The effect of vitamin E-deficient diets on the growth of the chick

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THE EFFECT OF VITAMIN E-DEFICIENT DIETS
ON THE GROWTH OF THE CHICK

by

Joseph Wheeler Kelly

A Thesis Submitted to the Graduate Faculty
for the Degree of

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INTRODUCTION

Within the last decade the results of investigations pertaining to vitamin and mineral requirements have aided materially in solving problems in poultry nutrition. However, the amount of research on vitamin E is somewhat meager as compared to the information accumulated on other members of the vitamin family. Consequently, little is known regarding the functions of vitamin E in the metabolism of the growing chick.

In order to study a vitamin E deficiency in chicks some investigators have employed simplified or purified diets, while others used natural feeds treated with ferric chloride in ether solution.

Due to the variation in composition and treatment of the diets used to study avitaminosis E in chicks, it is rather difficult to interpret and compare the results that are available.

The purpose of this investigation was to determine, in so far as possible, the effect of vitamin E-deficient diets on the growth of chicks.

It was planned to render diets deficient in vitamin E by: (1) treating with ferric chloride in ether solution, (2) by extracting the fat content, (3) by extraction and treatment with ferric chloride, (4) by rancidification of natural fat and lard, and (5) by extraction and rancidification.

Diets so treated were supplemented with various levels of wheat germ oil or other sources of vitamin E, to determine the growth stimulating activity of the vitamin.
In view of the evidence regarding resorptions and sterility in rats which had been fed extremely low fat or high fat and ferric chloride treated diets, it seems reasonable to assume that when chick rations are treated likewise they too are rendered E-deficient.
REVIEW OF LITERATURE

Evans and Bishop (15) in a search of the influence of diet on the precise mechanism of the estrous cycle of the rat, found that females reared on what was considered an adequate simplified diet would sooner or later lack entirely the ability to reproduce. The nutritional element that was apparently essential to reproduction was at first designated as the X factor. Sure (46) also recognized the necessity of the hitherto un-recogined vitamin necessary for reproduction in rats, and proposed that the term vitamin B be adopted to represent this new dietary factor.

Sure (47) and Evans and Burr (16) demonstrated that the reproductive dietary factor is a fat-soluble vitamin, and that it may be extracted from feeds with ether and other fat solvents. Evans and Burr (16) as well as Sure (48) found that cod liver oil was low in vitamin E. When coconut oil was substituted to the extent of two per cent for lard in an E-deficient diet, female rats failed in reproduction, according to Evans and Burr (16). These authors also reported that the introduction of large percentages of lard in the diet accelerated sterility in rats. Mattill (31) found that the oxidative changes that accompany the beginning and development of rancidity in unsaturated animal fats tend to destroy vitamins A and E. The progress of rancidity is hastened by several well recognized conditions, and especially by the catalytic action of ferrous iron. Weber, Irwin, and Steenbock (53) concluded that 30 per cent of a
moderately rancid lard is able to destroy at least three times the amount of vitamin E which would produce litters of rats on a fresh lard diet.

Waddell and Steenbock (51) found that vitamin E could be destroyed by treating natural food materials with ferric chloride. The treatment consisted of adding to the diet 1 per cent ferric chloride in an ether solution. The ether was dissipated by the use of a fan at room temperature. The vitamin A content of the iron treated ration was not appreciably affected, and for rats the palatability was not greatly reduced. Later work by Waddell and Steenbock (52) indicated that the sterility producing effect in rats of the iron treated ration, was evidently brought about by intimate contact of the iron and the ether soluble material in the ration.

Evans et al (18) succeeded in preparing three allophanates from the non-saponifiable portion of wheat germ oil. The melting point of the third allophanate ranged between 158-160°C. The alcohol obtained from this allophanate, for which they proposed the name alpha-tocopherol, was active biologically. When it was given in a single dose of 3 milligrams to vitamin E-deficient rats they could bear young. In 1938 Karrer et al (29) and Smith et al (45) synthesized alpha-tocopherol. Evans et al (19) found the synthetic alpha-tocopherol to be as effective in producing healthy litters of rats as alpha-tocopherol derived from natural sources.

Evans (17) showed that the administration of wheat germ oil concentrate to male rats which had received a vitamin E-deficient diet for
eight months, caused a marked stimulation in growth. In 1937 Emerson and Evans (14) obtained further evidence that wheat germ oil was capable of stimulating growth in rats, and the factor appeared to be in the non-saponifiable portion rather than in the fatty acid fraction. Olcott and Mattill (36) likewise found that growth in vitamin E-deficient rats is apparently stimulated by vitamin E. Evans, Emerson and Emerson (20) obtained evidence that alpha-tocopherol stimulated growth in rats that had plateaued in rate of growth on a vitamin E-low diet. Ferric chloride treated wheat germ oil containing approximately one-tenth of the E activity of the original oil stimulated growth when fed to female rats which had plateaued in rate of growth on a vitamin E-low diet, according to Evans and Emerson (21).

Adamstone (1) examined dead chick embryos from eggs produced by pullets receiving a diet composed of natural feeds that had been treated with an ethereal ferric chloride solution. He observed that the vitelline circulation was destroyed by an intensive proliferation of cells in the mesoderm which resulted in subsequent "degeneration" of the areas involved.

In order to study a vitamin E deficiency in chicks, Pappenheimer and Goettsch (37) fed Diets 107 and 108 composed of milk powder, casein, starch, yeast, cod liver oil, a salt mixture and filter paper. Chicks maintained on these diets developed ataxia, tremors, retraction or twisting of the head, clonic spasms of the legs and stupor. The symptoms may appear suddenly, between the 18th and 25th day, and may end in death. The lesions found
in the cerebellum of affected chicks consisted of edema, necrosis and hemorrhages. Histopathologic studies of the lesions produced by Diets 107 and 108, were made by Wolf and Pappenheimer (54). They studied 169 chicks and found cerebellar lesions in 153, cerebral lesions in 48, and medullary or midbrain lesions in 19.

Dunlap (12) described a disorder occurring under field conditions which was popularly referred to as "crazy chick disease." The symptoms described for this ailment resembled those of nutritional encephalomalacia, but the histological findings revealed kidney instead of brain lesions. Pappenheimer and Goettsch (38) found that nutritional encephalomalacia may be induced in chicks up to the age of approximately two months. The percentage incidence of the disease becomes progressively less as the preliminary feeding period on a diet of natural feeds is increased. Keenan et al (30) and Hogan and Boucher (24) fed chicks simplified diets, and produced a brain disorder or degeneration described as being similar to that produced by Pappenheimer and Goettsch (37).

Pappenheimer and Goettsch (39) discovered that the brain degeneration of chicks was prevented by replacing part of the lard of Diet 108 with either cottonseed, corn, or peanut oil. Adamstone (2) announced that chicks fed a ration treated with ethereal ferric chloride caused pathological lesions of the visceral organs. The same year Sloan, Card and Adamstone (44) fed "deutectomized" chicks a diet treated with ethereal ferric chloride to destroy vitamin E, and high mortality resulted during the first three
or four weeks. These chicks grew slower than normal chicks on the same diet. The "deutectomized" chicks manifested symptoms of the deficiency at least two or three weeks earlier than those retaining the yolk material. The removal of the yolk did not eliminate the variation between chicks within a lot, in so far as mortality and growth was concerned.

Pappenheimer and Goettsch (40) fed Pekin ducklings Diet 108, and produced nutritional myopathy. Apparently the skeletal muscles were the only structures affected. No lesions were observed in the brains, spinal cords and sciatic nerves examined.

Adamstone (3) produced definite visceral lesions in chicks by feeding a ferric chloride treated ration. The lesions appeared as white, creamy or grayish spots or lumps in the affected organs. The lesions appeared in the liver, heart, pancreas, gizzard, spleen and lungs. On the basis of structure the lesions were classified as lymphoblastomas (Feldman, 1932), since they were composed of a stroma made up of reticular tissue associated with accumulations of lymphocytes. In a similar manner, Goettsch and Pappenheimer (22) treated Diet 20, composed for the most part of natural feedstuffs, with ferric chloride in ether solution to destroy vitamin E. This diet was proved to be low in vitamin E by appropriate rat tests, but when fed to chicks it did not produce mortality or encephalomalacia. These workers showed further that even when Diet 108 was supplemented with cereal grains, fresh greenstuffs, and certain animal products, the chicks were not completely protected against the brain degeneration. They have demon-
strated that there is a protective factor present in the non-saponifiable fraction of certain edible oils such as corn oil, cottonseed oil, hydrogenated cottonseed oil (Crisco), peanut oil and soy bean oil.

Delaplane, Stuart and Hart (11) described a disease in chicks occurring under field conditions which resembled encephalomalacia in its neurological symptoms. Shortly afterwards, Jungherr (27) reported the occurrence of a brain disease in chicks fed commercial rations under field conditions. Histopathologic studies revealed that the disease was indistinguishable from experimental encephalomalacia.

Ni (32) induced encephalomalacia in chicks by feeding Diet 108. The disease was prevented by feeding Chinese gelatin. Even though the fat was extracted from the gelatin it still remained effective in preventing the brain disease.

In 1937, Elvehjem et al (13) differentiated between a vitamin B4 deficiency and encephalomalacia on the basis of symptoms and gross lesions, and stated that preliminary histological examinations did not reveal a marked degeneration of the brain in uncomplicated B4 deficiency.

Jungherr and Pappenheimer (28) fed Diet 108 to poults, and produced a selective necrosis of the smooth muscle of the gizzard wall. The brain and skeletal muscles were not affected.

Jukes, Babcock and Sidney (26) treated a normal diet with ferric chloride in ether solution and obtained slow growth of chicks. At 60 days of age the chicks averaged 360 grams in weight. One case of paraly-
sis appeared on the 57th day of the experiment.

Ni (33) indicated that the active factor contained in Chinese gelatin which prevents the occurrence of encephalomalacia is not destroyed by alkaline hydrolysis and subsequent ether extraction. The active factor in the gelatin apparently is not lipoidal in character.

Dam, Glavind, Bernth and Hagens (9) prevented encephalomalacia in chicks fed Diet 108, by giving synthetic dl-alpha-tocopherol. Daily doses of this substance increasing proportionally to the weight of the animals in quantities of 0.0075 milligram per gram of body weight completely protected the chicks. Subsequent work by Dam and Glavind (10) showed that the accumulation of fluid in the subcutaneous tissue in "alimentary exudative diathesis" could be prevented by dl-alpha-tocopherol.

Pappenheimer, Goettsch and Jungherr (41) confirmed the findings of Dam et al (9) that alpha-tocopherol would protect chicks against encephalomalacia. In the first experiment two groups of chicks were protected with a total dosage of 4.95 and 9.90 milligrams of alpha-tocopherol prepared from vegetable oil. In the second experiment two groups of chicks were given a total dosage of 5 and 10 milligrams of synthetic dl-alpha-tocopherol, and they were completely protected against the brain disorder.

An experiment conducted by Ni (34) appeared to indicate that the greater the amount of vitamin A or carotene added to Diet 108, the more rapid and severe was the onset of encephalomalacia. It was suggested that vitamin A hastens the oxidation of all traces of vitamin E. As
sources of vitamins A and D he used cod liver oil, halibut liver oil, and a solution of carotene and ostarin. Bird and Culton (8) induced a generalized edema in a large percentage of chicks fed a simplified diet. A considerable proportion of the chicks died between the ages of 3 and 9 weeks. This condition was prevented with dl-alpha-tocopherol by administering doses of such size as to approximate 7.5 micrograms per gram live weight per day. The alpha-tocopherol was dissolved and suitably diluted in nondestearinated U. S. P., cod liver oil. Destruction of alpha-tocopherol by cod liver oil was not evidenced under the conditions of this experiment.

Pappenheimer (42) completely prevented nutritional myopathy of ducklings by administering 4 milligrams of synthetic alpha-tocopherol daily. It was also stated that preliminary experiments indicated that soy bean oil and wheat germ oil in adequate dosages protected ducklings against the development of muscle degeneration produced by Diet 108.

Thompson (49) modified the Almquist and Stokstad basal Diet E, for the purpose of destroying the anti-gizzard-erosion factor. Instead of extracting the sardine meal and dried brewer's yeast, these substances were soaked in ether for 24 hours. This diet was designated as Number 44, and it was stated that every chick fed this diet developed encephalomalacia. The diagnosis was made on the basis of histopathologic studies.

In 1940, Holmes and Cravens (25) studied the effect of feeding wheat germ oil to chickens. The addition of 0.1 per cent of this oil to a normal ration did not significantly affect growth, mortality, age to sexual
maturity or egg production. During the same year, Russell, Taylor, and Polskin (43) found that the extensive removal of substances soluble in diethyl ether from an ordinary poultry growing mash did not retard growth of chicks significantly up to 14 weeks of age, when care was taken to provide the vitamins removed by the extraction process. The dietary fat was reduced to 0.1 per cent or less. Assuming that vitamin E was removed from the ration to a large extent by extraction, and that no attempt was made to supply this factor, it could be concluded that vitamin E is not necessary for growth. No internal abnormalities were observed in these chicks.

Norris (35) stated that evidence has been obtained at Cornell University, which confirms the results of Dam, of Copenhagen, Denmark, and of Pappenheimer and associates (41) that either natural or synthetic alpha-tocopherol prevents the development of nutritional encephalomalacia. Prevention of this disease was obtained by mixing the alpha-tocopherol in the lard portion of the diet, supplying it orally, and by injecting it into the peritoneal cavity. It was also stated that by storing a normal ration at room temperature for three months it was possible to produce a small amount of encephalomalacia in chicks.

Hammond (23) observed that encephalomalacia of chicks may be produced by adding three per cent or more of cod liver oil to their diet. Twenty chicks showing poor muscular coordination were given an oral administration of 5 milligrams of synthetic alpha-tocopherol every other day for
seven days. This treatment cured 19 of the 20 chicks affected. Nineteen of the 20 negative controls became completely paralyzed and died within eight days.

Adamstone (4) concluded that nutritional encephalomalacia can be produced readily in young chicks by feeding a natural ration treated with ferric chloride in ether solution to destroy vitamin E if heat is used to evaporate the ether. The disease does not occur when the ether is allowed to evaporate spontaneously in the cold. The destruction of vitamin E by ferric chloride is a necessary preliminary, since the use of ether alone in treating the food and its subsequent evaporation with heat produce no results. Hence, it is concluded that nutritional encephalomalacia is caused by at least two conditions occurring simultaneously. These conditions are: (1) a deficiency of vitamin E, and (2) the lack of some other heat-labile substance or substances, or the failure or inability of the animal to utilize some substances, if present, under conditions of vitamin E deficiency. In another study Adamstone (5) found that chicks receiving an iron treated ration supplemented with halibut liver oil developed a condition having many characteristics of anemia. Grossly, the liver was marked by numerous dark mahogany brown nodules, which occurred throughout the whole organ, and were visible on any cut surface. Adamstone (6) obtained evidence that supposedly normal chick embryos show spontaneous hemorrhage and a histologic picture which is apparently identical with that encountered in vitamin E-deficient embryos.
Adamstone (7) also found that chicks receiving a diet treated with ferric chloride in ether solution, and supplemented with cod liver oil or sardine oil develop tumors that are always confined to the lower part of the intestine or rectum. Sometimes the tumors extend into the oesophagus or for a short distance into the colon. They arise as a direct result of the occurrence of intestinal ulcers. The exact cause of this disturbance remains to be determined.

The references cited here indicate that when chicks are maintained on certain simplified diets, brain degeneration develops in some cases. It was not until 1938, that vitamin E was successfully used to prevent the development of this disease. The results of the original findings have been confirmed by several investigators.

Various workers have attempted to induce encephalomalacia in chicks, through the use of ethereal ferric chloride treated diets; one reported positive results. Lymphoblastomas were reported as a result of feeding an iron treated diet.

Some evidence is accumulated which points out that a disease of chicks occurring under field conditions is indistinguishable, both in symptoms and pathologic changes, from nutritional encephalomalacia.

Evidence has been presented by one investigator that encephalomalacia was produced by storing a practical ration at room temperature for a certain period of time. Another worker produced encephalomalacia in chicks through the use of high levels of cod liver oil. A group of affected
chicks were treated successfully with alpha-tocopherol.

Several species of fowl appear to be affected differently when restricted to the same synthetic diet. That is, chicks develop a brain degeneration, ducklings a skeletal muscle degeneration, and poults a degeneration of the gizzard muscle. The administration of alpha-tocopherol prevents the development of the first two disorders, but similar information on the latter condition is not available.

According to this information it would appear, therefore, that one manifestation of a vitamin E deficiency in chicks is encephalomalacia.

Several investigators have demonstrated that chicks grew poorly when fed ethereal ferric chloride treated and simplified diets. One report indicated that a normal diet supplemented with a small per cent of wheat germ oil did not stimulate growth in chicks. Evidence is lacking showing the effects of supplementing various levels of wheat germ oil and alpha-tocopherol to diets treated by several methods to inactivate vitamin E. It seems questionable, then, whether this vitamin is definitely concerned in the growth of the chick.
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EXPERIMENTAL

Materials and Methods

All experiments were conducted with Single Comb White Leghorn chicks. The chicks used for experiments 1 to 8 inclusive were from mass-mated flocks at the college poultry farm. Those used for experiments 9, 10, and 11, were secured through the courtesy of the Blue Ribbon Hatchery, Atlanta, Georgia.

Experiments 1 to 8 inclusive were conducted in the brooder house at the Iowa State College Poultry Farm, and the remaining experiments were initiated in a brooder house at the University of Georgia Poultry Farm.

Shortly after removing the chicks from the incubator they were selected on the basis of general vigor, individually wing banded, and then placed in electrically heated battery brooders equipped with wire floors. Three extra chicks were included in each lot for the first week. The extra chicks were selected at random and removed after that time. Individual weights were recorded at biweekly intervals.

Feed and water were kept before the chicks at all times during the experimental period. Artificial light was provided at night. The chicks were started on the experimental diets when they were placed in the brooder. Feed consumption records were kept for each lot.

Enough feed was prepared to complete each feeding period.
A post mortem examination was made of the chicks that diet except in those cases indicated, and beginning with experiment 3, upon termination of each trial all chicks were killed and examined for gross lesions. Histopathologic or bacteriologic studies were made in some cases.

The formulas of all rations used in these experiments and their estimated chemical analyses are given in Appendix Tables 1 and 2.

The regular feedstuffs included in Diets I, II, III, IV, and Diet E were secured from local feed dealers.

The riboflavin concentrate used in Diet I was a commercial product.* Dried brewer's yeast was used in Diets I and IV as a source of riboflavin.** Diets II and 108-A contained dried brewer's yeast from another source.*** The oils used for experiments 1 to 5 inclusive contained 3000 International units of vitamin A and 400 A.O.A.C. units of vitamin D per gram. For experiments 6, 7, and 8 the oil contained 1000 International units of vitamin A and 65 A.O.A.C. units of vitamin D. The cod liver oil**** used for experiment 9 contained 1800 International units of vitamin A and 290 A.O.A.C. units of vitamin D. The oil added to the diets for experiments 10 and 11, contained 1000 International units of vitamin A and 100 A.O.A.C. units of vitamin D.

The bleached spruce sulphite pulp used in Diet 108-A as a source of filler was obtained from the Forest Products Laboratory, Madison, Wisconsin.

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* Labocon, supplied by Borden & Co., New York  
** Fleischmann's  
*** Anheuser-Busch Corporation, St. Louis, Missouri  
**** Supplied through the courtesy of E. R. Squibb & Sons, New York, N.Y.
The wheat germ oil* administered to chicks for the first four experiments, was purchased on the market, while that** used in all succeeding experiments was obtained from the Department of Animal Chemistry and Nutrition, Iowa State College.

Synthetic dl-alpha-tocopherol*** was used in these experiments also after it became available.

The solvent used for fat extraction purposes was a normal pentane (Skelly A) which had a boiling point that ranged between 85 and 100°F.

The object of experiment 1 (Table 1) was to determine the effect on growth of a ferric chloride treated diet, and to observe the effect of feeding a high level of wheat germ oil and to determine whether the product would stimulate growth on the treated or untreated diets.

Diet I was formulated to be naturally low in vitamin E, but at the same time to be adequate in other well known essentials. The protein, calcium and phosphorus content was high enough to obtain fair growth. The ingredients were finely ground prior to mixing. The yeast, riboflavin concentrate and cod liver oil were mixed in the rations after treatment.

The original Waddell-Steenbock**** (51) method for the destruction or inactivation of vitamin E was slightly modified in order to make possible the recovery of the ether. The feed, including the fat soluble material was brought in contact with ferric chloride in ether solution.

* Archer-Daniels-Midland Company, Minneapolis, Minnesota
** Supplied through the courtesy of General Mills, Inc.
*** Supplied through the courtesy of Merck and Company Inc., Rahway, N.J.
**** The feeds for experiments 1, 2 and 3, were treated through the courtesy of Dr. B. H. Thomas of the Dept. of Animal Chemistry and Nutrition.
The ether was then evaporated off, leaving the iron compound in contact with the feed.

The ration was treated in batches of approximately 35 pounds. The feed to be treated was weighed and 1 per cent of ferric chloride by weight of the feed was dissolved in ether. The ether solution of ferric chloride was then mixed with the feed and allowed to stand at room temperature on an average of 12 hours. The feed was completely covered with the solution. A ten-gallon milk can was used to contain the feed and ether solution. The vessel contained a vertically fitted spiral one-fourth inch copper tubing with an inlet and outlet at the top. The cover had an outlet for the ether. To recover the ether the can was placed in a water bath heated with steam. The arrangement was such that steam was also passed through the copper coil within the can. From 8 to 12 hours were required to recover most of the ether. The feed was then removed from the can and spread out in shallow metal pans to allow the last traces of ether to evaporate. The pans of feed were next placed in a steam heated drying room having an average temperature of approximately 57°C. The feed remained in this room for two weeks. The purpose was to induce a high degree of rancidity, which, according to previous reports, inactivates vitamin E.

After removing the feed from the drying room the required amount of yeast and vitamin G concentrate were thoroughly mixed in the feed by hand. To reduce the possibility of destroying vitamin A when in contact with rancid fat, the cod liver oil was mixed in a small quantity of feed every
other day. Thus the opportunity for the destruction of vitamin A was
minimized.

The wheat germ oil was administered daily with a pipette. It was
planned to give the oil at the rate of 0.2 cc. per chick per day; gradually
increasing the quantity to 5 cc. per day by the eighth week. Based on
average feed consumption for the eighth week 5 cc. of oil per day would
amount to approximately 8 to 10 per cent of the feed intake. Smaller
quantities were given proportionally when a maximum level of 1 cc. per
chick per day was desired at the eighth week.

The second experiment (Table 2) was initiated to determine whether
the addition of 1 per cent lard to the ethereal ferric chloride solution,
the diet being the same, would induce mortality in the negative control.
It was also desirable to know whether a reduction in the wheat germ oil
intake would lower the mortality in the positive control. Wheat germ oil
was also given in a smaller quantity to chicks on the untreated ration, to
determine its effect on growth and mortality. Other than these exceptions
noted this trial was similar to experiment 1.

The object of the third experiment (Table 3) was to determine whether
the various combinations of ferric chloride, lard, and wheat germ oil would
affect growth or cause mortality on the untreated diet, and also to de-
terminate if 0.2 cc. of cod liver oil given orally every other day would in-
crease the viability of chicks on the high mortality producing diet.
Since the combination of wheat germ oil and the ethereal ferric chloride
treated feed appeared to be toxic to chicks when the former was given orally, intraperitoneal injections were administered with the hope of reducing the mortality. The wheat germ oil was autoclaved for two hours at 15 pounds pressure before it was administered. Each chick received 0.5 cc. during the second, third and fourth weeks. The diet used for this trial was the same as that used for experiment 2.

The object of experiment 4 (Table 4) was to ascertain the influence of extracted rancidified diets on growth and mortality. Diet II was formulated to be especially high in the B-G complex factors, and this diet contained a greater variety of feedstuffs than Diet I.

The fat content for the different batches of extracted feed varied from 0.6 to .95 per cent. It was also desirable to know whether natural rancidified fat possessed any growth promoting factors that were not present in rancid lard. Beginning with the third week, wheat germ oil was administered orally to three lots of chicks. Beginning with 0.2 cc. per day, the dosage was gradually increased to 1 cc by the end of the fourth week. From the fourth to the eighth week inclusive the chicks received 1 cc of wheat germ oil per day.

The extractor was very small, therefore, only 8 or 9 pounds of feed per batch could be extracted. Each batch of feed was extracted for 80 hours. The feed was heated at times to 95°C., before the extraction process was completed. A composite sample of the extracted feed analyzed .77 per cent fat. The natural fat and lard were made rancid by aeration.
The fats were placed in 10 liter suction flasks, which in turn were placed on a water bath and held at about 82°C., during the process of aeration.

The rancid fats were carefully rubbed into the extracted feeds. An effort was made to keep the fat content of the feed for the various lots about the same. The fat was mixed in the diet before it was spread in shallow pans and stored or aged for two weeks at about 100°F. This time interval permitted the feeds to become rancid as determined by odor. Based on an estimate, enough feed was weighed from the storage cans assigned each lot to feed the chicks for two days. The cod liver oil was mixed in the feed every two days. This procedure seemed necessary to lower the destruction of vitamin A. The feeds were supplemented with 0.5 per cent of fortified cod liver oil.

Experiment 5 (Table 5) was for the most part a duplication of experiment 4. A new extractor was designed to accommodate 25 pounds of feed per batch. The extraction was accomplished in the cold, thus removing the heat factor to which the feed was subjected in experiment 4. Diet II was extracted for 80 hours. The average fat content of the extracted feed was about 0.46 per cent. The fat content of the diets received by the various lots was similar except in those lots receiving wheat germ oil. The extracted rancidified diets were aged for two weeks at 100°F., to increase the degree of rancidity. Wheat germ oil was administered at a higher level but in a similar manner as described in trial 4, the object being to determine whether higher levels would stimulate or retard growth. Cod liver
oil was mixed in the diets at the same level and in a similar manner as in trial 4.

Since coconut oil is regarded as a poor source of vitamin E and wheat germ oil as a good source of this vitamin, it was desirable to know if one was superior to the other in stimulating growth. Experiment 6, (Table 6) was designed to obtain this information. Diet II was used for this trial. The fat content of the feeds was approximately the same in all lots. Instead of mixing the cod liver oil in the feed it was given orally. Each chick received 0.2 cc. or approximately 0.14 of a gram per day the first four weeks, and 0.28 gram thereafter. Hence, this method eliminated the possibility of destruction of vitamin A by coming in contact with rancid fats except in the digestive tract of the bird.

The object of experiment 7 (Table 7) was to ascertain the effects upon growth and mortality of subjecting Diet II to similar treatments previously applied to Diet I, as well as to extend the treatments.

The reasons for including the various lots in this experiment are given as follows:

1. Lot 1 was included to observe the effects of a low fat diet. The fat content was approximately 0.46 per cent. The coconut oil was added because the dry feed caked to the roof of the mouth, probably due in part to the high yeast content.

2. Lot 7 was used to show the effects of extraction, rancidification and reextraction. The feed was first extracted as in the case of lot 1.
Then 6 per cent of slightly rancid lard was rubbed into the feed; it was stored at 100°F., for two weeks in an effort to destroy the vitamin E content of the remaining or unextracted fat. The rancid fat was extracted, and 0.25 per cent coconut oil was added.

3. Lot 2 included a diet that was similar in fat content to lots 1 and 7. It was treated with 1 per cent ferric chloride in ether solution. Certainly it would appear that the ferric chloride would destroy all traces of vitamin E in a diet containing less than 0.5 per cent fat if this method is as effective as previous work indicates.

4. Lots 3 (a) and 3 (b) were included to determine whether an ethereal ferric chloride and a low heat treatment of 100°F., would produce high mortality when supplemented with wheat germ oil. The dosage of wheat germ oil was started at the rate of 0.2 cc. and gradually increased to 5 cc. by the end of the eighth week. The oil was not given until the chicks were eight days of age.

5. Lot 4 was used to determine the effects of low heat, and the ethereal ferric chloride and lard treatment. One per cent lard was dissolved in the ferric chloride ether solution and mixed with the feed.

6. Lots 5 (a) and 5 (b) were used to test the effects of the ethereal ferric chloride and high heat treatment. Wheat germ oil was given at the same level to chicks in lot 5 (a) as indicated for lot 3 (a).

7. Lots 6 (a) and 6 (b) were included to observe the effects of the ethereal ferric chloride and lard treatment plus high heat on growth and
mortality. Lot 6 (a) was supplemented with vitamin E. The synthetic dl-
alpha-tocopherol was suitably diluted with ethyl-laurate. A tuberculin
syringe equipped with a long blunted needle was used to administer the
vitamin orally. Each chick received approximately 0.1859 milligram daily
or a total dosage of approximately 10 milligrams by the end of the eighth
week.

8. Lot 13 served as the control.

All the feed treated at 100°F. was stored in an incubator for two
weeks; the feed held at 57°C was stored in a steam heated drying room
for the same period of time.*

Each chick in all lots received 0.2 cc. or approximately 0.14 gram
of cod liver oil daily for the first four weeks, and 0.28 gram the last
four weeks of the experimental period.

Experiment 8 (Table 8) was initiated to check the results of other
workers that encephalomalacia produced by Diet 108 can be prevented by
alpha-tocopherol. It was also desirable to know if rancid and nonrancid
natural fat substituted for lard would increase or decrease the incidence
of the disease.

Pappenheimer et al (41) pointed out that Diet 108 was modified in
order to better the mineral content. The fiber content was also reduced
to 5 per cent. The new diet, Diet 108-A, was used in all their feeding
experiments after the summer of 1937. The formula and estimated analysis

*Solvent was recovered by heating the can containing the feed with hot
water. The temperature of the water ranged from 25 to 45°C. It required
about 29 hours to recover most of the solvent.
for this diet are given in Appendix Tables 1 and 2.

During the first week only of the experimental period, the chicks received a mixed diet consisting of 50 parts of Diet 108-A and 50 parts Diet II extracted and rancidified with lard.

The materials included in Diet 108-A as used in this trial are believed to be similar in quality to those used in the original diet. Instead of mixing fresh fat in the diet it was made slightly rancid, which was another modification. At the beginning of the experiment the diets for each lot were mixed and stored under refrigeration. The cod liver oil was not mixed in the stored feed. The oil was mixed in a small amount of feed every other day for each lot receiving the modified diet. The natural fat included in the diets of lots 9 and 11 was extracted from Diet II.

The synthetic dl-alpha-tocopherol was diluted with ethyl-laurate. A tuberculin syringe equipped with a long blunted needle served to administer vitamin E orally. Each chick of lot 10 received approximately 0.1859 milligram daily or a total dosage of approximately 10 milligrams by the end of the eighth week.

The object of including Diet E in this trial was to determine whether alpha-tocopherol would prevent the occurrence of encephalomalacia. Thompson (49) observed that all the Barred Plymouth Rock chicks on this diet developed encephalomalacia during the fifth week. The formula and chemical analysis of Diet E are given in Appendix Tables 1 and 2.
The same batch of feed as used by Thompson (49) was used in this experiment six weeks later. It was further modified by adding 1 per cent cod liver oil. The oil was mixed in small quantities of feed every other day. Lot 12 (a) received the same amount of alpha-tocopherol as lot 10.

Since all the chicks in lots 8 and 9 either died or became paralyzed on these diets, it was decided to use the same diets which had aged for thirty days longer to observe whether the incidence of mortality would be lowered by administering cod liver oil orally. Each chick received 0.14 of a gram per day the first four weeks, and 0.28 gram thereafter.

Because the administration of alpha-tocopherol failed to protect the chicks against symptoms of encephalomalacia in trial 8, experiment 9 (Table 9) was designed to make further observations on the protective effect of larger dosages.

Diet 108-A was prepared with fresh lard and allowed to stand at room temperature for seven days. The diet had developed only a very slight rancid odor by this time. The feed was stored under refrigeration. Instead of mixing the cod liver oil in the diet for all lots it was given orally to two lots. Each chick of lots 1 and 2 received approximately 0.18 gram every other day during the first four weeks and 0.36 gram thereafter. The chicks of lots 3 and 4 received an equivalent amount mixed in the feed. The alpha-tocopherol was administered in gelatin capsules. The chicks in lots 1, 3, and 5, received a total dosage of 25 milligrams during the feeding period. Beginning with the second week the chicks received
a dosage of 1 milligram every other day for the duration of the experimental period.

Lots 5 and 6 were included in this trial to test the effects of storage on an extracted feed. Diet II was extracted as previously described to remove most of the fat, then 21 per cent of lard was thoroughly rubbed in the feed. The feed was then held at room temperature for four weeks in a feed can to permit a high degree of rancidity to develop. The object of adding the high level of lard was to destroy the amount of vitamin E contained in the small per cent of unextracted fat left in the feed. The lard was then removed from the feed by extraction. Subsequently the feed was stored in a can with a loosely fitted lid, where it remained at room temperature for seven months. Two weeks prior to feeding 6 per cent of rancid lard was rubbed into the feed. The diet was supplemented with 2 per cent cod liver oil mixed in the feed every other day. Alpha-tocopherol was administered to the chicks of lot 5, as indicated for lots 1 and 3.

In view of the results obtained by Hammond (23) that high levels, 3 per cent or above, of cod liver oil induced encephalomalacia, it was reasoned that by treating Diet 108-A similarly a high incidence of mortality should be obtained. Therefore, experiment 10 (Table 10) was initiated to determine the effect on mortality of high levels of cod liver oil. Alpha-tocopherol was given to prevent the development of the nutritional disease.
Diet 108-A was mixed and stored in cans at room temperature. The excess cod liver oil was added at the expense of corn starch. The diet had a slightly rancid odor at the end of two weeks at which time the cod liver oil was added. All the oil was mixed in the feed except for 0.5 per cent which was added freshly every other day. The alpha-tocopherol was administered as described for experiment 9.

An attempt was made to induce encephalomalacia with a diet composed of natural ingredients. Twenty-one per cent lard was mixed in Diet III. The feed was then placed in a can covered with a lid. The feed was stored at room temperature for five months. The lid was removed from the can at various intervals in order to stir and aerate the feed. At the end of five months the feed did not have an acrid odor. Lots 3 and 9 received this diet. Lot 9 received alpha-tocopherol as a supplement.

Experiment 11 (Table 11) was designed to determine whether the addition of high levels of cod liver oil to diets composed of natural feedstuffs would induce symptoms and gross lesions of encephalomalacia.

For this study two diets were used. Diet III was composed of a wide variety of feedstuffs as compared to Diet IV, which is more or less simplified. The formulas and estimated analyses of these diets are given in Appendix Tables 1 and 2.

Diet IV should be inferior to Diet III in its vitamin E content due to the kind, as well as the limited number, of ingredients included in the diet.
The feedstuffs included in these diets were finely ground, which should be conducive to rancidification and destruction of vitamin E. The total amount of cod liver oil was mixed in the diets with the exception of one-half of 1 per cent. The feeds were stored at room temperature for two weeks prior to feeding. Next, the remaining one-half of 1 per cent of oil was mixed in the diets every other day before feeding. By this procedure the total amount of oil was included in each diet. The levels of cod liver oil studied were 1.5, 3.5 and 6.5 per cent.
RESULTS

A statistical study of the mean weights of males and females within a lot failed to show, except in a few lots, sex differences in the rate of growth in these experiments. Therefore, a weighted mean was taken to compare the growth rate. The significance of the differences between lots, as determined by the "t" test, are indicated in the tables.

Table 1 shows the effect of diet treatment and supplement on weight gains and per cent mortality. It is obvious that the iron treatment lowered the growth rate, and that the mortality in this lot was very high. Eighty per cent of the chicks in lot 1 died. An oral administration of wheat germ oil to a level of 5 cc. appeared toxic. However, the same level of oil did not lower the mean weight appreciably nor produce mortality on the untreated diet. The results appear to indicate, then, that the dosages of wheat germ oil given failed to stimulate growth either on the treated or untreated diets.

The affected chicks of lot 1 showed the following symptoms: droopiness, eyes closed, loss of appetite, ruffled feathers, weakness and finally death. The dead chicks were examined for gross lesions. The most constant findings were enlarged gall bladders, gizzard erosion and grayish red-brown kidneys, which appeared enlarged in some cases. The majority of the chicks died between the fifth and eighth week of age.

The skin, shanks and beak of all the chicks receiving the iron-treated ration were depigmented.
Table 1.
The Effect of Ethereal Ferric Chloride Treatment and Wheat Germ Oil Supplement to Treated and Untreated Diets on Mean Weight and Mortality

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Treatment and supplement</th>
<th>No. chicks per lot</th>
<th>Chicks surviving 8th wk.</th>
<th>Per cent mortality 8th wk.</th>
<th>Mean weight both sexes (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Treated with ethereal FeCl₃ + 57°C + w.g.o. to 5 cc level</td>
<td>10</td>
<td>2</td>
<td>80</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>Same diet without w.g.o.</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>275**</td>
</tr>
<tr>
<td>3</td>
<td>Untreated plus w.g.o. to loc. level</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>415</td>
</tr>
<tr>
<td>4</td>
<td>Untreated plus w.g.o. to 5 cc level</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>431</td>
</tr>
<tr>
<td>5</td>
<td>Untreated control</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>471</td>
</tr>
</tbody>
</table>

** Wheat Germ Oil

Highly Significant
The results of diet treatment and supplement for experiment 2 are recorded in Table 2. The mean weight of the lot receiving the diet treated with ethereal ferric chloride and 1 per cent lard is definitely lower than the control. The same diet supplemented with wheat germ oil to a 2 cc. level produced 83 per cent mortality by the fourth week. The same amount of vitamin E supplement was administered to chicks on the untreated ration; it failed to increase the mean weight when compared to the control. Based on average feed consumption for the fourth week, the level of wheat germ oil administered amounted to approximately 3 or 3.5 per cent of the feed intake.

Even though the amount of wheat germ oil was reduced considerably as compared to the level given in experiment 1, the mortality remained about the same. Experiment 2 was terminated because of the excessive mortality in lot 2.

Since the same batch of iron and lard treated feed was employed for experiments 2 and 3, a description of the post mortem findings will be given under the latter trial.

The object of experiment 3 was to determine whether various combinations of ferric chloride, lard, and wheat germ oil would affect growth or produce mortality; and to observe whether oral administrations of cod liver oil would increase the viability of the chicks on the high mortality producing diet. The effects of the diets on mean weight and mortality are presented in Table 3.
Table 2.

The Effect of Ethereal Ferric Chloride and Lard Treatment and Wheat Germ Oil Supplement on Mean Weight and Mortality

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Treatment and supplement</th>
<th>No. chicks per lot</th>
<th>Chicks surviving 4th wk.</th>
<th>Per cent mortality 4th wk.</th>
<th>Mean weight both sexes (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Treated with ethereal FeCl₃+ 1% lard+ 57ºC.</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td>Same diet+w.g.o.* to 2 cc. level</td>
<td>12</td>
<td>2</td>
<td>83</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>Untreated diet+w.g.o. to 2 cc. level</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td>Untreated control</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>123</td>
</tr>
</tbody>
</table>

* Wheat Germ Oil

\( ^* \) No statistical analysis because of mortality.
The mean weights for the lots receiving the ethereal ferric chloride, lard treated diet (1 to 4 incl.) are lower than those for the control lot. The addition of 5 per cent of wheat germ oil, 1 per cent of an aqueous solution of ferric chloride and 1 per cent of rancid lard to the untreated diet failed to affect the mean weight of lot 5 as compared to the control. When other combinations of ferric chloride and rancid lard or rancid lard alone were tried on the untreated diet, growth was not affected as indicated by the weights for lots 6 and 7.

For the first time mortality occurred on the unsupplemented ethereal ferric chloride treated diet as shown by lot 1. The combination of wheat germ oil and the iron-treated diet still produced the highest per cent mortality. Apparently the oral administration of cod liver oil, 0.2 cc. every other day, retarded or reduced the incidence of mortality. The administration of wheat germ oil intraperitoneally failed to reduce the incidence of mortality. Since 25 per cent mortality occurred in the lot receiving no wheat germ oil, 75 per cent in the lot receiving intraperitoneal injections, and none in lot 5 receiving ferric chloride, rancid lard, and wheat germ oil with an untreated diet it is questionable then as to whether or not ferric chloride is toxic. The results still indicate that chicks receiving an ethereal ferric chloride treated diet are unable to tolerate the dosage of wheat germ oil that is tolerated by chicks on an untreated diet. The oral administration of cod liver oil afforded some protection against mortality.
### Table 3.
The Effect of Ferric Chloride and Lard Treatments and Wheat Germ Oil Supplement on Mean Weight and Mortality

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Treatment and supplement</th>
<th>No. chicks per lot</th>
<th>Chicks surviving 4th wk.</th>
<th>Per cent mortality 4th wk.</th>
<th>Mean weight a both sexes (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Treated with ethereal FeCl₃ + 1% lard + 57°C.</td>
<td>12</td>
<td>9</td>
<td>25</td>
<td>51</td>
</tr>
<tr>
<td>2</td>
<td>Same diet + w.g.o.* to 2 cc. level</td>
<td>12</td>
<td>2</td>
<td>83+</td>
<td>43</td>
</tr>
<tr>
<td>3</td>
<td>Same diet + w.g.o. to 2 cc. level + cod liver oil orally</td>
<td>12</td>
<td>8</td>
<td>33+</td>
<td>38</td>
</tr>
<tr>
<td>4</td>
<td>Same diet + 0.6 cc. w.g.o. intraperitoneally the 2nd, 3rd, and 4th wks.</td>
<td>12</td>
<td>3</td>
<td>75</td>
<td>53</td>
</tr>
<tr>
<td>5</td>
<td>Untreated + 5% w.g.o. + aqueous FeCl₃ + 1% rancid lard</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>81</td>
</tr>
<tr>
<td>6</td>
<td>Untreated + aqueous FeCl₃ + 1% rancid lard</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>7</td>
<td>Untreated + 1% rancid lard</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>87</td>
</tr>
<tr>
<td>8</td>
<td>Untreated control</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>94</td>
</tr>
</tbody>
</table>

* Wheat Germ Oil
* No statistical analysis because of mortality.
The chicks of lot 2, experiment 2, and of lots 2, 3, and 4 of experiment 3, showed the following general symptoms: A large percentage of the chicks developed a straddling stance, loss of appetite, weakness, muscular incoordination and death. A post mortem examination was made of most of the chicks in these lots. The most common gross lesions observed were subcutaneous edema, hydropericardium, ascites, yellow liver, grayish red-brown kidneys, gizzard erosion and dilatation of the heart. Large quantities of fluid accumulated under the skin around the abdominal region, and in the pericardial and peritoneal cavities. The exudate was clear and yellow or serous in some cases, while in others it was jelly-like in consistency. The yellow livers seldom appeared to be enlarged. The brains were not examined. The chicks examined in lot 1, experiment 3, showed lesions similar to those described with the exception of yellow livers. The chicks in lots 5, 6, 7, and 8, of Table 3 were killed and examined for gross lesions. The gizzards of these chicks were eroded or ulcerated to some extent in every lot. Bacteriological examinations of a number of diseased chicks failed to establish the fact that the disease was caused by pathogenic organisms.*

The greatest mortality occurred during the third and fourth weeks.

Because of the excessive mortality experienced with Diet I when treated, and due to the presence of gizzard lesions in chicks fed the control diet of experiment 3, Diet II was formulated to continue the study of vitamin E deficiency in chicks.

* The bacteriological examinations were made through the courtesy of Dr. E. F. Waller of the Veterinary Pathology Department now at the University of New Hampshire.
Table 4.

The Effect of Extracted Rancidified Diets, and Wheat Germ Oil Supplement on Mean Weight and Feed Utilization

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Treatment and supplement</th>
<th>No. chicks per lot</th>
<th>Chicks surviving 6th wk.</th>
<th>Per cent mortality 6th wk.</th>
<th>Mean Weight both sexes (grams)</th>
<th>Grams of feed per gram gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Extracted + rancid lard + w.g.o.* to 1 cc. level</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>152 **</td>
<td>3.4</td>
</tr>
<tr>
<td>2</td>
<td>Same diet without w.g.o.</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>171 **</td>
<td>3.1</td>
</tr>
<tr>
<td>3</td>
<td>Extracted + rancid natural fat + w.g.o. to 1 cc. level</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>140 **</td>
<td>3.6</td>
</tr>
<tr>
<td>4</td>
<td>Same diet without w.g.o.</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>184 **</td>
<td>3.0</td>
</tr>
<tr>
<td>5</td>
<td>Unextracted + w.g.o. to 1 cc. level</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>386</td>
<td>2.0</td>
</tr>
<tr>
<td>6</td>
<td>Unextracted control</td>
<td>12</td>
<td>11</td>
<td>8+</td>
<td>396</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* Wheat Germ Oil
** Highly Significant
The mean weights, feed utilization, and per cent mortality resulting from feeding extracted rancidified diets are presented in Table 4.

The growth rate of the chicks fed the control or nonrancid diet is decidedly superior to the growth of the chicks fed the rancid diets. The wheat germ oil supplement failed to stimulate growth when given at the level of 1 cc. The chicks receiving the rancidified diets appeared to be less efficient in feed utilization than the controls.

The improvised fat extractor heated the feed to 95°C. Therefore, the heat factor could have been partly responsible for the poor growth obtained on the rancidified diets, irrespective of the vitamin E deficiency.

When the experiment was terminated the chicks were killed and examined for gross lesions. The only significant lesion found was gizzard erosion, which affected a high percentage of the chicks in lots 1, 2, 3, and 4. The incidence of gizzard erosion was negligible in lots 5 and 6.

In the case of Experiment 5 the feed was extracted in the cold, thus eliminating the heat factor to which the feed was subjected in trial 4.

The mean weight, feed utilization and per cent mortality resulting from feeding extracted rancidified diets are tabulated in Table 5. There was no appreciable difference between the mean weights of the six lots of chicks and the weight difference that is apparent between lots 1 and 2 and the remaining four lots might be explained by an outbreak of coryza which was confined entirely to lots 1 and 2, in which over 50 per cent of the chicks were affected. The chicks grew as well, and utilized their feed as efficiently on rancidified diets as the controls. The growth in all lots was decidedly superior to that of any of the lots in experiment 4.
<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Treatment and supplement</th>
<th>No. chicks per lot</th>
<th>Chicks surviving 8th wk.</th>
<th>Per cent mortality 8th wk.</th>
<th>Mean weight (grams) both sexes</th>
<th>Grams of feed per gram gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Extracted + rancid natural fat + w.g.o.* to 2 cc. level</td>
<td>12</td>
<td>10</td>
<td>16+</td>
<td>471**</td>
<td>2.8</td>
</tr>
<tr>
<td>2</td>
<td>Same diet without w.g.o.</td>
<td>12</td>
<td>11</td>
<td>8+</td>
<td>461**</td>
<td>2.8</td>
</tr>
<tr>
<td>3</td>
<td>Extracted + rancid lard + w.g.o. to 2 cc. level</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>557</td>
<td>2.7</td>
</tr>
<tr>
<td>4</td>
<td>Same diet without w.g.o.</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>545</td>
<td>3.0</td>
</tr>
<tr>
<td>5</td>
<td>Unextracted + w.g.o. to 2 cc. level</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>533</td>
<td>3.0</td>
</tr>
<tr>
<td>6</td>
<td>Unextracted control</td>
<td>12</td>
<td>10</td>
<td>16+</td>
<td>518</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* Wheat Germ Oil
** Affected with Coryza
At least a part of this increase in rate of growth may be attributed to the decrease in temperatures during extraction. The mortality recorded in lots 1, 2, and 6 was not attributed to diet treatment.

The results of supplementing wheat germ and coconut oils to extracted rancidified and control diets are summarized in Table 6. It is evident that there is no difference between the mean weights, feed utilization and mortality of the lots studied. It is evident that there is no difference in the effect of coconut oil and wheat germ oil upon rate of growth.

The results on the incidence of mortality produced by subjecting Diet II to various treatments are recorded in Table 7. A study of this table indicates that when Diet II was treated with ethereal ferric chloride and lard and then stored in a drying room at 57°C. for two weeks no mortality occurred in lot 6 (b). These results are similar to those obtained when Diet I was subjected to similar treatment for 2, but not in the case of experiment 3, where mortality was evident. It is further shown that when wheat germ oil was administered at the 5 cc. level in lots 3 (a) and 5 (a) no excessive amount of mortality occurred. Growth was very poor in these lots. The massive doses of wheat germ oil appear to be responsible for the slow growth rate of these chicks. The treatments applied to the diets for lots 2 and 7 were considered as being severe enough to inactivate the residual E content. However, growth rate was not affected to any great extent by the treatment.
### Table 6.

The Effect of Supplementing Extracted Rancidified and Control Diets with Wheat Germ and Coconut Oils

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Treatment and supplement</th>
<th>No. chicks per lot</th>
<th>Chicks surviving 8th wk.</th>
<th>Per cent mortality 8th wk.</th>
<th>Mean weight both sexes (grams)</th>
<th>Grams of feed per gram gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Extracted + rancid natural fat + w.g.o. to 2 cc. level</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>597</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>Extracted + rancid natural fat + coconut oil to 2 cc. level</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>519</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>Extracted + rancid lard + w.g.o. to 2 cc. level</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>552</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td>Extracted + rancid lard + coconut oil to 2 cc. level</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>586</td>
<td>2.5</td>
</tr>
<tr>
<td>5</td>
<td>Unextracted + w.g.o. to 2 cc. level</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>561</td>
<td>2.6</td>
</tr>
<tr>
<td>6</td>
<td>Unextracted + coconut oil to 2 cc. level</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>514</td>
<td>2.7</td>
</tr>
</tbody>
</table>

* Wheat Germ Oil
/ Significant
Table 7.

The Effect of Various Diet Treatments on Mortality

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Treatment and supplement</th>
<th>No. chicks per lot</th>
<th>Chicks surviving 8th wk.</th>
<th>Per cent mortality both sexes 8th wk.</th>
<th>Mean weight (grams)</th>
<th>Grams of feed per gram gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Extracted + 100°F. + 0.25% coconut oil</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>562</td>
<td>2.7</td>
</tr>
<tr>
<td>7</td>
<td>Extracted then added 6% rancid lard - held 2 wks. at 100°F. then reextracted + 0.25% coconut oil</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>483**</td>
<td>2.8</td>
</tr>
<tr>
<td>2</td>
<td>Unextracted + ethereal FeCl₃ + 100°F. + 0.25% coconut oil</td>
<td>12</td>
<td>11</td>
<td>8+</td>
<td>487**</td>
<td>2.7</td>
</tr>
<tr>
<td>3(a)</td>
<td>Unextracted + ethereal FeCl₃ + 100°F., w.g.o.* to 5 cc. level</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>432**</td>
<td>2.4</td>
</tr>
<tr>
<td>3(b)</td>
<td>Unextracted + ethereal FeCl₃ + 100°F. No. w.g.o.</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>538</td>
<td>2.6</td>
</tr>
<tr>
<td>4</td>
<td>Unextracted + ethereal FeCl₃ + 1% lard + 100°F.</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>521</td>
<td>2.6</td>
</tr>
<tr>
<td>5(a)</td>
<td>Unextracted + ethereal FeCl₃ + 57°C. + w.g.o. to 5 cc. level</td>
<td>6</td>
<td>5</td>
<td>16+</td>
<td>330**</td>
<td>2.1</td>
</tr>
<tr>
<td>5(b)</td>
<td>Unextracted + ethereal FeCl₃ + 57°C. No w.g.o.</td>
<td>6</td>
<td>5</td>
<td>16+</td>
<td>489*</td>
<td>2.7</td>
</tr>
<tr>
<td>6(a)</td>
<td>Unextracted + ethereal FeCl₃ + 1% lard + 57°C. + alpha-tocopherol</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>564</td>
<td>2.5</td>
</tr>
<tr>
<td>6(b)</td>
<td>Unextracted + ethereal FeCl₃ + 1% lard + 57°C. No alpha-tocopherol</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>539</td>
<td>2.6</td>
</tr>
<tr>
<td>13</td>
<td>Unextracted control</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>592</td>
<td>2.7</td>
</tr>
</tbody>
</table>

* Wheat Germ Oil
** Significant
*** Highly Significant
Before the experiment terminated one chick in lot 2 and one in lot 5 (b) became paralyzed. These chicks were apparently affected with fowl leucosis. The diagnosis was based on histopathologic studies.* The chick that died in lot 5 (a) was not examined due to its state of decomposition.

The results on the incidence of mortality produced by encephalomalacic producing diets are given in Table 8. The incidence of mortality was just as high in lot 9, receiving the natural fat rancidified as lot 8 which contained rancid lard. Therefore, it is evident that there are no protective factors present in natural rancidified fat against the nutritional disease. The chicks of lot 10 failed to survive even though they received a daily dosage of 0.1859 milligram of alpha-tocopherol. When freshly extracted natural fat was used as the source of fat in Diet 108-A it failed to completely protect the chicks against the deficiency.

Diet E had a previous record of producing a high incidence of encephalomalacia, but when it was supplemented with 1 per cent of cod liver oil and alpha-tocopherol, no encephalomalacia resulted. Only 33 per cent mortality resulted in lot 12 (b) the negative control, when additional cod liver oil was added whereas the same diet produced symptoms of the deficiency in 100 per cent of the chicks. A histopathologic study was made of the brain from one of the two chicks that became paralyzed in lot 12(b). The sections studied from this brain revealed the major lesions of encephalomalacia as described by Pappenheimer et al (41).

* The histopathologic studies were made through the courtesy of Dr. S. H. McNutt of the Veterinary Research Institute.
### Table 8.

The Effect of Supplementing Encephalomalacic Producing Diets With Alpha-tocopherol and Cod Liver Oil

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Treatment and supplement</th>
<th>No. chicks per lot</th>
<th>Chicks surviving 8th wk.</th>
<th>Per cent mortality 8th wk.</th>
<th>Mean weight (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Diet 108-A modified - rancid lard Control*</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Diet 108-A modified - natural fat rancified</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Diet 108-A rancid lard + alpha-tocopherol</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Diet 108-A modified - natural fat not rancified</td>
<td>6</td>
<td>4</td>
<td>33 +</td>
<td>315</td>
</tr>
<tr>
<td>12(a)</td>
<td>Diet E modified + alpha-tocopherol</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>378</td>
</tr>
<tr>
<td>12(b)</td>
<td>Diet E modified + no alpha-tocopherol</td>
<td>6</td>
<td>4</td>
<td>33 +</td>
<td>371</td>
</tr>
<tr>
<td>14</td>
<td>Same feed as lot 8 aged 30 days longer + cod liver oil orally</td>
<td>11</td>
<td>6</td>
<td>45 +</td>
<td>140</td>
</tr>
<tr>
<td>15</td>
<td>Same feed as lot 9 aged 30 days longer + cod liver oil orally</td>
<td>11</td>
<td>5</td>
<td>54</td>
<td>197</td>
</tr>
</tbody>
</table>

* Cod liver oil mixed in feed for lots 8, 9, 10, 11, 12 (a) and 12 (b)

* No statistical analysis because of mortality.
When cod liver oil was given orally to chicks of lots 14 and 15 which received the same diets fed lots 8 and 9, the mortality ranged between 45 and 55 per cent. Hence, the incidence of mortality was reduced considerably by giving the cod liver oil orally.

The highest incidence of mortality occurred between three and five weeks of age in lots 8, 9, and 10.

With few exceptions, the gross examination of the brains did not show the presence of edema, hemorrhages, and necrosis, which develop in the cerebellum of chicks affected with encephalomalacia.

The brains of at least 4 chicks from each of lots 8, 9, and 10 were prepared for histopathologic studies. Several sections were taken from each brain for observation. No significant lesions were found in the sections studied.

The most consistent gross changes observed were grayish red-brown kidneys and a slight dilatation of the ureters with what appeared to be urates. The kidneys appeared to be enlarged in only a few cases.

The brains of the chicks of lots 14 and 15 were not examined for gross and microscopic lesions.

The purpose of experiment 9 was to confirm the work of previous investigators that alpha-tocopherol would protect chicks against encephalomalacia. Table 9 reveals the protective action of vitamin E against the deficiency disease. The lard used in Diet 108-A was not as rancid as that used in
The Protective Effect of Alpha-tocopherol Against Encephalomalacia

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Treatment and supplement</th>
<th>No. chicks per lot</th>
<th>Surviving chicks 8th wk.</th>
<th>Per cent mortality 8th wk.</th>
<th>Mean weight both sexes (grams)</th>
<th>Grams of feed per gram gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diet 108-A modified + alpha-tocopherol + cod liver oil orally</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>272</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Same diet without supplement + cod liver oil orally</td>
<td>10</td>
<td>5</td>
<td>50</td>
<td>290</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Same diet + alpha-tocopherol + cod liver oil mixed in feed</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>255</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>Same diet without supplement + cod liver oil mixed in feed</td>
<td>10</td>
<td>6</td>
<td>40</td>
<td>241</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Diet No. II extracted - 21% lard added and held 4 wks. at room temperature then reextratcted and stored 7 months at room temperature. Six per cent rancid lard added prior to feeding. Cod liver oil mixed in feed. Supplemented with alpha-tocopherol</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>357</td>
<td>3.8</td>
</tr>
<tr>
<td>5</td>
<td>Same diet without alpha-tocopherol</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>379</td>
<td>3.8</td>
</tr>
</tbody>
</table>

*No statistical analysis because of mortality.*
the same diet for trial 8. The incidence of mortality was practically
the same in the negative control lots. The administration of a total
dosage of 25 milligrams of alpha-tocopherol completely protected the chicks
against the brain deficiency disease.

It is also noted that the difference in the incidence of mortality is
slightly in favor of the lot receiving the cod liver oil mixed in the feed
rather than the lot receiving it orally.

The symptoms displayed by the affected chicks were similar to those
previously described. An examination of the brains of the chicks that
died in lots 2 and 4, revealed the presence of gross lesions. The lesions
varied with each brain as to the nature and extent of involvement. The
lesions observed were edema, flattened convolutions of the cerebellum,
hemorrhages less than one millimeter in diameter, and soft yellowish areas
one-half centimeter or less in diameter. The lesions were located in the
cerebellum more often than in the cerebrum. According to symptoms and le-
sions observed, these chicks appeared to be affected with encephalomalacia.

When Diet II was rancidified and reextracted and stored for seven
months it failed to produce mortality. It is further observed that a dosage
of 25 milligrams of alpha-tocopherol did not increase the weight above that
attained by the negative control.

The mean weights obtained on both diets indicate poor growth.

The purpose of experiment 10 was to induce a high incidence of en-
cephalomalacia by using excessive levels of cod liver oil with Diet 108-A.
Table 10.

The Protective Effect of Alpha-tocopherol and Cod Liver Oil Against Encephalomalacia

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Treatment and supplement</th>
<th>No. chicks per lot</th>
<th>Chicks surviving 8th wk.</th>
<th>Per cent mortality 8th wk.</th>
<th>Mean weighta (grams)</th>
<th>Feed per gram gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diet 108-A modified, added 3.5% cod liver oil</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Same diet + alpha-tocopherol orally</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Diet 108-A modified, added 6.5% cod liver oil</td>
<td>10</td>
<td>7</td>
<td>30</td>
<td>308</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Same diet + alpha-tocopherol orally</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>293</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>Diet No. III + 21% lard and stored 5 months at room temperature. Cod liver oil 2%</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>238</td>
<td>2.7</td>
</tr>
<tr>
<td>9</td>
<td>Same diet + alpha-tocopherol orally</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>259</td>
<td>2.8</td>
</tr>
</tbody>
</table>

a No statistical analysis because of mortality.
The results on the incidence of mortality are recorded in Table 10.

The mortality began in lots 1 and 7 the first part of the fifth week and continued to the latter part of the eighth week. In lot 2 the mortality began the sixth week and subsided the first day of the eighth week.

The symptoms manifested by these chicks and the gross lesions of the kidneys were similar to those described in experiment 8, whereas the gross brain lesions were similar to those described under experiment 9. The symptoms and gross lesions of the brain resembled those described for encephalomalacia.

Poor growth resulted as a consequence of adding 21 per cent lard to Diet III and storing it at room temperature for five months, but no mortality was produced in lot 3. The mean weight of the chicks receiving a total dosage of 25 milligrams of alpha-tocopherol appears to be similar to the mean weight of the negative control.

Experiment 11 was initiated in an effort to induce encephalomalacia or mortality by supplementing Diets III and IV, composed for the most part of natural feedstuffs with 3.5 and 6.5 per cent of cod liver oil. The results recorded in Table 11 show that no mortality occurred during the experimental period. The chicks failed to show any symptoms of muscular incoordination.

It was observed that a relationship existed between the intensity of shank color and the level of cod liver oil added to the feed. The yellow color of the beaks and shanks of most of the chicks receiving 6.5 per cent of cod liver oil in the feed was highly suppressed as compared to the
Table 11.

The Effect of Supplementing High Levels of Cod Liver Oil to Diets Composed of Natural Feed Ingredients

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Treatment and supplement</th>
<th>No. chicks per lot</th>
<th>Chicks surviving 8th wk.</th>
<th>Per cent mortality 8th wk.</th>
<th>Mean Weight (grams)</th>
<th>Grams of feed per gram gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Diet IV + 3 1/2% cod liver oil</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>364</td>
<td>3.6</td>
</tr>
<tr>
<td>5</td>
<td>Same diet + 6 1/2% cod liver oil</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>343 **</td>
<td>3.2</td>
</tr>
<tr>
<td>6</td>
<td>Same diet + 1 1/2% cod liver oil</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>438</td>
<td>3.3</td>
</tr>
<tr>
<td>10</td>
<td>Diet III + 3 1/2% cod liver oil</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>530</td>
<td>3.3</td>
</tr>
<tr>
<td>11</td>
<td>Same diet + 6 1/2% cod liver oil</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>547</td>
<td>3.1</td>
</tr>
<tr>
<td>12</td>
<td>Same diet + 1 1/2% cod liver oil</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>577</td>
<td>3.5</td>
</tr>
</tbody>
</table>

** Highly Significant
controls. Suppression of shank and beak color occurred on both rations. This confirms the findings of Hammond (23) who observed that excessive quantities of cod liver oil suppressed shank color in chicks.

The mean weights of the lots receiving 3.5 and 6.5 per cent cod liver oil supplemented to Diet III were lower than the control lot, but the difference was highly significant only in the case of lot 5. Diet IV was supplemented with the same levels of cod liver oil, but with this diet the differences in weight were not significant.
DISCUSSION

Previous work by Underbjerg (50) in which the diet was treated with ethereal ferric chloride, indicated that the method used for the destruction of vitamin E was effective, since feeds so treated, when fed to rats, produced typical resorptions. Therefore, the method described by Underbjerg (50) was used for the treatment of the feeds for the initial experiments.

The results of this study show that ferric chloride treated diets lower the growth rate of chicks, which agrees with the observation of Jukes and Babcock (26) and of Sloan, et al (44). No mortality or nutritional encephalomalacia resulted by this method of treatment, even though the feed was subjected to high and low heat treatments for long periods of time. These observations are not in agreement with the findings of Adamstone (4) who produced nutritional encephalomalacia with a ferric chloride treated diet exposed to a temperature of 34.6°C.

When chicks were fed simplified diets treated with ferric chloride to which lard had been added, some mortality occurred, but when a more complex diet was treated in a like manner, no mortality resulted. This observation indicates that the results obtained depend upon the composition of the diet prior to treatment, rather than upon the treatment alone. When the fat content of the feed was removed to less than 0.5 per cent, and then the diet was treated with ferric chloride, the growth of the
chicks was reduced to some extent, but diets so treated failed to induce symptoms of a vitamin E deficiency other than slow growth. No other observations are available on this type of treatment in studying a vitamin E deficiency in chicks.

Results are presented which show that when feed is subjected to high heat during the process of removing the fat, the rate of growth of chicks is decreased. When the fat of a diet is extracted in the cold to less than 1 per cent, the growth rate is not appreciably lowered. No mortality resulted from the effects of these treatments. These observations agree with the work of Russell, et al (43).

An attempt was made to inactivate vitamin E in natural feeds by extracting the fat in the cold to less than 0.5 per cent and then adding either rancidified lard or natural fat. The findings indicate that the growth rate was not lowered below that of the controls; and the source of fat, when rancidified appeared to be immaterial insofar as growth was concerned. No mortality resulted as a consequence of feeding diets so treated.

Data are presented showing that feeds which have been extracted, rancidified with lard; and then stored for seven months did not produce mortality. The rate of growth was decreased slightly. Twenty-one per cent lard was added or mixed with a diet composed of natural feedstuffs, and stored in a can at room temperature for five months. The feeding test revealed very poor growth at eight weeks of age, but no mortality occurred nor were there any symptoms of encephalomalacia. Norris (35) re-
ported the production of encephalomalacia by storing a normal ration at room temperature. As to whether the variance in results is due to the methods of storing or to other factors is not determined.

Wheat germ oil was administered to chicks orally to determine whether it would stimulate growth in chicks. When the oil was given to chicks receiving a simplified diet treated with ferric chloride or with ferric chloride and lard in ether solution, a high mortality was produced. The amount of oil given daily was approximately 8 to 10 per cent of the diet, and it was increased gradually until 2 and 5 cc. levels were reached. These dosages amounted to approximately 3 to 3.5 per cent of the feed intake at 8 weeks for the 2 cc. level and between 8 to 10 per cent for the 5 cc. level. The chicks receiving an untreated diet could tolerate these amounts of oil. Wheat germ oil did not produce a high mortality when the chicks received a treated diet containing a wide variety of feedstuffs.

Growth of chicks was not stimulated when wheat germ oil was supplemented to E-deficient or normal rations. Holmes and Cravens (25) supplemented a normal ration with 0.1 per cent of wheat germ oil and obtained similar results.

One experiment was initiated to test the effectiveness of wheat germ oil as a growth promoting substance as compared with coconut oil. This was done to determine whether the results obtained might be due to the fat content of the diet or to the addition of the vitamin E in wheat germ oil. Both oils appeared to be equal in their effect upon growth.
A study was made on the protective effect of alpha-tocopherol against nutritional encephalomalacia. Two diets were used for this study that were known to produce the brain degeneration. Diet 108-A was modified by using rancid lard and rancid natural fat to determine whether the rancid natural fat possessed a protective substance against the brain disease. No such substance was evident, as a complete mortality resulted. Diet E which previously had been completely encephalomalacia producing was modified by adding 1 per cent of cod liver oil. When 0.1859 milligram of synthetic alpha-tocopherol per chick per day was administered orally, the chicks were completely protected against encephalomalacia. At this level, a total dosage of 10 milligrams per chick was administered over the eight week period. However, when Diet 108-A, plus the same amount of tocopherol was fed, complete mortality resulted, but when the total amount of tocopherol fed with Diet 108-A was increased to 25 milligrams per chick for the eight week period, the chicks were protected. This difference in protective level could not be attributed to the method of administration, and it must therefore be due to differences in the diets. It would seem that the protective level of synthetic dl-alpha-tocopherol for chicks lies between 10 and 25 milligrams when administered over a period of eight weeks. This is in contrast with the results of Pappenheimer, et al (41) who found that chicks were protected against encephalomalacia when a total of 4.95, 5.0 and 10.0 milligrams were administered over periods of five weeks each.
It is possible that this difference in levels required for protection may be explained by the possibility of differences in the rancidity of the fats used, since these authors did not quote iodine numbers. It is probable that higher levels of alpha-tocopherol are required with extremely rancid feeds.

It was assumed that the vitamin A content of the cod liver oil was being destroyed by coming into contact with the large quantity of rancid fat.

The incidence of mortality was then reduced approximately 50 per cent on the same batch of feed by giving the cod liver oil orally. The incidence of mortality was reduced when cod liver oil was administered orally to chicks receiving a ferric chloride and lard treated feed. Also, when cod liver oil was given orally to chicks receiving extracted rancidified diets rate of growth was again improved. All of this evidence points to the fact that the oral feeding of cod liver oil improved the rate of growth regardless of the diet used. This substantiates the assumption above that some factor or factors in cod liver oil, probably vitamin A, was destroyed in part when the cod liver oil was mixed into the feeds. This was confirmed by the resulting mortality, for mortality was reduced quite uniformly when the cod liver oil was pipetted, as compared to those lots which were fed cod liver oil mixed into the feed.

Evidence pointing toward a factor in cod liver oil which might hinder the utilization of vitamin E has been reported by Hammond (23), who found
that when 3 per cent or more of cod liver oil was fed, nearly one-third of the chicks developed encephalomalacia. This cod liver oil was presumably added to diets made up with natural feedstuffs. However, in this work when either 3 1/2 or 6 1/2 per cent of cod liver oil was added to diets made up of natural feedstuffs, no cases of encephalomalacia developed.

When the diet was changed, and synthetic Diet 108-A was used, all of the chicks fed the 3 1/2 per cent level of cod liver oil developed definite symptoms of the deficiency, either with or without an additional supplement of 25 milligrams of synthetic alpha-tocopherol fed each chick over the eight week period. These results are in essential agreement with those of Hammond (23) in that a destruction or inactivation of vitamin E is indicated when this level of cod liver oil is fed, provided, however, that synthetic, rather than natural diets are used. The extension of this investigation, however, does not confirm the results of Hammond (23). That is, when 6 1/2 per cent of cod liver oil was added to Diet 108-A approximately 30 per cent of the chicks developed encephalomalacia. Thus an increase in the level of cod liver oil apparently afforded some protection, since all of the chicks on the 3 1/2 per cent level of cod liver oil were affected. When the 25 milligrams of alpha-tocopherol were added to the Diet 108-A with the 6 1/2 per cent of cod liver oil, no cases developed, indicating a further protective action of the tocopherol with cod liver oil at the extreme level. It is not known whether Hammond's (23) investigations were carried to such extreme levels of cod liver oil. Since cod
liver oil is very low in vitamin E, and since increases of cod liver oil from 1 or 2 per cent to 3 per cent result in a definite increase in the symptoms ascribed to vitamin E deficiency, it is hardly reasonable to explain these results in terms of additions of vitamin E with the higher amounts of cod liver oil. Therefore, it must be assumed that either the level of other vitamins, and particularly vitamin A, must be responsible for this protection against encephalomalacia, or that more than one factor is involved in the production of the conditions described as nutritional encephalomalacia.
CONCLUSIONS

1. Rate of growth in chicks is not affected by vitamin E (synthetic dl-alpha-tocopherol) or by wheat germ oil.

2. High levels of cod liver oil (3.5 and 6.5 per cent) did not produce encephalomalacia when added to diets made up of natural feedstuffs.

3. The addition of 3.5 per cent of cod liver oil to Diet 108-A (modified) produced 100 per cent of encephalomalacia in either the presence or absence of synthetic dl-alpha-tocopherol.

4. The addition of 6.5 per cent of cod liver oil to Diet 108-A (modified) produced only 30 per cent of encephalomalacia when no synthetic dl-alpha-tocopherol was fed, and none when 25 milligrams of the tocopherol were administered orally over a period of eight weeks.

5. The requirement of the chick for synthetic dl-alpha-tocopherol for the first eight weeks lies between 10 and 25 milligrams.

6. Wheat germ oil added at levels of 2 and 5 cc. to simplified diets made up of natural feedstuffs, but treated with ethereal ferric chloride, increased mortality.

7. Wheat germ oil added at levels of 2 and 5 cc. to diets made up of natural feedstuffs had no effect upon mortality.

8. The treatment of feeds with ethereal ferric chloride reduced the rate of growth of Single Comb White Leghorn chicks fed these diets.
9. The rate of growth of Single Comb White Leghorn chicks fed diets extracted at temperatures approximating 95°F. was less rapid than that of chicks fed diets extracted at approximately 55°F.

10. Either extraction with pentane (Skelly A) or treatment with ethereal ferric chloride may be used to remove or inactivate vitamin E in diets made up of natural feedstuffs.
SUMMARY

In this work, vitamin E-deficient diets were prepared (1) by treating with ethereal ferric chloride, recovering the ether by heating, then aging the feed for two weeks at 57°C; (2) by similar treatment except that 1 per cent of lard was added in the ether solution; (3) by extraction with normal pentane (Skelly-A) and then aging for two weeks at 37.8°C., (a) with sufficient lard to replace the amount of fat extracted, (b) with 1 per cent of ferric chloride and 0.25 per cent coconut oil, (c) with 0.25 per cent coconut oil, (d) with the extracted natural fat rancidified and readded to the feed, (e) 21 per cent lard added and aged four weeks at room temperature, reextracted and stored at room temperature for seven months; 6 per cent rancid lard mixed into the feed and aged two weeks longer; (4) 21 per cent lard added to untreated feed and stored at room temperature for five months; (5) Diet 108-A of Pappenheimer, et al, modified; (6) Diet B as modified by Thompson.

These diets were fed to Single Comb White Leghorn chicks in a series of experiments. Supplements of wheat germ oil, synthetic dl-alpha-tocopherol, and cod liver oil were added at varying levels, both by mixing into the feeds and by oral administration.

Growth and mortality were used as the criteria of judging the effectiveness of feed treatments, and, in addition, the symptoms and gross lesions which have been described, together with histologic studies, were
used to determine vitamin E deficiency.

In general, the results obtained indicate that the treatment of feeds with ethereal ferric chloride decreases the rate of growth of chicks whereas, the addition of ferric chloride in aqueous solution to the feed had very little if any effect upon the rate of growth. Those feeds which were extracted at high temperatures produced a lower rate of growth than those which were extracted at lower temperatures. The results indicate that either method of treatment, that is, treatment with ethereal ferric chloride, or extraction with normal pentane (Skelly A) may be used for the destruction or removal of vitamin E from natural diets to be used in studying deficiencies of this vitamin, provided that adequate diets are used. Apparently, rate of growth in chicks is not affected by vitamin E, since the diets extracted at the lower temperatures did not reduce rate of growth as compared with the unextracted control diets, and supplements of wheat germ oil or synthetic dl-alpha-tocopherol neither increased nor decreased rate of growth, except when massive doses of wheat germ oil were used, in which case a decrease in rate of growth was apparent.

When a simplified diet, made up of natural feedstuffs, was treated with ethereal ferric chloride, the addition of wheat germ oil at 2 and 5 cc. levels decidedly increased mortality. This was not the case when the wheat germ oil was added to the untreated control. Neither was this the case when wheat germ oil was added to the extracted diets. Therefore, there is an effect of dietary treatment in combination with the supplemen-
tation of wheat germ oil. The symptoms of nutritional encephalomalacia were produced in chicks fed the modified Diet 108-A. This deficiency was prevented by the oral administration of 25 milligrams of synthetic dl-alpha-tocopherol over a period of eight weeks, but 10 milligrams failed to protect. Therefore, the requirement of the chick for synthetic dl-alpha-tocopherol is between 10 and 25 milligrams for the first eight weeks of life, depending upon the composition of the diet.

The addition of rancidified natural fat did not affect the incidence of encephalomalacia in chicks.

High levels of cod liver oil (3.5 and 6.5 per cent) did not cause the production of encephalomalacia when those levels were used with diets made up of natural feedstuffs. However, when Diet 108-A (modified) was used, all chicks developed the deficiency when fed the 3.5 per cent level, either in the presence or the absence of dl-alpha-tocopherol. When the 6.5 per cent level was used without the tocopherol, 30 per cent of the chicks developed the deficiency, and complete protection was afforded by the use of 25 milligrams of dl-alpha-tocopherol fed over a period of eight weeks.
LITERATURE CITED


27. Jungherr, E. A field condition resembling nutritional encephalomalacia in chicks. Sci. 84;559-560. 1936.


34. Ni, T. G. Nutritional encephalomalacia and some factors accelerating its onset. Chinese Jour. Physiol. 15;161-166. 1940.


ACKNOWLEDGMENTS

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<table>
<thead>
<tr>
<th>Ingredients</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground yellow corn</td>
<td>56.75</td>
<td>21.75</td>
<td>40.00</td>
<td>57.00</td>
</tr>
<tr>
<td>Ground whole oats</td>
<td>26.00</td>
<td>10.00</td>
<td>15.00</td>
<td></td>
</tr>
<tr>
<td>Wheat bran</td>
<td>10.00</td>
<td>15.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat middlings</td>
<td>24.00</td>
<td>7.00</td>
<td>15.00</td>
<td>24.00</td>
</tr>
<tr>
<td>Soy bean oil meal</td>
<td>3.00</td>
<td>3.00</td>
<td>4.00</td>
<td></td>
</tr>
<tr>
<td>Fish meal</td>
<td>3.00</td>
<td>4.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried buttermilk</td>
<td>14.00</td>
<td>10.00</td>
<td>5.00</td>
<td>14.00</td>
</tr>
<tr>
<td>Alfalfa meal</td>
<td>5.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfalfa leaf meal</td>
<td></td>
<td></td>
<td>4.00</td>
<td></td>
</tr>
<tr>
<td>Yeast, dried brewer's</td>
<td>2.00</td>
<td>10.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Riboflavin concentrate</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground oyster shell</td>
<td>2.00</td>
<td>1.50</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Meat and bone scrap</td>
<td>1.75</td>
<td>4.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Cod liver oil: variable
Manganese chloride: 37 grams
Manganese sulphate: 10 grams

Diet 108-A (Modified)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Lbs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried skim milk, Merrell-Soule</td>
<td>15.0</td>
</tr>
<tr>
<td>Casein, commercial</td>
<td>20.5</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>20.0</td>
</tr>
<tr>
<td>Lard</td>
<td>21.0</td>
</tr>
<tr>
<td>Yeast, dried brewer's</td>
<td>5.0</td>
</tr>
<tr>
<td>Salt mixture, given by*</td>
<td>6.5</td>
</tr>
<tr>
<td>Paper pulp</td>
<td>5.0</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>2.0</td>
</tr>
<tr>
<td>Total</td>
<td>95.0</td>
</tr>
</tbody>
</table>

* Given by, Pappenheimer, Goetsch, and Jungherr (41)
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>10.00</th>
<th>10.00</th>
<th>10.00</th>
<th>10.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cod liver oil</td>
<td>variable</td>
<td>variable</td>
<td>variable</td>
<td>variable</td>
</tr>
<tr>
<td>Manganese chloride</td>
<td>37 grams</td>
<td>37 grams</td>
<td>10 grams</td>
<td>10 grams</td>
</tr>
<tr>
<td>Manganese sulphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Dist 108-A (Modified)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Lbs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried skim milk, Merrell-Soule</td>
<td>15.0</td>
</tr>
<tr>
<td>Casein, commercial</td>
<td>20.5</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>20.0</td>
</tr>
<tr>
<td>Lard</td>
<td>21.0</td>
</tr>
<tr>
<td>Yeast, dried brewer's</td>
<td>5.0</td>
</tr>
<tr>
<td>Salt mixture, given by*</td>
<td>6.5</td>
</tr>
<tr>
<td>Paper pulp</td>
<td>5.0</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>2.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>95.0</td>
</tr>
</tbody>
</table>

* Given by, Pappenheimer, Goettsch, and Jungherr (41)

**Dist E (Modified)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Lbs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane extract of alfalfa, added, equiv. to</td>
<td>3%</td>
</tr>
<tr>
<td>Polished rice, ground</td>
<td>73.0</td>
</tr>
<tr>
<td>Ether-treated sardine meal</td>
<td>17.5</td>
</tr>
<tr>
<td>Ether-treated dried brewer's yeast</td>
<td>7.5</td>
</tr>
<tr>
<td>Salt and small amounts of Fe, Cu, and Mn</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100.0</td>
</tr>
</tbody>
</table>

| Cod liver oil                                  | 3.0  |
Table 2.
Estimated Chemical Analyses of Diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Protein (%)</th>
<th>Calcium (%)</th>
<th>Phosphorus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I*</td>
<td>14.94</td>
<td>1.03</td>
<td>0.56</td>
</tr>
<tr>
<td>II*</td>
<td>20.66</td>
<td>1.26</td>
<td>0.79</td>
</tr>
<tr>
<td>III*</td>
<td>16.80</td>
<td>1.62</td>
<td>0.87</td>
</tr>
<tr>
<td>IV*</td>
<td>14.96</td>
<td>1.03</td>
<td>0.56</td>
</tr>
<tr>
<td>108-A (Mod.)**</td>
<td>26.09</td>
<td>1.18</td>
<td>0.72</td>
</tr>
<tr>
<td>E (Mod.)***</td>
<td>19.70</td>
<td>0.61</td>
<td>0.60</td>
</tr>
</tbody>
</table>

* Calculated analyses. Tables 6 and 7 by H. W. Titus, Yearbook, "Food and Life" U.S.D.A. 1939.


*** "Chemical Analyses", Appendix Table 12, Thompson (49)