1930

Reproduction and lactation on simplified diets

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DP

14554

REPRODUCTION AND LACTATION ON SIMPLIFIED DIETS

BY

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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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ACKNOWLEDGMENT

I wish to thank Professor V. E. Nelson for his counsel and kind assistance in this work.

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REPRODUCTION AND LACTATION ON SIMPLIFIED DIETS

INTRODUCTION

The first concern of the first living organism was to feed itself in order that it might continue to live. Since this time, during all the course of evolution, hunger has remained the most dominant instinct. To it may be ascribed, directly or indirectly, practically all other changes which have taken place.

In the beginning, when food was relatively scarce, the desire of the organism was simply to procure food, whatever food might be available. Then, as other organisms arose and food became more abundant, the desire for food was enlarged to include the preference of certain foods over others. Other desires also arose, but all were so modified that food might still be available. If, for example, the organism desired warmer surroundings, but food became scarce with the rise in temperature, then a compromise was necessary; the organism established itself in cooler surroundings than it might desire in order that it might still obtain food. As the organism evolved into more complex organisms, the process of living became correspondingly more complex. Under these conditions, it becomes increasingly difficult to attribute any single act solely to the desire for food. Finally, if one considers life under present conditions, it is impossible to trace all the relationships between the desire for food and the many other desires and actions.

However, though we cannot at the present time analyze our every action with respect to the desire for food, that fact does not decrease the importance of food, or of understanding the effects of diet. Until the last twenty or thirty years, food has been something which must be procured and which, when procured, must be made palatable. Although many dietary needs were filled unconsciously through a natural craving for certain food, or a fortunate choice of foods, little was known of their relative values. Even when the chemical nature of foods was discovered, the situation was not materially improved. People were lulled into a false sense of security by the thought that they were getting the proper amount of fats, carbohydrates, proteins, minerals, and calories. Diseases which were caused by faulty diets were not recognized as such and, in most cases, no cure was known.

The advent of the use of small laboratory animals, such as the rat and guinea pig, by means of which the values of various foods might be quickly and easily tested, was the greatest single step in the discovery of the importance and the complexity of a proper diet. It may be said that this single step introduced a new era in nutrition. In the period since 1900, more was discovered about diet and its relation to health than had been discovered in all the previous years. Before this time, a diet was considered sufficient if it contained the proper amount of protein, carbohydrate, fat, and various minerals, although some distinction was made among different proteins and certain minerals were considered more important than others.

When the first comparatively crude experiments indicated that some unknown substance was necessary for life, nutritional investigation received a great stimulus. Much research was begun on this new work and discoveries were made rapidly. At the present time it is recognized that, in addition to protein, fat, carbohydrate, and minerals, a complete diet must include various accessory foodstuffs known as vitamins. Since practically all of these substances are necessary in any complete diet, it may be worth while to discuss each of them briefly.

Proteins.

The proteins are a class of substances which consist, in the main, of combinations of alpha amino acids or their derivatives. Some, such as mucin, also contain carbohydrate molecules; some, such as the nucleo proteins, contain purine bases. Others may contain small amounts of phosphorus, iron, copper, iodine, or zinc. Because of the complexity of the protein molecule, relatively little is known regarding its structure.

Protein is essential in the diet of all animals and cannot be replaced by any other substance. It is the most important constituent of all cells and is necessary for the life of the cell. In all cellular metabolism, cell proteins are broken down. These proteins must be replaced eventually from the diet. The amount of protein in the diet may vary rather widely. If the protein is a good one, as low as nine percent in the diet may be adequate. However, over long periods of time higher levels of protein give more satisfactory results. Some evidence has been submitted (94) to show that dietary regimes very high in protein may cause damage to the kidneys. This appears to depend upon the particular protein which is used (92).

It was discovered in the earlier nutritional experiments that not all proteins were of equal value. However, the exact nature of the deficiency was not apparent for some time, since most of the experimental diets contained unsuspected sources of proteins or of amino acids. Osborne and Mendel, who were among the most prominent of the workers along this line, persisted in the use of "Protein-free milk" as a source of minerals and, though they did not realize it, of vitamin B. This "Protein-free milk" was found to be of considerable value in supplementing certain proteins which were tested.

Summarizing, the nutritive value of a protein depends upon the amount and kind of amino acids present. The more nearly the protein corresponds in composition to the protein of the animal tissue, the more nearly adequate it is.

Carbohydrates and Fats.

Carbohydrates and fats are comparatively unimportant from the dietary standpoint. Primarily, they are quick sources of energy. In practically all diets, fats are present because they contain the necessary fat-soluble vitamins and are readily available. Carbohydrates are present merely to make up the bulk of the diet.

The amount of carbohydrate or fat in the diet may be varied widely with no apparent detrimental effedt (67,79). The whole diet, except the minimum necessities of minerals, protein, and vitamins, may consist of carbohydrate or of fat. Both fat and carbohydrate may be omitted from the diet, their places being taken by protein.

There is some evidence to show that very small amounts of fat may be necessary. Burr (7) has shown that, on diets extremely deficient in fat, rats develop a peculiar disease, characterized by lesions of the skin and caudal necrosis; and eventually they die. This disease may be prevented or cured by the inclusion of small amounts of fat or of certain fatty acids in the diet.

Minerals.

Most of the natural foods are deficient in many minerals. Even a combination of natural foods seldom furnished the right proportions of all the minerals which are needed. Synthetic diets, containing highly purified constituents, are much more deficient in minerals than are the natural foods. It is necessary, therefore, that synthetic diets be supplemented by minerals. For this purpose a mixture of pure salts, containing all the known essential elements in about the correct amounts, is used. In all cases where the effects of minerals are not to be studied, such a salt mixture is added to the diet.

Analysis of an animal body shows the presence of a great many inorganic ions. Some of these: Na, K, Ca, Mg, Cl, SO_4, and PO_4, are present in considerable quantities. These elements have several important functions such as maintaining osmotic pressure and muscle irritability, building skeletal structures, aiding in the processes of metabolism, and many others. Other elements, such as Fe, I, and Cu, are present in much smaller amounts but are none the less essential. Still others, such as Al, Si, Zn, As and many others, are present only in traces and may or may not have definite physiological functions. Most of the minerals which occur in the body in traces are also found in foodstuffs. It remains to be proved whether these traces of minerals play some definite role or whether they are present merely as impurities.

Vitamins.

The vitamins are best defined as accessory food substances, probably organic in nature, which are synthesized by plants but not by animals, and which have a physiological function out of proportion to the amount required by the animal. Since their chemical nature and their mode of action are largely unknown, they are designated by the letters of the alphabet. At present, vitamins A, B, C, D, E, and G are recognized. However, it is highly probable that others will be discovered.

Vitamin A. Vitamin A was discovered almost simultaneously by McCollum and Davis (81) and by Osborne and Mendel (93). They observed that certain fats and oils possessed a remarkable power of stimulating the growth of rats while other fats did not. This could not be explained on the basis of the glycerides present.

Vitamin A is classified as a growth promoting substance, though it is not clear how this is effected. It is probable that the action is brought about through the ability of vitamin A to maintain the integrity of the epithelial tissues. At any rate, deficiency of vitamin A causes a keratinization of the epithelial tissues, the most noticeable of which is the keratinization taking place in the cornea of the eye and known as xerophthalmia. Vitamin A is also thought to play some part in the ability of the body to resist disease. This is very largely conjecture, however.

The chemical nature of vitamin A remains to be discovered. It is not improbable that it is related to the sterols, for the most concentrated preparations of vitamin A consist almost entirely of sterols. The early view of Steenbock (104) that vitamin A was in some way related to carotin was not confirmed by other workers. However, recent work done by Euler and co-workers (21,22) shows clearly that, though carotin is not the same as the vitamin A of liver oils, it may take the place of vitamin A in the diet. Moore (87) has shown that rats, fed an excess of carotin, transform the latter into the colorless vitamin A of liver oils, in which form it is stored in the liver. Morton (personal communication), employing spectrographic methods, has shown that the vitamin A of butter fat consists partly of carotin and partly of the colorless form of the vitamin.

Vitamin A is non-saponifiable and is found in the non-saponifiable fraction of oils. It is oxidized readily, hence all efforts to concentrate it are carried out best in an inert atmosphere. The heat stability of vitamin A is high providing oxygen is not present. On this account, Drummond and Coward (18) have had considerable success in the concentration of the vitamin by fractional distillation. However, in some recent work, Drummond and Baker (17) found that the vitamin A in more concentrated preparations was destroyed by heat, so that this method was inadequate.

Vitamins B and G. Within the last five years that substance which we have known as vitamin B has been divided into two factors known under the various names of B_1 and B_2, B and F, B and G, F and G, and B and P-P. In this paper they will be referred to by the nomenclature which has been made standard in this country, namely vitamins B and G.

Vitamin B studies are complicated by two facts. First, much of the work done on the effects of vitamin B deficiency are now known to have been due to the combined deficiencies of vitamins B and G. Second, many of the effects of vitamin B deficiency can be reproduced by starvation and vitamin B is known to have a profound effect on the appetite.

Lack of vitamin B causes a disease of the nerves known as beri-beri or polyneuritis. This is specific for vitamin B. Lack of this vitamin also causes atrophy of the endocrine glands with the exception of the adrenals. It is not correct to say that vitamin B is not a growth promoting vitamin. Through its effect on the appetite, it usually causes a cessation of growth.

Although the exact chemical nature of vitamin B is not known, it is definitely established that it is a nitrogenous base, probably a cyclic nitrogen compound. At least, many of its chemical properties indicate this. Vitamin B possesses a number of interesting chemical characteristics. It is soluble in water and in dilute acids, alcohols, and acetone. It is readily absorbed from its aqueous solution at a pH depending upon the adsorbent, and released when the pH is changed. It is precipitated as an insoluble complex with such compounds as phosphotungstic acid, tannic acid, picric acid, and others. It also may be precipitated as a salt of a noble metal. All these reactions are useful in the concentration of the vitamin.

Vitamin B is destroyed by heating, the ease of destruction depending upon the pH at which it is heated. In acid solution it is stable, but in alkaline solution it is destroyed quickly. It is also susceptible to oxidation.

Vitamin G. Vitamin G is the fraction of the vitamin B complex which is the more heat stable. The property first ascribed to it was that of promoting growth. However, this effect, similar to that caused by vitamin B deficiency, is due indirectly to the effect on the appetite rather than to any direct effect on growth. The true function of vitamin G, as shown by Goldberger and associates, seems to be the prevention of a disease known as pellagra. This function has been observed in the case of rats, dogs, and human beings. There may be other functions which have been unnoticed as yet.

Vitamin G is probably similar in composition and structure to vitamin B. At any rate, the solubilities, the adsorption properties, and the behavior toward various precipitating reagents are very nearly the same. Both vitamins B and G are destroyed by long heating or by heat and alkalies. These latter facts, and the fact that the distribution of the two vitamins in nature is nearly the same, may account for their having been considered as only one substance.

Vitamin C. The occurrence and treatment of scurvy was noticed long before anyone thought of attributing it to the absence of a vitamin-like substance. The failure to recognize scurvy as a vitamin deficiency disease, even though the idea of vitamins had been accepted, may be ascribed to the lack of technic in producing experimental scurvy and to the lack of an appreciation of the differences in susceptibility of various species of animals.

Present knowledge indicates that vitamin C is required by man, monkey and guinea pig only. Repeated experiments on rats have shown that vitamin C does not supplement or take the place of any of the other vitamins and that it is not required by rats. For this reason, since this paper is concerned entirely with experiments performed on rats, vitamin C will not be discussed at length.

Vitamin D. Since the work in this paper is not concerned with vitamin D, it will be discussed very briefly. Rickets, the disease occurring in the absence of vitamin D, can be produced in experimental animals only when the ratio of calcium to phosphorus in the diet is definitely wrong. All the data of this paper were obtained on rations containing an adequate salt mixture which maintained the calcium to phosphorus ratio at approximately the correct value. Hence, the interpretation of the results cannot be influenced by any possibility of rickets.

Vitamin D is generally assumed to be concerned with the formation of bone and, possibly, teeth. However, the mechanism probably is not a direct one. It is thought that vitamin D may influence calcium and phosphorus assimilation by its effect on the pH in the intestine. Several investigators have shown that in severe rickets the feces are alkaline, but on administration of vitamin D, they become acid very quickly. Vitamin D may act through its effect on the calcium and phosphorus concentration of the blood. In cases of rickets, blood phosphorus is very low while the calcium remains at nearly normal levels. Since phosphorus is concerned in the metabolism of nearly all body tissues, it is to be expected that rickets will be accompanied by abnormal metabolism in tissues other than bone.

Chemically, vitamin D is a substance which is formed from ergosterol by the action of ultra-violet light. It may be that vitamin D and ergosterol are tautomeric isomers. However, when ergosterol has been transformed into vitamin D, it is no longer possible to crystallize it or, in any other way, to separate it from the unchanged ergosterol. For this reason the properties of the pure vitamin are little known.

Vitamin E. The function of vitamin E is to prevent sterility in both the male and the female animal. Thus far, rats are the only animals which have been used to demonstrate this function. The symptoms of the deficiency differ in the two sexes. In the case of the female, although ovulation occurs normally and the foeti are implanted and begin to develop, the placental function fails before parturition, causing the death and resorption of the foeti. This condition may be cured at any time by small amounts of vitamin E in the diet. In the male, it leads to destruction of the germ cells and eventually of the entire seminiferous epithelium, producing permanent sterility. Sterility in the male or failure of the female to produce young may occur for reasons other than the deficiency of vitamin E. In none of these cases are the symptoms, particularly those exhibited by the female, the same as those caused by lack of vitamin E. Evans and Bishop (28), who first definitely announced a specific vitamin for reproduction, followed the oestrus cycle very carefully by histological methods. They also point out that the curative dose is effective when given as late as the fifth or sixth day of pregnancy.

Vitamin E is fat soluble. It is found in a large number of natural foods, especially in the seeds and the green leaves of plants. The grains, such as corn, oats, and wheat, are good sources. It is found more in the germ than in the endosperm, wheat germ oil being the most potent source known. It is distributed through nearly all the tissues of the animal body, the muscle and the fat containing more than the viscera. Milk fat is a relatively poor source though it may vary widely, depending on the diet of the cow. According to Evans and Bishop (35), cod liver oil, which is rich in vitamins A and D, is notably deficient in E. However, Nelson and co-workers

(91) submit data which show that one brand of cod liver oil is a good source of the reproductive factor.

Relatively little is known about the chemical composition of vitamin E since the most concentrated preparations so far obtained seem to contain vitamin E only as an impurity. According to Evans (41), vitamin E is soluble in methyl and ethyl alcohols, ether, pentane, benzene, acetone, ethyl acetate, and in fats. Cold pentane is a particularly valuable solvent for use in concentrating the vitamin. It is not soluble in water, nor in dilute acids or alkalies. The vitamin is very stable to heat, light, oxidation, saponification and many ordinary chemical reactions. It may be distilled in steam or *in vacuo*. Prolonged treatment by most of the above processes destroys the vitamin slowly. Ordinary oxidation affects it only slightly but either it is destroyed during the oxidation of fats which are present in the ration, or the oxidation products of the fats render it incapable of exerting its usual action.

Other vitamins. It is not only possible but probable that other vitamins exist. Bezssonoff (6) suggests that vitamin C may consist of two separate factors. Williams and Waterman (141) believe that the vitamin B complex may contain at least three different vitamins. Evans and Burr, (44,45,46) working on very highly purified nutrients, find that growth is not normal unless the diet is supplemented with some natural food. McCay, Bing, and Dilley (80) find that young trout require something besides all the known dietary factors. This substance, present in raw liver, they call factor H. Several investigators have suggested that there may be a specific vitamin concerned with lactation. The fact that these new factors have already been suggested and the possibility that others may follow raise the question of the proper limitations of the vitamin field.

STATEMENT OF THE PROBLEM

Man is essentially a selfish animal. Most of his works are done with a view toward increasing his future wealth, happiness, or comfort; and so it is in experimental work in nutrition. Being in a position to choose which foods he will eat and which not, man wishes to eat those foods which will best insure good health and longevity. However, he cannot test on himself the effects of the various foods, for in that way he would be susceptible to dangers as well as benefits and many of the results would benefit him little. For this reason the experiments are performed on smaller animals, so that the whole life span may be studied in two or three years. Many of the results obtained are valueless or difficult to interpret; a few seem to have a definite application and value. These latter results are then applied to the problems of human nutrition.

This paper deals with some of the dietary factors concerned in the reproduction and lactation of rats. It is well known that the process of reproduction and the subsequent feeding of the young involves a severe strain on the mother, probably the most severe strain of any normal function. Both before and after birth, the tissues of the young are formed at the expense of the tissues of the mother. The only way in which the mother can be protected from this depletion is by furnishing her with a diet which is not only adequate, but which contains a considerable excess of certain factors. That is, the diet must be very high in quality though not necessarily so in quantity. It was part of the purpose of this work to discover some of the dietary factors which seemed to be the most important to the mother and the suckling young.

When a machine becomes too complicated or unwieldy, it ceases to be of great value. Similarly, if the science of nutrition becomes encumbered with innumerable new factors, each one responsible for some minor bodily function, then it becomes too complex a tool and people, not understanding all the details, will hesitate to use it. With this in mind, many of the known dietary factors were tested to see if some of the factors other than vitamin E might not supplement or take the place of vitamin E. The experiments to be described were performed on rats, but it is believed that many of the results obtained are directly applicable to the nutrition of human beings. Many of the results are also applicable to the nutrition of farm animals.

REVIEW OF LITERATURE

It is not possible to review in this paper all the literature pertaining to nutrition. Hence, only those references will be noted which have a direct bearing on the general subjects of reproduction and of lactation. For a more complete discussion and bibliography of the various aspects of nutritional chemistry, the reader is referred to "The Newer Knowledge of Nutrition", revised edition, by E. V. McCollum and Nina Simmonds.

The failure of rats to reproduce normally when fed purified diets was noticed by several investigators before a specific reproductive vitamin was proposed. These investigators attempted to explain their results in terms of the dietary factors which at that time were known to be essential.

McCollum and Davis (1915)(82) in a study of the mineral requirements of the rat found that reproduction was not normal on a diet of whole milk powder 10, casein 15, butter fat 5, agar-agar 2, dextrin 53, sucrose 15, and various additions of inorganic materials. They were certain that this was not due to the inorganic material but did not know how else to explain it.

Reynolds and Macomber (1921)(97,98) stated that decreasing the content of the diet in vitamin A, in protein, and in calcium caused markedly decreased fertility in rats. Prior to this time, Mattill and Conklin (1920)(77) made a study of whole milk diets and came to the conclusion that whole milk was lacking either quantitatively or qualitatively in some factor which was essential for reproduction. They ascribed it first to the quality of the milk proteins and then to an insufficient amount of vitamin B, since they were able to improve reproduction greatly by the addition of one percent of yeast. However, they were not wholly satisfied with either of these explanations.

In 1922-23 Evans and Bishop (28,30,31,32,33,34,35) published the first of their extensive data to prove the existence of a specific dietary factor which was necessary for reproduction. They first observed the reproductive behavior of a large number of normal rats on a diet of natural foodstuffs. They observed all phases of the oestrus cycle, conception, implantation, and the period of gestation, using histological methods very largely. Then they observed rats on diets of purified foodstuffs, using as their basal ration the following: casein 18, cornstarch 54, lard 15, butter fat 9, salts (McCollum) 4, and Brewer's yeast (Harris) 0.4 - 0.6 gram per rat per day. On this diet they found that growth was normal, but that the females were all sterile. Closer examination revealed that the oestrus cycle was normal, conception and implantation proceeded as usual, but that at the sixteenth or seventeenth day of the gestation period, the foeti died and were resorbed. They found that small amounts of lettuce fed daily to females that had had one or two resorptions would bring about a complete recovery of fertility. Though they were sure that their basal ration contained all the known dietary factors in sufficient amounts, they decided to make thorough tests before announcing a new vitamin.

They found that increasing the amount or quality of protein, carbohydrate, and vitamins A, B, C, and D did not bring better results. Therefore, they felt justified in making the statement that a new dietary factor, present in natural but not in purified foodstuffs, was necessary for reproduction. When the lard in the basal diet was replaced by an equal weight of butter fat, making a total of 24 percent in the diet, reproduction was partially successful. From this they concluded that butter fat contained a small amount of the reproductive factor. They also concluded that, since butter fat could partially overcome sterility, it would seem that the inorganic salts in the diet could not be a factor. Factor X, as they called it, was present in wheat, wheat germ, lettuce, meat, and oats.

The work thus far had been concerned entirely with sterility in the female. Evans (1925)(24,40) now investigated the effect of rations containing no vitamin E, as it was now called, on the male rat and found that sterility was produced. He found four stages in the development of this sterility. First, there is a normal amount of sperm but loss of fertilizing power. The sex responses are normal. Second, there is a complete loss of sperm. Third, there is loss of the power to form the vaginal plug. Fourth, there is complete loss of all sex interest. After passing the first stage, male sterility cannot be cured. Mason (69,70,71) made a careful study of this same question at about the same time and corroborated Evans' results. He reported that, while growth was better than normal, male rats invariably became sterile. With young rats this usually occurred in 60 to 150 days. The sterility, as proved by matings with normal females, checked with the amount of cellular degeneration as shown histologically. He also found that the fresh weight of the testes was a good index of the reproductive capacity; as the rats became sterile, the testes became much smaller and lighter.

Evans and co-workers (1924, 1925, 1926, 1927)(37,38,39, 41) made an extensive study of vitamin E. They found that the vitamin was stored in the tissues of the body for a period of several months and that it was transmitted by the mother to the young. They also investigated its physical and chemical properties and found it to be stable.

Mattill and Stone (1923)(78), working on milk rations, found that such rations were insufficient in protein and in vitamins for reproduction. When vitamin E was announced, they said that it was the only possible explanation of their results. Mattill, Carman and Clayton (1924)(75), still working on milk diets, found that the addition of two to five percent of nucleoproteins did not aid in reproduction, nor did a large increase in the percentage of yeast fed. On addition of five or ten percent wheat germ to the ration, practically all the females gave birth to young, though very few were raised. Diets high in fat required more of the reproductive factor than diets low in fat. Another paper by Mattill and Clayton (1926)(76) repeated much of the previous work and stated that ".....yeast certainly does not provide any significant amount of vitamin E."

Sure (1923)(105) was working on milk and milk powder diets also. He found that, while they gave normal growth, reproduction was very poor. Even when young were born the mothers failed to raise them. His ration contained 9.6% of milk proteins, 7% of butter fat, and an alcoholic extract of 15 grams of ether extracted wheat germ. In a later paper (1923)(106) in which he described the use of a ration of skimmed milk powder 50, ferric citrate 0.2, cod liver oil 2, and agar-agar 2, he reported the same results. He concluded that success in reproduction was not due to proteins or to vitamins A, B, or D, but to some unknown factor which was to be found in Georgia velvet bean pod meal, yellow corn, and rolled oats. He also found fertility to be good on a ration of skimmed milk powder 35, salts (#32) 1, cod liver oil 2, and polished rice 61.9; and he concluded that "Success with fertility.....must be ascribed to some new factor in polished rice that is essential for reproduction." In his third paper on this subject, Sure (1924-25)(107) demonstrated that vitamin E was present in the ethereal extracts of yellow corn, wheat germ, and hemp seed. About one kilogram of material was extracted in a large Soxhlet extractor with anhydrous diethyl ether until the ether had siphoned six to eight times. The extracted material, which still contained about 0.4 percent fat, would not support reproduction. The ethereal extracts gave good reproduction, but very few young were raised until the vitamin B content of the ration was increased greatly.

Anderegg (1924)(1), also working on milk powders, found that 50 percent of whole milk powder as the sole source of protein and vitamins gave normal growth and reproduction, although the mortality of the young was high. Anderegg and Nelson (1925)(1926)(2,3) supplemented a skimmed milk powder diet so that it conformed in composition to a whole milk powder diet. However, they were unable to duplicate the results received on the whole milk powder diet. Addition of yeast or wheat germ gave some reproduction in the first generation.

Daniels and Hutton (1925)(12,13), working on the nutritive properties of milk and milk powders, secured good reproduction by supplementing whole milk with ferric citrate, iodine, and ash of soy bean, lettuce, or yeast. Analysis of the ash showed the presence of aluminum and silicon. They then discovered that a supplement of equal parts of NaF, $KAl(SO_4)_2 \cdot 12H_2O$, $MnSO_4 \cdot 5H_2O$, and Na_2SiO_3 in a whole milk diet so that each rat received 1.5 milligrams of each salt per day gave excellent reproduction. They concluded that the deficiency in milk diets was entirely inorganic. Tso (1927)(135) found that butter fat gave fairly good results on reproduction provided very little lard was present in the diet. Ash of soy bean was slightly beneficial as regards reproduction but not as regards lactation. Lettuce was very beneficial in lactation.

Sure (1926)(108) reported somewhat similar results on skimmed milk diets, saying that he found two types of sterility. The first type appears in first generation females and is characterized by resorption of the foetus. The second type appears in the second generation and can be overcome by the addition to the diet of small amounts of $KAl(SO_4)_2 \cdot 12H_2O$, NaF, $MnSO_4 \cdot 5H_2O$, and Na_2SiO_3.

Several other investigators confirmed the results of Evans and of Sure. Kennedy (1926)(65) showed the reproductive factor to be present in the ether extract of rolled oats. She also found that a deficiency of calcium, or an excessively high percent of protein, would induce sterility even though vitamin E were present.

Miller and Yates (1924)(85) extracted corn with cold acidified water and found that it did not support reproduction. Unextracted corn, wheat germ, and green kale contained the reproductive factor.

Hogan and Harshaw (1924)(59) were slow in accepting the necessity for a specific reproductive factor, believing that a proper relationship of the other factors was all that was necessary. In a later paper (1926)(60) they accepted the views of Evans and Bishop. They found no evidence that sterility was due to anemia, or that vitamin E had any direct relation to the formation of hemoglobin. Prior to this, Hart, Steenbock and co-workers (1925) had attempted to show that natural foodstuffs play a part in iron assimilation and that this effect might be the reason that natural foods gave better reproduction.

Nelson and co-workers (1926)(90) investigated the amount of vitamin B necessary for growth and reproduction. Their work indicated that a much greater amount of vitamin B was required for reproduction than was required for growth. Miller (1927)(84), investigating the same problem, decided that the amount of vitamin B required for growth and for reproduction was about the same. A later paper by Guest, Nelson, and associates (55) confirmed the views of Miller as regards the amount of vitamin B for growth and reproduction.

Nelson and co-workers (1927, 1928)(89,91) got results which were quite the opposite of those obtained by many other investigators who said that cod liver oil was notably deficient in vitamin E. They obtained very good reproduction on a ration in which cod liver oil and yeast were the sole sources of vitamins. Evidently, this was due to the particular cod liver oil used by Nelson, since Sure (1927)(113), using a different brand of oil, got very poor results on reproduction. In another paper, Sure (1927)(115) found that butter fat contained much more vitamin E than did cod liver oil. He also stated that continuous fertility was the only satisfactory criterion of vitamin E. However, vitamin E could be stored from the stock ration for only one gestation. Nelson (1926)(90) had been able to obtain six generations of rats on a diet of casein 18, salts 3.7, butter fat 5, yeast 5, and dextrin 68.3.

Anderson (1926)(4) examined the effect of vitamin E on fat and nitrogen metabolism. He found no difference which could account for the effect on fertility. Hartwell (1926, 1927)(57,58) could find little correlation between vitamin E and growth. Hogan and Harshaw (1926)(60) and Hogan, Hunter and Shrewsbury (1927)(61) confirmed these results. Similarly, Grijns (1925)(52) found no relation between growth and fertility. However, Mason (1929)(71) thinks that there may be two factors present in vitamin E, one necessary for growth and one necessary for fertility. Evans (1928)(26) had also found that, on long time experiments, rats receiving vitamin E exhibited uniformly improved growth and vigor over those receiving no vitamin E.

Some work has been done on the relations of vitamins A and B to sterility. Evans and Bishop (1922 and 1928)(29,25) found that rats became sterile on diets low in vitamin A. This was due to ovarian rather than to uterine disorders. The Graafian follicles failed to mature, so that no fertilization and implantation occurred. Parkes and Drummond (1926)(96) observed the sterility of rats on vitamin A deficient diets but were unable to find any histological explanation.

Sure (1928)(123) observed a dietary sterility in rats receiving very little vitamin A but an abundance of E. This sterility was characterized by resorption of the foetus during gestation.

Coward (1929)(10) in an investigation of the oestrus cycle, showed that it was impossible to use the vaginal smear method to diagnose pregnancy in rats on vitamin A deficient diets. Mason (1930)(72) found that lack of vitamin A caused testicular degeneration, the severity of the change being proportional to the duration and severity of the eye trouble. Diets containing wheat germ oil caused less severe changes, due, probably to the presence of traces of vitamin A.

Parkes and Drummond (1925)(95) reported that, when male rats were fed a diet totally deficient in vitamin B, degeneration of the testes and sterility ensued in a short time. On less deficient diets these results were postponed either temporarily or permanently.

Mattill (1927)(73) and Evans (1928)(27) also investigated the effect of vitamin B deficiency on male rats. They found no testicular degeneration or loss of potency in rats fed a diet containing an abundance of vitamin E, but no vitamin B. On the other hand, Marrian and Parkes (1928)(68) using the two fractions of vitamin B, found that extreme atrophy of the testes occurred in pigeons fed a diet deficient in the antineuritic B_1. Vitamin B_2 or G did not seem to be necessary for pigeons.

Cunningham and Osborn (1929)(11), in a preliminary report, stated that hot air and infra-red rays can produce sterility in male rats.

Simmonds, Becker and McCollum (1927)(101) attempted to show that inorganic iron, particularly in the form of ferrous sulphate, could not be assimilated by the animal. However, if wheat germ oil were added to the diet, the ferrous sulphate proved satisfactory as a source of iron. Organic iron, such as ferric citrate, could be assimilated. They suggested that vitamin E might play some part in iron assimilation, and that death of the foetus might be due to a crisis in iron assimilation. Jones (1927)(64) showed very conclusively that the poor results obtained when ferrous sulphate was included in the diet were due to the catalytic destruction of the vitamin A by the ferrous sulphate, and not to the fact that it was not assimilated. Estill and McCollum (1927)(20) confirmed the results of Jones. In a later paper, Estill and McCollum announced the isolation from cod liver oil of a substance which inhibited the destruction of vitamin A by ferrous sulphate. This substance they suggested might be vitamin E. However, Simmonds, Becker and McCollum (1928)(102), in a general paper on the distribution of vitamin E, withdrew their contention that vitamin E was necessary for iron assimilation and that wheat germ oil protected against the effects of ferrous sulphate because of its vitamin E content. Mattill (1927)(74) was inclined to believe McCollum's first contention. He showed that the oxidation which accompanied the beginning and development of rancidity in fats tended to destroy vitamins A and E. The presence of hydroxyl groups, as measured by the acetyl value of the fat, delayed this action and wheat germ oil had a high acetyl value. However, Daniels and Jordan (1928)(15) believed that the effectiveness of wheat germ oil in preventing sterility was due to the presence of some substance (vitamin E) and not to a retardation of oxidation of vitamin A. Evans and Burr (1927)(42) found that the size of the curative dosage of vitamin E depended upon the amount and kind of fat present in the diet. In a second paper (43) they proposed the presence of an anti-vitamin E in certain fats. The amount of this anti-vitamin increased with rancidity. Fridericia (1925)(50) found that certain fats seemed to inactivate the vitamin A in a ration. Lard rendered at fairly high temperatures was one of these. Nelson and co-workers (1926)(90) observed xerophthalmia in rats fed a diet containing five percent of butter fat and 19 percent of lard.

Waddell and Steenbock (1928)(137) reported the selective destruction of vitamin E in a ration composed of natural foodstuffs by the addition of ferric chloride in ether solution to make one percent of ferric chloride in the ration. Vitamin A was not destroyed as shown by feeding experiments. They stated "All of the animals on the various rations grew well and appeared normal and vigorous". This seemed to indicate that vitamin A was held in a different way from vitamin E, or that the latter is peculiarly sensitive to the action of ferric chloride. Goettsch (1930)(51) employed a similar ration and found that it caused extreme distrophy of the voluntary muscles.

A number of investigators have suggested the possible existence of a lactation promoting factor. Evans (1924)(23) found that lactation was not complete on many diets of purified foodstuffs. Fresh leaves, wheat germ in large amounts, egg yolk, meat and some vegetable oils caused great improvement. Sure (1925, 1926)(108,109), investigating various vegetable and fruit oils, found them to be divided roughly into three classes. Wheat, cottonseed, yellow corn, and palm oils permitted normal lactation and prevented sterility. Peach kernel, soy bean, peanut and olive oils seemed to promote lactation but had no effect on sterility. Wheat germ oil seemed to contain two factors. One was heat stable and promoted fertility; the other was easily destroyed by heat and promoted lactation. Grijns (1926)(53,54) believed that there were at least two reproductive vitamins, one of which affected lactation only. This latter was probably an albumin producer. Sure (1927)(112) found that the reproductive factor of cottonseed oil was concentrated in the unsaponifiable material and that this material gave good reproduction when incorporated into a skimmed milk powder diet to the extent of 0.0175 percent. Twice this amount was beneficial to lactation. In a much later paper, Sure (1929)(126) expressed some doubt as to the exact role of wheat oil in lactation. He suggested that the wheat oil might act partly to prevent the oxidation of vitamin A and partly to supply vitamin A. Sure also stated that it was still possible that wheat oil contained a fat soluble lactation factor.

Guest, Nelson, Parks and Fulmer (1926)(55) investigated various grains as sources of vitamin B for growth, reproduction and lactation. Wheat, rye, barley, yellow corn and white corn gave good growth and reproduction, but very few young were reared even on the highest levels of the grains. They decided that the amount of vitamin B necessary for lactation was much greater than that required for growth and reproduction. Smith and Hendricks (1926)(103) found that rolled oats as a source of vitamin B did not give good growth until it was supplemented with brewer's yeast which had been autoclaved at 15 pounds pressure for six hours.

In 1927 it became generally admitted that vitamin B consisted of at least two factors. One was heat labile; the other was relatively heat stable. The first prevented polyneutitis or beri-beri; the second was thought to be growth promoting and later was found to prevent pellagra. The various facts concerning these discoveries are well summarized in an editorial review by Sherman (1928)(99). A great deal of investigation has been done on these two factors, only a part of which will be quoted here.

Sherman and Axtmayer (1927)(100), in announcing work on the two factors of vitamin B, said that wheat was richer in the antineuritic vitamin B than in the anti pellagric vitamin G. Milk they found to be richer in G than in B. Hunt (1928) (63) found that wheat and corn contained about the same amount of vitamin B and of vitamin G, but that vitamin B was present in larger amounts. Fifteen percent of wheat supplied enough of the antineuritic vitamin for growth if supplemented with autoclaved yeast, a good source of vitamin G. Aykroyd and Roscoe (1929)(5) showed that wheat and corn were low in vitamin G. White corn contained slightly more than yellow corn. The germ of wheat and corn contained slightly more vitamin G than the endosperm. Williams and Waterman (1927)(140) had previously stated that young rats required both of the vitamin B factors. Pigeons seemed to requie vitamin B, but not vitamin G, and also a third factor, possibly vitamin E. Chick and Roscoe (1928)(9) found that young rats required vitamin G and suggested a method of testing for it, using a concentrate as the source of vitamin B.

The amounts of vitamins B and G required for lactation have been investigated thoroughly. Miller (1927)(84) decided that the amount of the vitamin B complex required for lactation was no greater than that required for growth and reproduction. However, Sure (1927)(116) found that the lactating mother used the vitamin B complex very inefficiently and that a large excess of it must be furnished in the mother's diet in order that the milk will contain sufficient amounts for the young. On the other hand, Sure (1928)(118) found that vitamins A and D were used very economically and that only the minimum amounts needed to be furnished to the mother.

Vogt (1928)(136) found that the vitamin requirements of the nursing young are very great. As partial evidence he showed that the vitamin content of the colostrum was much greater than that of ordinary milk. The young, when born, are fortified by a vitamin reserve obtained from the mother. Sure (1928)(119,120) in a series of papers, again showed that the nursing young require a large amount of the vitamin B complex and that the lactating mother secretes it very inefficiently. The growth of young could be greatly stimulated by direct feeding of vitamin B concentrates. Sure (121) stated that much infant mortality may be due to an insufficiency of the vitamin B complex.

Williams, Waterman and Gurin (1929)(142) point out some danger in the use of the separate factors of the vitamin B complex. While vitamin G is the more heat stable factor, it is destroyed to some extent by autoclaving. If autoclaving is carried out so that all the vitamin B is destroyed, then considerable vitamin G is destroyed also. If conditions are such that vitamin G is not destroyed, then considerable vitamin B remains unchanged. Increasing the pH in autoclaving causes a more rapid destruction of both vitamins. The writers believed that many results had been influenced by these facts. Nevertheless, since no better method has been discovered, the results obtained when autoclaved yeast is the source of vitamin G must be used.

Evans and Burr (1928)(48), using separate sources of vitamins B and G, found that about five times as much vitamin B was required for lactation as for growth. Vitamin G was found to be unimportant. Sure (1928)(125) disagreed with Evans. He found that rice polishings, which contain more vitamin B than G, must be supplemented with autoclaved yeast before adequate lactation is secured. About three times as much vitamin B and vitamin G were required for lactation as were required for growth. In a second paper, Sure (1928)(122) found that "vitavose", a commercial concentrate containing mostly vitamin B, was entirely inadequate for lactation unless supplemented with autoclaved yeast. In a third paper (129) he found that diets containing enough vitamin B to protect adult mice from beri-beri would not protect their nursing young. Daniels, Jordan and Hutton (1929)(16), working on milk diets, decided that the poor growth of the suckling young was due not to a deficiency in the secretion of vitamin B in the milk, but to the low calorific value of the diet or to a distaste for food so that too little was eaten by the mother. Daniels, Hutton and Marks (1930)(14) reiterated that food consumption by the mother on a milk diet plays an important part in the well being of the suckling young. The antineuritic vitamin probably was not the limiting factor.

Sure and co-workers (1929, 1930)(127,128,129,133) are making a considerable study of the effects of uncomplicated vitamin B deficiency on the nursing young. To date, they have found that the nursing young of mothers on such a dietary regime develop marked hypoglucemia before any loss of weight becomes apparent, anhydremia, and a marked disturbance of the hematopoetic function. When vitamin B was fed there was a rapid increase in blood sugar and a regeneration of blood. This might be the mechanism which controls food consumption in case of vitamin B deficiency. Vitamin B deficiency was found to have no effect on the alkaline reserve of the blood of nursing rats. When liver glycogen was examined, an extremely marked decrease was noted in the case of those young whose mother received no vitamin B. Sure considered this the most marked of any change he had observed on vitamin B deficiency. Lack of the vitamin B complex caused a fatty metamorphosis of the livers of nursing young. This was probably due to fatty infiltration. Uncomplicated vitamin B deficiency caused no such change.

Evans and Burr (1928)(47) announced a peculiar dietary disease of the suckling young. If young were born to a mother on a vitamin E deficient diet, the young grew very well until two or three days before weaning. At this time the young very suddenly became paralyzed and most of them died. A few survived—although they showed signs of the paralysis all their lives—and were able to reproduce normal young, provided vitamin E was included in their diet. This disease of the young did not occur on diets containing vitamin E.

Waltner (1929)(139) investigated the effects of iron on blood, growth, fertility, and lactation. Reduced iron had a harmful effect on fertility and on lactation, but both of these effects might be cured by vitamin D. The work of Hart (1929)(56) and of Waddell, Steenbock and Hart (1929)(138) had assigned the function of iron assimilation to the presence of copper in the diet. Evvard, Nelson and Sewell (1928)(49) had shown that very small amounts of copper as copper sulphate were beneficial to the growth of rats and swine. Sure (1928) (132) found that copper had no supplementary value to a vitamin B concentrate in lactation studies.

Nelson, Heller and Fulmer (1925)(88) discovered that crude cane molasses was a good source of vitamin B. Beet molasses contained some vitamin B and sorghum a very small amount. The crude cane molasses gave much better reproductive results than did yeast. Hoyle (1929)(62) found no vitamins A, B, D, C, or G in West Indian or English honey. Kifer and Munsell (1929)(66) obtained identical results except that they did not attempt to differentiate between vitamins B and G.

The action of mineral oil on the fat soluble vitamins has been tested by certain investigatos. Burrows and Farr (1927)(8) found that mineral oil in large amounts (24 percent of the diet) was very toxic to rats. In smaller amounts (2.6 percent of the diet) it seemed to prevent the action of vitamin A, as some of the animals showed incipient xerophthalmia. Dutcher, Ely, and Honeywell (1927)(19) reported that even smaller amounts of mineral oil prevented the action of vitamin A when only a limited amount of the latter was present in the diet. However, the calcifying power of cod liver oil was not appreciably affected by mineral oil. Hawk (1929), in a paper presented at the joint meeting of the American Chemical Society, showed that mineral oil did lower the calcifying power of cod liver oil. Dutcher (1929 - personal communication) corroborated this. Moness and Christiansen (1929) (86) fed a vitamin A concentrate from cod liver oil dissolved in mineral oil and in olive oil so that the total volume approximated that of the cod liver oil from which the vitamin A concentrate was obtained. They could detect no differences on the two diets. The only explanation offered is that the mineral oil used by Dutcher and Burrows differed from that which they used.

SOURCE AND PREPARATION OF MATERIALS USED

There have been many instances when apparent discrepancies in results have been traced to slightly different sources of the same kind of material or to slightly different methods of treatment of materials. For this reason, the sources and the treatment of all the important materials used in the experiments discussed in this paper will be described.

Casein. Commercial casein, obtained from Wilkens-Anderson Company, was washed for two weeks with distilled water acidified with acetic acid. The water was drained off each day and replaced with fresh water. Toward the end of the two weeks period the acetic acid was omitted. After the final washing, the casein was dried in shallow pans over steam hot plates and then reground. This method has always given a product which is low in ash and practically vitamin free.

Dextrin. Cornstarch, obtained from the Penick and Ford Company, was moistened with 0.5 percent citric acid solution and autoclaved for three hours at 15 pounds pressure. The resulting product, which was only partially dextrinized and which dried easily, was dried in shallow pans over a steam hot plate and then ground.

Mineral Mixture. A modification of McCollum's salt mixture #185 was used in all of the experiments. The modification consisted only of the addition of two grams ofcrystallized potassium iodide to every three and one-half kilograms of salt mixture. In preparing the salt mixture, the sodium chloride and the sodium, potassium and calcium phosphates were thoroughly dried at a temperature of 130° to 140°C. The ferric citrate and the potassium iodide were added to the other four salts and the mixture ground very fine. These salts were then thoroughly mixed with the powdered calcium lactate and anhydrous magnesium sulphate.

Yeast. Except when otherwise stated, the yeast used in all experiments was a dried product obtained from the Fleischmann Company.

Autoclaved Yeast. Distilled water was added to dried yeast until a thick paste resulted. This paste was autoclaved for five hours at 15 pounds pressure. It was then dried over a steam hot plate and ground.

Wheat Germ. The wheat germ was a very clean product obtained from Washburn and Crosby.

Extracted Wheat Germ. Except in special cases which will be mentioned in the experimental data, wheat germ was extracted for 48 to 60 hours with anhydrous di-ethyl ether in a large continuous extractor. The ether siphoned about every hour. The extracted product contained less than 0.3% of ether soluble material. In a few cases, the extraction was performed using petroleum ether, boiling point 40° to 60°C., as a solvent.

Wheat Germ Oil. Except when otherwise specified, the wheat germ oil was obtained according to the aforementioned process using diethyl ether as the solvent. The ether solution of oil was filtered through a large pleated filter paper to remove the finely-divided wheat germ which had been carried over by the ether. Most of the ether was removed from the oil by ordinary distillation; the remainder was removed by allowing the oil to stand in an open beaker beside a steam hot plant over night or until no odor of ether could be detected. The amount of the oil obtained varied with different lots of wheat germ. Usually about 10 percent of the germ was ether soluble. The oil was kept in an ice chest in glass stoppered bottles.

Butter Fat. A high grade of butter, obtained at the college creamery, was melted at a low temperature and the supernatant fat filtered through a soft filter paper or a piece of cotton wool in a hot water funnel to remove any suspended curd. It was prepared fresh at least every two weeks. Rations containing butter fat were prepared fresh every two or three weeks.

Cod Liver Oil. Squibb's cod liver oil was used in all experiments. It was usually fed each day by mixing the required amount into a fresh lot of ration. The cod liver oil was kept in the ice chest except when in use.

Molasses. Several kinds of molasses were used in the various experiments. They were as follows:

(1) Two samples of crude cane or black strap molasses, obtained from C. U. Snyder and Company, Mobile, Alabama.

(2) Straight beet molasses. This molasses is that which is obtained after the first extraction of sugar.

(3) Steffen molasses. This molasses is obtained from the straight beet molasses after treating the latter with lime to precipitate more sugar. The excess lime is removed by the addition of carbon dioxide.

(4) Barium residue molasses. Steffen molasses is treated with barium hydroxide to precipitate a third lot of sugar. The barium hydroxide is removed by the addition of carbon dioxide.

Three samples of beet molasses were received from the Great Western Sugar Company, Fort Collins, Colorado.

(5) Sorghum molasses. This was a refined product obtained from the retail market.

(6) Refined cane molasses. This was obtained from the retail market.

Honey. This was purchased from the retail market.

Experimental Animals. Rats were used in all the experiments. They were obtained from the breeding colony in our laboratory. This stock colony was maintained on a diet of natural foodstuffs and the animals have always been healthy and vigorous. It is the practice to wean the young rats at the end of one month. Before the end of one week from the time of weaning, all rats of 50 to 60 grams are started on the various experiments. In a few cases, which will be specially noted, normal adult rats were taken from the breeding stock and placed on experiment.

Cages. The cages, 12 by 10 by 24 inches in dimensions, were of galvanized iron screen on a wood frame with a removable iron pan for the bottom. Wood shavings were used for bedding.

EXPERIMENTAL

General

Rats were used as the experimental animals in all of the experiments to be discussed in this paper. The rats — all healthy and vigorous animals — were taken from the stock diet of natural foodstuffs when they were between four and five weeks of age and weighing between fifty and sixty grams. Usually six rats, two males and for females, were placed on each of the diets to be tested. In some cases, larger numbers of animals were used. These were kept in correspondingly larger cages. The animals were observed every day, partly for their general experimental behavior and partly to see that they had an adequate supply of food and distilled water. At the beginning of each experiment, the animals were weighed weekly; later they were weighed every two weeks and finally, when the curve of growth was definitely established, they were weighed only once a month. The cages were cleaned and fresh shavings supplied at about ten day intervals, depending somewhat upon the number of rats in any one cage.

The experimental work is concerned with two general subjects: first, the influence of various substances on reproduction and second, the influence of various substances on lactation. In both types of experiments, the females were put into individual cages as soon as they showed advanced signs of pregnancy. The number of young born and their weight were recorded. Where reproduction alone was to be studied, these were the only data recorded. The number of litters and the number of young obtained from the rats on a certain diet were taken as an index of the amount of vitamin E present in that diet.

The ability of a diet to promote lactation was studied by allowing the mother to raise her young. In most cases, the size of the litter retained was limited to eight young. When fewer than eight young were born, all of the litter were retained by the mother. In a few cases, more than eight young were retained. In no case did the litter contain fewer than five or more than ten young. The young on the experimental diets were weighed when they were thirty days of age. The number of young weaned and their weaning weight were used as the criteria of the mother's ability to secrete milk adequately.

The composition of the rations used are shown in the appended tables. A ration of casein 18, salt mixture (McCollum's #185) 3.7, butter fat 5, Fleischmann's yeast 12 and dextrin 61.3 was employed as the basal or control diet. This diet has been found to give good growth over long periods of time. Previous work in this laboratory (90) has shown that 12 percent of yeast is nearly optimum for reproduction from the standpoint of vitamins B and G. Yeast is also free from any appreciable amount of vitamin E. Sure (1927)(115) has found butter fat to contain varying but demonstrable amounts of vitamin E. However, the results obtained on this control ration showed no fertility except the initial fertility normally shown by animals which have been transferred from a diet of natural foodstuffs. Most of the experimental rations consist of modifications of this basal ration and the reproductive results on the various rations are compared with the results obtained at about the same time on the basal ration. This basal ration is designated ration 25.

Experiments on Reproduction

Reproduction on diets containing cod liver oil or butter fat and yeast or wheat germ.

On ration 25, the basal ration, four females and two males showed better than normal growth for eight months. Reproduction, however, was not normal. One female had one litter, two females had two litters each and one female had three litters, making a total of eight litters, or forty young. The following year, a second lot of four females and two males were fed the basal ration for six and one-half months. Three females had one litter each and one female had two litters, a total of five litters, or thirty young. In both experiments, no litters were born during the last two months, indicating that reproduction was ended. While there was some reproduction on this ration, there was not enough to indicate that the butter fat contained any significant amount of vitamin E, the fertility of the first four or five months being due to the reserve of vitamin E in the tissues of the animals.

Rations 28 and 29, containing three and five percent of wheat germ oil, respectively, in place of an equal amount of dextrin of ration 25, might be called the positive control diets. That is, these rations contained an adequate supply of vitamin E so that the reproductive performance of rats fed these rations should be normal. The results bear out this contention. On ration 28, four females had a total of twenty-nine litters, or two hundred and forty young. All the animals in this lot were fertile until about the eighteenth month on the diet. Ration 29 was discontinued at the end of thirteen months, further data being considered unnecessary. During that time, four females gave birth to seventeen litters, or one hundred and twenty-seven young.

Ration 31 contained twelve percent of wheat germ, extracted with anhydrous diethyl ether, in place of the yeast of the basal diet. The extracted germ contained less than three-tenths percent of ether soluble material. If vitamin E is entirely fat soluble, this ration should have been deficient in that vitamin. This deficiency was confirmed by the results, which were poorer than those obtained on the basal diet. Four females, one of which died and was replaced, gave birth to a total of only three litters of fourteen young. Growth on this ration was normal during the seven and one-half months that the experiment was run.

Ration 32 was the same as ration 31 except that it contained cod liver oil (Squibb's) in place of butter fat. The results obtained on this ration are somewhat obscured by the high mortality of the females. One female had one litter and died just before the birth of the second litter. Another female had two litters and then died in pregnancy. A third female had two litters successfully, but died while giving birth to the third. The one remaining female was on the ration for eighteen months and gave birth to ten litters with a total of seventy-seven young. A second lot of two males and four females, one of which died and was replaced, was on this diet for nine months. During this time, the females gave birth to fifteen litters, or one hundred and five young.

In order to test ration 32 thoroughly, several generations were raised. Each generation was carried for about five months or until the succeeding generation was well established. Two males and two females of the second generation produced seven litters, or forty-two young, during seven and one-half months on the diet. In the third generation, two males and four females produced eight litters in five months. From the fourth generation, consisting of three males and seven females, eleven litters, or a total of ninety-five young, were obtained in four months. This experiment was terminated at a time when the fifth generation was about two months old. Reproduction had been entirely normal in the first four generations and further data were considered unnecessary.

The remaining experiments in this group are not expecially significant. Rations 34 and 35 contained three percent of wheat germ oil, a potent source of vitamin E, and reproduction was normal on these diets. Rations 36 and 37 contained twelve percent of unextracted wheat germ — also a good source of vitamin E — and reproduction was likewise normal on these two diets. The data are given in the tables. The results on ration 36, containing five percent of butter fat and twelve percent of wheat germ, are noteworthy. Three females were continued on this diet for twenty-six months in which time they gave birth to a total of thirty-eight litters, or two hundred and sixty-six young. Each of the three females was fertile at the end of twenty-six months. This indicates the possible length of fertility of the female rat.

<u>The effect of petroleum ether and of mineral oil on reproduction.</u>

Petroleum ether, boiling point 40° to 60°C., has been used a great deal in this laboratory as a solvent in fat extractions. It was decided to compare the wheat germ oil extracted by means of anhydrous diethyl ether with that extracted by means of petroleum ether.

Both samples of oil were prepared by the usual method, using a large continuous extractor. Several differences were noted. Petroleum ether was not as efficient a solvent as diethyl ether. Using the same sample of wheat germ, diethyl ether extracted 9.3 percent of fat, while petroleum ether extracted only 8.7 percent. This may have been due to the slower rate of extraction caused by the higher boiling point and consequently lower rate of distillation of the petroleum ether. It was also noticed that the odor of petroleum ether did not completely leave the wheat germ oil, even though the latter was heated for some time. This probably was due to a small amount of a high boiling point fraction of the petroleum ether. There was also some difference in the character of the two oils. That extracted by means of petroleum ether was lighter in color and slightly less viscous.

The results obtained when wheat germ oil, extracted by means of anhydrous diethyl ether, was incorporated in the ration have been described. Reproduction is normal. When three and five percent of wheat germ oil, extracted with petroleum ether, (Rations 26 and 27 respectively) were substituted for an equal amount of dextrin in the basal diet, decidedly different results were obtained. On ration 26, four females had one litter each with a total of thirty young. Growth was normal throughout the six months of the experiment. On ration 27, four females produced six litters, or thirty-two young, in eight months. Growth was practically normal.

The difference in the two sets of results was striking and an attempt was made to discover the cause. Since petroleum ether extracted less fat from wheat germ than did diethyl ether, it was thought that vitamin E might be concentrated in that fraction of the fat which was roadily soluble in diethyl ether, but difficultly soluble in petroleum ether.

To test this supposition, three kinds of wheat germ oil were prepared. The first was prepared according to the following method. About one kilogram of wheat germ was put into a five liter balloon flask, covered with petroleum ether and allowed to stand for three to five hours. At the end of this time the ether was drained off as completely as possible. Fresh petroleum ether was added and drained off; this process was carried out three times. The ether solution of fct was filtered to remove finely divided wheat germ and the ether removed by distillation. This method of extraction removed about eighty-five percent of the fat from the wheat germ. In ration 42, no attempt was made to remove the last traces of petroleum ether. In ration 43, it was removed by heating the oil for several hours in an open vessel at a temperature of about 120°C. on an electric hot plate. In ration 51, it was removed by heating at a temperature of about 70°C. under reduced pressure.

Another kind of wheat germ oil was obtained by extracting with anhydrous diethyl ether the wheat germ from which the fat had been partially removed by the aforementioned extraction with petroleum ether. This oil differed markedly from that obtained in the petroleum ether extraction, being much darker in color and very nearly solid. The actions of these various wheat germ oils were compared with that of the ordinary oil obtained by extraction of the whole wheat germ with anhydrous diethyl ether.

Ration 42 contained three percent of wheat germ oil obtained by partial extraction with petroleum ether. This oil still contained a trace of the solvent, most of which had been removed by distillation using steam as a source of heat. Reproduction on this diet was very poor. Four females gave birth to a total of only three litters, or seventeen young, during six and one-half months on the diet. Growth was normal.

Ration 43 was identical to ration 42 except that the wheat germ oil was heated until no trace of solvent could be detected. The reproductive results were entirely different on the two rations. Sixteen litters, a total of one hundred and twenty-four young, were obtained from three females. Three other females on this ration died without having given birth to any young.

In order to be sure of the effect of small amounts of petroleum ether in the diet, 0.5 percent of petroleum ether was added to a diet of casein 18, salt mixture 3.7, butter 5, yeast 12, wheat germ oil (extracted with diethyl ether) 3, and dextrin 58.3. This ration was allowed to stand exposed to the air for about two hours, when very little odor of ether could be detected. This was known as ration 45. Reproduction on this ration was negligible, two females each having one litter. The lack of reproduction may have been due to the inactivation of the vitamin A in the ration rather than to any effect on the vitamin E, for growth was below the normal rate and three of the six animals showed symptoms suggestive of xerophthalmia.

Believing that purified diets, having a low content of fiber, might cause constipation or similar troubles in rats, it was decided to observe the effect that mineral oil might have on reproduction. Hence, ration 44, consisting of the basal diet with one percent of dextrin replaced by mineral oil (Nujol), was fed to two males and four females. During the first six weeks of the experiment, three animals, two males and one female, died and were replaced. Growth was about normal or slightly below. No sign of pregnancy was seen at any time during the six and one-half months the experiment was being carried on.

In order to determine whether there was any concentration of vitamin E in that fraction of wheat germ oil which was more easily soluble in diethyl ether than in petroleum ether, low levels of these oils were tested. Ration 50 contained one percent of the whole wheat germ oil extracted by means of diethyl ether; ration 53 contained 0.2 percent of the same oil. Ration 51 contained one percent of the wheat germ oil secured by partial extraction with petroleum ether. Ration 52 contained one percent of the whole wheat germ oil obtained on extracting with diethyl ether the wheat germ which had been partially extracted with petroleum ether; ration 54 contained 0.2 per-cent of this same oil.

The results obtained on these five rations indicated no difference in concentration of the vitamin in any of the fractions of the oil. The four females of ration 50 produced a total of twenty-one litters, or one hundred and seventy-four young, in ten months. Those of ration 51 produced sixteen litters, or one hundred and nine young, in eleven months on the diet. On ration 52, four females produced a total of seventeen litters, or one hundred and forty-seven young in ten months. On ration 53, four females, one of which died after producing only two litters, produced a total of twenty-three litters, or one hundred and fifty-seven young in twelve months on the diet. Two of the females on ration 54 died, having produced only one litter each. Another female had no young. Two other females on this diet produced a total of thirteen litters, or one hundred and five young in eleven months. The most significant thing about these last five experiments is the small amount of wheat germ oil necessary to maintain fertility.

Reproduction on diets containing molasses or honey.

Nelson, Heller and Fulmer (1925)(88) observed that reproduction was more nearly normal on a diet containing crude cane molasses as a source of vitamin B than on diets containing yeast as a source of vitamin B. It was decided to investigate more thoroughly the vitamin E content of various kinds of molasses.

Ration 59 contained three percent of crude cane molasses. Four females, three of which died, produced a total of fourteen litters, or ninety-one young, in eleven months on the diet. On ration 60, containing five percent of crude cane molasses, four females gave birth to a total of twenty-four litters, or one hundred and sixty-five young, in the same period of time. On ration 75, containing one percent of molasses, one female had one litter of five young, while the other three females had no young.

The three kinds of beet molasses tested gave about the same amounts of reproduction. The data is given in the tables. In practically every case, more young were born than were produced on the control diet, but not nearly as many as were produced on the diets containing crude cane molasses. On diet 66, containing five percent of Steffen molasses, no young were born, although the animals appeared to be normal.

Ration 70, containing five percent sorghum, gave very good reproductive results. Four females, two of which died and one of which was apparently sterile, gave birth to a total of thirteen litters, or seventy-one young. The three percent level of sorghum (ration 69) gave wholly different results, four females producing a total of only two litters.

Rations 71 and 72, containing three and five percent, respectively, of honey, gave practically negative results on reproduction. Only one of the eight females on the two diets had young and that female had but one litter of five young.

About one year later, a second sample of crude cane molasses, known as sample #2, was tested. Although this sample appeared to be of exactly the same composition as the first sample, it gave entirely different results. Six females on ration 84, containing three percent of this second sample of molasses, had no young. On the five percent level (ration 85) six females had but two litters, or seven young, in seven months. Doubling the percentages of molasses present during the last month of the experiment caused no change.

Refined cane molasses (ration 86) was tested at the same time and also found to give entirely negative results. Six females on this ration had no young.

Since vitamin E is fat soluble, it was decided to test for it in the fat of the first sample of crude cane molasses. It was found necessary to dry the molasses in order to extract the fat. The drying process was carried out by mixing five hundred grams of molasses with three times that weight of dextrin and drying this mass in shallow pans over a steam hot plate. About two days of drying brought the mixture to such a state that it could be ground easily. This finely divided mixture of dried molasses and dextrin was extracted with anhydrous diethyl ether in a continuous extractor for two days. The molasses contained about 0.3 percent of fat, calculated on the wet basis.

Ration 76 contained an amount of the mixture of dried molasses and dextrin equivalent to five percent of the original molasses in the ration. The purpose of this experiment was to determine if the drying process had destroyed the vitamin E present in the molasses. Reproduction on the ration was very poor. Three of the females had no young, while the fourth female had one litter of four young.

Ration 82 contained the fat extracted from molasses in an amount equivalent to five percent of molasses. The fat was dissolved in ether, mixed with a weighed amount of dextrin, and the ether allowed to evaporate completely. This method facilitated the preparation of the ration. Since the dried and unextracted molasses had not given reproduction, the fat from this molasses should not have done so. This was found to be the case. One of the eight females gave birth to a litter of seven young; the other females were apparently sterile.

Ration 83 contained dried and ether extracted molasses equivalent to five percent of the original molasses. This diet also should not have given reproduction. However, the eight females on this diet gave birth to thirteen litters. Twelve litters, or eighty-eight young, came from only three of the females. Of the remaining five females, one died in pregnancy and the others died apparently sterile.

Effect of inorganic substances on reproduction.

Evans and Bishop (1927)(41) have reported that the ash of wheat germ, or of wheat germ oil, was ineffective in curing or preventing sterility of rats. However, it was decided to test the effect of the ash of wheat germ oil when used with our basal ration.

Two kinds of ash were used. The first, which is designated as ash of wheat germ oil No.1, was obtained by ashing the oil in porcelain crucibles. The ashing process was not carried to completion, some carbon being unburned, because the heat necessary to oxidize the carbon caused the ash to melt and fuse with the porcelain. The second kind of ash, designated as ash of wheat germ oil No.2, was obtained by ashing the oil in platinum dishes. This ash was very nearly white in color. Since much of this latter ash became fused, it was considered advisable to grind it in an agate mortar.

Ration 47 contained the ash of wheat germ oil No.1, equivalent to three percent of wheat germ oil. Reproduction was very successful. One female died at the birth of her first litter; the other three females had six, six, and seven litters of young, respectively, with a total of one hundred and three young. From the young of ration 47 a second generation of two males and two females was started. The fact that both of the original males on this ration died and were replaced was not considered significant at the time. The second generation grew normally and each of the females had two litters. After this they apparently became sterile. A third generation of four males and three females was started before the second generation ceased to reproduce. No young were obtained from these rats during five months on the diet.

In order to be sure that the ash of wheat germ oil was responsible for reproduction, another group of animals, consisting of four males and eight females, was fed ration 47. Two similar groups of twelve animals each were fed ration 77 which contained ash No.2 in place of ash No.1. The results obtained on these animals were entirely negative. Two females died in pregnancy, one female had a litter of two young, the others had no young. Another group of four males and eight females (lot #78) was fed the basal diet. None of the females on this diet produced young.

Before the preceding experiments had been completed, still another group of animals were started on ration 47 and a similar group on ration 25, the basal diet. There were fifteen males and thirty-five females in each of these groups. None of the animals on either of the diets reproduced. This indicated very clearly that the ash of wheat germ oil did not maintain fertility.

When it was thought that vitamin E might be found among the mineral constituents, it was decided to feed the ash of the crude cane molasses which had given good reproduction. Ration 81 contained ash of this molasses equivalent to five percent of molasses. The inefficiency of this ash to maintain fertility is shown by the fact that no young were obtained from the group of four males and eight females.

Vitamin E requirements of the male and female.

The extraordinary lack of fertility on so many diets and in so many animals led to a great deal of speculation. It was decided to test as many as possible of these animals to see whether the males or the females or both sexes were sterile. The following method was used. Part of the male rats on any one diet were placed with normal females from the breeding stock. If any of the females became pregnant or gave birth to young after a normal period of time, then at least one of the males under observation was still fertile. The rest of the males and some of the females on this same diet were fed the ration of natural foodstuffs which is fed to the breeding stock. Their reproductive behavior was likewise noticed. Normal males from the breeding stock were put in with the remainder of the females on this diet and their reproductive behavior observed. A very large number of rats from rations 25, 47, 77, 81, 82, 84, 85, and 86 were tested in this manner. In the case of rations 47, 77, and 81, the ash which had been included in the diet was omitted, the animals receiving the basal diet for the duration of the experiment.

Twenty-one males from eleven different groups of animals were allowed to run with normal females from the breeding stock for sixty days. Previous to this time, the males had been on the experimental diets for seven months. At the end of that period none of the females had shown any sign of pregnancy.

Thirteen males and twenty-eight females from these same rations were fed the stock diet of natural foodstuffs for sixty days. No pregnancies were observed during this time, indicating that these males were also sterile.

About one hundred females from these groups were allowed to run with normal males from the breeding stock for periods of one hundred to three hundred days. The majority of these females were still able to give birth to young, some of them producing as many as eight litters. These results follow in detail.

Two females which had been on the basal diet for seven months were with normal males from the breeding stock for one hundred days. In that time, each female gave birth to three litters of young. About twenty-five females, which had been on the basal diets for periods varying from seven to twelve months, were with normal males for periods varying from three hundred to one hundred and fifty days. These females produced a total of sixty-seven litters. One of the females had eight litters, another six, two had five litters each, several had four, three, two, or one, depending largely upon the length of time they were with the normal males. Five of the females did not reproduce and all of the females became sterile after they had been on the synthetic diet for a total of sixteen months.

Forty females which had been on diets containing the ash of wheat germ oil were tested with normal males with very similar results. Three females produced four litters in a period of one hundred days. Three other females had no young in an equal period of time. Five females, one of which died soon after the start of the experiment, produced twenty-one litters, or one hundred and forty young, in seven months. Two females of the second generation had four litters each in six months. Two females of the third generation produced five litters in six months. The thirty-five females which were in the group of fifty animals were with normal males for periods varying from three hundred to one hundred and fifty days. During that time, they produced a total of thirty-nine litters of young. One of the females had six litters, several had four litters each. Six of the females had no young and all of the females ceased to reproduce by the end of the fifteenth month on the experimental diet.

Since rats are coprophagous by nature, it was thought that the females on these diets might have obtained vitamin E from the feces of the males which had been on a diet of natural foodstuffs. In order to test this theory, some of the females from the large groups on rations 25 and 47 were placed with normal males in cages having screen bottoms. These cages allowed the feces to drop through so that they were not available to the rats. Nine females of ration 25 were tested in this manner. They produced nine litters of young in one hundred days. Similarly, six females from ration 47 produced three litters in one hundred days.

Eight females on ration 81, containing the ash of crude cane molasses, did not reproduce during the first seven months that they were on the experimental diet. When four of these females were placed with normal males, they produced eight litters of young in one hundred days. Although the experiment was ended after that time, reproduction apparently was not ended.

Ration 82, containing the ether extract of crude cane molasses, likewise gave no reproduction. After being on the experimental diet for seven months, four of the eight females were placed with normal males for one hundred and thirty days. Although two of the females died, eight litters of young were produced before the experiment was ended. One female gave birth to five litters.

Six females on ration 84, containing three percent of the second sample of crude cane molasses, had no young during seven months. At the end of this time, four of the females were placed with normal males for one hundred and sixty-five days. Although two of the females died soon after the beginning of the experiment, a total of thirteen litters of young were produced. One female had six litters and another had five.

On the five percent level of this same molasses (ration

85) seven females had a total of only two litters in seven months. Four of the females were with normal males for one hundred and sixty-five days and produced twenty-two litters of young.

Six females on ration 86, containing five percent of refined cane molasses, did not reproduce in seven months. When two of these females were with normal males for one hundred days, they produced eight litters of young.

Summarizing this group of data, a total of seventy-three females which had been fed the basal diet or modifications of the basal diet produced a total of only nine litters of young in about seven months. When these females were allowed to mate with normal rats from the breeding stock during periods varying from three to ten months, a total of two hundred and eight litters were produced.

Effect of ferric chloride in the diet.

Waddell and Steenbock (1928)(137) reported the complete destruction of vitamin E in a ration composed of natural and varied foodstuffs by the addition of ferric chloride in ether solution to make one percent of ferric chloride in the diet. It was decided to investigate the effect of some diets, to which ferric chloride had been added, in order to determine whether the males or the females became sterile first.

Ration 97 consisted of casein 18, salt mixture 3.7, cod liver oil 5, wheat germ 12, dextrin 60.3, and ferric chloride (hydrated) 1. The ferric chloride was ground very fine and mixed thoroughly with part of the dextrin before being added to the rest of the ration. The results obtained on this experiment were rather astonishing. Six of the fourteen animals on this diet died before the end of six weeks showing distinct signs of xerophthalmia. The weight of the other animals remained practically constant.

The results were so different from those reported by Waddell and Steenbock that a further investigation was made. Another group of rats were fed ration 97, using a different sample of ferric chloride. Six of the twelve animals died within six weeks, exhibiting typical signs of xerophthalmia.

A second group of twelve animals were fed a diet identical to the one just mentioned except that a different method of mixing the ferric chloride in the ration was used. The ferric chloride was dissolved in diethyl ether, the addition of a small amount of water being necessary to cause complete solution, and the ether solution of ferric chloride was mixed with the rest of the ration. The ration was allowed to stand for twenty-four hours in order to evaporate the ether. The animals on this ration grew much better than the ones on the two preceeding rations, but their growth was still decidedly below normal. Five males on this diet gained an average of one hundred and sixteen grams in five months, while seven females gained an average of seventy-one grams.

A third group of animals was used to test for any possible deleterious effects of the diethyl ether. The ration was the same as that used in the previous experiment except that pure ether, containing no ferric chloride, was added and allowed to evaporate. The ether apparently did no harm. Five males gained an average of two hundred and seventeen grams in five months, while seven females gained an average of one hundred and forty grams. These females also produced a total of thirteen litters of young.

Since Waddell and Steenbock had reported good growth on their diets containing ferric chloride, it was decided to investigate the effect on growth of a diet exactly like one used by Waddell and Steenbock. Ration 110, consisting of five parts of a basal mixture (crude casein 5, yellow corn 71.5, linseed oil meal 15, alfalfa meal 2, butter fat 5, bone ash 1, and sodium chloride 0.5) and one part of whole milk powder, was chosen as being representative.

Growth on this diet was very good. Eight rats, four males and four females, gained an average of one hundred and thirty-one grams in eleven weeks. Three females each gave birth to one litter of young.

Ration 109 was the same as ration 110 except that enough ferric chloride in ether solution was added to make one per-cent of the diet. The result obtained on this diet was entirely different. Two of the females gained very little and eventually died; the rest of the animals gained an average of sixty grams in eleven weeks. There was no reproduction.

A method was now devised to show whether the actual ingestion of the ferric chloride, or the effect of the ferric chloride on some substance (vitamin A) present in the ration, was the reason for such poor growth. One hundred grams of cod liver oil were mixed with twenty grams of ferric chloride dissolved in diethyl ether and this mixture poured over two kilograms of dextrin in an open dish. These weights gave about the same proportions as were normally present in a ration. The ether was allowed to evaporate and the mixture left exposed to the air for three to four days. At the end of that time, the cod liver oil and most of the ferric chloride were extracted from the mixture of dextrin, cod liver oil and ferric chloride by means of diethyl ether in a continuous extractor. The ether solution of fat and ferric chloride was washed in a large separatory funnel, first with distilled water acidified with hydrochloric acid and then with distilled water, until the wash water gave no test for iron, using the ferrocyanide and the thiocyanate tests. The cod liver oil was somewhat darker in color than the original and had slightly more odor. Analysis showed the presence of 0.2 percent iron.

Ration 105 contained casein 18, salt mixture 3.7, ether extracted wheat germ 12, dextrin 61.3 and cod liver oil (treated with ferric chloride as previously described) 5. The cod liver oil was mixed in the ration daily. Growth on this ration was poor. Eight rats, four males and four females, gained an average of thirty-eight grams in eleven weeks. Toward the end of this period, the weight of the rats became nearly constant.

Ration 106 was identical with ration 105 except for part of the treatment of the cod liver oil. The cod liver oil received the same treatment throughout as that in ration 105 except that the ferric chloride was omitted. The process apparently caused little change in the oil. The eight rats, four males and four females, gained an average of one hundred and twenty-one grams. There was no reproduction.

Ration 107 contained casein 18, salt mixture 3.7, extracted wheat germ 12, dextrin 58.3, butter fat 5 and wheat germ oil 3. The wheat germ oil was treated with ferric chloride according to the previously described process. Sixty grams of wheat germ oil were substituted for the one hundred grams of cod liver oil. The rats on this ration grew very poorly; in fact, some of them did not even maintain their initial weight. Four of the eight animals died before the end of two months.

In contrast to the results obtained on ration 107 are those obtained on ration 108 which contained wheat germ oil receiving an identical treatment except that no ferric chloride was used. The eight rats on this diet gained an average of one hundred and twenty-two grams in eleven weeks.

Evans (24,40) and Mason (69,70,71) have shown that the testes of males rats fed a diet deficient in vitamin E undergo a rather rapid and severe cellular degeneration. Mason also reported that the size of the testes decreases a great deal. Mason (72) found that deficiency of vitamin A also causes degeneration. It was decided to examine histologically the testes of the animals on the diets containing ferric chloride.

The testes of two animals from rations 97, 105, and 109 were fixed, imbedded, mounted on slides and stained with Delafield's hematoxylin and eosin. For purposes of comparison, similar slides were made from normal rats, from rats on the basal diet and from rats on diets containing high percentages of fat which were known to induce sterility. All these rats were of very nearly the same age.

The testes of the rats on ration 97 seemed very nearly normal. One rat had some tubules which had degenerated almost completely and in which no sperm were seen. The rest of the tubules appeared practically normal. There was no definite decrease in the size of the testes of animals on this diet.

The testes of rats on ration 105 had degenerated a great deal. One testis had a few tubules which contained sperm, the other testis showed none at all. These testes were small.

The testes of rats fed ration 109 were also below the normal size. The histological pictures were quite contradictory. In the case of one rat, the testes appeared very nearly normal and contained many sperm. In the case of the other rat, there was a great deal of degeneration and practically no sperm were seen.

The testes of rats fed the basal ration showed some degeneration. In one case, there was a little degeneration, but most of the tubules appeared practically normal. In the other case, about forty percent of the tubules appeared normal, the rest had greatly degenerated and contained no sperm.

The testes of rats fed a diet of casein 18, salt mixture 4, yeast 12, lard 15, butter fat 9, and dextrin 42 showed almost complete degeneration. In the case of one rat, about five percent of the tubules were somewhat normal and contained sperm.

On a similar diet in which the percentage of lard was increased to twenty-two and the nine percent of butter fat was replaced by two percent of cod liver oil, there was much less degeneration of the testes. In the case of one male, about eighty percent of the tubules were nearly normal. The testes of the other animal showed about seventy percent degeneration, but the rest of the tubules contained sperm.

Experiments on Lactation

Lactation on diets containing wheat germ, wheat germ oil and molasses.

Sure (108,109) found that some vegetable oils seemed to have the property of promoting lactation. Wheat germ oil was one of these. Since the effect of wheat germ oil on reproduction was being studied, additional data were obtained on the ability of the mothers to rear their young on various diets.

On two groups of rats fed the basal diet (ration 25), results on lactation were very different. In one case, eight litters, or a total of forty young, were allowed to remain with the mothers. Twenty-eight of these were weaned at an age of thirty days, their average weight being thirty-one grams. In the other group only one young from five litters, or thirty young, was reared. Most of the young died in the first five days after birth. The number of young in a litter varied from four to seven.

When three percent of wheat germ oil (extracted by means of diethyl ether) was added to this diet, lactation was greatly improved. Twenty-nine litters, or two hundred and forty young, were allowed to remain with the mother. Only forty one of these young died, a mortality of seventeen percent. The young which were weaned averaged thirty-eight grams in weight. The number of young in a litter varied from five to ten.

Ration 29, containing five percent of wheat germ oil, should have given better results. However, it did not. The percent of mortality was twenty-eight and the young averaged thirty-three grams in weight when weaned.

Data on ration 31, containing extracted wheat germ as a source of vitamin B, could not be obtained since the animals on that diet did not reproduce. When cod liver oil was substituted for the butter fat (ration 32), many young were obtained and lactation was studied. With one group of animals, fifteen litters, or one hundred and fifteen young, remained with the female. Thirty-eight young, or thirty-three percent, died. In contrast to the results obtained on the basal diet, most of the young died between the sixteenth and twenty-first days of their life. Prior to this time, they had appeared normal and vigorous. Another lot of animals on this same diet gave better lactation. Eight litters, or fifty-three young, were allowed to remain with the mothers. Fifty-one of these young were weaned, a mortality of four percent. The average weight of the young from both lots of animals was thirty-five grams.

Ration 34 contained five percent of butter fat, twelve percent of extracted wheat germ and three percent of wheat germ oil. Nine litters produced fifty-four young, of which fifteen died. This was a mortality of twenty-seven percent. The young weaned averaged only twenty-three grams in weight. When the butter fat was replaced by cod liver oil, (ration 35) fewer young were weaned, but the average weight of the young was much higher. From fifteen litters, or one hundred and twenty-four young, only forty-six were weaned, a mortality of sixty-three percent. The average weight of the young weaned was forty-four grams. Death of the young occurred between the sixteenth and the twenty-first day.

Ration 36 contained five percent of butter fat and twelve percent of wheat germ. Thirty-nine litters, or two hundred and sixty-five young, remained with the mothers. One hundred and fifty-five young were weaned, a mortality of forty-two percent. The average weight of the young was thirty-five grams. As usual, when cod liver oil was substituted for butter fat (ration 37), the mortality of the young was higher and the average weight of the young weaned was higher. Ninety-eight of the one hundred and forty-one young which were allowed to remain with the mothers, died. Most of the young died between the sixteenth and twenty-first days. The mortality was seventy percent. The average weight of the young weaned was forty grams.

The first sample of crude cane molasses, which gave good results on reproduction, was tested for its ability to promote lactation. On ration 59, containing three percent of molasses, fifty-one young were allowed to remain with the mothers. Twenty-three of these young died, a mortality of forty-five percent. Most of these young died very soon after birth. The average weight of the young weaned was thirty-seven grams.

Ration 60, containing five percent of molasses, gave better results. Only five of the forty-seven young died, a mortality of eleven percent, and the average weight of the young weaned was forty-six grams. These are the best results obtained on any of the highly purified diets.

Lactation on diets containing various grains.

Guest, Nelson, Parks and Fulmer (55) found that, while reproduction was good on diets containing wheat, rye, barley, yellow corn, or white corn as sources of vitamin B, very few of the young were reared. They concluded that the failure of lactation probably was due to a deficiency of vitamin B. It was decided to make further tests on the lactation promoting properties of these grains and of oats. The tests were made on adult animals taken from the breeding stock, rather than on animals which had been raised on the experimental diets. In every case, the number of young in a litter varied from six to eight. The results thus obtained were found to check with those obtained by Guest and associates.

Ration 90 contained seventy-three and three-tenths percent of white corn as a source of vitamins B and G. Lactation, as evidenced by the ability of the mothers to rear their young, was very poor. Seven litters contained a total of forty-six young. Only nine were weaned, a mortality of eighty percent. The average weight of the weaned young was twenty-one grams. The mothers had been on the experimental diet for at least one month before the young were born. However, when pregnant females, which had been fed the stock ration of natural foodstuffs, were fed the experimental ration beginning on the day that the young were born, much better results were obtained. From four litters, or twenty-six young, twenty-two were weaned. There was a mortality of only fifteen percent. The average weight of the young was thirty-five grams. Evidently some substance essential for lactation, which was not present in white corn in sufficient amounts, was stored by these animals long enough to enable them to rear one litter of young.

Ration 91, containing seventy-three percent of yellow corn, gave even poorer results. Six litters, or forty young, were born, but none were reared. These females had been on the experimental diet for at least one month prior to the birth of the young. When females were changed from the stock diet to ration 91 the day their young were born, results were very little improved, the mortality being ninety-four percent.

Ration 92, containing seventy-three percent of barley, gave about the same results as white corn. From seven litters, or forty-five young, twelve young were weaned, a mortality of seventy-three percent. The average weight of the young was thirty-five grams. These females were on experiment one month before the birth of the young. Four litters, containing thirty-three young, were raised by females taken directly from the stock diet. Twenty-six of these young were weaned, a mortality of twenty-one percent. The average weight was thirty-seven grams.

Results obtained on ration 93, containing hulled oats as a source of vitamins, were very much better. Out of the fifty-eight young retained by the mothers, fifty-one were weaned. The average weight of the weaned young was thirty-eight grams. The mothers had been on the experiment for one month prior to the birth of the young.

Lactation on ration 94, containing seventy-three percent of rye, was rather poor. Seven litters produced a total of forty-four young. Seventeen young were weaned, a mortality of fifty-seven percent. The average weight of the young was only twenty-three grams. The mothers were on the experimental ration one month before the birth of the young.

The mortality of the young on ration 95, containing seventy-three percent of wheat, was fifty-seven percent. Twenty-nine young were weaned out of a total of sixty-nine young from eleven litters. The average weight of the young weaned was forty-five grams. The mothers were on experiment for one month before birth of the young. When the females were taken directly from the stock diet at the time of the birth of the young, nineteen young were reared out of a total of twenty-three, a mortality of seventeen percent. The average weight of the young was forty-two grams.

Ration 96 contained seventy-three percent of a mixture of equal parts of white corn, yellow corn, barley, oats, rye, and wheat, or about twelve percent of each grain. From nine litters, or a total of sixty-one young, which were born from mothers on this diet, forty were weaned, a mortality of thirty-five percent. The average weight of these young was thirty-five grams. The mothers were on the experimental ration for one month prior to the birth of the young.

Better results were obtained when the mothers had been taken directly from the stock diet. From four litters, or a total of twenty-six young, twenty-one were weaned, a mortality of nineteen percent. The average weight of these young was forty-four grams.

Several investigators have shown that the grains are rich in vitamin B but not in vitamin G. It has also been shown that young rats require a large amount of both of these factors. Therefore, it was decided to supplement the rations containing the various grains with yeast, a very good source of vitamin G. The yeast was fed at a ten percent level, replacing an equivalent amount of the grain which was present. These rations are designated as rations 90A, 91A, 92A, and so forth, and correspond to rations 90, 91, 92, and so forth.

Yeast was found to have a very high supplementary value in these diets. On ration 90A, thirty-two young were reared out of thirty-four young from five litters. The average weight of the young was forty-five grams. Mortality on ration 91 was one hundred percent. On ration 91A, fifty-seven young were weaned out of sixty-five young from ten litters, a mortality of only twelve percent. The average weight of the weaned young was fifty-two grams. On ration 92A, the mortality of the young was reduced to fifteen percent, while the average weight of the young was raised to forty-seven grams. On ration 94A, the mortality was reduced from fifty-seven to seven percent and the average weight of the young increased from twenty-three to thirty grams. On ration 95A, the mortality was decreased from fifty-seven percent to four percent and the weight of the young increased from forty-five to fifty-one grams. On ration 96A, the mortality decreased slightly to twenty-three percent and the weight of the weaned young was raised from thirty-five to fifty-one grams.

The least conclusive results were obtained on the ration containing hulled oats. The addition of ten percent of yeast to ration 93 caused an increase in the mortality from twelve to twenty-five percent, while the average weight of the young increased from thirty-eight to forty-seven grams. However, since the results obtained on the oats ration were very good, it was hardly to be expected that yeast could have much supplementary value.

If, as seemed probable, vitamin G was the limiting factor in these diets, then autoclaved yeast should have practically the same supplementary value as the untreated yeast. This was found to be the case. On ration 90B, containing ten percent of autoclaved yeast, nineteen young were weaned out of a total of twenty-seven, a mortality of thirty percent. The average weight of these young was forty-three grams.

On ration 91B, thirty-five young were weaned from a total of thirty-seven, a mortality of six percent. The average weight of these young was fifty-eight grams. These results probably were influenced by the fact that four of the five females tested were taken directly from the stock diet.

Somewhat similar results were obtained on rations 92B and 93B. On the former ration, fifteen young were weaned out of a total of twenty, a mortality of twenty-five percent. These young averaged forty-three grams in weight. On ration 93B, fourteen out of fifteen young were weaned, a mortality of seven percent. The average weight of these young was forty-four grams.

The behavior of the young which died seemed to be characteristic of these rations. The young grew well for about the first two weeks, sometimes longer, and appeared sleek and healthy. At the end of this time, they died within a period of one or two days. Autopsy showed that the stomach and intestines always were empty at the time of death.

DISCUSSION OF RESULTS

Sure (107), Evans and Bishop (33,34), and many other workers have demonstrated that vitamin E is present in wheat germ oil and not in the extracted wheat germ. Evans and Bishop (28) concluded that there was a small amount of vitamin E in butter fat. Sure (115) stated that butter fat contained varying but demonstrable amounts of vitamin E. According to Evans and Bishop (28), cod liver oil was notably deficient in vitamin E. Sure (113) corroborated this. However, Nelson and associates (89,91) obtained very good reproduction through several generations on a ration in which cod liver oil and yeast were the only sources of vitamins.

Very little needs to be said about the effect of cod liver oil, wheat germ and wheat germ oil on reproduction as shown in the data of this paper. It seems undeniably true that the particular kind of cod liver oil used in these experiments is a good source of vitamin E. Sure (107) reported that the extracted wheat germ used in his experiments contained 0.4 percent of fat and that this amount was not sufficient to maintain the fertility of the experimental animals. The extracted wheat germ used in these experiments contained about 0.3 percent of residual fat. Hence, it is reasonable to believe that this sample of extracted wheat germ contained less vitamin E than did the sample used by Sure.

The results obtained on ration 31, in which butter fat and extracted wheat germ were the only sources of vitamins, prove fairly conclusively that this germ contained very little vitamin E. That the maintenance of fertility was not due to the greatly increased amount of vitamins A or D present in cod liver oil has been proved by Sure and by Evans and Bishop. The brands of cod liver oil used by them, which did not give reproduction, were biologically tested and shown to be rich sources of vitamins A and D. The only possible conclusion is that the brand of cod liver oil used in this laboratory is a good source of vitamin E.

Wheat germ oil is a potent source of vitamin E. This was demonstrated by the reproduction obtained when various percents of it were incorporated into a ration in which butter fat and yeast were the only other sources of vitamins. Practically normal reproduction was obtained on the 5, 3, 1, and 0.2 percent levels of wheat germ oil. Just what the minimum level of wheat germ oil for normal reproduction is probably depends upon the kind of ration into which it is incorporated and the particular sample of some of the other ingredients of the ration, such as the butter fat.

When wheat germ oil, extracted by means of petroleum ether, failed to give reproduction, an explanation was sought. Although it had been noticed that the odor of the petroleum ether was not entirely dissipated from the oil even after heating to about 75°C. for several hours, little significance was attached to this fact. It was suggested that the odor and an accompanying taste might cause the ration to be unpalatable. However, no marked difference in food consumption was noticed (although records were not kept) and the growth of the animals was normal. The possibility of a concentration of vitamin E in a fraction of the wheat germ oil which was difficultly soluble in petroleum ether was disproved when no appreciable difference could be detected in the reproduction of the animals which were receiving the various fractions of the wheat germ oil.

While the results obtained on ration 45, to which 0.5 percent of petroleum ether was added, do not prove that the effect of the petroleum ether was to inactivate or render unavailable the vitamin E of wheat germ oil, they do prove that a small amount of petroleum ether in the diet may have a great effect. Burrows and Farr (8) and Dutcher, Ely and Honeywell (19) have shown that the presence of mineral oil in the diet prevents the assimilation of limited amounts of vitamin A. Hawk has reported that mineral oil also limits the calcifying power of cod liver oil. It would be logical to assume that petroleum ether, since it has the same chemical nature as mineral oil, would have a similar action.

It was observed that the rats on the diet containing petroleum ether (ration 45) suffered from an eye trouble and did not grow at a normal rate. In other words, 0.5 percent of petroleum ether (much of which evaporated) was able to inactivate or in some manner render unavailable the vitamin A in ten times as much butter fat. It is impossible to say whether the petroleum ether had any effect on the vitamin E of the ration, since the deficiency of vitamin A would be sufficient in itself to prevent reproduction. However, it is very probable that the vitamin E and the vitamin A might be similarly affected. Rations 26, 27 and 42 contained wheat germ oil which contained a trace of petroleum ether. The rate of growth of rats fed these diets was normal over fairly long periods of time. Although some of the vitamin A may have been rendered unavailable, the diets contained sufficient amounts for normal growth. We may conclude, therefore, that more vitamin A is required for reproduction than is required for normal growth, or that vitamin E is very easily destroyed or rendered unavailable by the action of small amounts of petroleum ether.

Mineral oil has the same action as petroleum ether, though the former does not seem to act in quite as low concentrations as does the petroleum ether. Ration 44 consisted of the basal diet with one percent of dextrin replaced by mineral oil. Growth on this diet was nearly as good as on the basal diet, but no young were born. Since each of four females on the basal diet produced one litter of young, while the four females on ration 44 had no young in the same period of time, it appears that mineral oil in some way prevents reproduction.

One sample of crude cane molasses appeared to be a good source of vitamin E. At any rate, when three or five percent of this molasses was incorporated into the basal diet in place of an equal amount of dextrin (rations 59 and 60), very good reproduction was obtained. At the end of eleven months — at which time these experiments were ended — all of the females which remained were fertile. Three kinds of beet molasses and refined sorghum also contained appreciable amounts of vitamin E, though not as much as the first sample of crude cane molasses. There seemed to be considerable variability in the behavior of the animals on rations 63 to 70 (inclusive), which contained different kinds of molasses. Several of the females died in pregnancy or in parturition. However, more reproduction occurred; and fertility was maintained for a longer period on these diets than was the case in rats fed the basal diet. Therefore, we feel justified in saying that the three kinds of beet molasses and sorghum contained vitamin E. Honey, on the other hand, gave no reproduction and apparently contained no vitamin E.

The attempt to secure reproduction with the fat from crude cane molasses as a source of vitamin E was disappointing. As a preliminary step, it was necessary to dry the molasses. The dried molasses was found to have no power to prevent sterility. The fat which was extracted from this dried molasses likewise had no anti-sterility properties. However, the dried and fat-free molasses gave fairly good reproduction, the females remaining fertile for eleven months. These contradicting results are difficult to explain. One hesitates to involve a toxicity factor, although it is possible that the drying process had caused the formation of some substance which promoted sterility and which was extracted by the ether. The most probable explanation is based on results which were later obtained on many of the same rats. It was found that the males on many of these synthetic diets became permanently sterile near the beginning of the experiment, while the females remained fertile for a much longer period of time. It is possible that one or more of the males on the diet containing the extracted molasses (ration 83) remained fertile for some time. The reproduction observed on this diet would then be due to the fertility of one or more males.

At the same time that the aforementioned experiments were being carried out, a second sample of crude cane molasses and a sample of refined cane molasses were being tested. The effect of the ash of wheat germ oil and of the ash of molasses on reproduction was also being tested, using a large number of animals. For purposes of comparison, a large number of animals were fed the basal ration (number 25). These animals were on the synthetic diets for seven months or longer and none of them reproduced. In seeking an explanation of this sterility, it was discovered that all the males on these various diets (rations 25, 47, 77, 80, 81, 82, 84, 85, and 86) were permanently sterile, but that the females, when mated with normal males, remained fertile for several months.

If the sterility of the males is the direct cause of the total lack of reproduction on this group of rations, it is difficult to explain why the females are able to remain fertile for so much longer periods. It seems hardly possible that enough vitamin E could be supplied to the female through the feces of the males which had been on a diet of natural foods. It was found that reproduction occurred when the animals were in a cage with a screen bottom which prevented access to the feces. It was also found that reproduction occurred even though the males had been away from the stock diet for eight months. This is an extremely long time for the males to store vitamin E in their tissues, especially since they must constantly eliminate enough to maintain the fertility of the females in that cage. It should also be remembered that only a small percentage of the feces are eaten, as they are thrown out when the cages are cleaned. Therefore, it seems highly improbable that the repeated instances of long fertility of females on synthetic diets was due to vitamin E which was eliminated by the male. There is, of course, the possibility that the groups of males used in these experiments were in some way physically unfit. However, this possibility seems very remote when one considers the large number of animals used. It does seem probable that the vitamin E requirement of the male and the female are quite different. Either the male requires a much large amount of vitamin E than does the female, or the male requires a different factor — possibly some fraction of what we now know as vitamin E.

The results obtained on the first group of rats which were fed ration 47, containing the ash of wheat germ oil, probably were due to the fertility of one or both of the males present. Both of the original males of this group died and were replaced by young males. For some reason, at least one of the latter animals remained fertile for about seven months, during which time a large number of litters were born. It is possible that the sample of butter fat used at that time was unusually rich in vitamin E.

Waddell and Steenbock (137), in reporting the destruction of vitamin E but not of vitamin A by ferric chloride, stated "All of the animals on the various rations grew well and appeared normal and vigorous". The work done in this laboratory does not confirm the work of Waddell and Steenbock. On none of the rations containing ferric chloride did growth even approach the normal rate. This was true of a case in which the ration was identical to one used by Waddell and Steenbock. Some of the animals died with symptoms of vitamin A deficiency after being on the diet about six weeks.

There are two possible explanations to account for the discrepancies between the results obtained in the two laboratories. First, the samples of ferric chloride used may not have been exactly the same. Second, the age at which the animals were started on the experimental diets may have caused different results. Waddell and Steenbock used animals which were two months old and had been on a stock diet during that time. The animals used in this laboratory were from four to five weeks old and consequently had a much smaller vitamin reserve in their tissues.

The most astonishing of the results obtained when ferric chloride was used was that secured on ration 107. This ration contained three percent of wheat germ oil which had been treated with ferric chloride and then washed nearly free from the ferric chloride. Analysis showed the presence of 0.3 percent of iron in the wheat germ oil. This gives a concentration of 0.04 percent of ferric chloride hexahydrate in the ration. Yet the rats fed this diet grew very poorly and showed decided signs of vitamin A deficiency. Several reasons have been suggested to account for these results. First, the ferric chloride or some of the degradation products formed from the fat by the action of the ferric chloride may act to destroy the vitamin A present in the ration. Second, the ferric chloride may destroy the vitamin E completely; and very small amounts of vitamin E may be necessary for growth and well being. Third, the ferric chloride may have caused the formation of toxic substances. The author favors the theory that the ferric chloride causes the formation of oxidative catalysts or of peroxides which, in turn, causes the destruction of the vitamin A.

Histological examination of the testes of male rats which were on these diets showed that degeneration was taking place. This may have been due to the deficiency of vitamin A in the rations. Rats on diets known to produce sterility showed more testicular degeneration than did the rats on diets containing ferric chloride.

Evans (23), Sure (108,109), and Grijns (53,54) have suggested that there may be a specific vitamin necessary for lactation. Sure stated rather specifically that wheat germ oil was one of the oils which prevented sterility and promoted lactation. The results obtained in this laboratory do not indicate very clearly that wheat germ oil may have such function. To be sure, on rations 28 and 29 which contained three and five percent, respectively, of wheat germ oil, a higher percentage of the young were weaned and the average weight of the young on weaning was higher than was the case on the basal diet. However, these results did not show any unusual success with lactation.

The results obtained on lactation when wheat germ was substituted for the yeast of the basal diet did not indicate the presence of a lactation promoting factor in wheat germ oil. In fact, slightly better results were obtained on a ration containing no wheat germ oil than on rations containing it. The mortality of the young from two groups of mothers on ration 32 was twenty-four percent, while the mortalities on rations 35 and 37 were sixty-three and seventy percent, respectively. The composition of these diets was the same except that ration 32 contained twelve percent of extracted wheat germ, ration 35 contained twelve percent of extracted wheat germ and three percent of wheat germ oil, and ration 37 contained twelve percent of unextracted wheat germ. The average weaning weight of the young showed some variation. On ration 32 it was thirty-five grams; on ration 35, forty-four grams; and on ration 37, forty grams. Although the weaning weight of the young increased as the amount of wheat germ oil in the ration increased, the mortality of the young was much higher on the rations containing wheat germ oil. These results can hardly be interpreted to mean that wheat germ oil has any great effect on lactation.

Some difference was noted between the lactation secured on diets in which the source of vitamin A was butter fat or cod liver oil (rations 34, 35, 36, and 37). Rations 34 and 36, containing butter fat, gave a fairly low percent mortality, but the weight of the weaned young was low. The corresponding cod liver oil rations caused a higher mortality, but those young which were weaned were much larger and healthier.

Molasses, previously shown by Nelson and associates to be a good source of vitamin B, has been shown in this paper to be very beneficial in lactation. This result may be due to the presence of a lactation promoting factor or it may be due to an addition to the vitamins B and G content of the ration.

Various investigators have shown that grains are much richer in vitamin B than in vitamin G. Sure (119,120) has shown that the nursing young of the rat requires rather large amounts of both vitamin B and vitamin G and that the lactating mother uses these vitamins very inefficiently. The very poor lactation obtained on diets 90 to 96, which contained the various grains as a source of vitamins B and G, were probably due to an insufficiency of vitamin G. This is proved by the fact that the addition of yeast caused a very marked increase in the ability of the mothers to rear their young. The percent mortality of the young was greatly lowered and the weaning weight was greatly increased on the diets containing yeast. The fact that autoclaved yeast — which is a good source of vitamin G but not of vitamin B — had a similar supplementary value to these diets was even more conclusive proof that the only deficiency of these grain rations was vitamin G. The fact that autoclaved yeast was not quite the same in value as the untreated yeast was probably due to the partial destruction of the vitamin G in the autoclaving process. Williams, Waterman and Gurin (142) have reported that such destruction might occur.

Whatever the factor may have been that was required to insure proper lactation, it cannot have been stored by the mothers for any length of time. When the mothers were taken from the stock diet and placed on the experimental diets the day the young were born, most of the young were successfully reared. However, when the mothers had been on the experimental diet for one month or more, the diets were not adequate for lactation. The young of all the mothers on these diets grew very well for the first two weeks. During this time, no difference was apparent in any of the young. If yeast was added at this time — but not before — the young continued to grow normally. If no yeast was added, most of the animals died suddenly. Vogt (136) found that the vitamin requirements of the nursing young were very great, but that they were fortified by a vitamin reserve taken from the mother when they were born. The sudden death in the case of so many of the young is believed to mark the depletion of their vitamin G reserve.

If vitamin G is the chief deficiency of these rations and if the death of the suckling young is due entirely to this deficiency, then the various mortalities may be used as an indication of the relative amounts of vitamin G which are present in these grains. The results indicate that yellow corn is very deficient in this vitamin; white corn, barley and rye contain a small amount; wheat contains a larger amount, and oats still more.

SUMMARY

Reproduction was normal on diets containing five percent of cod liver oil and twelve percent of extracted wheat germ.

Very little reproduction was obtained on diets containing a source of vitamin E (wheat germ oil) and small amounts of petroleum ether. Mineral oil (Nujol) also prevented reproduction.

One sample of crude cane molasses gave very good results in reproduction. Three kinds of beet molasses and sorghum gave more reproduction than was obtained on the basal diet. Diets containing honey did not give reproduction. A second sample of crude cane molasses and a sample of refined cane molasses did not give reproduction. The ash of wheat germ oil and the ash of crude cane molasses were inefficient in preventing sterility.

On several synthetic diets the male rats became permanently sterile soon after the beginning of the experiment. Female rats on the same diets were able to produce young over a long period of time.

Animals fed diets containing one percent of ferric chloride, or diets containing cod liver oil or wheat germ oil which had been treated with ferric chloride, did not grow normally. Some of the animals exhibited signs of vitamin A deficiency.

Lactation on diets containing wheat germ oil was not appreciably better than on diets containing no wheat germ oil. The first sample of crude cane molasses had a beneficial ef-

fect on lactation. Rations containing yellow corn, white corn, barley, rye and wheat as sources of vitamins B and G do not give normal lactation. The deficiency in these grains is corrected by the addition of yeast or of autoclaved yeast. Oats as a source of vitamins B and G gave fairly good lactation.

CONCLUSIONS

1. Squibb's Cod Liver Oil is a good source of vitamin E.

2. Small amounts of petroleum ether and of mineral oil may prevent reproduction by inactivating or rendering unavailable the vitamin A and the vitamin E of a diet.

3. Some samples of crude cane molasses are good sources of vitamin E. Three kinds of beet molasses and sorghum contained a small amount of vitamin E.

4. Vitamin E is not contained in the mineral constituents of wheat germ oil or crude cane molasses.

5. Male rats require either a larger amount of vitamin E than do the females, or a different vitamin — possibly some fraction of what we now know as vitamin E.

6. Ferric chloride prevents growth and reproduction by destroying the vitamin A and possibly the vitamin E which are present in the diet.

7. Evidence is not sufficient to show that wheat germ oil contains a vitamin which is necessary for lactation.

8. Crude cane molasses is beneficial to lactation.

9. Yellow corn, white corn, barley, rye and wheat do not contain sufficient vitamin G for lactation.

BIBLIOGRAPHY

1. Anderegg, L. T. Diet in relation to reproduction and rearing of young. J. Biol. Chem., 59:587 (1924).

2. Anderegg, L. T. and Nelson, V. E. Whole and skimmed milk powders as food. Ind. Eng. Chem., 17:451 (1925).

3. Anderegg, L. T. and Nelson, V. E. Milk powders as food. Ind. Eng. Chem., 18:620 (1926).

4. Anderson, E. M. Preliminary analyses of the tissues and of the metabolism of animals suffering from dietary sterility. Anat. Record, 32:227 (1926).

5. Aykroyd, W. R and Roscoe, M. H. The distribution of vitamin B_2 in certain foods. Biochem. J., 23:483 (1929).

6. Bezssonoff, N. L'effet antiscorbutique est-il du à deux substances différentes? Bull. soc. chim. biol., 9:568 (1927).

7. Burr, G. O. and Burr, M. M. A new deficiency disease produced by the rigid exclusion of fat from the diet. J. Biol. Chem., 82:345 (1929).

8. Burrows, M. T. and Farr, W. K. The action of mineral oil per Os on the organism. Proc. Soc. Exptl. Biol. Med., 24:719 (1927).

9. Chick, H. and Roscoe, M. H. Dual nature of water-soluble vitamin B. II. The effect upon young rats of vitamin B_2 deficiency and a method for the biological assay of vitamin B_2. Biochem. J., 22:790-799 (1928).

10. Coward, K. H. The influence of vitamin A deficiency on the estrus cycle of the rat. J. Physiol., 67:26-32 (1929).

11. Cunningham, B. and Osborn, G. Infra-red sterility: Preliminary report. Endocrin., 13:93 (1929).

12. Daniels, A. L. and Hutton, M. K. Fertility of the white rat on purified rations. Proc. Soc. Exptl. Biol. Med., 23:225 (1925).

13. Daniels, A. L. and Hutton, M. K. Mineral deficiencies of milk as shown by growth and fertility of white rats. J. Biol. Chem., 63:143 (1925).

14. Daniels, A. L., Hutton, M. K. and Marks, C. Relation of food consumption of milk-fed rats to the growth of the suckling young. Proc. Soc. Exptl. Biol. Med., 27:759 (1930).

15. Daniels, A. L. and Jordan, D. P. Influence of wheat germ oil on fertility of rats fed fat-free rations. Proc. Soc. Exptl. Biol. Med., 26:185 (1928).

16. Daniels, A. L., Jordan, D. and Hutton, M.K. The development of the suckling young of milk-fed rats. J. Nutrition, 2:19-29 (1929).

17. Drummond, J. C. and Baker, L. C. Further studies of the chemical nature of vitamin A. Biochem. J., 23:274 (1929).

18. Drummond, J. C. and Coward, K. H. Chemical studies of the vitamin A in cod liver oil. J. Soc. Chem. Ind., 43: 544-5 (1924).

19. Dutcher, R. A., Ely, J.O. and Honeywell, H. E. Vitamin studies. XV. Assimilation of vitamins A and D in presence of mineral oil. Proc. Soc. Exptl. Biol. Med., 24:953-5 (1927).

20. Estill, H. W. and McCollum, E. V. The separation of a substance from oils which inhibits the destruction of vitamin A by ferrous sulfate. J. Biol. Chem., 75:157-62 (1927).

21. Euler, B. v., Euler, H. v. and Hellström, H. A-Vitaminwirkungen der Lipochrome. Biochem. Z., 203:370 (1928).

22. Euler, H. v., Karrer, P. and Rydbom, M. Beziehung zwischen A-Vitaminen und Carotinoiden. Ber., 62B:2445 (1929).

23. Evans, H. M. Unique dietary needs for lactation. Science, 60:20 (1924).

24. Evans, H. M. Invariable occurrence of male sterility with dietaries lacking fat soluble vitamine E. Proc. Nat. Acad. Sci., 11:373 (1925).

25. Evans, H. M. The effects of inadequate vitamin A on the sexual physiology of the female. J. Biol. Chem., 77:651-4 (1928).

26. Evans, H. M. Relation of vitamin E to health and vigor. J. Nutrition, 1:23-8 (1928).

27. Evans, H. M. The effect of inadequate vitamin B upon sexual physiology in the male. J. Nutrition, 1:1 (1928).

28. Evans, H. M. and Bishop, K. S. On the existence of a hitherto unrecognized dietary factor essential for reproduction. Science, 56:650 (1922).

29. Evans, H. M. and Bishop, K. S. On an invariable and characteristic disturbance of the reproductive function in animals reared on a diet poor in fat soluble vitamin A. Anat. Record, 23:17 (1922).

30. Evans, H. M. and Bishop, K. S. On the relations between fertility and nutrition. I. J. Metabol. Res., 1:319 (1922).

31. Evans, H. M. and Bishop, K. S. On the relations between fertility and nutrition. II. The ovulation rythm in the rat on inadequate nutritional regimes. J. Metabol. Res., 1:335 (1922).

32. Evans, H. M. and Bishop. On the relations between fertility and nutrition. III. The normal reproductive performance of the rat. J. Metabol. Res., 3:201 (1923).

33. Evans, H. M. and Bishop, K. S. The production of sterility with nutritional regimes adequate for growth and its cure with other foodstuffs. J. Metabol. Res., 3:233 (1923).

34. Evans, H. M. and Bishop, K. S. Existence of a hitherto unknown dietary factor essential for reproduction. J. Am. Med. Assoc., 81:889 (1923).

35. Evans, H. M. and Bishop, K. S. On the existence of a hitherto unknown dietary factor essential for reproduction. Am. J. Physiol., 63:396 (1923).

36. Evans, H. M. and Bishop, K. S. The cause of reproductive upset in dietary deficiencies due to lack of vitamin A. Anat. Record, 25:129 (1923).

37. Evans, H. M. and Bishop. Stability and solubilities of the food substance or vitamin X required for reproduction. Anat. Record, 27:203 (1924).

38. Evans, H. M. and Burr, G. O. Preliminary steps in the isolation and concentration of vitamin X. Anat. Record, 27:203 (1924).

39. Evans, H. M. and Burr, G. O. Distribution of vitamin X in natural foods. Anat. Record, 29:356 (1924-5).

40. Evans, H. M. and Burr, G. O. The antisterility vitamine fat soluble E. Proc. Nat. Acad. Sci., 11:334 (1925).

41. Evans, H. M. and Burr, G. O. The antisterility vitamine fat soluble E. Memoirs of the Univ. of Cal., Vol.8, (1927).

42. Evans, H. M. and Burr, G. O. Vitamin E. The ineffectiveness of curative dosage when mixed with diets containing high proportions of certain fats. J. Am. Med. Assoc., 88:1462 (1927).

43. Evans, H. M. and Burr, G. O. Vitamin E. II. The destructive effects of certain fats and fractions thereof on the antisterility vitamin in wheat germ and in wheat germ oil. J. Am. Med. Assoc., 89:1587-90 (1927).

44. Evans, H. M. and Burr, G. O. A new dietary deficiency with highly purified diets. Proc. Soc. Exptl. Biol. Med., 24:740-3 (1927).

45. Evans, H. M. and Burr, G. O. New dietary deficiency with highly purified diets. II. Supplementary requirements of diet of pure casein, sucrose, and salts. Proc. Soc. Exptl. Biol. Med., 25:41 (1927).

46. Evans, H. M. and Burr, G. O. New dietary deficiency with highly purified diets. III. The beneficial effect of fat in the diet. Proc. Soc. Exptl. Biol. Med., 25:390 (1928).

47. Evans, H. M. and Burr, G. O. Development of paralysis in the suckling young of mothers deprived of vitamin E. J. Biol. Chem., 76:273-97 (1928).

48. Evans, H. M. and Burr, G. O. On the amount of vitamin B required during lactation. J. Biol. Chem., 76:263 (1928).

49. Evvard, J. M., Nelson, V. E. and Sewell, W. E. Copper salts in nutrition. Proc. Iowa Acad. Sci., 35:211-5 (1928).

50. Fridericia, L. S. Inactivating action of some fats on vitamin A in other fats. J. Biol. Chem., 62:471 (1924-5).

51. Goettsch, M. The dietary production of dystrophy of the voluntary muscles. Proc. Soc. Exptl. Biol. Med., 27:564 (1930).

52. Grijns, G. Voeding en vruchtbaarheid. Verslag. Akad. Wetenschappen Amsterdam, 34:874 (1925).

53. Grijns, G. and de Haan, K. Voeding en vruchtbaarheid. II. Verslag. Akad. Wetenschappen Amsterdam, 35:485 (1926).

54. Grijns, G, de Haan, K. and van der Loeff, J. A. Voeding en vruchtbaarheid. III. Verslag. Akad. Wetenschappen Amsterdam, 37:540 (1926).

55. Guest, A. E, Nelson, V. E., Parks, T. B. and Fulmer, E. I. Diet in relation to reproduction and rearing of young. II. Am. J. Physiol., 76:339 (1926).

56. Hart, E. B. Iron in nutrition; a new inorganic factor as a supplement to iron in hemoglobin building. Wisc. Med. J., 28:60 (1929).

57. Hartwell, G. A. Growth and reproduction on synthetic diets. I. Biochem. J., 20:1273 (1926).

58. Hartwell, G. A. Growth and reproduction on synthetic diets. II. Biochem. J., 21:1076 (1927).

59. Hogan, A. G. and Harshaw, H. M. Nutritional requirements for normal reproduction. J. Metabol. Res., 5:111 (1924).

60. Hogan, A. G. and Harshaw, H. M. Some relations between fertility and the composition of the diet. Missouri Agr. Expt. Sta., Res. Bull., #94, (1926).

61. Hogan, A. G., Hunter, J. E. and Shrewsbury, C. L. The relation of diet to bodily capacity and to capacity to withstand unfavorable circumstances. Missouri Agr. Expt. Sta. Bull., 256:501 (1927).

62. Hoyle, E. The vitamin content of honey. Biochem. J., 23:54 (1929).

63. Hunt, C. H. The complex nature of vitamin B as found in wheat and corn. J. Biol. Chem., 78:83-90 (1928).

64. Jones, J. H. The relation of the inorganic constituents of a ration to the production of ophthalmia in rats. J. Biol. Chem., 75:139-46 (1927).

65. Kennedy, W. P. Reproduction and diet in the rat. Quart. J. Exptl. Physiol., 16:281 (1926).

66. Kifer, H. B. and Munsell, H. E. Vitamin content of honey and honey comb. J. Agr. Research, 39:355 (1929).

67. Levine, H. and Smith, A. H. Growth experiments on diets rich in fat. J. Biol. Chem., 72:223 (1927).

68. Marrian, G. F. and Parkes, A. S. The effects of inanition and vitamin B deficiency on the testis of the pigeon. J. Roy. Micr. Soc., 48:257 (1928).

69. Mason, K. E. A histological study of sterility in the albino rat due to a dietary deficiency. Proc. Nat. Acad. Sci., 11:377 (1925).

70. Mason, K. E. Testicular degeneration in albino rats fed a purified food ration. J. Exptl. Zoöl., 45:159 (1926).

71. Mason, Karl E. The effect of purified diets and their modifications on growth and testicular degeneration in male rats. J. Nutrition, 1:311 (1929).

72. Mason, Karl E. The specificity of vitamin E for the testis. I. Relation between vitamins A and E. J. Exptl. Zoöl., 55:101-22 (1930).

73. Mattill, H. A. The relation of vitamins B and E to fertility in the male rat. Am. J. Physiol., 79:305-15 (1927).

74. Mattill, H. A. The oxidative destruction of vitamins A and E and the protective action of certain vegetable oils. J. Am. Med. Assoc., 89:1505 (1927).

75. Mattill, H. A., Carman, J. S. and Clayton, M. M. The Nutritive properties of milk. III. The effectiveness of the X substance in preventing sterility in rats on milk rations high in fat. J. Biol. Chem., 61:729 (1924).

76. Mattill, H. A. and Clayton, M. M. Vitamin E and reproduction on synthetic and milk diets. J. Biol. Chem., 68:665 (1926).

77. Mattill, H. A. and Conklin, R. E. The nutritive properties of milk with special reference to reproduction in the albino rat. J. Biol. Chem., 44:137 (1920).

78. Mattill, H. A. and Stone, N. C. The nutritive properties of milk with special reference to reproduction in the albino rat. J. Biol. Chem., 55:443 (1923).

79. McAmis, A. J., Anderson, W. E. and Mendel, L. B. Growth of rats on "fat free" diets. J. Biol. Chem., 82:247-62 (1929).

80. McCay, C. M., Bing, F. C. and Dilley, W. E. Factor H in the nutrition of trout. Science, 67:249 (1928).

81. McCollum, E. V. and Davis, M. The necessity of certain lipins in the diet during growth. J. Biol. Chem., 15:167 (1913).

82. McCollum, E. V. and Davis, M. The influence of the composition and amount of the mineral content of the ration on growth and reproduction. J. Biol. Chem., 21: 615 (1915).

83. McCollum, E. V., Simmonds, N. and Becker, J. E. Studies on salt ophthalmia. III. Proc. Soc. Exptl. Biol. Med., 24:952 (1927).

84. Miller, H. G. Vitamin B requirements for successful reproduction and rearing of young. Am. J. Physiol., 79:255-9 (1927).

85. Miller, H. G. and Yates, W. W. The relation of natural foodstuffs and their treatment on growth and reproduction. J. Biol. Chem., 62:259 (1924-5).

86. Moness, E. and Christiansen, G. Assimilation of vitamin A when dissolved in liquid petrolatum. J. Am. Pharm. Assoc., 18:997-8 (1929).

87. Moore, Thomas. The relation of carotene to vitamin A. Lancet, (1929) II, 380-1.

88. Nelson, V. E, Heller, V. G. and Fulmer, E. I. Molasses as a source of vitamin B. Ind. Eng. Chem., 17:199 (1925).

89. Nelson, V. E., Jones, R. L., Adams, G, and Anderegg, L. T. Cod liver oil as food. Observations on the existence of vitamin E. Ind. Eng. Chem., 19:840-4 (1927).

90. Nelson, V. E, Jones, R. L., Heller, V. G., Parks, T. B. and Fulmer, E. I. Diet in relation to reproduction and rearing of young. I. Observations on the existence of vitamin E. Am. J. Physiol., 76:325 (1926).

91. Nelson, V. E., Ohrbeck, E., Jones, R. L. and Taylor, M. W. Cod liver oil for reproduction. Am. J. Physiol., 85: 476-81 (1928).

92. Newburg, L. H. and Curtis, A. C. Production of renal injury in the white rat by the protein of the diet. Dependence of the injury on the duration of feeding, and on the amount and kind of protein. Arch. Internal. Med., 82:801 (1928).

93. Osborne, T. B. and Mendel, L. B. The relation of growth to the chemical constituents of the diet. J. Biol. Chem., 15:311 (1913).

94. Osborne, T. B., Mendel, L. B., Park, A. E. and Winternitz, M. C. Physiological effects of diets unusually rich in protein or inorganic salts. J. Biol. Chem., 71:317 (1927).

95. Parkes, A. S. and Drummond, J. C. The effect of vitamin B deficiency on reproduction. Proc. Roy. Soc., 98B:147 (1925).

96. Parkes, A. S. and Drummond, J. C. The effects of fat-soluble vitamin A deficiency on reproduction in the rat. Brit. J. Exptl. Biol., 3:251 (1926).

97. Reynolds, E. and Macomber, D. Defective diet as a cause of sterility. A study based on feeding experiments with rats. J. Am. Med. Assoc., 77:169 (1921).

98. Reynolds, E. and Macomber, D. Certain dietary factors in the causation of sterility in rats. Am. J. Obsted. Gynec., 2:379 (1921).

99. Sherman, H. C. Nutritional significance of our present knowledge of the multiple nature of vitamin B. J. Nutrition, 1:191 (1928).

100. Sherman, H. C. and Axtmayer, J. H. A quantitative study of the problem of the multiple nature of vitamin B. J. Biol. Chem., 75:207 (1927).

101. Simmonds, N., Becker, J. E. and McCollum, E. V. Relation of vitamin E to iron assimilation. J. Am. Med. Assoc., 88:1047 (1927).

102. Simmonds, N., Becker, J. E., and McCollum, E. V. The distribution of vitamin E. J. Nutrition, 1:29 (1928).

103. Smith, M. I. and Hendricks, E. G. Some nutrition experiments with Brewer's yeast with especial reference to its value in supplementing certain deficiencies in experimental rations. U. S. Pub. H. Rep., 41:201 (1926).

104. Steenbock, H. White corn versus yellow corn and a probable relation between the fat-soluble vitamine and yellow plant pigments. Science, 50:352 (1919).

105. Sure, B. Dietary requirements for reproduction. I. The nutritive value of milk protein from the standpoint of reproduction. J. Biol. Chem., 58:661 (1923).

106. Sure, B. Dietary requirements for reproduction. II. The existence of a specific vitamin for reproduction. J. Biol. Chem., 58:693 (1923).

107. Sure, B. Dietary requirements for reproduction. III. The existence of the reproductive dietary complex (Vitamin E) in the ethereal extracts of yellow corn, wheat embryo, and hemp seed. J. Biol. Chem., 62: 371 (1924-5).

108. Sure, B. Dietary requirements for reproduction. IV. Solubility of the reproductive dietary complex (Vitamin E) in various organic solvents. J. Biol. Chem., 63:211 (1925).

109. Sure, B. Dietary requirements for reproduction. V. The role of various vegetable and fruit oils in fertility and lactation. J. Biol. Chem., 69:29 (1926).

110. Sure, B. Dietary requirements for reproduction. VI. The types of sterility produced on a skimmed milk powder reproduction deficient diet. J. Biol. Chem., 69:41 (1926).

111. Sure, B. Dietary requirements for reproduction. VII. The existence of a lactation promoting factor in the unsaponifiable matter from wheat oil. J. Biol. Chem., 69:53 (1926).

112. Sure, B. Dietery requirements for reproduction. VIII. Further studies of a skimmed milk powder reproduction deficient diet. J. Biol. Chem., 74:37-44 (1927).

113. Sure, B. Dietary requirements for reproduction. IX. Cod-liver oil versus wheat oil as sources of vitamin E. J. Biol. Chem., 74:45-53 (1927).

114. Sure, B. Dietary requirements for reproduction. X. Vitamin B requirements for normal lactation. J. Biol. Chem., 74:55 (1927).

115. Sure, B. Dietary requirements for reproduction. XI. The potency of butter fat in vitamin E. J. Biol. Chem., 74:71-84 (1927).

116. Sure, B. Dietary requirements for reproduction. XII. The inefficiency of the lactating mother to secrete vitamin B in the milk and the relation of such phenomenon to infant mortality. Science, 66:265-6 (1927).

117. Sure, B. A detailed study of the role of vitamin B in Anorexia in the albino rat. J. Nutrition, 1:49 (1928).

118. Sure, B. Dietary requirements for fertility and lactation. XIII. Storage of fat-soluble vitamins for lactation, with some observations on the cod-liver oil requirements of nursing young. J. Biol. Chem., 76:659 (1928).

119. Sure, B. Dietary requirements for fertility and lactation. XIV. A quantitative biological method for the study of vitamin B requirement for lactation. J. Biol. Chem., 76:673 (1928).

120. Sure, B. Dietary requirements for fertility and lactation. XV. The inefficiency of the lactating mother (Mus norvegicus albinus) in secreting vitamin B in the milk, and the relation of this phenomenon to infant mortality (detailed report). J. Biol. Chem., 76:685 (1928).

121. Sure, B. The relation of vitamin B deficiency to infant mortality. Am. Med., 34:786-92 (1928).

122. Sure, B. Dietary requirements for fertility and lactation. XVI. Potency of 'vitavose' versus dehydrated yeast in vitamin B. Proc. Soc. Exptl. Biol. Med., 25:603-5 (1928).

123. Sure, B. Dietary requirements for fertility and lactation. XVII. A dietary sterility associated with vitamin A deficiency. J. Agr. Research, 37:87-92 (1928).

124. Sure, B. Dietary requirements for fertility and lactation. XVIII. The vitamin A content of wheat oil. J. Agr. Research, 37:93 (1928).

125. Sure, B. Dietary requirements for fertility and lactation. XX. A differentiation of the vitamin B complex in rice polishings as evidenced in studies of lactation. J. Biol. Chem., 80:297 (1928).

126. Sure, B. Dietary requirements for fertility and lactation. XXI. Further studies of the role of wheat oil in lactation. Proc. Soc. Exptl. Biol. Med., 27:148 (1929).

127. Sure, B, Kik, M. C. and Walker, D. J. Vitamin requirements of nursing young. VI. Anhydremia associated

with disturbance in hematopoietic function in nursing young of the albino rats suffering from a deficiency of the vitamin B complex. J. Biol. Chem., 82:287 (1929).

128. Sure, B. and Kik, M. C. Vitamin requirements of nursing young. VIII. Effect of vitamin B deficiency on alkaline reserve of blood of nursing young of albino rat. Proc. Soc. Exptl. Biol. Med., 27:860 (1930).

129. Sure, B. and Schilling, S. J. Vitamin requirements of the nursing young. II. The production of beriberi in the nursing young (Mus norvegicus albinus) associated with hemorrhages. Am. J. Diseases Children, 35:811 (1928).

130. Sure, B. and Smith, M. E. Effect of vitamin deficiencies on carbohydrate metabolism. I. Hypoglucemia associated with anhydremia and disturbance in hematopoietic function in nursing young of the albino rat suffering from uncomplicated vitamin B deficiency. J. Biol. Chem., 82:307 (1929).

131. Sure, B. and Smith, M. E. Vitamin requirements of nursing young. IX. Effect of vitamin B deficiency on glycogen content of liver of nursing young of albino rat. Proc. Soc. Exptl. Biol. Med., 27:861 (1930).

132. Sure, B. and Stuart, E. H. Dietary requirements for fertility and lactation. XIX. Does copper supplement vitamin B for lactation. J. Biol. Chem., 80:289 (1928).

133. Sure, B., Walker, D. J. and Thatcher, H. S. Vitamin requirements of nursing young. X. Effect of a deficiency of vitamin B complex and vitamin B on histological changes in liver of nursing young of albino rat. Proc. Soc. Exptl. Biol. Med., 27:862 (1930).

134. Sure, B., Walker, D. J. and Stuart, E. H. Vitamin requirements of nursing young. III. A quantitative biological method for the study of vitamin B requirements of nursing young of the albino rat. J. Nutrition, 1:139 (1928).

135. Tso, E. The effects of synthetic diets on fertility and lactation. Proc. Soc. Exptl. Biol. Med., 24:465 (1927).

136. Vogt, E. Über die Bedeutung der Vitamine für die erste Lebenszeit. Klin. Wochschr., 7:1941 (1928).

137. Waddell, J. and Steenbock, H. The destruction of vitamin E in a ration composed of natural and varied foodstuffs. J. Biol. Chem., 80:431 (1928).

138. Waddell, J., Steenbock, H. and Hart, E. B. Iron in Nutrition. X. The specificity of copper as a supplement to iron in the cure of nutritional anemia. J. Biol. Chem., 84:115 (1929).

139. Waltner, K. Über die Wirkung grosser Mengen Eisen. II. Über die Wirkung des Eisens auf Blut, Wachstum, Fertilität und Lactation. Biochem. Z., 205:467 (1929).

140. Williams, R. R. and Waterman, R. E. The composite nature of vitamin B. Proc. Soc. Exptl. Biol. Med., 25:1 (1927).

141. Williams, R. R. and Waterman, R. E. The tripartite nature of vitamin B. J. Biol. Chem., 78:311 (1928).

142. Williams, R. R., Waterman, R. E. and Gurin, S. The effect of pH control in the autoclaving of yeast with respect to the vitamin B factors. J. Biol. Chem., 83:321 (1929).

Table I

Composition of diets

Ration number	Casein	Salt mixture No. 185	Butter fat	Cod liver oil	Yeast	Wheat germ	Extracted wheat germ (diethyl ether)	Wheat germ oil (diethyl ether)	Wheat germ oil (petroleum ether)	Dextrin
25,25A,78,87	18	3.7	5		12					61.3
26	18	3.7	5		12				3	58.3
27	18	3.7	5		12				5	56.3
28	18	3.7	5		12			3		58.3
29	18	3.7	5		12			5		56.3
31	18	3.7	5				12			61.3
32,32A,32(2), 32(3), 32(4)	18	3.7		5			12			61.3
34	18	3.7	5				12	3		58.3
35	18	3.7		5			12	5		58.3
36	18	3.7	5			12				61.3
37	18	3.7		5		12				61.3

Table II

Composition of diets

Ration number	Casein	Salt mixture No. 185	Butter fat	Yeast	Extracted wheat germ *	Wheat germ oil No. 1	Wheat germ oil No. 2	Wheat germ oil No. 3	Wheat germ oil No. 4	Petroleum ether	Mineral oil (Nujol)	Dextrin
42	18	3.7	5	12		3						58.3
43	18	3.7	5	12			3					58.3
44	18	3.7	5	12							1	60.3
45	18	3.7	5	12				3		0.5		57.8
46	18	3.7	5		12							61.3
50	18	3.7	5	12				1				60.3
51	18	3.7	5	12			1					60.3
52	18	3.7	5	12					1			60.3
53	18	3.7	5	12				0.2				61.1
54	18	3.7	5	12					0.2			61.1

*Wheat germ was partially extracted with petroleum ether
Oil #1 was that oil obtained by the afore mentioned extraction
Oil #2 was oil #1 which had been heated to 120°C to remove petroleum ether
Oil #3 was the ordinary wheat germ oil
Oil #4 was the oil obtained by extracting with diethyl ether the wheat germ which had been partially extracted with petroleum ether

Table III

Composition of diets

Ration number	Casein	Salt mixture No. 185	Butter fat	Yeast	Crude cane molasses No. 1	Straight beet molasses	Steffen molasses	Barium residue molasses	Sorghum	Honey	Dextrin
59	18	3.7	5	12	3						58.3
60	18	3.7	5	12	5						56.3
63	18	3.7	5	12		3					58.3
64	18	3.7	5	12		5					56.3
65	18	3.7	5	12			3				58.3
66	18	3.7	5	12			5				56.3
67	18	3.7	5	12				3			58.3
68	18	3.7	5	12				5			56.3
69	18	3.7	5	12					3		58.3
70	18	3.7	5	12					5		56.3
71	18	3.7	5	12						3	58.3
72	18	3.7	5	12						5	56.3
75	18	3.7	5	12	1						60.3

Table IV

Composition of diets

Ration number	Casein	Salt mixture No. 185	Butter fat	Yeast	Crude cane molasses (dried)	Ether extract of crude cane molasses	Crude cane molasses (ether extracted)	Crude cane molasses No. 2	Refined cane molasses	Ash of crude cane molasses	Ash of wheat germ oil No. 1	Ash of wheat germ oil No. 2	Dextrin
76	18	3.7	5	12	5								56.3
77	18	3.7	5	12								3*	61.3
81	18	3.7	5	12						5*			60.0
82	18	3.7	5	12		5*							61.0
83	18	3.7	5	12			5						56.3
84	18	3.7	5	12				3					58.3
85	18	3.7	5	12				5					56.3
86	18	3.7	5	12					5				56.3
47,47(2) 47(3),88	18	3.7	5	12							3*		61.3

*In these cases, an amount of the ash or of the extract was added which was equivalent to the amount shown in the table

Table V

Composition of diets

Ration number	Casein	Salt mixture No. 185	Butter fat	White corn	Yellow corn	Barley	Hulled oats	Rye	Wheat	Yeast	Autoclaved yeast
90	18	3.7	5	73.3							
90A	18	3.7	5	63.3						10	
90B	18	3.7	5	63.3							10
91	18	3.7	5		73.3						
91A	18	3.7	5		63.3					10	
91B	18	3.7	5		63.3						10
92	18	3.7	5			73.3					
92A	18	3.7	5			63.3				10	
92B	18	3.7	5			63.3					10
93	18	3.7	5				73.3				
93A	18	3.7	5				63.3			10	
93B	18	3.7	5				63.3				10
94	18	3.7	5					73.3			
94A	18	3.7	5					63.3		10	
95	18	3.7	5						73.3		
95A	18	3.7	5						63.3	10	
96	18	3.7	5	12.2	12.2	12.2	12.2	12.2	12.2		
96A	18	3.7	5	10.5	10.5	10.5	10.5	10.5	10.5	10	

Table VI

Reproduction on diets containing

cod liver oil or butter fat and yeast or wheat germ

Ration Number	No. of females	No. of males	No. of females died	No. of males died	No. of litters	Total young born	Curve of growth	Months on diet
25	4	2	0	0	8	40	N	8
25A	4	2	0	0	5	30	N	6½
28	4	2	1	0	29	240	N	18
29	4	2	1	0	17	127	N	13
31	4	2	2	1	3	14	N	7½
32	4	2	3	0	18	149	N	18
32A	5	2	1	0	15	105	N	9
32(2)	2	2	0	0	7	42	N	7½
32(3)	4	2	1	0	8	48	N	5
32(4)	7	3	2	0	11	95	N	3-5
34	4	2	2	1	9	54	N	7½
35	4	3	0	1	16	129	N	11
36	4	3	1	2	42	277	N	26
37	4	2	1	0	16	141	N	14

Table VII

Effect of petroleum ether and mineral oil on reproduction

Ration Number	No. of females	No. of males	No. of females died	No. of males died	No. of litters	Total young born	Curve of growth	Months on diet
42	4	2	0	0	3	17	N	6½
43	6	2	4	1	16	124	N	10½
44	5	4	1	2	0	0	-	6½
45	4	2	2	2	2	8	-	6½
46	5	2	2	0	12	91	N	10
50	4	2	1	0	21	174	N	10
51	4	2	1	0	16	109	N	11
52	4	2	1	0	17	147	N	10
53	4	2	2	0	23	157	N	12
54	5	2	2	1	15	123	N	11

Table VIII

Reproduction on diets containing

various kinds of molasses

Ration Number	No. of females	No. of males	No. of females died	No. of males died	No. of litters	Total young born	Curve of growth	Months on diet
59	4	2	3	1	14	91	N	11
60	4	3	1	2	24	165	N	11
63	4	3	3	0	9	68	N	8½
64	4	2	1	0	8	43	N	8½
65	4	2	2	1	7	37	N	8½
66	4	2	1	0	0	0	N	8½
67	4	2	1	1	3	11	N	8½
68	4	2	1	0	8	51	N	8½
69	4	2	1	0	2	13	N	8½
70	4	2	2	0	13	71	N	8½
71	4	2	0	0	0	0	N	8½
72	4	2	3	0	1	5	N	8½
75	4	2	0	0	1	5	N	8½
76	4	2	2	0	1	4	N	7½
82	8	4	1	1	1	7	N	7
83	8	4	5	0	13	96	N	13
84	6	3	0	0	0	0	N	7
85	6	4	0	1	2	7	N	7
86	6	3	0	0	0	0	N	7

Table IX

Reproduction on diets containing

molasses, ash of molasses and ash of wheat germ oil

with special reference to length of fertility of the female

Ration Number	No. of females	No. of males	No. of females died	No. of males died	No. of litters	Months on diet	Females placed with normal males: No. of females	No. of days	No. of litters
47	4	4	2	2	20	10	0	-	-
47(2)	2	2	0	0	4	7½	2	180	8
47(3)	3	3	0	0	0	5	2	180	5
							(3	100	0
77	16	8	4	1	2	7	(5	210	21
47	8	4	2	1	0	7	3	100	4
78	8	4	2	1	0	7½	2	100	6
81	8	4	0	0	0	7	4	100	8
82	8	4	1	1	0	7	4	130	8
84	6	3	0	0	0	7	4	165	12
85	7	3	0	0	2	7	4	165	21
86	6	3	0	0	0	7	2	100	7
87	35	15	3	0	0	7-12	5-22	300-150	67
88	35	15	4	0	0	7-12	5-26	300-150	39

Table X

Lactation on diets containing

wheat germ, wheat germ oil and molasses

Ration Number	No. of Litters	No. of Young	No. of Young died	Percent Mortality	No. of Young weaned	Average weaning weight
25	8	40	14	35	26	31
25A	5	30	29	97	1	28
28	29	240	41	17	199	38
29	17	127	34	28	93	33
31	3	14	14	100	0	
32	15	115	38	33	77	35
32A	8	53	2	4	51	35
34	9	54	15	27	39	23
35	15	124	78	63	46	44
36	39	265	110	42	155	35
37	16	141	98	70	43	40
59	8	51	23	45	28	37
60	7	47	5	11	42	46
83	7	42	5	12	37	51

Table XI

Lactation on diets containing various grains

Ration Number	No. of Litters	No. of Young	No. of Young died	Percent Mortality	No. of Young weaned	Average weaning weight
90*	7	46	37	80	9	21
90**	4	26	4	15	22	35
90A	5	34	2	6	32	45
90B	4	27	8	30	19	43
91*	6	40	40	100	0	-
91**	5	32	30	94	2	30
91A	10	65	8	12	57	52
91B	5	37	2	6	35	58
92*	7	45	33	73	12	35
92**	4	33	7	21	26	37
92A	7	46	7	15	39	47
92B	3	20	5	25	15	43
93*	8	58	7	12	51	38
93A	6	44	11	25	33	47
93B	2	15	1	7	14	44
94*	7	44	27	57	17	23
94A	4	28	2	7	26	30
95*	11	69	40	57	29	45
95**	3	23	4	17	19	42
95A	9	59	2	4	57	51
96*	9	61	21	35	40	35
96**	4	26	5	19	21	44
96A	6	40	9	23	31	51

* The mothers had been on the experimental diet for at least one month prior to the birth of the young.

** The mothers were taken from the stock diet and placed on the experimental diet the day the young were born.