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Diet in relation to reproduction and to rearing of young rats

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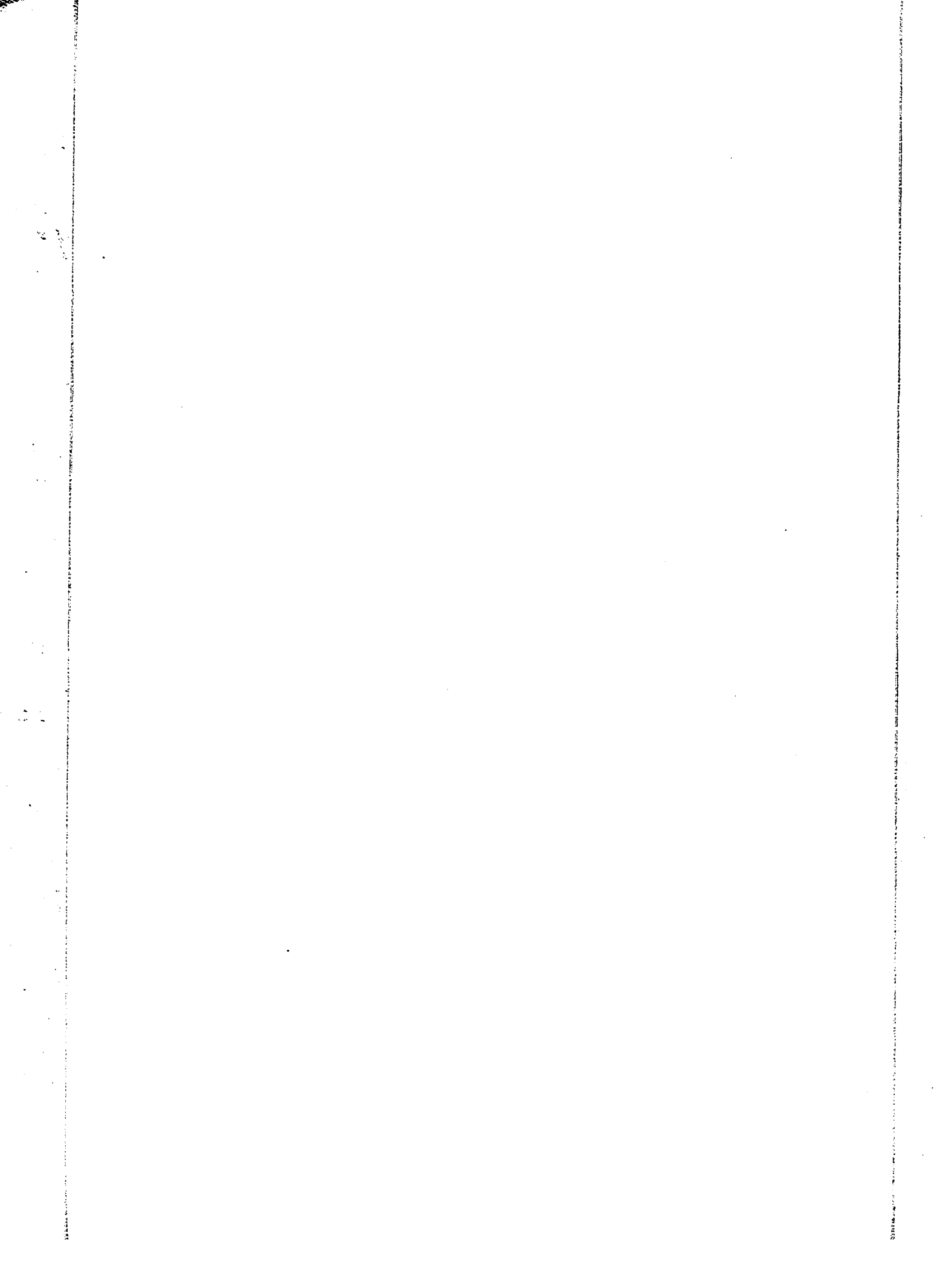
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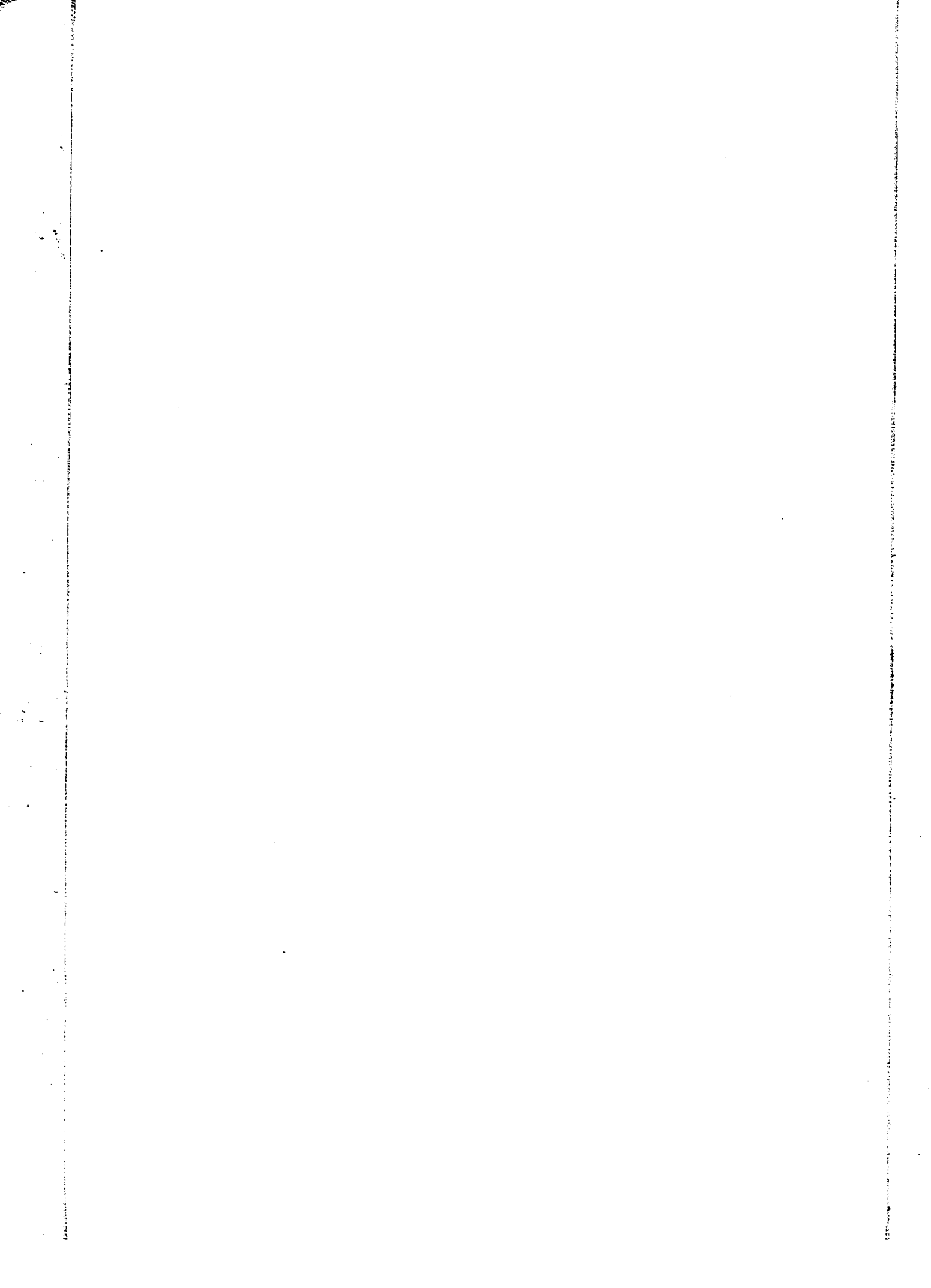
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DIET IN RELATION TO REPRODUCTION AND TO
REARING OF YOUNG RATS

BY

Paul D. Wilkinson

A Thesis submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject
Physiological and Nutritional Chemistry

Approved

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INTRODUCTION

A Brief Discussion of the Problem of Nutrition

For many years man has put a large amount of time on the study of the problems of nutrition. As with most of his investigations the problems were approached from the economic point of view. That is, he was interested in finding the cheapest food that would enable his stock to grow faster and give the best quality of meat. It is interesting to note that, for possibly centuries, the nutritional requirements of man himself were of secondary consideration. Apparently man was more interested in feeding his stock than in feeding his children. It was only when a large group of human beings was under the direct supervision, control, or pay of some organization that their health, from a nutritional point of view, was considered. This was due to the fact that a sick man did not give the service paid for by his employers, and in institutions cost considerably more to care for than a well man.

The marvelous advance in science that has been taking place for the last fifty years has been accompanied by a universal desire, not only to lighten man's labors but also to free him from bodily ills. Scientific investigations of various dietaries, along with historical study of the incidence of various diseases, have led to the discovery of dietary essentials hitherto unsuspected. As a result of the tremen-

dous amount of investigation that has been carried on for the last quarter of a century, the materials that must be included in the diet have been classified as follows: proteins or amino acids, minerals or salts or inorganic substances, vitamins A, B, C, D, E, G, possibly a lactation factor and water. These various factors will be discussed in detail later.

It will be observed that fats and carbohydrates have been left out of the list of dietary essentials. Osborne and Mendel (1), feeding a highly extracted diet, obtained results warranting the conclusion that, if true fats are essential for nutrition during growth, the minimum necessary must be exceedingly small. On the other hand Burr and Burr (2) conclude from their experimental evidence that a rigid exclusion of fat results in a new deficiency disease. Carbohydrates are valuable chiefly as a source of energy, and they can be replaced as such by fat or protein. When so replaced, the body has the ability to synthesize the carbohydrate that is necessary for tissue metabolism. Because of its cheapness carbohydrate is used as much as possible in nutritional experiments. However, according to Newburgh and Marsh (3), high protein diets may cause renal injury, due to the passage of large amounts of amino acids. Other investigators have reported lesions and kidney disturbance with high protein diets. On the other hand Osborne and Mendel (4) obtained satisfactory growth with diets containing ninety per cent protein. And, too, the Eskimos, who consume very little

carbohydrate, are known as a particularly hardy and healthy race. In this instance the carbohydrate is largely replaced by fat as well as protein.

Protein.

Protein has long been recognized as the most prominent component of the body tissues, except the skeleton. Since Carl Voit (5) showed that an animal receiving adequate protein was in nitrogen equilibrium, research on protein has gone on apace and the literature on the subject is voluminous. Among the many objectives of this research, two are of outstanding interest to the present day student of nutrition. They are, first, the number and kind of amino acids necessary for the repair of tissue wastes, and second, the physiological effects of nutrition with the protein intake at different planes. The number of amino acids that have been recovered from proteins has been increasing from year to year. Gortner (6) in his recent book lists no less than thirty-one. As nobody has succeeded in completely analyzing protein, due to the formation of humin, there still remains the possibility of more amino acids being discovered. It is indeed fortunate that all of these acids are not essential in appreciable quantities. Much work has been done to find those essential to normal metabolism and the foods that contain them. This work has been greatly hampered by the difficulty in obtaining adequate quantities of the acids in a pure form. The problems involved have not, by any means, been solved as yet.

Under carefully controlled conditions, it has been found that the protein intake necessary to maintain a nitrogen balance can be reduced to a very low level. However, as a considerable excess of a good quality of protein has not been proven injurious, it is usually customary, in nutritional experiments, to feed somewhat of an excess over the minimal requirements. A number of natural foods, such as eggs, milk, mixtures of certain grains etc. have been found to contain adequate protein for growth. Casein has been found to be the most satisfactory of the purified proteins from the standpoint of availability and of composition. Mendel (7) has found an eighteen percent level of casein as the sole protein to give satisfactory growth. This level of casein as the source of adequate protein has been used by a great many investigators, and is the level used in the experiments described in this thesis.

Minerals.

As we study the history of food supply, we find that one of the dominating influences on the immigration of people is the accessibility of salt. It is also known that animals will undergo the most severe hardships in order to reach a salt supply. Following this lead, those experimenting in nutrition always saw to it that their experimental animals had salt (sodium chloride) included in their diet. Next, in order, were included calcium and phosphorus, because they were recognized

as essential to bone development. Then came iron, magnesium, potassium, and sulphur, because analysis showed that they were present, in appreciable quantities, in all animals.

With gradual increase in purity of the experimental diets, contaminating but essential elements were eliminated. This led to an era of severe investigation of the metallic, inorganic, or mineral requirements of the animal. Many combinations have been, and are being, tried. Such elements as fluorine, copper, and manganese are being recognized as exerting a profound influence on metabolism. Some of the elements not ordinarily listed as essential have been included in many so-called purified diets as impurities.

The salt mixture used in this series of investigations is one worked out by McCollum and co-workers (8,9). It is termed, by him, Salt Mixture 185, and will be so called in this thesis. While this salt mixture may not be optimum, it does give satisfactory growth and reproduction, when used in conjunction with other highly purified food materials. Many investigators have used this salt mixture in experiments covering numerous generations.

Vitamins.

The vitamins A, B, C, D, E, and G, have been recognized and acknowledged by the American Society of Biological Chemists. It is probable that other vitamins will be discovered. A very brief resumé of the known vitamins follows.

Vitamin A. Vitamin A was discovered, almost simultaneously, by McCollum and Davis (10) and by Osborne and Mendel (11). It was found as a growth promoting factor occurring associated with fats and lipoid substances. The evidence, so far, indicates that it is synthesized only by plants and not by animals. It is a necessary factor in the diets of all animals that have been investigated up to the present time. The vitamin A content of animal products is dependent upon the food material fed the animal. The primary effect of vitamin A deficiency is a keratinization of the epithelial tissues and a resulting failure of growth. Xerophthalmia is a secondary infection.

Investigators have used numerous food materials as a source of this vitamin in synthetic diets. Those foods that are most easily available, and also give the most consistent results, are butter fat and cod liver oil. Vegetable foods are not used because they also contain considerable amounts of other vitamins -- notably B and G. In this series of experiments a five percent level of butter fat is used as the source of vitamin A. Its preparation will be described under "Experimental".

Vitamin B. Vitamin B is also referred to in the literature by the letters B, F, and B-P. This confusion is a result of the separation of vitamin G from substances known to contain the heat labile, beri-beri preventative factor discover-

ed by Eijkman (12). The anti-neuritic factor known as vitamin B was the first of the vitamins to be discovered and has probably been investigated more thoroughly than any of the other vitamins. While it has not been isolated, it has been purified to the point where a daily dose of about 0.015 mgm. is sufficient to protect pigeons from polyneuritis. Vitamin B, besides preventing polyneuritis or beri-beri, also exerts a profound effect on the appetite. It should be noted that the nervous disorders characteristic of vitamin B deficiency cannot be induced by starvation. Among those foods most commonly used as a source of vitamin B in experimental rations are yeast, rice polishings, and wheat embryo.

Vitamin C. Vitamin C, the anti-scorbutic factor, will not be discussed, as it has been definitely shown by a number of investigators that the rat not only does not need this vitamin in its diet, but that it also has the ability of synthesizing it.

Vitamin D. While a number of investigators recognized rickets as a dietary disease, it remained for McCollum (13) and co-workers to demonstrate the existence of vitamin D and its relation to rickets. This demonstration stimulated an enormous amount of work which has resulted in a much better understanding of methods for the prevention and cure of rickets. A number of investigators noted at approximately the same time that cholesterol acquired anti-rachitic properties

when irradiated with ultra-violet light. This has more recently been shown to be due to the action of the light on ergosterol.

Realizing that rickets is closely associated with calcium and phosphorus metabolism investigators succeeded in showing that with a properly balanced calcium and phosphorus intake, the onset of the disease could be avoided. Such a balance is approximately furnished in the diet of the rat by a three and seven-tenths percent level of McCollum's salt mixture 185 (8).

Vitamin E. Vitamin E was discovered by Evans and Bishop (14,15) and was defined by them as being a substance, the absence of which causes death and resorption of normally implanted foeti. Experiments on this vitamin have so far been confined to rats. After the female has given evidence of vitamin E deficiency, administration of small quantities of the vitamin usually results in restoring the ability to produce normal litters. On the other hand permanent sterility of males on a vitamin E deficient diet has been repeatedly observed. The vitamin has been found to be very widely distributed in natural foods, the most potent substance probably being the oil extracted from wheat germ.

References to some of the investigations carried on in connection with this vitamin will be found under "Review of Literature".

Vitamin G. Vitamin G is also referred to in the litera-

ture as B₂ and P-P. Its property of preventing pellagra was long attributed to vitamin B. The discovery of this vitamin is credited to Goldberger (16,17) and his associates. The vitamin is necessary for growth and is usually associated with vitamin B. It can be readily prepared free of vitamin B, due to the fact that it is heat stable, while vitamin B is readily destroyed by heat. At present no adequate method has been devised for destroying vitamin G in the presence of vitamin B. Foods of vegetable origin are usually rich in vitamin B and poor in vitamin G. The reverse holds true for foods of animal origin. However, yeast seems to be rich in both.

Lactation.

Investigation of diets by feeding experiments on rats has been carried on for a number of years. In the early experiments the animals were carried for only a few months to observe variations in growth curves. Using highly purified diets it finally became possible to obtain normal growth, but trouble was experienced in raising several generations.

Shortly after Evans reported vitamin E, Guest, Nelson, Parks, and Fulmer (18), working on grains, reported difficulty with lactation and suggested that the amount of vitamin B required for growth may not be ample for lactation. They also suggested a possible optimum relative concentration of the several dietary factors involved. Sure and a number of others have reported experiments that support these observations. Some of this work will be referred to later.

Statement of the Problem

In the present day of rapid communication and transportation, food materials that formerly had little economic importance in this country are rapidly becoming of national significance. Such rapid adoption of a new food material has a certain element of danger. Scientists now recognize the fact that all foods, both new and old, should have their dietary value rigidly investigated. Such an investigation might lead to a general adoption or rejection of a given food material. At any rate, its nutritional value would at least be known.

In the study of such individual foodstuffs the rat has proved of great value. The short life span of this animal coupled with the fact that its nutritional requirements are similar to other mammals makes it very suitable for experimental work. The comparatively small size of the rat and its consequent limited food consumption makes it a desirable animal to use when highly purified materials are fed. The usual practice is to feed a highly purified diet supplemented by the food material under investigation. In studies of this type it is customary to start with young rats and observe growth, reproduction and lactation. Investigations involving the use of the rat and highly purified diets have resulted in the discovery of a number of the dietary essentials previously enumerated. Although the existence of vitamin E has been definitely established, it is recognized that other factors

may influence reproduction. A number of investigators have also reported difficulty with lactation, even though a diet thought to contain adequate amounts of all the known essentials was used.

The experiments described in this thesis were designed with the idea of investigating various food materials. The rat was the experimental animal and, although growth was studied in some cases, particular attention was paid to reproduction and lactation. It was hoped that a clearer understanding of the dietary influences on reproduction might be obtained. In view of the fact that human mothers are having difficulty feeding children from the breast it was further desired that the way might be pointed to a diet that would give superior lactation.

REVIEW OF LITERATURE

Human beings and farm animals are dependent upon seeds and grains for a considerable portion of their food supply. Many of these substances have been the subject of rigid dietary investigations within recent years — especially the grains — because of their great economic importance. Soy beans, which have been used as a food by Chinese and Japanese for hundreds of years, are becoming of great importance in the United States. There are many varieties of soy beans known and the color of the bean varies from the light yellow of the Manchu to the black of the Sable. Grantham (19) reports the analysis of seventeen varieties, and he does not include all. In his series of analyses he found that those varieties having the most protein had the least oil. Neumann (20) gives a more complete analysis of soy beans and reports the average composition as being: water 10.93%, protein 33.58%, fat 17.06%, carbohydrate 28.76%, roughage 4.85%, and ash 4.81%. In a series of metabolic investigations on human subjects he found soy bean bread slightly inferior to rye-wheat bread. Daniels and Nichols (21) found that a sixty per cent level of soy bean furnished adequate protein and a sufficient amount of vitamins A and B for normal growth. In the absence of butter fat they observed failure of young. The soy beans used in their experiments were cooked at fifteen pounds pressure. Somewhat later Daniels and Hutton (22),

feeding milk alone, failed to get reproduction. They found that supplementing the milk with soy beans or soy bean ash corrected the deficiency. They also obtained reproduction when they used, instead of soy bean ash, a mixture of manganese sulphate, sodium fluoride, aluminum sulphate, and sodium silicate. McCollum, Simmonds, and Pitz (23) observed that a twenty-five percent level of soy bean gave adequate vitamin B for growth. They cooked their soy beans one and one-half hours at fifteen pounds pressure. Osborne and Mendel (24) found that good reproduction and normal growth of young could be obtained on a diet containing fifty percent of soy bean as the only source of protein and vitamin B. The diet they used included eighteen percent of butter fat. Plimmer, Raymond, and Lowndes (25) in establishing comparative vitamin B values of foodstuffs gave soy beans a value of thirteen, as compared to yeast with a standard value of one hundred. They propose a thirty percent level of soy bean, as the sole source of vitamin B, as being necessary for maintenance.

Guest, Nelson, Parks, and Fulmer (18) made extensive studies on various grains as the only source of vitamin B. They employed a highly purified diet consisting of casein, salt mixture, butter fat, various amounts of grain, and sufficient dextrin to make one hundred percent. The grains tested were: wheat, rye, barley, white and yellow corn. They fed these grains at levels all the way from five to seventy-three percent. They found that with a sufficient amount of any one

of these grains in the diet the rats grew and reproduced normally but that lactation was poor, which fact was manifested by a high mortality of the young. For instance, they found that, on the wheat rations, the mortality of the young from the first generation females varied from thirty-five to one hundred percent, on the rye diets young of the first generation had a mortality between forty-one and one hundred percent, on barley the mortality varied between twenty-one and one hundred percent, on white corn the young of first generation females gave a mortality of seventy-one to one hundred percent, and in the case of yellow corn the mortality of the young from the first generation varied between fifty-one and ninety-three percent.

Meat of various kinds has always been and always will be one of the main articles in the diet of man. The literature on the role of meat in the diet is very extensive. However, most of it has little application to the problem at hand, which consists of a study of the supplementary value of the various organs for lactation. Muscle meats, as reported in the literature, seem to have little dietary value outside of their protein content. Watson (26), feeding one hundred percent ox flesh, reported poor reproduction and lactation. He concluded that an increased meat consumption has a bearing on the failure of mothers to nurse their young. Osborne and Mendel (27) showed beef muscle to be deficient in vitamins A, B, and C. On the other hand they found liver to be a good source of vit-

amins A and B. Cooper (28), working on polyneuritis, made preparations from animal tissues that would cure the disease. His results show the presence of vitamin B in a number of foodstuffs, among which are to be found brain, liver, and egg. McCollum and Davis (29) while endeavoring to prepare purified food substances demonstrated that pig liver and codfish testicles contain vitamin A. They also found that heart contains less vitamin A than kidney. McCollum, Simmonds, and Parsons (30) in a study of the nutritive value of animal tissues fed a twenty-five percent level of thoroughly cooked kidney, liver, and muscle tissue as the sole source of protein. They obtained four generations and report liver and kidney as containing vitamins A and B. Hitchcock (31) tried supplementing the diet of nursing mother rats with meat. He does not report the composition of the balance of the diet, but from the text it appears to have been his regular stock ration. He observed an increased rate of growth of the young. Rosenheim and Webster (32) report the vitamin A content of the liver fat of sheep, calf, and ox to be about ten times that of Newfoundland cod-liver oil. Evans and Burr (33) found that a diet consisting of pure casein, sucrose, salts, and high levels of vitamins A, B, D, and E was inadequate for growth. Upon supplementing this diet with lettuce, liver, or lard they obtained good growth. Laqueur, Wolff, and Dingemans (34) observed that hog liver contained less vitamin A than the liver of cattle. They also report a variation of the vitamin A content of the

livers with the season. Aykroyd and Roscoe (35) using Chick and Roscoe's method (36) for determining vitamin B₂ found that wheat, maize, and dried peas were relatively poor in this vitamin, while ox liver, yeast, and fresh whole milk were all good sources of it.

Feeding experiments necessarily require a very carefully planned diet. Investigators are continually seeking single food substances which will supply only one factor of a complete diet. In case of vitamin A this problem is met by most experimenters by feeding butter fat or cod liver oil. However, with vitamins B and G there is considerable difficulty because of the frequent occurrence of the two vitamins in the same food material. At the present time no method has been devised for destroying vitamin G without affecting vitamin B. Yeast has been used to a great extent as a source of vitamin B ever since Hopkins (37) first used it in dietary studies in 1912. Kennedy and Palmer (38), using a highly purified diet, showed that yeast stimulated growth in rats. They observed failure of reproduction but did not attempt to account for it. Somewhat later Palmer and Kennedy (39) looking for another source of vitamin B found that extracted wheat embryo when used in a highly purified diet as the sole source of vitamins B and G did not give adequate growth. Chick and Roscoe (40) in studies on the then so-called vitamin B found yeast to contain both vitamins B and G. They report wheat embryo as being rich in vitamin B but poor in vitamin G. They also observed that

vitamin B could be prepared in a fairly pure form by Peter's (41) method and that it could be destroyed by heating at 120° for four to five hours. However, Williams, Waterman, and Gurin (42) warn against too great confidence in the current practice of autoclaving yeast to destroy vitamin B and leave vitamin G. They show that results may be greatly influenced by differences in pH. Sherman and Axtmayer (43) made a study of the multiple nature of the so-called vitamin B and found yeast to contain both factors in about equal amounts, whole wheat to be richer in vitamin B, and milk to be richer in vitamin G. In an interesting series of experiments they fed a highly purified diet containing neither vitamin B nor vitamin G and obtained practically no growth. Supplementing this diet with eight percent of autoclaved yeast gave very poor growth, while eight percent of whole wheat gave slightly better results. When, however, the purified diet was supplemented with four percent of autoclaved yeast and four percent of whole wheat practically normal growth was obtained. Evans and Burr (44) in a study of the dual nature of vitamin B found that both commercial casein and corn starch contain a growth promoting factor. They also suggest that possibly lard contains a new growth promoting factor. Hogan and Hunter (45) proposed destroying vitamin G without affecting vitamin B by irradiation with ultra violet light but their work was not substantiated by Kennedy and Palmer (46).

The literature on the problem of lactation has become

very extensive within recent years. Most of the work has led to the conclusion that there is an intimate relation between the consumption of vitamins B and G and the growth and well being of the suckling young. Osborne and Mendel (47) as a result of their experiments on vitamin B, concluded that animals do not have the ability to store vitamin B and that it must be continuously supplied in the diet. Evans (48) proposed the possibility of a new dietary factor or possibly larger quantities of certain dietary elements as being necessary for lactation. He concluded that if there is a lactation factor it is not soluble in fats. Sure (49) reports work indicating that the vitamin B requirement for lactation is somewhat greater than that required for growth. Hartwell (50) as a result of a study of the diet of the lactating rat concluded that vitamin B was intimately associated with protein metabolism. Similarly, Nelson (51), also working on the relationship of the diet to lactation, reports an increased amount of vitamin B as being necessary with a high protein diet. On the other hand, Sherman and Glay (52), working specifically on the relationship of the vitamin B requirement to protein intake, came to the decision that an increased protein intake did not require an increase in the amount of vitamin B supplied in the diet. Nelson, Jones, Heller, Parks and Fulmer (53) using a highly purified diet in which yeast was the sole source of vitamin B, concluded that a higher level of this vitamin was necessary for lactation than was needed for growth.

In a subsequent paper Guest, Nelson, Parks, and Fulmer (18) suggest that if such a high vitamin B requirement is necessary for lactation, then the human mother of today is very close to the border line as regards the needs for vitamin B during lactation. Sure (54) proposes the possibility of a lactation promoting factor in the unsaponifiable matter from wheat oil. Rose and McCollum (55) feeding different proportions of various seeds and vegetables obtained good growth and reproduction but poor lactation. Sure (56), in a series of studies on lactation, decided that vitamins A and B were both necessary for lactation, and that the rat had the ability to store the fat soluble vitamin A. Sure (57) further concluded that copper does not supplement vitamin B for lactation. Evans and Burr (58) used a highly purified diet supplemented by lard, yeast, and cod liver oil in their experiments on lactation. They obtained good growth but found it necessary to relieve sterility by the use of wheat germ oil. In order to get satisfactory lactation they added, in the form of tikitiki, five times the usual amount of vitamin B required for growth. They believe that it is a large excess of vitamin B that is necessary for lactation instead of such a large excess of both vitamins B and G. Evans and Burr (59) in a later paper state that paralysis in suckling young can be prevented by a sufficient quantity of vitamin E (Wheat germ oil). They conclude that a new dietary substance is not needed for lactation. More recently Daniels, Jordan, and

Hutton (60) found lactation unsatisfactory in milk fed rats. They observed that deficient lactation could not be stimulated by feeding an excess of vitamin B.

Rice polishings have long been used in feeding experiments as a source of vitamin B. Interest in this food material has been renewed by the discovery of vitamin G and its differentiation from the beri-beri preventative factor. Sure (61), using a highly purified diet, reports a twenty percent level of rice polishings as containing insufficient vitamin G for adequate lactation. Munsell (62) in a preliminary report suggests that rice polishings are rich in vitamin B but relatively poor in vitamin G.

Considerable work has been done on the beneficial effect of fat in the diet. However, Fredericia (63) has found lard, when incorporated into the diet, to exert a destructive action on vitamin A. Levine and Smith (64) found a diet containing as high as sixty-four percent lard to give good growth. They did not report on reproduction. Osborne and Mendel (65) worked on the relation of the rate of growth to the diet. They obtained optimum growth when nine percent of butter fat and fifteen percent of lard was incorporated into the diet and yeast and lettuce fed daily. Evans and Burr (66) observed that when fats are used in a diet the action of vitamin E is decreased by rancidity of such fats. More recently Mattill (67) reports that certain vegetable oils exert a protective action against the oxidative destruction of vitamins A and E.

A great many investigators have found milk and milk preparations unsatisfactory in studies on reproduction and lactation. Anderegg (68) found milk powder to be unsatisfactory as a complete food in a series of experiments on reproduction and rearing of young. Brigl, Euler, and Held (69) report American semi-solid buttermilk as being deficient in both vitamins A and B.

Kennedy and Palmer (38), in their study of yeast, observed failure of reproduction but apparently did not realize that such failure could be due to the lack of a new dietary essential. The discovery of vitamin E by Evans and Bishop (14) stimulated an enormous amount of research work on reproduction. No attempt will be made here to review all of the work that has been done, but it will be well to mention a few papers of outstanding interest. Shortly after the discovery of vitamin E, Sure (70) reported on its presence in various grains and their products. He failed to find the vitamin in milk. On the other hand, Daniels and Hutton (71) believed that their experiments indicated the presence of vitamin E in both milk and butter fat. Hartwell (72) and Sure (73) observed at about the same time that cod liver oil was lacking in vitamin E. Hogan and Harshaw (74) observed that it took six to eight months for females to become sterile in the absence of vitamin E. Nelson and co-workers (75) have found that when butter fat is replaced by cod liver oil in synthetic diets better reproduction is obtained. Most of the properties

of vitamin E so far determined are the result of extensive experiments by Evans and Burr (76). Somewhat later Evans (77) emphasizes the view that vitamin E holds an important relation to general nutritional well being. He (78) also points out the difference between the degeneration of the reproductive system caused by inadequate vitamin A and the fertilization and subsequent resorption characteristic of a lack of vitamin E. Foust and Nelson (79) found that high levels of casein delay reproduction and interfere with lactation as is evidenced by the stunted growth of young.

For a more complete bibliography and review of the literature on nutrition the reader is referred to McCollum and Simmond's recent book (80).

EXPERIMENTAL

Preparation of Materials

Casein.

Commercial casein placed in large glass battery jars was allowed to stand in contact with a one percent solution of acetic acid for eight to nine days. The acid solution was drained off through cheese cloth and replaced with fresh acid daily. At the conclusion of the washing process the casein was placed in enameled trays and dried over a hot plate, care being taken to prevent charring. The commercial casein was obtained from the Wilkins-Anderson Company of Chicago, Illinois.

Dextrin.

Commercial corn starch, made by the A. E. Staley Manufacturing Company of Decatur, Illinois, was worked into a stiff paste with a solution of seventy-five grams of citric acid in fifteen liters of water. This paste was placed in enameled pans and autoclaved at fifteen pounds pressure for two and one-half hours. It was then thoroughly dried on the hot plate and ground.

Butter Fat.

A good grade of creamery butter was bought on the market, melted below one hundred degrees centigrade, and the supernatant fat decanted through a filter. The butter fat was prepared as needed.

Salt Mixture 185.

The salt mixture devised by McCollum and Davis (8) was prepared as directed and used throughout these experiments at three and seven-tenths percent level. The salt mixture furnishes sodium, potassium, calcium, magnesium, iron, phosphate, sulphate, chlorine, and iodine.

Extracted Wheat Embryo.

Wheat embryo, obtained from the Washburn-Crosby Company of Minneapolis, Minnesota, was subjected to continuous extraction with anhydrous diethyl ether. The extraction was carried out in a percolator of about one kilogram capacity for from twenty-four to forty-eight hours. Animals raised on a twelve percent level of this material as a source of vitamins E, B, and G did not reproduce.

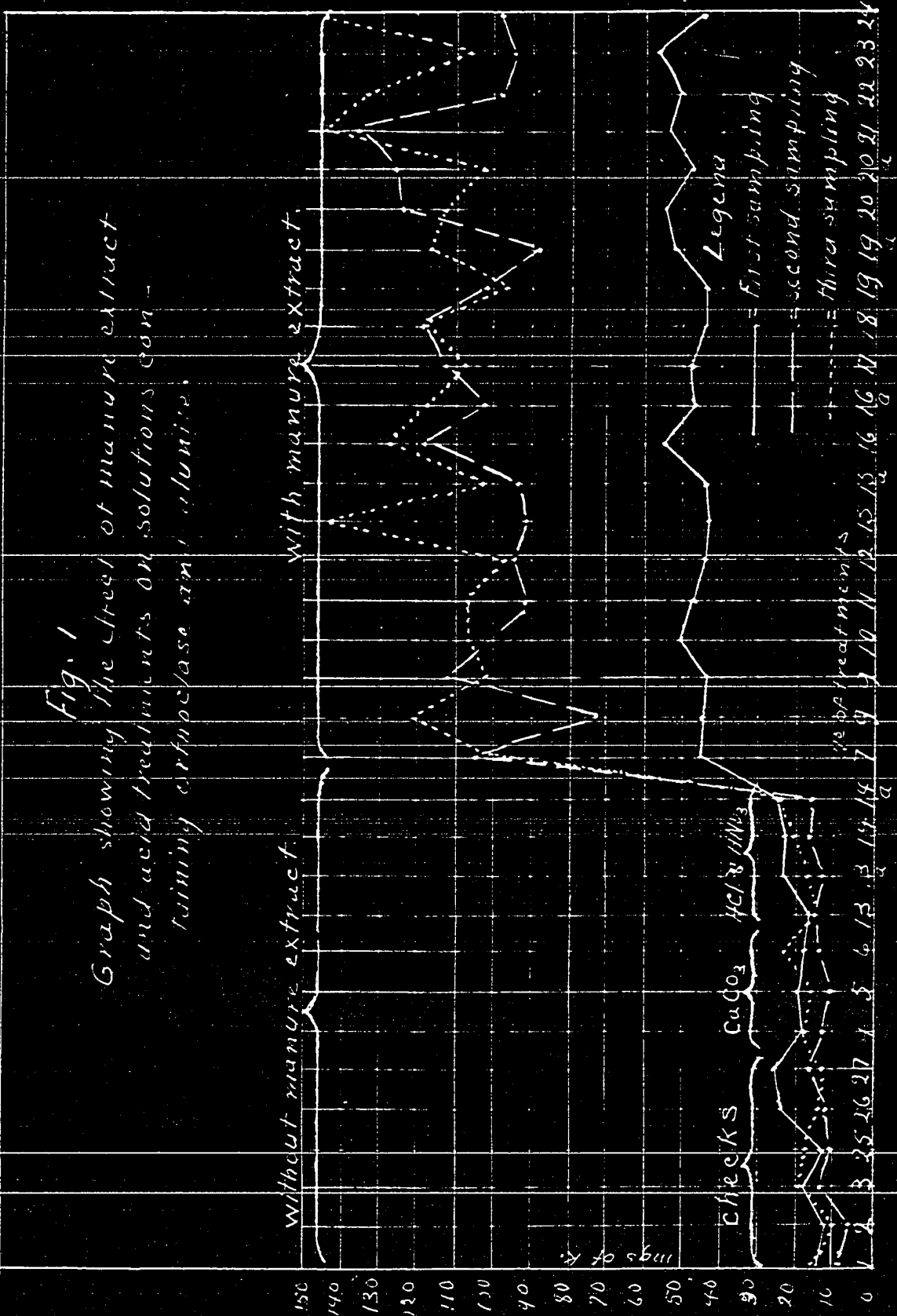
Wheat Embryo Oil.

The ether extract from the preparation of extracted wheat embryo contained the oil. The ether was driven off and the residual oil used in the experiments. About eleven percent of the original wheat embryo was recovered as oil.

Alfalfa Flour Ash.

Commercial alfalfa flour was placed in porcelain crucibles and ashed to constant weight at a dull red heat in a muffle furnace. Alfalfa flour yielded twenty-two and three-tenths percent ash.

Fig. 1
 Graph showing the effect of manure extract
 and acid treatments on solutions con-
 taining urease and alunine.



lain crucibles in a cold muffle furnace and the temperature gradually raised to five hundred degrees. The residue, a gray powder, was forty-four percent of the original. This residue will hereafter be spoken of as kelp ash.

Rice Polishings.

The rice polishings used in these experiments were obtained from the Standard Rice Company, Inc. of Stuttgart, Arkansas. The guaranteed average analysis was: crude oil or fat eleven percent, protein eleven percent, crude fiber two percent, carbohydrates sixty-one percent.

Autoclaved Rice Polishings.

The rice polishings just described were autoclaved at fifteen pounds pressure for three hours.

Yeast.

The yeast was obtained, in the dry state, from the Fleischman Company.

Autoclaved Yeast.

Autoclaving was done at fifteen pounds pressure for three hours.

Salts 12.

This mixture was prepared by mixing equal quantities by weight of NaF, $MnSO_4 \cdot 4H_2O$, Na_2SiO_3 and $AlK(SO_4)_2 \cdot 12H_2O$. The material was thoroughly ground and mixed, and used as required. It was observed to turn brown upon standing for several weeks.

Lard.

Ordinary lard obtained from the local shops was used.

Autoclaved Lard.

The lard was autoclaved for one hour at fifteen pounds pressure.

Buttermilk Powder.

Commercial buttermilk powder was used.

Cod Liver Oil.

Squibbs cod liver oil was used without any additional treatment.

Meat.

The various organs used were obtained for the most part from the Meat Laboratory of Iowa State College. They were fed as soon as possible after removal from the animal. Until mixed in the ration they were kept in the refrigerating room of the meat laboratory and in no case used if there was the slightest evidence of decomposition or putrefaction.

Dried Liver.

The liver that was fed dry was prepared by covering the bottom of shallow enameled pans with a very thin layer of the material, placing these pans on wooden supports above a hot plate and drying in a stream of air from an electric fan.

Egg Yolk.

Fresh eggs were placed in boiling water for fifteen minutes, cooled, and the yolks separated.

Extracted Liver.

Five hundred gram lots of dried liver were placed in a flask and refluxed twenty-four hours with eight hundred cubic centimeters of anhydrous ether. The ether was decanted through a filter and the residue washed with three hundred cubic centimeters of fresh ether. This process of refluxing and washing was repeated three times. The residue, hereafter spoken of as extracted liver, was warmed to remove any residual ether. It was found to contain eighty-three percent of the dried liver.

Liver Extract.

The filtrates and washings from the preparation of the extracted liver were combined and the ether removed. The residue, a heavy viscous substance, almost black, was sixteen percent of the original dried liver. This substance will be spoken of as liver extract.

Care of Animals

The animals were kept in wooden cages covered with galvanized wire screening. The bottom of the cage was a galvanized iron pan that could be readily removed for cleaning. The bedding supplied was wood shavings, and was renewed as often

as necessary to keep the cages reasonably clean. Pregnant females were removed to large individual cages and the bedding not disturbed until the litter was weaned. Food was kept in the feed cups so that the animals always had access to it. Distilled water was available at all times. All cages were inspected daily.

A number of the experiments were started at Indiana State Teachers College, Terre Haute, Indiana, on young rats shipped from Iowa State College. The experiments that were not concluded at Terre Haute were finished at Ames. The animals were returned to Ames by express. As this transfer resulted in no apparent change in the animals no subsequent mention will be made of it.

Presentation of Data

1. Soy Beans as the Sole Source of Vitamins B and G.

Three varieties of soy beans were used in this series of investigations in order to ascertain if any differences might be manifested by the different kinds. The varieties used were: Manchu, a yellow bean; Virginia, a brown bean; and Sable, a black bean. The beans were prepared as previously described and each of the three varieties used was fed at ten, twenty, forty, and seventy-three and three-tenths percent levels, as the only sources of vitamins B and G. The balance of the ration consisted of: casein, eighteen percent; salt

mixture 185, three and seven-tenths percent; filtered butter fat, five percent; and sufficient dextrin to make one hundred percent.

The animals weighed from forty-five to fifty-five grams when placed on the different experiments. Six females and three males were put on each level of each variety of soy bean. When an animal died, it was usually replaced by one of like sex weighing from forty-five to fifty-five grams. No replacements were made toward the end of the experiments. A record of each animal contained the following data: growth, number of litters, number of young given to mothers to be weaned, number of young that died during the weaning period, average weight of the young when weaned, mortality of adult males and females, and the length of time the animal was on experiment. A compilation of this data will be found in table I.

Growth curves for all lots were practically the same. However, the animals on the ten percent levels, particularly those on Virginia soy bean, did not appear in the best nutritive condition. Of the ten females placed on this ration (ten percent Virginia soy bean), six died before the experiment was discontinued. Very few females in this series of experiments died in parturition or shortly after the birth of young. Only three females from the seventy-three and three-tenths percent level of Manchu soy bean and the same number of females from the forty and the seventy-three and three-tenths percent levels of Sable bean produced more than one litter.

Soy Beans

Table 1

Number	% Soy Bean	No. of Females	No. of Males	Growth	No. of Females Died	No. of Males Died	No. of Litters	No. of Young Recovered	No. of Young Given to Mothers	No. of Nursing Young Died	% Mortality of Nursing Young	Av. Weight of Young When Weaned (28 days)	Days of Experiment
1	10	6	3	+	0	0	37	259	35	21	60	24	345
2	20	7	3	+	1	0	28	185	24	12	50	27	342
3	40	6	3	+	0	1	26	163	29	9	31	23	325
4	73.3	6	3	+	0	1	15	92	16	7	43	29	325
5	10	7	3	+	2	0	36	224	36	36	100	—	328
6	20	6	4	+	1	1	34	230	36	36	100	—	328
7	40	6	3	+	0	0	9	66	18	13	72	25	325
8	73.3	6	3	+	1	3	10	63	12	3	25	33	325
9	10	10	4	+	6	1	15	79	18	18	100	—	325
10	20	7	3	+	2	1	15	77	30	25	83	40	325
11	40	6	3	+	1	0	12	78	28	20	71	36	325
12	73.3	7	3	+	2	0	9	54	24	8	33	24	325

1, 2, 3, 4 Manchus; 5, 6, 7, 8 Sablo; 9, 10, 11, 12 Virginia.

Similar results were obtained on the ten percent level of Virginia bean. The three females on this level of Virginia soy bean had two, six, and three litters respectively. On the forty and seventy-three and three-tenths percent levels of Virginia bean, only two females from each lot produced more than one litter, and reproduction on the other two levels of this bean was poor. On each of the other lots of the different varieties of soy beans five or more females produced more than one litter. The litters varied from two to nine for each female. Reproduction was good on the ten, twenty, and forty percent levels of Manchu soy bean. On these levels of Manchu soy bean twelve females had each from four to seven litters in a period of three hundred and twenty-five days. Reproduction was also good on the ten and twenty percent levels of Sable soy beans, ten females on these levels producing from four to nine litters per female in three hundred and twenty-five days. Table II shows the number of litters for each female.

Table II

Litters per Female on Soy Beans

% Bean:	Manchu	Sable	Virginia
10	:8,6,5,7,8,3 (37)	:0,6,7,9,7,5,2 (36)	:0,0,0,1,1,1,1,2,6, (15)
20	:6,7,1,2,6,6,0 (28)	:8,6,6,5,3,6 (34)	:0,1,2,2,3,3,4 (15)
40	:7,6,7,3,3,0 (26)	:0,0,0,2,3,4 (9)	:0,1,1,1,4,5 (12)
73.3	:0,1,1,2,6,5 (15)	:0,0,1,2,3,4 (10)	:0,0,0,0,1,2,6 (9)

Some of the females were allowed to raise their young. In order to place an equal burden on all females the number of young given to an animal to raise was limited to six. If six or less young were born the female was permitted to have the entire litter. The percent mortality of the nursing young decreased as the percentage of soy bean increased. The Manchurian soy bean seemed to give slightly better results on rearing of the young than the other two varieties. The poorest results in this respect were obtained on the ten and twenty percent levels of Sable and the ten percent level of Virginia soy beans, the mortality being one hundred percent on these three experiments. In all other cases the mortality varied from twenty-five to eighty-three percent. The mortalities on the different levels of Manchurian soy bean were sixty, fifty, thirty-one, and forty-three percent for the ten, twenty, forty, and seventy-three and three-tenths percent levels respectively. The mortalities for the same levels of Sable soy bean were one hundred, one hundred, seventy-two, and twenty-five percent, whereas for the Virginia soy bean the mortalities were one hundred, eighty-three, seventy-one and thirty-three percent. When the young were weaned their average weight was considerably below normal, in all cases, except one litter of five, which was reared on the twenty percent level of Virginia soy bean. The litter averaged forty grams at twenty-eight days. The young from the other mothers on the various levels had an average weight of from twenty-three to thirty-six grams when weaned at twenty-eight days.

2. The Supplementary Value for Lactation of Various Animal Tissues.

Satisfactory lactation was not obtained in any of the preceding experiments where soy beans were the sole source of vitamins B and G. It was therefore deemed advisable to ascertain if the soy bean ration could be supplemented by a single foodstuff so that lactation would be normal. Animal tissues were chosen as the supplementary food for several reasons. If an animal needs a special dietary substance for lactation it seems reasonable to suppose that such a substance is likely to be present in the normal tissues of other animals. The presence of an abbatoir on the campus made it possible to obtain and feed various organs without resorting to any preservative process other than keeping them cold.

The evidence of many previous workers seems to indicate that animals do not have the ability to store vitamins B and G. Pregnant females from the breeding stock were therefore used as the experimental animal. The stock animals had been raised on a mixture of feeds that has proven comparatively adequate over a period of years. The litters were delivered from one to five days after the animals were placed in individual cages on the experimental diets. Each female was given six young to wean. This number, six, was arbitrary but seemed sufficient for any one mother to care for and should place an equal burden on all the females so that interpretation of the results ought to be more easy of accomplishment. The animals

had access to distilled water and the experimental rations at all times. The young were considered weaned at thirty days. Records were kept for each experiment of the number of litters born, the number of young given to mothers to wean, time of death of young, number of young weaned, and the average weight of the young on each ration when weaned. Observation has shown that occasionally females kept on the growing ration refuse to nurse their young and even kill them at birth. For this reason the calculation of the percent mortality of the young is based on the number surviving on the eighth day.

The control diet consisted of the following: casein, eighteen percent; salt mixture 185, three and seven-tenths percent; filtered butter fat, five percent; Virginia soy bean, fifteen percent; and dextrin, fifty-eight and three-tenths percent. The mortality of the young on this diet was eighty-eight percent and the average weight of the four young that were weaned was twenty-two grams. The other rations were similar in composition to the control diet, except that part of the dextrin was replaced by the various organs under investigation. The organs tested were the following: lung, spleen, pancreas, brain, kidney, thymus, and heart. They were obtained from hogs and cattle. The organs were finely ground and mixed into the rations while fresh. Hog lung and spleen were fed at thirty percent levels and the rest of the organs at twenty-five percent levels. Moisture determination on the various organs gave the following results: hog lung, eighty-

seven and seven-tenths percent; hog spleen, seventy-five and six-tenths percent; hog liver, seventy and four-tenths percent; hog kidney, seventy-four percent; hog brain, seventy-three and five-tenths percent; hog pancreas, sixty-four and two-tenths percent; and beef heart, seventy-eight percent. It is believed that the slight variation in moisture content of the various organs does not appreciably affect the comparative results as given in table III.

The percent mortality of young from the mothers receiving the various organs was for hog lung, ninety-four; for hog spleen, seventy-one; for hog pancreas, forty-seven; and for beef thymus, sixty. The average weights of the young when weaned from mothers receiving the same organs was: twenty-two, thirty-one, forty-five, and twenty-six respectively. Hog pancreas seemed to give slightly better results than beef pancreas. The average weight of young when weaned from mothers on beef pancreas was lower (forty grams) and the percent mortality lower (forty). The percent mortality of these young, however, was still considerably higher than on many other rations. Brains, both beef and hog, did not give quite as good results as pancreas. The percent mortality on hog brain was forty-five, whereas on beef brain it was twenty-six. The young from mothers on hog brain averaged thirty-two grams when weaned, while the young from mothers receiving beef brain averaged thirty-three grams when weaned. The mortality of young from mothers receiving beef heart was seventy-

Table III

15% Soy Bean + Various Organs

Meat	:No. : :Lit-:ters:	No. : :Young:	Died: :0-7:	Died: :8-14:	Died : :15-30:	% Mortal- :ity after: :7th day	:No. : :Wean-:ed	:Average :weaning :wt. gms.
0	8	48	15	0	29	88	4	22
30% Lung (Hog)	8	48	14	13	19	94	2	22
30% Spleen (Hog)	8	48	0	0	34	71	14	31
25% Pancreas: (Hog)	8	48	6	0	20	47	22	45
25% Pancreas: (Beef)	8	48	0	5	14	40	29	40
25% Thymus (Beef)	8	48	0	6	24	60	20	26
25% Brain (Hog)	9	54	1	9	14	45	30	32
25% Brain (Beef)	8	48	6	1	10	26	31	33
25% Kidney (Hog)	9	54	6	4	2	14	36	63
25% Heart (Beef)	9	54	6	15	20	73	13	42
25% Liver (Beef)	9	54	4	12	2	28	36	61
25% Liver (Hog)	10	60	17	8	3	25	32	67
25% Egg Yolk:	5	30	13	0	4	23	13	59

three percent while the young that were weaned averaged forty-two grams. The best results in this series of experiments were obtained on diets supplemented with twenty-five percent of either hog kidney, beef liver, hog liver, or egg yolk. The young from animals on hog liver, beef liver, kidney, and egg yolk all had about the same low percentage mortality and the average weaning weights on all four of these supplementary food materials was far above those obtained when the other organs were used. The mortalities of the young on these better supplemental foods were, hog liver, twenty-five percent, beef liver, twenty-eight percent, hog kidney, fourteen percent, and egg yolk, twenty-three percent, while the average weaning weights were sixty-seven grams, sixty-one grams, sixty-three grams, and fifty-nine grams, respectively.

The effect of feeding different levels of hog liver was next tried. The same basal ration containing fifteen percent of soy bean was used, and the other rations contained hog liver from five to thirty percent in place of an equivalent amount of dextrin. A compilation of the results appears in table IV. The mortality on the basal ration was eighty-eight percent, on the five percent level of hog liver fifty percent, on the fifteen percent of hog liver thirty-nine percent, twenty-five percent on the twenty-five percent of hog liver, while only three percent mortality was obtained on the thirty percent hog liver. The average weaning weights in the same order were: twenty-two grams, thirty-eight grams, sixty-one

grams, sixty-seven grams, and sixty-eight grams.

Table IV

15% Soy Bean + Liver (Hog)

% Liver	No. Litters	No. Young	Died 0-7 days	Died 8-14 days	Died 15-30 days	% Mortality after 7th day	No. Weaned	Average wt. gms.
0	8	48	15	0	29	38	4	22
5	8	48	6	0	21	50	21	38
15	10	60	9	17	3	39	31	61
25	10	60	17	8	3	25	32	67
30	7	42	12	0	1	3	29	68

The extensive experiments of Guest, Nelson, Parks, and Fulmer (18) have been previously discussed. Recalling their difficulty with lactation, it was decided to try supplementing one of their diets with various levels of hog liver. The diet selected consisted of casein, eighteen percent, filtered butter fat, five percent, salt mixture 185 three and seven-tenths percent, wheat, thirty percent, and dextrin forty-three and three-tenths percent. This ration was fed as a control to a number of females with young, as in the preceding experiments. Another group of rats received this same ration with five percent of hog liver in place of an equal quantity of dextrin, whereas two other lots received fifteen and twenty-five percent of liver for equivalent quantities of

dextrin. The data obtained are presented in table V. The

Table V

30% Wheat + Liver (Hog)

% Liver	No. Litters	No. Young	Died: 0-7 days	Died: 7-14 days	Died: 15-30 days	% Mortal- ity after: 7th day	No. Weaned	Average weaning wt. gms.
0	7	42	5	4	8	32	25	24
5	4	24	4	5	9	70	6	34
15	6	36	16	2	0	10	18	51
25	6	36	12	0	2	8	22	50

mortalities on the rations in the order named were thirty-two (for the control), seventy, ten, and eight percent. The average weaning weights were: twenty-four, thirty-four, fifty-one, and fifty grams respectively.

It became obvious from the preceding experiments that the lactation promoting value of a diet where hog liver was the sole source of vitamins B and G should be tried. Consequently a diet consisting of casein, eighteen percent, filtered butter fat, five percent, salt mixture 185, three and seven-tenths percent, various levels of hog liver, and sufficient dextrin to make one hundred percent, was used. The levels of hog liver incorporated into the rations were, five, fifteen, twenty-five, and forty percent. The mortalities of the young in the experiments (table VI) were found to be one

hundred percent on the five percent level of hog liver, fifty-

Table VI

Liver (Hog)

% Liver	No. Litters	No. Young	Died: 0-7 days	Died: 8-14 days	Died: 15-30 days	% Mortality after 7th day	No. Weaned	Average weaning wt. gms.
5	6	36	0	0	36	100	0	—
15	9	54	11	12	11	53	20	55
25	8	48	0	7	8	31	33	55
40	7	42	0	1	4	12	37	66

three percent on the fifteen percent level, thirty-one percent on the twenty-five percent level, and on the forty percent level the mortality was twelve percent. In the same order the average weaning weights (omitting the five percent level on which no young were weaned) were: fifty-five grams, fifty-five grams, and sixty-six grams.

In the first series of experiments egg yolk was found to give good results on lactation when a twenty-five percent level supplemented a fifteen percent level of soy bean (table II). Therefore, two lower levels of egg yolk, supplementing the same basal ration, were tried on separate lots of animals. The results appear in table VII. The five percent level of egg yolk gave a mortality of forty-two percent with an average weaning weight of thirty-six grams, while on the fifteen

percent of egg yolk the mortality and average weaning weight of the young were found to be sixty-one percent and thirty grams respectively.

Table VII

15% Soy Bean + Egg Yolk

% Egg Yolk	No. Litters	No. Young	Died: 0-7 days	Died: 8-14 days	Died: 15-30 days	% Mortality after 7th day	No. Weaned	Average weaning wt. gms.
0	8	48	15	0	29	88	4	22
5	4	24	0	4	6	42	14	36
15	4	24	6	3	8	61	7	30
25	5	30	13	0	4	23	13	59

Further investigations were carried out using various preparations from liver. It was first thought desirable to feed dried liver as a supplement to the fifteen percent soy bean ration. The liver was placed in a thin layer on shallow pans and put in an oven regulated to maintain a temperature of one hundred degrees centigrade. The liver was left in the oven over night and in the morning it was observed that the temperature control had stuck and the temperature had risen to one hundred and twenty degrees centigrade. As such a temperature would effectively destroy vitamin B, it was decided to try the supplemental action of this dried liver. The fifteen percent soy bean ration was used with dried liver equi-

valent to thirty percent of the fresh material replacing thirty percent of the dextrin. The mortality of the young from mothers on this ration was one hundred percent. These results and the results of subsequent experiments in this series of investigations are given in table VIII.

Table VIII

Miscellaneous

Composition	No. : Lit- :ters:	No. : Young:	Died : :0-7 :days:	Died : :8-14 :days:	Died : :15-30 :days:	% Mortal- :ity after :7th day :	No. : Wean- :ed :	Average :weaning :wt. gms.
Liver dried at 120°C.	5	30	0	12	18	100	0	—
Liver dried on hot plate:	8	48	1	3	13	34	31	45
Liver ext. + Ext'd.liver:	6	36	0	6	12	50	18	40
Liver ext.	5	30	6	0	17	57	7	18
Ext'd liver	4	24	2	0	14	58	8	40
12% Yeast	8	48	0	6	0	12	42	42
12% Yeast + 1.3% Kelp ash	4	24	1	0	0	4	23	33

Dried liver, prepared as described under "Preparation of Materials", was incorporated into a ration made up in the same proportion as that in which the inadvertently heated material was used. This ration when fed to another lot of animals gave

a mortality of young of thirty-four percent and an average weaning weight of forty-five grams.

Three lots of rats were put on experiment using the extracted liver and liver extract previously described. The fifteen percent soy bean ration was again used as a basal ration. The ration fed to the first lot of animals contained both extracted liver and liver extract equivalent to thirty percent of fresh liver. This material replaced thirty percent of dextrin. The second lot of rats received a ration in which extracted liver equivalent to thirty percent of the fresh material replaced thirty percent of dextrin, and the ration of the third lot had thirty percent of the dextrin replaced by liver extract equivalent to thirty percent of fresh liver. The percent mortality of the young whose mothers received both liver fractions in the diet was fifty percent, whereas those young whose mothers received the extracted liver in the ration had a mortality of fifty-eight percent, while those on the remaining ration, which contained liver extract as a supplement, had a mortality of fifty-seven percent. The average weaning weights in the same order were forty grams, forty grams, and eighteen grams.

Yeast is reported by a number of investigators as containing considerable amounts of both vitamins B and G. Consequently a series of experiments was designed to test its value in stimulating lactation. Females from the breeding stock were used in the same manner as in the preceding ex-

periments. One lot was put on a ration of the following composition: casein, eighteen percent, filtered butter fat, five percent, salt mixture 185, three and seven-tenths percent, dried yeast, twelve percent and dextrin, sixty-one and three-tenths percent. A second lot received the same ration except that one and three-tenths percent of kelp ash replaced an equivalent amount of dextrin. It was thought desirable to find if inorganic material such as is found in kelp would supplement a substance, such as yeast, which is reported to contain adequate amounts of vitamins B and G. With yeast as the sole source of vitamins B and G the percent mortality was found to be twelve and the average weaning weight of the young to be forty-two grams. When the diet was supplemented with kelp ash the mortality was four percent and the average weaning weight thirty-three grams.

In the preceding studies on lactation three of the females died before their young were weaned. As the death of the females could not be attributed to any fault in the diet, no record was made of them or their young in compiling the data. There was one of these animals on each of the following rations: fifteen percent soy bean supplemented with thirty percent liver, forty percent liver, and fifteen percent soy bean supplemented with extracted liver.

3. Rice Polishings as the Sole Source of Vitamins B and G.

The experiments on rice polishings were carried out in

a manner similar to those on soy beans. Young rats weighing from forty-five to fifty-five grams were used as the experimental animal, six females and three males being placed on each experiment. The experimental rations consisted of: casein, eighteen percent, salt mixture 185, three and seven-tenths percent, filtered butter fat, five percent, various levels of rice polishings, and sufficient dextrin to make one hundred percent. The levels of rice polishings used were ten, twenty, fifty, and seventy-three and three-tenths percent. In the early days of the investigation when an animal died it was replaced by one of like sex weighing from forty-five to fifty-five grams. A record of each animal contained the following data: growth, number of litters, number of young, number of young given to mothers to be weaned (six per female), number of young that died during the weaning period, average weight of the young when weaned, mortality of adult males and females, and the length of time the animal was on experiment. The results are given in table IX.

Table IX

Rice Polishings

% Rice Polishings	No. of Females	No. of Males	Growth	No. of Females Died	No. of Males Died	No. of Litters	No. of Young Recovered	No. of Young Given to Mothers	No. of Young Died	% Mortality of Nursing Young	Ave. Weight of Young when Weaned (28 days)	Days of Experiment
10	7	3	+	1	0	32	256	18	3	16	43	267
20	6	3	++	0	1	30	232	18	7	38	36	267
50	6	3	+	0	0	31	229	18	2	11	33	267
73.3	7	3	+	1	0	26	199	12	1	8	37	267

Growth on the twenty percent level was more rapid than normal. On the other three levels the growth curves appeared to be normal. With the exception of two females that died during parturition all of the females on the different levels of rice polishings produced three or more litters. The mortalities of the young were all sixteen percent or below with the exception of those whose mothers received twenty percent of rice polishings. The mortality of young on this level was thirty-eight percent. The highest average weaning weight of young was obtained on the ten percent level, the average weight of young when weaned from mothers receiving this ration being forty-three grams. The weaning weights of the young on the other three levels of rice polishings were thirty-

six, thirty-three, and thirty-seven grams for the twenty, fifty, and seventy-three and three-tenths percent levels respectively. Observation of animals on the growing ration shows average weaning weights of between forty and forty-five grams. Hence it would appear that the lowest level of rice polishings used gives the best results in so far as lactation is concerned.

Various workers have postulated a high level of vitamin B as being necessary for lactation. It was thought desirable to find out what would be the effect on the animals if the vitamin B in the rice polishings was destroyed. The rations were therefore altered to include autoclaved rice polishings in place of the untreated rice polishings that had been previously fed. The animals that had received the untreated rice polishings were transferred without interruption to the new diet. The same data were collected as before and is presented in table X.

Table X

Autoclaved Rice Polishings

% Autoclaved Rice Polishings	No. of Females	No. of Males	No. of Females Died	No. of Males Died	No. of Litters	No. of Young Recovered	No. of Young Given to Mothers	No. of Nursing Young Died	% Mortality of Nursing Young	Ay. Weight of Young When Weaned (28 days)	Last Litter	Days of Experiment
10	6	3	2	3	6	32	18	14	77	40	26th day	91
20	6	3	1	1	4	15	6	1	16	22	41st day	91
50	6	3	0	0	9	54	18	0	0	39	84th day	91
73.3	6	3	0	0	10	47	16	3	18	24	86th day	91

It was observed within a few days that the nutritional well-being of the animals, particularly the males, was being seriously affected. This was especially true of those on the ten and twenty percent levels. On the ten percent level the last male died on the forty-third day. The ability of the mothers to raise their young was decidedly less on the ten and twenty percent levels but little difference was noted on the higher levels. Reproduction stopped on the twenty-sixth day on the ten percent level and on the forty-first day on the twenty percent level of autoclaved rice polishings. On the other two levels reproduction persisted until the experiments were discontinued.

4. Butter-milk Powder as the Sole Source of Vitamins B and G.

A number of investigators have used milk and milk preparations in various nutritional studies. They have found the dietary properties for reproduction and lactation to be generally poor for such substances. The dietary value of butter-milk powder was not found reported in the literature so a series of experiments was designed to test such values of this substance. Young animals, weighing from forty-five to fifty-five grams were used as in the preceding experiments. Five females and three males were placed on each experiment. As before, those animals that died in the early days of the experiment were replaced by young of the same sex. The levels of butter-milk powder used were forty, sixty, and seventy-three and three-tenths percent, the balance of the ration consisted of: casein, eighteen percent, salt mixture 185, three and seven-tenths percent, cod liver oil, five percent, and sufficient dextrin to make one hundred percent. The cod liver oil was fed daily by mixing it into a proportionate amount of the experimental ration. A record of each animal contained the following data: mortality of adults, growth, number of litters, number of young, number of young given to mothers to wean (not more than six per litter), mortality of young and the length of the experiment. The results will be found in table XI.

Table XI

Butter-milk Powder

% Butter-milk Powder	No. of Females	No. of Males	Growth	No. of Females Died	No. of Males Died	No. of Litters	No. of Young Recovered	No. of Young Given to Mothers	No. of Nursing Young Died	% Mortality of Nursing Young	Last Litter	Days of Experiment
40	5	3	+	0	0	7	42	6	6	100	245th day	298
60	5	6	+	0	3	15	115	24	24	100	282nd day	298
73.3	5	3	-	3	0	0	0	-	-	-	-	298

Normal growth was obtained on both the forty and sixty percent levels of butter-milk powder. The seventy-three and three-tenths percent level gave subnormal growth but all of the animals reached maturity. On the forty percent level only one female produced more than one litter, and she had four. Of the five females on the sixty percent level of butter-milk powder two did not have any litters and the other three had seven, six, and two litters. No litters were produced on the seventy-three and three-tenths percent level. Lactation on the two levels tried was very poor, all of the young dying uniformly from the fifteenth to the eighteenth day.

5. The Supplementary Value of Kelp for Growth, Reproduction, and Lactation.

Kelp is a very abundant seaweed that contains considerable amounts of iodine and potassium as well as the other elements characteristic of plants. Because of its availability it was decided to investigate its dietary properties, particularly as a supplementary food material. Young animals weighing from forty-five to fifty-five grams were used in this series of experiments. The levels of kelp fed were one, three, eight, ten, and twenty-five percent. On the one, three and eight percent levels the balance of the ration consisted of casein, eighteen percent, salt mixture 185, three and seven-tenths percent, filtered butter fat, five percent, yeast, twelve percent, and sufficient dextrin to make one hundred percent. In the rations containing ten and twenty-five percent kelp, the yeast was replaced by an equal amount of extracted wheat embryo. A compilation of the data obtained in these experiments is given in table XII.

Table XII

Kelp + Yeast

% Kelp	No. of Females	No. of Males	Growth	No. of Females Died	No. of Males Died	No. of Litters	No. of Young Recovered	No. of Young Given to Mothers	No. of Nursing Young Died	% Mortality of Nursing Young	Av. Weight of Young Weaned (28 days)	Days of Experiment
1	7	3	++	1	0	21	137	18	1	6	51	266
3	6	4	++	0	1	22	170	18	0	0	46	266
8	6	3	+	0	0	34	255	18	1	6	48	266
10	5	3	+	1	0	20	147	73	49	69	26	354
25	5	3	+	2	0	14	87	62	41	64	33	354

Better than normal growth was obtained with the animals on the one and three percent levels of kelp, while the animals on the eight, ten, and twenty-five percent levels gave normal growth curves. All the females in this series of experiments gave birth to two or more litters. Lactation on the one, three, and eight percent levels of kelp was better than that obtained on the growing ration. On these levels of kelp the mortalities of young were, in the order named, six, zero, and six percent, while the weaning weights at twenty-eight days were fifty-one, forty-six, and forty-eight grams respectively. It will be recalled that on the above levels of kelp

yeast was used as the sole source of vitamins B and G. Lactation was somewhat poorer on the ten and twenty-five percent levels of kelp, the mortalities of the young being sixty-nine and sixty-four percent, while the average weaning weights were twenty-six and thirty-three grams respectively. The last two levels of kelp were fed with extracted wheat embryo as the source of vitamins B and G.

After the two hundred and sixty-sixth day autoclaved yeast was used in making up the rations containing the one, three, and eight percent levels of kelp; the results are shown in table XIII. It will be observed that reproduction stopped

Table XIII

Kelp + Autoclaved Yeast

% Kelp	No. of Females	No. of Males	No. of Females Died	No. of Males Died	No. of Litters	No. of Young Recovered	No. of Young Given to Mothers	No. of Nursing Young Died	% Mortality of Nursing Young	Av. Weight of Young Weaned (28 days)	Last Litter	Days of Experiment
1	6	3	1	0	1	6	6	6	100	—	15th day	86
3	6	3	4	0	1	7	0	—	—	—	1st day	86
8	6	3	0	1	3	26	6	1	16	37	30th day	86

very soon after the change in diet was made, the last litter being obtained on the fifteenth day from the females on the diet containing one percent kelp, on the first day from those on the three percent kelp diet, and on the thirtieth day from those on the eight percent kelp diet. The quality of lactation as evidenced by the few young that were tried was considerably below that obtained before the yeast was autoclaved. There was one hundred percent mortality of the one litter whose mother received the one percent level of kelp, and while the mortality of the young whose mother received the eight percent level was only sixteen percent, the average weaning weight of thirty-seven grams was somewhat below that obtained before the yeast was autoclaved.

6. The Supplementary Value of the Ash of Foods in Stimulating Reproduction.

Young rats were again used as the experimental animal. Five to eight females and three males were used in each experiment. The diets used in these experiments all contained casein, eighteen percent, salt mixture 185, three and seven-tenths percent, filtered butter fat, five percent, twelve percent of either yeast or extracted wheat embryo as a source of vitamins B and G, various amounts of ashed substances, and sufficient dextrin to make one hundred percent. Table XIV shows a summary of the results.

Table XIV

Mineral Supplements

Number	No. of Females	No. of Males	Growth	No. of Females Died	No. of Males Died	No. of Litters	No. of Young Recovered	Last Litter	Days of Experiment
1	8	3	+	1	0	1	7	150th day	180
2	7	3	+	1	0	5	28	113th day	247
3	7	4	+	5	1	21	231	231st day	390
4	6	3	+	0	1	3	11	110th day	247
5	6	4	+	1	0	5	16	176th day	261
6	6	3	+	0	0	12	63	242nd day	261
7	5	3	+	1	0	0	0	—	180
8	5	3	+	0	0	2	9	150th day	180
9	5	3	+	0	0	0	0	—	180
10	6	3	+	2	0	2	9	97th day	242
11	5	3	+	3	0	1	6	140th day	311

Satisfactory growth was obtained on all rations. As a control, ration number one contained twelve percent of yeast with no additional supplement. The results of a similar control using twelve percent of extracted wheat embryo in place of yeast as a source of vitamin B and G, will be found in group 11.

Previous experiments with kelp had given satisfactory reproduction. In order to determine if the minerals of the kelp were responsible for reproduction, different lots of rats were fed rations two, three and four, which contained twelve percent of yeast and various amounts of kelp ash. Ration number two had 0.43% of kelp ash as a supplement. This amount of ash was equivalent to feeding one percent of kelp. Of the seven females placed on this ration only two produced litters. One female had one litter and the other four litters. The last litter was obtained on the one hundred and thirteenth day. Ration number three contained one and three-tenths percent of kelp ash which was equivalent to a three percent level of kelp. Reproduction on this level of kelp ash was somewhat better, the seven females having one, two, three, three, three, four, and five litters. However, reproduction had stopped on the two hundred and thirty-first day. Ration four contained three and five-tenths percent of kelp ash (equivalent to eight percent of kelp). Of the six females on this ration three did not have any litters and the other three had one litter each. No litters were observed after the one hundred and tenth day.

It will be recalled that satisfactory reproduction was obtained with soy beans on the ten and twenty percent levels of three different varieties. Again the question as to the value of the mineral content for reproduction was investigated. In this instance two varieties of soy bean were ashed,

the Manchu and the Sable. The ash of these beans, fed at a one percent level, was equivalent to about twenty percent soy bean. In both of these experiments a twelve percent level of extracted wheat embryo was used as a source of vitamins B and G. Experiment number five contained the ash of the Manchu soy bean. Of the six females used in this experiment one produced three litters, two produced one litter each and three females did not produce litters. The last litter was obtained on the one hundred and seventy-sixth day. Those females receiving one percent of Sable bean ash (experiment number six) showed slightly better results. The six females used in this experiment all produced litters, the numbers of litters per female being one, one, two, two, three, and three. Reproduction had stopped on the two hundred and forty-second day.

Previous experiments in this laboratory had shown that animals receiving alfalfa flour as the sole source of vitamin E reproduced normally. It was therefore thought advisable to try the supplementary effect of the ash of alfalfa flour. The ash was fed to three groups of animals represented in table XV, as groups seven, eight, and nine. Group seven received one and one-tenth percent of alfalfa flour ash (equivalent to a five percent level of alfalfa), groups eight and nine received two and two-tenths percent of the ash. Groups seven and eight received a twelve percent level of extracted wheat embryo as the sole source of vitamins B and G. For group nine, twelve percent of yeast replaced the extracted

wheat embryo as a source of these vitamins. No litters were obtained from any of the five females on the one and one-tenth level of alfalfa ash. The experiments were discontinued on the one hundred and eightieth day. Of the five females in group eight only two produced litters and they had only one litter each. No litters were observed after the one hundred and fiftieth day. The ninth group of animals, which received, like group eight, two and two-tenths percent of alfalfa ash but had the extracted wheat embryo of their diet replaced by yeast, did not produce any litters.

Daniels and Eutton (22) found that rats on milk alone failed to reproduce but when a mixture of pure salts (salts 12, Preparation of Materials) was added to the milk a diet was obtained that gave normal reproduction. It was thought desirable to test these salts with a purified ration. Consequently a group of animals (table XIV, number 10) was fed a ration similar to those used in the other experiments of this series. A twelve percent level of yeast was used as a source of vitamins B and G. The diet was supplemented by a 0.06% level of salts 12. Of the six females used in this experiment only one gave birth to young, and she only had two litters, the last one on the ninety-seventh day of the experiment. The experiment was discontinued at the two hundred and forty-second day.

Fresh males were added to groups two, four, five, and six on the two hundred forty-seventh, two hundred forty-

seventh, two hundred sixty-first and two hundred and sixty-first days respectively. The results for the ensuing ninety-seven days are given in table XV. It will be recalled that

Table XV

Addition of Fresh Males

	No. of Females	No. of Males	No. of Females Died	No. of Males Died	No. of Litters	No. of Young Recovered	Last Litter	Days of Experiment
0.43% Kelp Ash	6	3	1	0	5	25	76th day	97
3.5% Kelp Ash	6	3	0	1	4	20	72nd day	97
1.0% Manchu Ash	6	3	1	0	3	19	48th day	97
1.0% Sable Ash	6	3	2	1	4	29	72nd day	97

reproduction had stopped on these four experiments. The addition of fresh males resulted in the females in all groups again producing litters. The first two groups represented in table XV had been receiving yeast and the second two groups had been receiving extracted wheat embryo as a source of vitamins B and G. Of the five litters obtained from the first

group three came from one female and one each from two other females. All of these females had produced litters in the previous experiment. In the second group four females each produced one litter. Two of them had not previously had any young in the preceding experiment. One female of the third group produced two litters and another one litter. The latter female had not previously produced a litter. In the fourth group the four litters were produced by three females. The one that had two litters had not had any litters before. The other two had had litters.

7. The Effect of Lard on Growth, Reproduction, and Lactation.

Lard has been reported as having a destructive action on certain vitamins. It was therefore decided to test the effect of adding lard to various rations. Young animals were used as in the preceding experiments. The first group received a control ration consisting of casein, eighteen percent, salt mixture 185, three and seven-tenths percent, and extracted wheat embryo, ten percent. A daily addition of five percent of cod liver oil was made to the ration as it was fed to the animals. The balance of the ration was dextrin. The results are given in table XVI. Contrary to expectations twenty litters were obtained from the four females (each female had three or more litters) put on this diet, and while the percent mortality (sixty-five percent) of the young was high their average weaning weight (forty-four grams) was

Table XVI

The Effect of Lard

Number	No. of Females	No. of Males	Growth	No. of Females Died	No. of Males Died	No. of Litters	No. of Young Recovered	No. of Young Given to Mothers	No. of Nursing Young Died	% Mortality of Nursing Young	Av. Weight of Young Weaned (28 days)	Days of Experiment
1	4	3	+	0	0	20	135	69	46	65	44	355
2	5	4	+	2	0	0	—	—	—	—	—	372
3	8	3	+	3	0	5	31	19	19	100	—	215
4	4	3	+	1	0	22	135	73	68	93	26	354
5	6	5	+	1	2	11	77	36	29	67	37	225

good. The second group of rats received a ration containing the same amounts of casein, salt mixture, extracted wheat embryo, and cod liver oil. Fifteen percent of lard replaced an equal amount of dextrin. The entire ration was made up in one kilogram quantities as needed. None of the five females on this ration produced any young. A third group of animals received the same ration as the second group. However, the cod liver oil was not mixed into the ration until forty-eight hours before feeding. The five litters obtained on this ration were produced by three females, two of them yielding two litters each. The young given to the mothers to wean suffer-

ed a one hundred percent mortality. Two females on this ration died in parturition. The ration fed to the fourth group of animals was exactly the same as that fed to the third group, except that the cod liver oil was added to the ration daily. The four females all produced more than four litters each for a total of twenty-two litters. However, lactation was poor, the percent mortality of young being high (ninety-three percent) and the average weaning weight (twenty-six grams) being poor. The last ration fed in this series of experiments was identical with that fed the second group of animals with the exception that previous to mixing in the ration the lard was heated for one hour at fifteen pounds pressure. On this ration three females had two litters each, one female had one litter and another female had three litters. One female died in parturition after having had two previous litters. The young from females on this ration had a mortality of sixty-seven percent and an average weaning weight of thirty-seven grams.

DISCUSSION OF RESULTS

Other workers have reported that, in order to get normal growth, it is necessary to use twenty-five percent or more of soy beans, as the sole source of vitamins B and G. In the experiments described in this paper normal growth was obtained with a ten percent level of each of three varieties of soy beans used. The difference between these results and those obtained by other investigators is probably due to the fact that others cooked their soy beans under fifteen pounds pressure while the soy beans used in this investigation were cooked at atmospheric pressure. It has been definitely shown by a number of workers that high temperatures will destroy vitamin B. Previous investigations on soy beans did not differentiate between vitamins B and G since the dual nature of vitamin B was unknown at that time.

Three varieties of soy beans were used in this series of investigations in order to ascertain if any difference might be manifested by different kinds. Little difference in the dietary values of the different varieties was observed, although the Manchu soy bean did give somewhat better results on reproduction and lactation than either the Virginia or Sable soy beans. Growth, contrary to expectations, was found to be normal on all the levels of soy bean used. It was expected that if the ten percent level gave normal growth, then the higher levels would show an improved rate of growth.

However, those animals on the ten percent levels did not appear in the best nutritive condition. Guest, Nelson, Parks, and Fulmer (18) observed in their studies on various grains that many females died in parturition or shortly after the birth of young. The results of this investigation show that only a few females died during this period on the soy bean rations, not nearly as many as reported by the above authors on the grain rations. The reason for this difference in mortality the writer does not know. There was a decided decrease in reproduction on the higher levels of soy bean as compared to the normal reproduction obtained on the lower levels. This peculiar result is difficult to explain. It may be that on the higher levels the animals have a high intake of protein of such a quality that normal metabolism is interfered with in a way as to effect reproduction.

Various workers have postulated a high level of vitamin B as being necessary for lactation. The results of these experiments indicate this to be true. However, something else seems to be necessary for normal lactation. In this series of investigations normal growth was obtained on the ten percent level of soy bean. One would think that forty to seventy percent of soy bean would furnish sufficient additional vitamins B and G for normal lactation. The high mortality of nursing young and the low weight when weaned indicates that there may still be something, possibly of an unknown nature, which is necessary for normal lactation and consequently for

a complete diet.

With this idea in mind, it was decided to investigate the supplementary values of various organs. The results obtained (table III) indicate that lung, spleen, and thymus have little or no supplementary value. Pancreas, brain, and heart gave an improved rate of growth and a lower mortality of young, but the results were far from being optimum. With liver and kidney, however, a low mortality and an extraordinary rate of growth of young was obtained. No record of such rapid growth of young as was obtained in these experiments was found in the literature. Diets which were believed to contain a large excess of vitamins B and G have been fed. Young from mothers receiving such a diet weigh much less at thirty days than those whose mothers received the liver supplements, such as described in this thesis. The young also had a remarkably sleek and well-nourished appearance. One of the males whose mother received fifteen percent soy bean supplemented by thirty percent liver (table IV) weighed ninety grams when weaned at thirty days.

A comparison of tables IV and VI shows that liver as the sole source of vitamins B and G does not give quite as good results on the different levels as when it is supplemented by fifteen percent of soy bean.

The evidence seems to indicate the presence of a new dietary substance in liver and kidney, such substance being necessary for optimum lactation.

The results obtained when dried liver was used in the ration were not as good as those obtained when the fresh material supplemented a fifteen percent level of soy bean (compare table IV thirty percent liver and table VIII, number 2). In the preparation of the dried material an endeavor was made to avoid temperatures sufficiently high to destroy vitamin B. It would appear that the substance that stimulates such remarkable growth of young is destroyed or impaired by relatively low temperatures or dehydration.

The results obtained when the fractions prepared by extraction with ether were fed (table VIII) indicate that such a separation is not satisfactory. Better growth of young was obtained when the combined fractions were fed than that observed when the ether extract and extracted liver replaced fresh liver in separate experiments. However, those animals receiving the extracted liver in the ration showed better lactation, as evidenced by the growth of young, than those receiving the ether extract.

Sure (61) reports that a twenty percent level of rice polishings as the sole source of vitamins B and G is necessary for normal growth. The results obtained in this investigation (table IX) indicate that a ten percent level of rice polishings not only furnish adequate vitamins B and G for growth but also enough of these vitamins for reproduction and lactation. The growth of nursing young, whose mothers received this level of rice polishings, while not optimum, was

as good as the growth reported by some investigators as normal. When the change was made to autoclaved rice polishings the poor health of the adults and the decrease in lactating ability of the females as evidenced by the young indicated, as was expected, an inadequate supply of the heat labile vitamin B. The rapid onset of reproduction failure on the lower levels of autoclaved rice polishings was probably also due to an inadequate supply of vitamin B.

The poor reproduction and lactation record (table XI) of those animals receiving powdered butter-milk as the sole source of vitamins B and G is more difficult to account for. The poor reproduction is probably due to a very low level of fat soluble vitamin E in the butter-milk rations that were fed. Butter-milk has also been reported as being low in vitamin B, which in the light of present knowledge would account for the failure of lactation. Just why the high level of powdered butter-milk resulted in complete failure of reproduction while some reproduction, although poor, was obtained on the lower levels is not known. It will be noticed that similar results were obtained with soy beans, reproduction being decidedly poorer on the higher levels. The high protein intake on the higher levels of powdered butter-milk and soy beans may upset metabolism in such a way as to lower the ability of the animals to reproduce. High protein intake has been reported as injurious by a number of workers.

It is evident from the results obtained (table XII) that

kelp has a very good supplementary value for reproduction and lactation. Where the one, three, and eight percent levels of kelp supplemented a twelve percent level of yeast, good reproduction, and lactation better than that obtained when mothers received growing ration, was observed. On a similar ration containing no kelp (table XV, number one) but all the other elements of the ration in the same proportion, only one litter was obtained from eight females in one hundred and eighty days, whereas on the rations containing kelp all of the females had two or more litters each. The ten and twenty-five percent levels of kelp supplemented a twelve percent level of extracted wheat embryo instead of a similar amount of yeast as in those rations containing the lower levels of kelp. The animals receiving the rations containing kelp and extracted wheat embryo also reproduced normally, but lactation on the other hand was poor. Chick and Roscoe (40) report wheat embryo as being very low in vitamin B. It would seem, therefore, that kelp at ten and twenty-five percent levels does not contain sufficient vitamin B to supplement a twelve percent level of extracted wheat embryo so that normal lactation might be obtained.

The low vitamin B content of kelp is also indicated by the failure of reproduction (table XIII) and the generally poor appearance of the animals when autoclaved yeast was used in place of untreated yeast in the above experiments where the rations contained one, three, and eight percent of kelp.

The ash of various foods, when used as a supplement to a diet adequate in all known elements except vitamin E, did not permit of normal reproduction. The ration containing a one and three-tenths percent level of kelp ash seemed at first to be adequate but reproduction had stopped on the two hundred and thirty-first day of a three hundred and ninety day experiment. Daniels and Hutton (22) failed to get reproduction using milk alone, but did get reproduction when the milk was supplemented with the ash of soy bean or purified salts. In experiments described in this thesis a diet believed adequate in all essentials except vitamin E was supplemented with soy bean ash. The results (table XIV, number 5 and 6) indicate that the soy bean ash does not supplement an inadequate amount of vitamin E so that normal reproduction is obtained. Similarly another purified diet supplemented with the same salts used by Daniels and Hutton did not give compatible results (table XIV, number 10). The addition of fresh males to four of the experiments just described (table XIV, numbers 2, 4, 5 and 6) resulted in reproduction being stimulated (table VI). It is therefore concluded that the males on the original experiments had become sterile while the females had remained fertile. Other investigators have also found that males become sterile much sooner than females.

Fredericia (63) found the presence of lard in the diet destroys vitamin A. In a series of experiments previously described ((7) The Effect of Lard on Growth, Reproduction,

and Lactation) it was found that a fifteen percent level of lard did not affect growth. However, when incorporated into the ration reproduction was impaired. Such was not the case when cod liver oil (the source of vitamin A) was mixed into the ration just before feeding. Neither did reproduction fail when the lard was autoclaved prior to mixing it in the ration.

SUMMARY

Rats grow at a normal rate with soy bean as the only source of vitamins B and G.

Reproduction was normal on the lower levels of soy bean but on the higher levels the rats were not as prolific as on the lower levels.

Satisfactory lactation was not obtained on any level of soy bean.

Manchu soy bean appeared to give somewhat better lactation results than either Virginia or Sable bean.

Hog lung and spleen, as well as beef thymus, did not supplement a fifteen percent level of soy bean so as to appreciably improve lactation.

Hog and beef pancreas and brain, and beef heart supplemented the soy bean ration so an improvement in lactation was apparent.

Hog and beef liver, hog kidney and egg yolk supplemented the soy bean ration in such a way that very marked improvement in lactation was obtained. The young showed a better rate of growth than is ordinarily considered normal.

Thirty percent of wheat as the only source of vitamins B and G did not permit of satisfactory lactation.

The thirty percent wheat ration was markedly improved for lactation by the addition of liver.

The possibility of there being a new dietary substance

necessary for lactation has been emphasized.

If such a substance exists it probably is destroyed by dehydration or temperatures of about one hundred degrees centigrade.

Extraction with ether does not furnish an adequate means of separating the factor or factors that stimulate lactation.

Satisfactory growth, reproduction and lactation were obtained when a ten percent level of rice polishings was the sole source of vitamins B and G.

With powdered butter-milk as the sole source of vitamins B and G reproduction and lactation were poor on all levels tried (forty to seventy-three percent).

A twelve percent level of yeast, more so than a similar level of extracted wheat embryo was decidedly improved for reproduction and lactation by the addition of kelp.

The ash of soy bean, kelp or alfalfa does not improve the reproduction value of a purified ration.

Fresh lard when incorporated into the ration impairs its value for reproduction. Such impairment may be at least partially prevented by autoclaving the lard prior to mixing it in the ration.

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