

STUDIES ON
THE AMINO ACID COMPOSITION OF MUSCLE TISSUES
OF BUFFALO, GOAT AND POULTRY,
WITH REFERENCE TO LYSINE, ARGININE, LEUCINE,
ISOLEUCINE AND PHENYLALANINE



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"Ad Maiorem Dei Gloriam"

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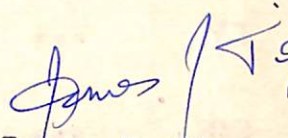
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C O N T E N T S

| CHAPTER | | PAGE |
|---------|------------------------------|---------|
| I | INTRODUCTION. | 1 |
| II | REVIEW OF LITERATURE ... | 10 |
| III | MATERIALS AND METHODS... .. | 49 |
| IV | RESULTS AND DISCUSSION.. ... | 71 |
| V | SUMMARY. | 102 |
| | BIBLIOGRAPHY | 1 - xi. |

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CHAPTER - I.

INTRODUCTION.

I N T R O D U C T I O N

"Over the greater part of the earth and for at least three-fourths of our two billion planetary population, the will to eat is the primary urge of the eternally hungry man".

(Bowman, 1935).

From the dawn of history, the primal instinct of hunger has given man a compelling interest in the problems of securing and maintaining his food supply. First, as a hunter and then as agriculturist and herdsman, he acquired a simple but constantly enlarging knowledge of production and conservation. From such simple beginnings has arisen one of the most important, complex, and interesting of the social and economic problems of the present time, the World's food supply. With these impressions, Prescott and Proctor (1937) pinpointed the significance of the pressing need of World's food supply, on more scientific lines.

In general consideration of World's food problem, the Food and Agriculture Organization of United Nations, examined, all the means of adding to supplies of food, and published the known facts in series, in connection with Organization's "Freedom from Hunger Campaign". In relation to future needs for nutrients, Sukhatme (1961) of the said organization, looked forward over the periods of, roughly, the next 10, 20, and 40 years; which shows that, if recommended allowances are to be complied with, even to moderate extent, one of the main needs will be, for production of protein-rich foods to increase at a rate, relatively much greater

than of cereals and starchy roots (cited by Harvey and Rigg, 1964). Obviously, in developing countries, where population pressures are greatest, the most serious difficulty lies in the quality of diet which is characterized by low level of protective foods. The diet of an average Indian lacks with these, which are so important and essential for human life.

✓ From practical standpoint of human nutrition, Finn (1954) stated that there is no relatively simple unit, such as the calorie, available for the measurement of the quality of diets, and the protein content is perhaps the best available indicator, because most foods rich in protein are also comparatively good sources of other essential nutrients. Since this is particularly true of foods of animal origin, the animal protein intake is probably better than the total protein intake as an indicator.

✓ The joint F.A.O./W.H.O. Expert Committee on Nutrition (1953) uses the term protein malnutrition, "to indicate in general, a state of ill health occurring where diets are habitually poor in protein, while they are more adequate in calories The concept includes the effects of deficiency in the quality of protein consumed, of imbalance of amino acids and deficiency of factors such as Vitamin B₁₂, commonly found in foods in association with animal protein and concerned with protein metabolism". (cited by Thomson and Duncan, 1954).

✓ Tracing the history, the name protein (Greek : preeminent, or first) was suggested by Berzelius to Mulder and given by the latter in 1838 to the complex organic nitrogenous substances found in the cells of all animals and plants. Proteins

occupy a central position in the architecture and functioning of living matter.

✓ Proteins are indispensable constituents of the living protoplasm and participate as such in all vital processes. According to Geiger (1961), the specific structure of the various body proteins imparts particular properties which are determinants of growth, neuromuscular and mental functions, of enzymatic and hormonal processes, immunological phenomena, physicochemical properties of tissue fluids, of many other organ functions and indirectly of reproduction and heredity.

✓ Since, protein is the principal constituent of the organs and soft structures of the animal body, a liberal and continuous supply is needed in the food throughout the life for growth and repair, and thus the transformation of food protein into body protein is very important part of nutrition. Apparently, no two proteins seem to be exactly alike in their physiological behaviour but from the standpoint of nutrition, the important distinguishing feature of the various proteins is their amino acid make up.

In Howe's observations (1951), an amino acid, to an organic chemist, is any of a large group of compounds, which contains one or more amino groups and one or more acid groups. To the biochemist, however, this term applies only to a very limited number of compounds, all of which have in common an amino group in the α -position to a carboxyl group. In addition, they are products of hydrolysis of proteins or very closely related to these products. Proteins as such are polymerized units of amino acids, which vary in relative amounts and sometimes in kind from protein to

protein.

Those amino acids which can be synthesized by the body from the other constituents of the ordinary diet at a rate adequate for nutritional demands are called "dispensable or nonessential" amino acids; those which cannot be so synthesized but must be present in the diet are called "indispensable or essential" amino acids. These terms refer solely to the presence of these amino acids in the diet.

Magendie (1816) recognized that the nitrogen present in the body had its origin in nitrogen compounds present in the food thereby it became established that proteins were the nitrogen compounds essentially concerned. Subsequently, Magendie (1841) in his "gelation report" impressed that gelation could not take the place of meat protein in the diet. Three decades later, Escher found that the nutritive value of gelation for dogs could be improved by the addition of tyrosine. But almost no quantitative data on the distribution of amino acids were available before 1900, as such structure proteins from diverse sources were being regarded in general as equal in nutritive value. Gradually, it became apparent that nitrogen metabolism is concerned, not with a single entity (protein) but with each of the components of the protein i.e. amino acids.

Osborne and Mendel (1914) remarked, "The current trend of investigation of chemistry of nutrition is emphasizing the significance of the amino acids as the fundamental factors in all problems in which hitherto the role of protein has been involved The question of protein synthesis has now become a

problem of biochemical department of amino acids". (cited by Rose, 1957). They further showed in the same year that certain proteins, which resulted in nutritive failure in rats when used alone, were rendered satisfactory by the addition of missing amino acids.

Meat is the properly dressed flesh derived from cattle, swine, sheep or goats, sufficiently mature, and in good health at the time of slaughter, but is restricted to that part of the striated muscle which is skeletal or that which is found in the tongue, diaphragm, heart or oesophagus. The principal value of food animals lies in the meat proper, namely in the striated muscles, which in accordance with their location and coarse anatomical structures, possess different values as human food. The chief chemical constituents of the muscles are the proteins and gelatin that yield nitrogenous bodies. Fat, carbohydrates and salts are the components that vary in quantities.

One of the most dramatic changes in nutritional concepts in medical opinion regarding meat in the diet resulted from an experiment conducted by the Russell Sage Foundation in the year 1928. The arctic explorers, Vilhajmer Stefansson and Karsten Anderson, lived for a year on a diet consisting exclusively of meat. These studies provided proof of the value of meat in the diet of man (cited by Singh, 1967).

Nutrition researches for the space travellers are being conducted in U. S. A., U. S. S. R., and other countries. Finkelstein (1960) recommended sample menu during flight, which included meat too, as the source of animal protein. The spectacular scientific achievement, during the rendezvous mission of Apollo-8 (1968),

rendered a chance to the astronauts, for enjoying the Christmas delicacy (turkey meat), in the distant space. Following in the series, a giant leap of mankind, exhibited by the moonlanders (1969), revealed the precise precautions undertaken by the Nutrition Scientists, to pack the Astronauts' bags with bacon and sausage.

The domestic goat "a poorman's cow" is widely distributed, because ^{of} its ability to thrive on sparse vegetation in both temperate and warm climates, and to withstand dry conditions better than cattle. It forms the primary source of meat in this country for obviously prevailing sentiments. On the other hand, buffaloes comprise 25% of Nation's bovine population which approximates half the world's figure. With the advent of exotic breeds of poultry in the country, further stride in the development of this particular industry as a whole, consequent to this, improvement upon the supply of poultry products, may indicate promise for the imminent future.

Customarily, in scientific livestock feeding, the various protein supplements of animal origin, obtained as by-products from dairy, meat, industries and fishery plants, are primarily incorporated in the usual rations of pigs and poultry. The role of amino acids in health, clinical and dietotherapeutic procedures is immense. The latest trend has been the inclusion of lean meat in human nutrition, as a delicious component for balancing the diet, which on economic consideration, seems, comparatively, the cheapest source of animal protein. Incessant inflow of new concepts on amino acid nutrition, invite the scientists to the different problems

coupled with divergent views, but meat in particular, has yet a predominant place as an item and supplement.

The discovery that many of the amino acids composing body proteins must be supplied as such by food proteins, explains why different foods and rations of same protein content have different values in nutrition i.e. they differ in protein quality. Various procedures have been adopted to determine it but essentially, they can be categorised into these three :-

(i) Biological value :- The biological value of a protein is a measure which is based upon a nitrogen balance technique, and can be regarded as the percentage of the absorbed nitrogen, from the given dietary protein which is retained in the animal body. However, its determination requires distinction to be made between exogenous and endogenous components of excreta. Dynamic concept of metabolism makes such quantitative distinction difficult.

(ii) Growth measurements :- Growth measurements are used for the determination of gross protein value and based upon a comparison between the mean growth of animals on diets containing a given protein with that achieved by the reference protein. The results are expressed as "protein efficiency ratio" or "growth promoting value". These methods are essentially dependent on the availability of a particular amino acid in the test protein but they do not usually comply with the necessary conditions of sound bioassay.

(iii) Chemical score :- A comparison of the quantitative distribution of the essential amino acids in a feed with the relative amounts needed by the body per unit of feed provides a method of estimating protein quality. Such a measure has been referred to as

a "Chemical Score".

With the recent development of relatively simple methods for quantitative determination of amino acids in protein hydrolysates viz. the microbiological, enzymatic and chromatographic methods, chemical scoring of proteins can be done conveniently and thus the protein quality known.

Amino acids can be limiting either by deficiency or by excess and is most effective at an intermediate level depending on the relative proportions of other amino acids in the diets, (Rerat et al., 1956). Further, the prevalent argument is that there is need for further researches on a reference protein and its ability to satisfy the needs of an organism for amino acids, because balancing the amounts of these in the diet may be advantageous than merely increasing the intake of protein, (Allison, 1956).

Mitchell's hypothesis (1950) paved the way to determine the amino acid requirements of the various species by amino acid assays of the entire carcass, or even approximately by assay of dominant tissues such as muscle. Such assaying procedure has been realized promising, in assessing the specific effect and the value of the nutrients.

The present investigation was undertaken, in an effort to know the quantities of arginine, lysine, phenylalanine, leucine and isoleucine, all belonging to the essential group, in muscle tissue of buffaloes, goats and hens in the adult stage, and to find out whether they maintain the same pattern. Incidentally, the nitrogen content of the muscle tissues in the species in

question, were studied as a complementary analytical work.

The paper chromatographic technique was utilised for estimation of the different amino acids.

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CHAPTER - II.

REVIEW OF LITERATURE.

REVIEW OF LITERATURE

ESSENTIAL AND NON-ESSENTIAL AMINO ACIDS.

From nutritional standpoint, the amino acids are the components which differentiate the different proteins. These structural components in an organism have been categorised into two groups, particularly, on the basis of the prevalent dietary needs of the species.

Kossel and Kutscher (1900-1901) presented a method for the determination of basic amino acids, in which precipitation of the group followed by subsequent separations of the amino acids were involved. Emil Fisher (1901) described the ester procedure for the isolation of monoamino acids. These two methods were responsible to great extent, for accumulation in the subsequent years, of reasonably reliable informations about the composition of the proteins, such as deficiency of lysine in gliadin, inadequacies of lysine and tryptophan in zein.

By 1932, only three amino acids had been shown unequivocally to be indispensable dietary components for the growing rat. They were tryptophan (Willcock and Hopkins, 1906-07; Osborne and Mendel, 1914), Lysine (Osborne and Mendel, 1914), and histidine (Ackroyd and Hopkins, 1916; Rose and Cox, 1924). In addition, evidence had been presented in support of the idea that cystine or methionine, or both, must be supplied. Jackson and Block (1931; 1932) and Weichselbaum *et al.* (1932) had made the important observation that a large part of the cystine may be replaced by methio-

-nine, but it was not clear whether the substitution was possible because methionine was transformed into cystine, or because either of the two amino acids might perform independently certain functions in which both ordinarily took part. Nor was it known whether the reverse replacement of methionine by cystine could occur. (cited by Rose, 1957).

McCoy et al. (1935-36), Meyer and Rose (1936) with their simultaneous discovery and identification of threonine, made it feasible for the first time to attack the problem of Nitrogen requirements of the organism by giving diets containing mixtures of highly purified amino acids in place of proteins.

Rose (1957) while reviewing the past achievements in this field of research, remarked, "Once an abundant supply of threonine had been acquired by its isolation from proteins, the demonstration of the nutritive importance of each amino acid became relatively simple".

During the investigations with the weanling rats by the use of diets designed to be otherwise adequate for the normal growth of rats, in which the sole source of nitrogen was supplied by amino acids, Rose, Osterling and Womack (1948) showed that only ten of the nineteen or twenty amino acids ordinarily found in proteins were necessary for maximum gain of weight. Those amino acids were valine, leucine, isoleucine, methionine, threonine, lysine, phenylalanine, tryptophan, histidine and arginine. The exclusion of anyone of them from the diet with the exception of arginine, lead to diminished appetite, loss of weight, profound nutritive failure and eventual death, as such they were classified as

indispensable ones for rats.

Summarising the earlier statements, Rose (1957) explained that an indispensable dietary component was one which could not be synthesized by the species in question from materials ordinarily available to the cells at a rate commensurate with the needs for optimum growth.

On the basis of a critical study of a large body of data on chick amino acids requirements, an indispensable amino acid was defined by Almquist (1951) as "One which must normally be obtained from the gastrointestinal tract in order that synthesis of body proteins may take place".

Almquist (1951) further observed that the most recent analysis of body tissue showed the presence of practically all of the dispensable amino acids to the extent of 40 per cent or more of tissue protein. Thus an emphasis, that the "dispensable" amino acids were physiologically indispensable to the formation of the characteristic proteins of the animal.

White et al. (1959) explained that the terms "essential" and "non-essential" related only to dietary requirements and had no meaning with respect to the relative importance which the amino acids might have been in metabolism. Those amino acids which were essential in the diet represented compounds whose carbon skeleton could not be readily synthesized by the body.

NUTRITIVE VALUE OF ANIMAL PROTEINS

Meat as a dietary source of protein has a significance of its own, since it encompasses the vitamins, particularly Vitamin B₁₂, minerals and trace elements, fair amount of fat, within its structural folds, made up of both essential and non-essential amino acids. Better digestibility and utility, palatability and stability, are the additional characteristics which count for its nutritive value.

The assay of nutritive value of a protein is usually conducted by the study of comparative growth rates, gains in body weight or in body protein per unit of protein ingested, biological values, nitrogen balance, and tissue protein regeneration. Chemical scoring to obtain the essential-amino-acid-index is also utilised for such purpose. However, the value of a given protein is subject to variation with the level of total protein fed, the presence of other kinds of protein in the diet or substances which interfere with digestion, as well as adequacy of all other growth or health factors. It also varies with the calorie intake.

Mendel (1923) originally suggested "The efficiency of the individual protein in this respect (satisfying the nutritive needs of the body) must depend on the minimum of any indispensable amino acid that it will yield".

Beach Bernstein and Macy (1941) were the first to estimate amino acid needs from amino acid analysis of food intake required to produce normal growth. Beach, Munk and Robinson (1943)

also observed that protein structure, which make up of voluntary muscle tissue is similar in animals. However, Block and Bolling (1944) suggested, "The more nearly the mixture of essential amino acid ingested approaches in composition the amino acid mixture needed for structural and functional purposes each day, the less the mixture is needed or in other words the higher is its biological value or nutritive value. Thus a perfect protein or amino acid mixture would be one which would replace ^{weight} ~~weight~~ for weight, tissue protein or supply new material for growth and lactation etc. leaving no excess to be utilised for other purposes".

Mitchell and Block (1946) proposed a method based on amino acid assay for estimating the biological value of proteins for growth. The amino acid content of whole egg proteins "almost perfectly utilizable in rodent metabolism" was used as a standard of reference.

✓ Almquist (1953) summarising the principles of amino acid balance stated, "when a dietary protein is partly deficient in one essential amino acid the fraction which contains amino acids in optimum proportions to the deficient amino acid is the only part available for growth. Increasing the amount of the unbalanced protein will increase the amount of balanced fraction and hence permit increased growth upto the natural limit of growth rate. Increasing the amount of the deficient amino acid increases the proportion of the protein available for growth until the whole of protein so used is so used or the natural limit is reached. If slightly deficient protein is given at a high level so that maximum growth response is obtained, addition of the deficient

amino acid will increase the efficiency of utilization of the feed".

✓ 2. Trials conducted with growing chicks followed by carcass analysis, Prince, Taylor and Russell (1953) observed that 33 per cent of the Nitrogen in the feed was retained when the supplementary protein was from both vegetable and animal sources. Retention varied within the range from 27 per cent for Tryptophan to 43 per cent for lysine. One third of the unrecovered essential amino acids in the carcass was found in the excreta. On parallel trials with another ration (16.5% crude protein) having all the supplementary proteins from animal by-products, they noticed 56 per cent of the Nitrogen as retained in the carcass and between 39 to 71 per cent for individual amino acids. Although the first ration was deficient in methionine and threonine and the second one contained only 68 per cent of the recommended allowance of arginine, there was no significant difference between the amino acid composition of the chicks receiving the different rations.

✓ During the study of feeding of premature infants, Ferriera et al. (1953) showed that human milk alone was not the best food for rapid growth but excellent results could be obtained by the addition of 1 per cent of a mixture of amino acids and salts. In case of weanling rats, Sure and his associates (1953) noted the improvement in the nutritive value of cereal grains with the addition of individual amino acids and condensed fish solubles, as indicated by the economy of food utilization. In the same year, struck recorded brown pigmentation of fat and appearance of faecal smell in pork carcasses due to protein deficient ration.

Since the ϵ - NH_2 group of lysine was thought to form a nutritionally unavailable complex when intact proteins are damaged during processing, Carpenter and Ellinger (1955) impressed that free NH_2 groups could be estimated by reaction with di-nitro-fluorobenzene and available lysine values could be obtained which may be utilised for the prediction of the protein quality of animal products.

✓ Richter and Oslage (1955) in the feeding trial with the pigs found that a low protein diet had a low point count for carcass quality, and pale coloured meat that gave up water more readily.

✓ Maynard and Loosli (1956) observed that on the basis of the studies with rats and the much fewer ones with pigs and chicks, certain generalizations had been made regarding the comparative biological value of individual foods and combinations. Animal products as a class were superior to foods of plant origin. At the top were milk and eggs followed by liver, kidney and other glandular organs. Muscle meat ranked somewhat lower but was above most seeds and other vegetable products.

✓ Meyer (1957) noted that increasing amount of animal protein in the ration, reduced the feed requirement per kg. gain and also raised the daily gain of weight. In the same year, Chrzaszcz could find small difference in the meat quality of sussex, Rhode Island Red, white leghorn, and Greenleg chickens and their crosses when fed on the same rations. But in crosses, meat had more dry matter particularly in breast muscle.

With a single group of adult rats Nasset (1957) compared whole egg with two mixtures of amino acids. He observed that the amino acid mixture based on the mean minimum requirement of 9 essential amino acids was significantly inferior in maintaining Nitrogen balance to either egg protein or the amino acid mixture which simulated egg protein. However, Deshpande, Harper and Elvehjem (1957) with weanling rats obtained maximum growth when intact protein in the form of casein, fibrin and beef formed part of the supplement of the basal diet. The growth was not as good when the protein was replaced by equivalent quantities of essential and non-essential amino acids.

Schuphan and Weinmann (1958) estimated the crude protein and 8 essential amino acids in large number of food plants commonly used in human diets. The biological values expressed as E. A. A. - index were calculated and compared with beef and pork which had values 78 and 76. Values for 7 cereals were from 55 in wheat to 73 in rice; for 5 legumes from 49 in unripe peas to 72 in soyabeans; for 20 vegetables from 41 in Kohhrabi stems to 72 in potatoes.

Watts et al. (1959) reported that average Nitrogen balance and availabilities of the amino acids to human subjects were similar for egg and pork with the exception of tyrosine. Except for isoleucine, the availabilities of the amino acids in the milk and egg diet were equal to, or slightly higher than, those of the egg diet.

Hryniewiecki and Krawczak (1960) conducted an experiment in which two groups of 42 and 41 rats received for 3 months diets

with protein of animal or plant origin; for the first the protein came from casein, dried milk, and meat and bone meal, for the second from wheat flour and soyabean meal. Rats given animal protein gained on average 19 g. in 90 days, those on plant protein lost 27 g., with the greatest loss in the third month. Those on the animal protein maintained their serum total protein value whereas, in the other case there appeared a decrease on an average from 7.58 to 6.75 per cent.

✓ Arpai et al. (1960) found that the taste of meat from pigs fed on animal protein was superior. They noted that the muscles held more of its juice in comparison to those fed on plant protein.

Bigwood with Martin (1960) noted that there was no significant difference between breeds (lactating jersey and Friesian cows) in proportions of amino acids in the total protein of the milk. In the meat of those breeds about 90 per cent of total Nitrogen was protein Nitrogen, compared with 98 per cent in milk protein. The eight essential amino acids were 39.65 and 38.75 per cent of total amino acids on a molar basis in milk and meat respectively. Kofra'nyi and Muller - Wecker (1960) reported that the biological value of dried milk for human subjects was about the same as that of eggs.

Eastone and Long (1960) observed that since collagen is deficient in several amino acids, its presence in large amounts in bonemeal and meatmeal might be the reason for significant reduction in the nutritional value of the total protein of a meal. Their suggestions for the estimation of hydroxyproline that collagen

generally contains, could form the basis of a simple method of assessing protein quality of meatmeals and bonemeals. About fish-meal Pritchard and Smith (1960) observed that the length of time between death and processing of whale had no effect on the nutritive quality of the meal except the rapid increase of the free fatty acid content of the oil in the meal.

✓ Kerte'sz and Csire (1961) stated that the lower cost of vegetable protein feeds could not compensate for lower efficiency of feed conversion. Such a statement was on the basis of their findings on the groups of Hungarian Yorkshire pigs which were fattened from 30 to 150 kg. liveweight on rations with equal amounts of protein either all vegetable or with milk. Pigs getting 15 per cent milk protein reached 90 kg. - 61 days and 150 kg. - 83.7 days sooner than those getting vegetable proteins only. To reach these weights respectively, pigs getting only vegetable protein required 38.9 and 31.6 per cent more protein and 13.2 and 18.9 per cent more starch equivalent. They considered that milk to supply 10 per cent of total protein would be adequate.

Matsumo (1961) estimated the free amino acids (8 essential one) in serum of blood obtained from human subjects receiving vegetable proteins only and also from persons taking 54 per cent of total protein from animal origin. He obtained the values after a meal which indicated the difference in the quality of ingested protein.

Owings and Balloun (1961) studied the effect of protein source and amino-acid-supplementation on the intestinal microflora and plasma amino acids in the chicks. Addition of arginine to

diets with casein as the source of protein increased weight gains and improved efficiency of feed utilization but there was no improvement when it was added to diets with casein and zein. Presence of 1.8 per cent arginine in the casein diet did not affect the concentration of arginine in the blood but there were changes in other amino acids. It was suggested that arginine affected the absorption of the amino acids and enabled the chick to utilise them less completely. When lysine was added to the diet the value in the blood was increased, sometimes as much as 100 per cent and at the same time arginine was decreased. Either amino acid, or both added to the diet, generally increased microbiological count.

Heller et al. (1961) studied the availability of lysine from beef, ham or lamb subjected to heat, during the growth experiments in weanling rats. The results showed that 75 to 90 per cent of the lysine was utilised from meats before or after normal cooking at 200 to 400°F. in an electric oven. Lysine was equally available from the inner or outer portions of such cooked meats. Autoclaving at 250°F for 16 hours reduced availability of lysine to 40 to 50 per cent.

Bender (1961) determined the nutritive value of proteins by chemical analysis and the relation between the chemical score (C.S.) and the biological value (B.V.) of proteins was investigated. He found that defatted egg protein, which is usually considered to be the "ideal protein" contained an excess of several amino acids. The amino acid composition of a hypothetical "ideal protein" for the rat was obtained by reducing the concentration of those present in excess in a mixture simulating egg protein. The mixture had a

net protein utilization (NPU) of about 100 when given to rats. When each amino acid was omitted in turn and the NPU was plotted against C. S. Three different curves were obtained and the essential amino acids could be classified in 3 groups. Amino acids were estimated in several proteins and NPU predicted from the C.S./B.V. curve agreed well with that obtained by carcass analysis.

During the trials with rats Kik (1962) demonstrated that a diet with protein supplied by chicken meat, and rice gave better growth than either at the same protein level. Dark chicken meat had a slightly higher nutritive value than light and both were better than whole egg. Chickens contained much more tryptophan, lysine, threonine and cystine plus methionine than rice and probably that was mainly responsible for the supplementary effect.

Purichal et al. (1962) studied growth and utilization of feed, in the experiments lasting for 28 days, on young pigs given diets in which dried skimmed milk and soyabean, fishmeal or cottonseed meal supplied 20 per cent protein. Two pens, each of 4 pigs, were assigned to each treatment. On the 26th day, pooled samples of blood were obtained from animals in each pen and amino acids with the exception of tryptophan were estimated by ion-exchange chromatography in the protein free plasma and diets. They noted that growth and efficiency of feed utilization increased and plasma urea decreased in the order - meatmeal - cottonseed meal - fishmeal - soyabean meal - dried skimmed milk. The extent to which the concentration of individual amino acids exceeded that in the diet varied with particular acid as well as with the type of proteins, but concentrations in plasma were in general greater

with those proteins that promoted best growth.

Cahilly et al. (1963) studied the effects of various levels of dietary lysine on muscle protein of growing swine for biological value. They concluded that biological value of the meat for growing rats did not differ between groups.

Dvorák and Uognarova (1965) reported the mean values for available lysine in 8 beef cuts which ranged from 6.53 to 9.15 g. per 16 g. Nitrogen and in pork cuts from 5.66 to 8.94. They observed that such variation was due to unequal distribution of the connective tissue protein. From the ratio of tryptophan to hydroxyproline, it was concluded that the available lysine in raw meat is in indirect proportion to the content of connective tissue. Reduction on heat processing depended on the time of heating and the proportion of meat products lost. Smoking at 320° and treatment with nitrite reduced lysine but glucose or starch had no effect.

Lewis (1966) made comparisons of a chemical and a biological method of protein evaluation. The amino acid composition of unheated and excessively heated pork protein was determined together with biologically determined net protein utilisation values (NPU). Comparison of biologically determined NPU and chemically determined protein score of unheated and heat treated pork protein showed that the unavailability of essential amino acids, rather than their destruction, is responsible for the marked lowering of protein utilization following excessive heat treatment.

CARCASS ANALYSIS AND PROTEIN NUTRITION

Procedure which involves the killing of the animals and the analysis of certain specific tissues or of the body as a whole has been commonly referred to as slaughter experiment. In studies of the protein and amino acid requirements for growth or of the comparative value of different protein sources, it is important to know the specific effect in terms of protein tissue formed.

Munk et al. (1945) proposed a method that amino acid analysis of the tissues produced may afford a measure of amino acid requirements, for, growth were different from those for egg production.

Mitchell (1950) suggested, " an effective method of determining the requirements of a growing animal for these (essential) amino acids may be to determine, first the requirement in grams per day of some one amino acid, such as lysine, and then to estimate the requirements of the others from the proportion existing between the essential amino acids and lysine in the body of the animal, these proportions to be determined by amino acid assays of the entire carcass, or, approximately, even by amino acid assays of a dominant tissue such as muscle. This method may be as accurate as methods in current use for measuring amino acid requirements. It has the distinct advantage of avoiding the confusion concerning the utilization of the D-isomers that are commonly fed in racemic mixtures. The usual assumption that the utilization of these isomers is an " all or none " proposition

has been shown to be wrong in some instances atleast, since partial utilization has been established".

Schweigert and Guthneck (1953) proposed a technique while in quest of a method to find the utilization of amino acids from foods by the rats. Adult rats depleted of protein for 12 to 15 days were used for the study of quantitative utilization of lysine from foods. When graded amounts of L-lysine were added to a lysine deficient basal ration of purified amino acids or of sesame meal, ammonium citrate and certain amino acids. The weight gains of the depleted animals in 4, 8 and 11 days were proportional to the amounts of lysine given. Food products of known lysine content (estimated microbiologically) were then added to the basal diet to provide the graded levels of L-lysine, and from the weight gains the percentage lysine utilized from the foods was calculated. They recommended the technique with certain limitations for the study of utilization of amino acids from foods.

Williams and Coworkers (1954) estimated the amino acid requirements for growth, by assay of whole carcasses of the rat, chick, pig at different stages of growth. They found that not only the patterns of amino acid composition was comparable within each species at different stages of growth but also remarkably similar among the species. Most of the differences between two sets of figures they explained as due to the use of amino acids of the food for purposes other than protein formation eg g. of methionine as a methyl donor, or tryptophan for nicotinic acid formation, or to possible inadequacies of nutritional requirements. The relatively similar requirements among species and the close agreement

of the calculated values as those determined by nutritional studies were the basis for their support that the carcass analysis procedure is a valid method for evaluating growth requirements for most, if not all, of the "essential" amino acids.

By analysis of whole body Drolshagen (1958) estimated the nitrogen, lysine and histidine retention in albino rats. He selected an inbred-strain in matched littermate pairs (50-60 g. weight) of male rats, pre-fed for 8 days and finally 6 pairs weighing nearest to average were put under trial. One rat of each pair was killed for analysis at the start of the experiment, the remaining ones were fed for 30 days limited to same feed intake daily. In the third trial supplement of butryllysine was given to supply 2.54 g. L-lysine/Kg. feed. He obtained good agreement for percentage contents of Nitrogen, lysine and histidine. Addition of lysine in the third trial, caused an increase of 10 per cent greater weight gain and good agreement in percentage results which confirmed that all three components continued to be retained in the same proportions. Consequently, he noted that when lysine was limiting it affected retention of other amino acids and nitrogen.

Goetani et al. (1959) studied the distribution of methionine ^{35}S in some of muscles of rats in protein depletion after giving isocaloric diet and intraperitoneal injection of L-methionine ^{35}S 10 $\mu\text{c.}$ per 100 g. body weight. They observed no percentage total sulphur in the protein of several muscles, and also in the rats depleted of protein as there was no decline of specific activity of total sulphur, methionine or cystine. Masseter had a distinctly lower specific activity in the depleted rats;

that of cystine remained constantly low but methionine tended to rise.

According to Cardi et al. (1960) the effect of protein depletion on the amino acid composition of sarcoplasm proteins of gastrocnemius muscle in rats may cause some change, chiefly the decrease in the relative proportions of isoleucine, leucine, lysine and tryptophan. In their findings, Myosin (alkali soluble) fraction remained devoid of such alterations but distinct loss in the initial weight was noted, particularly, the weight of gastrocnemius muscle lowered down upto 35 per cent in 21 days period. They opined that protein preferentially broken down in such conditions, were those richest in lysine.

Fry and Standelman (1960) studied the effect of dietary methionine on the methionine and cystine content of poultry meat. A basal ration of maize and fish-n-fifty fortified with vitamins and minerals was given to day old chicken for 12 weeks alone or supplements with 0.05 or 0.50 per cent DL-methionine. Methionine was estimated in muscle after 6, 9 and 12 weeks and liver and heart after 12 weeks. The addition of methionine had no significant effect on the rate of growth or on the methionine and cystine contents of muscle and liver and produced only slight increases in the case of heart. Cystine but not methionine in muscle increased with age of the bird. Further, they investigated in the same year, the effect of cooking on the carcass part and observed that the increases occurred in both amino acids due to the loss of other nitrogenous components, and it thus suggested the stability of the amino acids.

Summers and Fisher (1961) adopted the carcass analysis for the evaluation of protein. In their preliminary experiments, they found the ratio of body water to Nitrogen under different feeding conditions, remained constant in the chicken. In the final procedure, they used diets with 13 per cent protein to quadruplicate groups of 5 chickens, at the same time provided N-free diet to the control group. Such a method was used for obtaining net protein values for the growing chickens and simultaneously, by analysis of the carcasses 5 sources of protein could be evaluated. Ericson (1961) observed that although the amino acid composition of the body is similar in all species, there are marked differences in the proportions of amino acids required for Nitrogen balance.

Holler and Hill (1962) noted the marked effects in a group of 10 improved Landrace pigs after prolonged protein deficiency. There was slightly positive Nitrogen balance in deficient pigs and the muscles contained 74.8 per cent average moisture in contrast to 70.3 per cent in the controls. Significant decrease of protein content ($N \times 6.25$) i.e. 74.8 against 83.4 per cent in dry matter was also noted. In the deficient group; 8 essential amino acids fell by 8 to 18 per cent only phenylalanine non-significantly.

The effect of acute amino acid deficiencies on carcass composition was investigated by Lyman and Wilcox (1963), by group feeding the Long Evan rats, while conducting the two separate trials. The animals were forcibly fed with about 10 g. daily in two portions of a synthetic diet in which single amino acid could be replaced by sucrose. Rats on complete diet gained 5 to 8 g. in 10 days but those on diets lacking in histidine, methionine,

phenylalanine or threonine lost 10.5, 17.5, 11.0 and 14.0 g. respectively. There was no difference in gross body composition. In the second trial, rats deprived of isoleucine suffered severest weight loss, 24 g. and in the deprived group where valine, lysine, tryptophan and leucine were absent, the range of this loss was 11 to 14 g.

Holmes et al. (1963) studied the amino acid composition of broiler cockrels in relation to the dietary amino acid requirement. They fed a diet with 25 per cent protein and 1370 Kcal. metabolisable energy to 16 pairs of Chunky Chick Cockrels from day old for 10 weeks and killed at 1 day and 2, 6 and 10 weeks of age, gutted and analysed the carcass by ion-exchange chromatography except for tryptophan. According to their statement the compositions of the carcasses were constant, with the only variation that seemed to be due to increased contribution of feather protein. Only for glycine there was more than 47 per cent of intake retained.

In their classical experiment for growth on selected lines of white Rocks, Leporie et al. (1963) reported the proximate amino acid composition of eggs and chicks as follows :-

- (i) In proximate composition of eggs, differences between the high weight and low weight lines were significant for fresh and dry weights, and the amounts of protein, ash and carbohydrate values for the former being the greater.
- (ii) Ratio of weight of yolk to weight of white was similar except that on percentage basis none was significant.
- (iii) On the basis of the tabulated findings for amino acids

in two analysis of separate eggs and chickens, calculating the efficiencies of conversions, it could be concluded that differences in efficiencies were greatest for lysine, histidine and arginine. Values were greatest in the high weight line but on subsequent tests with large number of specimens, differences were statistically significant for histidine only.

- (iv) Energy utilised by embryo was calculated to be 26.9 and 28.1 Kcal in the respective lines, but with the larger chickens in the high weight line, utilization was more efficient.

Cahilly et al. (1963) studied the effects of various levels of dietary lysine on certain blood phenomena, muscle development and muscle protein biological value of growing swine. With 24 weaned pigs, the basal diet containing 0.3 per cent lysine was compared with diet supplemented with 95 per cent L-lysine monohydrochloride to give 0.6, 0.9 and 1.2 per cent total lysine. The diets had 16 per cent protein reduced to 14 per cent at 75 lb. live-weight and to 12 per cent at 120 lb. live-weight with corresponding reductions in lysine. Experimental results of the above investigations were as follows :-

- (i) Blood from pigs receiving very little lysine had lower Hb. value and decrease in total serum protein and albumin.
- (ii) The diets with least lysine gave significantly lower growth rate and efficiency of food utilization, and animals did not reach slaughter weight of 180 lbs. and were slaughtered at 139 lb. live-weight. The increase in total lysine

to 0.9% of the diet, it increased the area of the eye muscle and proportion of lean cuts, total lean and primal cuts. There was also an increase in protein content of the longissimus dorsi and semitendiosus muscle as total lysine rose.

Gruhn and Anke (1965) observed that the changes in crude nutrients, amino acids and minerals in poultry during development from day old chicks to pullet. Composition of plucked carcass and of feathers and down was estimated for sussex X Leghorn chickens at intervals of 2 weeks from hatching to 12 weeks of age. In plucked carcass, they reported, that there were with aging increases in dry matter from 23.7 to 31.0 per cent, crude protein from 13.4 to 17.5 per cent and ash from 1.8 to 4.9 per cent of fresh weight. Fat was least at 14 days of age, 4.7 per cent and increased to 7.8 per cent. Most amino acids and P, K, Na and Zn in ash were noted as fairly constant but glycine tended to fall and calcium to rise slowly with age.

AMINO ACID REQUIREMENTS FOR DIFFERENT SPECIES

Rose and Co-workers (1942-1952) investigated the amino acid requirement of man and further, in an excellent review, Rose (1957) summarised their findings. Man needs the same list of amino acids for maintenance as rats with the exception of histidine.

Manynard and Loosli (1962) through their concise statement, impressed in their book, "Animal Nutrition", that many data had been secured on the quantitative requirements of the amino acids and it continued to be very active field of study. The general

procedure had been to feed a diet designed to be adequate except in the amino acid to be tested and to add varying levels of it to different groups to arrive at the level which is sufficient to result satisfactory growth. Weight increase had been the usual criterion, but in some experiments the nitrogen balance measure had been employed.

In the following tables, recommendations by various authors of requirements of essential amino acids for man and some other animals are reproduced.

Table showing essential and non-essential amino acids in Human Nutrition.

| | Essential Amino Acids. | | | Non-essential amino acids |
|-----------------|----------------------------|---------------------------|---|---------------------------|
| | Minimum daily requirement* | Recommended daily intake* | Daily intake with self selected diets** | |
| L-tryptophan | 0.25 gm. | 0.5 gm. | 0.92 gm. | Glycine |
| L-phenylalanine | 1.10 | 2.2 | 3.66 | Alanine |
| L-lysine | 0.80 | 1.6 | 6.35 | Serine |
| L-threonine | 0.50 | 1.0 | 2.86 | Cystine |
| L-valine | 0.80 | 1.6 | 4.61 | Tyrosine |
| L-methionine | 1.10 | 2.2 | 1.89 | Aspartic Acid |
| L-leucine | 1.10 | 2.2 | 6.00 | Glutamic Acid |
| L-isoleucine | 0.70 | 1.4 | 4.62 | Proline |
| | | | | Hydroxyproline |
| | | | | Citrulline |
| | | | | Histidine |
| | | | | Arginine |

* Rose's values are calculated for normal males.

** These values were computed from data on self-selected diets of Women (Putrell *et al.*, J. Nutr: 1952, 46, 299). In evaluation of these data the phenylalanine sparing action of tyrosine and the methionine sparing action of cystine have to be considered.

Cf. Wohl and Goodhart : Modern Nutrition in Health and Disease, 2nd Ed. 1960, page 115.

Table showing essential amino acid requirement for optimum growth of Chickens, Turkeys, Swine, and Rats.

(Data based on N.R.C. reports)
(Percent of diet).

| Amino acid | Starting chicks. | Starting poults | Pigs(27 to 70 lbs.) | Weanling rats. |
|------------------|------------------|-----------------|---------------------|----------------|
| Dietary protein. | 20.0 | 28.0 | 16.0 to 18.0 | 20.0 |
| Arginine. | 1.2 | 1.6 | 0.20 | 0.2 |
| Glycine. | 1.0 | 1.0 | None | None |
| Histidine. | 0.3 | ? | 0.20 | 0.4 |
| Isoleucine. | 0.6 | 0.84 | 0.60 | 0.5 |
| Leucine. | 1.4 | ? | 0.60 | 0.9 |
| Lysine. | 1.0 | 1.5 | 0.65 | 1.0 |
| Methionine* | 0.45 | 0.52 | 0.60 | 0.6 |
| Phenylalanine** | 0.7 | ? | 0.50 | 0.9 |
| Threonine. | 0.6 | ? | 0.40 | 0.5 |
| Tryptophan. | 0.2 | 0.26 | 0.20 | 0.2 |
| Valine. | 0.8 | ? | 0.40 | 0.7 |

* Cystine will replace methionine for chicks as long as the ration contains 0.45 per cent methionine. It can replace one-half the methionine requirement listed for pigs and one-fourth that listed for the rat.

** Tyrosine will replace phenylalanine for chicks as long as the ration contains not less than 0.7 per cent phenylalanine. It can replace 30 per cent of the phenylalanine listed for pigs and one-half that listed for the rat.

Cf.- Maynard and Loosli : Animal Nutrition, 5th Ed. 1962, page 393.

The mixture of amino acid provided by the data in the table must be supplemented by non-specific nitrogenous sources of the non-essential acids. The data apply to the period of rapid growth and to the protein levels listed and assume an anergy intake adequate for rapid growth.

Sym (1938) reported that rumen content possess proteolytic properties but it was the work of Pearson and Smith (1943) that first clearly showed both synthesis and breakdown of protein occurred in the rumen. Loosli et al. (1949) obtained the specific evidence that microbial action in the rumen can synthesize from urea, all of the 10 amino acids which are essential for rat growth. McDonald (1948; 1952) established that the breakdown of protein in the rumen was of quantitative importance and demonstrated that the resultant and product, ammonia re-entered in the rumen as salivary urea, thus framing "Nitrogen Cycle".

As an important corollary of protein synthesis in the rumen, Annison and Lewis (1959) stated that the essential amino acid requirement cannot be demonstrated for ruminants. On the other hand, the young suckling ruminant, in which the rumen is largely non-functional requires many of the essential amino acids.

METHODS FOR THE DETERMINATION OF AMINO ACIDS

For many years, methods for the determination of the basic amino acids followed the patterns originally set by Kossel, namely, precipitation of the group with phosphotungstic acid, followed by separation of the individual amino acid for example as

their silver salts. The results so obtained are now known to be minimal values. The more recent methods often do not require preliminary separation of the basic amino acids.

There are various methods applicable to the determination of several amino acids. The general methods employed are as follows :-

1. Specific Precipitants:

Block and Bolling (1945) observed that most of the accumulated data on amino acid composition of proteins were obtained by isolation methods. These depended upon the precipitation by reagents supposedly specific for single amino acid or groups of related amino acids. Further purification by recrystallization yielded fractions which, after having been characterized as pure by elemental analysis and melting point could be weighed. Thus, information concerning the amounts of the amino acids in the original protein is obtained. The method involves laborious processing.

2. Microbiological Methods:

Hawk, Oser, Summerson (1954) described that the fundamental principle involved in microbiological assays is to measure the response of bacteria, yeasts, or molds to graded increments of the sample and of a standard solution added to media furnishing all the nutrients required by the micro organism, except the amino acid under assay. The graded response may be measured by the increase in population of the micro-organisms (i.e. turbidometrically) or by their products of metabolism (acid or CO_2 production). The methods are advantageous, less expensive, simple

but not devoid of limitations.

3. Enzymatic Decarboxylation:

Olcott (1951) expressed that in this method of amino acid determination, enzyme systems that have the property of specifically decarboxylating one amino acid are employed. An aliquot of a neutralized protein hydrolysate is mixed with such an enzyme system in a Warburg or Van Slyke apparatus and the carbon dioxide evolved is measured by standard techniques. The total amount liberated is equivalent to the amino acid present in the hydrolysate. The method seems to be rapid and capable of accuracy provided the enzyme preparation is available.

4. Chromatography:

Chromatography has been defined as " a procedure of analysis by percolation of fluid through a body of comminuted or porous rigid material" (Gordon, Martin, Synge 1944). They developed a system of partition chromatography which was used by Tristram (1946) for determining the monoamino, monocarboxylic acid contents of several proteins. In the method, the amino acids in protein hydrolysate are acetylated with acetic anhydride. The resulting acetamino acids are then subjected to separations that involve partitions between an aqueous phase and a non-aqueous solvent mixture. The aqueous phase is held stationary in the form of a column of wet silica gel. A suitable indicator is adsorbed on the silica. The acetamino acids are then dissolved in the non-aqueous solvent and the solution is permitted to percolate down through the column. As it does so, the different amino acids move at

different rates and so separate into bands that are detectable by means of colour changes in the indicator. As bands move out of the column, they are caught in separate containers and then subjected to further separation procedures, or to direct determination by titration.

(1) Paper chromatography:

Consden, Gordon and Martin (1944) used for the first time, partition chromatography on a sub-micro scale. Cellulose in the form of a strip of filter paper is used as the stationary phase. Majority of the methods which have been devised for paper chromatography are based on the principle, "separation of substances from a mixture by the passage of solvent in a definite direction and selective fixation" (Weil 1953). "A drop of solution containing a mixture of substances is placed on a piece of filter paper one end. Next, this end is placed in a suitable solvent within a closed container. The solvent passes the spot where the solution had been applied. Each substance in this mixture will ideally move along with the solvent at a unique rate, so that after a while all of the components of the mixture will occupy a distinct position somewhere along the path of the solvent" (Block and Zweig 1958).

For qualitative and quantitative estimations of amino acids paper chromatography is widely used.

(b) Adsorption technique:

For basic amino acids. The technique necessitates an adsorbent mixture and a filter aid.

(c) Ion-exchange chromatography:

The synthetic ion-exchange resins are essentially solid acids or bases. They possess the capacity to hold bases or acids with which they come in contact. They are attractive analytical reagents and because of the displacement phenomena by which a strong acid displaces a weak one or a strong base a weak base (Olcott 1951).

(d) Gas chromatography:

Zlatkis Or'o and Kimball (1960) demonstrated that amino acids can be converted by ninhydrin into volatile aldehydes. These were separated by gas chromatography and converted to methane which was subsequently estimated in a thermal conductivity cell.

5. Ionophoresis:

Martin and Synge (1945) defined ionophoresis a "process concerned with the movement in an electrical field of relatively small ion", electrophoresis as "movement of large molecules and particles", but the distinction between them is ignored. Blackburn (1965) expressed that electrophoretic method was capable of determining amounts of amino acids in micro quantities.

The advantages of the high-voltage electrophoresis method are for their speed and high resolving power. Both, the paper chromatographic technique and electrophoresis are equally efficient except that the latter requires less time.

6. Isotope dilution method:

Shemin (1945) stated, "this method is based upon the

principle that the usual laboratory procedures for isolating amino acids do not separate isotope containing molecules from their normal analogues. If a known amount of an amino acid, that has been labeled by the incorporation of a stable or radioactive isotope element, is added to a protein hydrolysate, and then the same amino acid is isolated from the mixture and purified, a determination of the concentration of isotope in the sample will give a direct measure of the amount of the amino acid originally present in the hydrolysate. The decrease in the concentration of the labeled element from that present in the added amino acid to that present in the isolated amino acid indicates the extent to which the labeled amino acid has been diluted".

The technique has so far been applied only with amino acids labeled with N^{15} . The measurement of N^{15} requires mass-spectrograph, an instrument which is not generally available, although the method has the possibility of being the most accurate and reliable.

AMINO ACIDS IN MUSCLES

A number of muscle fibres make the lean part of meat. These fibres differ in length in various types of meat and are generally longer in older than in young animals. Principal solids of meat are muscle proteins, which are usually divided into sarcoplasm (water-soluble fraction), myosins (alkali-soluble fraction), and collagen. On hydrolysis, such distinctions disappear, as the hydrolytic end-products are none but the constituent units, the amino acids. The knowledge of the quantitative presence of these

units in meat and their availability to the organism, facilitates while formulating the human diet and also ideal ration for livestock.

Schweigert, et al. (1945); Greenhut et al. (1946); Greenut, Sirney and Elvehjem (1948); and Greenhut, Schweigert and Elvehjem (1946) determined many amino acids by microbiological assay in the meats of lamb, pork, beef and veal. The average values were on percentage basis for leucine 7.7, isoleucine 5.7, valine 5.2, tryptophan 1.3, phenylalanine 3.9, threonine 4.3 and lysine 8.0. The range of variations in the contents were also noticed for methionine, which varied from 2.2 to 3.3 per cent, arginine 6.4 to 6.5 per cent and histidine 3.1 to 3.4 per cent.

Schweigert et al. (1949) presented the data on the leucine, valine, isoleucine, phenylalanine, threonine, histidine, arginine, lysine, methionine and tryptophan content of fresh and cooked pork and lamb cuts. Schweigert, Bennett, Guthneek (1951), on further study included the data on glutamic acid, aspartic acid, tyrosine, proline, glycine, serine, and cystine. They concluded that the amino acid composition of the proteins of different cuts of pork or lamb were similar and were not destroyed during cooking. The composite data for the 12 each of the pork and lamb samples were reported as 84.9 and 85.1 per cent respectively of total nitrogen accounted for by the eighteen amino acids determined.

Greenwood, Kraybill, and Schweigert (1951) reported the amounts of eighteen amino acids in six different cuts of fresh and cooked, choice and utility grades of beef after microbiological

veal muscle cuts and beef organs. Expressed in grams per 100 gram of protein calculated to 16 per cent Nitrogen they recorded the average of values of 1.1, 5.0 and 5.2 per cent, respectively. Kidney, liver and tongue of beef gave higher values than the muscle meats. They also tested the efficacy of the hydrolytic procedure by adding crystalline amino acids to the samples and obtained 98-100 per cent recovery.

Effect on storage was studied by Swanson and Sloan (1953) in ten New Hampshire fowls which were killed at 11 to 12 months of age and after dressing stored at -5°F . for 40 weeks. Samples of breast and leg meat were taken from each bird at 8 weeks interval for the estimations. They noted that breast had higher Nitrogen content throughout and absence of any change in total Nitrogen content of either. Water soluble Nitrogen increased significantly in leg and breast and also in NPN fraction which indicated proteolysis during storage. The free L-amino Nitrogen however decreased which they suggested as an indication of continued Nitrogen catabolism beyond the amino stage.

Williams Miranda (1956) estimated histidine and threonine in beef, mutton, veal and pork and reported for histidine as 0.411, 0.375, 0.306 per cent of fresh material. Corresponding values for threonine were 0.480, 0.347, 0.612 and 0.298 per cent respectively.

Scott (1959) analysed meats from 5 Broad Breasted turkey toms and 5 hens and tabulated the findings as percentage of protein for all the essential amino acids and a few non-essential ones. There was little sex difference in the values. The

determinations. The percentages of those amino acids in the crude protein of different cuts and grades of beef were similar. The amounts of amino acids in the cuts of utility beef were higher than in choice cuts, owing to the higher percentage of protein and associated lower content of fat in the utility cuts. They noted that approximately 88 per cent of the total nitrogen was accounted for by the nitrogen from the eighteen amino acids determined and also confirmed that the amino acids were stable to cooking.

Violante et al. (1952) estimated the four non-essential amino acid contents of muscle meat by using the microbiological method. The average values of the skeletal muscle tissues of veal, lamb, pork and beef for glutamic acid, aspartic acid, tyrosine and proline contents were 16.5%, 100%, 3.5 and 4.0% of the crude protein respectively. In beef organs the amino acids were 14.9, 10.4, 3.7, 5.0 per cent, respectively. Recoveries of added amino acids from meat hydrolysates averaged 96-100 per cent.

After hydrolysing the muscle tissues of beef and veal for 22 hours with hydrochloric acid, Bigwood et al. (1953) analysed the amino acids by ion-exchange chromatography. They reported that ammonia, 18 amino acids and β -alanine constituted 95 to 96 per cent of total Nitrogen in beef and 92 to 93 per cent in veal. The amount of protein ($N \times 6.25$) was about 20 per cent more. The data for proline, glycine, and arginine in beef were below and for aspartic acid, threonine, methionine, tyrosine and histidine above the comparable microbiological data of Greenwood et al. (1951).

Alexander et al. (1953) determined the alanine, cystine, glycine and serine content of 18 samples of beef, lamb, pork and

histidine content was noted to be greater than that of lean meat. On comparing the results with values reported for other meats, milk and eggs, he recommended that turkey meat with its high protein content was a rich source of essential amino acids.

Bigwood (1960) in his classical study of the metabolism of Nitrogen and sulphur in the ruminant, determined the amino acids in cow's milk, beef and cattle feeds in the rumen. Estimations for these items were done separately, devoting greater attention towards the study of methionine and lysine. Samples of shoulder and leg of beef were obtained directly from the slaughter house. The muscle tissues were then analysed and in both the ultimate findings loin had significantly more alanine and tyrosines than other cuts.

Dahl (1962) noted that the distribution of 19 amino acids in the degenerated muscle of pig the same as that of the normal pig.

During the broiler trials lasting for 90 days, Saakjan (1963) compared pure bred Erevan chickens and their crosses with Pancyrev or New Hampshire breeds. Carcasses of groups of 6 birds were taken for the comparative studies. Meat of all had about 69 per cent moisture and in Pancyrev XErevan group crude protein was the highest. He further concluded that there was no difference between the breeds.

Dahl (1963) traced the occurrence of free methionine in the skeletal muscle of cattle, pigs, horse and found 181-257 mg. per cent, 167 to 244 mg. and 152 to 268 per cent in crude protein. He further pointed that as an index of quality, free

methionine content of meat was no better than its hydroxyproline content.

Miller et al. (1965) studied the free amino acid content of chicken muscle from six broilers on a commercial ration and six hens on commercial laying ration. All the birds were of the same strain, age and sex. Light meat had less free amino acids than dark meat except for lysine and histidine. Taurine was higher in the dark meat of both hens and broilers. They could not find any relation between tenderness and also in the general pattern of free amino acid concentration, or the concentration of any single free amino acid.

DIETARY AMINO ACIDS IN BLOOD AND TISSUES

The theories on protein metabolism explain that the proteins of blood are in dynamic equilibrium with each other and with tissue proteins. Rapid rate of disappearance of labeled plasma proteins on parental administration from blood in normal animal indicates the interchange with mobile pool of tissue protein.

Eagle (1955) studied the specific amino acid requirements of a mammalian cell and found that the concentration of essential amino acids needed for maximum growth varied and below certain level rate of growth fell sharply. None of essential amino acid could be replaced by D-isomers, but the D-isomers did not actively inhibit growth. While Piez and Likins (1957) opined that lysine was apparently the source of hydroxyproline of collagen which was converted while in the bound form. However, Longenecker et al. (1959) described a procedure by which amino acid adequacy of a diet for dog could be evaluated through a study of the free amino acids in the plasma of animal fed the protein being assayed.

Geiger's (1960) observations in the book "Modern Nutrition in Health and Disease" on the metabolism of proteins have been summarised as follows :-

That the absorbed amino acids are carried with portal blood to the liver, partly retained there for the specific requirement, but the rest enter the general circulation from which they are rapidly removed by several tissues involving specific selection mechanisms. The liver and kidney are most active while skeletal muscle is less efficient. The fate of amino acids in each tissue varies according to the momentarily prevalent requirement. In starvation and semi-starvation, the dietary amino acids are utilized primarily as a source of energy to compensate the caloric deficit.

Earlier theories have been disapproved which assumed that tissue proteins do not regularly enter the metabolic systems but instead are used up slowly by daily "wear and tear" and are

mobilized only when exogeneous supply is deficient. New concept refers that a dynamic equilibrium exists between tissue proteins and dietary amino acids, as shown by the isotope studies with labeled amino acids which indicate the tissue proteins, not as rigid structures but there is the steady "give and take" process between the two.

For optimal protein synthesis, all constituent amino acids must be present simultaneously in adequate quantities. Absence of one or more essential amino acids or in unsatisfactory quantities, the rest unutilized ones are irreversibly metabolised. Thus, it is a loss to the specific purpose because such dietary excess can neither be stored nor used again in the body. Any delay in supplementation of the missing amino acids decreases utilization so that, when 4 to 6 hours intervene between feeding the incomplete mixture and the missing amino acids, there is detectable interference with protein synthesis. Single labeled amino acids disappear from metabolic system within 4 hours. The metabolic fate of amino acids other than synthesis, include deamination, decarboxylation, transformation in other compounds i.e. as precursors of some hormones, vitamins, co-enzymes.

Gaetani et al. (1961) studied the distribution of lysine ^{14}C in plasma, liver, heart, and gastrocnemius muscle of rats and concluded that in protein depletion protein synthesis becomes concentrated in the liver. Tonkinson and Coworkers (1961) observed the effects of dietary lysine on free amino acid concentrations in blood plasma of growing turkey, when supplied with graded amounts of lysine. Growth and efficiency of utilization of feed were similar

but less with the increase of lysine its concentration
with the corresponding decrease of arginine, aspar-
non-signifi methionine and serine. They suggested that the
plasma of of arginine indicated for limiting growth but
no lysine, ma concentrations in plasma concentration for
value" for retention of egg yolk at 2 weeks and high energy
pigs, fed for der birds.

wheat gluten Olsen (1961) studied the effect of starvation
in poultry and found that the removal of feed
upto 2 weeks concentration of lysine and threonine in the
lated soyabean est concentration in 24 to 36 hours. The protein
and then the ver concentrations of all amino acids than the
calculated to the same year, Hill et al. observed that in 4
protein with r lysine deficient low protein diet, there was
lacking one ess rate except a decrease of lysine in plasma by
valine. Weight g of lysine to such diet, restored plasma lysine
and the birds wer

(1963) obtained the Jackson and Friedrich (1962) observed the
in 1961 as they found nitrogen supplements on growth and on
less amount of miss. plasma and muscle of weanling rats fed a low
the depression of w that on addition of threonine plus trypto-
of amino acids which ightly and retardation by unessential

Puchal and mented. They noted that there was an incre-
plasma amino acid pat amino acids in plasma of rats given supple-
teins and compared the in, largely, but not exclusively through
proteins, thus the amino given and non-comitant reduction in the
essential amino acids. There was similar

in all diets but with the increase of lysine its concentration rose in plasma with the corresponding decrease of arginine, aspartic acid, glycine, methionine and serine. They suggested that the low concentration of arginine indicated for limiting growth but variations in plasma concentrations in plasma concentration for others as due to retention of egg yolk at 2 weeks and high energy requirements in older birds.

Hill and Olsen (1961) studied the effect of starvation and non protein diet in poultry and found that the removal of feed resulted increased concentration of lysine and threonine in the plasma, giving highest concentration in 24 to 36 hours. The protein free diet caused lower concentrations of all amino acids than the complete diet. In the same year, Hill et al. observed that in 4 weeks old chicks, on lysine deficient low protein diet, there was no effect on growth rate except a decrease of lysine in plasma by 64 per cent. Addition of lysine to such diet, restored plasma lysine level to normal.

Swendseid, Hickson and Friedrich (1962) observed the effects of non essential nitrogen supplements on growth and on amino acid content in plasma and muscle of weanling rats fed a low protein diet. They found that on addition of threonine plus tryptophan, growth increased slightly and retardation by unessential Nitrogen sources was prevented. They noted that there was an increase in free unessential amino acids in plasma of rats given supplementary unessential Nitrogen, largely, but not exclusively through increase in the amino acid given and non-comitant reduction in the ratio of essential to unessential amino acids. There was similar

but less marked changes in the free amino acids in the muscles.

The views of Bender and Doell (1962) related to the non-significant difference in lysine and arginine content of plasma of 24 male rats fed for 14 or 28 days on diet containing no lysine, or 33 per cent or 100 per cent of Bender's "target value" for lysine. The same was the case as noted in the pairs of pigs, fed for 8 weeks on a commercial diet which was based on wheat gluten, or a lysine free diet based on zein.

In the trials, white Plymouth Rock pullets were given, upto 2 weeks of age, a basal diet containing 9.5 per cent of isolated soyabean protein, supplemented with DL-Methionine and glycine and then the same diet, supplemented with mixtures of amino acids calculated to be equivalent to 25 per cent of isolated soyabean protein with respect to the contribution of L-amino acids but each lacking one essential amino acid, arginine, isoleucine, lysine or valine. Weight gains, feed consumption, were recorded for one week and the birds were killed. During such studies Hill and Olsen (1963) obtained the results in conformity of their earlier work in 1961 as they found reduced weight gain, feed consumption, and less amount of missing amino acid in plasma. They concluded that the depression of weight gain was due to relatively large amounts of amino acids which underwent deamination or excretion.

Puchal and Co-workers (1962) described the specific plasma amino acid patterns for a series of different dietary proteins and compared them with that in plasma of animals fed milk proteins, thus the amino acids that may be responsible for

restricting protein synthesis in the animals could be observed.

Muramatsu et al. (1963) in the tracer study demonstrated the loss of activity of heart, muscle and testis proteins showed less change with changing diet protein levels.

Zimmerman and Scott (1965) showed the inter-relationship of plasma amino acid levels and weight gain in the chick as influenced by suboptimal and superoptimal dietary concentrations of single amino acid. In the same year, Richardson et al. found no significant change in plasma free amino acids in pigs after 12 hours and 24 hours fasting. They could not detect any significant change in free amino acid in plasma taken 3, 6 and 9 hours after feeding.

The use of free amino acid concentrations in blood plasma may aid to detect deficiencies and excesses of dietary amino acids was advocated by Dean and Scott (1966). Their study on diets containing insufficient lysine or valine resulted in a fall of these limiting amino acids in blood plasma of chicks. While diets in which lysine and valine were first and second limiting as shown by growth trials, a fall of both in plasma was observed, lysine being most reduced. Excess of lysine caused, a striking increase in plasma lysine, but relatively small changes in other amino acids. Suboptimum amounts of valine and threonine with excess of lysine, tyrosine, and isoleucine resulted in low plasma levels of the first two and high levels of the other three.

*

CHAPTER - III.

MATERIALS AND METHODS.

MATERIALS AND METHODS

PREPARATION OF STANDARD AMINO ACID SOLUTIONS:

The techniques of Cowgill and Pardee (1962) were adopted with some modifications for preparing the standard solutions and their subsequent applications on the chromatograms for development.

0.2 milli mole of each of the amino acids was weighed accurately, in a clean and dry test tube that was weighed previously. The quantities for individual amino acids were as follows:-

| | | |
|-------|--|------------------|
| (i) | Lysine monochloride (E. Merck) - | 36.53 milligram. |
| (ii) | Arginine monochloride (E. Merck) - | 42.14 ,, |
| (iii) | DL-B-Phenylalanine (B. D. H.) - | 33.04 ,, |
| (iv) | L-Leucine (containing iso-leucine) - (B. D. H.) - | 26.38 ,, |

The quantity weighed for both leucine and isoleucine was equivalent to 0.2 milli mole of leucine and this quantity was also pooled with that of 0.2 milli mole phenylalanine. These steps were taken for the following reasons :-

- (a) All the three amino acids viz. leucine, isoleucine and phenylalanine belong to the essential group.
- (b) Leucine and isoleucine have the same molecular weight as such their locations on the developed chromatogram are nearer to each other. On the other hand, the phenylalanine spot too, is generally nearer to those two spots.
- (c) All of them yield the same initial colour with

ninhydrin, after colour development (blue-purple).

- (d) Non availability of leucine and isoleucine in pure form.

Then to each test tube, added 10 ml. of 10 per cent isopropanol in distilled water containing dilute HCl. Shaken the tubes carefully till the amino acids got dissolved. Presence of HCl helped this process. Then further added 10 ml. of this solvent to each test tube to make the three amino standard solutions i.e. lysine, arginine, and phenylalanine, leucine containing isoleucine (one solution). 1 microlitre of each of the standard solution contained 0.01 micro mole of the respective amino acids except for leucine and isoleucine. Stoppered the test tubes and kept for the preparation of standard curves.

The weight of the amino acids contained in standard solutions used for the preparation of standard curves. (Table 3.1 presented on page no. 51).

PREPARATION OF REFERENCE AMINO ACID MIXTURE:

Since, the amino acid solutions were already prepared for the purpose of utilising them for standard curves, 10 ml. from each of the three standard solutions were pooled in a clean and dry test tube. It was prepared for applying it by the side of the unknown mixtures or hydrolysates in the chromatogram, for the sole purpose of identification of the resolved amino acids.

T A B L E - 3.1

The weight of the amino acids contained in solutions
used for the preparation of standard curves.

| Quantity (in micro moles). | Lysine (Microgram) | Arginine (microgram) | Leucine containing isoleucine and pheny- lalanine (microgram). |
|----------------------------------|-----------------------|-------------------------|--|
| 0.01 | 1.8264 | 2.1066 | 2.9638 |
| 0.02 | 3.6528 | 4.2132 | 5.9276 |
| 0.03 | 5.4792 | 6.3198 | 8.8914 |
| 0.04 | 7.3056 | 8.4264 | 11.8552 |
| 0.05 | 9.1320 | 10.5330 | 14.8190 |
| 0.06 | 10.9584 | 12.6396 | 17.7828 |
| 0.07 | 12.7848 | 14.7462 | 20.7466 |
| 0.08 | 14.6112 | 16.8528 | 23.7104 |
| 0.09 | 16.4376 | 18.9594 | 26.6742 |
| 0.10 | 18.2640 | 21.0660 | 29.6380 |
| 0.11 | 20.0904 | 23.1726 | 32.6018 |
| 0.12 | 21.9168 | 25.2792 | 35.5656 |
| 0.13 | 23.7432 | 27.3858 | 38.5294 |
| 0.14 | 25.5696 | 29.4924 | 41.4932 |
| 0.15 | 27.3960 | 31.5990 | 44.4570 |
| 0.16 | 29.2224 | 33.7056 | 47.4208 |
| 0.17 | 31.0488 | 35.8122 | 50.3846 |
| 0.18 | 32.8752 | 37.9188 | 53.3484 |
| 0.19 | 34.7016 | 40.0254 | 56.3122 |
| 0.20 | 36.5280 | 42.1320 | 59.2760 |

LABORATORY FOR CHROMATOGRAPHY:

All the chromatographic work were done in the laboratory spared for the purpose, which was free from ammonia and fumes. The room had minimum of window space and was located in the centre of the building, which prevented sudden draft and radical changes of temperature. These conditions permitted to obtain the developed chromatograms with regular spots of amino acids and with straight solvent-fronts.

The following equipments were used for the estimation of the amino acids in the present investigation :-

- (i) Two dimension paper chromatography cabinet (Sirco make) size - 24"x12"x24", which had the glass and wood construction (paraffined) along with 3 solvent troughs, 3 bended glass supports, and 6 anti-siphon rods.
- (ii) A packet of 100 sheets of whatman chromatographic filter paper no.-1 (size - 46 cms. X 57 cms.).
- (iii) Hair dryer, glass atomizer, table lamp.
- (iv) Pipettes, micropipettes, funnels, separating funnels and other routine glass wares.
- (v) Working table with glass plate.

There was also an almireh containing different chemicals all belonging to 'AnalaR' grade.

CHROMATOGRAPHIC METHOD:

Utmost cleanliness was observed during the chromatographic work. The glass wares along with the glass plate, solvent troughs and rods were thoroughly washed with hot soap water and distilled water. The sequence for cleaning of micropipettes was,

hot soap water, distilled water, and acetone. It was a routine practice that the appliances after cleaning were perfectly dried before use with adequate care to prevent contamination. During work, both the hands were being kept clean as far as practicable. Clean rubber hand-gloves were also being used when necessary particularly while touching the chromatograms.

The method of Block and Zweig (1958) was adopted with some modifications according to the convenience. One dimension descending paper chromatography with a single solvent system was employed throughout the chromatographic work. It involved the following main procedures :-

- (i) Preparation of sample (Hydrolysates).
- (ii) Application of the sample to the filter paper.
- (iii) Development of the chromatogram.
- (iv) Drying of the chromatogram after development and detection of spots.
- (v) Quantitative elution of the nihydrin treated amino acids.

The technique followed for the preparation of the hydrolysates has been described under the head "preparation of samples".

(1) Application of the sample (spotting) to the paper.

A sheet of filter paper was taken out from the packet and placed over the table with the care so that it lay traught on the glass plate. A line 8 cm. distant and parallel from the shorter edge, was faintly drawn with a light pencil. Eight tiny

pencil points spaced at 5 cm. interval were faintly marked on the same line and a small circle (4 mm. dia) was made on each point. Two points on either extremes had the locations of 2.5 cm. away, from the respective edges. While doing so perfectly clean hands were used and also touching the filter paper was avoided.

The micropipette used was 100 micro litres in capacity having subdivisions indicating 1 micro litre and without automatic delivery device. Prior to taking up the work of spotting, manual manipulations of the other micropipettes were practised, to get used to their applications in the actual procedure of spotting the sample.

The usual procedure for spotting was as follows :-

One of the clean, dry micropipette was dipped in the solution which was to be applied on the paper. The micropipette was allowed to get filled by capillary action. Sucking from mouth was not practised as there was possibility of contamination. When the desired quantity of the solution travelled in the pipette, it was then withdrawn, and the outer sides were wiped off, with a clean and dry ordinary filter paper. To prevent the loss of the solution to the glass plate during spotting, a watch glass was put below the chromatographic paper. The watch glass was being moved on that line after each spotting. The solutions were applied at the designated points already marked and encircled with the soft pencil along with the identification marks below each one of them. The "modus operandi", adopted while spotting the solution of the specified volume was, by gently touching the paper, a number of

times. While doing so, usually an angle of 45° was formed between the micro pipette and the paper. After each such operation, the material was allowed to dry with the exposure to ordinary table lamp. Since, spot size, the uniformity of spot and shape of it, can affect the subsequent spot of the developed chromatogram such meticulous care was taken with each application so that ultimately, each spot was small, round and uniform. Average size of the spot was 3.5 to 4 mm. in diameter. The number of the applications on the paper depended upon the samples but care was taken not to exceed more than eight, in all. The spots were then allowed to dry completely. After it, the paper was folded sharply along a line 5 cm. from the edge. Such folding kept the distance of the spots 2.5 cm. from the fold. Finally, the paper was transferred to the chromatographic cabinet where it was put in the manner as described below :-

Both edges near the fold were caught hold, by hand protected by clean rubber gloves and the paper was pushed gently in the glass trough which was resting on the upper grooves of the cabinet. The paper was fixed by the help of glass support which had bended ends. During the operation care was taken that paper rested over the antisiphon rod which too, was placed parallel to the trough on the upper grooves. The paper was thus in a hanging position and the antisiphon rod was in a position to control the excess flow of the solvent. Then the paper was allowed to equilibrate in the solvent vapour escaping from the beaker, kept on the floor of the cabinet.

(2) Development of the chromatogram:

Partridge's solvent (n-Butanol : acetic acid : water; 40 : 10 : 50) was the only solvent used throughout the chromatographic work. The solvent has the following advantages :-

- (1) It has appreciable resolving power for amino acids.
- (ii) It has the non-toxic properties and can be handled with ease.
- (iii) The solvent system does not destroy the amino acids while drying the developed chromatograms.
- (iv) In this solvent system the chromatographic paper do not require lengthy pre-equilibration time.
- (v) It can be comparatively easily prepared.

(a) Preparation of the solvent :

400 ml. of n-butyl alcohol was taken in a one litre volumetric flask. To it 100 ml. of glacial acetic acid was added and shaken for a while. Distilled water was added in small amounts upto the mark, and mixed the contents well. During such a procedure butanol and acetic acid got saturated with water and their saturation point was ascertained by the absence of shining bubble like droplets at the junction of the two layers of the mixture. Two layers of this mixture were separated, by the help of a separating funnel. The contents of the lower layer in the separating funnel was collected in a clean and dry bottle and stoppered it. 50 ml. of this solution was always kept in a beaker in the chromatographic chamber in order to saturate the inner atmosphere with this solvent vapour. The supernatant part containing butanol, acetic

acid mixture, was also collected in a separate clean and dry bottle and stoppered it for future use as solvent.

(b) Development of the chromatogram:

Enough of this solvent was poured into the troughs by means of a pipette through the holes in the upper lid of the closed chamber, so that the troughs were $\frac{3}{4}$ th full. During solvent-development of the chromatogram it was an usual practice to check whether the trough contained ample quantity of the mixture. When necessary, some more was poured. After 20 - 22 hours, when the solvent-front had travelled a distance of 40 - 42 cms. and approx., 2 - 3 cms. away, from the other edge, development was stopped. The developed chromatogram was taken out with a clean hand covered with gloves and kept for drying in the room.

(3) Locating the spots in the chromatogram:

Methods of Giri, Radhakrishnan and Vaidyanathan (1952) was adopted for drying and colour development of the chromatogram and also during subsequent elution of the ninhydrin spot for quantitative estimation.

The developed chromatogram was hanged with plastic clips. Perfect drying of the paper was ensured by exposure to ordinary table lamp for 10 - 15 minutes. Then the paper was sprayed with 0.5 per cent ninhydrin in 95 per cent acetone. Spraying was done with the glass atomizer, 50 ml. capacity, having a rubber bulb which was connected to the glass tube of the glass container, by small connecting rubber tube. The following procedures were adopted during the spraying operations:-

Glass container with the colouring reagent was caught hold with the left hand and kept in slightly tilted position, at the distance of 15 - 18 inches from the developed chromatogram. Gently, the rubber bulb was manipulated, with the result, thin spray of the colour reagent appeared which were allowed to fall on the right corner of the paper above. Gradually, the container was moved to the left on the same line but continuing the operation, till it reached on the other corner. Same procedure was repeated below this application, and continued till the entire paper was sprayed with the reagent which left a wetting mark on the paper. Care was taken, not to over flood the chromatogram to prevent formation of diffused and ill-defined spots. The spraying technique was followed as described, since it prevented overflowing of the reagent. The whole process took 10 - 15 minutes and consumed about 30 ml. of the reagent. Then the sprayed chromatogram was allowed to remain for another 15 - 20 minutes till it got finally dried. After it, the sprayed paper was folded and transferred to the hot air oven and kept at 65°C for 30 minutes, with particular care for the temperature control. Precautions were taken, to put clean and dry, but ordinary papers, as linings to the inner walls of the oven. The chromatogram was then taken out and put in the hanging position. Examined the resolutions of the sample and located the ninhydrin spots against the corresponding known amino acid (reference amino acid).

(4) Elution of the ninhydrin spots :

Distinct zones of ninhydrin spots that had different

colorations viz. bluish purple, pink, slight red, yellow were formed depending upon the amino acid. The spots, on identification against the reference amino acids, were cut, one after another and put into separate clean and dry test tubes, already marked with glass pencil for their identity. Care was taken to hold paper-cuts with clean forceps not by bare fingers, and while cutting the paper clean and dry scissors were used. The paper cuts from the place of original application of the sample and from some randomly selected portion of the paper were taken for blank determination.

To each test tube 4 ml. of 75 per cent Ethyl alcohol containing 0.2 mg. of copper sulphate was added. Thoroughly shaken till the ninhydrin colour was eluted from the paper. The optical density of the coloured solution was measured using a klett - Summerson photoelectric colorimeter with green filter (540 m. μ). Colorimetric readings were noted and averaged. For the standard amino acid solutions these were plotted on the graph paper, against the respective quantities of amino acids originally taken for application on the paper, the points were joined with a straight line. The colorimetric readings obtained from the unknown mixture or hydrolysate were utilised, for evaluation of the quantities, with the help of the standard curves.

To know the quantity of amino acids in the unknown mixtures and hydrolysates the standard curves were always used. The amino acid on identification in the chromatogram followed by elution, indicated its quantitative transfer to the solvent (i.e. 4 ml. 75 per cent Ethyl alcohol containing 0.2 mg. of $\text{Cu SO}_4 \cdot 5\text{H}_2\text{O}$).

The optical density of the solution, when known by the colorimetric reading, shows the concentration of the amino acid or unknown substance in the solution. It was known by reference to the standard curve prepared for that particular amino acid.

COLLECTION OF MEAT SAMPLES:

The muscle tissues of buffaloes and goats were collected from the local slaughter floors of Phulwarisharif and Rajabazar. The animals were reported to have been killed approximately 30 minutes earlier, by Halal method, with bleeding as far as it was practicable. The muscle tissues were chosen from the middle third of the longissimus dorsi muscle. To prevent loss of water, during preparation and subsequent handling, about 25 grams (approx.) of fat free, connective tissue free muscle tissues were taken to the laboratory.

Breast muscles from white leghorn hens were obtained from the slaughtered birds from the Department of Physiology, Bihar Veterinary College, Patna. Apparently, the birds were healthy and no abnormal characteristics appeared in them, after a lapse of 20 minutes from death. The same quantity of the sample was taken following the same procedures, as in the case of buffaloes and goats. The live birds were reported to be layers.

ESTIMATION OF MOISTURE, DRY MATTER, AND FAT CONTENT:

Fat percentage and dry matter content of the corresponding samples were estimated as recommended in A.O.A.C. method with some minor modification.

Weighed 25 gms. of meat, in a previously weighed and dried petri dish along with its lid. Dehydrated the sample in the steam oven at $95 - 97^{\circ}\text{C}$ till a constant weight was obtained. Calculated the dry matter. Moisture content was estimated as usual, by difference.

Dehydrated samples were then properly ground in a clean and dry glass pestle and mortar, and shifted it to the oven for re-heating for a few minutes. Cooled the contents in the desiccator and weighed 5 gms. of it. Transferred the entire quantity to the soxhlet's thimble and extracted with petroleum ether (range 40 to 60°C) for 20 hours in soxhlet's apparatus. Then taken the contents from the thimble in a glass basin and evaporated ether in the steam oven. The dehydrated ether extracted muscle was again ground, as before and put in the glass test tube and stoppered it. The separated fat was freed from ether, weighed it and calculated the percentage.

PREPARATION OF SAMPLES (HYDROLYSATES):

The method of Block and Zweig (1958) was adopted for the preparation of the hydrolysate from dehydrated, ether extracted muscle. 100 mg. of the sample was accurately weighed in a previously weighed test tube. 10 ml. of 6 N HCl was added and stoppered with rubber cork along with the reflux tube. It was clamped and put in the boiling water bath for hydrolysis, which was carried for a period of 24 hours spread over 3 consecutive days. The hydrolysate was ultimately transferred quantitatively, to clean and dry basin by repeated washing with 5 ml. of distilled water each time.

Placed the basin along with the contents in the steam oven for the removal of excess of hydrochloric acid present in the hydrolysate. Allowed to remain the basin till thin films of amino acid hydrochlorides appeared. Transferred the basin to the desiccator and kept over calcium chloride for 24 hours. Then added 20 ml. of warm water to the basin containing the dehydrated hydrolysate, thoroughly mixed it by gentle shaking and filtered slowly with care to avoid any loss by spilling. The filter paper over the funnel was carefully washed repeatedly with distilled water, but the total volume of water did not exceed 15 ml. The filtrates already collected in the clean and dry basin was then shifted to the steam oven as before and the content was evaporated to dryness. After it, placed the basin in the desiccator over calcium chloride to ensure perfect drying and cooling, and left it for 12 hours. Then known volume of 10 per cent isopropanol in distilled water was added and with gentle but persistent shaking the amino acid crystals were dissolved. Transferred the content to a clean and dry test tube with the identification mark, stoppered it and shifted for the analysis.

ESTIMATION OF TOTAL NITROGEN:

The estimation of total nitrogen in dehydrated, ether extracted muscle was done in the following manner :-

Digestion :

Micro-Kjeldahl digestion by the Arnold Gunning method was adopted. 100 mg. of the material was weighed and transferred to the micro-kjeldahl digestion flask. 3 ml. of conc. sulphuric

acid, 10 drops of 5 per cent copper sulphate solution, 1 gm. of potassium sulphate and a few glass beads were added to it. Digestion was continued till mixture became clear. Added 1 ml. of conc. H_2SO_4 and further heating for an hour was allowed to ensure complete digestion. It was cooled and quantitatively transferred to a volumetric flask 50 ml. capacity by rinsing 3 - 4 times with distilled water. Added distilled water, after shaking it and diluted upto the mark. 5 ml. of diluted digest was utilised for the estimation of ammonia, by distillation and titration was given below:-

Distillation of ammonia and titration
for the estimation of Nitrogen:

The method of Meeker and Wagner (1940) was adopted for this purpose. The method involves absorption of ammonia in the boric acid solution. The exact strength and volume of this solution are not important since the excess basic acid does not affect titration. 4 per cent boric acid was used during the process. On distillation the solution of boric acid at once turns alkaline to methyl red due to the presence of ammonia. To turn the colour of the solution to original colour, standard sulphuric acid was added, which was equivalent to the amount of ammonia distilled in the boric acid solution.

20 ml. of 4 per cent boric acid solution containing Meeker's indicator, comprising methyl red and methylene blue was taken in 100 ml. conical flask having mark at 50 ml. and 80 ml. levels. Care was taken to dip the tip of the condenser in the boric acid solution. It prevented the escape of ammonia in the atmosphere.

5 ml. of the diluted digests was pipetted in the Markham's still. Saturated solution of sodium hydroxide was added till the solution in the inner chamber of Markham's still became blackish.

Initially the steam was allowed to pass at a slow rate and gradually it was increased to an optimum speed. The distillate was collected upto 50 ml. mark in the receiving flask. Frequent trouching of the condenser and continuous flow of cold water through the rubber tubes, ensured that it was not hot. The receiving flask was lowered down and the tip of the condenser was washed with distilled water collecting the washings in the receiving flask. The receiver was kept for titration with $N/35 \text{ H}_2\text{SO}_4$.

Distillation was stopped and the inner chamber was cleaned with water and the fluid was removed by back suction so as to make it ready for further use. Before the distillation of other digests blank distillations were done to remove the presence of ammonia if any. Titration was done from a micro-burette of 10 ml. capacity having graduation of 0.05 ml. The end point was judged by comparing the colour with control flask having 20 ml. boric acid with indicator, diluted to 80 ml. The volume in the experimental flask was also made up to 80 ml. by means of distilled water to match the volume of the control (comparing flask). Finally, the volume of $N/35 \text{ H}_2\text{SO}_4$ needed to match the colour was noted for calculation. Calculated values of Nitrogen were tabulated and on further calculations, the crude protein contents were also noted.

REAGENTS USED

The following reagents were used in the present investigation:-

(a) Reagents used for the estimation of amino acids

- (1) 10% Isopropanol in distilled water (containing dilute HCl.).

10 ml. Isopropanol, 1 ml. 6N.HCl, 89 ml. distilled water. (when it was used for hydrolysates HCl was being omitted, and distilled water was taken in 90 ml. quantity).

- (2) 6 N.HCl.

- (3) Partridge's solvent.

n-butanol: glacial-acetic acid: water (40: 10:50)
Detail description for preparations of Partridge's solvent are under the title "Development of Chromatogram".

- (4) Ninhydrin solution (for colour development).

0.5 gm. ninhydrin ($C_6H_4CO.CO.CO.H_2O$) was dissolved in 100 ml. of acetone ('AnalaR').

- (5) 75% Ethyl alcohol (containing 0.2 mg. $CuSO_4.5H_2O$. in 4ml)

50 mg. $CuSO_4.5H_2O$, was dissolved in 210 ml. of distilled water. To this solution 790 ml. of Ethyl alcohol was added to make it one litre.

(b) Reagents used for the estimation of total Nitrogen.

- (1) Copper sulphate 5% solution.

- (2) Boric acid 4% solution.

40 g. boric acid in some hot distilled water and volume made upto 1 litre.

(3) Meeker's indicator.

25 mg. of methylene blue and 100 mg. of methyl red in 100 ml. of absolute alcohol. 2 ml. of this indicator was added to 1 litre of boric acid 4% solution.

(4) Standard sulphuric acid.

N/35 H_2SO_4 .

(5) Strong alkali.

60 g. sodium hydroxide pellets in 80 ml. distilled water.

METHODS FOR CALCULATIONS.

During the various quantitative estimations of the muscle tissues, different calculations were made as follows :-

1. Dry matter, Moisture and Fat content:

(a) Dry Matter per cent.

$$\frac{\text{Weight of D.M.}}{\text{Weight of fresh muscle tissue}} \times 100 = \text{D.M. per cent.}$$

(b) Moisture per cent.

$$\frac{\text{Weight of muscle tissue} - \text{Weight of D.M.}}{\text{Weight of muscle tissue}} \times 100 = \text{Moisture per cent. of fresh muscle tissue.}$$

(c) Fat per cent(in D.M.).

$$\frac{\text{Weight of ether extract}}{\text{Weight of D. M.}} \times 100 = \text{Fat per cent of D. M.}$$

2. Total Nitrogen in dehydrated, ether extracted muscle:

The mode of calculation was as given below :-

Since, 50 ml. of total digest was prepared from 100 mg. of dehydrated, ether-extracted muscle.

Therefore, 5 ml. of it, contained the amount of N., present in 10 mg. of the sample. A.

Since, 1 ml. of N/35 H_2SO_4 is equivalent to 0.4 mg. of Nitrogen.

So A = ml. of N/35 H_2SO_4 X 0.4 mg. B.

or in 100 mg. of dehydrated, ether-extracted muscle contains B X 10 mg. of Nitrogen. C.

or 100 g. of sample contains C. grams of Nitrogen.

or 100 g. of sample contains C. X 6.25 gms. of crude protein.

3. Amino acid content in dehydrated, ether-extracted muscle:

First, the amount of amino acid was estimated in 10 micro litres of hydrolysate and then, from this estimated amount the amino acid content present in 2 ml. of hydrolysate was known by calculations. Standard curves and Table 3.1, were used during such calculations.

For instance, in case of lysine, when the colorimetric reading indicated 80 on the scale, the reading was referred to the standard curve which showed 0.08 micro moles of that amino acid. Evidently, Table 3.1 as quoted above, pointed to 14.6112 micrograms of lysine. However, from practical standpoint the last two decimal places were omitted. This value indicated the quantity of amino acid present in 10 micro litres of hydrolysate. To know the total quantity of amino acid present in 2 ml. of hydrolysate, it required usual multiplications and divisions to arrive at the values, which

were then represented either in milligrams or grams of the amino acid. To simplify the matter, it can be represented as follows:-

Since 2 ml. of hydrolysate was prepared from 100 mg. of dehydrated, ether-extracted muscle.

Therefore 1 ml. of it represents 50 mg. of the sample.

or 10 micro litres of it represents 0.5 mg. of the sample.

When G represented the total amount of amino acid present in 10 micro litres of hydrolysate, and I the amount of amino acid in 2 ml. of the hydrolysate.

Then $I = G \times 200$ Microgram (i.e. amount present in 2 ml. hydrolysate or 100 mg. of the sample).

$$= \frac{G \times 200}{1000} \text{ mg.}$$

or $\frac{G \times 100}{1000}$ mg. of amino acid present in 1 ml. of the hydrolysate or 50 mg. of the sample.

$\frac{G \times 200 \times 10 \times 100}{1000}$ mg. = Total amount of amino acid in 100 g. of dehydrated, ether-extracted muscle.

$\frac{G \times 200 \times 10 \times 100}{1000 \times 1000}$ g. = Grams of amino acid in 100 g. of dehydrated, ether-extracted muscle R .

4. Various ways of representing the amount of amino acid content in the sample.

During the present work of the determination of amino acids in the muscle tissues, the values were represented as follows :-

- (1) Grams of amino acid per 100 gram of dehydrated, ether-extracted muscle.

R - represents this value.

- (ii) Grams of amino acid per 16 grams of Nitrogen in the dehydrated, ether-extracted muscle.

$$\frac{\text{Grams of amino acid in 100 g. of dehydrated, ether-extracted muscle}}{\text{Total Nitrogen in grams in 100 g. of dehydrated, ether-extracted muscle}} \times 16 = \frac{R}{C} \times 16 \text{ gms.}$$

- (iii) Amino acid content in grams of Nitrogen per 100 g. of dehydrated, ether-extracted muscle.

Calculations were made on molar basis. The number of atoms of Nitrogen present in the amino acid, was multiplied by 14 and the product was divided by the molecular weight of the amino acid, in question. Then the value was expressed in grams.

For example :-

$$\text{Amino acid Nitrogen for lysine} = \frac{2 \times 14}{182.65} \dots\dots P \text{ gm.}$$

The total quantity of amino-acid-Nitrogen ^{was} calculated by multiplying P with the amount of amino acid estimated in the hydrolysate.

For example :-

$$\begin{aligned} \text{Amino acid N. in estimated lysine} &= \frac{2 \times 14}{182.65} \times 8.30 \\ &= 1.27 \dots\dots Q \end{aligned}$$

- (iv) Amino acid content in gram of Nitrogen per 100 g. of ^{Nitrogen} ~~of~~ dehydrated, ether-extracted muscle.

$$\frac{\text{Amino acid Nitrogen}}{\text{Total Nitrogen in 100 g. of dehydrated, ether-extracted muscle.}} = \frac{Q}{C} \times 100.$$

- (v) Amino acid in grams per 100 grams Nitrogen.

$$\frac{\text{Amino acid in g. in 100 g. of dehydrated, ether-extracted muscle.}}{\text{Amount of Nitrogen in 100 g. of dehydrated, ether-extracted muscle}} = \frac{R}{C} \times 100.$$

STATISTICAL METHODS.

(1) 'F' test :

The data of the present study were subjected to 'F' test for statistical inference. The following is the skeleton of the analysis of variance.

The skeleton of analysis of variance table :-

| Source of variation | d. f. | S. S. | M. S. | F. |
|------------------------|---------|-------|-----------------------|-------------------|
| Between species. | $s - 1$ | B | $\frac{B}{s-1} = X$ | $\frac{X}{Y} = Z$ |
| Within species (Error) | $n - s$ | A-B | $\frac{A-B}{n-s} = Y$ | |
| Total :- | $n - 1$ | A | | |

Where n = is total number of samples.

s = is total number of species.

(2) S. E. :- The following formula was used to obtain the standard error of the different estimates.

$$S. E. = \sqrt{\frac{\sum X^2 - \left(\frac{\sum X_1}{n}\right)^2}{n(n-1)}}$$

Where $\sum X^2$ = crude sum of square.

$\frac{(\sum X_1)^2}{n}$ = Correction factor.

n = Total number of observations.

EXPERIMENTAL

Choice of solvent

It is a well established fact in the field of chromatography that choice of the developing solvent plays a central role in the ultimate efficiency of the process of resolution and the selection of solvent for this work was based on the findings of previous works.

CHAPTER - IV.

RESULTS AND DISCUSSION.

It is well known that all common organic solvents could be classified into two main groups, i.e. polar and non-polar, as the developing solvent. Kowalsky (1967) explained the proper solvent mixture for the separation of various compounds. He (1967) recommended the use of butanol, acetic acid, water mixture, as a solvent. Levy and Smith (1962) used the same mixture as a solvent. Levy and Smith (1962) also recommended it, as one of the solvents of choice, for the analysis of amino acids on paper.

Taking one from others, it was considered proper, to use Kowalsky's solvent throughout the chromatographic work. The advantages enumerated earlier, made it more alluring for the researcher.

RESULTS AND DISCUSSION

Choice of Solvent :

It is a well established fact in the field of chromatography that choice of the developing solvent plays a central role in the ultimate efficiency of the process of resolutions and the solvent ultimately chosen in this work was based on the findings of previous works.

Wolwood (1949) preferred the use of Butanol : Acetic acid : water mixture, as solvent. He stated that when the two layers separated, the upper layer served as the moving phase and an aliquot of lower layer was placed in the chromatogram chamber. Redfield and Baron (1952) observed that all common amino acids could be separated with different aliphatic alcohols and water mixture, as the developing solvent. Kowkabany (1952) explained that slower moving solvents produced rounder and less diffused spots. Giri et al. (1952) appreciated the use of butanol, acetic acid, water mixture, as a solvent. Levy and Chung (1953) used the same mixture with success. Block and Zweig (1958) also recommended it, as one of the solvents of choice, for the analysis of amino acids on paper.

Taking cue from others, it was considered proper, to use Partridge's solvent throughout the chromatographic work. The advantages enumerated earlier, made it more alluring for the purpose.

Fixation of location of Amino Acid in a Chromatogram :

The specific distance travelled by the particular amino acid during the solvent development, indicates its resolution from the mixture of amino acids or hydrolysates applied on the chromatographic filter paper. Different amino acids have different positions in the chromatogram. Their locations on the paper along with the distance travelled by the solvent front, assists in calculating its Rf. values. Calculations of the Rf. values have been appreciated as the guidelines for identification of the amino acids. However, Zimmerman (1953) enumerated a number of points that may cause variations in Rf. values. Block and Zweig (1958) cautioned that such calculated values must not be depended upon entirely. They further recommended that while applying the aliquot of a sample on paper, a good number of spots of reference standard amino acids solution (mixture) may be applied. The application should be such, as to facilitate identity of unknowns. They also advocated that such applications in the same chromatogram cancelled the probable experimental variations.

Therefore, in the beginning, it seemed to be a necessity, to have an idea of the approximate locations of each amino acid of the standard solution. The Rf. values were calculated by the following recognized formula :-

$$\text{Rf.} = \frac{\text{Distance travelled by substance}}{\text{Distance travelled by solvent front}}.$$

Two separate standard amino acid solutions were prepared

for phenylalanine and leucine containing isoleucine, taking 0.2 milli moles of the amino acids, in each case. For lysine and arginine, there was no necessity to prepare fresh one. Two filter papers were taken and numbered as 'A' and 'B'. In filter paper 'A' four standard amino acid solutions were applied in quantities of 5 and 10 micro litres. Duplicates were also made in filter paper 'B'. Filter paper 'A' was developed only once, but 'B' was run for the second time after 16 hours drying in between two solvent stages in a room for better separation. For test of the procedures, there was no difference.

Both the solvent fronts were measured. Various ninhydrin spots were also measured (distance from the point of application to the ninhydrin spot). Rf. values were calculated. The calculated values are represented in Table 4.1 on page 74.

Apparently, the locations of lysine, arginine, phenylalanine, leucine and isoleucine were in the descending order. The Rf. value for each one was different (for each amino acid). In the case of leucine and isoleucine due to incomplete resolutions, same values were taken, for both.

It was noticed that the quantity of amino acid (in 10 micro litres) made bigger ninhydrin spots, whereas, the spot-size in 5 micro litre aliquot showed small areas.

The location of lysine and arginine were further apart in each chromatogram. The intervals between leucine and phenylalanine were comparatively closer.

T A B L E - 4.1

Table showing the distance of solvent fronts, amino acids in a single run and re-developed chromatograms with the calculated Rf. values.

| Solvent develop-ment. | Description | Lysine | | Arginine | | Leucine con- taining iso- leucine | | Phenylalanine. | |
|---------------------------|---|---|------|----------|------|---|------|----------------|------|
| | Application (in micro litres) | 5 | 10 | 5 | 10 | 5 | 10 | 5 | 10 |
| Single Run 'A' | Distance trave- lled by solvent front (in cms.) a. | 33.5 cms. | | | | | | | |
| | Distance trave- lled by the amino acid (in cms.)... b. | 3.8 | 3.8 | 5.3 | 5.3 | 22.5 | 22.5 | 19.7 | 19.7 |
| Room temp. 34°C. | Rf. = $\frac{b}{a}$ | 0.11 | 0.11 | 0.15 | 0.15 | 0.67 | 0.67 | 0.59 | 0.59 |
| | Distance trave- lled by solvent front (in cms.) a. | 46.0 cms. - 1st run. *46.4 cms. - 2nd run. | | | | | | | |
| Re-deve- loped 'B'. | Distance trave- lled by the amino acid (in cms.) b. | 8.0 | 8.0 | 11.1 | 11.2 | 37.9 | 37.9 | 34.7 | 34.7 |
| Room temp. 34°C. | * Rf. = $\frac{b}{a}$ | 0.17 | 0.17 | 0.23 | 0.23 | 0.81 | 0.81 | 0.76 | 0.76 |

* The distance of solvent front in chromatogram in second run (46.4 cms.) was taken for calculation of * Rf. values.

Assessment of the resolution of the constituent amino acids from a mixture :

To test the efficiency of Partridge's solvent, steps were taken to examine the resolutions of different amino acids. Two filter papers, designated as No. I and No. II were taken. The applications of an aliquot of 10 micro litres were made to 6 spots only in this order i.e. Mixture - Lysine - Mixture, - Arginine - Mixture - Phenylalanine and Leucine containing Isoleucine. In this work, reference amino acid mixture and three standard solutions were used. Applications were made on the same pattern, as it was done in Chromatogram No. I in the case of second Chromatogram. Chromatogram No. II was re-developed, after 16 hours drying in between the two solvent stages. Separated amino acids were identified by the help of known amino acids (reference). Measurement of Rf. values and visual assessment of separations were done, for both the chromatograms after colour development.

Table 4.2 indicates the values obtained from such study, as presented in page no. 76.

The Chromatogram No. II showed the distinct separation of arginine and lysine and the distance in between were wider. The distance between leucine and isoleucine were negligible. Between phenylalanine and leucine comparatively narrow separation was observed.

The solvent re-development technique of Pasieka and Logan (1966) was thus verified and found to be more effective. The results indicated for its further application in the subsequent analysis.

Preparation of standard curve :

During the preparation of standard curves all the procedures, as detailed in the chapter materials and methods, were scrupulously followed. Solvent re-development technique was, however introduced, for it was found to provide better separations of amino acids.

A standard amino acid solution for lysine was taken and an aliquot of 2, 4, 6, 8, 10, 12, 16, and 20 micro litres were applied on Whatman chromatographic filter paper, one after another. Duplicate applications of these quantities were also spotted on separate sheet of paper. Utmost care was taken while application of the different volumes of the solution. The chromatograms were developed with Partridge's solvent, for 20 hours and then re-developed after 16 hours of drying in between the two solvent stages. Colour development with 0.5% ninhydrin in 95% acetone provided regular, uniform, ninhydrin spots. The ninhydrin spots were then cut and eluted. Optical density measurements of the elutriates were made and the colorimetric readings were recorded. The values of different elutriates obtained from the second chromatogram were also noted. Ultimately, the average values were calculated. These were plotted on the graph paper against the corresponding quantities of amino acid applied initially, on the chromatographic paper. A straight line was drawn which consequently, provided the standard curve. It was observed that aliquots which had more quantity of amino acid during application on the paper, provided bigger spots.

Same procedures were repeated in case of arginine, leucine

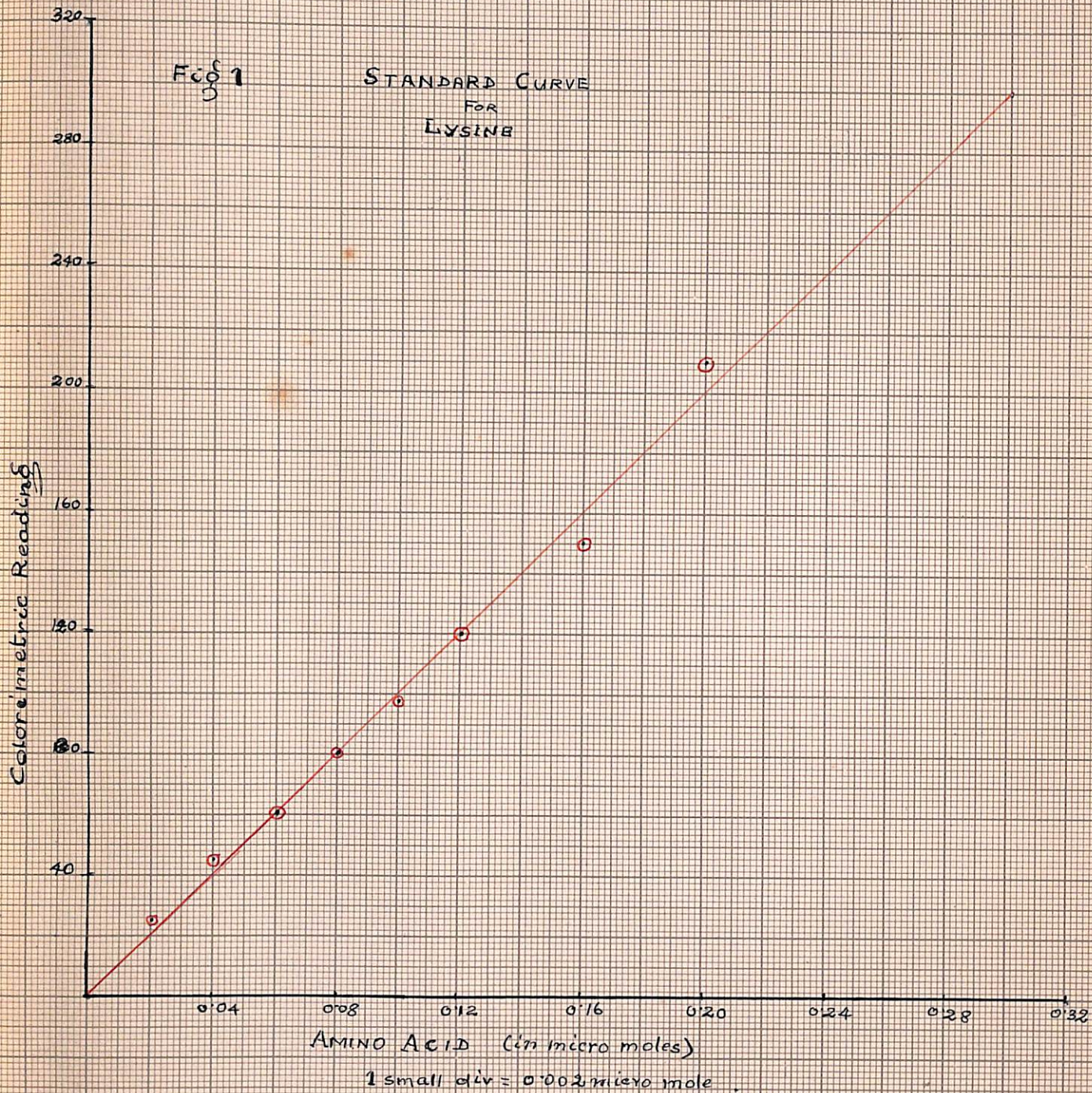
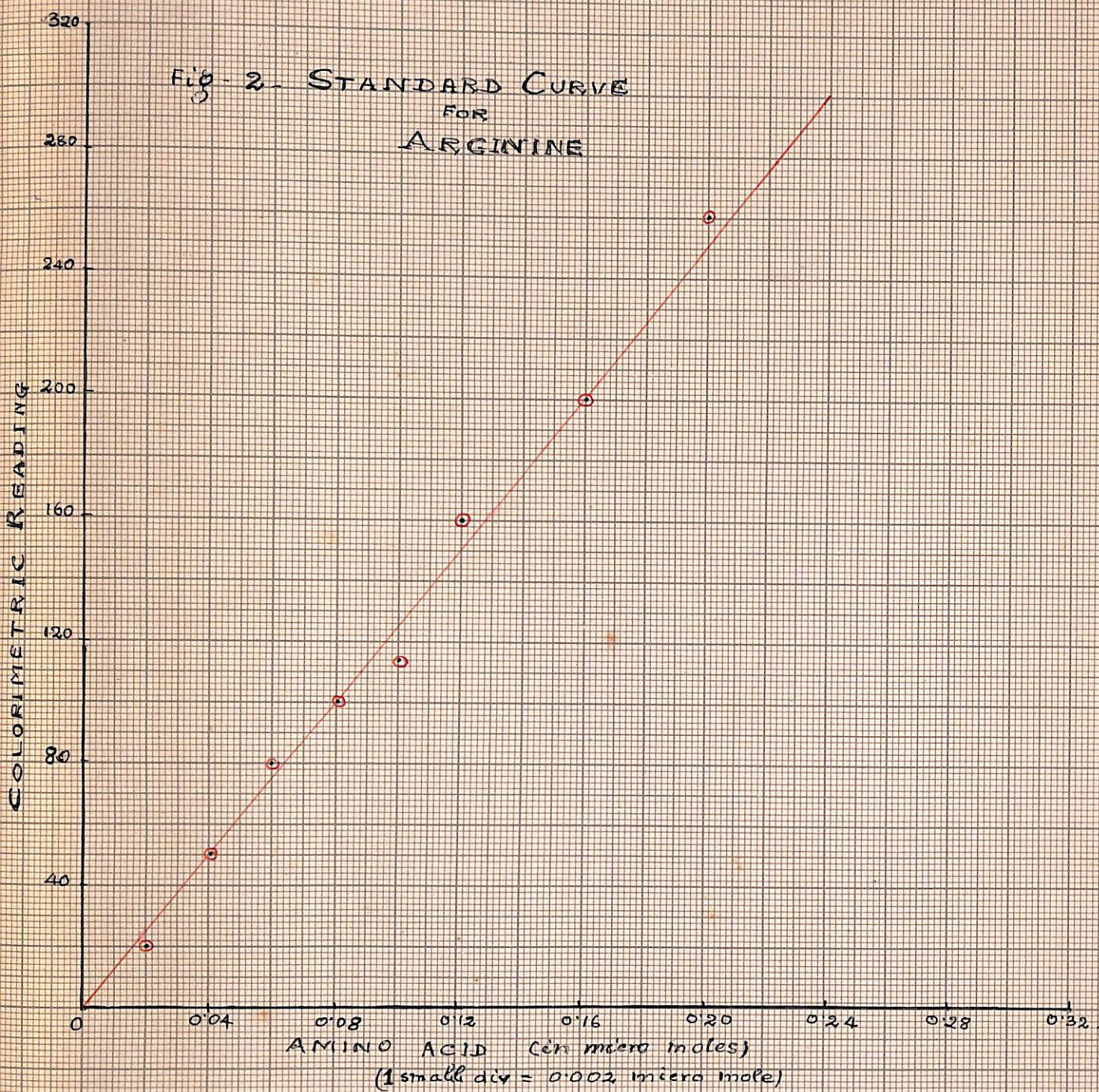
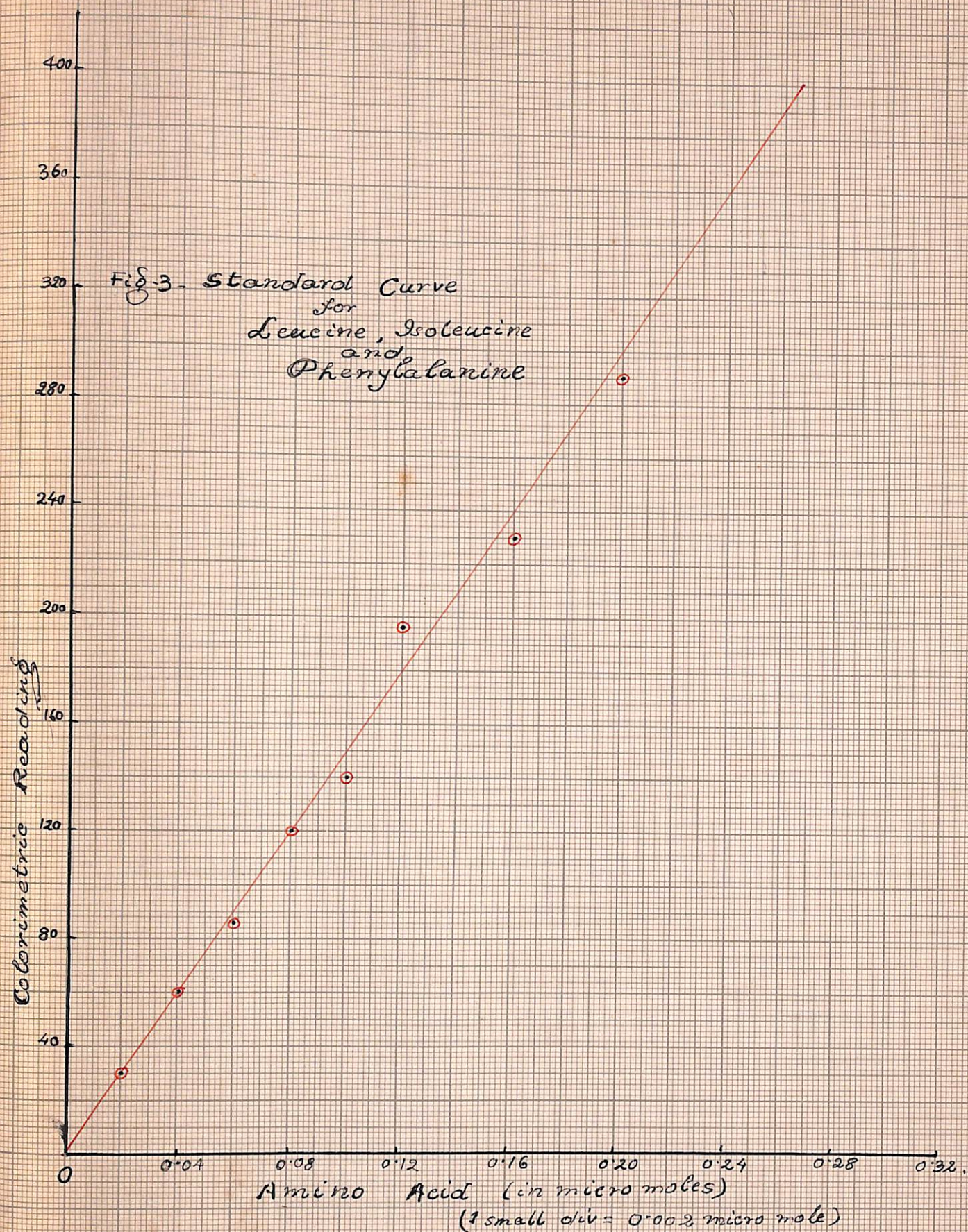


Fig. 2. STANDARD CURVE
FOR
ARGININE





containing isoleucine and phenylalanine. The three standard curves thus obtained were used for reference during estimations of amino acids in muscle hydrolysates.

The standard curve for lysine, arginine and leucine - isoleucine - phenylalanine have been represented graphically in figure 1, 2, and 3 respectively.

Determination of optimum concentration of hydrolysate for use in the chromatographic work:

Earlier experience pointed to the necessity of keeping the volume of applications on chromatographic paper to a minimum while containing the optimum amount of amino acids. Therefore, for rest of the analytical work, with muscle hydrolysates, an aliquot of 10 micro litres were taken, on every occasion. During chromatographic analysis optimum concentration of a mixture is essential for better resolutions of different amino acids. And it was also observed during the preparation of standard curves that there existed a correlation between the amounts of amino acids and the corresponding sizes of ninhydrin spots. As a consequence, particular attention was devoted to the determination of their optimum concentration in the hydrolysates for analytical purpose.

Three hydrolysates were prepared from dehydrated, ether extracted muscle of hen, weighing exactly 100 mg. The hydrolysates were dissolved in 1 ml., 2 ml. and 5 ml. of 10% isopropanol in water. Transferred the contents in the respective clean and dry test tubes, marked as A., B., and C. respectively. Test tube A. contained 1 ml., B. 2 ml., and C. 5 ml. of the hydrolysates.

An aliquot of 10 micro litre was taken from A. and applied it on the chromatographic paper. Duplicate application for the same was also made. An application of 10 micro litre of reference amino acid mixture was spotted by the side of the duplicate. In this order, the spots were made for rest of the hydrolysates on the same paper, with fixed volume of 10 micro litre every time. The chromatograms were re-run and colour developed. After colour development, the chromatogram was put in the hanging position and was examined through naked eyes for the resolutions of the amino acids. As stated earlier, the spots formed from the aliquot of a reference amino acid mixture were used, while identification of resolved amino acids from the hydrolysates. Following were the observations with the three hydrolysates :-

A. Lysine and arginine:

Ninhydrin spots were bigger than other hydrolysates but due to trailing, appeared not to be fully separated.

Leucine, isoleucine, and phenylalanine:

A big, single spot was noticed without showing clear resolution.

B. Lysine and arginine:

Ninhydrin spots were of medium size and they had regular shapes. Trailing was absent.

Leucine and isoleucine and Phenylalanine:

Each spot appeared separated but they were very close to each other.

C. Separations were clear. The presence of irregular spots (diffused spots) were noticed.

After such examination, the different ninhydrin spots for lysine and arginine, falling on the line formed by aliquots of B. and C. were cut, and kept in separate labeled test tubes. Same was repeated for leucine, isoleucine and phenylalanine spots but they were taken together and kept in test tubes allotted for them. Though the separations were incomplete, in Aliquot A., yet one of the ninhydrin spots for lysine from it was cut (on approximation of the size of it) and placed in a test tube for elution. Elutions were done in the manner as described earlier. Optical density measurement of the elutriates were made in Klett-Summerson colorimeter with green filter (540 m μ). The values of the estimated amino acids are tabulated below :-

T A B L E - 4.3

Different concentrations of amino acids in
muscle hydrolysates
of hen.

| Hydrolysates | Observations | Lysine | Arginine | Leucine, iso- leucine, phe- nylalanine. |
|--------------|--------------|-------------------|----------|---|
| | | mg./ml. | mg./ml. | mg./ml. |
| A. | I | Not estimated. | - | - |
| | II | - | - | - |
| B. | I | 4.346 | 3.454 | 7.202 |
| | II | 4.492 | 3.328 | 6.905 |
| C. | I | 1.461 | 0.928 | 2.96 |
| | II | 1.369 | 1.262 | 2.696 |

On the perusal of the above table it will be observed that for B, the estimated values of lysine and arginine are in close agreement between the duplicates. For leucine, isoleucine, and phenylalanine, some variability is noticed. The values obtained for the hydrolysate C, some variations are seen in all the observations.

Taking into consideration the results of visual examination and the estimated values, it was finally concluded that when the hydrolysate is dissolved in 2 ml. of 10% isopropanol, optimum concentration in 10 micro litres could be obtained. Therefore, for further estimations, this volume of isopropanol 10% was used while preparing the hydrolysates for spotting.

Tests for Recovery :

According to Olcott (1951), one of the unresolved problems in the analytical chemistry of proteins, concerns the extent of destruction of amino acids during the hydrolytic procedure. He further observed that the interaction of amino acids upon each other, during hydrolysis had not been appreciated so far. Keeping these in view it was thought to proceed with a test for the recovery of added lysine in dehydrated, ether-extracted muscle.

In two separate test tubes 100 mg. of the dehydrated, ether-extracted muscle were taken. In test tube B, 1 ml. of standard solution for lysine was added. Therefore test tube B contained the muscle sample plus 1.826 mg. of added lysine. In both the test tubes 10 ml. of 6 N HCl. was then added and hydrolysed them under

reflux. Both the hydrolysates were taken in 2 ml. of the 10% isopropanol. An aliquot of 10 micro litre was taken from test tube A (without added amino acid) and applied on the chromatographic paper in duplicate. Same volume of the hydrolysate was taken from test tube B (containing added amino acid) with a separate pipette and applied on the paper in duplicate. Spots from reference amino acid solution, were also made after each duplicate applications. It was then chromatographed. After drying it, colour developing was done with ninhydrin as described earlier. The ninhydrin spots were cut and eluted and estimations were made for lysine. Results obtained are given below :-

T A B L E - 4.4

Table showing the recovery of lysine.

| Particulars | Amount of lysine. |
|--|-------------------|
| Amount of amino acid in test tube 'A'. | 8.84 mg. |
| Amount of amino acid added. | 1.826 mg. |
| Amount of lysine recovered from 'B'. | 10.55 mg. |
| Percentage of recovery. | 93.64% |

The results of recovery test indicate the possibility of obtaining reliable results from the hydrolysates.

Since, Schein and Berg (1946) observed that basic amino acids were quite stable under the conditions for complete hydrolysis

of protein by acids, it was concluded that the loss of added amino acid (lysine) might be due to experimental errors. This view receives support from the work of Block and Zweig (1958). They agreed that the elution technique when adopted during chromatographic work, for substances which were clearly separated, an accuracy of the procedure may be better than $\pm 5\%$.

Estimation of amino acids in muscles of hen, goat and buffalo:

The procedures adopted for preparation of hydrolysates have been already described in the chapter "Materials and Methods".

Obtained dehydrated, ether-extracted breast muscle samples of 3 laying hens and prepared the hydrolysates. Each hydrolysate was dissolved in exactly 2 ml. of 10% isopropanol in water and finally transferred them to the respective clean and dry test tubes. The test tubes were then stoppered and shifted to the laboratory meant for chromatography.

A sheet of chromatographic filter paper was taken out from the packet and put on the table with care so that it laid traught over the glass plate. Eight pencil dots were made on a line 8 cms. away the narrow edge. 10 micro litres of the aliquot from each hydrolysate was applied in duplicate. Care was also taken to spot 10 micro litres of the reference amino acid solution after the first and second duplicate spots. After re-developing and colour developing the chromatogram, it was put for removing the ninhydrin spots. With the aid of the ninhydrin spots of the respective amino acids obtained as a result of the resolution of the reference amino acid mixture, and the same amino acids in the hydrolysate were

identified. The spots for leucine, isoleucine and phenylalanine cut together, but spots for lysine and arginine were taken separately. After elution, each eluate was measured for its optical density in Klett-Summerson photoelectric colorimeter using green filter (540 m μ).

Thus duplicate readings for lysine and arginine were obtained for each hydrolysate. For leucine, isoleucine and phenylalanine, the values indicated cumulative figures. The readings were then referred to the respective standard curves and the quantities of the amino acids were thus known. The average values for the particular amino acid was thus obtained and expressed in different standard forms by calculations.

For illustration, the actual duplicate readings and calculations made for ascertaining the content in 2 ml. of hydrolysate are indicated in Table 4.5 below, in respect of lysine.

T A B L E - 4.5

Table showing the details of calculation for ascertaining lysine content in 2 ml. of muscle hydrolysate.

| Volume used for spotting (micro litres) | Eluate | Colorimetric reading | Amount obtained on reference curve (in micro moles). | Weight in micro gram. | Average (micro gram) | Total lysine in 2 ml. of hydrolysate (mg.) |
|---|--------|----------------------|--|-----------------------|----------------------|---|
| 10 | I | 235 | 0.236 | 43.8 | 43.99 | $\frac{43.99 \times 200}{1000}$ $= 8.798 \text{ mg.}$ |
| 10 | II | 245 | 0.246 | 44.91 | | |

Same procedures were adopted to estimate lysine, arginine and leucine, isoleucine, phenylalanine in hydrolysates prepared from the muscles of goat and buffalo.

Estimated values of lysine, arginine and leucine, isoleucine, and phenylalanine are being presented in Table 4.6. The results of proximate analysis are also being incorporated in the same table.

MOISTURE AND DRY MATTER IN MUSCLE TISSUE:

Measurement of the total body water is useful for its own sake but it also has ~~then~~ advantage that it may be used to estimate the other components of the body. The other components in nutrition, are termed as "dry matters". Since, there is variability in the water-fraction of a body, a measure of the moisture percentage gives the idea of the presence of the dry matter. An embryo possesses this fraction at the maximum. During different stages of growth, it gradually decreases to the extent, that at maturity, appears to indicate a constant percentage of it. Gruhn and Anke (1965) observed the increase in dry matter 23.7 to 31.0 per cent of fresh cut in chickens from day old to 12 weeks of age.

Maynard and Loosli (1956) impressed that there exists variations in water-for their quantitative distribution in the body. Such variation is found less in degree, in muscle, as it remains within the range of 72 to 78 per cent. Ratio of body water to Nitrogen under different feeding conditions remains constant, as observed in chickens. Summers and Fisher (1961), however noted a deviation of this ratio on protein depletion. The findings of Holler and Hill (1962) in pigs related to the increase in moisture with simultaneous, decrease in protein content, when the animals were depleted of protein.

In the present investigation, Table 4.6 shows the estimated values for the percentage of moisture, in case of hen, goat and buffalo, within the range i.e. 72 to 74%, 72 to 73% and 73 to 75% respectively. The range of variation, as witnessed in table,

points to the fact that the active growth stage was passed by all the animals.

FAT CONTENT IN DRY MATTER OF MUSCLE:

Lipides occur as essential constituents in every cell in the body. While depot fat serves primarily as a source of energy and as a non-conducting layer under the skin. There are variations in fatty acid distribution in different species. Variations in the fat depot according to their locations in the body of an animal, are also found. Deposits of fat are also seen between the muscle fibres and such characteristic feature has been termed as "marbling." Further, the amount of fat in the carcass is the most variable factor but the composition of the body on fat free basis seems to be constant.

Albritton (1955) presented the following standard values for fat content, present in different cuts of various species, with medium fat :-

| | | | | | |
|---------|---------------------|-------------------------------------|----|----|----|
| Beef | (a) Chuck | - 16 gm./100 gm. of edible portion. | | | |
| | (b) Flank | - 18 gm./100 gm. of edible portion. | | | |
| Lamb | (i) Leg roast | - 17.5 gm./100 gm. | ,, | ,, | ,, |
| | (ii) Shoulder roast | - 25 gm./100 gm. | ,, | ,, | ,, |
| Pork | - Ham | - 31 gm./100 gm. | ,, | ,, | ,, |
| Chicken | - Roaster | - 12.6 gm./100 gm. | ,, | ,, | ,, |

Since, a cut taken as a whole, contains an abundant extramuscular fat, the figures quoted above do not reflect the exact amounts present in muscle tissues. However, they indicate the probable quantities of fat which remain in the muscle tissues.

Table 4.6 shows the average percentage of fat in the form of ether-extract in goat 15.67% and in buffalo 13.92%. These values indicate the presence of constant amount of fat in the longissimus dorsi muscles of both the species. Whereas, the average percentage i.e. 9.75% ether extract for poultry distinctly point to the fact that breast muscle in birds contain lesser quantity of it.

Lewis (1966) pointed to the marked aversion of the modern consumer to fat. He emphasized that such an attitude can encourage increasingly for the production of poor quality of meat so far as flavour and tenderness of the lean components are concerned.

The lower fat content of poultry meat as found in this investigation, may be one of the causes of preference of poultry meat by many people.

TOTAL NITROGEN AND CRUDE PROTEIN IN ETHER EXTRACTED MUSCLE:

From Table 4.6 it appears that the estimated values of total nitrogen in dehydrated ether-extracted muscles of hens, are relatively high as compared to goats and buffaloes. The data indicate inverse relationships between nitrogen content and fat for each of the observations. However, such a trend is absent between fat and moisture. The nearly constant ratios between moisture and nitrogen in all the species point to the fact that the animals appear to be in optimum protein nutrition, as suggested by Holler and Hill (1962), Summers and Fisher (1961). Estimated total nitrogen for goats and buffaloes of 13.96% and 14.20% of ether extracted

muscle when calculated to fat free basis, are similar to those obtained by Lawrie (1961) in Friesian bulls and by Gruhn (1965) in pigs.

The content of crude protein of 92.87% of dehydrated ether extracted muscles of hen suggests that poultry meat is a richer source of animal protein. Duncan's Summary (1962) relates to the fact, "The total protein of Chicken breast meat is considered to contain a higher proportion of first class protein than other meats except some pork. Its high total protein and low fat content are considered to make it an ideal food for invalids". Percentage of crude proteins for goats and buffaloes on the same basis are 87.25% and 88.75% respectively which show that they also contain abundant protein.

The N.P.N. fraction, (not estimated in the present work) in meat is subject to variation, and that the value ranges from 10 to 14%. Lawrie (1961) reported 0.40% of N.P.N. in fresh longissimus dorsi muscle of beef animal when the total Nitrogen was 3.39% of the muscle. The cumulative figures for amino acid nitrogen for 16 amino acids in pork and lamb cuts were presented by Schweigert et al. (1949) as 84.9 and 85.1 per cent of total nitrogen, respectively. The balance amount regarded by them was non-protein-nitrogen. Similar findings by Greenwood et al. (1951) were noted in different cuts of beef. Eighteen amino acids showed 88% of total nitrogen present in the different cuts. In case of Jersey and Friesian cows, this value has been found to be 90% of the total nitrogen by Bigwood (1960).

The amounts of ether extract and crude protein present

in fresh muscle of hen, goat and buffalo were calculated from the estimated values. The basis of calculation is given below:-

Since 100 gm. of D.M. of the muscles of hen contained 9.75 gm. of Ether Extract.

Therefore, 27.01 gm. of D.M. of the muscles of hen contained $\frac{9.75 \times 27.01}{100} = 2.63$ g. Ether Extract. So 100 gms. of fresh muscle tissue contained 24.38 gm. of Ether Extracted D.M.

Since 100 gm. of Ether Extracted D.M. of muscle of hen contained 92.87 gm. Crude protein.

Therefore, 24.38 g. of Ether Extracted D.M. contained $\frac{92.87 \times 24.38}{100} = 22.64$ gm. of Crude Protein.

T A B L E - 4.7

Table showing moisture, dry matter, fat and crude protein per 100 gm. of fresh muscle tissue from Hen, Goat and Buffalo.

| Species | Moisture (gm.) | Dry matter (gm.) | Ether Extract* (gm.) | Crude protein* (gm.) |
|----------|-------------------|------------------------|---------------------------|---------------------------|
| Hen. | 72.98 | 27.01 | 2.63 | 22.64 |
| Goat. | 73.09 | 26.90 | 4.21 | 19.80 |
| Buffalo. | 74.52 | 25.48 | 3.55 | 19.45 |

* Calculated values.

The calculated values as seen in Table 4.7 are found to be similar with the estimated values of beef cuts and lamb cuts as presented in tables of standard values by Albritton (1955) and Wohl and Goodhart (1960).

T A B L E - 4.8

Table showing average percentage composition of muscle of hen, goat and buffalo.

| Description | Poultry Average | Goat Average | Buffalo Average |
|--|--------------------|------------------|--------------------|
| Moisture. | 72.98 \pm 1.32 | 73.09 \pm 0.35 | 74.52 \pm 0.79 |
| Dry matter. | 27.01 \pm 2.98 | 26.90 \pm 0.35 | 25.48 \pm 1.91 |
| Ether Extract (in D.M.) | 9.75 \pm 0.15 | 15.67 \pm 0.79 | 13.92 \pm 0.52 |
| Total Nitrogen. (Min Ether Extracted D.M.) | 14.86 \pm 0.09 | 13.96 \pm 0.11 | 14.20 \pm 0.05 |

AMINO ACIDS IN MUSCLES OF POULTRY, GOAT AND BUFFALO:

Stein (1946) emphasized the necessity of having available two kinds of amino acid analytical techniques, "primary standard" and "routine". Primary method need not necessarily be a convenient, or rapid method, nor adaptable to handling of small quantities of protein. Olcott (1951) pointed to "isotope dilution method" which closely approached to the criteria of primary standards. The rest of the methods classified as routine may have some inherent

experimental errors. Most of the estimated values for amino acids were obtained from microbiological assay, which too have certain limitations viz. changes in micro-organisms, synthetic potentialities of the organisms, presence of inhibitors in the hydrolysates. Estimated values for amino acids obtained by microbiological assay have been usually taken as standard for comparison of the values obtained by other methods.

(1) LYSINE:

On perusal of Table 4.6 it is observed that the estimated values of lysine calculated to 16 g. Nitrogen of dehydrated, ether extracted muscles are similar in three hens. The values are 9.37 g., 8.85 g. and 8.65 g. A slightly higher value is, however, noticed for one of the hens which may be due to greater amount of protein present in the muscle.

The estimated lysine content, calculated to 16 g. N. of dehydrated, ether extracted muscle in goats are 8.57 g., 8.64 g., 8.32 g. and for buffaloes 8.61 g., 8.14 g. and 8.25 g. respectively. These values show that there is similarity in the amount of lysine as present in the longissimus dorsi muscles of different goats and buffaloes. The average values for goats and buffaloes are 8.51 g. and 8.33 g. which indicate the marked similarity between these species.

No data have been available for lysine content of goat and buffalo muscle, as also that of laying hens. The estimated values of lysine in goat and buffalo obtained during the present work are also similar with the estimated values, as reported by

Greenwood et al. (1951) in beef cuts and Schweigert et al. (1949) in lamb and pork cuts.

The result of statistical analysis of lysine content in poultry, goat, buffalo are shown in Table 4.10. However, a table showing the average quantity of amino acids estimated during the present study along with their standard errors are also being presented in Table 4.9.

T A B L E - 4.9

Table showing the average quantity of amino acids (grams) present in muscle of hen, goat and buffalo.

| Species | Lysine Average | Arginine Average | Leucine, Isolucine and Phenylalanine Average. |
|----------|-------------------|---------------------|---|
| Hen. | 8.95 \pm 0.2 | 6.49 \pm 0.23 | 15.41 \pm 0.1 |
| Goat. | 8.51 \pm 0.1 | 6.24 \pm 0.18 | 15.40 \pm 0.08 |
| Buffalo. | 8.33 \pm 0.14 | 6.02 \pm 0.04 | 15.26 \pm 0.3 |

T A B L E - 4.10

Analysis of variance table for lysine.

| Source of variation | D. F. | S. S. | M. S. | F. |
|----------------------------|-------|-------|-------|------------|
| Between species. | 2 | 0.16 | 0.08 | 1.14 N. S. |
| Within species (error). | 6 | 0.42 | 0.07 | |
| Total:- | 8 | 0.58 | | |

Inference:- Since the calculated value of F is less than table value at $n_1 = 2$ and $n_2 = 6$, it is concluded that the result is non-significant. As 'F' value is non-significant, it reveals that the three species (hen, goat and buffalo) do not differ significantly with respect to lysine content of muscle.

(11) ARGININE :

The estimated values of arginine calculated to 16 g. Nitrogen of dehydrated, ether extracted muscle tissue of hens are 7.14 g., 6.55 g., 6.37 g., For goats the values were 6.60 g., 6.04 g., 6.10 g. and for buffaloes 6.07 g., 6.02 g. and 5.98 g. There is no marked variation in different hens, so also with goats and buffaloes. The average values for hens, goats and buffaloes are 6.49 g., 6.24 g., and 6.02 g. respectively. No data have been available for arginine content of goat and buffalo muscle, as also, that of laying hens. The estimated values for goats and buffaloes are also similar to the values as reported by Greenwood et al. (1951) for beef cuts and Schweight et al. (1949) for pork and lamb cut.

The results of statistical analysis in Table 4.11 as given below show non-significant difference between hen, goat and buffalo with respect to arginine.

T A B L E - 4.11

Analysis of variance table for arginine.

| Source of variation | D.F. | S. S. | M. S. | F. |
|-------------------------|------|-------|-------|-----------|
| Between species. | 2 | 0.25 | 0.125 | 1.44 N.S. |
| Within species (error). | 6 | 0.52 | 0.087 | |
| Total :- | 8 | 0.77 | | |

(111) LEUCINE, ISOLEUCINE AND PHENYLALANINE:

It has been already stated earlier that due to practical considerations these three amino acids were estimated together. Therefore, the estimated values in Table 4.6 for leucine, isoleucine and phenylalanine indicate cumulative amounts of these amino acids.

On perusal of Table 4.6 it is seen that the hens contain 15.27 g., 15.78 g. and 15.20 g. in 16 g. Nitrogen of dehydrated, ether-extracted muscle. The average value for the species is 15.41 g. In the case of goats and buffaloes the estimated values are 15.33 g., 15.56 g., 15.32 g. and 15.63 g., 15.53 g., 14.66 g., indicating an average of 15.40 g. and 15.26 g. respectively.

The estimated values in hens, goats and buffaloes, on statistical analysis do not show any significant variation between the species. The table of statistical analysis is presented below:-

T A B L E - 4.12

Table showing analysis of variance for
Leucine, Isoleucine and phenylalanine.

| Source of variation | D. F. | S. S. | M. S. | F. |
|----------------------------|-------|-------|-------|------------|
| Between species. | 2 | 0.15 | 0.09 | 0.94 N. S. |
| Within species (error). | 6 | 0.57 | 0.095 | |
| Total :- | 8 | 0.85 | | |

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It is clear that due to practical difficulties the values were estimated together.

For leucine, isoleucine and phenylalanine of these amino acids.

It is seen that the values are

Nitrogen of dehydrated

for the species is

The estimated values

15.53 g., 14.66 g.,

respectively.

and buffaloes, on

that variation between

is presented below:-

For
phenylalanine.



0.94 N.S.

LEUCINE
ISOLEUCINE
PHENYLALANINE

LO

(iii) LEUCINE, ISOLEUCINE AND PHENYLALANINE:

It has been already stated earlier that due to practical considerations these three amino acids were estimated together. Therefore, the estimated values in Table 4.6 for leucine, isoleucine and phenylalanine indicate cumulative amounts of these amino acids.

On perusal of Table 4.6 it is seen that the hens contain 15.27 g., 15.78 g. and 15.20 g. in 16 g. Nitrogen of dehydrated, ether-extracted muscle. The average value for the species is 15.41 g. In the case of goats and buffaloes the estimated values are 15.33 g., 15.56 g., 15.32 g. and 15.63 g., 15.53 g., 14.66 g., indicating an average of 15.40 g. and 15.26 g. respectively.

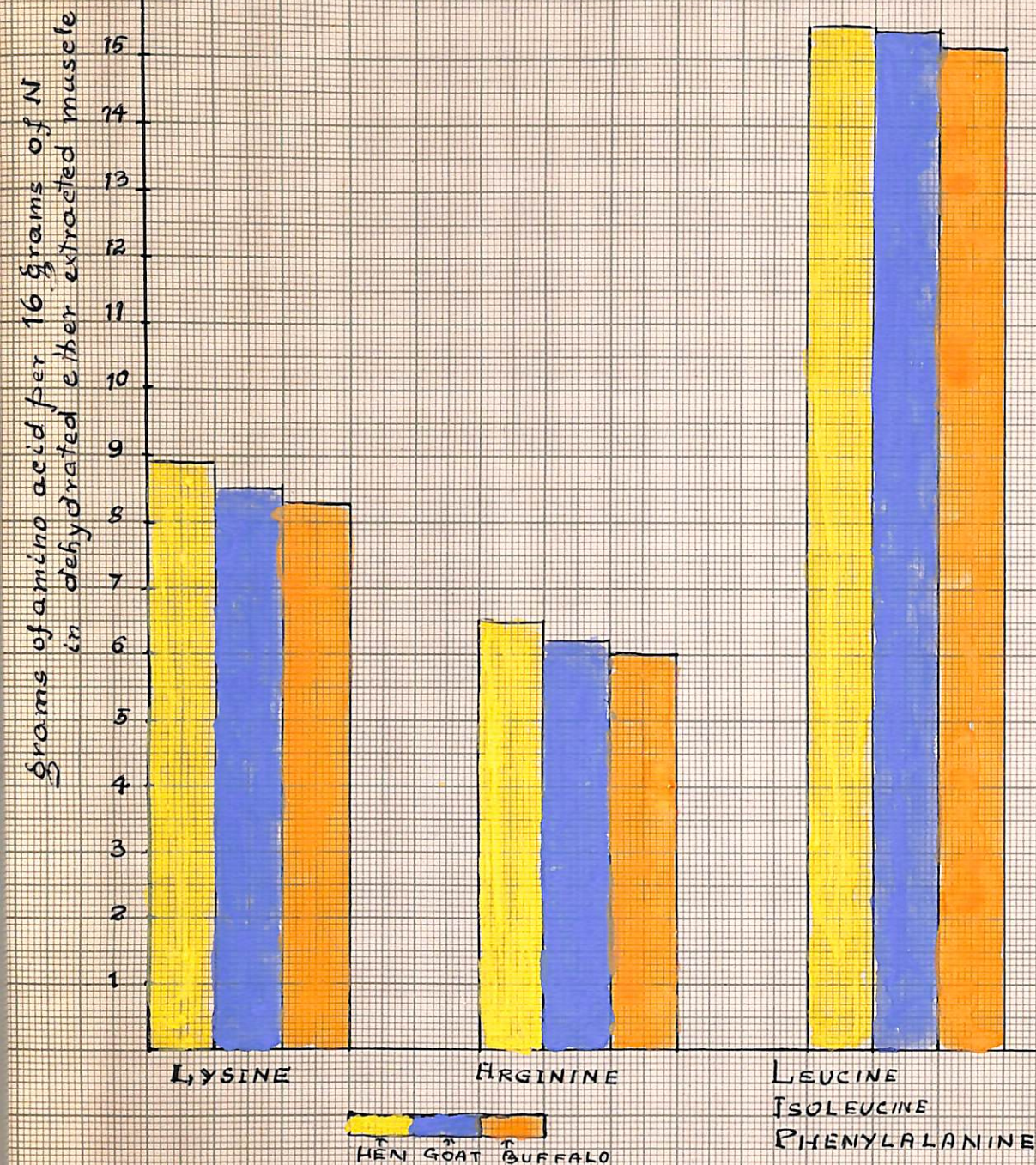
The estimated values in hens, goats and buffaloes, on statistical analysis do not show any significant variation between the species. The table of statistical analysis is presented below:-

T A B L E - 4.12

Table showing analysis of variance for
Leucine, Isoleucine and phenylalanine.

| Source of variation | D. F. | S. S. | M. S. | F. |
|----------------------------|-------|-------|-------|------------|
| Between species. | 2 | 0.18 | 0.09 | 0.94 N. S. |
| Within species (error). | 6 | 0.57 | 0.095 | |
| Total :- | 8 | 0.85 | | |

Fig-4. HISTOGRAMS SHOWING LYSINE, ARGININE
LEUCINE, ISOLEUCINE AND
PHENYALANINE CONTENT
IN HEN, GOAT AND
BUFFALO



No data have been available for leucine, isoleucine and phenylalanine content of goat and buffalo muscle, as also that of laying hens. The estimated values for these amino acids are, however, 18% lower than the total estimated values, observed by Greenwood et al. (1951) in beef cut and Schweigert et al. (1949) in lamb and pork cuts. The probable cause of such lower result may be due to different experimental procedures adopted in two cases.

COMPARATIVE DATA:

Bigwood (1960) reported that there was no variation in the amino acid content in the shoulder and leg muscles in beef animals. His findings along with the observations of Schweigert et al. (1949) affirm that there is no variation in the amino acid content in different muscles.

Williams and Co-workers (1954) observed that the amino acid composition is similar for all species. Ericson (1961) also agrees that different species do not differ in amino acid composition. In the present study, it has been observed that the muscles of three species, i.e. poultry, goat and buffalo do not differ in amino acid composition with reference to lysine, arginine and also leucine, isoleucine and phenylalanine. The average estimated values of these amino acids from different species have been plotted in Figure IV.

The estimated values of lysine, arginine and total of leucine, isoleucine and phenylalanine from the muscle tissues of hen, goat and buffalo summarised in Table 4.13 have been calculated on the basis of the different accepted forms.

Albritton (1955) presented the standard values of amino acid composition of certain other protein foods. Lysine content for casein, egg albumin, and wheat gluten in his table are 50.6 mg., 45.0 mg., 12.6 mg. per 100 mg. Nitrogen respectively. The estimated values in the present study for hen, goat and buffalo are 55.85 g., 53.22 g. and 52.04 g. per 100 g. Nitrogen in dehydrated, ether-extracted muscle. A study on the values, point to the characteristic feature that wheat gluten is the poorest source, whereas, casein and egg albumin are at par with muscle, with regard to lysine content. The arginine content for those proteins in his table are 24.4 mg., 37.4 mg. and 23.0 mg. per 100 mg. Nitrogen, respectively. The estimated values in the present work are 41.85 g., 39.40 g., and 37.67 g. per 100 g. Nitrogen in dehydrated, ether-extracted muscles of hen, goat and buffalo, respectively. Comparing the estimated values with Albritton's values, it would appear that muscle contains more arginine than casein and wheat gluten but more or less similar to that in egg albumin.

Bose et al. (1958) reported arginine, lysine, leucine, isoleucine and phenylalanine content of fishes of Bay of Bengal. They represented the values, on the basis of amino acid content in gms. of Nitrogen per 100 gms. of fat free dried tissue. The values for these amino acids were higher in fishes than the estimated values obtained during the present study.

Williams et al. (1954) estimated the amino acid content of rat, chick and pig carcasses at different stages of growth. They concluded that not only the pattern of amino acid composition are

comparable within each species but also remarkably similar. Scott (1959) observed that there was little sex difference in turkey toms and turkey hens in the amino acid composition and percentage of protein.

Price et al. (1953) reported that there was marked similarity in the patterns of the essential amino acids in total protein of the chick carcasses. Holmes et al. (1963) observed that amino acid composition of broiler cockrels was constant. Their observations are presented below (Table 4.14) along with the results obtained in the present work.

T A B L E - 4.14

Comparative values of lysine, arginine, leucine, isoleucine, phenylalanine content of chickens at different stages of growth.

| Amino Acid | Ether Extracted chick carcasses | | | | Estimated values in hen*** |
|----------------|--|--|--|-------------------------------------|----------------------------|
| | Day old* gm./16 gm. Nitro- gen. | Week old** (Amino acid content calculated to 16% N | 4-5 week** (Amino acid con- tent cal- culated to 16% N. | 10 week* gm./16 gm. Nitrogen. | |
| Lysine. | 7.75 | 6.8 | 6.4 | 7.25 | 8.95 |
| Arginine. | 6.69 | 6.6 | 6.7 | 6.63 | 6.49 |
| Leucine. | 7.07 | 7.3 | 7.1 | 6.50 | |
| Isoleucine. | 4.17 | 4.2 | 4.5 | 4.29 | 15.41 |
| Phenylalanine. | 4.39 | 4.4 | 4.1 | 3.78 | |

* The values are from the work of Williams et al. (1954).

** The values are from the work of Price (Jr.) et al. (1953).

*** The values obtained during the present work.

On comparing the different values in Table 4.14, it is observed that the estimated value of arginine is similar to the values obtained by other workers. The total estimated value of leucine, isoleucine and phenylalanine in hen, also closely tallies with the cumulative values for these amino acids present at different stages of growth. The estimated value for lysine in the present work is somewhat higher than in both day old and 10 weeks old chicks obtained by Williams et al. (1954). The reason for this higher value is not forthcoming. Whether this higher value is associated with maturity is worth investigating, more so, because of similar higher values obtained both with adult goat and buffalo.

*

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*

CHAPTER - V.

SUMMARY.

S U M M A R Y

1. Quantitative determinations of lysine, arginine, leucine, isoleucine and phenylalanine (all belonging to essential group) were done by paper chromatographic technique.
2. The method of Block and Zweig (1958) was adopted with some modifications.
3. Partridge's solvent containing n-butanol : acetic acid : water in the proportions of 40 : 10 : 50 were prepared. Upper layer of the mixture was used as a solvent and lower layer was being kept in the chromatographic cabinet, for saturation of the chamber with solvent vapour.
4. One dimensional, descending chromatography was resorted to. Solvent development was stopped after 20 - 22 hours, when the solvent front moved a distance of 40 - 42 cms.
5. The chromatogram was developed with 0.5% ninhydrin in 95% acetone.
6. Noticed the ninhydrin spots and the colour was eluted and their optical density determined in photoelectric colorimeter.
7. Rf. values of the amino acids under investigation were determined. Aliquots of 5 and 10 micro litres were taken from each of the standard amino acid solution of lysine, arginine, leucine containing isoleucine, and phenylalanine containing 0.05 micro mole and 0.1 micro mole for each of the amino acids.
8. Resolution of the constituent amino acids from the

mixture was studied and was found to be satisfactory for lysine and arginine but ineffective with regards to leucine, isoleucine and phenylalanine, which however, were clearly differentiated from lysine and arginine. Re-development technique was found to provide better resolution of the amino acids.

9. Standard curves for the individual amino acid were prepared with amounts of the acid ranging from 0.02 to 0.2 micro mole of each.

10. The method of Block and Zweig (1958) was adopted for the preparation of the hydrolysate from dehydrated, ether extracted muscle. To 100 mg. of sample 10 ml. of 6 N HCl was added and hydrolysed under reflux for a period of 24 hours. For collection of samples, breast muscle was selected in case of hen and longissimus dorsi in case of goat and buffalo.

11. Muscle hydrolysate when dissolved in 2 ml. of 10% isopropanol and an aliquot of 10 micro litres when applied, provided better resolution of amino acids.

12. Recovery test with lysine added to the muscle hydrolysate came to above 93%.

13. Three samples of muscle protein hydrolysate were prepared from each of hen, goat and buffalo and chromatographed in duplicate.

14. The average lysine content in 16 g. Nitrogen of dehydrated, ether extracted muscle, were 8.95 g., 8.51 g., and 8.33 g. for hen, goat and buffalo, respectively. Similarly, the average values of arginine were 6.49 g., 6.24 g. and 6.02 g. respectively.

Total leucine, isoleucine and phenylalanine on an average, for these species were 15.41 g., 15.40 g. and 15.26 g. respectively.

15. On proximate analysis, average percentage of moisture in the muscles of hen, goat and buffalo were 72.98, 73.09, and 74.52 and the average dry matter per cent in the same muscles were 27.01, 26.90, and 25.48 respectively. The average percentage of ether extract on dry matter basis were 9.75, 15.67 and 13.92 respectively, for these species. Crude protein of ether extracted muscle on an average, were 92.87%, 87.25%, and 88.75%, respectively.

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