COMPARISON OF THE EFFECTS OF AN ADEQUATE AND A LOW PROTEIN DIET ON GROWTH AND REPRODUCTION IN THE RAT.

by

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ABSTRACT OF THESIS.

From a survey of the literature concerning the effects of low protein diets upon the reproduction of the rat and upon the growth of the body as a whole as well as that of the blood, it was concluded that further investigation was required. Comparatively few long term experiments, more especially those extending through several generations, had been undertaken on the reproduction of the rat fed low protein diets. Some divergence of opinion existed concerning the relative priority between the body and haemoglobin proteins, when the dietary supply of these were inadequate, and concerning the haemopoetic role of individual amino acids under conditions of low protein intake. Very recent work had suggested, rather than proved, the presence of certain ill defined endocrinal disturbances in the low protein rats which probably mediate the effects of low protein diets upon growth and reproduction.

The experiments undertaken showed how the deleterious action of the low protein diets on growth and reproduction became increasingly severe as the diets were fed for longer periods, whether during a single life time or throughout several generations (three). Lysine was the limiting amino acid of the low protein diets whose effects were gradually

counteracted as increasing levels of protein (fishmeal) were incorporated into the diet. The results suggested that under conditions of low protein intake the body proteins competed successfully against that of haemoglobin for available protein, and that no single amino acid had a special haemopoetic role. The work concerned with the growth of the endocrine glands of the malnourished animals supported the concept of an altered endocrinal balance of such animals and pointed to the presence of an under-functioning adrenal cortex.

The significance of the results is discussed, especially in their application to human nutritional problems.

PREFACE.

The work herein presented is submitted as a thesis for the degree of Ph.D. of the University of London.

The research was undertaken in the Human Nutrition Research Unit of the Medical Research Council under the direction of Professor B.S. Platt, C.M.G., Director of the Unit and Professor of Nutrition, London School of Hygiene and Tropical Medicine, The University of London.

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SURVEY OF THE LITERATURE.

SURVEY OF THE LITERATURE.

Introduction.

It is now recognised that malnutrition due to a deficiency of protein is common in many undeveloped countries. Phillips (1951) has listed the average intake of vegetable and animal protein per capita in 58 countries. Using the arbitrary figure of 30 grams of animal protein as a desirable intake, he notes that this level is equalled or exceeded in only 18 out of the 58 countries. With the increasing world population the need for obtaining the maximum "protein harvest" from the world is of vital importance. Fish and shellfish are thought to contribute about 2% of the total food consumption of the world's human population, and of this amount approximately 90% is taken from the seas of the Northern Hemisphere, the far larger expanses of the Southern Hemisphere remaining essentially untouched (Weiss 1953). Since more than 71% of the earth's surface is water covered, and an acre of sea is estimated to produce three times as much as an acre of land (Sure 1956), fish provides a virtually untapped food reserve. Within recent years F.A.O. and U.N.I.C.E.F. have been investigating the possibility of edible fish flour as a protein supplement to the diet of these malnourished communities. (Waterlow 1953).

The effects of protein malnutrition in these countries are found particularly amongst young children, where shortly after weaning they are given a diet largely composed of foods such as cereals or starchy fruits and roots containing small amounts of protein of poor quality. The resultant disease is known in different parts of the world by different names and in Africa it is called Kwashiorkor. The symptoms of the illness have been extensively reviewed (Brock and Autret 1952, Platt 1952, Trowell et al 1954) and common amongst these are mental apathy, anorexia, failure of growth, muscle wasting, anaemia, low serum albumen and a decreased formation of pancreatic enzymes.

It was in the light of the above human nutritional problems that the effect of a low protein diet on the growth and reproduction of the rat was undertaken. For this reason the diets used were of similar type to that eaten in many parts of Africa, and fishmeal was given as a protein supplement. The growth of the rat was investigated from measurements of body weight as well as from that of the blood. The growth of some of the endocrine glands was also studied.

Before considering the experimental work on reproduction and growth, it is appropriate to take into

account several factors which can affect the results obtained. These may be separated into two main headings the "nutritional factors" which include both the quantity as well as the quality of the protein deficiency, and the "physiological factors" which are concerned with the state of the animal and the duration of the experiment.

The quantity of the protein in the diet available to the rat is modified in several ways, for example the amount of non protein calories in the diet may exert an important protein sparing action (Monro 1951). The availability or digestibility of the dietary protein may be modified by cooking which often helps to break down the less readily digestible coverings of cellulose or fibrous connective tissue surrounding the protein. On low protein diets the fact that the rat can utilise non protein nitrogen e.g. ammonium nitrogen (Sprinson et al 1949) may become significant.

Perhaps more important than the magnitude of the deficiency is the pattern of the amino acid deficiency. Much of the work written gives a percentage figure for the minimum protein requirements of the rat for growth or reproduction, but no mention is made of the quality of the protein. The important work of Rose has defined

the ten essential amino acids required for rat growth and more recently the importance both of the simultaneous administration (Geiger 1950) and of the relative proportions in the food (Elvehjem et al 1955) of these amino acids has been stressed.

The physiological state of the animal is frequently overlooked when considering the effect of a low protein diet. Such factors as age, sex and pregnancy may influence the results. McCollum and Simmonds (1929) state that experiments extending over a significant part of the life cycle yield more valid impressions of the effects of protein intake than do relatively short term studies. The protein requirements are higher for the male than for the female rat and are increased during phases of tissue anabolism as in the actively growing young animal and during pregnancy and lactation. Albanese (1950) points out that in an adult animal where protoplasmic growth is minimal the amino acids required especially for hair growth-cystine and methionine- dominate the picture, so that the lysine requirement of the adult animal is less prominent than in the young growing rat.

The concept of the dynamic state of the body's constituents (Schoenheimer 1946) makes the protein

reserves present in the rat's body of significance when considering low protein diets. These reserves may counteract the effect of a low protein diet when fed for a short time to adult rats. In this connection the increased amino acid requirements for growth of protein-depleted adult rats is of interest. (Benditt et al 1950).

Thus it is seen that when considering the effect of a low protein diet particular attention should be paid to the nature of the amino acid deficiency and to the duration of the experiment, more valid results being obtained from the more chronic experiments.

Experimental-Body Weight and Reproduction.

Stephen Babcock 1843-1931, in order to discover what contribution various feeding stuffs were making to the animal's needs, conceived the idea of trying out rations made up entirely from a single plant and began his experiments on cattle. Needless to say when one cow died he met with considerable opposition from the animal husbandrymen supplying the cattle and had to abandon the work. Later his idea was carried out extensively by his younger colleagues (Hart et al 1911), who placed four groups of young cows on four rations derived respectively from wheat, oat, corn (maize) and a mixture of the three.

By mixing proper proportions of leaves and stalk of the plant with its seed or some protein concentrate of the seed, all four rations were given the same protein and energy content. Growth was completed on all four rations but the reproduction and lactation records differed. The calves born on the wheat ration either died at birth or soon after, and their mother's milk yield was reduced by about a third. This experiment showed how the diet of the mother while sufficing for her growth might seriously affect the viability and vigour of her offspring. It was concluded that wheat contained something toxic or perhaps lacked some nutrient.

Later with the classic work of Osborne and Mendel it was shown that the wheat protein was lacking in lysine. These authors fed a diet where gliadin was the only protein to adult rats and found that grown rats could be maintained for long periods (546 days) but ungrown rats failed to grow (Osborne et al 1913). They studied such rats till they were 550 days old (Osborne et al 1915), and then by changing the diet observed a resumption and completion of growth which would normally have been completed by 300 days. On adding lysine so that the protein quota contained 2 or 3 per cent lysine, growth was resumed by the rat which had been stunted through

the influence of the lysine-poor diet. (Osborne et al 1916). This lysine deficiency of wheat protein was confirmed by the work of others (Harris et al 1943, Rosenberg et al 1952), and the deficiencies so far as rat growth was concerned of other amino acids in wheat such as arginine, histidine, valine and threonine (Pearson 1936, Sure 1953) have also been shown.

There is a considerable amount of work concerning the effects of low protein diets on the growth and reproduction of the rat extending through one generation only. As early as 1918 Hart et al found that rats fed a wheat protein diet failed to wean their young and concluded that this was due to an insufficient quantity rather than quality of the milk produced. Simmonds (1924) studied the effects of protein, ranging from 9-67% of the diet, on the reproduction and lactation of the rat, and states that lactation was not generally affected by protein contents higher than 21%. These experiments were concerned primarily with the effects of high protein diets. Guilbert and Goss (1932) found that with dietary protein contents of 7.5% to 8% the rat's growth was slightly reduced but the oestrous cycles were normal. With protein contents of 3.5 to 5% the cycles either ceased or became long and irregular. The dietary proteins used were derived from

a mixture of milk and wheat or from egg-albumin and wheat. Measurements of food intake showed that inanition was not the cause. When the sole source of protein was wheat gliadin however, the testicles were undersized and an 18% protein in the diet was not sufficient for the maintenance of normal oestrous cycles which were restored to normal by the inclusion of 0.6% lysine (Courrier et al 1932, Pearson 1936). Doubt has been cast on the vaginal smear method, from a histological study of the uteri and ovaries of rats fed a 7% protein diet (Freed et al 1939). In this experiment the vaginal smears indicated oestrous in contradiction to the ovaries and uteri, which were frequently atrophic.

Macomber (1933) investigated the reproduction of rats fed diets containing 5, 10, 16 and 20% protein derived from casein. He found little effect on pregnancy but as the protein fell, lactation, as indexed by the number and weight of the young weaned and the maternal weight loss, was impaired with decreasing protein levels. A criticism of this work was that adult rats were used, and that the same rats were shifted from one protein diet to the next in order to study its effects, so that the animals' varying protein reserves must have influenced the results.

McCoy (1940) by the replacement of dextrin by casein to give the relatively high protein levels of 15, 25 and 40%, found that the average weaning weight was highest in the 25% protein group and lowest in the 15% protein group. Using paired feeding, growth and carcass nitrogen were greater on the 40% than on the 15% protein level, and on the latter diet carcass fat was highest.

More recently Nelson and Evans (1953), studied the effect of various casein contents to give dietary protein levels of 0, 3, 12, 18, 24 and 30%, and found that 5% casein was critical for normal reproduction in the rat. Pregnancy on the diets below 5% was characterised by the high incidence (70%-100%) and early occurrence of reabsorptions, a marked decrease in maternal body weight and a reduction in the birth weights. Using a high protein diet and paired feeding, food restriction as the cause of these effects was eliminated. These authors point out that previous work concerning the effects of low protein diets on reproduction were carried out before modern knowledge of Vitamin B complex was available. In these experiments B vitamins were included in the diets and adult rats were first placed on the low protein diet from the day of mating. Curtiss (1953) also studied the

effect of low protein diets (3 and 5% casein) on rat reproduction, but the diets used, which were also first fed to adult rats, were deficient in choline.

The nitrogen balance method has been used by Morse and Schmidt (1944), who found that rats on diets containing 2.4% nitrogen (15% protein) stored amounts of nitrogen sufficient for the foetus but those on diets containing 1.2% nitrogen (7.5% protein) did not. During lactation rats on 15% protein lost nitrogen but were able to rear their young. At lower protein levels rats were unable to rear their young unless they had unusually large reserves of nitrogen at the beginning of lactation. Adult rats were used for this work. The protein content of the body before pregnancy of rats reared on an 18% protein diet has been compared with that just prior to delivery after 18 days on diets of different casein levels (11, 16, 27 and 43%) fed from the age of 90 days. (Poo et al 1940). The ratios of protein intake to increment of body protein indicated most efficient use of the protein at 16%.

Another approach to the effect of low protein diets on reproduction has been made in considering at which stage during pregnancy was the need for protein most critical. In experiments where pregnant rats were fed a nitrogen-free

diet or one containing 10% gelatin as the protein source a need for nitrogen was shown during the earliest stages of pregnancy. Rats placed on the experimental diets earlier than 13 days before term failed to have viable young, but when given the diets from 13 to 8 days before term living young were obtained which were underweight and nitrogen deficient. This was confirmed by Nelson and Evans (1953) whose results also pointed to a critical need for protein during the earliest stages of pregnancy in contrast to the reproductive success which resulted when the protein deficient diets were introduced shortly after implantation. Using self selection methods rats, given access to casein, sucrose, dried yeast, olive oil and salts, chose a diet with 22.3% of the calories derived from protein during the last five days before mating, 28.1% during the last five days of pregnancy and 23.8% during the last five days of lactation. (Richter et al 1938). The choice of diet may have been influenced by palatability.

Comparatively little work has been done on the effects of protein through more than one generation, more especially when diets very low in protein content are considered.

Conner and Sherman (1936) studied the effect of a milk-wheat diet through two successive generations but

there was no mention of any difference from one generation to the next. It was not until the work of Slonaker (1931a, b, and c, 1933) that this was more thoroughly investigated. Slonaker used a basal diet where 10.3% of the energy came from protein. The protein content was then altered by the addition of meat scraps to give percentages of 10, 14, 18, 22 and 26 while keeping the energy producing qualities of the diet constant. He concluded that a diet containing 18% or more protein calories led to an increase in sterility, delayed birth of first litter, shortened breeding period and effected a reduction in the number of young and litters born. He considered, however, that this 18% was best for prenatal development of the young and for the survival of the young to weaning. He recommended a diet with 26% calories from the protein during lactation. With successive generations there was a tendency for delayed eruption of the incisor teeth and opening of the eyes, for lower birth weights but increasing rates of growth and mortality, and a marked decrease in maternal weight loss during lactation. This work of Slonaker has been criticised (Macomber 1933) on the grounds that as the protein contents were increased

by the addition of meat scraps the percentage of other dietary components was decreased. This was especially important in the case of vitamins A, D and E where the amounts in the basal diet were suboptimal.

Kao et al (1941) studied the reproduction and lactation performance of three generations of rats on diets containing varying amounts of protein at two liberal levels of calcium. The protein contents were varied from 14% on the Sherman diet A (5/6 wheat + 1/6 dried milk) by the addition of casein to bring the protein contents up to 19 and 25%. No marked changes were observed between the generations. With increasing protein levels the initial growth rates were increased but from the age of 6 months there was no difference between the 19 or 25% protein diets, nor was there any difference between these two diets in the average number of young born or weaned. In these experiments as in those of Van Duyne's (1941), the reproductive capacity and ability to rear the young was superior to that obtained by Slonaker on the same dietary protein levels. This supports the criticism mentioned earlier concerning the inadequacy in respect to nutrients other than protein of Slonaker's diets.

Goettsch (1948, 1949) from her study of the reproduction of rats through several generations concluded

that the minimum requirement of a rice-bean-casein dietary protein was 17%, and that the limiting amino acid of such a mixture was methionine. Results obtained from three generations on protein contents of 11.9%, 14.3%, 16.7% and 19.1% showed no difference in the gestation performance between successive generations, but the litters were artificially reduced in size so this would probably offset some of the effects of the low protein diet. Rats receiving 14.3 % protein grew approximately as well as the controls but the growth of the second generation was significantly reduced.

Some interesting results were obtained by Damodaran (1950, 1951, 1952), who increased the protein content of a basal diet containing 70% of rice flour to which sugar salts, oils and vitamins were added, by the replacement of an equivalent amount of sugar by protein. The three diets used were the basal or the basal supplemented with 3% pulse protein or with 15% casein as a control. Results were obtained from three generations of rats. As compared with the first generation, the low protein animals of the second generation had reduced rates of growth, lower basal metabolic rates and a reduction in the size of the litters born which resulted in an increase in the birth

weight of the new born and in the percentage number weaned.

Recently Schultze (1956) studied the reproduction of rats fed diets where the protein was derived from synthetic amino acid mixtures. The amino acids were patterned after those recommended by Rose for growing rats, as well as after those contained in casein. Neither of these diets supported normal lactation or growth of the young and there was no evidence with successive pregnancies or generations of a progressive deterioration in the ability to reproduce or lactate. Fatty livers were observed in these rats during lactation which suggests some dietary complication such as an amino acid imbalance (Deshpande et al 1955) or the lack of some unrecognised nutrient.

From this survey of the literature it is seen that comparatively little has been done concerning the effect of a low protein diet on growth and reproduction when the diet is first introduced to the young animal as opposed to the adult. Further, the effect of a very low protein diet through several generations seems only to have been recorded by Damodaran and Goettsch, but the latter worker artificially reduced the size of the litters during lactation. The lowest protein diet used by Slonaker was a

10% good quality meat protein which is a relatively mild restriction, and in any case his diets have been severly criticised; and the lowest protein diet of Kao was 14% which was also of a relatively good quality protein mixture.

Experimental-Anaemia of Low Protein Rats.

It is well established that a low protein diet adversely affects the growth of the blood elements so that anaemia results. Rats were placed after weaning onto a diet containing 3.5% lactalbumin and compared with the animals fed the control diet containing 18% lactalbumin (Orten and Orten 1943). After 76 days the haemoglobin concentration in the low protein group was 11.5g/100ml as compared with that of 15.8g/100ml in the controls. The anaemia was cured by increasing the amount of protein eaten while the caloric content remained unchanged, showing that the anaemia was the result of a protein rather than a caloric deficiency. The anaemia was characterised by a subnormal haemoglobin content of the blood and a normal erythrocyte count. Metcoff et al (1937) found in acute protein deficiency in the rat there was an associated haemoconcentration and a significant decrease in total circulating haemoglobin, while in

chronic protein deficiency the total circulating haemoglobin was still slightly decreased but the haemoglobin concentration remained unaltered.

There appears to be some divergence of opinion concerning the relative priority of haemoglobin over other body proteins, when the supply of dietary protein is inadequate.

Pearson (1937) compared the respective merits of various proteins e.g. liver, casein, albumin etc., on curing the anaemia of young rats fed a milk diet. He concluded that the formation and maintenance of normal haemoglobin values was more vital than growth and that the animal would utilise its body protein for this purpose provided adequate amounts of copper and iron were given. A criticism of this work was that no measurements of total haemoglobin were made, only that of the blood samples. Buechler et al (1949) fed two groups of anaemic protein depleted rats a diet containing 9 and 18% casein respectively. These diets were fed ad libitum to some animals, while others were given only 60% of the quantity of those allowed to feed freely. In the animals fed the restricted diets the haemoglobin rose but the increase in body weight was considerably less

than that of animals allowed free access. The criticism again of this work was that no account was taken of changes in total haemoglobin; and it was not certain that the increase in total haemoglobin was greater than the general increase in body protein in this experiment of caloric restriction.

Whipple and his colleagues from their well known work concerning the ability of dogs to recover from anaemia caused by repeated haemorrhage in association with a low protein diet, concluded that haemoglobin formation takes precedence over the formation of other body proteins, including plasma proteins (Whipple 1942).

Hallgren (1953) confirms that under the condition of haemorrhage in addition to that of low protein intake, haemoglobin had priority. He states however that under the conditions of a low protein diet alone, this was not the case. Hallgren worked with rats fed a diet deficient only in protein of which egg albumin was the source, and found that the total fall in haemoglobin was greater than that of the carcass protein of such rats.

Varying results have been obtained concerning the role of specific amino acids in the synthesis of haemoglobin.

Whipple et al (1940) showed that certain individual

amino acids given to dogs made anaemic by repeated haemorrhages caused an increase in new haemoglobin production over the basal level. Threonine, glycine, glutamic acid, aspartic acid, cystine, histidine, phenylalanine and proline produced an increase in haemoglobin output of 23-24 grams above control levels for a two week period. Leucine, methionine, lysine and tryptophan caused an average increase of 20 grams; and alanine, tyrosine, valine, isoleucine, arginine, and hydroxyproline an increase of 10-17 grams. No correlation was found between the quantity of amino acid in globin and its regenerative capacity.

Sebrell (1949) working with young rats fed a protein free diet for ten days before being subject to a severe haemorrhage, investigated the effects of various amino acid mixtures on the blood regeneration. He found apparent variations in the importance of the amino acids on blood formation, where valine and histidine were shown to be especially concerned. A diet deficient in any one of the ten essential amino acids except arginine, caused some interference with the haemoglobin regeneration of these rats.

On the other hand, Orten et al (1945) who studied the effect of administering various amino acids on the

haemoglobin content of rats maintained on low protein diets consisting of 3.5% lactalbumin, found no consistent sustained increase in haemoglobin values following the individual supplementation with any one of the ten essential amino acids. He concluded that a combination of amino acids in as yet undetermined proportions was essential for the in vivo fabrication of haemoglobin. These results were confirmed by Benditt et al (1947) who on administering amino acid mixtures to protein deficient rats produced an equal haemoglobin regeneration as that produced by a 9% casein diet. If any one of the amino acids however was missing from the mixture, there was no significant increase in haemoglobin.

These divergent results support the view of Hallgren concerning the different conditions of haemoglobin priority and synthesis in animals subject to haemorrhage and a low protein diet, as compared with that of a low protein diet alone.

With reference to the effect of lysine supplementation on the anaemia of rats fed low protein diets, Hogan (1941) found that by administering 2-4 times the normal requirement of lysine the anaemia of rats fed a deaminized casein diet was prevented. These authors speculate as to whether this very large amount of lysine was required to

remedy the lysine deficiency of casein, or whether as seems more likely it was required to detoxify the anaemia-producing factor isolated from deaminized casein. (Smith et al 1936).

Evidence has been presented that lysine deficiency in rats results in anaemia. (Harris et al 1943. Gillespie et al 1945). These workers maintained rats on a diet in which the protein was supplied in the form of zein. One group of animals was given a daily supplement of tryptophan and lysine, a second group received only tryptophan. In the group receiving lysine the red cell count and the haemoglobin content of a sample of blood was considerably higher than that of the group without the lysine supplement. In a third group of animals it was demonstrated by feeding an adequate diet in restricted amounts, that the anaemia was not due to inanition. A criticism of this work was that no measurements were made of total haemoglobin content.

It has been shown that a low protein diet causes anaemia. Some controversy exists as to the priority of haemoglobin over other body proteins under conditions of low protein intake, and further work is required to elucidate the role of individual amino acids in curing the anaemia of low protein rats. The importance of measuring total haemoglobin rather than just the haemoglobin content of blood samples has been stressed.

Endocrinal Aspects.

Some of the effects of a low protein diet already mentioned, that is on the growth of the body, on the growth of the blood and on reproduction, may be the direct result of an insufficiency of protein, or they may be the indirect consequence of endocrinal dysfunction resulting from the protein restriction. That body growth and reproduction can be profoundly influenced by the endocrine glands has been well known for many years. The influence of the endocrine glands on haemopoesis has attracted less attention, but nevertheless this has also been established. (Gordon 1957, Crafts et al 1957).

It is only fairly recently that experimental work has suggested a general endocrinal disturbance resulting from a low protein diet, the exact nature of which disturbance is still obscure, and which has attracted very little comment in works concerned with protein malnutrition.

One approach to the problem is by considering the size and structure of the various endocrine glands, following a restriction of dietary protein. Different factors such as the age of the subject, the duration and severity of the deficiency as well as the amino acid pattern of the diet may influence the results. For

example, when rats were held at constant weight by underfeeding from birth there was an increase in the testis weight to a maximum of 374%, if the diet restriction was begun at the age of three weeks there was a less marked increase of 34%, while if the experiment was started at the age of ten months there was little change in testis weight (Stewart 1918, 1919). In relatively short acute experiments such as complete starvation for 7-13 days there was an increase in adrenal weight; conversely with chronic underfeeding the loss in adrenal weight was usually relatively greater than that of the body. (Mulinos and Pomerantz 1941a). With reference to the amino acid pattern of the deficiency, Samuels (1952) force feeding isocaloric diets made up of synthetic amino acid mixtures, found that a tryptophan deficiency did not affect the adrenal weight, while an isoleucine deficiency resulted in an adrenal enlargement.

It can be surmised from these divergent effects that while a study of endocrine size under conditions of low protein may provide a clue to the functional state of the gland, in itself, it is not a sufficient criterion of endocrinal activity. A more detailed account of previous work on the effects of a low protein diet upon the size of the endocrine glands is dealt with in the appropriate experimental section (p.116).

Mulinos and Pomerantz (1939, 1940, 1942) concluded that chronic inanition leads to a condition of "pseudohypophysectomy", as such animals showed marked atrophic changes in the genital tracts of both sexes, in the thyroid, adrenal cortex, thymus and pituitary. It is likely that a restriction of the food intake (by 50%) would cause a deficiency of factors other than calories as implied by these authors.

Samuels (1952) found that whenever the diet was inadequate for the maintenance of nitrogen balance, irrespective of whether the caloric content was high or low, there was an atrophy of the secondary sex organs. These glands responded to small doses of gonadotrophins. Adamstone (1950) still obtained degenerative changes in the seminiferous tubules when a tryptophan deficient diet (acid hydrolized casein) was force fed to rats aged 6 weeks, and Maun (1945a, b, 1946) also found that the testicular atrophy was due at least in part to factors other than inanition, since it was more severe in diets deficient in phenylalanine, leucine or histidine than in the paired fed controls. The cessation of oestrous cycles on low protein diets mentioned earlier was also shown to be the result of protein rather than caloric restriction (Guilbert and Goss 1932). Rivero-Fontan et al (1952) divided adult rats into three groups: a control group was fed an adequate

diet ad lib, a starvation group fed the control diet was restricted to 1/4 of the food intake of the former group, and a calorie restricted group was also given 1/4 of the food intake of the controls. The diet used in this latter case however, was isocaloric with and contained 4 times the protein content of the control diet. The experiment was terminated after 15 days. The level of protein in the hypocaloric diets did not influence the weight loss of the pituitary, or thyroid, but that of the uterus and seminal vesicles was significantly greater on the low protein diet. The atrophy of the ovary and testicles also tended to be greater on this diet. The weight of the adrenals remained the same in all three groups.

The above survey shows that the effect of a low protein diet upon the sex endocrine glands was due primarily to a protein rather than a caloric restriction. This has also been shown to be the case with the adrenal (Samuels 1952) where diets adequate in calories but insufficient for the maintenance of nitrogen balance caused adrenal changes similar to those after hypophysectomy- total decrease in weight and high cholesterol content. There was also a reduction in thyroid weight (Samuels 1952) when diets containing adequate calories but insufficient

tryptophan or phenylalanine were force fed to rats. The average rate of radio active iodine uptake was slightly less in the phenylalanine deficient animals than in the controls, but this difference was not significant. No mention was made of iodine uptake rates on the tryptophan deficient diets (Samuels 1952). Animals on diets insufficient to maintain nitrogen balance have partial thyroid atrophy, variable loss in thyroid weight and a reduced basal metabolic rate. (Jackson 1925).

The effect of a low protein diet upon the gonads would appear to be largely the result of a reduction in the gonadotrophin output from the anterior pituitary, since the atrophic sex glands of rats on nitrogen deficient (Samuels 1952) or nitrogen free diets (Yamamoto et al 1950) responded to small doses of gonadotrophin; though there was some direct effect also, as the hypertrophy of the glands was proportional to the nutritive value of the protein. (Yamamoto 1950). The implantation of a normal male hypophysis reactivated the ovaries of lysine deficient rats (Courrier 1932).

The effect of a low protein diet on the adrenals under basal conditions also appears to be the result of a reduction in the adrenal cortical stimulating hormone

(A.C.T.H.) released from the hypophysis. Changes in adrenal weight and cholesterol content of the same type as found following hypophysectomy have been noted in rats on diets low in protein content (Gershberg 1952, Sayers 1949, Samuels 1952). The administration of A.C.T.H. to such rats (Gershberg 1952) caused an increase in adrenal and a decrease in thymus weights indicating that the adrenals were not refractory. Rats fed diets of low protein (8% casein) for 10 weeks, unlike their controls, were unable to maintain their blood glucose levels during a subsequent fast. This fasting hypoglycaemia was abolished by the administration of A.C.T.H. (Handler 1951). The percentage fall in the circulating eosinophil count 4 hours after adrenalin administration was decreased in the animals on the low protein diet. After administration of A.C.T.H. however the reduction in eosinophil counts was equally marked in rats on both high and low protein diets (Handler et al 1950). In this latter paper Handler found that the ingestion of the low protein diets resulted in the disappearance of the hypertension of partially nephrectomised rats. The administration of small doses of A.C.T.H., essentially without effect on the blood pressure of the unoperated or operated controls, raised the blood pressure of the operated rats on the low protein diet to levels comparable

with the operated animals on the high protein diet.

Samuels (1952) believes that under normal conditions the amount of A.C.T.H. released by rats on low protein diets is reduced, but that the pituitary contains adequate amounts of trophic hormone which can be released under the stimulation of severe stress. He confirms the earlier results of Sayers (1949) where the adrenal response to severe stress (scalding) was the same - increase in weight, reduction in ascorbic acid and cholesterol contents - for the rats fed low protein or control diets. Samuels found that the restoration of the original cholesterol subsequent to stress was delayed however in the low protein animals. Sayers states that the malnourished rats had approximately the same store of A.C.T.H. as the normal animals, and after scalding there was a reduction of 50% in both groups. These results are not in conflict with Samuels' view, since the amount stored under resting conditions is not necessarily a guide to the amount released.

Selye and co-workers have found that the corticotrophic action of A.C.T.H. was enhanced by diets abnormally high in protein, but this action was no longer present in hypophysectomised animals (Henriques et al 1949). Under basal conditions there was no difference in the adrenals

of rats fed 15% or 30% casein protein diets, but the response to stress e.g. cold, unilateral adrenalectomy, muscular exercise, etc. was greater on the higher level of protein. Ascorbic acid and cholesterol contents and adrenal size were the criteria used. (Moya et al 1948, Constantinides et al 1950). This difference was later confirmed with diets of 16% and 22% casein (Dumm et al 1955). Selye and others found that rats chronically treated with anterior pituitary extract if fed a very high protein diet (30% casein), developed high blood pressure, kidney lesions and enlarged adrenals. These effects were not apparent, however, on a 15% casein diet (Hay et al 1948, Dontigny et al 1948). This work of Selye provides further evidence for the influence of dietary protein on adrenal function.

A characteristic feature of adrenal insufficiency, used diagnostically for Addison's Disease, is the reduction in the rate of excretion of ingested water (Chester Jones 1957). This delayed excretion is found also in low protein rats (Leslie et al 1947, Heller 1947). Hegsted (1950) found that the effect of a low protein diet in causing an increase in the rat's plasma volume was enhanced by exposure to low temperatures. Since there was no correlation with the level of plasma protein, the possibility

of adrenal influences was suggested. Sinclair (1953) also supports the involvement of the adrenal cortex in nutritional oedema.

Another experimental approach used is to counteract the effects of a low protein diet by the administration of hormones.

The reactivation of the sex organs following the administration of gonadotrophins has already been mentioned, and this may take place at the expense of body weight (Yamamoto 1950). The accessory reproductive organs of the malnourished male have also been stimulated by testis hormone (Moore et al 1931). The work of Nelson and Evans (1954) is of special interest in this connection. These authors by the administration of oestrone and progesterone were able to maintain the pregnancy of rats fed a protein free diet. Using the dose of 2 μ oestrone or 4 mg of progesterone daily during pregnancy the number of females with living foetuses was increased from about 10% to 80%, but the average number of live foetuses per rat was only 6 in comparison with the normal of 8-9. The combined administration of oestrone and progesterone maintained live foetuses in 100% of the animals and the number of living young per rat was nearly normal. Food intakes were measured and shown not to contribute to the results

since the injected animals actually ate less than their controls.

The work of Handler concerning the role of A.C.T.H. in combating the effect of a low protein diet on fasting hypoglycaemia and lack of blood pressure response to nephrectomy has already been mentioned. Aschkenasy (1954) was able to prevent the lethal effect of protein inanition on the survival of the adrenalectomised rat by the administration of cortisone.

Using adult female rats (7-10 months old) fed isocaloric diets of varying protein contents ranging upwards from 6% casein, Gordon et al (1948) found that the amount of nitrogen retained and the increment of weight gained under the influence of a 5 day injection period with growth hormone, varied concordantly with the dietary protein, being optimal on a 24% casein diet. Geiger (1953) working with adult male rats put 15 days on a protein free diet and then 15 days on a 24% casein diet, found that physiological doses of growth hormone had no effect on body weight during protein re- or de-pletion, but that once predepletion weight was reached, the growth increment was greater for the animals receiving growth hormone than for the controls.

Aschkenasy et al (1953) deprived rats of protein for 60 days and then allowed a slow recovery by feeding 7% casein and investigated the effects of Hormones on the repair of the resultant anaemia. Thyroxine stimulated the production of all types of blood corpuscles; testosterone stimulated the production of erythrocytes, neutrophils and lymphocytes but retarded the production of eosinophils and caused numerous deaths; A.C.T.H. stimulated the production of erythrocytes only; oestrogen inhibited the production of erythrocytes and stimulated the production of white blood corpuscles other than neutrophils; growth hormone in small doses was erythropoetic and in large doses inhibited erythropoesis.

When rats previously subject to protein deficiency were repleted the simultaneous injections of adrenal and thyroid hormones speeded up the restoration of normal urinary levels of amino acids, urea and total nitrogen, as well as the liver content of certain proteolytic enzymes. (Protasova 1953). No mention was made of which adrenal hormones were used.

The preceding survey would indicate that there is some interference of normal endocrinal function on a low protein diet, but much more work is required however to put this concept onto a firm experimental basis and to

elucidate the exact nature of the disturbances. For example Nelson and Evans concluded from their experiments that the harmful effect of protein deficiency on reproduction was due to a failure in hormone production. However it could be that protein deficiency increased either the demand for the hormones or their rates of destruction. In fact some authorities believe that the amount of circulating oestrogen may be actually increased in protein malnutrition owing to an impairment of the liver's destructive mechanism (Biskind 1946).

The role of the adrenal cortex in protein malnutrition is also rather obscure. Some authors believe that the activity of the adrenals is increased on low protein diets; the adrenal hypertrophy observed by McCarrison resulting from protein deficiency (1918-1935) is thought by Sinclair (1953) to be accompanied by an over secretion of the mineralo-corticoids. Sayers (1949) reports that neither the liberation of A.C.T.H. nor its formation is altered from normal in the protein deficient rat, and concludes that the functional capacity of the adrenal cortical system is only slightly affected in contrast to the more marked effect on the pituitary gonadal axis. Samuels (1952) believes that the amount of A.C.T.H. liberated is reduced under conditions of

low protein intake but that under stress it is capable of being released in normal amounts. Contrary to this are the results of Handler concerning the stresses of starvation and partial nephrectomy which show different effects on low and adequate dietary protein levels, and the work of Selye concerning the increased response to A.C.T.H. on very high protein diets.

Summary.

Protein malnutrition is a present day human nutritional problem which has existed in many areas of the world for generations. For this reason a study of the effects of protein shortage and how they may be counteracted is of interest, more especially when the deficiency has been present for long periods. Practically no work has been undertaken on the growth and reproduction of the rat fed low protein diets for several generations. When the protein supply is restricted the body and the blood compete for the available protein and there is controversy as to which is the more successful in this competition. There is also controversy as to the role of specific amino acids in counteracting the anaemia of protein deficiency. Recent work has suggested that growth and reproduction are affected by protein deficiency by way of an altered endocrinal balance, though further work is required to elucidate the nature of this altered balance.

PART II

EXPERIMENTAL.

EXPERIMENTAL.

Introduction.

The starting point in this work was the experiments of Balfour (1952), who found that it was impossible to breed from rats fed a diet of similar composition to that eaten by the Africans in Gambia. In view of the lack of work previously done on the growth and reproduction of rats through successive generations as compared with short term experiments, it was decided to supplement a Gambia-Type Diet with enough protein to enable the rats to breed.

The first section deals with the animals and diets used.

The second section outlines the experiments undertaken in order to study the reproduction of rats through several generations on low protein diets, where body weight is taken as the criterion for growth.

In the third section work involving the measurements of red blood cell concentrations as a further aspect of the growth of the low protein animals is reported.

In the final section the growth of some of the endocrine glands of the low protein rats is considered as a clue to their functional state.

ANIMALS AND DIETS.

Animals.

The animals used throughout this work were those obtained from the Unit's Colony of Glaxo Albino Rats inbred by brother-sister mating, and they were housed on wire screen cages.

Diets.

Three main diets were used in this work, namely: stock diet, basal diet and basal diet supplemented with various levels of fishmeal. All diets were fed ad libitum.

The stock diet, which was supplemented with liver (5g. per rat per week), was diet 41 supplied by Glaxo. (Bruce 1950).

The basal diet was one where the food-stuffs corresponded in amounts with those of a typical diet of a rural area in the Gambia (Applied Nutrition Unit 1945) but were in fact of European origin. (Table 1 (a) and (b).

Table 1 (a) Composition of Basal Diet.

Food Constituent.	% Composition.	% Protein Content *
Wheat	80	10.6
Potato starch	10	0.0
Haricot bean	5	1.3
Lucerne meal	2	0.5
Arachis oil	2	0.0
Salt mix	1	0.0
Total Protein	as grams per cent.	12.4
	as grams per 100 cals.	3.3

*Values given are derived from the literature.

Table 1 (b) Composition of Gambian Diet

Food Constituent.	% Composition.	% Protein Content.
Sorghum flour	91	11.5
Groundnuts	6	1.5
Dried leaves	2	0.5
Salt	1	0.0
Total Protein	as grams per cent. as grams per 100 cals.	13.5 2.8

The various fishmeal levels were achieved by substituting it for a corresponding amount of potato starch in the basal diet. Table 2 summarises the dietary data.

Table 2. Percentage Composition of Diets.

Constituent	Basal Diet	2% Fishml. Basal Diet	5% Fishml Basal Diet	10%Fishml Basal Diet	Stock Diet
White fishmeal	0	2	5	10	8
Potato starch	10	8	5	0	0
Dried skimmed milk	0	0	0	0	3
Dried yeast	0	0	0	0	1
Liver supplement	0	0	0	0	5g/rat/ week
Haricot bean powder	5	5	5	5	0
Whole meal flour	80	80	80	80	46
Sussex ground oats	0	0	0	0	40
Lucerne meal	2	2	2	2	0
Ground nut oil	2	2	2	2	0
Cod liver oil	0	0	0	0	1
Salt mix (Glaxo)	1	1	1	1	1

Vitamins A, D, and B complex were added to all four

experimental diets in the following amounts:

Vitamin Additions per 100 grams of Diet.

	I.U.
Vitamin A (in Arachis Oil)	700
Vitamin D (in Arachis Oil)	360
	m.g.
Vitamin B. complex (Copping et al 1951)	
Choline	30.0
Inositol	10.0
P.A.B.A.	10.0
Nicotinic acid	10.0
Ca-d-pantothenate	0.5
Riboflavin	0.4
Pyridoxin	0.1
Thiamine	0.1
Folic acid	0.02
Biotin	0.02

The composition of the salt mix supplied by Glaxo was as follows:

Salt Mix (de Loueiro 1931)

	gm.
Sodium chloride	22
Calcium phosphate	130
Potassium citrate	125
Magnesium sulphate	30
Iron citrate	5
Trace element mixture*	0.70

* Trace Element Mixture

Potassium iodide	12
Potassium fluoride	10
Manganese sulphate	2
Cuprous iodide	1
Potash alum	1
Zinc sulphate	1

The wheat and beans were ground in the laboratory. The experimental diets were mixed as required, and 50% of water was added to form a dough, which was rolled out, cut into biscuits then cooked in an oven for 24 hrs. at 50°C.

The values for the protein content of the diets (obtained from the literature) were as follows:

Table 3. Protein Content of the Diets (on dry weight basis)

Protein Content (Nx6.5)	Basal Diet	2% Fishmeal Diet	5% Fishmeal Diet	10% Fishmeal Diet	Diet 41 + Liver
as grams %	12.4	13.8	16.0	19.5	19.9
as g.per 100 cals.	3.7	3.7	4.4	5.4	4.9

Fairly good agreement with the above values was obtained from chemical analysis of the nitrogen content of the basal diet, which was estimated at the beginning and end of the experiments, and was found to be 12.2%. The protein content of the fishmeal was 63.6% which would give a protein content of 18% instead of 19% in the 10 per cent. fishmeal diet. (Table 3). The method used for the nitrogen determinations was the Markham Process (Kabat and Mayer 1948). The water content of the diet varied from 6-8% depending on the length of time from baking.

Fig.1 shows how the amino acid content of the diets

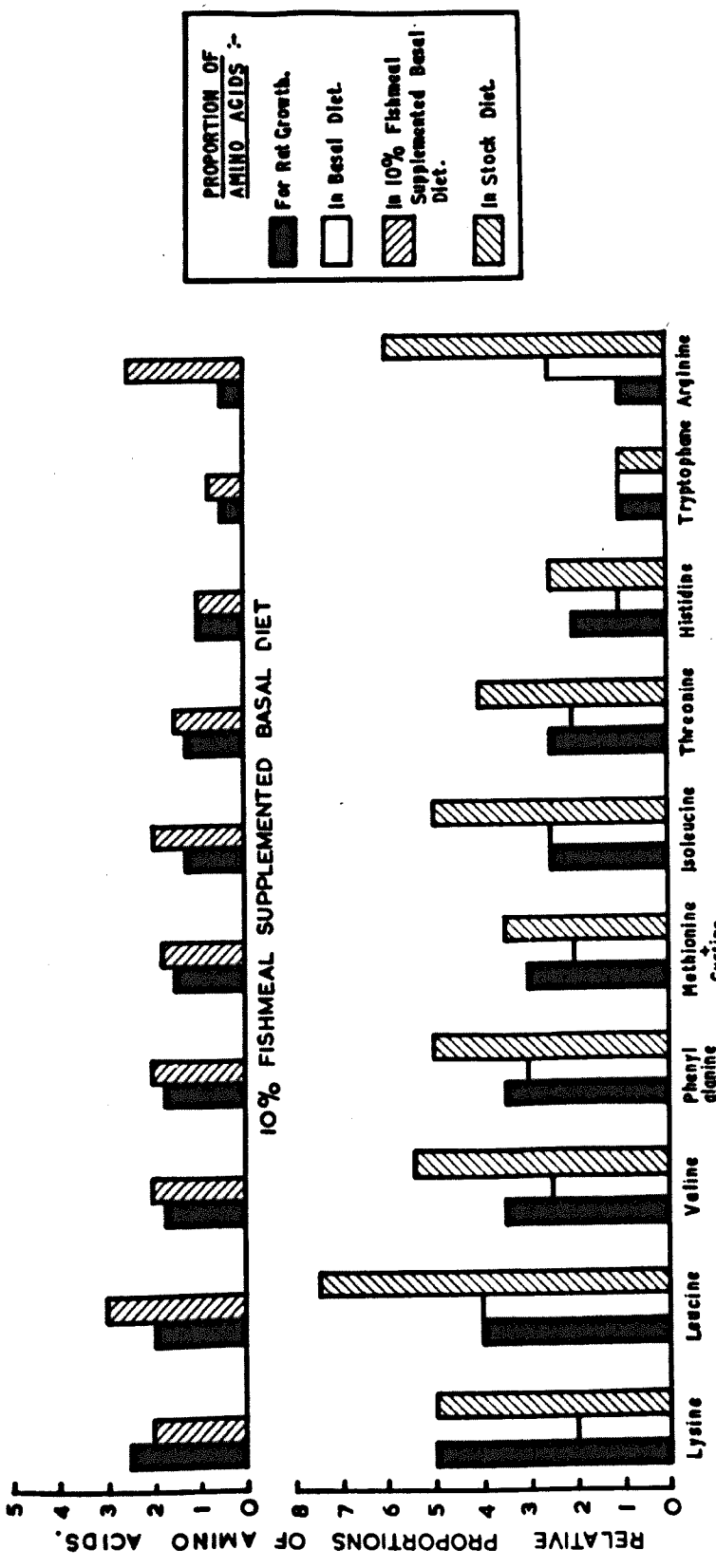


Fig. 1. Amino Acid Proportions In Experimental Diets Compared With Those Utilised For Rat Growth.

(Block and Bolling 1951) compared with the proportions required for rat growth (Rose 1937). Since the tryptophan content of the basal and stock diets was the same as Rose's value, this was taken as unity, and the relative proportions of the other amino acids determined accordingly. On the same basis histidine was taken as unity for comparing the fishmeal diet. It can be seen from these approximate values that the basal diet was deficient in the majority of essential amino acids, lysine being limiting. The 10 per cent fishmeal diet was also slightly deficient only in lysine. The experiment described on page 98 confirms the lysine deficiency of the basal diet, but that of the fishmeal diet was found to be deficient for male growth only.

As far as could be ascertained from the known dietary requirements of the rat (Sturtevant 1949 , Russel 1948), the experimental diets were deficient only in protein. Vitamins A, D and B complex were added to the diets as was the mineral salt mix containing the trace elements; arachis oil is a good source of unsaturated fatty acids (Hilditch 1940) and whole wheat and lucerne meal are rich in vitamin E (Harris et al 1950). The specific effects of certain vitamin deficiencies are usually recognisable (Platt 1945) and none of these were observed.

The possibility of the diets being deficient in

vitamin B12 and animal protein factors is discussed on page 136. The depression in growth observed on the basal diet was in no way altered by the inclusion of vitamin B12. (page 55).

The results obtained on the low protein diet are complicated to some extent by those introduced by the resulting reduction in food intake. The factors concerned in the regulation of the appetite have been extensively reviewed (Lepkovsky 1948, Mayer 1955) and it has been shown that the presence of nine essential amino acids is required for the maintenance of the rat's food intake (Frazier et al 1947). The diminished food intake is therefore indicative of a deficiency in the diet so it was considered immaterial for the purpose of this work - the effect of a low protein diet - whether the effect was wholly direct or partially caused by the reduced appetite which is in itself the result of the protein deficiency. In any case, if the growth of the rat is severely limited as when the protein intake is reduced, the requirements of other nutrients which are dependent on the animal's size and rate of growth, will be correspondingly reduced.

The experimental diets were cooked primarily to render the starch more digestible (Pozerski 1933) and to simulate Gambian conditions. It is probable that the

cooking process reduced the functional value of the protein (Duckworth 1952, Patton 1950) mainly by reducing the lysine content, because this amino acid acid is especially susceptible to heat.

GROWTH AND REPRODUCTION OF SUCCESSIVE GENERATIONS OF RATS FED LOW PROTEIN DIETS.

(a) PRELIMINARY EXPERIMENT.

The following two experiments were undertaken in order to confirm that the basal diet was similar in its general effect to that used by Balfour (1952), and that the use of fishmeal as a protein source would counteract these effects. In the first experiment reproductive performance was studied. The rats used were adult stock animals which were put on to the experimental diets, in contrast to the second experiment where the diets were introduced to weaning stock rats.

Exp. 1: A COMPARISON OF STOCK DIET AND BASAL DIET A, WITH AND WITHOUT 10% FISHMEAL, FOR RAT REPRODUCTION-ADULT RATS.

Stock rats aged approximately 2½ months were fed one of the following three diets: basal diet A, a 10% fishmeal supplemented basal diet A, or, as a control, the stock diet. Each group was composed of four female and one male rat housed together in a cage; as the rats became pregnant they were isolated and given wood wool to make their nests.

The experimental diets in these preliminary experiments differed from those described earlier (page 36), in that B vitamins were not added, potato flour was used in place of potato starch and the fishmeal did not replace an equivalent weight of potato starch, but was added directly to the basal diet.

The reproductive results are summarised in the following Tables 4 and 5. The "birth pup weights" were measured within 12 hours after birth.

Table 4 .Reproductive Performance - Pups Produced.

Diet	No. of Rats	No. of Litters	No. of Pups			Litter at Birth	
			Born	Weaned	% Weaned	Mean No. of Pups	Mean Pup Weight (g)
Basal Diet A	4	4	24	4	17	6.6*±1.8	5.1±0.2
10%Fishml + Basal Diet A	4	4	21	9	43	5.3±1.6	5.3 ± 0.5
Stock Diet	4	4	27	19	71	6.8±2.1	4.8 ± 0.9

Table 5. Reproductive Performance - Dams.

Diet	No. of Rats	Weight of Dam		Time taken to Produce 1st Litter (from introduction of male) dys.
		Initial g.	Increase(To postpartum wt.) g.	
Basal Diet A	4	139.3* ± 3.3	33.5 ± 6.4	42 ± 4
10%Fishml. + Basal Diet A	4	134.5 ± 4.4	51.3 ± 3.7	43 ± 6
Stock Diet	4	138.0 ± 9.1	56.8 ± 3.8	38 ± 5

* Values given are the mean together with the standard deviation of the mean.

The number and weight of the pups born were unaffected by the diets, yet the weight gain during pregnancy

of the mothers was probably significantly greater on the stock diet than on the basal diet ($p = 0.03$), there being no significant difference between the fishmeal and stock diets. This supports the general concept (Jackson 1925, Burke 1943) that within certain limits the adult female is drained of protein in order to preserve the foetus.

On applying the Chi-Square Test to the weaning success on the various diets, the results using stock diet were significantly better than that on the basal diet ($P = 0.01$), there being no significant difference between the fishmeal and stock diets. The litter from the basal diet mother survived only a week after weaning, the pups becoming very feeble with alopecia developing behind the ears and on the back of the neck and shoulders. The pups weaned on the other two diets survived normally until killed at 7 weeks.

Fig. 2 illustrates the growth of the three male rats. The weight gain from the age of 15-20 weeks was practically the same for the fishmeal and stock diet, 58 and 56 grams respectively, that on the basal diet being much less, 36 grams. The set-back to the weight gain on the experimental diets found over the first week or so, is presumed to be the result of the drop in appetite following the introduction of an unaccustomed diet.

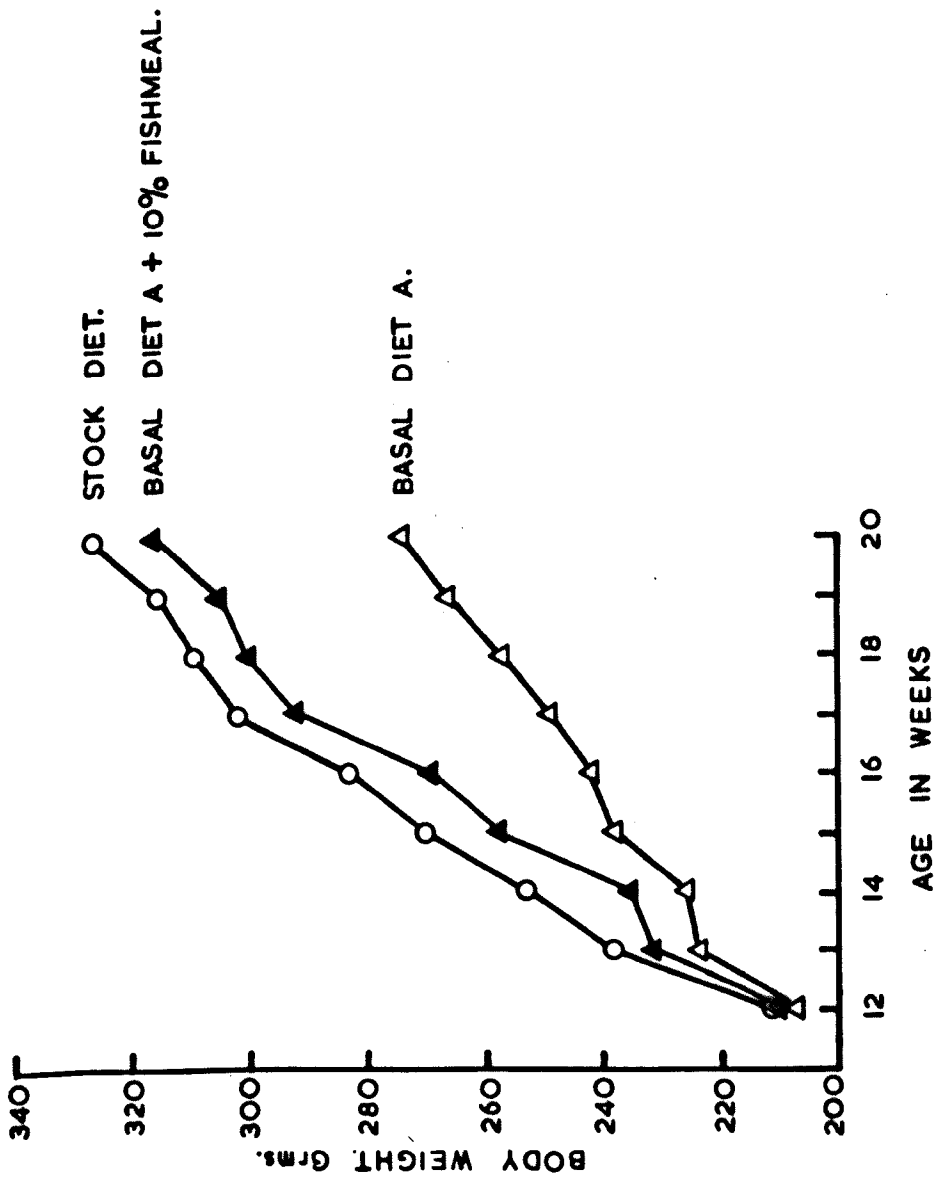


Fig.2. Growth Of Three Litter Mate Male Stock Rats, Fed From The Age Of 12 Weeks; Basal Diet A, Basal Diet A + 10% Fishmeal Or Stock Diet.

Exp. 2. A Comparison of Stock Diet and Basal Diet A with/without 10% Fishmeal for Rat Reproduction - Weanling Rats.

Three groups each of six female weanling stock rats were fed the same diets as in the previous experiment, and as before each group at the age of about 2½ months was mated with one of three stock litter mate males. Prior to mating the food intakes were measured collectively for each cage of three rats.

During the first two weeks of the experiment the diets were fed as a cooked porridge in order to imitate African conditions, but this had to be abandoned because of souring, and the diets were then fed in the usual biscuit form.

Some of the dams were killed immediately on their being found with no live litters and their mammary glands removed and fixed in formalin for subsequent staining with Haematoxylin and Oil-Red-O for the presence of fat.

Fig. 3 and Table 6 summarise the results on growth during the initial period. The results for the first few weeks were neglected because of the drop in appetite due to diet souring.

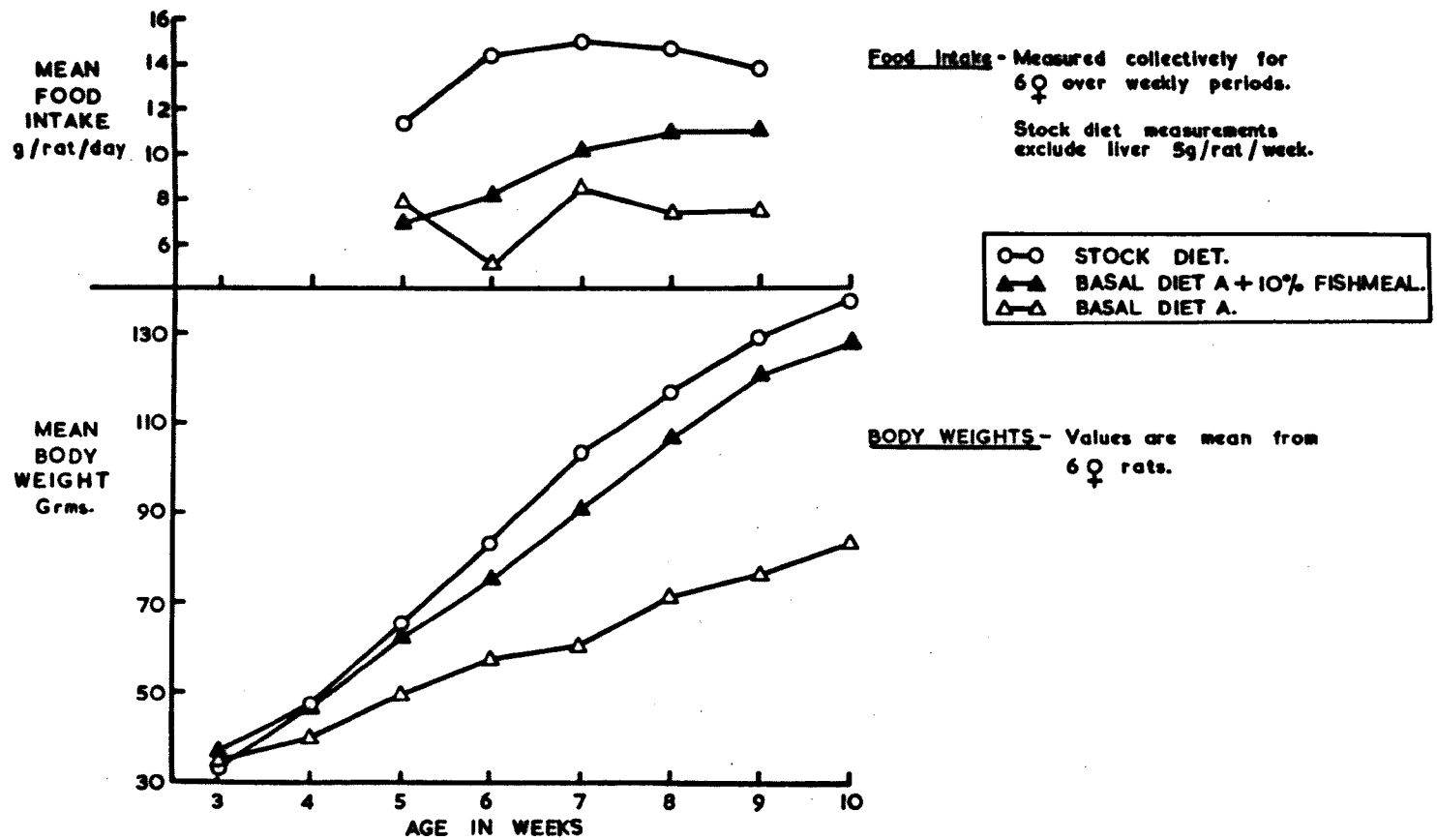


Fig. 3. Growth And Food Intake Of Female Rats Fed From Weaning, Basal A, Basal A + 10 % Fishmeal Or Stock Diets.

Table 6. Increase in Rat Weight from the Age of 6-10 Weeks.

Diet	No. of Rats	Mean Increase in Body Weight from Age 6-10 Weeks g.
Basal Diet A	6	25.2 ± 1.0 *
10% Fishmeal + Basal Diet A	6	53.7 ± 1.9
Stock Diet	6	54.0 ± 1.7
	Fisher's P for groups compared	
Basal and Fishmeal Diets	< 0.001	
Fishmeal and Stock Diets	> 0.9	

* Values given are the mean together with its standard deviation.

The weight gained on the fishmeal diet was significantly greater than that on the basal diet ($p < 0.001$), there being no significance between the fishmeal and stock diets ($p > 0.9$). The values on food utilisation expressed as grams of food per gram increase in body weight over the ages of 6-9 weeks were 11, 6 and 9 on basal, fishmeal and stock diets (excluding liver), respectively. The poorer utilisation of the basal diet indicates that the depression in growth was not just the result of the lower food intake. The main cause of the poor utilisation of the basal diet was probably its amino acid imbalance, since the essential amino acids can only be utilised efficiently when supplied

simultaneously in the correct proportions (Geiger 1950). Similar effects have been observed by other workers (Ellis and Hankins 1935), and the results of Mitchell (1935) suggest that on higher protein levels the more balancedration results in less wastage of energy as heat loss. The impaired production of digestive enzymes on low protein diets (Knox et al 1956) may also be a conditioning factor. Whether any significance can be attached to the smaller difference in food utilisation between the fishmeal and stock diets is doubtful. Strictly speaking these two values are not comparable as the diets were fed in different forms. In the case of the pelleted stock diet the error due to food loss on crumbling was greater than with the biscuit diet, which would tend to raise the value obtained for the former diet.

The results on reproduction, Tables 7, 8 and 9, show that the basal diet was unsuitable for rearing rats as none of the litters could be weaned, as compared with a 70% weaning success on the fishmeal and stock diets. The Chi-Square Test showed that the difference between the fishmeal and basal diet was significant ($p = 0.01$).

The litter size ($p = 0.01$), birth pup weight (p 0.001), and post partum dam weights ($p = 0.02$), were all significantly reduced on the basal diet, where the females also took

significantly longer to become pregnant ($p < 0.001$), as compared with the animals on the fishmeal diet. Again there was no significant difference between the results obtained on the fishmeal and stock diets.

Table 7. Weaning Success on the Various Diets.

Diet	No. of Litters	No. of Pups Born	No. of Pups Weaned	% No. of Pups Weaned
Basal Diet	6	10	0	0
10% Fishmeal + Basal Diet	6	48	34	71
Stock Diet	6	44	31	71

Table 8. Weight and Size of the Litters.

Diet	No.of Litters	Litter at Birth		Litter at Weaning	
		No. of Live Pups per Litter	Mean Pup Weight g.	No. of Live Pups per Litter	Mean Pup Weight g.
Basal Diet	6	1.7 ± 0.6*	3.6±0.1	0	-
10%Fishml. + Basal Diet	6	8.0 ±0.7	4.9±0.9	5.7 ± 0.9	27.7 ± 6.7
Stock Diet	6	7.3 ± 0.8	4.8±0.2	5.2 ± 1.7	30.7 ± 0.7

* Values are the mean together with its standard deviation.

Table 9. Weight and Fertility of the Dams.

Diet	No. of Rats	Time Taken to Produce Litter (from introduction of male) (days)	Dam Weight	
			Post partum (g.)	Weaning Gain (g.)
Basal Diet	6	82 ± 8 *	134.8 ± 8.4	-
10%Fishml. + Basal Diet	6	36 ± 4	159.8 ± 5.1	14.2 ± 4.5
Stock Diet	6	31 ± 2	175.5 ± 6.3	20.8 ± 4.6

* Values are the mean together with its standard deviation.

The suggestion that the failure of the rats fed basal diet to wean their litters was due to a cessation of lactation (Balfour 1952), seems unlikely as there was no sign of any diminished amount of fat staining colloid within the alveoli of the mammary glands obtained from the rats which failed to rear their young, when compared to control animals, though of course the "let-down" reflex may have been involved (Waller 1938). ~~(Newton 1950).~~ In inanition it is known that despite mammary gland atrophy secretion continues (Meynier 1906, 1908). It is thought that as with humans, the very small birth weight gave the young little chance of surviving (Nichols 1945).

Reduction of the litter size on the basal diet is

thought to be primarily due to the increased number of foetal reabsorptions known to occur when the protein in the diet is reduced (Guilbert and Goss 1932).

Any direct effect of the low protein diet on reducing the birth weights would be enhanced indirectly by the lowered weight of the low protein mothers, whose size is known to influence the birth weights (King 1915, Hammond 1944).

The longer time taken by the rats fed basal diet to produce a litter may be the result of several factors known to occur on low protein diets, such as the delay in reaching sexual maturity (Slonaker 1930), failure of the low protein rats to mate though in oestrous and prolonged irregular oestrous cycles (Guilbert and Goss 1932). It is possible that the gestation period may also be prolonged as is known to be the case with inanition (Barry 1920).

On comparing the results of this with the previous experiment, the detrimental effect of the basal diet on reproduction is much more marked when the dam has been fed the diet from infancy than only in adult life. This is not surprising as in the latter case not only has the dam greater protein reserves to call upon, but the actual protein requirement of an adult animal are less than that of the younger more rapidly growing animal. Indicative of

this is the significantly lower ($p = 0.01$) post partum dam weights on this experiment as compared with the preceding one.

The results obtained from these two preliminary experiments show that the basal diet alone, like the Gambian diet, is unsuitable for rat reproduction, and that a 10% fishmeal supplemented basal diet compares favourably with stock diet.

Effect of Vitamin B12 Supplementation of the Experimental Diets.

Though lucerne meal is rich in vitamin B12 (Hartman 1949, Peeler 1949), it was recognised that the basal diet might be deficient in this vitamin, so that the results obtained previously on the addition of fishmeal may have been due to its vitamin B12 content* rather than that of its protein.

Six litters each of two male and two female weanling rats were divided into four groups, composed of three male and three female rats respectively. The groups were fed one of the following diets ad libitum: basal diet with and without vitamin B12, or 10% fishmeal diet with and without vitamin B12.

* Dr. J.E. Ford kindly determined the B12 content of the fishmeal which was 0.05µg/g.

Doses of vitamin B12 equivalent to 0.3µg per day, which is in excess of the rats requirements (Sebrell and Harris 1954), were given orally as a solution of vitamin B12 triturate (Mannitol) in water twice weekly.

The animals were housed in individual cages and their weekly weights and food intakes measured. The composition of the diets was the same as that described in the first section (page 36) but the wheat was from a different source.

Table 10. Effect of Vitamin B12 Supplementation of Experimental Diets.

Diets	No. of Rats M.	No. of Rats F.	Initial Rat Weight g.	Increase Rat Wt., age 3-11 wks. g/rat/8wks.	Total Food Intake, Age 3-11 wks. g/rat/8wks.	Food Utilisation Age 3-11 wks. g.food/g inc. body weight.
Basal Diet	3	3	32.5±1.3*	8.3±2.0	268.2±15.1	42.7±11.0
Vit.B12 + Basal Diet	3	3	32.2±1.4	7.2±2.1	254.2±10.8	30.7±9.4
10%Fish-meal + Basal Diet	3	3	32.2±1.5	111.0±10.8	572.8±32.3	5.3±0.3
Vit.B12 +10% Fishmeal Basal Diet	3	3	32.5±1.1	111.8±7.3	558.3±29.6	5.1±0.3
				Fisher's P for means compared		
Basal Diet with/without vit. B12.				0.7	0.5	0.4
10% Fishml.B.D.with/without vit. B12.				0.9	0.8	0.6

* Values given are the mean together with its standard deviation.

As seen from the results (Table 10 and Fig. 4), the addition of vitamin B12 had no effect upon the growth or food intake of the animals on either of the diets. Nor was there present any sex difference within each dietary group concerning the effect of vitamin B12 on growth. It is concluded therefore, that the vitamin B12 content of the fishmeal was not the prime cause of the response obtained on its inclusion in the basal diet.

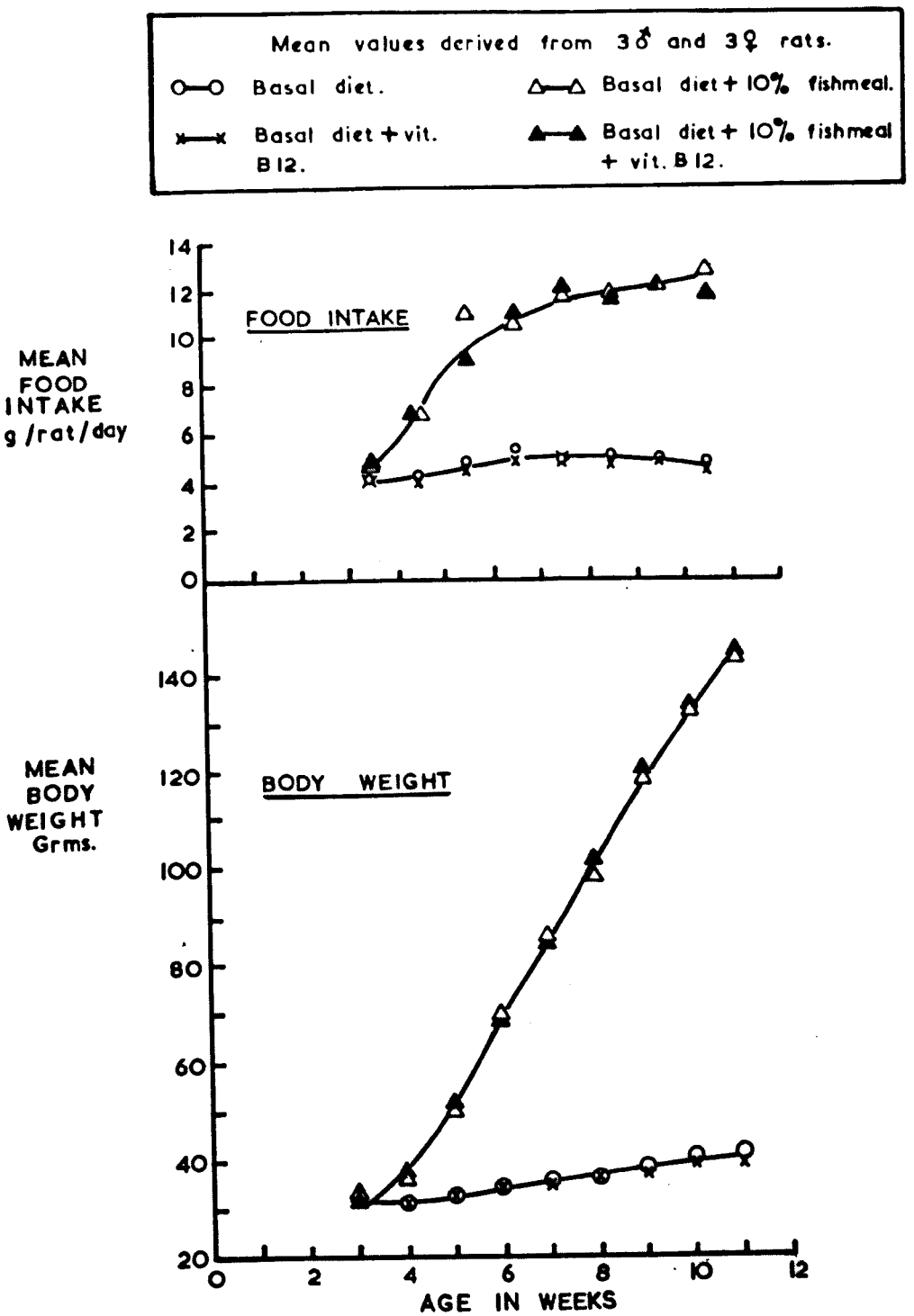


Fig.4. Results Obtained From Vitamin B12 Supplementation Of Experimental Diets.

(b) GROWTH AND REPRODUCTION OF SUCCESSIVE GENERATIONS.

The results of the preliminary experiments indicated that a suitable fishmeal level for the rearing of low protein rats lay between a 0 and a 10 per cent supplementation of the basal diet.

It is well known that the protein requirements are increased during pregnancy and lactation, and the work of Seegers (1937) and Nelson (1953), point to a critical need for protein during the earliest stages of pregnancy. It was therefore decided to try out various levels of fishmeal supplements added to the basal diet during pregnancy and lactation.

1. First Generation.

Litters of five weanling female Stock rats were divided in the ratio of 4:1 between two groups fed the basal and stock diets respectively. At the age of 20-21 weeks the rats fed basal diet were subdivided into four groups fed basal, 2, 5 or 10% fishmeal supplemented basal diets respectively, (for composition see page 36), and one of five litter-mate adult stock males was introduced into the cage containing each dietary group. The rats were mated later than is usual for breeding purposes, in view of the lighter weight of the low protein animals. As the rats became pregnant they were housed individually and given wood wool to make their nests. The supplemented diets

were continued until the female young were weaned onto basal diet for the production of the next generation, their mothers being returned to the basal diet for a fortnightly rest period before again repeating the above dietary changes for the next pregnancy. The stock animals were maintained on their diet throughout, and all rats were weaned at three weeks.

Stock male rats were also weaned onto the basal diet, and from the age of 10 weeks the males were bred with stock females, both rats being fed the basal diet. The females were occasionally removed for an hour or so and given some stock diet. When the difference in size between the two sexes became considerable, the female, whose fertility was subsequently tested using a stock male, was replaced by another stock female aged about $2\frac{1}{2}$ months.

The results on growth are summarised in Tables 11, 12 and 13, and Figs. 5 and 6. The food intakes were measured collectively for several rats in a cage.

The growth of the rats fed basal diet was considerably less than that on stock diet, the weekly increase in body weight over the initial period being about 3 g. as compared with 16 g. on the latter diet. The growth of the female rats fed basal diet was probably significantly greater than that of the male ($p = 0.04$). This greater susceptibility of the

male to low protein diets has been noted by other workers (Hartwell 1925, Jones 1951). Records kept for over a year (Fig. 6) suggest that the normal initial growth spurt was absent rather than delayed in the low protein animals, and it seems likely that hormonal mechanisms are involved (Kim 1952).

Table 11. Increase in Body Weight from Age 5-15 Weeks.

Diet	No. of Rats	Sex	Mean Gain in Body Weight g/10 Weeks
Basal diet	23	F	31.2 ± 1.9*
Stock diet	8	F	110.4 ± 6.4
Basal diet	10	M	23.7 ± 1.1
Stock diet	3	M	211.3 ± 6.1

	Fisher's P for Groups compared
Basal and Stock diet males	< 0.001
Basal and stock diet females	< 0.001
Basal diet males and females	0.04
Stock diet males and females	< 0.001

* Values given are the mean together with the standard deviation of the mean.

Table 12. Food Utilisation of the Rats from the Age of 4-15 Weeks

Diet	No. of Rats.	Sex	Total Food Intake (g/rat/11wks)	Gain in Body Weight (g/rat/11wks)	Food Utilisation (age 4-15 Weeks)
Basal diet	23	F	473.2	34.9	13.6
Stock diet	8	F	956.2	130.9	7.3
Basal diet	10	M	441.0	26.6	16.6
Stock diet	3	M	1267.7	245.0	5.2

Table 13. Age at which Vaginal Membrane became Open.

Diet	No. of Rats	Age when Vaginal Membrane Opened. (Weeks)
Basal diet	18	10.6 ± 0.08 *
Stock diet	8	5.9 ± 0.02
Fisher's P for groups compared		< 0.001

*Values given are the mean together with its standard deviation.

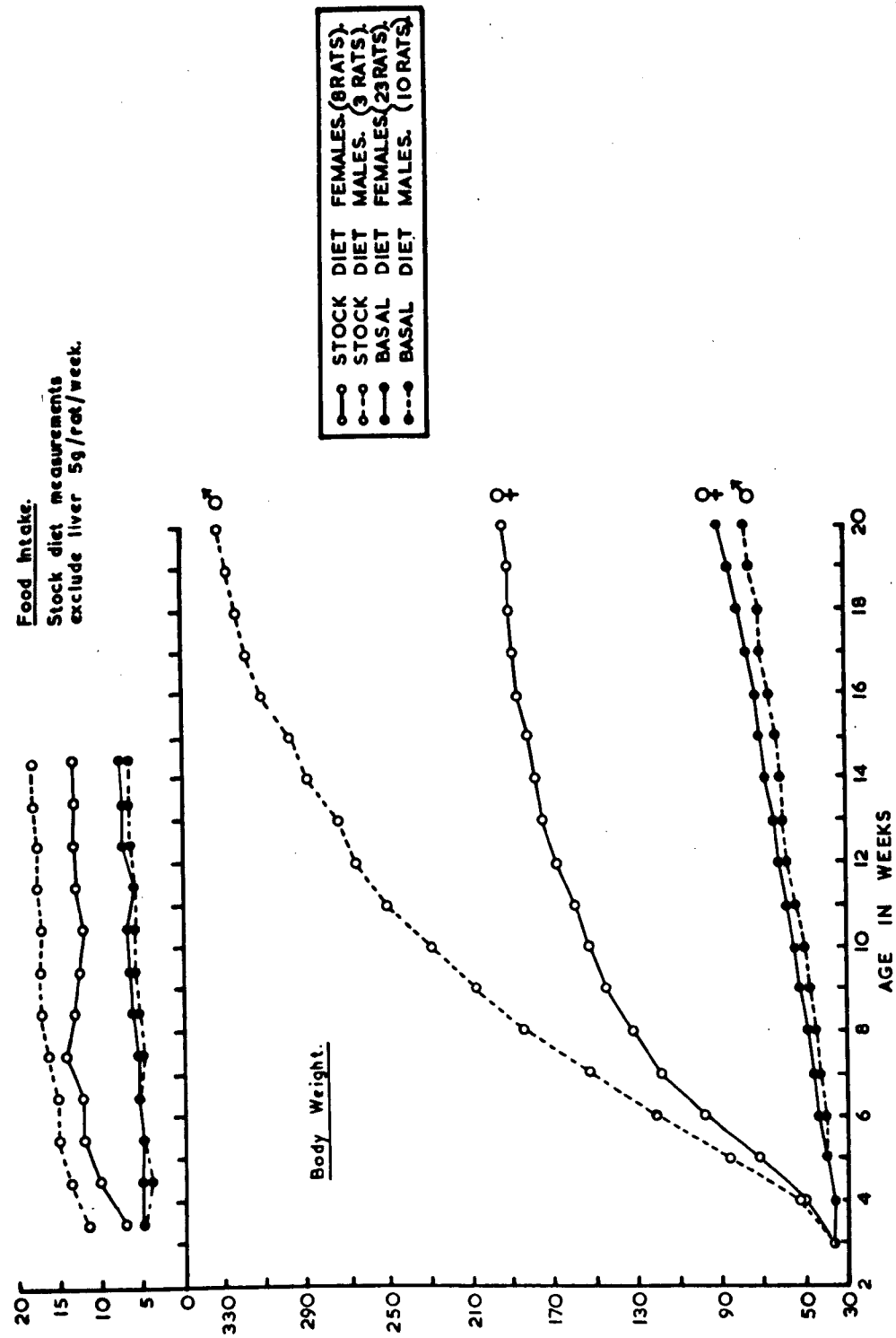


Fig. 5. Growth And Food Intake Of Rats Fed Basal And Stock Diets From Weaning. (Short Term).

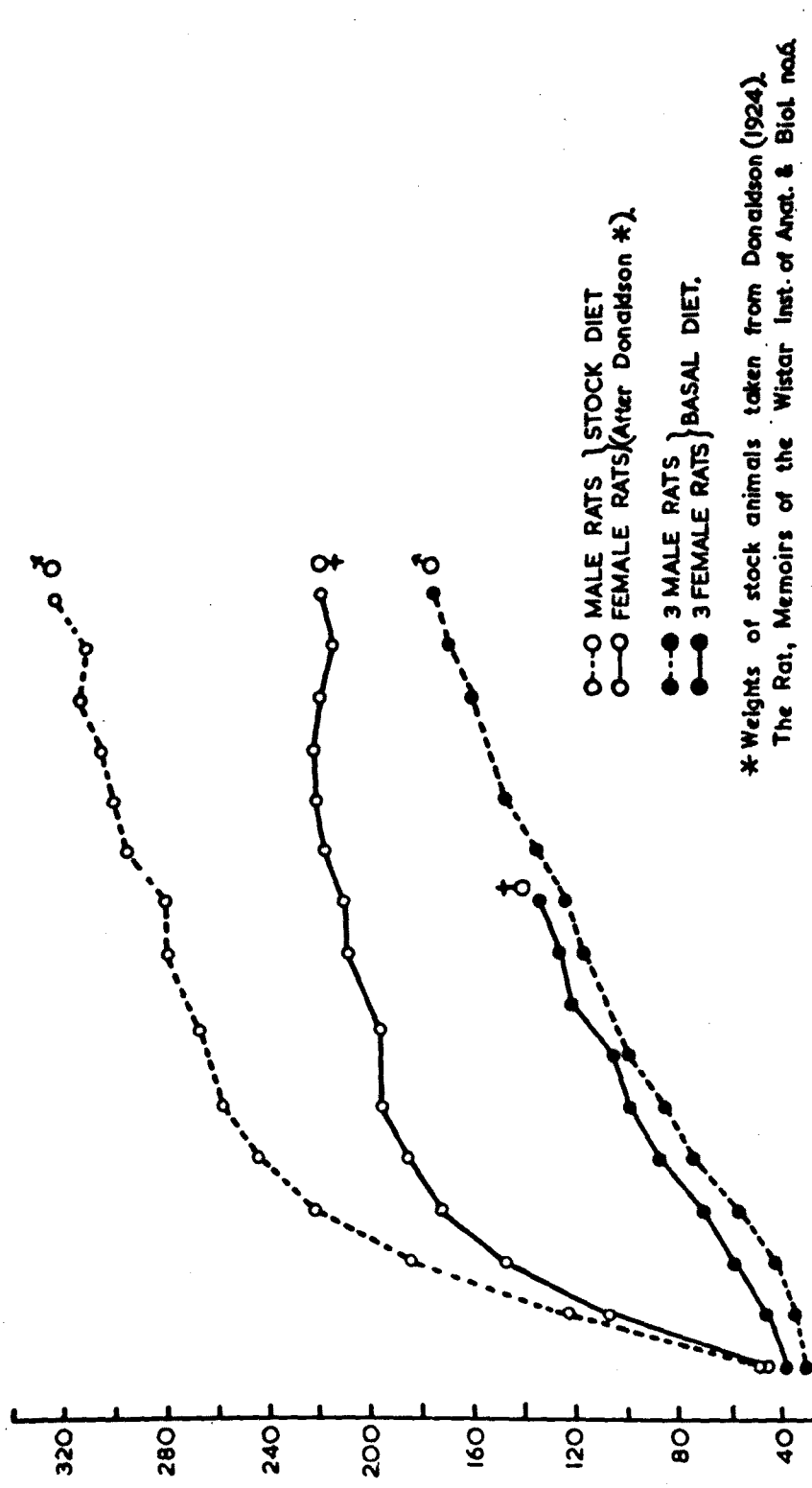


Fig. 6. Growth Of Rats Fed Basal And Stock Diets (Long Term).

The males fed stock diet ate more than the females while both sexes ate considerably more than the low protein rats. The factors involved in the poorer utilisation of the basal diet have already been discussed (page 50).

The female rats fed basal diet took longer to mature as indicated by the time of vaginal opening; and the replacement of the soft short baby hair by the coarser, longer adult type was also delayed in both sexes on the deficient diets. None of the five male rats fed basal diet tested were fertile by 10 months though the testicles had already descended. It is concluded that the diet was inadequate for male fertility. The females eventually became fertile at a later than normal age (Table 17). Slonaker and Card (1918, 1923) found that pubescence was delayed more in the male than in the female on low protein vegetable diets, and the higher protein requirements of the male rat (Zucker and Zucker 1944) is probably a factor influencing these results as well as those found for rat mortality (Table 14). The death rate was lower on stock than on basal diet where the mortality was highest amongst the males.

The deaths occurred mostly in the first few weeks following weaning. Before death the rats frequently became ill and lethargic sitting hunched up in a corner

Table 14. Rat Mortality.

Diet	No. of Rats	Sex	No. of Deaths	% Mortality
Basal diet	19	M	9	47
Basal diet	28	F	5	18
Stock diet	3	M	0	0
Stock diet	9	F	1	11

with staring fur, their skin extremities (ears, feet, etc.) usually were extremely pale. The most common post mortem findings were, throughout this work, signs of respiratory infection as indicated by greyish patches on the lungs which were also frequently inflamed; fluid was often found in the respiratory tract, but the lungs invariably floated in water. Other occasional results were that of intestinal disturbance revealed by an inflamed gas filled intestine and loose faeces, although no blockages were detected; or the presence of gut bleeding, the coffee grain appearance of the blood suggesting a stomach origin though no definite lesions were detected.

Fig. 7 shows how in a general way the reproductive results improved as the fishmeal contents of the diets were increased. The more detailed results from which the summary in this and in the following experiments was derived is given in the Appendix on page 149.

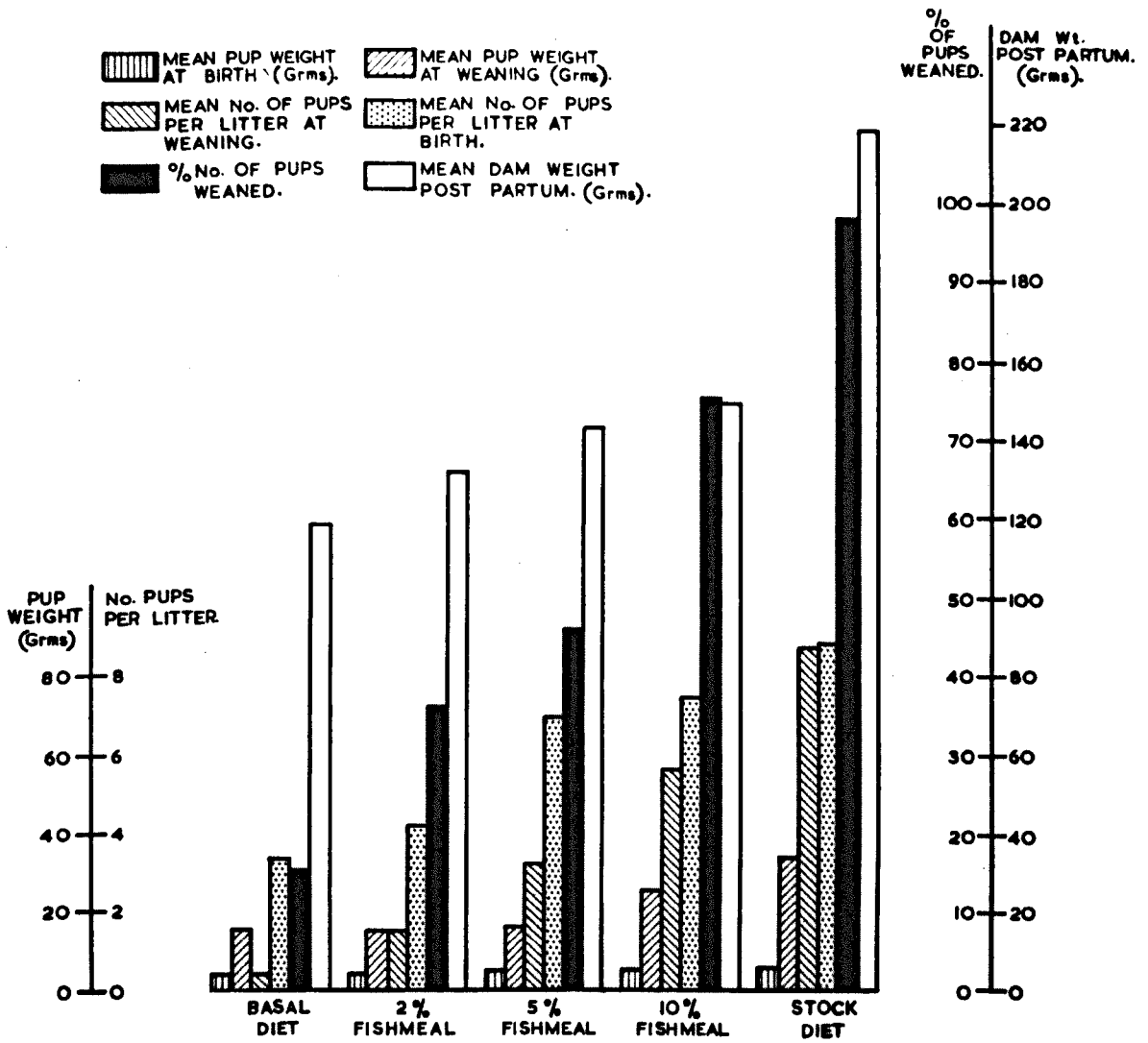


Fig. 7. Reproductive Performance Of Rats Fed Basal Diet, With Increasing Levels Of Fishmeal Supplementation During Pregnancy And Lactation.

The striking increase in weaning success (Table 15) with increasing fishmeal levels is illustrated graphically in Fig. 8.

Table 15. Weaning Success on the Various Diets.

Diet during Pregnancy and Lactation.	No. of Litters	No. of Pups Born	No. of Pups Weaned	% No. of Pups Weaned
Basal diet	4	13	2	15
2% Fishmeal Basal diet	12	50	18	36
5% fishmeal Basal diet	12	83	38	46
10% fishmeal Basal diet	12	89	67	75
Stock diet	12	106	104	98

The percentage number of pups weaned ranged from 36% on the 2% fishmeal level to 75% on the 10% level; on the basal diet only two of the 13 pups born were weaned and these both died soon after. The Chi-Square Test showed that the differences were significant between the stock and 10% fishmeal diets, ($p < 0.001$) and between the 5 and 10% fishmeal diets ($p = 0.01$). The reduction in the quantity of milk produced by the mothers on low protein diets (Mueller and Cox 1946) is probably a factor influencing these results.

Tables 16 and 17 show the main effect of the low protein diets on the reduction of the size of the litters, the influence being less striking on the mean pup weight at birth. By weaning, the differences in the weights because appreciable.

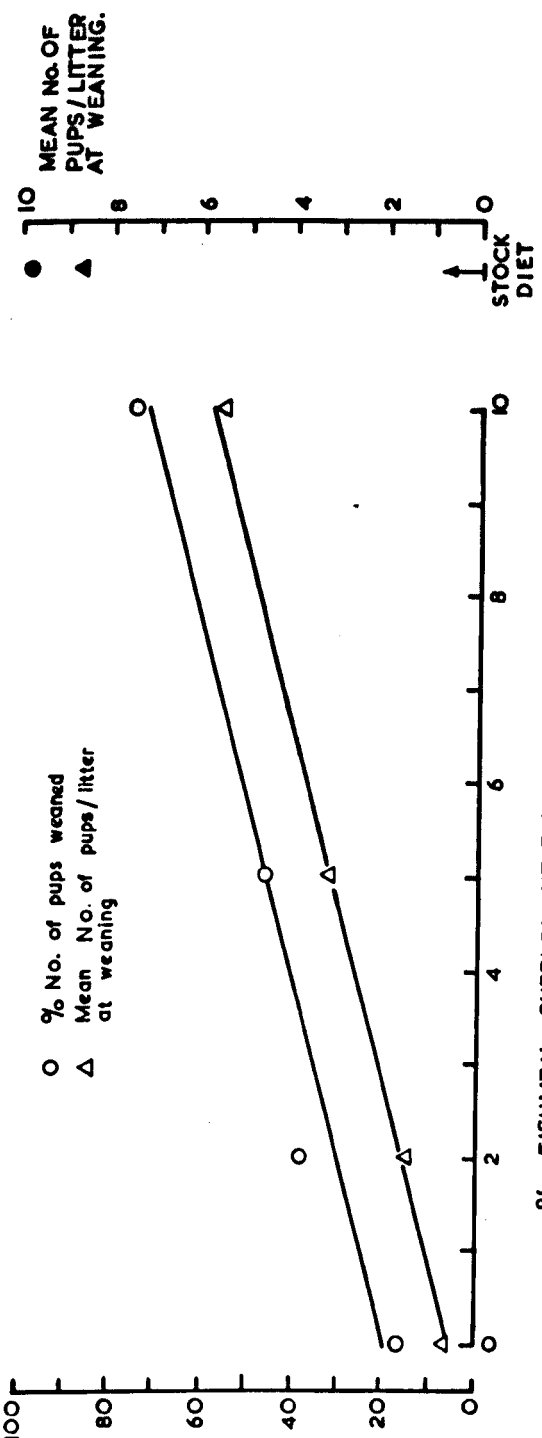


Fig. 8. Effect Of Increasing Levels Of Fishmeal Supplementation During Pregnancy And Lactation On The Number Of Pups Weaned.

Both those between the stock, 10 and 5% fishmeal levels were highly significant ($p < 0.001$, though the differences were not statistically different on the poorer diets.

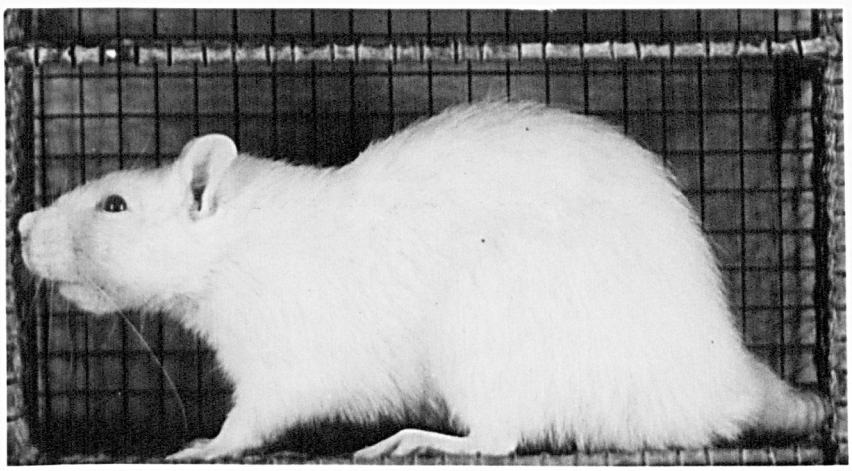
Table 16. Weight and Size of Litters.

Diet during Pregnancy and Lactation	No. of Litters	Litter at Birth		Litter at Weaning	
		No. of Pups per Litter	Mean Pup Weight g	No. of Pups per Litter	Mean Pup Weight g
Basal diet	4	3.3±0.9*	3.9±0.4	0.4±0.5	15.5±0
2% fishmeal basal diet	12	4.2±0.7	4.3±0.7	1.5±0.7	14.6±1.2
5% fishmeal basal diet	12	6.9±0.3	4.7±0.2	3.2±1.0	15.8±1.6
10% fishmeal basal diet	12	7.4±0.5	4.7±0.2	5.6±0.8	25.5±1.1
Stock diet	12	8.8±0.5	5.6±0.2	8.7±0.5	34.0±1.6

*Values given are the mean together with the standard deviation of the mean.

The rats on basal diet though thin with little subcutaneous fat and reduced in size appeared well and active. Their whiskers and nails were frequently broken off short and they often had patches of hair which had been nibbled short on the head flanks and abdomen (Plate 1). There was commonly yellow staining in the region of the urethral opening presumably because the animals failed to clean themselves adequately.

Male rat (aged 14 months) fed basal diet from weaning



Male rat (aged 11 months) fed stock diet from weaning

(Background is of 1 cm. squares)

PLATE 1

To illustrate Poor Hair Coat of Low Protein Animal.

Table 17. Weight and Fertility of the Dams.

Diet during Pregnancy and Lactation	No. of Litters	Time taken for production of first litter (from introduction of male) days.	Dam weight post partum g.
Basal diet	4	69 ± 4 *	118 ± 9
2% fishmeal basal diet	6	48 ± 8	131 ± 5
5% fishmeal basal diet	6	27 ± 1	142 ± 6
10% fishmeal basal diet	6	27 ± 2	149 ± 6
Stock diet	6	26 ± 1	219 ± 7

* Values given are the mean together with its standard deviation.

The skin colour of the basal rats was paler than that of their controls. All these symptoms were much more marked during the initial growing period. The low protein animals were not as strong as those on stock diet and the penile prolapse sometimes found in the deficient male was thought to be also indicative of this low muscle tone.

2. Second Generation.

All the female animals derived from the various supplemented basal diets of the previous experiment were weaned onto unsupplemented basal diet. When these rats were 20-21 weeks old they were sub-divided and their diets changed

to the same level of fishmeal supplementation which had been given to their mothers. None of the pups descended from basal or 2% fishmeal supplemented mothers had survived, so there were two groups fed the 5% and 10% levels of fishmeal respectively. To each of these groups was added one of three adult litter-mate stock males; the third male was used for the control group of females fed stock diet throughout this and the previous experiment, which group was also mated when aged about 20 weeks. The only difference from that of the previous experimental method was that because of the high post-weaning mortality thought to be caused by the immaturity of the suckling pups of low protein mothers (Slonaker 1938), the pups subsequently born on this experiment were weaned at five instead of three weeks, the supplemented diet being changed to basal for the final week to make the dietary transition for the pups a more gradual one. The stock animals were weaned as before at three weeks.

It was thought that the relative immaturity of the low protein pups at weaning was a factor in their subsequent high mortality rate (Table 18). Whereas the normal rat by weaning has already begun to nibble at its parent's diet (Murphy and Dunn 1949), the low protein pup at weaning, in contrast to that on stock diet, had frequently to be pulled away forcibly from clinging to the mother's teat.

Table 18. Mortality amongst the Young Female Rats.

Diet	Supplemented Diet of Mother	No. of Pups Weaned at 3 wks.	% Mortality on No.Weaned	
			Age 3-5 weeks.	Age 5-10 weeks.
Basal diet	2% fishmeal basal diet	9	67	33
Basal diet	5% fishmeal basal diet	18	50	17
Basal diet	10% fishmeal basal diet	31	55	16
Stock diet	Stock diet	19	21	0

Table 19 shows that, as might be expected, the lighter pups within each group were less likely to survive.

Table 19. Comparison of Weaning Weights of Pups which Survived with those that Died.

Diet	Diet of Mother	Mean Pup Weaning Weight in Grams	
		Survived beyond Age 5 Weeks	Dead by Age 5 Weeks
Basal diet	2% Fishmeal basal diet	14.0	14.3
Basal diet	5% Fishmeal basal diet	18.7	15.0
Basal diet	10% Fishmeal Basal diet	28.1	24.6
Stock diet	Stock diet	32.9	30.3

Table 20 and Fig 9 illustrate that the dietary history of the mother has considerable influence on the growth rate of the pups. Of all those pups fed basal diet from weaning, those derived from 10% fishmeal supplemented dams grew at a significantly greater rate than those from 5% fishmeal.

Individual rats underweight at birth tend to remain underweight during later growth (Dunn 1908), and with cats the birth weight determined the subsequent rate of growth (Scott 1957). It is thought that interuterine factors rather than those present during lactation also influence the results obtained here. This is supported by the fortuitous results obtained earlier (page 46), where despite the initial setback in growth due to souring of the diets amongst the 10% fishmeal rats, their subsequent growth was parallel to that of the control animals and did not as in this case continue at a decreased rate. Schultze (1955) found that the offspring of mothers fed amino acid mixtures and vitamin B12 in lieu of protein, grew at a reduced rate even when fed an adequate diet, or when changed to normal foster mothers for suckling, though the latter did increase the growth rate slightly.

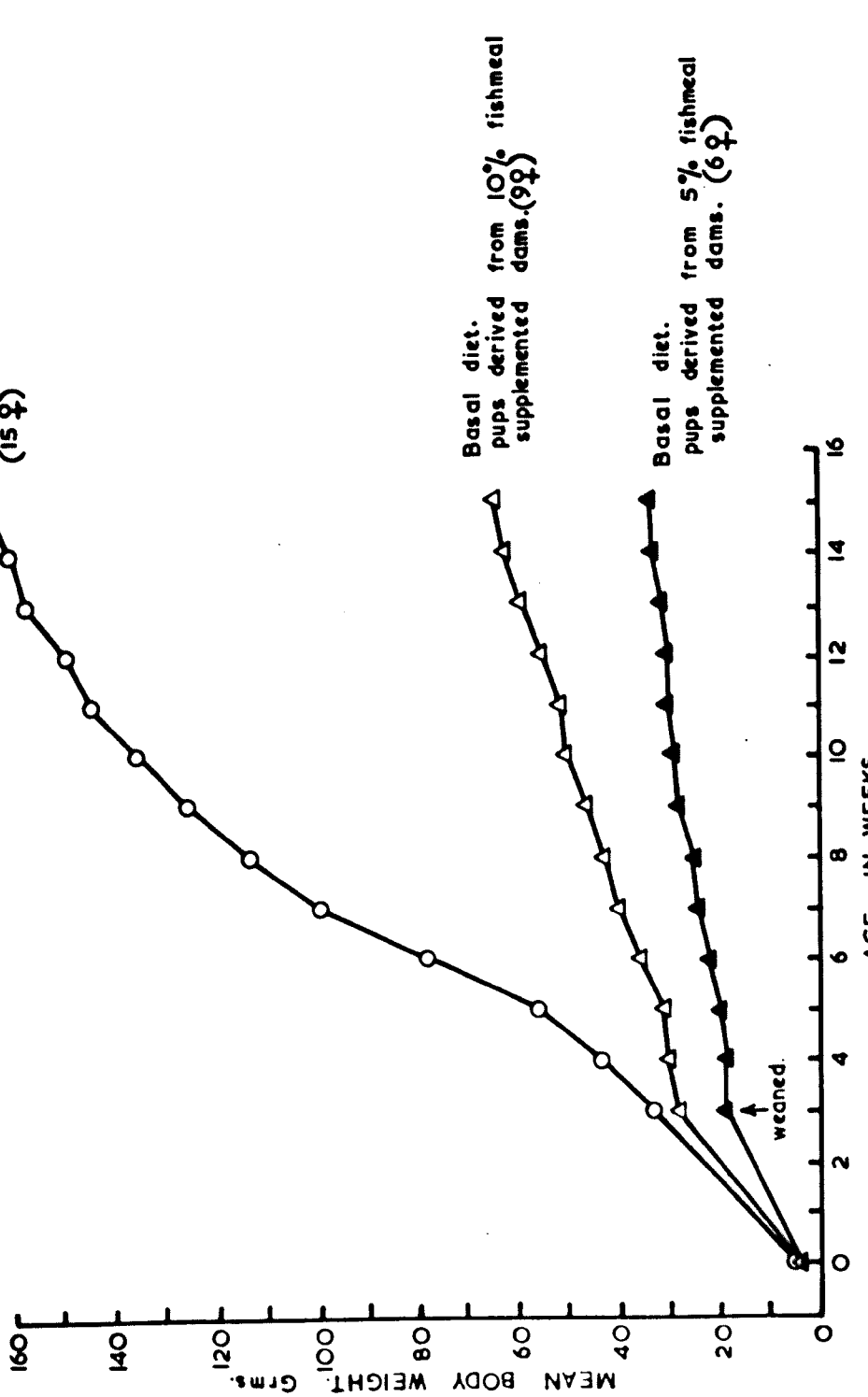


Fig. 9. Growth Of 2nd. Generation Of Rats Fed Stock And Low Protein Diets.

Table 20. Increase in Female Body Weight from Age 5-15 Weeks.

Diet	Diet of Mother during Pregnancy and Lactation	No. of Rats	Body Weight Gain from Age 5-15 Weeks g/ 10 Weeks
Basal diet	5% Fishmeal basal diet	6	12.8 ± 2.1 *
Basal diet	10% Fishmeal basal diet	9	32.6 ± 3.2
Stock diet	Stock diet	15	107.6 ± 10.2

	Fisher's P for groups compared
Pups derived from 5 and 10% fishmeal dams	< 0.001
Pups derived from stock and 10% fishmeal dams	< 0.001

*Values given are the mean together with its standard deviation.

With increasing levels of fishmeal supplementation the results on reproduction (Tables 21, 22 and 23) improved in much the same way as in the previous experiment. Plates 2 and 3 illustrate the differences in size of the pregnant female and the weanling pups which they produced.

Table 21. Weaning Success on the Various Diets.

Diet of Dam during Pregnancy and Lactation	No. of Litters	Length of Lactation Period Weeks	No. of Pups Born	No. of Pups Weaned	% No. of Pups Weaned
5% Fishmeal basal diet	8	5	36	14	39
10% Fishmeal basal diet	16	5	99	88	89
Stock diet	14	3	121	117	97

Table 22. Size and Weight of the Litters.

Diet of Dam during Pregnancy and Lactation	No. of Litters	Litter at Birth		Litter Aged 3 Weeks	
		No. of Pups /Litter	Mean Pup Weight g.	No. of Pups /Litter	Mean Pup Weight g.
5% Fishmeal basal diet	8	4.5 ± 0.7*	4.6 ± 0.2	5.3 ± 1.0	13.1 ± 2.0
10% Fishmeal basal diet	16	6.2 ± 0.3	5.1 ± 0.1	5.7 ± 0.3	22.2 ± 1.2
Stock diet	14	8.6 ± 0.8	5.6 ± 0.2	8.4 ± 0.8	37.9 ± 2.0
		Litter at Weaning			
5% Fishmeal basal diet	8	1.8 ± 0.6	17.6 ± 3.3		
10% Fishmeal basal diet	16	5.5 ± 0.3	31.9 ± 2.1		
Stock diet	14	Weaned	at 3 weeks		

*Values given are the mean together with its standard deviation.

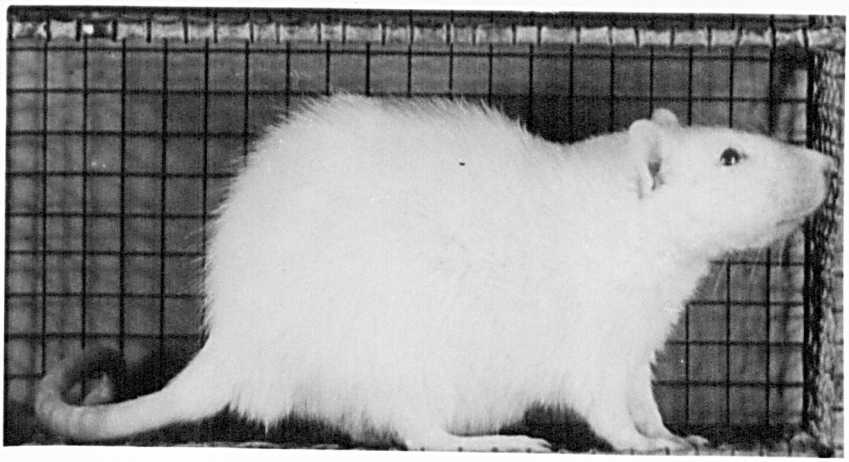
Rat fed 5% fishmeal supplement
to basal diet during pregnancy.

Rat fed 10% fishmeal supplement
to basal diet during pregnancy.

Rat fed stock diet throughout.

(Background of 1 cm. squares)

PLATE 2. Difference in Size of Pregnant Rats fed Various Experimental Diets.



Upper rat- female aged 5 wks. 3 days, weight 13 g., mother had 5% fishmeal supplement.

Middle rat -female aged 5 wks. 3 days, weight 30g., mother had 10% fishmeal supplement.

Lower rat- female aged 4 weeks, weight 40g., stock diet.

N.B. All low protein rats weaned at 5 weeks onto basal diet, stock rats weaned at 3 weeks.

(Background of 1 cm. squares.)

Plate 3. Difference in Size of Pups Produced by Dams Fed Various Experimental Diets.

Table 23. Dam Weight and Fertility.

Diet of Dam during Pregnancy and Lactation	No. of Dams	Time Taken to produce 1st Litter (from adding male) days.	Dam Weight Post Partum grams
5% Fishmeal basal diet	4	51 ± 11*	121 ± 6
10% Fishmeal basal diet	8	36 ± 4	145 ± 7
Stock diet	7	28 ± 2	212 ± 4

*Values given are the mean together with its standard deviation.

A comparison of the results on growth and reproduction between the various generations is given on page 85.

3. Third Generation.

The low protein females produced on the preceding experiment were weaned onto basal diet and then treated in the same manner as their parents in order to produce a further generation, some stock females being kept as before for use as controls. None of the rats derived from 5% fishmeal supplemented dams survived to breeding.

The results on mortality and growth are summarised in Tables 24 and 25, and the results on reproduction in Tables 26, 27 and 28. As before these results show the superiority of stock diet.

Table 24. Mortality amongst the Young Female Rats.

Diet	Diet of Mother during Pregnancy and Lactation	Age at Weaning Weeks	No.of Rats Weaned	% Mortality on No. Weaned	
				0-5 Wks from Weaning	5-10 Wks. from Weaning
Basal diet	5% Fishmeal basal diet	5	5	80	20
Basal diet	10% Fishmeal basal diet	5	47	51	4
Stock diet	Stock diet	3	17	12	6

Table 25. Increase in Female Body Weight from Age 5-20 Weeks.

Diet	Diet of Mother during Pregnancy and Lactation	No. of Rats	Body Weight Gain g/15 Weeks
Basal diet	10% Fishmeal basal diet	21	17.9 ± 1.7 *
Stock diet	Stock diet	14	117.7 ± 4.4

Table 26. Weaning Success on the Various Diets.

Diet of Dam during Pregnancy and Lactation	No. of Litters	Length of Lactation Period Weeks	No.of Pups Born	No.of Pups Weaned	% No. of Pups Weaned
10% Fishmeal basal diet	16	5	87	55	63
Stock diet	16	3	137	129	94

* Values given are the Mean together with its standard deviation.

Table 27. Size and Weight of the Litters.

Diet of Dam during Pregnancy and Lactation.	No. of Litters.	Litter at Birth		Litter aged 3 Weeks.		Litter at Weaning	
		No. of Pups per Litter.	Pup Weight. g.	No. of Pups per Litter.	Pup Weight. g.	No. of Pups per Litter.	Pup Wt. g.
Basal diet + 10% fishmeal	16	5.4 ± 0.5 *	5.0 ± 0.1	3.6 ± 0.6	21.8 ± 1.5	3.4 ± 0.3	31.5 ± 2.0
Stock diet	16	8.6 ± 0.4	5.4 ± 0.2	8.0 ± 0.4	37.0 ± 1.2	Weaned at 3 weeks	

Table 28. Weight and Fertility of the Dams.

Diet of Dam during Pregnancy and Lactation	No. of Dams	Time taken to produce 1st Litter (from addition of male) (days)	Dam Weight Post Partum (g)
Basal diet + 10% fishmeal	8	85 ± 13 *	147 ± 6
Stock diet	8	36 ± 3	222 ± 13

*Values given are the mean together with its standard deviation.

4. Fourth Generation.

The growth of the low protein female rats born on the previous experiment and weaned onto basal diet was followed to the age of 10 weeks, (Fig.10), when the experiment was terminated. The mortality during this period (Table 29) was very high.

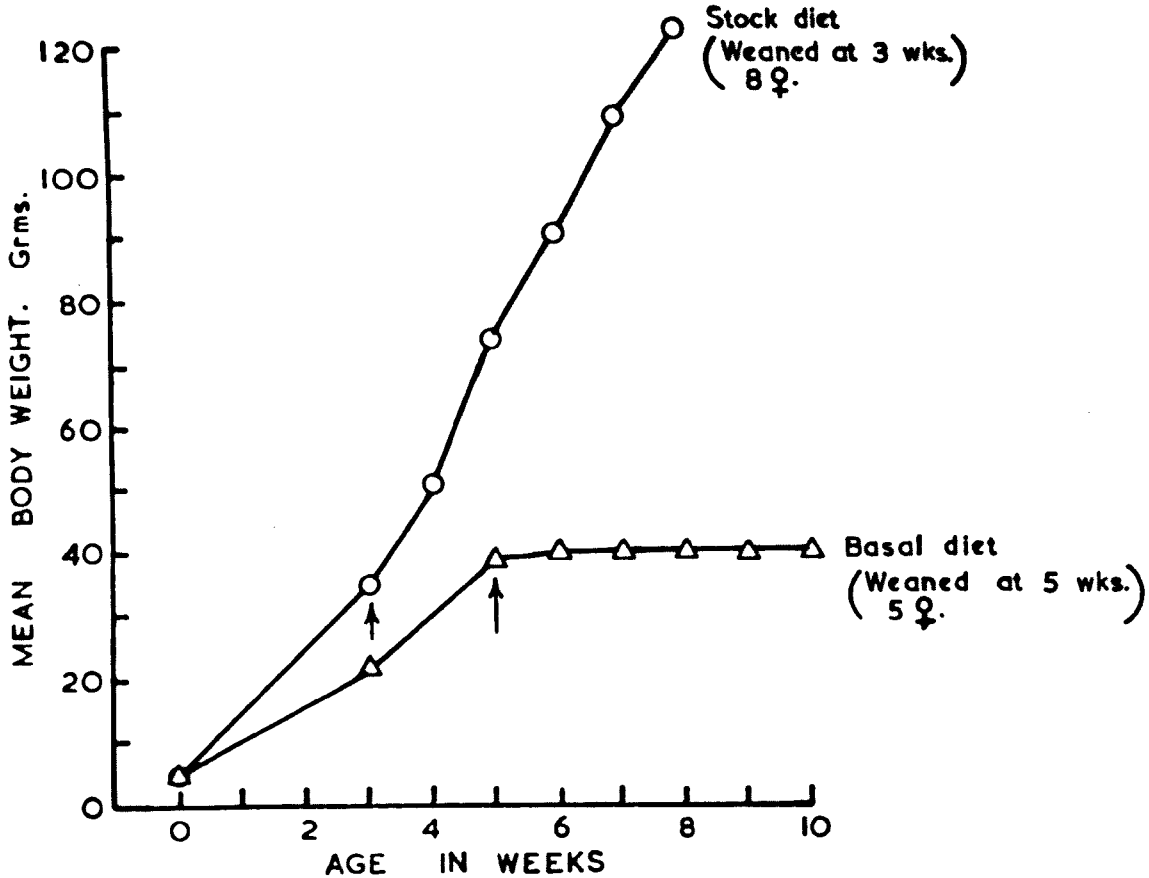


Fig. 10. Growth Of 4th Generation Of Rats Fed Stock And Low Protein Diets.

Table 29. Mortality amongst the Young Female Rats.

Diet	Diet of Mother during Pregnancy and Lactation	Age when Weaned (Weeks)	No.of Rats Weaned	%Mortality during first 5 weeks after weaning. (on No. weaned)
Basal diet	10% Fishmeal basal diet	5	31	84
Stock diet	Stock diet	3	10	20

5. Generations Compared.

Table 30 summarises the data from the various generations. With each succeeding generation the birth weight of the pups remained unchanged, but there was a progressive reduction in the size of the litters born. The differences between the first and second generationat the 5% and 10% fishmeal levels were probably significant ($p = 0.05$), though the difference between the second and third was not ($p = 0.2$). These results could be indicative of some adaptive mechanism whereby the pup weight was maintained at the expense of litter size. It is well known that the birth weight of an animal is inversely related to litter size (Hammond 1944). Damodaran (1952) also using diets of low protein derived from vegetables obtained a reduction in litter size and an increase in the pup birth weight with a successive generation. The post partum dam weights on the 10% fishmeal levels were much the same for each generation, so this was not influencing the results.

On the other hand there was a progressive delay in each generation in the time taken to produce the first litter, so that the greater age of the mothers may have been a factor. Student's Test (Fisher 1950) showed that these age differences were significant between the second and third generation ($p = 0.002$) given 10% fishmeal, but that between the first and second generation were probably not significant ($p = 0.055$). With the 5% fishmeal diet this age difference between the first and second generation was probably significant ($p = 0.03$), there being no significant difference between the successive generations on stock diet, ($p = 0.5$ and 0.3). Slonaker's results (1938) where meat scraps were used to give protein contents ranging upwards from 10%, would appear to support this delay in maturity, for though he found no consistent effect on the age of first parturition, the pups tended to have their eyes open later and there was a delay in the eruption of their incisor teeth with successive generations.

The results on the litter size at three weeks were too variable to draw any conclusions but the weight of the suckling pup aged three weeks diminished with each generation. The difference between that of the first and third generation at the 10% fishmeal level was highly significant ($p = 0.001$), but the differences between the second and third ($p = 0.7$), or first and second on the 5% fishmeal ($p = 0.3$), or 10%

fishmeal ($p = 0.1$), were not statistically significant.

At birth there were negligible weight differences between the respective generations but during the lactation period the rate of growth of the deficient animals became increasingly reduced with successive generations as illustrated by the weight of the pup at three weeks. The reduction in growth rate continued after weaning as shown in Fig. 11, so that despite the higher mean weight of the five week old pups obtained from second generation mothers (Table 30) where a longer weaning period was used, their subsequent growth rate was inferior to that of the preceding generation. These differences in weight gains from the age of 5-20 weeks were highly significant ($p = \angle 0.001$). Both Damodaran (1952) and Schultze (1955) also found a decrease in the growth rates with successive generations. The latter author used diets containing adequate amino acids as the protein source and states that the inclusion of vitamin B12 did not overcome this growth retardation. On the other hand Slonaker (1938) using meat scraps, obtained a tendency for increased growth with successive generations. The above results suggest that animal protein may supply certain unknown growth factors.

The slight variations in the growth rate of the stock rats, where the order of superiority was: generation 1, 3, then

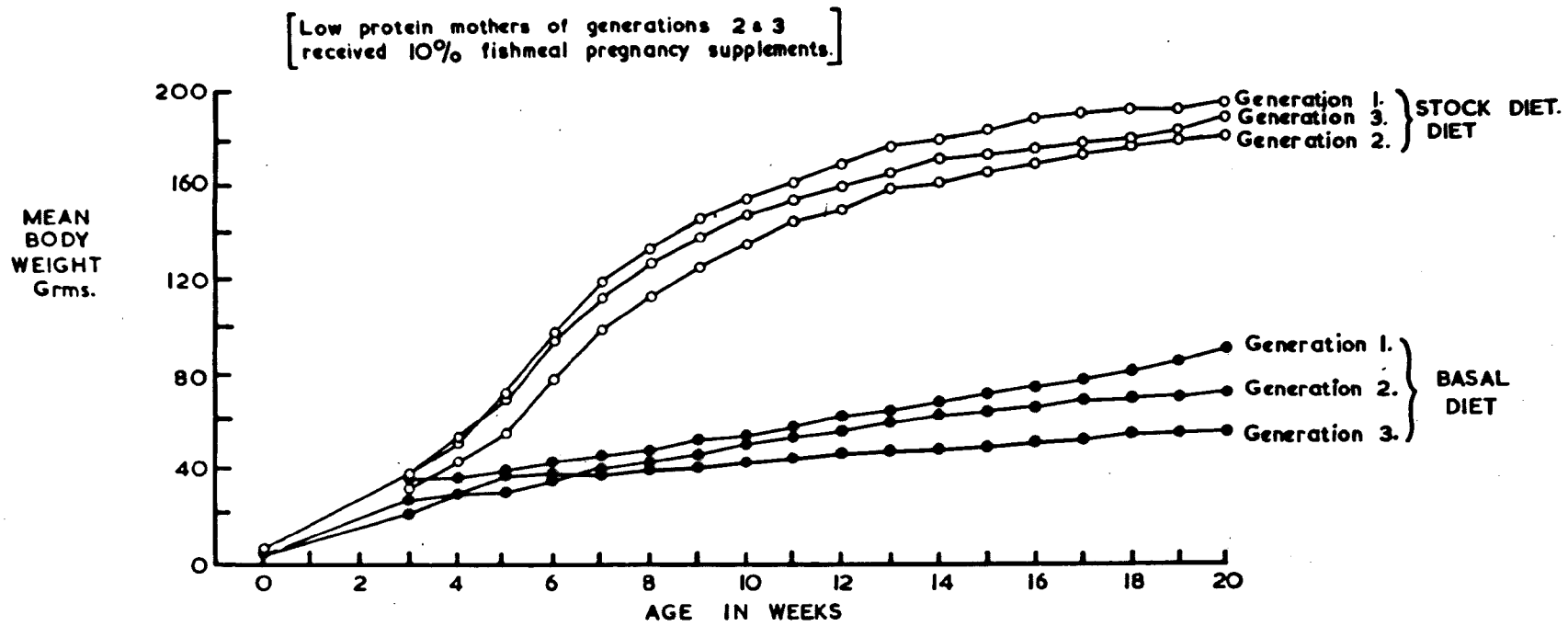


Fig. II.

Growth Of Successive Generations Of Female Rats On Low Protein And Stock Diets.

2, were not statistically significant. Seasonal differences are known to affect the growth of rats (Campbell 1945).

It would appear that there was a tendency for the mortality rate to increase with successive generations, but this was masked to some extent by the introduction of a longer weaning period (Table 30). The mortality rate by the age of 10 weeks amongst animals derived from 10% fishmeal supplemented dams of the second generation was significantly less ($p < 0.001$) than that of the next generation, the pups being weaned at five weeks in both cases. On the other hand, on comparing the mortality rate obtained from the first two generations of 10% fishmeal supplemented dams, where the three week lactation period of the first generation was changed to a five week period in the second, the mortality rate was significantly reduced ($p = 0.01$) on the introduction of the longer lactation period, despite this being a successive generation. There was no significant difference on the 5% fishmeal diet between the mortality rates of these two generations ($Chi^2 = 1.2$).

Slonaker (1938) found a tendency for increased mortality with successive generations, though his diets have been criticised by other workers (Macomber 1933) for

their suboptimal levels of vitamins A, D and E.

On the 10% fishmeal diet the death rate between the ages of 3-5 weeks was reduced from 50% to zero when the rats were weaned at five instead of three weeks, the onset of the high death rate coming subsequent to weaning in both cases. On the 5% fishmeal level the longer weaning period did not delay the onset of this high death rate.

The quantity of milk produced is reduced on low protein diets (Mueller and Cox 1946), so that the above results could be interpreted to mean that the inclusion of a 5% level of fishmeal did not improve the lactation performance sufficiently for the pups to benefit from a longer lactation period, as was the case with the inclusion of the 10% fishmeal. The value of a prolonged period of breast feeding amongst protein malnourished communities has been stressed by Platt (1953).

Table 30. Generations Compared.

Diet during pregnancy and lactation	5% Fishmeal		10% Fishmeal Basal Diet			Stock Diet		
Generation number	1	2	1	2	3	1	2	3
Mother and litter								
Time taken for production of 1st litter (days)	27	51	27	36	85	26	28	36
Body weight of dam post partum (grams)	142	121	149	145	147	219	212	222
No. of pups born / litter	6.9	4.5	7.4	6.2	5.4	8.8	8.6	8.6
Body weight of pup at birth (grams)	4.7	4.6	4.7	5.1	5.0	5.6	5.6	5.4
No. of pups per litter at 3 weeks	3.2	5.2	5.6	5.7	3.6	8.7	8.4	8.0
Body weight of pup aged 3 wks. (grams)	15.8	13.1	25.5	22.2	21.8	34.0	37.9	37.0
% no. of pups surviving to 3 weeks	46	72	75	92	67	98	97	94
Female pups produced								
Length of lactation (weeks)	3	5	3	5	5	3	3	3
No. of pups born	41	20	42	50	51	22	18	11
%total no.deaths 0-10wks.on no. born	85	95	79	54	90	32	22	27
No. deaths 0-3 weeks	23	5	11	3	17	0	1	1
No. deaths 3-5 weeks	9	10	17	0	3	0	2	1
No. deaths 5-10 weeks	3	4	5	24	26	7	1	1
Body weight aged 5 weeks (grams)	20.3		31.4	36.9	39.0	56.1	69.8	73.6
Body weight aged 10 weeks (grams)	29.0		50.3	43.2	40.4	134.9	146.9	
Difference	8.7		18.9	6.3	1.4	78.8	77.1	

(d) SUMMARY OF RESULTS OBTAINED.

1. The effect of the basal diet was similar to that of the Gambian diet (Balfour 1952) in that, when it was fed to rats, it produced a severe retardation of growth and made it impossible to wean successfully any animals produced.

2. The normal sex difference in growth rates was absent amongst the rats fed basal diet. The weekly gain in body weight of these rats for the first four months was about 3 and 2 grams for the females and males respectively, as compared with 11 and 21 grams for the animals fed stock diet.

3. The amount of basal diet eaten was reduced as was the efficiency of its utilisation for body weight gain.

4. The rats fed basal diet took longer to grow their adult hair coat and the disappearance of the vaginal membrane was delayed, as was the age at which the female produced her first litter.

5. The male rats weaned onto basal diet were still infertile by 10 months.

6. When female rats were fed the basal diet first as adults, the age of parturition, and weight and size of the litters at birth were unaffected, though the maternal weight gain during pregnancy was significantly reduced and no pups survived to weaning.

7. The size of the litters born to the rats fed basal diet from weaning, the weight of the pup at birth, and the dam weight post partum were all significantly reduced. Out of a total of 23 pups born, only two survived to weaning and these died soon afterwards.

8. The rats fed basal diet appeared healthy and active, their skins were paler than normal, and patches of alopecia developed at some time on the bodies of many of the animals.

9. The low protein basal diet supplemented with a 10% level of fishmeal compared favourably with stock diet as far as the results from rat growth and female reproductive performance were concerned.

10. The addition of vitamin B12 produced no alteration either in the amount of food eaten or in the gain in body weight of the rats fed basal or 10% fishmeal supplemented basal diets.

11.a. With increasing levels of fishmeal supplementation, (2, 5 and 10%) of the basal diet during pregnancy and lactation there was a graded improvement in the size of the litters born, the number and weight of pups weaned, and the dam weights post partum, to values approaching but still inferior to those found with stock diet. The addition of fishmeal also increased the pup weight at

birth although to a subnormal value, and reduced the age of first parturition; in the case of the 5 and 10% fishmeal levels the latter was reduced to normal.

11 b. P. T. O.

12. Using a 10% level of fishmeal supplementation to the basal diet during pregnancy and lactation, at least three successive generations could be obtained; with the 5% level only two were achieved and with the 2% level only one.

13. With each successive generation there was a reduction in the litter size at birth, in the weight of the suckling pups aged three weeks, in the future growth rates of the rats and in the time taken to produce their first litters.

14. Using the 10% fishmeal supplement, the mortality rate amongst the pups weaned at five weeks from mothers of the second generation was significantly less than amongst those weaned at three weeks from first generation mothers. With the introduction of this longer lactation period the onset of the high death rate occurring between the ages of 3-5 weeks was postponed until after weaning.

15. Using the 5% level of fishmeal there was no significant difference between the number of deaths which occurred amongst pups weaned at 3 or 5 weeks from mothers of the first and second generations respectively, nor was

there any alteration in the distribution of deaths occurring between the age of 3-5 and 5-10 weeks.

11 b. **The post weaning growth rate of the pups derived from 10% fishmeal supplemented dams was superior to that of the pups derived from 5% fishmeal supplemented dams, despite the fact that both groups of pups were fed basal diet from weaning at 3 weeks of age.**

ANAEMIA OF LOW PROTEIN RATS.

1. Packed Red Cell Volume of Blood Samples.

To find out whether the chronic low protein rats were anaemic, tail vein blood samples were taken from rats of various ages in the colony. No rat was bled more than once. The samples were spun for an hour at 3,000 rev/min. in sealed Wintrobe haematocrit tubes to determine the packed red cell volume, using heparin as the anticoagulant. Samples were taken from rats of both sexes fed basal diet from weaning at 5 weeks. These rats had been born to mothers similarly fed the basal diet from weaning, but during pregnancy and for the first 4 weeks of the lactation period the mothers' diet had been changed to the 10% fishmeal supplemented basal diet. The total lactation period of the mothers had been 5 weeks, the basal diet being reinstated during the final week. Rats obtained from the colony of stock diet animals (weaned at 3 weeks) were used as controls.

The results obtained are shown in Fig. 12. It can be seen that no differences between the two groups were apparent until after the low protein animals had been weaned, when there was a drop in the values obtained from the malnourished animals only. Following this there were indications of a gradual rise in the red cell volume, but by 11 months the values were still below those of the stock animals.

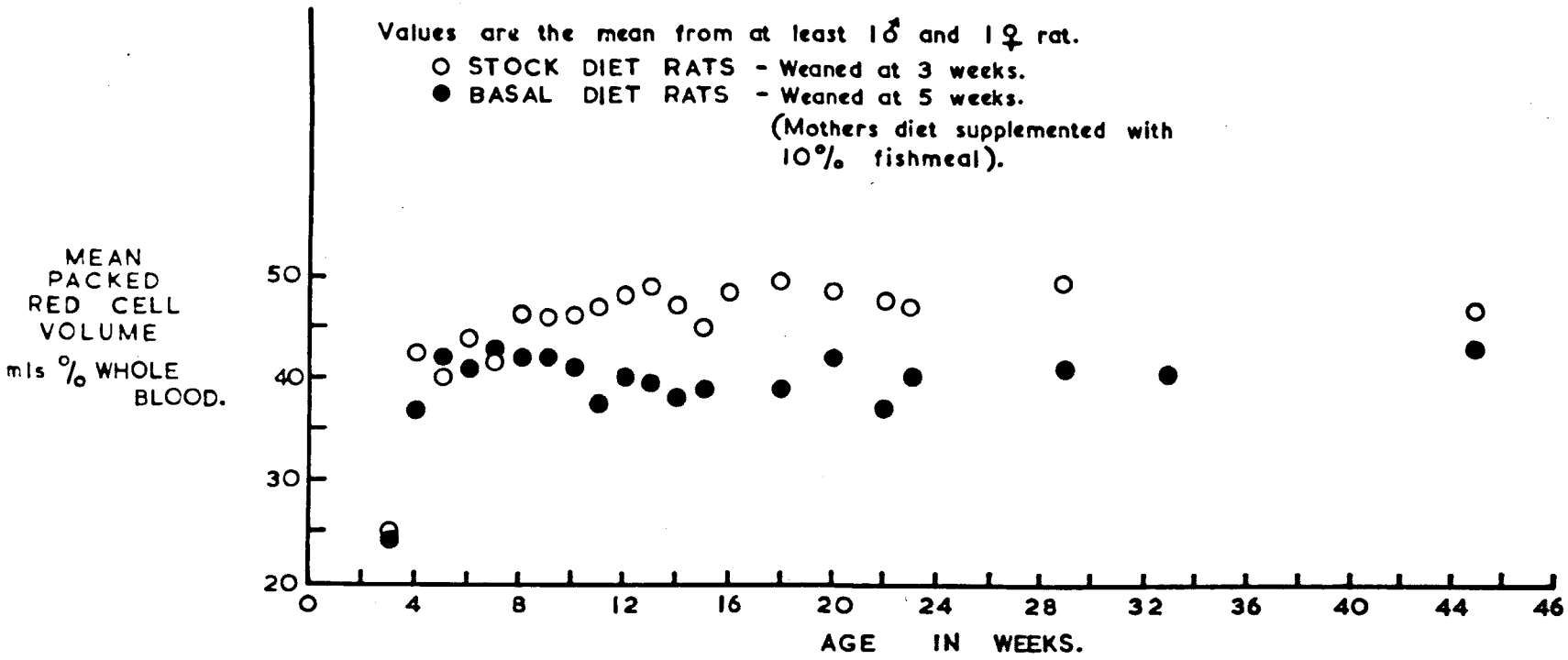


Fig.12. Packed Red Cell Volume Of Blood Samples Taken From Rats On Basal And Stock Diets.

Damodaran (1952) using diets of low protein from vegetable origin, found that in rats there was a gradual decrease in circulating haemoglobin until the age of 4 months, after which there was a continual rise to normal values at 9 months; this probably reflects the greater amino acid requirements of the young rat, where lysine is especially concerned (Albanese 1950), as compared with the adult.

2. The Effect of Lysine Supplementation upon the Growth and the Total Red Cell Volumes of the Rats fed the Experimental Diets.

A criticism of the previous results is that they may not represent a true picture of the total red cell volume of the malnourished animals owing to the possibility of concurrent changes in plasma volume affecting the values obtained from blood samples. It was therefore decided to determine the total red cell volume of the deficient animals.

The opportunity was also taken to verify that the limiting amino acid in the experimental diets was lysine (page 41).

Stock weanling rats of both sexes were divided on a litter-mate basis into the following four dietary groups: basal diet with or without lysine and 10% fishmeal basal diet with or without lysine. The composition of the diets was the same as that described on page 36, except that the

wheat was from a different source. In view of the harmful action of heat upon lysine (Reinius 1956) and because of the necessity for the simultaneous availability of the amino acids (Cannon et al 1950), a solution of *l*-lysine monohydrochloride in water was painted onto the diets after cooking. Since an excess of dietary lysine can have harmful effects (Russel et al 1952, Elvehjem et al 1955) the amount added was such as to adjust the theoretical content to 1.0g per cent (Rose 1937).

The rats were housed individually and their body weights and food intakes measured at weekly intervals for a period of two months, after which the blood haematocrit values were determined as before, as well as the total plasma volume using the Evans Blue Dye Method (Wang et al 1949, Metcoff et al 1944).

The rat was anaesthetised with ether and the hair over the groin shaved off, a blood sample was taken from the tail vein as before, for a subsequent haematocrit determination. The femoral vein was exposed and into it was injected over a period of a minute, a warmed 0.1% solution of T 1824 evans blue in the following amounts:

Rat Weight (g.)	Amount of Dye Injected (mg.)
100-200	0.8
50- 100	0.4
25-50	0.2

The needle was left in situ for about a minute, then quickly withdrawn and a piece of cotton wool pressed to the site. The syringe was weighed before and after the injection. Between 3.75 and 4.25 minutes after the injection, a blood sample was taken by cutting into the apex of the heart; 0.2 ml. of the plasma obtained in this way was pipetted into 4 mls. of distilled water and duplicate samples read off at 575 μμ in a colorimater, which was set at 100% transmission for a blank sample of plasma (obtained from tail vein). The total red cell volume was then calculated from the following formula:-

$$\text{red cell volume} = \frac{\text{mg dye injected}}{\text{mg/ml in plasma sample}} \times \frac{\text{packed red cell volume}}{\text{haematocrit plasma volume}}$$

Two sources of error in this method where the haematocrit value is used, are that the red cell concentration of the blood varies according to the size of the vessel in which the blood is flowing, and that some of the plasma is trapped between the corpuscles following centrifuging. The amount of trapped blood plasma is very small in the rat (Wadsworth 1957), and since the source of blood was the same in the two dietary groups, the second factor also does not affect the comparisons made.

The results on growth are summarised in Fig. 13 and Table 31. The inclusion of lysine in the basal diet

Table 31.

(a) Mean Values for Rat Growth from Age of 3-10weeks

	Basal Diet				10% Fishmeal Supplmt. Basal Diet			
	Without lysine		With lysine		Without lysine		With lysine	
No. of rats Sex	4 male	5 female	4 male	6 female	4 male	6 female	6 male	4 female
Rat weight increase (g.per 7 weeks)	8.8± 0.7 *	9.6 ± 1.6	35.3± 8.3	41.2± 6.9	123.0± 1.9	86.7± 9.1	153.7± 5.6	92.0± 2.8
Food intake (g. per rat per day)	5.3± 0.6	5.1± 0.2	6.7± 0.5	7.7± 0.3	11.2± 0.2	10.0± 0.6	13.2± 0.5	11.0± 0.5
Food utilisation (g.food/g body wt.inc.)	29.5± 2.3	29.8± 5.6	10.6± 1.8	10.9± 2.2	4.5± 0.1	5.6± 0.3	4.2± 0.2	5.9± 0.2

(b) Fisher's P for two groups compared

	Sex	Basal Diet with/without lysine	10% Fishmeal Basal Diet with/without lysine
Rat wt.increase (g.per 7 wks.)	male	0.02	0.0015
	female	0.0015	0.2
Food intake (g.per rat/day	male	0.1	0.01
	female	< 0.001	0.3

* Values given are the mean together with the standard deviation of the mean.

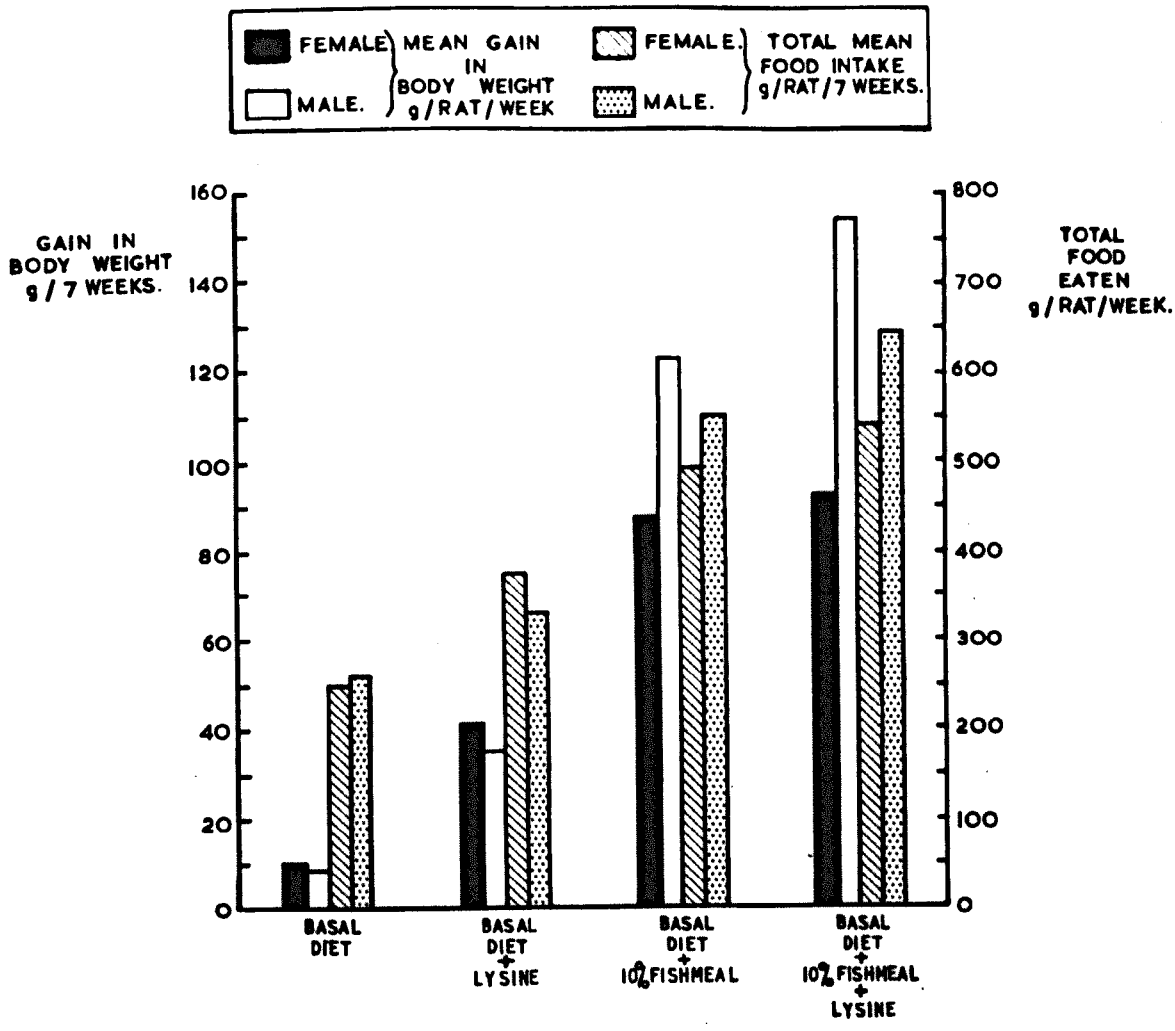


Fig. 13. Effect Of Lysine Addition To Basal And 10% Fishmeal Basal Diets, On Growth.

produced a striking increase is growth for rats of both sexes, but the rate was inferior to that obtained using the fishmeal diet, and the typically greater growth of the male was still not present. This increase was statistically significant. The growth increase with lysine was due primarily to a greatly improved food utilisation, though there was also present a slight increase in food intake.

On adding lysine to the 10% fishmeal diet, there was a significant increase in the growth of the males only, due, not to an increase in food utilisation which remained unchanged, but to an increase in the food intake of the male rats. This result is rather surprising in the light of the classic concept of the necessity for the simultaneous availability of the amino acids for utilisation, (Geiger 1950), where the increased supply of a previously limiting amino acid enables the organism to use more of the other amino acids and therefore increases the food utilisation. The results of Sure (1953) may be pertinent, as he also obtained an increase in growth of the male rat only due to an increased appetite, on the addition of vitamin B12 to a lysine supplemented wheat diet. The growth response obtained from protein and vitamin B12 are synergistic (Hartman 1949), so that the inclusion of lysine in the diet may render available more of its vitamin B12 content for utilisation by the male rat, whose requirements are

Table 32.

(a) Mean values for rat blood volumes at age of approx.11 weeks.

	Basal Diet		10% Fishmeal Basal Diet	
	without lysine	with lysine	without lysine	with lysine
	Values derived from:	Values derived from:	Values derived from:	Values derived from:
	4 male, 5 female rats	4 male, 5 female rats	4 male, 5 female rats	5 male, 4 female rats
Packed red cell volume (% whole blood sample)	37.3 ± 1.5 *	44.3 ± 1.1	50.6 ± 1.4	49.8 ± 0.7
	3 male, 3 female rats	3 male, 4 female rats	3 male, 3 female rats	5 male, 4 female rats
Total plasma volume (mls/100g body wt.)	4.3 ± 0.1	3.5 ± 0.1	3.1 ± 0.1	2.9 ± 0.2
Total red cell volume (mls/100g body wt.	2.7 ± 0.1	2.8 ± 0.1	3.2 ± 0.2	3.0 ± 0.1
Mean rat weight (g)	41.7 ± 1.3	79.7 ± 5.8	147.2 ± 10.2	170.2 ± 13.5

* The values given are the mean values together with the standard deviation of the mean.

(b) Fisher's P.for above dietary groups compared.

	Basal Diet with/without lysine	10% Fishml. diet with/without lysine	Basal diet with Lysine and 10% Fishml. Diet
Packed red cell volume	< 0.01	0.5	< 0.01
Total plasma volume	< 0.01	0.5	Basal diet with lysine, and both 10% F.M.diets: 0.02
Total red cell volume	0.6	0.5	Both basal diets, and both 10% Fishmeal diets: 0.02

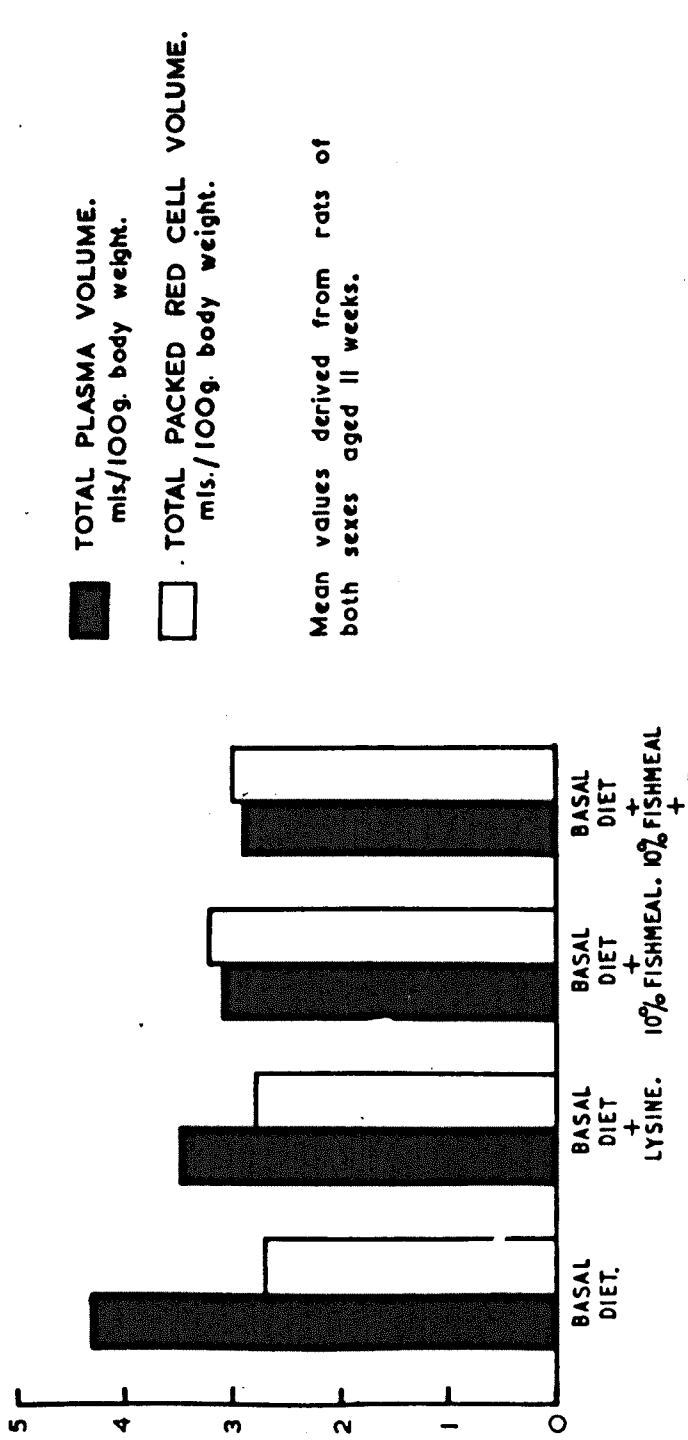


Fig. 14. Effect Of Lysine Supplementation Of Basal And 10% Fishmeal Basal Diets, On The Plasma And Red Cell Volumes Of The Rat.

presumably greater than those of the female.

As seen from Table 32 and Fig. 14, the total red cell volume of the rats fed basal diet was significantly below ($p = 0.02$), and the relative plasma volumes significantly above ($p = 0.02$) those obtained from rats fed the 10% level of fishmeal.

After adding lysine to the basal diet there was no increase in total red cell volume despite the increase in rat growth, which does not support the view (Pearson 1937; Whipple 1942) concerning the priority of haemoglobin over other body proteins when the protein content of the diet is inadequate. An explanation of the results obtained here may be in the different pattern of amino acids required for haemoglobin synthesis where valine and histidine are especially concerned, (Sebrell 1949), as compared with those required for body protein synthesis. Orten (1945) also obtained no improvement in the anaemia of low protein rats when single amino acids were added to the diet, but there was no mention of body weight.

With the addition of lysine to the basal diet there was a reduction in the relative plasma volume which accounted for the raised haematocrit value. It is probable that the results of others (Harris, Neuberger el al 1943) who have claimed to remedy the anaemia by the addition of

lysine to a wheat protein diet can also be explained in this way, since their conclusions were based on haematocrit values, no determination of total red cell volume being made.

Enlarged relative plasma volumes associated with protein malnutrition have been observed by other workers (Hallgren 1953, Walters 1947, Allison 1957). Changes occur in the fluid compartments of normal rats aged 2 months; the adult having a relatively lower plasma volume than the young rat. A formula for calculating the plasma volume based on the body weight has been devised by Wang et al (1949). Though the values obtained here were less than the calculated values, presumably because of the experimental technique used, the differences were all of the same order with the exception of the lysine supplemented basal diet (Table 33).

Table 33. Observed and Calculated Plasma Volumes.

Diet	Basal Diet	Basal Diet + Lysine	10% Fishmeal Basal Diet	10% Fishmeal Basal Diet + Lysine
Observed plasma vol. (mls/100g body wt)	4.3	3.5	3.1	2.9
Calculated plasma vol.(mls/100g body wt.)	5.6	5.4	4.4	4.3
Difference	1.3	1.9	1.3	1.4

It seems likely therefore that the larger plasma volume of the basal diet rats, unlike those of the "lysine basal diet" animals, was due to a retention of their infantile characteristics.

The addition of lysine to the 10% fishmeal basal diet produced no alteration in the measured blood values.

Summary of Results Obtained.

1. The packed red cell volume of a sample of blood, taken from rats fed the basal diet from weaning from malnourished mothers (10% fishmeal pregnancy supplement), was normal while the pups were suckling (at ages of 3-5 weeks). Thereafter the value fell below that of the stock animals and, though there was a tendency for a gradual increase from the age of about 4 months, by 11 months the values obtained from the basal diet animals were still below those of stock rats of the same age.

2. Stock rats fed basal diet for 2 months from weaning had significantly lower total relative red cell and higher total relative plasma volumes than their controls fed the 10% fishmeal basal diet.

3. The addition of lysine to the basal diet fed for 2 months to stock animals from weaning, resulted in a significant reduction in the relative plasma volume, no change in total red cell volume, and an increase in the body weight, though the normal superior growth of the male remained absent. There was also a considerable increase in food utilisation accompanied by some increase in food intake.

4. The addition of lysine to the 10% fishmeal supplemented basal diet fed for 2 months to stock animals from weaning, resulted in no significant alteration of the plasma or red cell volumes or in female body weight gain, though there was an increase in the weight gain of the male rats due to an increase in food consumption.

ENDOCRINAL ASPECTS.

Introduction.

Many of the results obtained in this work, such as the failure of the low protein animals to reproduce satisfactorily, the absence of the normal sex difference in growth rates, and the higher mortality and infertility found amongst the deficient male animals when compared with the malnourished females, suggested that the effect of the low protein diet upon growth and reproduction might be mediated by the endocrine system. This hypothesis was further supported by the work of others who have remedied to some extent the effect of a low protein diet on reproduction (Nelson and Evans 1954) and in causing anaemia (Aschkenasy et al 1953) by the administration of hormones.

The concept that alcoholism is caused by nutritional deficiencies which may be also genetical in origin has arisen primarily from the work of Williams (1947, 1950) and Mardones (1951). Since more recent literature has suggested an endocrinal involvement in alcoholism (Smith 1950, 1951, Goldfarb 1949, Gross 1945-46), it was thought that alcohol might provide a useful tool to illustrate endocrinal disturbances in the low protein rats, since the two factors concerned in alcoholism, the nutritional and the endocrinal, were probably related. This idea was originally suggested by the cravings both for meat and for alcohol found amongst malnourished communities.(Platt 1957)

The Effect of Dietary Protein and the Influence of Alcohol on the Size of the Rat's Endocrine Glands.

Stock rats of both sexes were weaned either onto basal diet (group A) or the 10% fishmeal supplemented basal diet (group B). At the age of 12-13 weeks these rats were killed with coal gas and the following organs removed for weighing: right testis or right ovary, right adrenal, both thyroids and the thymus. An approximation to the area of the pituitary in situ was obtained by multiplying its maximum width by the minimum length as measured by a travelling microscope[x]. Since the left adrenal is heavier than the right (Tadokoro 1954), single organs, bilaterally placed, were removed from the same side of the animal.

In order to compare rats on a weight as well as an age basis, a few of the basal diet rats (group C) were allowed to survive until they reached the same weight as those of the same sex of group B fed the fishmeal diet. Similarly some young stock rats (group D) were killed when their weight was equal to the rats in group A fed the basal diet. The size of the endocrine glands of these rats was measured as before.

Two further groups of female stock rats were weaned onto either the basal diet (group E) or the 10% fishmeal basal diet (group F) as before, but both these groups were

x after having removed the bone etc. to expose this organ lying freely on the brain.

allowed access to a 10% solution of ethyl alcohol in addition to their water supply. The positions on the cage of the bottles containing water and alcohol were interchanged daily. When these rats reached about the same weight as their dietary controls not fed alcohol(groups A and B), the rats were killed and the endocrine glands measured in the same manner as previously.

Effect of Dietary Protein.

Tables 34 (a) and (b) summarise the data obtained from the rats of groups A, B, C and D.

On comparing the rats aged three months of groups A and B, the fresh weights of all the organs were smaller in the rats fed the low protein basal diet. On comparing relative dimensions, however, the thyroid and pituitary were significantly larger in the low protein group (for males $p = 0.02$ and < 0.001 respectively, for females $p = 0.01$ for both glands); the adrenals were also larger but the difference was only statistically significant amongst the females (for females $p = 0.01$; for males $p = 0.75$). This sex difference was also observed by Sarason (1943) who, on completely starving rats for 7 days, obtained a moderate adrenal hypertrophy which was greater amongst the females than amongst the males. There was no significant difference between relative thymus or ovarian weights but the testis weight was lighter in the low protein groups ($p < 0.001$).

TABLE 34a. Effect of the Level of Dietary Protein on the Rat Endocrine Glands(Fresh Weight)

Group	No. of Rats	Sex	Rat Weight (g)	Rat Age (dys.)	Dimensions of Organs-expressed as fresh weight or size				
					Testis or Ovary (g)	Adrenal (g)	Thyroids (g)	Thymi (g)	Pituitary (mm²)
D(Stock)	3	M	47* ± 1	24.7±5.5	0.18 ± 0.02	0.0093± 0.0004	0.0037± 0.0004	0.136± 0.009	0.050 ± 0.003
A(Basal)	6	M	50 ± 2	91.2±2.6	0.20 ± 0.05	0.0083± 0.0008	0.0065± 0.0019	0.064± 0.006	0.056 ± 0.008
B(10%FM)	10	M	196±19	92.8±1.8	1.37± 0.04	0.0174± 0.0011	0.0104± 0.0014	0.230± 0.013	0.091± 0.005
C(Basal)	2	M	198±25	508.5 ± 48.5	1.35± 0.28	0.0125± 0.0014	0.0088± 0.0006	0.058± 0.008	0.098± 0.020
D(Stock)	3	F	36± 4	19.3 ± 2.9	0.017± 0.007	0.0073± 0.0008	0.0034± 0.0004	0.077± 0.029	0.050± 0.003
A(Basal)	3	F	42± 4	84.2± 1.9	0.011± 0.002	0.0097± 0.0007	0.0059± 0.0007	0.074± 0.002	0.052± 0.003
B(10%FM)	6	F	143± 4	93.0± 4.6	0.041± 0.004	0.0206± 0.0034	0.0115± 0.0031	0.238± 0.012	0.095± 0.006
C(Basal)	2	F	152± 3	408.0± 1.0	0.047± 0.009	0.0179± 0.0009	0.0129± 0.0026	0.129± 0.0	0.118± 0.009

* Values given are the mean together with the standard deviation of the mean.

Table 34b. Effect of the Level of Dietary Protein on the Rat Endocrine Glands(Relative wt.)

Group	No. of Rats	Sex	Rat Weight (g)	Rat Age (dys.)	Dimensions of Organs-expressed as per 1000g body weight. Testis or Ovary	Adrenal	Thyroids	Thymi	Pituitary ($mm^2 g^{-1}$)
D(Stock)	3	M	47	24.7	* 3.9± 0.5	0.120 ± 0.041	0.078 ± 0.008	2.92 ± 0.14	1.06 ± 0.01
A(Basal)	6	M	50	91.2	4.0 ± 0.9	0.167± 0.016	0.131 ± 0.038	1.27 ± 0.11	1.13 ± 0.07
B(10%FM)	10	M	196	92.8	7.0 ± 0.1	0.088 ± 0.004	0.052 ± 0.006	1.18 ± 0.06	0.46 ± 0.02
C(Basal)	2	M	198	508.5	6.8 ± 0.6	0.063 ± 0.001	0.045 ± 0.002	0.29 ± 0.0	0.49 ± 0.04
D♀Stock)	3	F	36	19.3	0.51 ± 0.26	0.204 ± 0.023	0.095 ± 0.016	2.06 ± 0.68	1.39 ± 0.10
A(Basal)	5	F	42	84.2	0.26 ± 0.02	0.236 ± 0.019	0.145 ± 0.023	1.80 ± 0.06	1.26 ± 0.08
B(10%FM)	6	F	143	93.0	0.28± 0.02	0.141 ± 0.021	0.080 ± 0.005	1.67 ± 0.11	0.67 ± 0.04
C(Basal)	2	F	152	408.0	0.30 ± 0.05	0.118 ± 0.009	0.085 ± 0.019	0.84 ± 0.01	0.78 ± 0.05

* Values given are the mean together with the standard deviation of the mean.

On comparing the older rats fed basal diet of group C with their weight controls of group B fed the fishmeal diet, none of the differences were significant except that of the thymus which was larger ($p = 0.01$) in the younger animals of group B.

Similarly on comparing the rats fed basal diet of group A with their weight controls of group D fed stock diet, the thymus was again the only gland showing statistical differences, it being smaller ($p = < 0.001$) in the older low protein rats of group A.

From the results using weight controls it appears that the endocrine glands of the low protein rats are reduced relatively to the body weight, with the exception of the thymus, whose atrophy with age continued. The normal growth pattern of the thymus and other lymphatic tissues according to Dougherty (1952) "Does not merely reflect changes in body weight during aging but exhibits a type of growth unique among the tissues of the body."

From the results using age controls of three months, when the control rats were relatively mature, it was seen that in the low protein animals the pituitary, adrenal and thyroid were relatively resistant to the "reducing" action of the diet, that the thymi and ovaries were reduced proportionally to the body weight, but the testis alone had a reduced relative weight.

The relative resistance of the pituitary-adrenal-thyroid axis to protein malnutrition has been noted by other workers, though variable results have been obtained as to whether the weight change, and more especially that of the adrenal, increased or decreased. McCarrison (1918-35) working mainly with pigeons given rice diets, obtained adrenal hypertrophy and a tendency for pituitary enlargement, and the order of decreasing severity of weight loss of the remaining organs was thyroid, ovary, testicles then thymus. Mclennan and Jackson (1933) on feeding a diet containing no protein to adult rats for about 17 days found that the adrenal weight remained nearly constant, the hypophysis and testis lost relatively less weight than the body as a whole, the thyroid loss was proportional to that of the body, while the thymus loss was greater. Samuels (1952) force feeding an isoleucine deficient diet observed adrenal enlargement, no loss in thyroid weight and an atrophy of the secondary sex organs and thymus. Lafon (1939) working with diets deficient in cystine and lysine observed no alteration in the size of the pituitary or adrenal despite a decrease in the weight of the ovary and thymus. Aschkenasy (1953) states that on feeding rats a non protein diet the first organs which became emaciated were the lymphoid organs and accessory genital glands, the testicles, thyroid and adrenals being harmed later.

The greater vulnerability of the testis as compared with the ovaries and thymus was not observed by other workers (Limson and Jackson 1932, McCarrison 1918-35) and Lafon (1939) even obtained an increase in the relative weight of the testis. There is a greater delay for the male than for the female rat to reach maturity on a low protein diet (Slonaker and Card 1918, 1923), and this is reflected in the longer time taken here for the males than for the females in group C to equal their controls in body weight. This probably accounts for the greater reduction in the testis than in the ovarian weights, where at this same age the deficient female is further developed sexually than the male.

Fig. 15 illustrates the relationship between the relative adrenal and thymus weights of the two groups A and B, aged three months. In the low protein rats there was a significant positive correlation between the relative thymus and adrenal weights ($r = 0.96$ for which $p < 0.001$). This correlation was not present in the control group B. The criticism that this correlation may have been a normal one with age, made apparent by the larger age scatter in the low protein group is over-ruled if only the female rats, where there was much less age scatter, are considered. In the low protein females there was a significant positive correlation between relative thymus and adrenal weights ($r = 1.0$ for $p < 0.01$), despite the fact that there was no positive

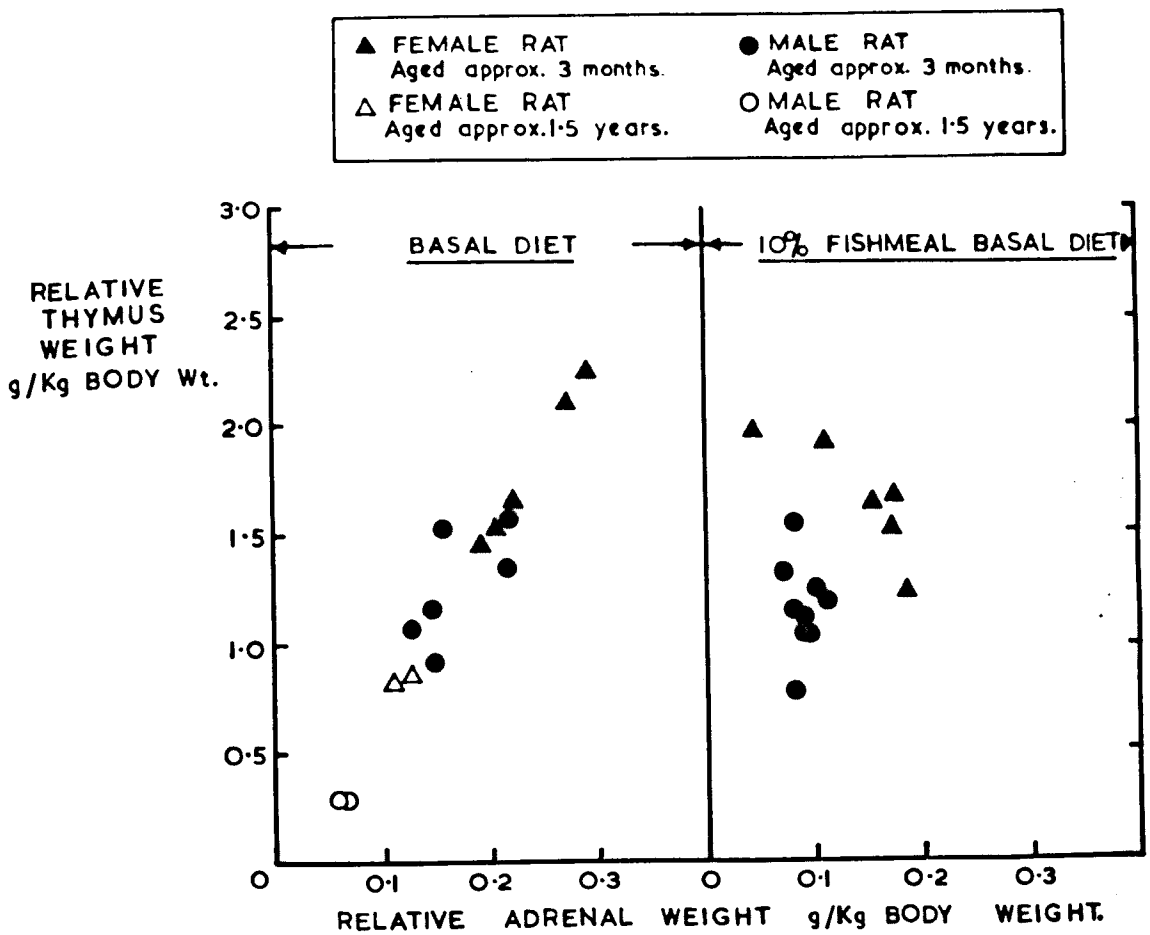


Fig. 15. Adrenal-Thymus Weight Relationships Of Rats Fed Basal And 10% Fishmeal Supplemented Basal Diets.

correlation between age and thymus weights. In the control group of females there was a just significant correlation ($r = -0.8$ for $p = 0.05$) which unlike that of the low protein rats was of negative sign, there being again no correlation between age and thymus weights.

It is unlikely that this correlation represented a normal stage of development which was delayed in the low protein animals, as the same linear correlation still held for the four basal diet rats of group C aged 1½ years (Fig. 15).

In both dietary groups there was a positive correlation between relative thyroid and adrenal weights. (Basal rats: $r = 0.8$ for $p < 0.01$; control rats $r = 0.7$ for $p < 0.01$).

Inhibition of thymus growth is affected by the adrenal cortex (Dougherty 1943) by the gonads (Evans and Simpson 1934) and by the thyroids in the young animal (Best and Taylor 1950). There is a linear relationship between adrenal cortical activity and thymus weight so that it has been used as an assay method for adrenocorticoids (Stephenson 1954). It seems likely therefore that the altered adrenal thymus weight relationship of the low protein animals represents an altered level of adreno-cortical activity, though the possibility of no change in adrenal function but an altered activity of the other endocrine glands, such that adrenal influences predominate, or some

combination of these two effects, cannot be ruled out.

The fact that the thymus body weight ratio is unchanged on the low protein diet is not inconsistent with this theory, since the thymus to body weight ratio of adrenalectomised animals was found to be the same as that of their untreated well nourished controls (Stoerk 1944).

Effect of Alcohol.

The above work concerning the adrenal-thymus weight relationship of the low protein animals suggested an alteration of adrenal function. Since the size of an endocrine gland taken singly is not a sufficient criterion of its activity, it was of interest to investigate any other differences between the dietary groups, such as their response to alcohol.

The figures for the relative weights and sizes of the organs (Table 35) show that the response to alcohol of the two groups E and F, fed the basal and 10% fishmeal diets respectively, differed. Amongst the low protein rats having access to alcohol, there was approximately a 50% reduction in adrenal and thymus weights, unlike the fishmeal group where the weights of these organs remained unchanged. With both high and low protein diets alcohol caused approximately a 60% reduction in thyroid weight and a delay, which was greater amongst the rats fed basal diet to reach the same weight as their controls.

Table 35.

Effect of Alcohol on the Endocrine Glands of Rats fed Two Levels of Dietary Protein.

Group	No. of Rats	Rat Weight (g)	Rat Age (dys)	Dimensions of Organs, expressed as per 1000g Body Weight				
				Adrenal	Thymus	Thyroids	Ovary	Pituitary 2 - 1 (mm g)
A(Low protein)	5	42.0 ± 3.5	84.2 ± 1.9	0.236 ± 0.019	1.80 ± 0.16	0.145 ± 0.023	0.26 ± 0.02	1.26 ± 0.08
E(Low protein + alcohol)	8	42.1 ± 2.2	105.6 ± 0.8	0.145(7) ± 0.005	0.94 ± 0.09	0.091 ± 0.002	0.19 ± 0.02	1.13 (7) ± 0.04
B(high protein)	6	143.3 ± 4.1	93.0 ± 4.6	0.141 ± 0.021	1.67 ± 0.11	0.080 ± 0.005	0.28 ± 0.02	0.67 ± 0.04
F(High protein + alcohol)	9	147.1 ± 4.4	103.8 ± 1.5	0.127(8) ± 0.007	1.54 ± 0.07	0.049 ± 0.005	0.26 ± 0.03	0.72 (8) ± 0.02
Fisher's P for Groups A and E compared.		>0.5	<0.01	<0.01	<0.01	0.01	0.15	0.25
Fisher's P for Groups B and F compared.		>0.5	0.02	0.4	0.3	<0.01	0.6	0.2

Footnotes: (i) the results given are the mean values together with their standard deviation
(ii) the figures (7) and (8) give the number of animals from which the mean value is derived when different from that stated in the second column.

The work of Sanchez Calvo (1941) and other (Hion Jon 1925, Uprus 1921), who obtained thyroid hyperfunction in rabbits following alcohol administration, and of Diethelm (1955) findings of hyperthyroidism common amongst alcoholics, makes it probable that the reduction in thyroid weight obtained here was also indicative of hyperthyroidism, although Aschkenasy-Lelu (1955) using adult male rats found no significant alteration in thyroid weight with alcohol. The increased katabolism resulting from increased thyroid activity with alcohol might cause the delay to reach the desired weight found for both groups of animals.

Acute thymus involution following the administration of thyroxine is mediated by the pituitary-adrenal system (Dougherty et al 1945). Patients with Addison's disease have an increased sensitivity to thyroid hormone (Soffer 1956), and increased sensitivity has also been noted by Zwemer (1927) in adrenalectomised rats. It seems possible therefore, that a very small dose of thyroid hormone, ineffective in a normal animal, might be effective in the presence of a low level of adrenal activity. The reverse has been shown to be true by Brown et al (1956), who found that in adrenalectomised rats maintained on tap water a small dose of cortisone, without effect in normal animals, increased the release of radio active iodine from the thyroid of the operated rats. These authors also state

that a similar acceleration has been observed in cases of Addison's disease treated with small doses of cortisone (Thorn 1950). It would seem credible therefore that the thyroid stimulation, resulting from the alcohol in both groups of animals, led to an increase in adrenal cortical activity, as indexed by the relative thymus and adrenal weights, in the low protein group only, since these animals unlike their controls had an underfunctioning adrenal cortex.

It is concluded from the literature that alcohol stimulates the adrenal cortex (Forbes 1951, 1953, Klieiwe 1955, Goldfarb 1949, Smith 1951), but if administration is prolonged eventually produces hypoadrenalism primarily of pituitary origin (Dowden 1952, Lovell 1951, Goldstein 1951), so that cortical steroid and adrenocortical stimulating hormones are beneficial in the treatment of alcoholism. (Smith 1950b, McAllister 1953, Voeglin 1953).

A possible explanation for the results obtained here might be that the low protein diet, in causing a low level of adrenal cortical activity, as suggested from the adrenal thymus weight relationship (page 117) and from the adrenal histology (page 124), rendered the organism more susceptible to the stimulatory action of alcohol on the adrenal cortex as shown by the reduction in thymus weight. This stimulatory action may be a direct one, or mediated by the pituitary, or by the thyroid-pituitary axis. This supports

the belief of Tintera and Lovell (1949), that pre-existing hypoadrenalism may predispose certain young males to the effect of alcohol. Smith (1950) also is of the opinion that endocrinal dysfunction antedates alcoholism. Results concerning pituitary implants are of interest, for like alcohol, they produce no effect on the adrenal weight of normal animals, while in chronically underfed animals such grafts restore the adrenal size to normal. (Mulinos and Pomerantz 1941b). The low protein animals tended to drink more alcohol than their controls, more animals than those originally used being required to make this difference statistically significant.

Histology of the Adrenal Cortex in Protein Deficiency.

The functional activity of an endocrine gland cannot be determined by its weight alone. For example Gley (1914) found that thyroidectomy in rabbits produced adrenal enlargement but not hyperfunction as the enlargement had resulted from a fatty degeneration of the cells.

The relative adrenal weights were larger in the low protein animals in the present investigation, but the fact that the thymi were enlarged proportionally to the adrenals suggested that the enlarged adrenal cortex was hypofunctional. In order to investigate this further some of the adrenals were examined histologically.

The rats used had been weaned onto basal or 10% fishmeal basal diets in groups A, B and C of the previous experiment. The right adrenal was fixed in Zenker Formal

solution and subsequently stained with Ehrlich's haemotoxylin and eosin, and the left adrenal was fixed in buffered formalin and stained for the presence of fat with oil red O.

Table 36. Number of Rat Adrenals Examined.

Diet.	10% Fishmeal basal diet.		Basal diet.			
Group	B (aged 3 months)		A (aged 3 months)		C (Body wt. = Grp. B)	
Sex	M.	F.	M.	F.	M.	F.
No. of rats	3	4	6	7	0	2

On comparing rats of equal age, the cortex of the low protein animals was narrower and composed of smaller cells than the controls. Plates 4 and 5 show a high power view of the outer part of the cortex, where the zona fasciculata cells of the rat fed basal diet (Plate 5) are much smaller, less regularly and more compactly arranged than in the controls (Plate 4).

A feature in the older low protein rats was the greater width of the transitional zone (Plate 6). This transitional zone lying between the zona glomerulosa and zona fasciculata is roughly co-extensive with the sudanophobic zone (Jones 1957), so a consideration of the

fat stained sections is relevant here. In normal rats the sudanophobic zone is conspicuous in both sexes prior to puberty. It persists in adult males but is absent (Greep and Jones 1950) or only very slightly apparent (Tadokoro 1954) in adult female rats. Plates 7, 8 and 9 show the fat stained adrenal sections. This sudanophobic zone was present in both the control and low protein male rats and as might be expected was much less marked in the three month old control female rats (Plate 7). However it was still conspicuous in both groups of female basal diet rats aged 3 months (Plate 8) and 1½ years (Plate 9). The functional significance of this zone is obscure; ovariectomy after puberty cannot reproduce it though castration or treatment with oestradiol in the male causes it to disappear (Gaunt 1953). It is generally believed that the persistence of this zone indicates a low level of cortical activity (Greep and Jones 1950, Tadokoro 1954).

The density of the fat staining material within the cortex of the various groups was too varied to draw any conclusions. Tadokoro (1954) notes that lipoid droplets show different responses even under the same conditions, and Cain et al (1950) also stress the variability of the lipoid content of the rat adrenals under normal conditions.

In the zona reticularis of the low protein rats aged

both 3 months and 1½ years there was a greater deposition of greenish yellow pigment than in the control rats aged 6 months. Plate 10 demonstrates two clumps of this pigment. These pigment granules, which are more numerous in older animals (Tadokoro 1954, Cowdry 1952), are known to increase under conditions of adrenal hypofunction (Tadokoro 1954). The Gillmans (1951) in their book on human malnutrition note the heavily pigmented zona reticularis which did not contain demonstratable iron in cases of pellagra. Chronic inanition in the rat also results in increased deposition of this pigment (Mulinos and Pomerantz 1940).

The low power views of the adrenal cortex show how the zona reticularis of the control rats (Plate 11) had a lace-like structure not present to the same extent in the basal diet rats of equal age (Plate 13) or weight (Plate 12). As can also be seen from Plate 12, the older low protein rats had an almost continuous band of eosinophilic cells lying between the reticularis and the medulla. These cells are normally found as isolated clumps, and these cells which are enlarged during oestrous (Anderson et al 1932, Bourne et al 1940), are described by Howard (1937-38) as being small groups of cells of the juvenile cortex which may fail to undergo differentiation into the zona reticularis and so persist as structurally isolated groups in the adult. He considers these cells analogous to the mouse X zone, and

describes an exceptional case of this zone being well defined in a castrated rat. The present results concerning the magnitude of this zone in the two older low protein females examined, is of especial interest in view of the report by the Gillmans (1951) that the foetal x-zone persisted in African infants dying of Kwashiorkor. On the other hand protein deficiency (Howard and Benua 1950) and caloric restriction (Deanesly 1938) in the mouse caused a suppression of the x zone.

No difference in the appearance of the zona glomerulosa was observed on comparing the various groups. This might be expected if the changes in the adrenal cortex were caused by an altered pituitary function, as according to the "Zonal Theory" (Greep and Deane 1949) the zona glomerulosa, unlike the remainder of the cortex, is largely independent of the control of adrenocortico-stimulating hormone.

The changes in adrenal structure - increased pigmentation and persistence of the sudanophobic zone-in the rats fed basal diet suggest a lowered level of cortical activity.

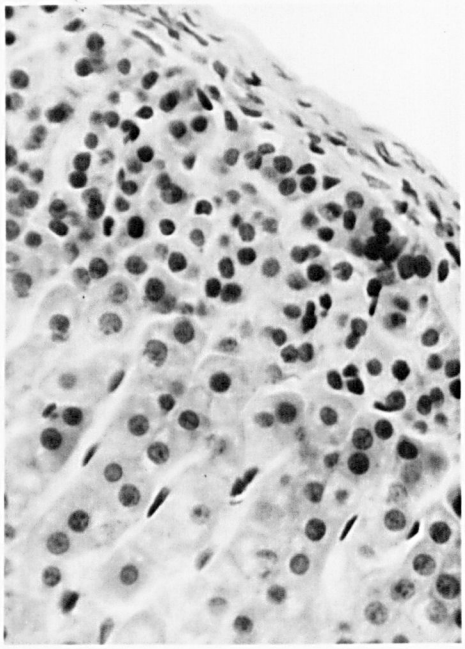


Plate 4
Control rat (aged 3 months)

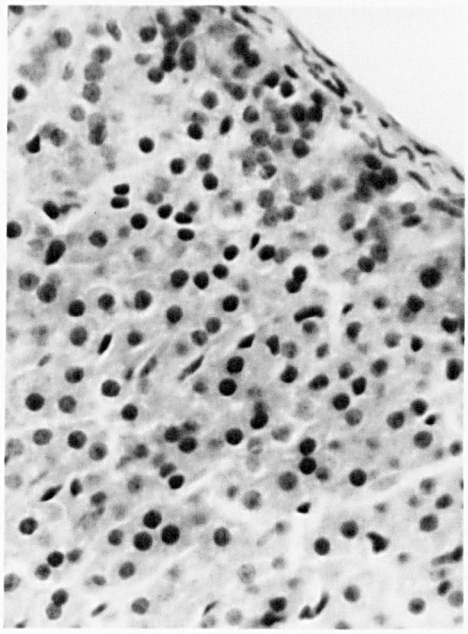


Plate 5
Low protein rat(aged 3 months)

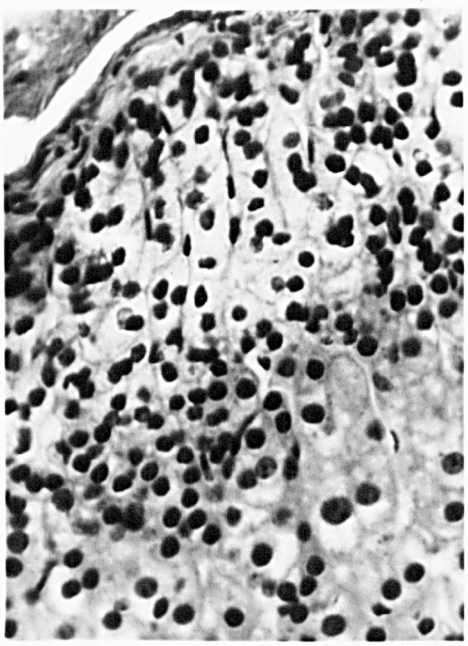


Plate 6. Low protein rat (aged 1½ years)

High Power Views of Outer Part of Adrenal Cortex (x500).

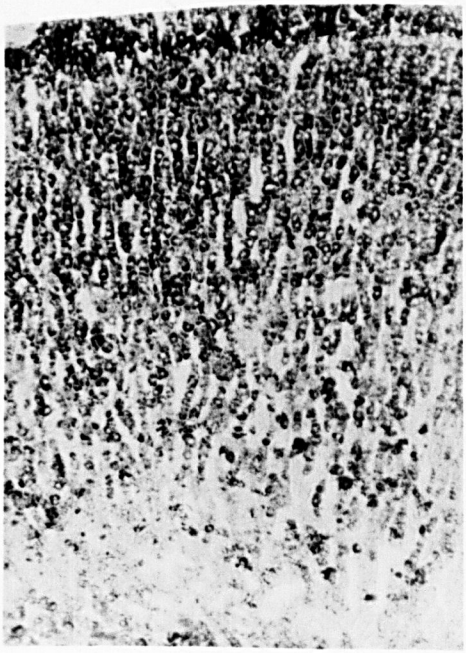


Plate 7.

Female Control Rat.(Aged 3 months). x 96.

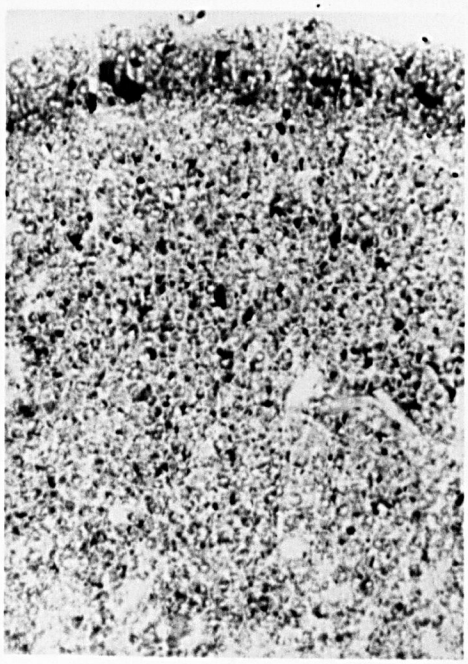


Plate 8.

Female Low Protein Rat.(Aged 3 months). x 120.

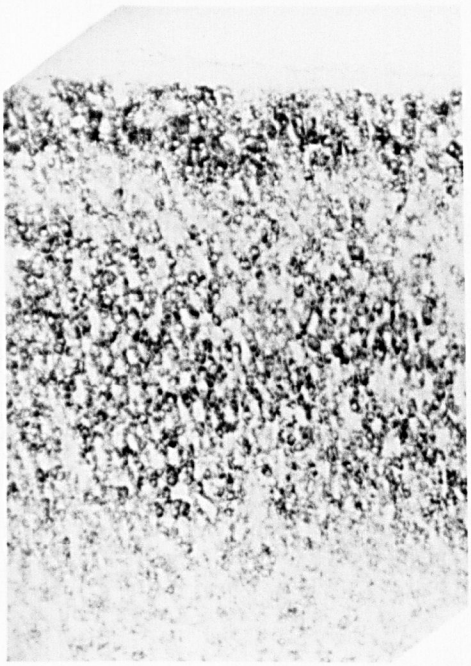
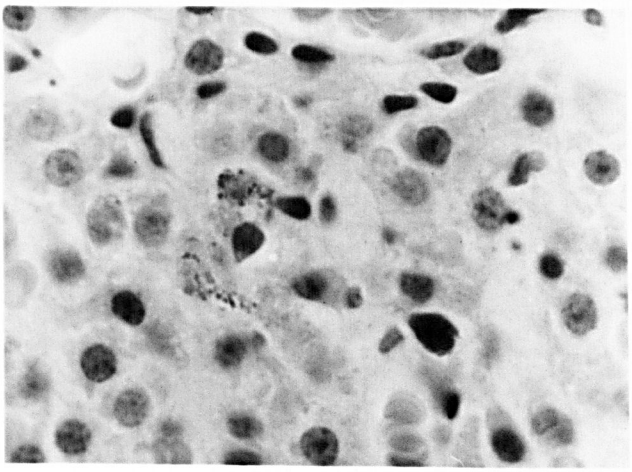


Plate 9.

Female Low Protein Rat.(Aged $1\frac{1}{2}$ years). x120.

Low Power Views of Adrenal Cortex Stained for Fat



(Two clumps of granules are situated almost centrally.)

Plate 10.

Area from Zona Reticularis of a Low Protein Rat to Show Pigment Granules.(x 1000).

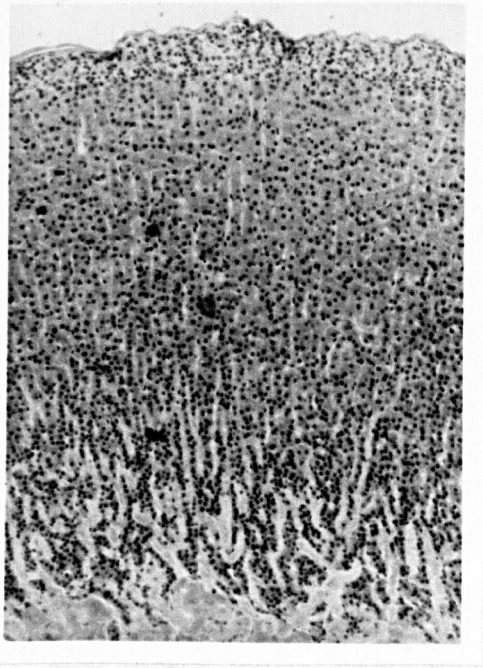


Plate 11.

Control Rat. (Aged 3 months).

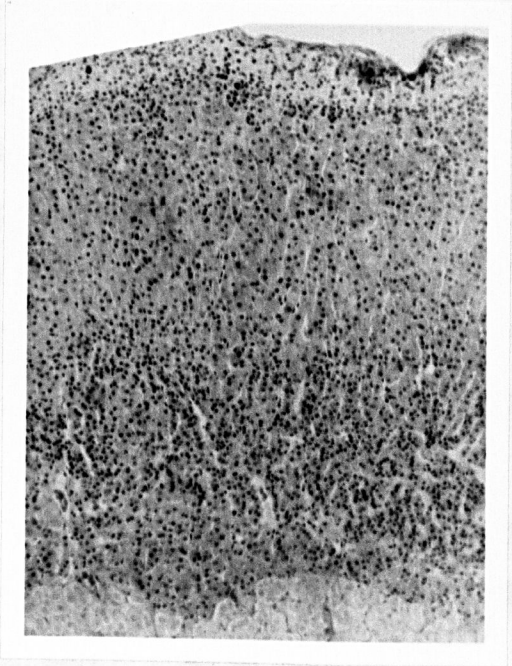


Plate 12.

Low Protein Rat.(Aged 1½ years

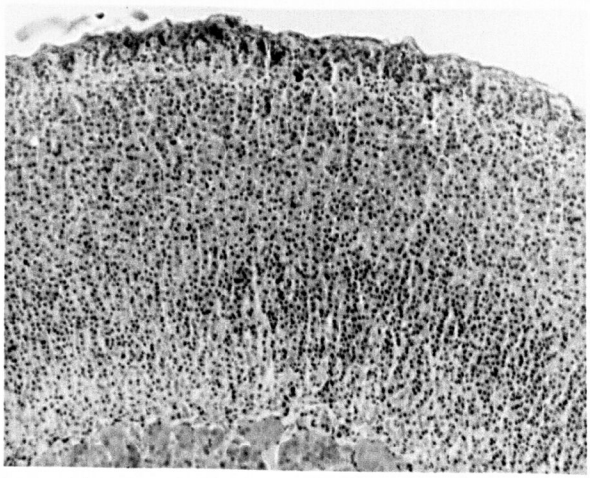


Plate 13.
Low Protein Rat (Aged 3 months).

Low Power Views of Adrenal Cortex. (x 120)

Summary of the Results Obtained.

1. On comparing the endocrine glands of rats aged three months fed basal diet from weaning, with stock rats of equal body weight, there was no difference in the weights of the testis, ovary, adrenal, thyroid or pituitary size but the thymus weight was significantly reduced in the older low protein rats.

2. On comparing rats aged three months fed the 10% fishmeal basal diet from weaning, with rats of equal weight fed the basal diet from weaning, there was again no difference in the weights of the endocrine glands examined above, except in the weight of the thymus which was significantly reduced in the older low protein rats.

3. (a) On comparing rats aged three months fed either the basal or the 10% fishmeal basal diets from weaning, the fresh weights of the testis, ovary, adrenal, thyroid, thymus and the size of the pituitary were all significantly smaller in the low protein animals.

   (b) On comparing the organ dimensions relative to body weight, however, the thyroid, pituitary and adrenals were all larger in the low protein group. These differences were all significant except for that of the male adrenal. There was no significant difference between the relative ovarian or thymus weights, and the relative weight of the testis was significantly less in the rats fed basal diet.

4. In the rats (aged 3 months and 1½ years) fed basal diet from weaning there was a positive correlation between the relative thymus and adrenal weights, not present in the rats fed the 10% fishmeal diet (aged 3 months). In both groups there was a positive correlation between relative thyroid and adrenal weights.

5. Femals rats were allowed from weaning access to 10% ethyl alcohol solution in addition to their usual water supply. These rats were fed either the basal or 10% fishmeal basal diets and killed when they reached the same weight as control rats aged three months fed the same diets without alcohol.
(a) In both the basal and 10% fishmeal groups alcohol resulted in about a 60% reduction in the relative thyroid weight, and a delay, which was greater in the low protein group, to reach the desired body weight. There was no significant alteration in the size of the pituitary.
(b) In the basal group only, alcohol produced about a 50% reduction in relative adrenal and thymus weights, there being no change with alcohol in these organ weights in the rats fed the fishmeal diet.

6. The adrenal cortices of rats aged three months fed either the basal or 10% fishmeal basal diets were examined histologically. The adrenals from two older (1½ years)

females fed basal diet of the same body weight as the "fishmeal" rats were also included.

(a) In rats of equal age the cortex was smaller both in total and cellular size in the rats fed basal diet.

(b) The transitional zone was especially conspicuous in the older female rats. The sudanophobic zone which was barely present in the female rats fed the 10% fishmeal diet, still persisted in the basal diet females aged 3 months and 1½ years. In the male rats this zone was present in both dietary groups.

(c) In the basal diet rats of both age groups there was an increased pigmentation of the zona reticularis which was more compact structurally than in the 10% fishmeal diet rats. The appearance of the eosinophilic cells lying adjacent to the medulla were more marked in the two older basal diet rats where they formed an almost continuous layer.

PART III

DISCUSSION.

Figure I showed how the amino acid content of the basal diet, which was considerably below that of the rat's requirements, was raised to optimal values by the inclusion of 10% fishmeal. The effects of the fishmeal were no doubt primarily the result of its protein content, but it must be admitted that these effects may have been enhanced by unknown factors allied to animal protein. In discussing the etiology of Kwashiorkor, Trowell et al (1954) stated that it was caused by a shortage of protein and possibly factors allied to protein.

The existence of such unknown factors is demonstrated from the fact that it has not yet been possible to make an experimental diet where the protein is derived from synthetic amino acids, and which compares favourably with a natural diet as far as rat reproduction is concerned. In a review of animal protein factors Cravens (1950) concluded that it is a complex of vitamin B12 and one or more unidentified factors. This concept was supported by a review (Anonymous 1953) dealing with the work of Schultze, who found that feeding soya bean oil meal, instead of purified protein with vitamin B12, had a curative effect on the reproductive failure which otherwise occurred in rats. It was concluded that a protein factor was necessary for rat lactation. Piccioni (1951) found that there was a high mortality amongst rats

from mothers receiving a diet containing a mixture of wheat, maize, barley, oats and rye supplemented with wheat germ, calcium lactate, salt and 5% of exhaustively washed casein in addition to fresh vegetables and yeast. Vitamin B12 was found to be ineffective in remedying this mortality when given to the second generation of animals, whereas a small quantity of cow's milk or of crude casein saved the animals. Wiesner and Yudkin (1951) from experiments on the reproduction of rats supplemented with liver concluded that the liver contained factors other than B12 which were required for successful rat reproduction.

To what extent vitamin B12 deficiency contributes to the cause of Kwashiorkor is as yet unknown (Trowell et al 1954). In the present experiments it is thought that the effect of the fishmeal addition was the result primarily of its protein content. Since the poor rate of growth and development found on diets deficient in animal protein can be corrected by vitamin B12 only when the diet is adequate in total protein (Wokes 1955), increasing the protein content of the basal diet would have the indirect effect of increasing the available B12 content of the diet. It is thought very unlikely that this increase of the dietary protein by the inclusion of fishmeal would render the rat deficient in vitamin B12, because fishmeal itself an animal protein rich in

vitamin B12, would contain both protein and B12 in balanced proportions. The experiment described on page 55 supported this view, as the inclusion of vitamin B12 with either the basal or the fishmeal supplemented basal diet produced no increment of growth. Further there was no deterioration in the reproductive performance with successive litters, as has been noted with vitamin B12 deficient rats (Lepkovsky et al 1951).

A survey of the literature revealed that comparatively little work had been done on the reproduction and growth of rats when fed deficient diets from infancy or for more than a single generation. The present experimental work demonstrated the importance of such chronic type experiments, for not only were there differences from one generation to the next in the effects of low protein diets, but when the female rat was first fed the deficient diet from weaning there was a reduction in the weight and size of the litters born, not present when the diet was first fed to the adult female. This the conclusion of Nelson and Evans (1953) that a 6% casein level was critical for normal rat reproduction depended on the fact that these workers first introduced the deficient diet to adult well nourished female rats. The more chronic type of experiment is especially important in relation to human problems, because in most malnourished communities deficient diets have existed for many generations.

The experiment where the fishmeal content of the basal

diet was increased from 0% to 2,5 and 10% during pregnancy showed in a striking manner how the number, weight and survival of the offspring improved with increasing levels of dietary protein, and confirmed the work of others (Nelson and Evans 1953). The number of rats born markedly increased as the dietary protein was raised from 16% to 20% (5% and 10% fishmeal) in contrast to the adverse effects found by Slonaker (1931) as his protein contents were increased from 14% to 18, 22 and 26%. In the present experiments the protein was increased by the replacement of an equivalent weight of starch in contrast to Slonaker's method of straight addition where the increased protein content would result in a proportionate decrease of the other nutrients.

The effects of the low protein diet upon the growth and number of rats born became more apparent with successive generations. This reduction of litter size on low protein diets only seems to have been recorded before by Damodaran (1952), and was not observed by Goettsch (1948, 1949) with successive generations of her low protein rats. It is of interest that when rats and their offspring were bred on the vitamin B12 deficient ration of 60% soya bean, the size of the litters born remained unaffected by the inclusion of vitamin B12 (Emmerson et al 1949). The retardation in growth with successive generations of low protein rats has been noted by others (Damodaran 1952, Goettsch 1949). With diets

deficient in vitamin B12 (Jaffe 1956), there was a reduction in the rate of growth of the second generation, but unlike the results obtained here, there was no further reduction with succeeding generations. The reverse type of result i.e. an increase in growth progressing through many generations of rats has been also observed by Mendel et al(1932). These results would indicate that nurture can influence hereditary characteristics to a marked extent. In fact De Castro (1952) stated that the pygmies of equatorial Africa lose their distinct anthropological features if transported to regions of better diet. Orr and Gilks (1931) on comparing two African tribes of similar origin who are near neighbours, found that the members of the tribe living mainly on milk and meat were far better grown than those of the other tribe subsisting on primarily vegetarian foods.

In a survey of the literature concerning the growth of the blood under conditions of low protein intake, it was noted that some controversy existed as to the priority of haemoglobin over other body protein formation when the supply of dietary protein was inadequate, as well as concerning the role of specific amino acids in alleviating the anaemia of low protein rats.

The experiments where the lysine supplementation of the basal diet caused a marked increase in body growth while having no effect on the relative total red cell volume

of the rats which remained below normal, suggested that the body proteins had priority over that of the blood, in contrast to the views of Whipple et al (1942) and in support of the work of Hallgren (1953).

The results did not confirm the conclusion of Whipple et al (1940) concerning the haemopoetic action of certain individual amino acids under conditions of low protein intake, and they were also contrary to the deductions of Harris, Neuberger and Sanger (1943) who claimed that the lysine supplementation cured the anaemia of rats fed a wheat protein diet. These authors based their results on haematocrit determinations only, but the experimental results obtained here indicated that the raised haematocrit value following the lysine supplementation was the result of a decreased plasma volume, there being no alteration in the total red cell volume. The work performed here supported the opinion of the Ortens (1945) that a combination of amino acids rather than any one specific acid, is required for the synthesis of haemoglobin. An unexpected result was found with the lysine supplementation of the 10% fishmeal diet, which resulted in an increase in male growth only, due to an increase in food intake, there being no alteration in the food utilisation. The possible mechanisms involved are discussed on page 103. It is of interest that the anaemia of the low protein rats did not develop until after weaning, as the time of onset of

Kwashiorkor is also frequently shortly after weaning. (Trowell et al 1954).

The concept just beginning to emerge, that the effects of inadequate protein intakes on growth and reproduction are the result of disturbances in the endocrine system rather than the direct effect of a low protein diet, was supported by the results obtained in the present work. These results substantiated the existence of an altered endocrine balance in the low protein rats.

The unusual adrenal-thymus weight relationship found in the low protein animals did not appear to have been previously recorded and, taken into consideration with the histological state of the adrenal cortex, supported the view of Samuels (1952) concerning the low level of cortical activity caused by inadequate protein intake, and did not support the views of other workers (Sinclair 1953), who believe that the adrenals are hyperactive under such conditions.

The effect of alcohol on the adrenal and thymus weights of the low protein animals only also supported the existence of this altered level of adrenal activity. As there is evidence to suggest that alcohol stimulates the secretion of adrenocortical stimulating hormone (page 123) and since alcohol restored the adrenal weights of the deficient animals to normal, it is thought that the low protein animals were

still capable under certain conditions, e.g. alcohol stimulation, of secreting greater amounts of adrenal cortical-stimulating hormone (A.C.T.H.). This supports Samuels (1952) who believes that though the amount of A.C.T.H. is reduced by a low protein intake; it is capable under stress of being released in normal amounts. The results obtained in this work with alcohol also supported the suggestion that the nutritional and endocrinal concepts of alcoholism (page 110) are related, because a deficiency of the nutritional factors by causing endocrinal dysfunction may predispose the individual to alcoholism.

The presence of such endocrine disturbances in human nutritional problems has been stressed by the Gillmans (1951) who call attention to the many similarities found in protein malnutrition with those typical of endocrine disorders. Salvador and co-workers (1953) concluded from their study of human malnutrition that the sex organs were hypofunctional largely as the result of the pituitary influence, as well as the adrenals and possibly the thyroid. These workers do not say what type of malnutrition was present, but owing to the relatively greater cost of protein foods, it is assumed that this was the prime deficiency. Evidence for their conclusions was obtained both from morphological studies as well as functional tests of the endocrine organs. The

p resence of gynocomastia common in African males has also pointed to the involvement of the endocrine system (Davidson 1954, Trowell et al 1954). Salvador et al (1953) emphasized that this occurred only during the recovery period and presented evidence that it was due to rhythmical variations in the output of gonadotrophins, and did not support the view held by previous observers concerning the involvement of the liver's inability to inactivate oestrogen in gynocomastia.

The work undertaken here has shown how the low protein diets compared unfavourably with an adequate diet in promoting total body growth, growth of the blood and reproduction; and the experiments, on such animals, concerned with endocrine gland growth suggest their involvement in these effects.

SUMMARY.

1. Rats fed the low protein Gambia type diet (basal diet) had reduced rates of growth, a delay in attaining maturity, impaired powers of food utilisation and an inability to produce a second generation, as compared with the more favourable performances obtained from the rats fed either stock diet, or basal diet supplemented with a ten per cent. level of fishmeal. The majority of these effects were more pronounced when the deficient diet was introduced from weaning rather than just prior to mating, and the males were affected more than the female low protein rats.

2. The inclusion of vitamin B12 with either the basal or the 10% fishmeal supplemented basal diet produced no alteration of rat growth or food intake.

3a. The increasing levels of fishmeal supplementation (2%, 5% and 10%) added to the basal diet during pregnancy and lactation produced a graded improvement in the reproductive performance, to values still inferior to those of the stock rats.

3b. The effects of these low protein diets upon litter size and growth became more severe with succeeding generations.

4. The haematocrit values of the basal diet rats subsequent

to weaning fell below, and at the end of a year were still inferior to, those of the stock animals. The basal diet rats (aged 11 weeks) had lower relative red blodd cell volumes (mls per 100g body weight) and higher relative plasma volumes than the rats receiving a 10% fishmeal dietary supplement.

5. The addition of lysine to the basal diet produced an increase in body weight and appetite, a more efficient utilisation of the food, a reduction in the relative plasma volume, but no alteration in the relative red cell volume. The addition of lysine to the 10% fishmeal supplemented diet produced no alteration in the above values except that it caused an increase in the growth of the male rats only, due to an improvement in the appetite of these rats.

6a. When rats fed either the basal or the 10% fishmeal basal diet were compared on an <u>equal body weight basis</u> (c.a.45g and c.a.170g) there was no significant difference in the dimensions of the ovary, testis, adrenal, thyroids or pituitary but the thymus was significantly lighter in the older low protein rats.

6b. On comparing such rats on an <u>equal age basis</u> (c.a.90 days), though the fresh weights of these organs were

all smaller in the low protein animals, the relative weights of the adrenal, thyroid and pituitary size were larger in the low protein group, those of the ovary and thymus were unaffected, and the relative weight of the testis was reduced in the low protein group.

6c. Amongst the rats fed the unsupplemented diet there was a positive correlation between the relative adrenal and thymus weights not present in the fishmeal group.

6d. There were histological changes present in the adrenal cortices only of the rats fed the unsupplemented basal diet, which were similar to those found in hypofunctional states of this gland.

6d. The response to alcohol ad lib. when given to female rats of these two dietary groups differed. In the low protein group only alcohol produced about a 50% reduction in relative adrenal and thymus weights. In both groups alcohol resulted in about a 60% reduction in the relative thyroid weight and a delay to reach the desired body weight, (approx. 40g and 145g for low and high protein rats respectively).

7. This work has shown the importance of fishmeal supplementation of the diet of the rat in counteracting the effect of protein malnutrition on several aspects

of growth and on reproduction. If this work on the rat is applicable to man, as is probable, it is of practical interest since fishmeal is a virtually untapped protein source.

FIRST GENERATION - REPRODUCTIVE RESULTS.

Supplemented Diet during Pregnancy and Lactation	First Litter										
	Litter at birth					Litter at 3 Weeks				Mean Dam Weight	
	No. of Litters	Mean No.pups /litter M.	F.	Mean pup weight M. (g)	F. (g)	Mean No.pups /litter M.	F.	Mean pup weight M. (g)	F. (g)	Post partum (g)	Loss/Gain 3 Weeks (g)
Basal diet	3	1.3	1.7	3.5	3.9	-	-	-	-	118	-
2% Fishmeal basal diet	6	2.5	1.0	4.5	4.0	0.2	0.3	18.0	16.5	120	+22
5% Fishmeal basal diet	6	3.7	3.5	4.5	4.1	2.0	1.2	12.2	12.5	128	+1
10%Fishmeal basal diet	6	3.8	3.3	4.4	3.8	2.0	2.0	26.3	25.4	137	+15
Stock diet	6	4.2	4.3	6.0	5.3	4.2	4.3	36.8	34.9	214	+11
	Second Litter										
Basal diet	1	3·0	1·0	4.7	5.0	2.0	0	15.5	-	118	-11
2% Fishmeal basal diet	6	2.5	2.3	4.3	4.0	1.3	1.2	14.2	13.1	142	-2
5% Fishmeal basal diet	6	3.3	3.3	5.3	4.9	1.3	1.8	19.8	18.5	157	-3
10% Fishmeal basal diet	6	4.0	3.7	5.1	5.0	4.0	3.2	26.4	25.8	162	+16
Stock diet	6	4.7	4.5	5.9	5.3	4.3	4.5	32.9	31.7	223	+16

SECOND GENERATION - REPRODUCTIVE RESULTS.

Supplemented Diet during Pregnancy and Lactation	No. of Litters	Litter at Birth: Mean No. Pups/ Litter	Litter at Birth: Mean Pup Weight (g)	Litter at 3 Wks.: Mean No. Pups/ Litter	Litter at 3 Wks.: Mean Pup Weight (g)	Litter at Weaning: Mean No. Pups/ Litter	Litter at Weaning: Mean Pup Weight (g)	Mean Dam Weight: Post Partum (g)	Mean Dam Weight: Loss or Gain 3 wks (g)	Mean Dam Weight: at Weaning 5 wks (g)
		M. F.	M. F.	M. F.	M. F.	M. F.	M. F.			
First Litter										
5% Fishmeal basal diet	4	2.8 1.8	4.3 4.0	1.8 1.8	13.4 13.4	1.5 0.5	19.5 13.0	111	-6.7	99
10% Fishmeal basal diet	8	3.1 3.6	4.9 4.7	2.8 3.3	20.6 20.0	2.5 3.3	30.9 30.0	131	-0.5	123
Stock diet	7	2.9 4.9	6.1 5.7	2.9 4.4	41.0 37.1	Weaned at 3 weeks		199	+22.7	-
Second Litter										
5% Fishmeal basal diet	4	1.3 3.3	5.1 5.0	1.0 2.0	12.8 12.3	0.8 0.8	15.0 16.3	132	-13.0	111
10% fishmeal basal diet	8	3.0 2.6	5.3 5.4	2.8 2.6	23.9 24.9	2.6 2.6	33.1 31.2	160	-12.6	140
Stock diet	7	5.3 4.3	5.2 5.1	5.1 4.3	35.7 35.8	Weaned	at 3 weeks	225	+19.6	-

THIRD GENERATION - REPRODUCTIVE RESULTS.

Supplemented Diet during Pregnancy and Lactation	No. of Litters	First Litter														
		Litter at Birth				Litter at 3 Wks				Litter at Weaning				Mean Dam Weight		
		Mean No. Pups / Litter		Mean Pup Weight (g)		Mean No. Pups / Litter		Mean Pup Weight (g)		Mean No. Pups / Litter		Mean Pup Weight (g)		Post partum	Loss or Gain 3Wks (g)	at Weaning 5Wks. (g)
		M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.			
10% Fishmeal basal diet	8	2.4	2.8	5.0	4.9	1.6	2.1	20.3	20.4	1.6	1.8	26.4	29.9	133	+2.6	128
Stock diet	8	4.1	4.4	5.4	5.0	3.9	4.4	37.2	35.7	Weaned at 3 Wks.				210	+26.5	-
		Second Litter.														
10% Fishmeal basal diet	8	2.1	3.6	4.9	4.9	1.4	2.1	20.4	23.5	1.4	2.1	31.1	33.0	161	+1.5	138
Stock diet	8	4.8	3.9	5.8	5.4	4.4	3.5	38.1	37.0	Weaned at 3 Wks.				233	+13.4	-

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