6.3
Moinmoin				
Raw	4.1	78	259	3.3
Cooked	4.1	74	237	3.3

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TABLE 14

Vitamins in the raw mixed and cooked diets.

Diet	(Per 100 g. of the diet)			
	Vitamin A I.U.	Vitamin B_1 mg.	Vitamin B_2 mg.	Vitamin C mg.
Eko and akara				
Raw	5070	0.54	0.19	4.04
Cooked	2460	0.23	0.09	Tr.
Pounded yam, efo and snails				
Raw	4005	0.23	0.14	0.14
Cooked	2700	0.05	0.08	Tr.
Elubo, egusi soup and panla				
Raw	2138	0.18	0.13	2.0
Cooked	1528	0.04	0.09	0.0
Fried plantain beef stew				
Raw	3136	0.06	0.15	40.0
Cooked	1872	Tr.	0.08	4.0
Gari, okro and dry fish				
Raw	5006	0.07	0.10	23.0
Cooked	3618	Tr.	0.06	8.0
Moinmoin				
Raw	7511	0.52	0.22	47.0
Cooked	5821	0.30	0.22	28.0

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3. **Quantitative estimation of Amino acids in the raw and cooked diets.**

Procedure.

One gram representative samples of the diets (the methods of preparation are given in page 38) were hydrolysed and treated as described on page 59.

The various amino acids present in the hydrolysates were determined as described on page 64 for the neutral and acidic amino acids, 150 cm. long column was used, while the basic amino acids were estimated in a 12 cm. column. The experiments were carried out in duplicate.

Results.

The results of the samples of raw and cooked diets are recorded in Table 15. These values are the average of the duplicate determinations which agreed closely and are calculated as ./100 g. of food (dry wt. basis). The results for seventeen amino acids are reported. Since the determinations were carried out in acid hydrolysates tryptophan was completely lost. The partial destruction of threonine and serine during acid hydrolysis of protein has also been reported by some workers (Prutos, 1963) Conversion of glutamine to glutamic acid and cystein to cystine also took place during hydrolysis and hence they appear only as glutamic acid and cystine. Similarly, no distinction was possible between asparagin and aspartic acid.

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Losses.

When amino acids in the raw mixed and cooked diets are compared, the results show only mild losses in the cooked diets in most cases. On the other hand small increases have been noticed especially in diet No. 1 and 6. This is due to a slightly higher percentage (about 1%) of N in the cooked form. The reason for increase in this percentage of N has already been given under the section on anlysis of diets page 94 . Many other factors such as the method of preparation, presence of metallic ions and the reaction of the cooking medium are also highly complex factors which can have an effect on the amino acid patterns and which need a more detailed study. However, from what has been reported it seems likely that the amount of total change brought about by cooking is hardly of any nutritional importance; except for lysine where the losses are as high as 30% in most of the diets.

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Amino acid	Moinmoin		Control
	Raw	Cooked	
Aspartic acid	961	1020	1670
Threonine acid	740	742	966
Serine acid	936	967	1400
Glutamic acid	2152	2697	5220
Proline acid	782	797	2655
Glycine acid	370	375	409
Alanine acid	1030	1125	758
Cystine acid	224	200	120
Valine acid	1040	1131	1670
Methionine acid	250	200	696
Iso-Leucine acid	851	756	1480
Leucine acid	1389	1360	2270
Tyrosine acid	524	522	1217
Phenylalanine acid	1275	1184	1319
Lysine acid	928	340	1500
Histidine acid	549	648	682
Arginine	900	972	919

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TABLE 15

Amino acids in Raw-Cooked, and Control diets (mg./100 g. of the food)

Amino acid	Eko and Akara Raw	Eko and Akara Cooked	Pounded yam, efo and snails Raw	Pounded yam, efo and snails Cooked	Eluba, egusi soup and pomo Raw	Eluba, egusi soup and pomo Cooked	Fried plantain and beef stew Raw	Fried plantain and beef stew Cooked	Gari, okro and dry fish Raw	Gari, okro and dry fish Cooked	Moinmoin Raw	Moinmoin Cooked	Control
Aspartic acid	1239	1304	684	679	802	776	755	824	565	560	961	1020	1670
Threonine acid	721	632	213	200	394	384	357	188	278	189	740	742	966
Serine acid	962	825	247	226	281	250	339	302	198	175	936	967	1400
Glutamic acid	2960	3142	1015	839	1159	1162	1228	1410	817	944	2152	2697	5220
Proline acid	795	795	368	329	554	532	399	356	391	454	982	797	2655
Glycine acid	341	364	366	335	502	486	502	520	354	360	370	375	689
Alanine acid	1054	975	174	143	544	548	418	400	362	400	1030	1125	758
Cystine acid	222	181	134	123	122	112	102	85	86	85	224	200	120
Valine acid	1036	1109	537	492	485	467	449	462	341	322	1040	1131	1670
Methionine acid	277	186	185	179	265	196	201	90	186	90	250	200	696
Iso-Leucine acid	888	912	355	325	461	468	424	433	525	350	851	756	1480
Leucine acid	1378	1384	642	588	687	688	663	641	484	496	1389	1360	2270
Tyrosine acid	545	533	219	202	246	242	275	270	273	210	524	522	1217
Phenylalanine acid	962	970	252	231	338	310	333	304	238	222	1275	1184	1315
Lysine acid	1202	625	463	243	798	347	707	246	562	174	928	540	1510
Histidine acid	55	548	108	99	313	260	281	286	208	220	549	648	682
Arginine	1184	1174	565	526	512	522	522	582	361	366	900	972	919

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4. The effect on rate of feeding raw mixed and cooked diets.

Procedure.

Rats bred and selected as described on page 87 were housed in individual cages. Out of the eight litter mates, four were given the raw mixed diet and the other four the same diet in cooked form. They were allowed a 3 days non-experimental period when they were fed ad lib on the experimental diets but no observations were taken. This was done in order to equilibriate the animals on the experimental diets. The process was repeated for all the six diets and the control diet simultaneously. The actual trial lasted for 3 weeks. The following observations were recorded for each rat individually.

Observations.

(i) Initial weight of the animal.

(ii) Weight gain/loss, every alternate day.

(iii) Food intake, every 24 hours.

(iv) Faeces passed in 24 hours were collected dried, weighed and stored.

(v) Urine passed in 24 hours was collected daily.

(vi) Final weight of the animals at the end of 3 weeks.

(vii) The nitrogen content of the raw mixed and cooked diets was determined at the beginning and at the end of each experiment.

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(viii) The samples of the dry pooled faeces of every animal were analysed for the total nitrogen content weekly.

(ix) The samples of the pooled urine (which were preserved in dilute sulphuric acid) were analysed for their nitrogen content every week, in case of each animal.

At the end of the first series of experiments, the experiments were repeated with a fresh batch of rats.

Results.

(a) **Weight gain studies.**

The results obtained are presented in Table 16 on page 107. The growth of the rats (mean of the 4 rats in every group) on the raw mixed, and cook diets, along with the control, has been plotted against time, in order to show the growth pattern, and the graphs are presented in figure 2 on page 106.

The weight gains were calculated as per gram of food intake and per gram of nitrogen intake. The data thus obtained eliminates the variations due to different quantities of food consumed by individual rats.

These data have been tested for any statistical significance using the standard "t" test. The results obtained, along with the means and standard deviations

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are reported in Tables 17 to 20. These have been classified as follows:-

Table 17. Shows the comparison of wt. gains/g. of food intake between raw and cooked diets.

Table 18. Shows the comparison of wt. gains/g. of food intake between raw, cooked and control diets

Table 19. Shows the comparison of wt. gain/g. of nitrogen intake between raw and cooked diets.

Table 20. Shows the comparison of wt. gain/g. of nitrogen intake in raw and cooked diets and ~~control diets~~ and control diets individually.

The results in Table 12 show that there is a significant difference in wt. gain/g. of food intake in case of diets No. 2, 4 and 6, when raw and cooked diets are compared; while the wt. gain/g. of food intake was not significant in the case of diets No. 1, 3 and 5. It is therefore evident that the quality of the food in diets No. 2, 4 and 6 has been considerably influenced during cooking in such a manner so as to cause an appreciable effect on the growth of rats. In diets No. 1 and 3, although there is not a significant difference of growth between the animals fed on raw or cooked diets. But this does not mean that the quality of the food has not been influenced. The differences in growth rate seem to have been

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masked by the high digestibility of the cooked food (Table 21). In diet No. 5 there was almost negligible growth and the animals just maintained their weight. Whereas on diet No. 2 they actually lost weight during the experimental period.

Comparison with control diet.

Gain in wt./g. of food in raw and cooked diets when compared with the control diet (Table 18) were significantly lower in all the diets except for diet No. 6.

Gain in wt./g. of nitrogen intake.

The results presented in Table 19 in which gains in wt./g. of nitrogen intake are compared in raw and cooked diets show similar significant differences as in the case of gains in wt./g. of food intake. It would appear that the significant difference in growth rate which arises on account of cooking may inpart be due to differences in the quality of nitrogen (protein) in raw and cooked diets. One measure of the difference in the quality of nitrogen (protein) is the digestibility of these proteins in the animal body. This has been examined and the results are presented in Table 21 and 22, under criteria for nitrogen utilisation.

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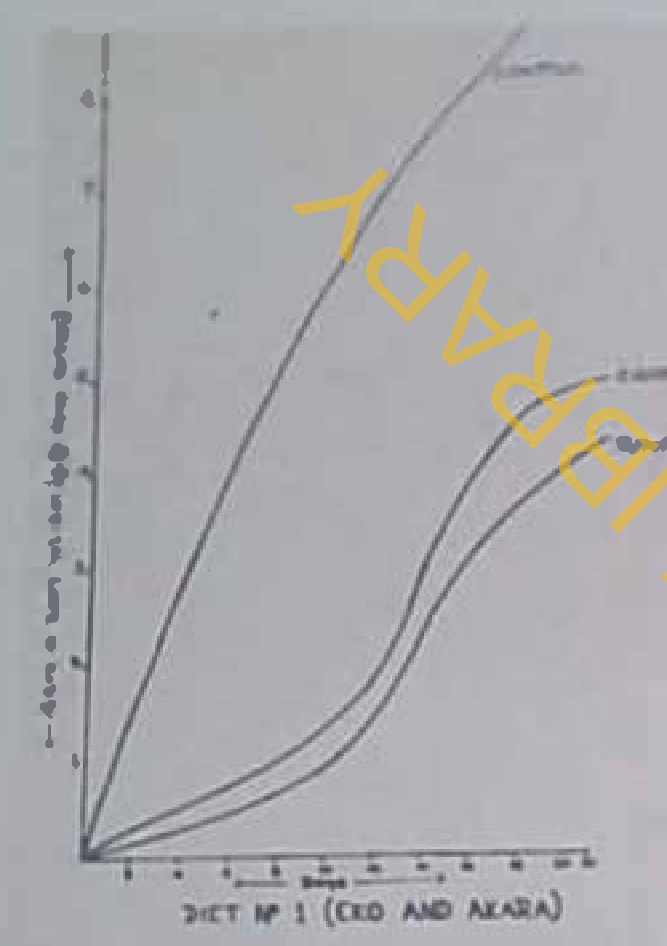

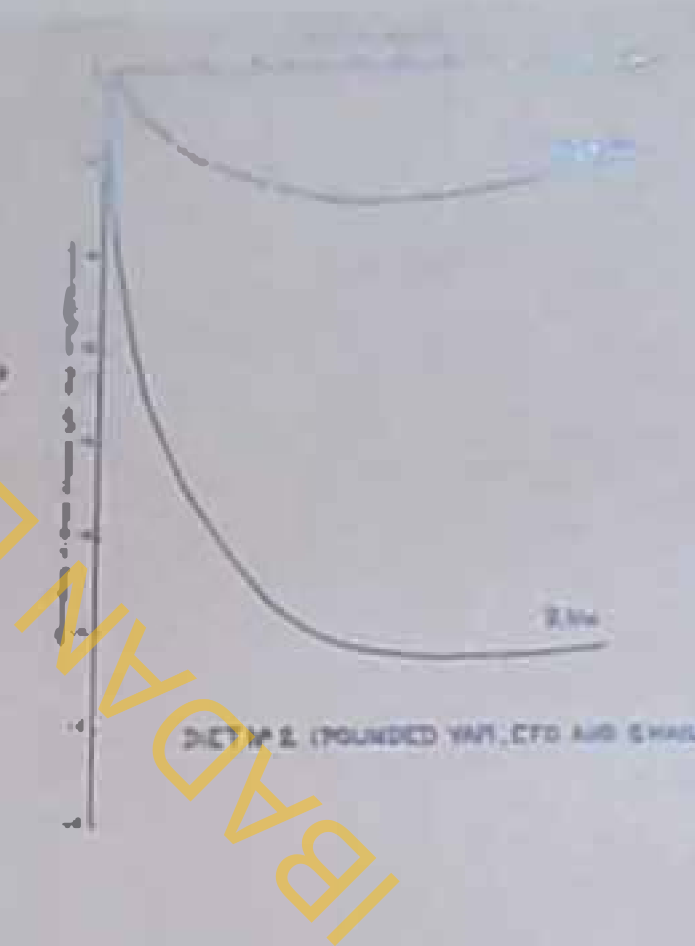

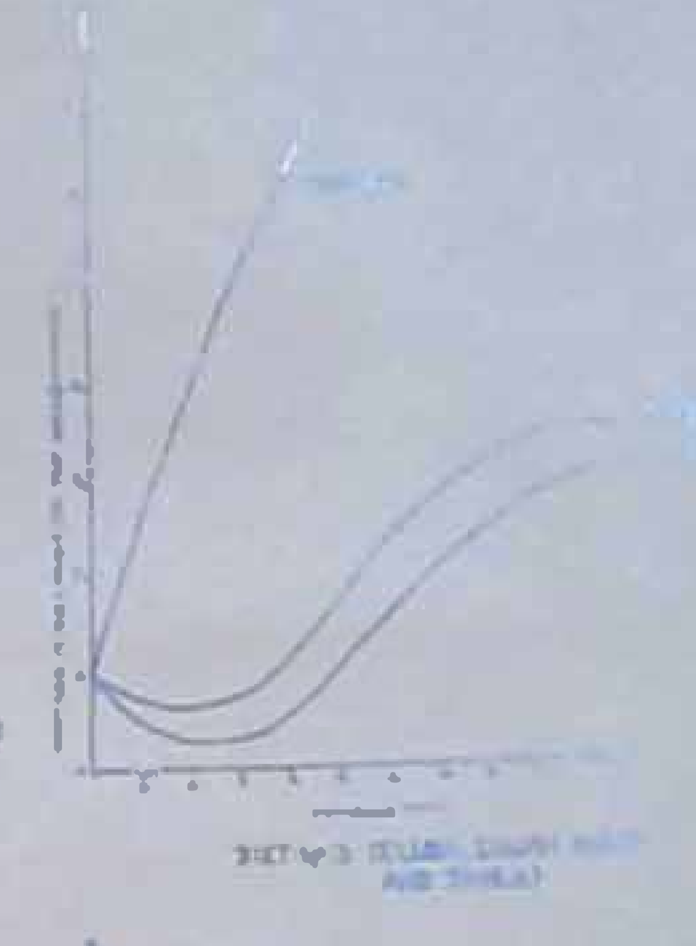

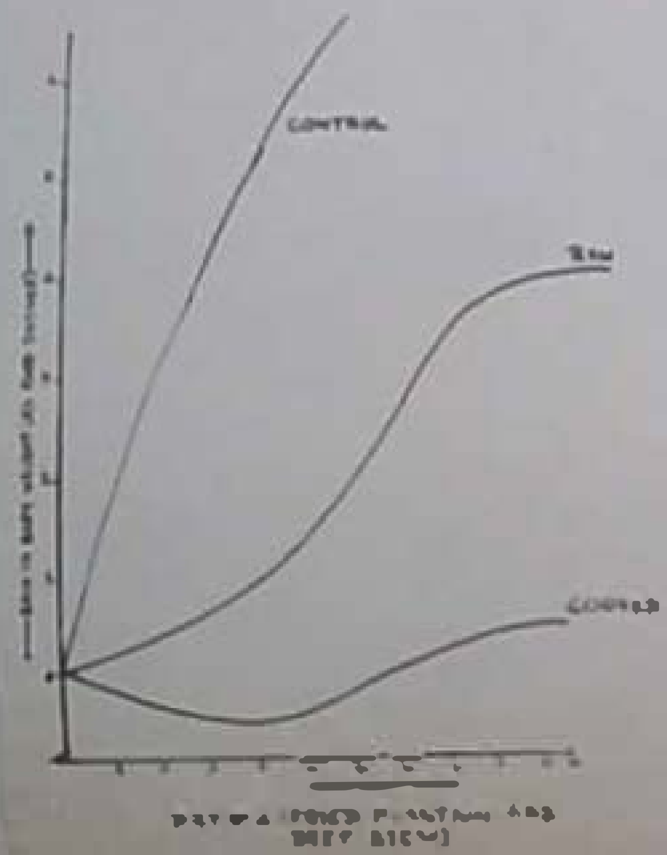

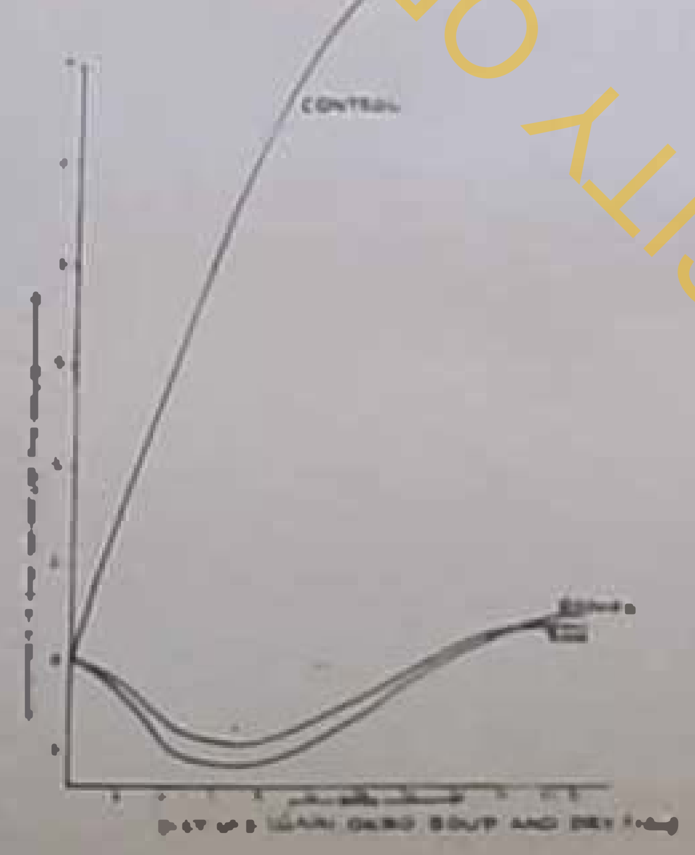

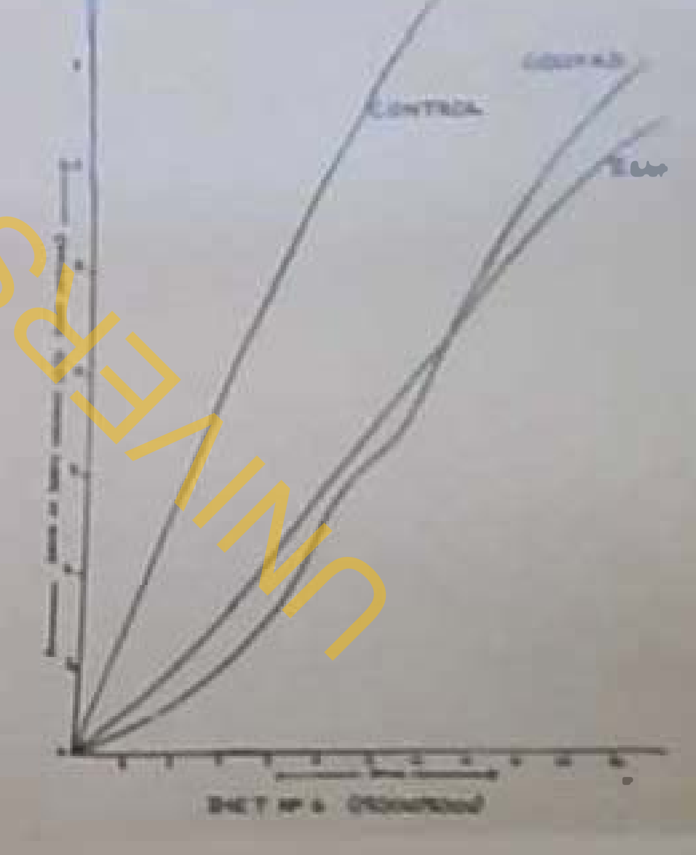

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TABLE 18

The food intake and gain/loss in weight of rats fed on raw mixed and cooked diets

Group No.	Diet	(Mean of 4 rats for 3 weeks) Nitrogen % dry wt. of diet	Food intake dry g.	Total nitrogen intake g.	Gain/loss in wt. g.
1	Eko and Akara				
	Raw	2.960	154.8	4.582	25.9
	Cooked	3.072	154.6	4.749	24.3
2	Pounded yam, efo and snails				
	Raw	1.200	97.8	1.173	-15.6
	Cooked	1.120	132.8	1.487	- 3.0
3	Elubo, egusi and panla				
	Raw	1.520	156.0	2.271	15.4
	Cooked	1.520	160.1	2.433	18.5
4	Fried plantain and beef stew				
	Raw	1.248	176.8	2.206	24.3
	Cooked	1.248	133.5	1.666	2.6
5	Gari, okro and dry fish				
	Raw	1.024	168.9	1.729	4.7
	Cooked	1.024	115.2	1.179	3.2
6	Moinmoin				
	Raw	2.912	145.3	4.231	23.7
	Cooked	3.136	140.8	4.415	38.7
7	Control	3.630	144.9	5.259	44.5

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TABLE 17

The comparison of gain in wt./g. of food intake between the raw and the cooked diets.

Diet	Mean gain in wt./g. of food intake of 4 rats g.	Standard deviation	"t"	Significance
Eko and akara				
Raw	0.166	0.039	0.383	Non significant
Cooked	0.157	0.012		
Pounded yam, efo and snails				
Raw	-0.161	0.031	7.459	Significant
Cooked	-0.023	0.008		
Elubo, egusi soup and panla				
Raw	0.098	0.012	1.294	Non significant
Cooked	0.116	0.021		
Fried plantain beef stew				
Raw	0.137	0.012	16.140	Significant
Cooked	0.012	0.006		
Gari, okro and dry fish				
Raw	0.029	0.001	0.555	Non significant
Cooked	0.030	0.003		
Moinmoin				
Raw	0.195	0.022	3.435	Significant
Cooked	0.275	0.034		
Control	0.306	0.077		

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TABLE 18

The comparison of gain in wt./g. of food intake between the test diets and the standard diet

Diet	Mean gain in wt./g. of food intake (mean of 5 rats) g.	Standard deviation	"t"	Significance
D.1 Raw Control	0.166 0.306	0.039 0.077	2.81	Significant
D.1 Cooked Control	0.157 0.306	0.012 0.077	3.31	Significant
D. 2Raw Control	-0.161 -0.306	0.031 0.077	9.74	Significant
D.2 Cooked Control	-0.023 0.306	0.008 0.077	73.9	Significant
D.3 Raw Control	0.098 0.306	0.012 0.077	4.63	Significant
D.3 Cooked Control	0.116 0.306	0.021 0.077	4.12	Significant
D.4 Raw Control	0.137 0.306	0.012 0.077	3.76	Significant
D.4 Cooked Control	0.012 0.306	0.006 0.077	6.59	Significant
D.5 Raw Control	0.029 0.306	0.001 0.077	6.23	Significant
D.5 Cooked Control	0.030 0.306	0.003 0.077	6.20	Significant
D.6 Raw Control	0.195 0.306	0.022 0.077	2.40	Non Significant
D.6 Cooked Control	0.275 0.306	0.034 0.077	0.68	Non Significant

D.1 = Eko and akara

D.2 = Pounded yam, efo and snails

D.3 = Klubo, egusi soup and pomo

D.4 = Fried plantain beef stew

D.5 = Gari, okro and dry fish

D.6 = Moinmoin

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TABLE 19

The comparison of Gain in wt./g. of Nitrogen intake between the raw and the cooked diets

Diet	Mean gain in wt./g. of nitrogen (mean of 4 rats) g	Standard deviation	"t"	Significance
Eko and akara				
Raw	5.62	1.013	0.81	Non significant
Cooked	5.11	0.401		
Pounded yam, efo and snails				
Raw	-13.30	0.931	17.33	Significant
Cooked	- 2.04	0.757		
Elubo, egusi and peals				
Raw	6.47	0.932	1.20	Non significant
Cooked	7.81	1.351		
Fried plantain and beef stew				
Raw	11.01	0.938	15.79	Significant
Cooked	1.76	0.387		
Gari, okro and dry fish				
Raw	2.94	0.243	1.13	Non significant
Cooked	2.83	0.323		
Moinmoin				
Raw	6.69	0.742	2.49	Significant
Cooked	8.07	0.606		
Control	11.31	0.569		

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TABLE 20

The comparison of gain in wt./g. of N intake between the test diets and the control diet.

Diet	Mean gain in wt./g. of N. intake (Av. of 4 rats)	Standard deviation	"t"	Significance
D.1 Raw Control	5.62 11.31	1.013 0.569	8.48	Significant
D.1 Cooked Control	5.11 11.31	0.401 0.569	15.40	Significant
D.2 Raw Control	413.30 11.31	0.831 0.569	42.30	Significant
D.2 Cooked Control	-2.04 11.31	0.757 0.569	24.40	Significant
D.3 Raw Control	6.47 11.31	0.932 0.569	7.91	Significant
D.3 Cooked Control	7.61 11.31	1.351 0.569	4.37	Significant
D.4 Raw Control	11.01 11.31	0.938 0.569	00.315	Non significant
D.4 Cooked Control	1.76 11.31	0.387 0.569	24.00	Significant
D.5 Raw Control	2.54 11.31	0.245 0.569	23.7	Significant
D.5 Cooked Control	2.83 11.31	0.323 0.569	22.4	Significant
D.6 Raw Control	6.69 11.31	0.742 0.569	8.56	Significant
D.6 Cooked Control	8.07 11.31	0.606 0.569	6.73	Significant

D.1 = Eko and akara

D.2 = Pounded yam, efo and snails

D.3 = Alubo, egusi soup and ponmo

D.4 = Fried plantain beef stew

D.5 = Gari, akara and dry fish

D.6 = Moinmoin

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(b) Criteria of Nitrogen Utilisation.

The following criteria of nitrogen utilisation were worked out on the basis of analysis of faeces and urine for total nitrogen content:-

1. Digestibility of protein

$$= \frac{\text{Nitrogen Absorbed}}{\text{Nitrogen Intake}} \times 100$$

$$\text{or D.P.} = \frac{(NI - NF)}{NI} \times 100$$

where NI = Total nitrogen intake in food

NF = Nitrogen excreted in faeces

D.P. = Percentage of digestible protein

2. $$\text{Biological value} = \frac{\text{N. Retained}}{\text{N Absorbed}} \times 100$$

$$\text{B.V.} = \frac{NI - (NF - \text{metabolic}) - (UN - \text{endogenous N})}{NI - (NF - \text{metabolic N})} \times 100$$

where

NI = Total nitrogen intake in food.

NF = Nitrogen excreted in faeces.

UN = Nitrogen excreted in urine.

The metabolic nitrogen in faeces and endogenous N in urine were determined by feeding non-nitrogenous isocaloric diet.

3. Net protein utilisation (NPU)

$$NPU = \frac{\text{N Retained}}{\text{N Intake}} \times 100$$

or = B.V. x D.P.

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(b) Criteria of Nitrogen Utilisation.

The following criteria of nitrogen utilisation were worked out on the basis of analysis of faeces and urine for total nitrogen content:-

1. Digestibility of protein

$$= \frac{\text{Nitrogen Absorbed}}{\text{Nitrogen Intake}} \times 100$$

$$\text{or D.P.} = \frac{(NI - NF)}{NI} \times 100$$

where NI = Total nitrogen intake in food

NF = Nitrogen excreted in faeces

D.P. = Percentage of digestible protein

2. Biological value $= \frac{\text{N. Retained}}{\text{N Absorbed}} \times 100$

$$\text{B.V.} = \frac{(NI - (NF - \text{metabolic}) - (UN - \text{endogenous N}) \times 100}{NI - (NF - \text{metabolic N})}$$

where

NI = Total nitrogen intake in food.

NF = Nitrogen excreted in faeces.

UN = Nitrogen excreted in urine.

The metabolic nitrogen in faeces and endogenous N in urine were determined by feeding non-nitrogenous isocaloric diet.

3. Net protein utilisation (NPU)

$$\text{NPU} = \frac{\text{N Retained}}{\text{N Intake}} \times 100$$

or = B.V. x D.P.

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Digestibility of protein.

The differences in the digestibility of food protein of raw and cooked diets have been worked out. The mean of 4 rats in every group fed on raw diet has been compared statistically with the corresponding group fed on the cooked form of the same diet.

From the results reported in Table 21 it can be seen that in diet No. 1 it is significantly higher in the cooked diet than in the raw. The weight gains/g. of food intake or per g. of nitrogen intake in this diet are not significant between the raw and cooked form. It is therefore likely that the higher digestibility of protein in the cooked form has only compensated for the other losses which the food might have suffered during cooking. In diet No. 2 the digestibility is significantly lower in the cooked form than it is in the raw. The diet produced only loss of wt. in the animals although this loss was significantly less when the diet was fed in cooked form. This suggests that there was either an improvement in the utilisation of absorbed nitrogen (Biological value) of the cooked form of diet or certain other factors caused a more efficient intake in the cooked form of the diet, and prevented as heavy a loss of wt. as was noticed when the diet was fed raw. In diet No. 3 the differences in digestibility, gain in wt./g. of food or per g. of nitrogen were all non-

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significant when the raw and cooked forms were compared. In diet No. 4 the digestibility is significantly higher in the cooked form; while the weight gains are higher when the diet is fed raw. It is therefore likely that factors other than the digestibility of the protein have played an important role in case of this diet. In diet No. 5 the digestibility is significantly lower in the case of the cooked diet but the wt. gains are non-significant when raw and cooked forms of the diet are compared. It is therefore likely that the better utilisation of factors other than protein have compensated for any lower digestibility of protein in the cooked form. In diet No. 6 the digestibility, wt. gains/g. of food and/g. of nitrogen were all better in the cooked form of diet.

Comparison with the control diet.

When compared with the control diet (Table 22) the digestibility of proteins in all the diets was significantly less except for the cooked form of diet No. 6 where this difference was non-significant.

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TABLE 21

The comparison of digestibility of protein of raw and cooked diets in the rat

Diet	Digestibility of protein % (Av. of 4 Rats)	standard deviation	"t"	Significance
Eko and akara				
Raw	78.8	0.973	7.89	Significant
Cooked	86.3	1.327		
Pounded yam, efo and snails				
Raw	73.6	3.310	4.07	Significant
Cooked	63.2	2.936		
Elubo, egusi soup and panla				
Raw	68.7	2.396	1.14	Non Significant
Cooked	71.2	2.931		
Fried plantain and beef stew				
Raw	60.0	4.932	2.72	Significant
Cooked	72.9	6.593		
Gari, okro soup and dry fish				
Raw	78.1	0.583	2.60	Significant
Cooked	73.1	3.276		
Moinmoin				
Raw	78.1	2.083	4.20	Significant
Cooked	88.8	3.892		
Control	92.4	3.444	—	—

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TABLE 22

The comparison of digestibility of protein between the test and control diets in the rats.

Diet	Mean digestibility of protein % (Av. of 4 rats)	Standard deviation	"t"	Significance
D.1 Raw Control	78.8 92.4	0.979 3.444	6.85	Significant
D.1 Cooked Control	86.3 92.4	1.327 3.444	2.86	Significant
D.2 Raw Control	73.6 92.4	3.310 3.444	6.82	Significant
D.2 Cooked Control	63.2 92.4	2.936 3.444	11.20	Significant
D.3 Raw Control	68.7 92.4	2.394 3.444	10.00	Significant
D.3 Cooked Control	71.2 92.4	2.931 3.444	8.12	Significant
D.4 Raw Control	60.0 92.4	4.932 3.444	9.33	Significant
D.4 Cooked Control	72.9 92.4	6.553 3.444	4.56	Significant
D.5 Raw Control	78.1 92.4	0.983 3.444	7.09	Significant
D.5 Cooked Control	73.1 92.4	3.276 3.444	7.03	Significant
D.6 Raw Control	78.1 92.4	2.083 3.444	6.15	Significant
D.6 Cooked Control	88.8 92.4	3.892 3.444	1.19	Non-Significant

D.1 = Eko and akara

D.2 = Pounded Yam, efo and Snails

D.3 = Elubo, egusi soup and panla

D.4 = Fried plantain beef stew

D.5 = Gari, okro and dry fish

D.6 = Moinmoin

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TABLE 23

Criteria of Nitrogen Utilisation in the raw and cooked diets.

Diet	Total N intake	Nitrogen Absorbed	Nitrogen Retained	Digestibility	(Biological value)	Net protein Utilisation
	(Average of 4 rats for a period of 3 weeks)					
Eko and akara						
Raw	4.582	3.873	3.315	78.8	85.6	72.3
Cooked	4.749	4.4.416	3.961	86.3	89.7	83.4
Pounded yam, efo and snails						
Raw	1.173	1.064	0.900	73.6	84.5	76.7
Cooked	1.487	1.211	1.058	63.0	87.3	71.1
Elubo, egusi soup and panla						
Raw	2.371	1.953	1.814	68.7	92.8	76.5
Cooked	2.433	2.083	1.925	71.2	93.3	79.1
Fried plantain and beef stew						
Raw	2.206	1.688	1.368	60.0	81.0	62.0
Cooked	1.666	1.490	1.308	72.9	87.7	78.5
Gari, okro soup and dry fish						
Raw	1.729	1.709	1.510	78.1	88.3	87.3
Cooked	1.179	1.098	0.861	73.1	78.3	73.0
Moinmoin						
Raw	3.503	2.986	2.580	78.1	86.3	73.6
Cooked	4.491	4.213	3.559	88.8	86.7	80.5
Control	5.259	5.162	4.191	92.4	83.1	81.5

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9. Protein levels in the blood sera of rats fed raw and cooked diets.

Procedure.

Total serum proteins were estimated in the pooled samples of serum obtained from the blood of rats after decapitation. All the rats fed on the experimental diets were killed at the end of 24 days and their blood collected. It was allowed to coagulate at 37 °C for one hour and then serum obtained by centrifugation. The sera of each group of rats (all of them being on the same diet) were pooled. Analyses for total proteins in duplicate samples of each pool were carried out by the method described in page 80.

Results.

The results of the total protein content in the serum of various groups of rats fed on raw mixed or cooked diets are presented in Table 24. It can be seen that the total serum proteins range between 5.3 to 6.4 g./100 ml., with the serum protein content of the control animals being at the top end of the scale.

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TABLE 24

Total serum proteins as estimated by the Biuret method

Diet	Total serum proteins g./100 ml.
Eko and Akara	
Raw	6.1
Cooked	6.4
Pounded yam efo and snails	
Raw	6.0
Cooked	5.3
Elubo, egusi and panla	
Raw	5.3
Cooked	5.5
Fried plantain and beef stew	
Raw	6.5
Cooked	6.1
Okro, dryfish and Gari	
Raw	5.3
Cooked	5.2
Moinmoin	
Raw	6.0
Cooked	6.2
Control	6.3

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6 (a) **Protein patterns in the blood sera of rats fed raw and cooked diets. (paper electrophoresis).**

Procedure.

In 0.07 ml. of serum from each pool the serum proteins were separated by paper electrophoresis. The method followed is described in page 81 to 83. The experiments were carried out in duplicate.

Results.

The paper strips showing the various fractions of the serum protein were cut and scanned. Table 25 gives the percentage of various protein fractions as found in the serum of various groups of rats fed on raw, cooked and control diets. From these results it can be seen that the concentration of various fractions of protein in the serum of rats fed on the raw and the cooked diets is almost the same. Further these concentrations are not very much different from the ones found in the case of rats fed on control diet.

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TABLE 25

Percentage of various proteins fractions in the sera of rats fed on raw, cooked and control diets.

Diet	Albumin	Globulins.		
		α_2	β	γ
Eke and Akara				
Raw	58.0	12.0	20.4	6.7
Cooked	59.2	11.4	18.5	6.5
Pounded yam, efo and snails				
Raw	57.3	10.4	22.0	5.8
Cooked	58.4	11.0	21.7	6.2
Elubo, egusi soup and ponmo				
Raw	58.2	10.8	23.6	6.1
Cooked	59.0	11.2	21.8	6.0
Fried plantain and beef stew				
Raw	57.2	11.5	20.6	6.4
Cooked	59.6	10.9	21.0	5.9
Gari, okro and dry fish				
Raw	58.1	10.4	21.6	6.1
Cooked	59.2	10.8	21.3	6.0
Moinmoin				
Raw	59.1	11.2	19.5	6.1
Cooked	59.4	11.1	20.4	6.2
Control	60.0	12.2	21.9	6.6

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6 (b) **Protein patterns in the blood sera of rats fed on raw and cooked diets. (starch gel electrophoresis).**

Procedure.

The same samples of blood sera as for paper electrophoresis were run on starch gel. The method is described in page 83.

Results.

The results are shown in plates 5-6. The main features of these separations are noticeable.

(a) The clear separation of various fractions of serum protein.

(b) The *similarity* of protein patterns in the sera of rats, whether fed raw or cooked forms of the diets.

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Protein patterns in the blood sera of rats fed on "Eko and Akara"

1 = raw diet
2 = cooked diet.

Protein pattern in the blood sera of rats fed on "Pounded yam, efo and snails"

3 = raw diet
4 = cooked diet.

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PLATE 5.

Protein patterns in the blood sera of rats fed on "K[illegible]o, egusi soup and panla"

5 = raw diet
6 = cooked diet

Protein patterns in the blood sera of rats fed on "Fried plantain and beef stew"

7 = raw diet
8 = cooked diet.

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Protein patterns in the blood sera of rats fed on "Gari, okro soup and dry fish"

9 = raw diet

10 = cooked diet.

Protein patterns in the blood sera of rats fed on "Moinmoin"

11 = raw diet

12 = cooked diet.

Protein patterns in the blood sera of rats fed on control diet (13).

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PLATE 7

CHAPTER 5

DISCUSSION

The various parameters examined to assess the nutritional value of six common Nigerian diets were:-

1. Recipes, method of preparation and the intake of various dietaries.
2. Chemical composition of ingredients, raw mixed diets, and cooked diets.
3. The amino acid composition of proteins of the raw mixed and the cooked diets.
4. Losses incurred during the preparation of food.
5. The effects of feeding these diets on rats.
6. Serum protein levels and serum protein patterns of experimental animals.

The results of all these criteria are explained at appropriate places in this thesis, but for a better evaluation it is necessary to discuss their importance and study an overall cumulative effect of all of them together.

1. Recipes, method of preparation and the intake of various dietaries.

While judging the nutritional adequacy of these diets it is important to keep in mind that these results were derived by feeding the diets to the animals in isolation.

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With man, the recipes would be subject to changes according to the taste of the individual, the location and the season; and last but not the least is the fact that the consumption of individual dishes within the same diet depends very much on the economic status of the individual taking the meals. There were for instance, wide variations in the amount of akara taken with eko Table 1, and in the amount of stew taken with pounded yam, gari, fried plantain or alubo (Table 2 to 5). This is not a matter of mere taste. The eleven persons selected for the test represented different income levels. It was noticed that those with a low income took comparatively smaller quantities of the costly protein supplements. In this connection, it may be noted that the addition of eggs in diet No. 6 has been described as optional by Williams (1962) depending mostly on the income of the family.

2. <u>Chemical composition of ingredients, raw mixed and cooked diets.</u>

The analysis of the ingredients used in the preparation of these diets is described in Table 10. The values presented are the means of the concordant duplicate analyses on a single sample of the food, of average quality, as bought in the market. Most of these foods had already been analysed and references are scattered through the literature

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(Bassir (1964), Oyenuga (1959), Oke (1965) Akinrele (1965). A consolidated information for some of the foods is also available in M.R.C. Bulletin No. 302 (1965). Slight variations in these results were noticed, which can be due to a number of factors such as varieties, treatment of food crops, handling and storage of food etc.

The chemical composition of the raw mixed and cooked diets varied according to the ingredients used. It was noticed that in the case of diets Nos. 1 and 6 (Table 1 to 6) where the protein source is of vegetable origin the protein percentage was higher while it was less in the case of other diets where the protein source was of animal origin. The latter, being expensive, are used in smaller quantities. A finding which supports results of Bassir (1953).

3. Amino acids.

Results of the quantitative determination of amino acids in raw mixed and cooked diets are reported in Table 15 page 101. The modification made in the original method of Stein and Moore (1956) (as adopted by Meanig, 1959) are described on page 55. A comparison of the amino acid patterns of the test diets with the FAO provisional pattern is given in Table 25

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TABLE 26

Comparison of essential amino acids in raw and cooked diets with the FAO provisional pattern

Diet	Amino acids mg./g. of Nitrogen									
	Iso-Leucine	Leucine	Lysine	Phenyl-alanine	Methionine	Total sulphur containing	Threonine	Tryptophan	Valine	Tyrosine
FAO provisional pattern	270	306	270	180	144	270	180	90	270	180
Eko and akara Raw	300	465	406	325	93	169	243	60	350	184
Cooked	296	450	203	315	60	121	205	60	361	173
Pounded yam, efo and snails Raw	296	535	402	210	167	277	285	54	448	182
Cooked	290	425	216	206	159	269	178	57	439	178
Elubo, egusi soup and panla Raw	303	451	525	222	174	249	259	59	319	161
Cooked	307	452	225	203	129	203	252	59	307	160
Fried plantain beef stew Raw	339	531	566	266	161	237	286	75	359	202
Cooked	346	513	197	243	72	135	150	75	270	205
Gari, okro and dry fish Raw	317	473	549	232	182	262	271	64	333	168
Cooked	343	486	170	217	888	167	185	64	320	205
Moinmoin Raw	292	470	318	438	83	159	254	65	357	180
Cooked	241	434	108	378	63	126	240	65	361	166
Control diet	407	625	498	362	191	214	266	83	460	335

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From a nutritional point of view the essential amino acids are the most important ones in any food protein. FAO in their nutrition studies No. 16 (1955) have given a provisional pattern of amino acids considered to be the optimum quantities of essential amino acids required in a protein. In Table 26, the quantities of essential amino acids as found in raw and cooked diets have been compared with the FAO provisional pattern. The values for tryptophan shown in this table are only the approximate values calculated from the amino acid composition tables of Orr and Watt (1957).

From the results, it is clear that almost all the diets are slightly low in the sulphur containing amino acids, (although diets No. 2, 3 and 5 are not deficient in methionine). Lysine is the limiting amino acid in the cooked form of all the diets; but there is no deficiency of this amino acid in the raw mixed diets. Tryptophan is the 3rd limiting amino acid but since its requirements are so small it may not cause much change. The concentration of all the remaining amino acids nearly agreed with the requirements as outlined in the FAO provisional pattern (1957).

One serious objection to the applicability of the results of amino acid estimation by this method is raised by workers like Bender (1966) who contend that the results achieved by strong acid hydrolysis may not be the same as in the animal

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body since the conditions of enzymatic hydrolysis are different. The objection seems valid unless proved otherwise. Nevertheless, there is reason to believe that the results of acid hydrolysis give a good picture of amino acid pattern, because when Basir (1964) estimated the amino acid patterns of his experimental diets and supplemented them with the deficient ones, obtained a positive response of the animals fed on the corrected diets.

4. Losses incurred during the preparation of the food.

Changes in the total content of various nutrients during the preparation of food have already been explained (page 94-95). The loss of vitamins, however, deserves special consideration. The loss of vitamin A (including carotene 25 - 50% from the cooking oil is a possible result of high temperature in the presence of atmospheric oxygen. Similar results have been reported by Maqsood, Haque and Khan (1963).

Thiamine is a vitamin which is stable to heat in acid medium. But much of it could be lost in cooking water due to leaching, excessive washing and sincing of the food material. The loss varies from about 5% as in the case of diet No. 6 to almost complete destruction as in diets Nos. 4 and 5. There was an initial high content of the vitamin, and steam cooking with no extra water in the case of diet No. 6, reduced the loss; while prolonged cooking, washing

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and cutting of the ingredients took place in preparation of diet No.4 and 5. The results agree in principle with the work of Roy & Rao (1963) and Cuendet (1954).

Although vitamin B_2 is stable to oxygen and to acid conditions it is lost by the influence of light and alkali. The losses observed were up to 50%; most of them were due to leaching and open pan cooking.

Another water soluble vitamin is C, which is also easily oxidised by atmospheric oxygen and heat. The losses in all the diets were high, (Table 14). They ranged from complete destruction to a minimum of about 40% destruction. These were primarily due to washing, soaking of food material in water and cooking in an open pan. Similar losses have been observed by other workers (Bender 1960), Mary (1949) and Oke (1961).

5. The effects of feeding these diets to rats.

One of the most practical methods of finding the nutritional value of a diet is measurement of the weight change which it will produce when fed to rats. The significance of these changes has been mentioned in page 104.

When raw mixed and cooked forms of the Eko and Akara diet were compared, it was evident that both of them produced considerable gains (25.9 g. in the case of raw, and 24.4 g. in the case of the cooked diet, in the weight

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of rats fed on them. The total food intake was nearly the same 134 g. in both cases. The percentage of digestible protein was about 10% higher in the cooked diet, the difference being statistically significant at 1% level.

However, when the diet was compared with the control balanced diet the weight gains were about 45% less than those of rats which were fed on the control diet; although the total intake of the Eko and Akara was about 7% higher.

This tends to show that both the raw and cooked forms of this diet are only capable of supporting the animal at a suppressed rate of growth, although the digestibility of protein is improved by the cooking.

When the Pounded yam, Efo and Snails diet, both in raw and cooked forms, was fed to rats, loss of weight resulted. The loss was higher in the rats which were fed on the raw diet. The digestibility of proteins was 73.6% with the raw diet while it was only 63.2% with the cooked diet. The total intake, 99.8g of the raw feed was lower than that of the cooked diet 132.8 g. This difference can be attributed to both the better digestibility and palatability of the diet. However, the total food intake of the cooked diet is not very different from that of the control.

The most obvious conclusion is that the diet is deficient in one or more essential nutrients and so fails

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to maintain the optimum growth rate in the rats.

Rats on both the raw and the cooked Elubo egusi soup and panla diets showed an initial lag of about one week before they started to gain any weight. After another week, the growth rate became almost steady. At the end of 3 weeks the average body weight gains were 18.5 g. and 13.4 g. respectively, for the animals fed on the cooked and raw mixed diets. The corresponding total food intake was 160 g. and 156 g. respectively. The digestibility of protein also did not show any significant difference. (68.7% and 71.2%).

When compared with the control, the food intakes of both the raw and the cooked diets were significantly high; while gain in weights and digestibility was low. On the whole, the diet was found to be nutritionally inadequate to support optimum growth.

The rats fed on the raw mixed fried plantain and beef stew diet showed an average body weight gain of 24.3 g. in 3 weeks while those receiving the diet in cooked form gained weight at a much slower rate, only up to 2 g. more than the initial weight of the rats. The total food intakes of the raw and cooked diets were 176.8 g. and 133.5 g. respectively, while the digestibilities of the proteins were 60% and 72.9%. The difference in the digestibility

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nearly accounts for the high food intake of the raw mixed diet. It is therefore probable that the difference in weight gains is due to the loss of certain growth promoting factor other than protein.

As compared with the control, the raw diet produced only half as much weight gain, and the cooked form produced almost negligible weight gains. The diet is therefore essentially a deficient one.

The rats both on the raw and the cooked gari, dry fish and okro diet showed only minor variations in body weight gains. At first they started loosing weight slowly until about 9th day when the average maximum loss was 5 g. in the case of the cooked diet and 2.5 g. in the case of the raw mixed diet. Thereafter, there was a gradual increase in the weights of rats so that at the end of 21 days the rats on the cooked diet had gained 5.5 g., while those on the raw mixed diet increased their weights by 4 g. on the average. As for the food intake, the rats on the raw diet took 168.9 g. on the average while those on the cooked took only 115.2 g. The digestibility of the proteins was also better 78.1% in case of the raw and against 73.1% in the case of the cooked diet. It therefore seems likely that growth on this diet was inhibited either by lack of some essential nutrient or by the presence of some growth-reducing factor which may be more active in the case of the

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raw mixed diet. When compared with the control, the results were much lower.

There was an average gain of 23.7 g. in the body weight of rats fed on the raw moinmoin diet, and 38.7 g. in the animals on the cooked diet. The rats consumed 120.3 g. and 140.8 g. of raw and cooked diets, respectively. The digestibility of the protein in the raw diet was 78.1% as against 88.8% in the case of the cooked diet. When compared with the control, the gain in weight on the cooked diet seems quite near the optimum weight gain as recorded with the control diet. In the case of the raw mixed diet, it is about 25% less than the control. It seems likely that cooking has improved the digestibility of the proteins and caused an increase in the food intake. Some important conclusions which can be drawn from the foregoing are:-

(a) All the diets tried in these experiments failed to produce optimum growth in rats.

(b) The gain in weight produced in the rats by feeding these diets roughly increased with the increasing amount of nitrogen (protein) intake.

(c) There is a definite improvement in the digestibility of proteins in the diets Nos. 1 and 6 (containing beans) and diet No. 4 (containing plantain and beef stew) on account of cooking.

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(d) Diets Nos. 6, 1, 4 (in raw form only), and 3, produced from good to medium gains in the weight of rats, while diet No. 4 (in cooked form) and diet No. 5 maintained the animals and did not produce any appreciable growth.

(e) The animals on diet 2 lost weight at first rapidly and then maintained themselves at the lower weight. The loss was more serious in the case of the raw mixed diet than in that of the cooked form of it. But this difference could have been due to the fact that rats on the raw diet ate very little food.

The conclusion (a)-(e) tend to show the following two main points:-

(i) The diets are deficient in one or more nutrients.

(ii) Enough protein is not being fed or the protein is deficient in certain essential amino-acids.

To prove these points, the levels of nutrients in the diets can be compared with the minimum levels of these nutrients required to produce maximum growth in rats as outlined by Cuthbertson (1939). The result of such a treatment is presented in Table 27. The deficiencies of amino acids which look so obvious in this table are mainly due to the low percentage of protein in the diet Nos. 2, 3, 4 and 5. They would be much less serious if the percentage

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TABLE 27

The comparison of various nutrients in raw and cooked diets with the standard requirements for rat

Nutrient	Standard requirement	Eko and akara		Pounded yam, efo and snails		Eluba, egusi soup and panla		Fried plantain and beef stew		Gari and okro and dry fish		Moinmoin		Control
		Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	
Protein %	12.5	18.5	19.2	7.5	7.0	9.5	9.5	7.8	7.8	6.4	6.4	18.2	19.6	22.6
Valine g./100 g.	0.7	1.036	1.109	[illegible]	0.492	0.485	0.467	0.449	0.462	0.341	0.322	1.040	1.131	1.670
Leucine "	0.8	1.378	1.382	[illegible]	0.588	0.687	0.688	0.663	0.641	0.484	0.496	1.389	1.360	2.270
Iso-Leucine g./100 g.	0.4	0.888	0.912	[illegible]	0.325	0.461	0.468	0.424	0.433	0.325	0.250	0.851	0.756	1.480
Methionine "	0.4	0.277	0.186	[illegible]	0.179	0.265	0.196	0.201	0.090	0.186	0.090	0.250	0.200	0.696
Threonine "	0.5	0.721	0.632	[illegible]	0.200	0.394	0.384	0.357	0.188	0.278	0.189	0.740	0.742	0.966
Phenylalanine g./100 g.	0.8	0.962	0.970	[illegible]	0.231	0.338	0.310	0.333	0.304	0.238	0.222	0.275	1.189	1.315
Tryptophan "	0.1	0.185	0.185	[illegible]	0.059	0.090	0.090	0.094	0.094	0.065	0.065	0.182	0.182	0.301
Lysine "	1.0	1.202	0.625	[illegible]	0.243	0.798	0.347	0.707	0.246	0.562	0.174	0.928	0.340	1.510
Histidine "	0.4	0.555	0.548	[illegible]	0.099	01313	0.260	0.281	0.281	0.208	0.220	0.549	0.648	0.682
Arginine "	0.2	1.184	1.174	[illegible]	0.526	0.512	0.522	0.582	0.582	0.361	0.366	0.900	0.972	0.919
Vitamin A (I.U./Kg.)	3000	50700	246000	[illegible]	27000	21380	15780	31360	16720	50060	36180	75110	58210	11500
Vitamin B_1 (mg./Kg.)	1.2 to 2.0	5.4	2.3	2.2	0.5	1.8	0.4	0.6	–	0.7	-	5.2	5.0	4.0
Vitamin B_2 (mg./Kg.)	2.5 to 5.0	1.8	0.9	1.3	0.8	1.3	0.9	1.4	0.8	1.0	0.6	8.2	2.2	20.0
Calcium (g./Kg.)	4.5 to 6.0	0.87	0.75	[illegible]	3.07	2.12	2.15	0.16	0.10	3.44	3.18	0.78	0.74	1.08
Phosphorus (g./Kg.)	3.5 to 4.0	2.89	2.78	[illegible]	1.50	1.63	1.57	1.23	1.18	0.72	0.70	2.59	2.37	0.42
Iron (mg./Kg.)	10 to 50	61.2	61.0	[illegible]	100.0	117.0	112.0	17.0	17.0	63.6	63.0	33.0	33.0	20.0

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of total protein in the diet were brought up to 12.5, or if the results were expressed as amino acids per gram of N. This has intentionally not been done in order not to mask the real value of the diets, as they are used.

The deficiencies of vitamins and minerals are also no less important. But if the protein levels of the diets were improved, it is likely that they would also improve. The major deficiencies of B vitamins, and their loss during preparation, require an improvement of the existing recipes, handling, and cooking methods. Similarly the low levels of calcium in diets Nos. 1, 4 and 6 need to be corrected before one can achieve optimum growth of the animals fed on these diets.

It must be pointed out that the table of nutritional requirements (Table 27) is not complete. There are certain other nutrients which are equally important (although in micro quantities) which could not be included in this study because of limitation of time.

6. Serum protein levels and serum protein patterns of experimental animals.

The results of these experiments as presented in Table 25 and plates in pages 123 to 125 did not show much variations from the standard. Two conclusions are therefore possible:-

(i) The diets do not adversely affect the serum protein levels and the protein patterns;

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or

(ii) 3 weeks is too short a period to produce any change of this nature.

Both of these seem tenable unless proved otherwise.

General Summary.

Of all the six diets tried in this study, Moinmoin proved to be the best; while Eko and Akara, Elubo egusi soup and ponle, Fried plantain and Beef stew, followed in a descending order. Okro dry fish and gari just supported the animals and did not produce any growth. Pounded yam, efo and snails made the animals to lose weight. None of the diets tried in this study could produce optimum weight gains. The most common deficiency noticed is the quantity of proteins of good composition. Deficiencies of vitamins and minerals were also present.

It is therefore recommended that the use of more good quality proteins may be of vegetable or animal origin along with vegetable salads (which seem conspiciously absent from Nigerian diet) and fruits should be encouraged in order to ensure good health.

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Contributions to knowledge

The present study has made the following important contributions to the knowledge of nutrition:-

(1) The levels of the major nutrients in all the foods (as sold in the market) used in the preparation of six common Nigerian diets have been determined.

(2) The nutritional values of the raw mixed diets, and of the cooked diets, have been determined, and the losses which take place during preparation of diets have been elucidated.

(3) A battery of special cages was designed for conducting trials on rats so as to find out the digestibility and biological values of food proteins.

(4) The digestibility, biological values and other criteria of nitrogen utilisation have been worked out by feeding these diets to rats. At the same time, by using rat growth as an index of nutritional adequacy, the nutritional values of these diets have been ascertained.

(5) The original method of Stein and Moore as adopted by Hennig (1959) was used for the quantitative estimation of amino acids, in the raw mixed and cooked diets, on the automatic amino acid analyser manufactured by Bender and Hobein Munchen W. Germany.

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(6) The levels and patterns of serum protein of rats fed on six common Nigerian diets have been determined, using a biuret method and the techniques of paper electrophoresis, and starch gel electrophoresis.

(7) A comparison of the nutritional values of these diets with the standard requirements of rats, and essential amino acid patterns of these diets with the FAO provisional pattern of amino acids in food proteins has been presented.

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