Effect of avitaminosis E on reproduction and vitamin E storage in the tissues and milk of goats

Gravers K. L. Underbjerg

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UMI
EFFECT OF AVITAMINOSIS E ON REPRODUCTION AND VITAMIN E
STORAGE IN THE TISSUES AND MILK OF GOATS

by
Gravers K. L. Underbjerg

A Thesis Submitted to the Graduate Faculty for the Degree of
DOCTOR OF PHILOSOPHY
Major Subjects
Dairy Husbandry - Animal Nutrition

Approved:

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Iowa State College
1939
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As to my personal obligations to others - too numerous to mention in detail or by names - I content myself to say with Goethe:

"...Bei den besten
Sass ich unter zufriednen Gasten;
Ihr Frohmahl hab ich unverdrossen
Niemals bestohlen; immer genossen!"

- "For den Nytte du formes!"
  - Bernhardt Bang.
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I. INTRODUCTION

A. Early Observances of the Importance of Reproduction
   and of Factors which Affect Fertility

Reproductive failure, sterility, infertility or barrenness in man and animals apparently has been known to occur down through the ages. Practically all primitive peoples at the dawn of history have been inspired by dread of the same evil, sterility, and all have sought to attain one end, fecundity. That this is true is revealed by the fact that one deity remained extremely popular among the early peoples. That deity was the spirit of sex and fertility, the very principle of life itself. Nearly all the early peoples worshipped some such mother goddess, for the power of reproduction among plants, beasts, and men remained unfailingly the most vital and engrossing power of all. To be able to control it meant to live; to fail meant to die. Little wonder, therefore, that everywhere in the world where phallic worship is prevalent, we find people groveling at the feet of some sex-dealing, life-breeding spirit.

In Babylonia and throughout the Levant the people seem to have bowed down to it inordinately, and sex rites in honor of Ishtar - or Astarte, Ashtareth, Isis, Cybele, Venus, and Aphrodite as the goddess was known in the various lands - were counted of primary importance.
Among the ancient Romans each man was believed to have what was called a *Genius*, a spirit personifying his virility; and each woman had what was called a *Juno*, a spirit personifying her power to conceive. The early Romans, like most other primitive peoples, were driven by their constant struggle against extinction to consider the power of reproduction a miraculous and highly divine thing.

Early comments concerning barrenness are found in the book of Genesis (21). Sarah, the wife of Abraham, was unable to bear children; and Jacob (32) may have known the whereabouts of abundant pastures or known the secrets of animal breeding and genetics, since it is stated there was a great increase of the herds and flocks under his management as herdsman for his uncle Laban. Aristotle (10) noted an increased fertility of sheep in a favorable environment. In more recent times Buffon (26), among others, remarked on sterility occurring in man. Darwin (40) commented on the fact that domestic animals breed oftener and produce more young than wild animals belonging to the same species. He attributed the increased fertility of the former to a long habituation to a regular and abundant food supply without the labor of seeking it.

B. Complexity of Factors which Affect Reproduction

To the livestock breeder sterility or reproductive failure among his herds and flocks is a problem of great economic importance and no extensive breeder of livestock has escaped its effects. Infertility and low fertility are usually regarded by the laity as caused by disease or
infection, inducing abnormal function of the reproductive organs of male or female. In a number of cases this is undoubtedly correct although often the cause of decreased fertility and many times complete sterility is improper nutrition. Men of science for years have been trying to find the causes for infertility and aberrations in the reproductive system in the male and female. In many cases they have substantiated the fact that reproductive abnormalities could be traced to pathological conditions which resulted from 1) infection, 2) genetic factors and 3) malnutrition, or 4) their combinations. The etiology of sterility presents, therefore, a most complex problem and the solution of the subject is not to be found in any one aspect. All etiological factors which have been cited above must receive due consideration in an investigation of the subject of sterility. To attempt to cover all phases of sterility and its associated phenomena is, however, beyond the scope of this paper.

The role played by the proper nutrition of animals has been emphasized especially during the past half century. Malnutrition, in the presence of sufficient food, caused by a lack of balance of certain components of the animal diet, such as proteins, carbohydrates, fats, minerals and vitamins or the presence of toxic substances, may interfere seriously with the metabolic activity and the functions of the various systems in the body of animals and man. An imbalance of such factors may affect the animal as a whole or the effect may be local and thus specifically affect the gonads and accessory organs of the genitalia. The accessory food factors play important roles in regard to the normal functions of the reproductive system. Certain avitaminoses affect the reproductive activity adversely
by reducing the vitality and shortening the breeding life of the affected animals by evoking specific inhibitions in the genital organs and on their rhythmic functions. It is an established fact that vitamin E is necessary in the diet for normal reproduction in rats (61) and mice (19) and for the hatchability of chicks (30).

Small laboratory animals (rats, mice) can be reared successfully on simplified dietary mixtures consisting of protein, fat, carbohydrate, salts and the vitamins. Lack of vitamin E, however, in such purified diets, although healthy adulthood is reached, sooner or later leads to sterility among the animals. The most striking aspects of the sterility among the animals subjected to avitaminosis E regimes are the different effects on the two sexes. Lack of vitamin E in the male ration apparently evokes permanent degeneration in the testes; e.g. histopathological changes in the germinal epithelium occur which interfere with spermatogenesis and finally involve the entire testicle (68,127,135). In the female, on the other hand, no degeneration of the ova can be demonstrated. Deficiency of vitamin E does not appear to impose any inhibition upon the ovarian and early uterine phase of reproduction. The inhibitory effects are imposed at a later phase on the developing embryo; sterility resulting from placental insufficiency (68,195). That such is the case is afforded by the restoration and in fact the complete normality of young in gestations when the curative dose of vitamin E is not administered until a few hours before implantation of the blastodermic vesicles - an administration as late as the fifth day after copulation. (Evans 68). If a high curative dose is given later than that date the fetal tissues apparently are beyond
repair. The mother, however, is able to store and make use of such a high dose of vitamin E in a subsequent gestation and deliver normal young at term. Thus it would seem that the effect on tissues in the female as a result of avitaminosis E apparently is transitory in nature and the fetal rather than the maternal tissues in the placenta show elective disorders when vitamin E is low or absent. Evans (57), however, has indicated that the maternal mechanism is abnormal in a strange tendency of the uterus in such females to form spontaneous deciduomas when pseudopregnancy is provoked by copulation with vasectomized males.

It has been assumed that a deficiency of this vitamin may occur extensively in farm animals under usual farm conditions which lower their fertility. No scientific experimental data exist, however, to indicate to what extent vitamin E does actually affect fertility among our domesticated mammals in practical intensive or semi-intensive farming, but it may be of considerable importance. The necessity of this vitamin in the diet has not been demonstrated for other mammals than those mentioned previously. Sterility due to experimentally induced avitaminosis E can be cured therapeutically in the female rat by the administration of vitamin E in the form of wheat germ oil (68), and also by administration of alpha-, beta-, and gamma-tocopherols or their allophejmates (12, 51, 52, 71, 74, 153). The success of wheat germ oil (vitamin E) therapy in the treatment of certain types of temporary sterility in domestic animals has been reported by Vogt-Moller and Bay (202), Tutt (194), and Jones and Swalt (96). Equally good results are reported with wheat germ oil (vitamin E) therapy in women suffering from habitual abortion by Vogt-Moller (200, 201), Juhass-Schaffer (102),
It has been pointed out in the preceding paragraphs that the causes of infertility in animals often may be due to a number of concomitant etiological factors related to nutritional deficiencies. Dairy cattle through generations have been selected according to their ability to consume feed and convert it into milk. While breeding and selecting for high production, other physiological factors important for the normal function of the reproductive system also may have been impaired. In order to reach their maximum production, they have been required to consume and digest widely differing by-products from different industries, such as linseed oilmeal, soybean oilmeal, cottonseed meal, coconut meal, peanut meal and molasses. This is especially the case in the milk sheds around big cities in the United States and in foreign countries. To make it still more difficult for the cows to fulfill the task of high production, by converting these processed products into milk, they are stanchioned and kept inside in confinement most of the year without exercise or access to sunshine and fresh air. (Europe). It is, therefore, no wonder that under such conditions, which are contrary to the natural environment of cows, maladjustment exists, and that the reproductive equilibrium may be altered through imbalance or lack of certain substances which apparently are necessary for fertilization and normal reproduction.

C. Purpose of the Study

Realizing the economic importance of sterility in farm animals, one
may reasonably hold the view that one of the greatest services which science could render to the livestock industry would be to provide the means of banishing the causes for reproductive failures. In view of the fact that avitaminosis E is one cause known to exert a deleterious effect on fertility in small laboratory animals, an accurate evaluation of its importance in farm animals would seem quite justifiable. Much valuable information could be obtained by studying the fertility of farm animals restricted to a vitamin E-deficient ration.

Some apparent cures of certain types of sterility in larger animals, as mentioned above, have resulted from wheat germ oil (vitamin E) therapy, and since it seemed probable that vitamin E might play an important role in the fertility of farm animals such as dairy cattle, the Iowa Experiment Station, 1933, initiated a project to determine if nutritional sterility could be induced experimentally in larger animals by a ration deficient in vitamin E. Though the ultimate intention was to use dairy cattle for experimental animals, it was thought wise to use first a smaller animal of similar type. Milk goats were selected, as they fit well into a laboratory procedure and since it was thought that they might give results comparable to those with cattle. Therefore, the first objective was to determine whether or not milk goats required vitamin E in the ration for successful reproduction. These goats reproduced normally for two years without exhibiting any abnormal functions (209). They were restricted all this time to a standard ration in which vitamin E successfully had been destroyed with an ethereal solution of ferric chloride (183). The treated ration was supplemented with vitamin E-free accessory food factors. In
view of our present knowledge of nutrition it was adequate in all respects except for the lack of vitamin E. Rats were used in evaluating the efficacy of the method employed to inactivate vitamin E. All rats employed produced resorption gestations with remarkable uniformity when restricted to mixtures of the ferric chloride treated goat ration.

The failure to induce nutritional sterility among goats by restricting them to a ration which invariably produced sterility in female rats naturally suggested that either the vitamin E reserves of goats are not readily decreased to the point of inducing reproductive abnormalities or vitamin E might possibly be synthesized by the goat. A study of the effect of avitaminosis E on the reduction of the amount of this factor occurring naturally in milk and certain tissues of goats was therefore undertaken. Comparative tests were made on the occurrence of this factor in the milk and tissues of goats reared under usual farm conditions and in the progeny of the parental goats under experimentally induced avitaminosis E. A further objective was to ascertain whether continued avitaminosis E during a reasonable length of time would induce nutritional sterility, and if so whether this vitamin was the causal agent which could prevent or cure this type of sterility in the goat.
II. REVIEW OF LITERATURE

A. Discovery of Vitamin E and its Confirmation

Some sixteen years ago several investigators in animal nutrition began to suspect that for normal reproduction, the rat required a dietary constituent not yet recognized or included within the generally accepted group of accessory food factors. Evans and Bishop (59, 60, 63, 64), in a search of the influence of diet on the precise mechanism of the estrus cycle of the rat, found that females reared on what was considered an adequate simplified diet would eventually lose the ability of reproduction. In 1922 Evans and Bishop (61) designated the nutritional element that was apparently essential for reproduction as the X factor. The experimental evidence which these workers presented, demonstrated that rats on a diet which was sufficient in regard to proteins, caloric content, minerals and the then known vitamins (A, B and D) became partly sterile in the first and completely sterile in the second generation.

The female sterility was of placental origin, since normal numbers of follicles matured and ruptured and the ova were fertilized and implanted. Abnormalities of the placentas were evidenced as early as the second day after their establishment by blood extravasations which continually increased in extent. Ultimately the products of conception were resorbed. This reproductive disturbance could be prevented by adding fresh lettuce
leaves, alfalfa or wheat germ to the ration. Increasing the butterfat content in the ration from nine to 2\% per cent also allowed normal fertility. These investigators also showed (62) that the X-substance was fat-soluble and different from the other fat-soluble vitamins A and D. Concurrently other workers, independent of Evans et al, published work which indicated a new factor different from the other known vitamins was necessary for the maintenance of fertility in rats.

Sure (186) fed rats on a diet consisting of milk, milk protein or skimmed milk powder plus additions of various amino acids as proteins, dextrin, agar-agar, salts, cod liver oil (A and D vitamins), and an alcoholic extract of ether-extracted wheat embryo which was used as B vitamin. On such diets the animals became sterile, but the sterility could be prevented by adding polished rice, yellow corn and rolled oats. Sure acknowledged (187) that the factor was identical with Evans' X-substance and proposed that the term "E" be adopted to represent this new dietary factor that influenced reproduction. Mattill and Conklin (138) had previously noticed that rats, maintained entirely on milk with addition of iron citrate and one per cent yeast, would not propagate successfully. They also noticed that the animals were inhibited in growth in the third or mature growth cycle. Mattill and his coworkers (129,134,135,137,140) continued their research on this problem to ascertain the cause for the reproductive failures, when rats were kept on these various milk diets, and showed that milk apparently was lacking in a factor which was necessary for normal development of the embryo in the female and for prevention of atrophy of the male gonads, thereby verifying Evans' and Bishop's findings (61).
The following years of investigation partly supported, partly refuted, the existence of a new vitamin essential for reproduction. Nelson and co-workers (145, 146, 147) and Anderegg (6) met the proposal of a vitamin necessary for reproduction with much criticism. The reason these investigators could not reproduce Evans', Sure's, and Mattill's experiments was probably partly due to the use of butterfat in their rat diet. By substituting lard for butterfat in their ration they produced typical signs of vitamin B deficiency after a period of "initial fertility". They probably would have found changes that presented convincing evidence had they examined the male gonads. However, on the basis of their experiments, they concluded that it was the lard per se which caused the sterility. That the latter can be the case, when rancid lard is used, was reported later by Kudrjaschov (111) and Kudrjaschov and Beljajeva (113). These workers allowed lard to stand exposed to the air at room temperature for 12 to 18 months. Such rancid lard, when incorporated in the diet for normal pregnant rats reared on a natural ration, uniformly prevented implantation of the fertilized ova or caused resorption of the formed embryo. They also isolated two active fractions from the rancid lard which had a sterilizing effect. The sterilizing substances did not destroy the fertilized ova, but markedly reduced the normal percentage of implantation. If implantation succeeded, resorption ensued. The sterilizing substances exerted their greatest influence during the period between the sixth and ninth day of gestation. Later, the embryo had a greater resistance toward these substances. This kind of sterility was not identical with that caused by vitamin B deficiency, since the animals had stores of this factor in their
bodies and when subsequently restricted to vitamin E-free rations they produced litters. Further this sterility was found to be refractory towards vitamin E therapy.

Histological studies (113) showed that decomposition products of rancid fats and oleic acid, when fed to normal rats in the first stages of pregnancy before implantation of the fertilized ova, had no deleterious effect on the uterus and its functions. The progravid changes in the uterus were normal. The embryo died either at the moment of implantation or early in the developmental stages following implantation. The trophoblast and fetal placenta were either poorly developed or not at all, whereas the maternal placenta was well developed. However, after interrupted pregnancy the maternal placenta was rapidly resorbed. When implantation of the fertilized ova failed, or when pregnancy with resorption of the embryo was completed, the uterus soon returned to normal and the sexual cycle was resumed. The sterilizing substances had no deleterious effect on the ovaries. Formation of corpus luteum and ripening of follicles were normal. The failure of normal gestation was in no way related to the degeneration of the corpus luteum.

Evans and Burr (68) had reported earlier that merely the high proportion of fat in the diet did not cause sterility but that the introduction of large percentages of lard in the diet accelerated sterility or nullified the presence of slight amounts of vitamin E. Later (69) they reported on a substance or substances present in lard, oleic acid and possibly some other fats in small amounts, which, when fed in large percentages, robbed definite and adequate amounts of wheat germ of all effectiveness in curing
sterility. They also reported (70) that these substances present in lard increased with rancidity and were led to believe that these substances acted as antivitamins and thereby neutralized the action of the fat soluble vitamins A and E.

Hogan and Harshaw (90), who used hydrogenated cottonseed oil as the source of fat in the diet, refused to recognize the existence of vitamin E until Sure (189) had shown that cottonseed oil contained vitamin E. Hogan and Harshaw (91) then substituted lard for cottonseed oil and reproduced Evans' results.

The existence of a reproductive vitamin was energetically investigated for seven years in America before it was considered by European investigators. Until 1928, vitamin E remained distinctly an American vitamin. Only rather sceptical expressions and opinions, which, however, never were supported by experimental observations, were set forth by European authorities in biology and medicine. Juhasz-Schaffer (98) gave as reasons, the great requirement for large numbers of animals, long durations of time before deficiency symptoms appeared and the material difficulties in preparation of vitamin E-free rations and last but not least, the many phases of the problem. The official expression of scepticism in regard to the existence of a vitamin for reproduction was given by Kreitmar in "Ergebnisse der Physiologie", 1930 (109), in which he criticised the experimental procedure in vitamin E investigations in these words: "Die Methode hat natürlich mit sehr viel Unsicherheitsfactoren zu rechnen und die Ergebnisse können nur bewertet werden, wenn sie in einen sehr grossen Tiermaterial durchgeführht worden sind. Ich konnte mich bisher von der
Existenz des Fortpflanzungsvitamin mit Sicherheit nicht "überzeugen."

Translation: ("The method, naturally, has to take into account many uncertain factors and the results can only be evaluated, if they are conducted on a very large number of animals. I can not as yet convince myself with certainty of the existence of a reproductive vitamin.")

He did not say whether he based his statements on unsuccessful experiments or whether he had undertaken any investigations in order to clarify this problem.

The first European investigations in regard to vitamin E originated in Italy. Bisceglie (20) described degenerative changes in the testes, as well as in the ovaries on a vitamin E-deficient diet. He reported a loss of weight in the animals from the beginning of his experiments. It must therefore be assumed that the rats used suffered from lack of other accessory factors in the diet, since avitaminosis E is characterized by ovarian integrity, good growth and vigor. The ovarian abnormalities and the diminution in growth probably were due to the fact that the ration contained only one per cent yeast.

Kudrjaschov, a Russian, (110) verified Mattill, Carman and Clayton's (135), Mason's (123,124) and Evans' (54) experiments with male rats. These workers caused sterility in male rats by feeding rations free of vitamin E, and investigated the pathological changes occurring in the testes due to avitaminosis E.

After 1930 vitamin E investigations gained momentum in Europe. Medical workers became interested. Juhasz-Schäffer (98,101), a Swiss, published some work, which essentially substantiated the American results in
regard to male and female sterility in the rat.

The lapse of time which occurred before some of the early investigators would recognize the existence of vitamin \( E \) was evidently due to many concomitant factors. Storage of vitamin \( E \) in the body only sufficient to produce one or two litters, or "initial fertility", among females reared on a vitamin \( E \)-free diet, was probably a factor which delayed the recognition of this vitamin. The use of older rats (50-60 g. at weaning) probably also played a part in "initial fertility", since their bodies contain a larger store of vitamin \( E \). Ringsted (165) has shown that, when large quantities of vitamin \( E \) are employed in the stock ration for breeding animals, weanling female rats (30 days old) from such mothers in their first gestation on vitamin \( E \)-free diets (60 days) in a large number of cases show "initial fertility". When a ration low in vitamin \( E \) was employed for the breeding animals, the offspring from such mothers on vitamin \( E \)-free rations for the same period of time as the former showed no "initial fertility". Weanling male offspring (30 days) from mothers on a ration containing excessive amounts of vitamin \( E \) showed a marked increase in the time before testicular degeneration appeared as compared to male offspring from mothers on rations containing small amounts of this factor. In the latter, testicular changes in the young were constantly and uniformly observed in 30 days on vitamin \( E \)-free diets. In the former, 90 to 120 days passed before testicular changes were noted.

More recent evidence would indicate that the destruction of vitamin \( E \) in the ration was a cause for the difference in the early results obtained by various workers. Also the role played by antioxidants in the
preservation of vitamin E may have been important in giving varying results. The presence of peroxide and peroxide like substances, which apparently accelerate autoxidation and destroy vitamin E as well as antioxidants, probably were factors of which the early workers were unaware.

Mattill (131) showed that vitamin E was destroyed by the oxidative processes, which are accompanied by the rancidification of fats and oils and that the rancidity could be retarded by the presence of compounds containing hydroxy groups. He concluded that vegetable oils, especially wheat germ oil, contain more hydroxy groups than animal fats, such as lard and cod liver oil, and hence delayed the autoxidation of fats and preserved vitamin E. When protected from oxidative changes, small amounts of butterfat sufficed for normal reproduction. The development of rancidity could be hastened by ferrous sulphate.

Cummings and Mattill (38) and Mattill and Crawford (139) further investigated the chemical changes accompanying rancidity and its importance in regard to the fat-soluble vitamins A and E. They found that the content of vitamin E in foodstuffs was dependent on the oxidizability and autoxidizability of the fat when present in a food mixture. The oxidizability and autoxidizability of a fat, on the other hand, were dependent on the number of double bonds in the fat and the presence of catalysts and antioxidants for the process of autoxidations. Whether oxidizability or autoxidizability played the greater role in the oxidative destruction of vitamin E was not determined.

As will be noted in the history of early investigations of vitamin E, the difficulties, which many of the workers encountered, were varied and
materially delayed the recognition of this new factor. However, as the experimental evidence accumulated, the nature of vitamin \( E \) became more completely understood and even the most hesitant investigators accepted the existence of this vitamin and its importance in the diet for normal reproduction. Evans and Burr (68) have published a very complete account of the discovery, occurrence and functions of the vitamin. Other more recent comprehensive reviews on the vitamin include those of Sherman and Smith (173), Evans (58) and Mattill (133). Foreign workers, who have reviewed the subject, are Juhasz-Schaffer (102), Verzar (197) and Bacharach (13).

B. Isolation of Vitamin \( E \)

In order to give an accurate definition of vitamin \( E \), it is necessary to isolate it and determine its chemical structure. Evans and Burr attempted to obtain vitamin \( E \) in its pure state, or at least to derive a concentrate from wheat germ oil. In their Memoirs (68, page 131) a graphic outline is given and a general plan of procedure to be followed in order to obtain the active substance. They were successful in securing a concentrate, which represented 0.25 per cent of the original wheat germ oil. Five mg. of this substance served as a cure in preventing sterility in female rats. Olcott and Mattill (154) have used a similar procedure for the preparation of a vitamin \( E \) concentrate from lettuce. They reported that the vitamin \( E \) concentrate of lettuce had the same solubilities as that of wheat germ oil and later (156) they obtained a concentrate from wheat germ
oil which, when fed to female rats in doses of three to five mg., produced litters.

Evans et al. (74) made further attempts to isolate vitamin E and succeeded in preparing three allophanates from the non-saponifiable portion of wheat germ oil, 1) with a m.p. at 25°, 2) m.p. 138° and 3) a m.p. at 158-60°. The alcohol regenerated from (1) had no vitamin E potency. The alcohol from the second allophanate had some vitamin E potency but less than that from the third allophanate. The alcohol from the third allophanate for which they proposed the name alpha-tocopherol, when given in a single dose of three mg., enabled vitamin E-deficient rats to bear young. Analysis of the third alcohol indicated a provisional formula for alpha-tocopherol of C_{29}H_{50}O_{2}.

Alpha-tocopherol showed a characteristic absorption band in the spectrum at 2980 Å. Bowden and Moore (22, 23) and Morton and Edisbury (143) earlier had associated bands in the spectrum with vitamin E. However, Olcott and Mattill (149) and Olcott (151) concluded that the band in the absorption spectrum of concentrates from wheat germ oil and cottonseed oil was not a property of the vitamin, but was caused by some other substance closely related to it and almost inseparable from it. They thought the substance might be the natural antioxidant associated with vitamin E (151). Karrer and Salomon (105) and Drummond et al. also have studied concentrates of wheat germ oil. Kimm (106), Sei-iche et al. (172) and Todd et al. (193) have studied the concentrates from rice oil and attempted to isolate vitamin E from these substances.

Fernholz (77) reported on the thermal decomposition of alpha-
tocopherol. The thermal splitting was a comparatively smooth reaction and durohydroquinone was a characteristic decomposition product along with a hydrocarbon. Independently McArthur and Watson (116) made a similar observation. The thermal decomposition was repeated successfully in other laboratories. Todd et al (193) repeated it, and more recently a contribution has appeared from Windaus' laboratory in Gottingen, which has a direct connection with the subject although it deals apparently with another factor of the vitamin E group, which was isolated from wheat germ oil by John (95). This new compound was named "Cumo-tocopherol". The analysis indicated an empirical formula of C_{28}H_{46}O_{2}. This substance is probably identical with Karrer's (105) neo-tocopherol and Emerson's et al (52) beta- or gamma-tocopherol. Fernholz (78) studied the degradation products of alpha-tocopherol obtained by oxidation with chromic acid. On the basis of his experiments he proposed a structural formula for alpha-tocopherol as follows:

Fernholz regarded alpha-tocopherol as a substituted 6-hydroxy chromane with a long aliphatic side chain attached to the pyran ring. Lately Smith et al (182) have synthesized alpha-tocopherol and as a result most
workers (182,50) agree on the structural formula proposed by Fernholz.

Vitamin E activity apparently is not confined to a single chemical compound, since three chemical substances have been prepared which possess vitamin E activity, the alpha-, beta- and gamma-tocopherols (51,52,74). Of the tocopherols, alpha- and gamma- apparently possess the greatest activity and beta-tocopherol the least. The approximate minimum effective doses of these compounds when administered to vitamin E-deficient female rats were: alpha-, 1-3 mg.; beta-, 3-5 mg.; and gamma-tocopherol, 1-3 mg. (153).

Bacharach (12) has biologically assayed the allophanates of alpha- and beta-tocopherol prepared from wheat germ oil concentrates. The mean fertility doses were 1.2 mg. for alpha-tocopherol and 1.9 mg. for beta-tocopherol. The values refer to the weights of the tocopherols which were calculated from the weights of their allophanates. Biological assays of various synthetic compounds of vitamin E have been made by Evans et al (73). The mean fertility dose of the alpha-tocopherol obtained by these workers was found to be 3 mg.

C. Physical and Chemical Properties of Vitamin E

Concentrated fractions of wheat germ oil, other vegetable oils and lettuce leaves have been studied extensively to determine the chemical and physical nature of vitamin E. Evans and Burr and co-workers have published (65,68) results in regard to the solubility and stability of vitamin E. From the extracts of wheat germ they obtained a golden yellow
oil which made up about 10 per cent of the germ. The extraction was complete since the oil was as effective as the corresponding amount of wheat germ, because 250 mg. of the latter was as efficacious as 25 mg. of the former as a minimum dose in securing reproduction in female rats. The extracted wheat germ contained practically no vitamin E. The extraction was best with ether; however, petroleum ether, acetone, benzene and absolute alcohol were found to be good solvents also. The solvent was afterward recovered in vacuo on a water bath.

The active material showed very little solubility in water. If lettuce leaves were covered with water for several hours an extract was obtained which protected rats against sterility. However, the extract was not a true solution, but rather an emulsion. The vitamin could be extracted with hot 95 per cent alcohol; this extraction was not complete. Evans and Burr obtained, however, a very active substance by extracting wheat germ oil with alcohol, but the residue also showed a relatively high activity. Likewise these workers obtained an active fraction from hydrogenated cottonseed oil (Crisco). The solubility of the vitamin E-fraction derived from wheat germ oil was much greater in pentane than the sitosterols already present in the oil, and by using this solvent the highest concentrated fraction was obtained. The vitamin was also soluble in absolute methyl alcohol, but in decreased concentration of this alcohol the solubility of the vitamin E-fraction diminished.

The active vitamin E-fraction was highly thermostable, but charring and ashing destroyed its activity. When the concentrated fraction was heated to 170° C. the activity of the substance remained unchanged.
heated in vacuo for two hours at 155° C. the material took on a dark color and a peculiar odor, although the activity remained unchanged. Further heating in vacuo to 220 to 250° C. showed it was still stable although less potent.

The vitamin appeared to be somewhat sensitive to light. The wheat germ oil was spread out in thin layers in a cooled porcelain dish and then exposed to ultraviolet light for 20 minutes. In this case the active substance did not seem to have lost its potency to any great degree. When the oil was exposed in very thin films (two mm.) for about 45 minutes, the oil acquired a peculiar odor without losing its color. Such oil had lost some of its activity. The destruction of the active substance apparently was partial.

The vitamin was not found to be sensitive to mild oxidation such as caused by atmospheric oxygen. Evans and Burr (68) investigated the stability of the active substance in wheat germ oil to aeration. The air was first washed, dried and heated to 97° C. before it came in contact with the oil. After aeration for four hours at 97° C. the oil became dark in color and acquired a rancid taste. Twelve hours aeration affected the oil more markedly. The potency decreased with increased aeration, yet at the close of 12 hours and 20 minutes the oil still had some potency.

Olcott and Mattill (156) confirmed Evans' results that vitamin E concentrates were stable to mild oxidation, benzylation and hydrogenation. They could not, however, confirm Evans and Burr's (68) result in regard to the effect of acetylation. Olcott and Mattill explained the difference as being due to the destruction of the antioxidant material.
by the acetylation, which in time allowed the destruction of the vitamin because of the development of rancidity. Olcott (149) showed that concentrates of vitamin E were rendered inactive by aeration with ozone. These workers also showed that bromination and oxidation with potassium permanganate inactivated vitamin E. They further demonstrated that treatment with perbenzoic acid, potassium amide, potassium ethylate and chlorine destroy vitamin E. However, chlorinated and brominated concentrates could be reactivated by boiling with zinc and hydrochloric acid in methanol. Cottonseed oil was as satisfactory as wheat germ oil for the preparation of active concentrates.

Preparations of active concentrates of wheat germ oil by saponification have been outlined by Evans and Burr (68). The vitamin separated out in the non-saponifiable portion. The active portion was concentrated in a fraction making up only three-tenths of one per cent of the original volume. Olcott and Mattill (154) have used a similar process for the preparation of a vitamin E concentrate from the lipids of lettuce and found the vitamin from this source had the same solubilities as that derived from wheat germ oil.

Vitamin E possesses a high degree of stability towards acid. Evans and Burr mixed portions of wheat germ oil with N/10 normal hydrochloric acid and 20 per cent hydrochloric acid and let the mixture stand at room temperature for 20 and 34 hours. The wheat germ oil was recovered from the emulsion by extraction with alcohol and ether. The oil still had retained its potency. These experiments show that vitamin E in wheat germ oil possesses a relatively high stability toward high temperatures,
ultraviolet rays, atmospheric oxygen, saponification with strong alkali, strong acids and hydrogenation. Other workers have verified Evans' and Burr's and Olcott and Mattill's findings and have prepared vitamin E concentrates from various vegetable oils.

D. Physiological Functions of Vitamin E

After scanning the experimental and well-founded interpretation of results obtained in regard to vitamin E by various workers, it becomes obvious that many questions in regard to the functions of vitamin E and its role in physiological processes remain unanswered. The mode of action of this vitamin in the body still is obscure. It is of interest to note that the male gonads react differently than the female gonads in avitaminosis E. While the former show progressive morphological changes in the germinal epithelium during avitaminosis E, no changes are noted in the latter. Whether the vitamin exerts its influence in the gonad itself or exerts its influence in other places in the organism; whether the effect of avitaminosis E mainly is local or a general one; whether this effect only is related to the genitalia or is related to other organs as well; are questions whose answers remain obscure.

1. Reproductive function

a. Mammals. The most notable abnormality occurring among certain mammals fed a ration lacking in vitamin E is failure of reproduction. Since the sterility, which occurs because of avitaminosis E, apparently is of
different pathogenesis in the two sexes, they will be discussed separately.

i. The male rat. A survey of the literature leaves no doubt that nutritional adequacy is important for the normal function of the testicles. Numerous factors have been mentioned in connection with partial and complete testicular degeneration. Apparently the male gonad is a highly labile organ and the germinal epithelium is highly sensitive to various factors. This high degree of sensitiveness is perhaps due to the great proliferation of cells which takes place here. No doubt, testicular degeneration has been produced experimentally by diets which unknowingly have been deficient in vitamin E. It is not the intention of the writer to cover all phases of these experiments as they would consume more space and time than is justifiable.

Mattill and Stone (140) apparently have caused sterility in the male rat by feeding a ration which seems to have been lacking only in vitamin E. However, at that time they were unaware of the existence of this vitamin and therefore did not investigate the histopathology of the testes. Since these investigations did not cast any light on testicular degeneration, no further comment will be made on this early work.

Mattill, Garman and Clayton (135) were the first workers to have investigated pathological changes in the testes due to avitaminosis E. They called the missing factor X but it was shown later to be the same factor which caused sterility in the female rat. The factor was afterward named vitamin E. Their description of the histological findings was limited mainly to the statement that there was a marked degeneration of the germinal epithelium and an increased proliferation of the interstitial tissue. In
advanced stages of degeneration no spermatozoa were observed in the lumen of the tubules. These workers showed that when five to 10 per cent of wheat embryo or green lettuce leaves were added to the ration no degeneration of the testes occurred.

Evans reported (54) sterility in male rats on vitamin E-free rations for the first time in 1925. Twenty-one-day-old weanling male rats from females on a normal ration were placed on vitamin E-free rations. The animals grew well but became sterile after 90 to 150 days. Evans studied both the animals' libidinousness and the number and motility of the spermatozoa in the vaginal plug or in the vaginal smear. On the basis of these physiological determinations, which were made in connection with morphological studies of the ejaculated spermatozoa, he divided the development of male sterility as a result of avitaminosis E into four stages:

Stage I - was characterized by normal abundance of spermatozoa in the vaginal plug, but the spermatozoa were unable to fertilize the ova. Later there appeared abnormal spermatozoa in parallel clumps. The sex interest was unchanged.

Stage II - manifested itself by absence of spermatozoa in the vaginal plug and a diminishing sex interest.

Stage III - was characterized by loss of ability to form vaginal plug and the animals frequently showed no sex interest.

Stage IV - was marked by loss of all sex interest.

In regard to the histology of the germinal epithelium, Evans reported: "Male sterility is sooner or later characterized by grave changes in the seminiferous epithelium and in advanced stages, complete loss of this epithelium."
Concurrently with Evans' work on male sterility, Mason (123,124) published a more detailed description regarding the cytological changes which took place in the degeneration of the seminiferous tubules. He divided testicular degeneration into five stages entirely on the basis of morphological criteria. In the first stage degenerative changes of the mature spermatozoa were noted, while the other parts of the germinal epithelium remained unchanged. The spermatozoa were the first elements to be affected by vitamin E deficiency, since they apparently were more sensitive because of their high degree of structural differentiation. In the second stage changes in the spermatids were apparent. The third stage was marked by the formation of giant cells from the spermatids. Also the spermatocytes showed signs of degeneration. In the fourth stage the germinal epithelium was markedly reduced. Formation of giant cells had ceased. The spermatocytes showed marked degenerative changes and the spermatogonial cells underwent desquamation and degeneration. The fifth and last stage showed similarity to the cryptorchid testicle, the germinal epithelium had disappeared and only the cells of Sertoli remained. The interstitial tissue appeared more prominent probably because of atrophy of the tubules. There was no hyperplasia of the interstitial tissue as a whole, nor hypertrophy of the single cells. Mason compared this type of testicular degeneration as a result of avitaminosis E to those caused by experimental cryptorchidism, degeneration caused by X-ray treatments, inanition, chronic cachexia or other avitaminoses.

Evans and Burr's memoirs (68) did not add much new information to the morphology in testicular degeneration. They merely expanded their four
stages to six and at the same time reported a histological study of the
testes. The most important details of the six stages follow:

Stage A. The number of spermatозoa and their motility were normal, but
the spermatозoa were unable to fertilize the оvа. The histology of the
testes was normal.

Stage B. The morphology of the spermatозoa was normal but they had be­
come immotile. Their histology was normal.

Stage C. The spermatозoa were shed frequently with small clumps of
cytoplasm of the cells of Sertoli to which the spermatозoa were fused in
parallel fashion. These masses of Sertoli cell debris and spermatозoa
were called cytoplasmic "cuffs".

Stage D. Spermatozoa were absent from the vaginal plug.

Stage E. The male was no longer able to form the vaginal plug and
displayed diminished sex interest.

Stage F. The male had lost all sex interest.

The length of the stages are mentioned. The preliminary fertility period
extended from the end of the second month to three and one-half months of
age. The periods or epochs A-C were still characterized by the presence of
sperms but with sterility intervening up to six and one-half months of age.
During epoch D spermatозoa were not present in the plug up to near the
fourteenth month of life.

Mattill (130) and Evans (55) have shown that lack of vitamin B-complex
did not cause any testicular degeneration when there was adequate vitamin A
in the ration. Mason (126) published new results in regard to testicular
degeneration caused by lack of vitamin A, vitamin-A and E and vitamin E in
the diet. As a result of these investigations Mason came to the conclusion that testicular degeneration due to avitaminosis A is similar and identical in morphology with avitaminosis E and the combined A and E avitaminoses. Vitamin A did not prevent changes caused by lack of vitamin A, and vitamin A and -E were both specific factors indispensable for the preservation of the anatomical integrity of the testes.

Kudrjashov (110) investigated the role of vitamin E in relation to production of testicular hormones. He studied the seminal vesicles and prostate as well as the testes and found that the onset of testicular degeneration began about the 90th to 100th day in rats on vitamin E-free diets; complete degeneration was accomplished after 160 to 200 days. He divided the testicular degeneration into five stages and was entirely in agreement with Mason's (123,124) findings. Kudrjashov noticed structural changes in the cells of Sertoli in the fourth and fifth stage. On the 145th to 160th day the rats showed a retardation in the secondary sex characteristics and from then on a loss in sex interest. On the 200th to 240th day the atrophy of the seminiferous tubules and the prostate was very marked and was identical with the atrophy which occurred in castrated rats with loss of the secondary sex characteristics. When the testis degenerated the germinal cells died. The interstitial tissue remained normal. He concluded, therefore, that the seat of the production of the male sex hormone was not in the interstitial tissue, but that the elements in the seminiferous tubules produced the male sex hormone. There was no mention of which cells in the germinal epithelium were responsible for the hormone production.
Juhasz-Schaffer has published a series of papers on vitamin E, among which there also is a histological study (93) of the testicular degeneration caused by avitaminosis E. The initiative to these investigations was partly due to divergences existing between certain European workers and the previously mentioned American investigators' results. On the basis of his investigations Juhasz-Schaffer could, on the whole, verify the American results in regard to the development of sterility in male rats. However, he criticised Mason's morphological description and did not agree as to the division of the development of male sterility into five stages. He suggested a new division of the development of sterility caused by avitaminosis E based on the appearance, motility and number of spermatozoa in the epididymis. As an expression for the normal function of the spermatozoa, he assigned positive rheotaxis and motility in 38°C warm Ringer's solution. On the basis of his observations and using this technique he divided the sterility into three stages.

Stage I, corresponded to the first six to 12 weeks of the experiment. The spermatozoa were morphologically normal with marked positive rheotaxis which later decreased. The spermatozoa were able to fertilize normal females; the litters, however, were small.

Stage II, the spermatozoa were morphologically normal, but reduced in numbers. The spermatozoa which had retained some motility had lost positive rheotaxis and the male soon became infertile. This stage ended in the 15th to 20th week.

Stage III. After 15 to 20 weeks on vitamin E-free rations short spermatozoa with two or more tails appeared. Formation of spermatozoa in
dwarf and giant forms was noted. The giant form was produced by the fusion of several spermatozoa. Furthermore, nuclei of spermatids and cell debris (from spermatocytes) which adhered to the heads of the spermatozoa were seen. These pathological forms disappeared after a short period and the tubules of the epididymis were entirely empty and the animals were completely sterile.

Juhasz-Schäffer's observations of the morphological changes in the testes deviate in several ways from earlier results obtained by other workers. As initial changes he described the appearance of irregular, radial clefts running from the lumen in the seminiferous tubules towards the basal membrane. The epithelium was otherwise of normal configuration. The formed spermatozoa fell into these clefts and formed peculiar star-shaped configurations. The introduction of spermatozoa into these clefts caused a decrease of spermatozoa in the lumen. The intercellular junction of the spermatids loosened and the intercellular spaces in the layers of the spermatocytes and spermatogonial cells increased in size, the cells became displaced among one another and were dislodged from the basal membrane. Subsequently, the appearance of the previously mentioned pathological forms of dwarf and giant cells and spermatozoa with two or more tails took place. The germinal epithelium did not disappear entirely even after eight to nine months on vitamin E-free diets. A few spermatogonial cells were left together with an entirely normal Sertoli syncytium. The interstitial tissue did not undergo any changes. As will be noted, there are divergences of opinion between Mason and Kudrjashev on one side and Juhasz-Schäffer on the other, in regard to the cytological
changes in detail, but in general there is agreement about the testicular
degeneration due to "vitaminosis $E$.

Korenchevsky (108) gave a short description of his histological find-
ings in the testes during $A$ and $E$ avitaminoses. His descriptions were in
good agreement with Mason's (123, 124, 126) findings on testicular degenera-
tion, and the morphological picture was alike in the two avitaminoses.
Mason, however, subsequently brought forth new results (127) on the same
problem. He pointed out that though testicular degenerations due to lack
of vitamin $A$ and $E$ or other factors in general morphologically might be
alike, the degeneration caused by avitaminosis of $A$ could be repaired ad
integrum. Degeneration of the seminiferous tubules due to lack of vitamin
$E$ seldom, if ever, could be repaired. These observations mark the out-
standing differences between degeneration in the testes caused by lack of
vitamin $E$ and degeneration caused by lack of other factors. Mason ex-
plained that the divergence between his earlier and later results was due
to insufficiency of vitamin $E$ in the sources used in his first experiments,
and that the vitamin $A$-free animals also were vitamin $E$-free to a certain
extent.

More recently Ringsted (165) sought to determine whether specific
morphological changes could be demonstrated in degeneration of the testes
accompanying vitamin $E$-deficiency. His work dealt with a detailed study
of the histology and cytology of the testes in avitaminosis $E$ as compared
with the normal state. He placed groups of animals receiving diets de-
ficient in vitamin $E$ for 15, 30, 45, 60, 120, 180, 270 and 460 days in
order to provide all phases of degeneration. The first degenerative
changes were produced more rapidly (within 30 days) than had previously been the case in former experiments. This was attributed to a low but sufficient content of vitamin E in the breeding animals in conjunction with a vitamin E-free basal diet, so that the young were born with small stores of vitamin E.

As a result of systematic study, Ringsted concluded that the degeneration did not occur in layers, but attacked several types of epithelia simultaneously with an intensity decreasing irregularly from the lumen to the basal membrane. No changes were seen in the vascular walls or lumen that could explain the variation in localization or degree. The specific pathological changes which occurred in the germinal epithelium in vitamin E-deficiency were: 1) the formation of the so-called "cytoplasm bladders", 2) dilated spermatozoa with large masses of residual clumps of cytoplasm and 3) the late occurring phenomenon of marked atrophy of the interstitial tissue. The "cytoplasm bladders" were considered to be formed from the spermatids while the latter were subjected to cytometamorphosis, since the former in rare cases were found to contain tails of developing spermatozoa. The dilation of the "cytoplasm bladders" was attributed to abnormal physico-chemical changes and their occurrence was ascribed to the failure of the Sertoli cells to resorb the cytoplasmic cuffs which surrounded the tails of the spermatids. The dilation of the spermatozoa was considered to be due to abnormal physico-chemical changes in the acrosomes and the large masses of cytoplasm, which were found to be affixed to the spermatozoa, arose from agglutination of the spermatozoa to the "cytoplasm bladders". It was acknowledged that certain degenerative phenomena
which occurred in vitamin E-deficiency were not specific for vitamin E-
disease (c.f. (127)). However, the histogenesis of the polymuclear giant
cells was thought to be the result of fusion of degenerated cells rather
than to mitosis accompanying plasmotomy. It was also noted that the damage
done to the tissues in avitaminosis E was much more intense than with other
nutritional deficiencies, and that the testes showed very little ability to
regenerate (c.f. (127)).

Ringsted recognized four stages in the degeneration of the testes,
epithelial changes being dominant in the first three and complete atrophy
of the germinal epithelium and of the interstitial tissue in the fourth
stage. The atrophy of the interstitial tissue was followed by edema.

ii. The female rat. In the female rat the sterility accompanying
vitamin E deficiency is characterized by failures in the placental func-
tions. The vaginal cycle, ovulation, fertilization of the ova, and im-
plantation of the ova are apparently normal and synchronous as in the con-
trols. The feti, however, are retarded in development, death of the em-
byros ensues, and the products of conception are resorbed. Ovulation re-
curs a few days after resorption and the normal estrus cycle is resumed.
Administration of vitamin E prior to, or within five days after coitus
will allow normal development of the feti. The stores of vitamin E in the
body tissues must be depleted before the symptoms of vitamin E-deficiency
will be manifested. An excess quantity of the vitamin above that which
is required for fertility apparently does not increase fecundity.

The intrauterine changes occurring in sterility during avitaminosis
E have been discussed by Evans and Burr (68). The first change which took
place during gestation occurred on the eighth day with a retarded growth of the ectodermal cavity. The following day this retarded development was more marked; especially the differentiation of the mesoderm seemed to be delayed. On the 10th day there was marked hypoplasia of the mesenchyme in the allantois and in caput and at the same time hypoplasia of the hematopietic tissue. The following day (11th to 12th day) was characterized by a universal hypoplasia of the feti with precedence in allantois and the hematopietic tissue, especially of the liver and yolk sac. On account of the latter, very few erythroblasts were seen in the heart and blood vessels, and on the 13th day the feti died because of asphyxia. Outwardly this was manifested in the gestation curve, as there was diminution in weight of the animal compared to that of the control. After the 15th day resorption of the feti took place and as a result, at the end of the 20th day, there remained no traces of the feti.

Paralleling the death of the feti, changes in the placenta took place. On the eighth to ninth day dilations of the blood capillaries in the uterine mucosa were noted in the mesometrial part of the uterine horn and often extravasated blood was found in the uterine cavity. The following day dilation of the maternal blood vessels and hypoplasia of the trophoblast and placenta in general were still marked. On the 13th day the most characteristic phenomenon was a hypoplasia of the allantois and a degeneration of the entodermal villi and the appearance of syncytial proliferations from the trophoblast. These cell proliferations later became giant cells. The following day, after the death of the feti, the maternal placenta continued to grow until the 16th day and remained unchanged until the 20th
day, when coagulation and necrosis began. Then the whole placenta was re-
sorbed, manifesting itself outwardly in diminution of the resorption ges-
tation curve in the first days of the puerperium. As to the cause of the
death of the feti, Evans and Burr assumed that the embryo starved and died
from asphyxia because of the retarded development of the yolk sac and lack
of nutrition from the umbilical veins. The weight curve in the resorption
gestation showed some unusual characteristics. After the death of the
feti on the 13th day, one should expect a decline in weight. The animal,
however, continued to gain until the 18th day. Evans and Burr (68, p.20)
in regard to this phenomenon said: "we assume that the continued gain in
weight of the mothers after fetal death is due in some obscure way to the
influence of the placenta as an endocrine organ on the metabolism of the
mother." Urner (195) called attention to this fact and did not think that
the gain in weight was due to the placenta alone, but that the increased
growth of the mammae played a part. Between the 16th and 18th day, the
placenta was resorbed and the mammary tissue underwent involution. Be-
cause of this there was a decrease in weight. From the 18th day there
was marked involution of the mammae and decided loss in weight.

Urner (195) has repeated Evans and Burr's work on the intrauterine
changes occurring in rats on vitamin E-free diets. He noted no hypo-
plasia of the feti before the 10th day, contrary to Evans and Burr's
seventh to ninth day. Otherwise Urner was entirely in agreement with the
aforementioned investigators. Juhasz-Schaffer (98, 101) also has re-
peated and substantiated Evans and Burr's results.

It will be noted that the sterility in avitaminosis E is quite
different in the two sexes. In the female the sterility is of placental origin and the germ cells do not seem to be directly affected. The sterility apparently is of a temporary nature and is readily curable by subsequent treatment with a good source of vitamin E. In males, on the other hand, lack of vitamin E leads to degeneration of the germinal epithelium in the seminiferous tubules and to degeneration of the interstitial tissue in the gonads. The animal finally loses all sex interest. Subsequent feeding of vitamin E-rich rations fails to repair the damage done to this specific organ.

iii. Other species of mammals. Only a limited amount of experimental work concerning the necessity of vitamin E in the diet for mammals other than the rat has been attempted.

Beard (19) has emphasized the necessity of vitamin E for mice and Juhasz-Schaffer (93) demonstrated testicular changes in the same species induced experimentally by avitaminosis E, although he did not express himself with certainty as to whether vitamin E was needed by this animal because only four mice were used.

b. Fowl. A study of the vitamin E requirements of poultry has been carried out by Card (29) and his associates at the Illinois Agricultural Experiment Station (30,31,32,33). Eight-weeks-old pullets were placed on a ration which had been treated with an ethereal solution of ferric chloride to destroy vitamin E. At the age of one year they were mated to normal males. Though a large percentage of the eggs were fertile when incubated, none of the embryos developed beyond the sixth day. Wheat germ oil was then added to the ration at the rate of one-half cc per bird daily.
Approximately 30 per cent of the eggs laid the following week hatched, and the hatchability of the eggs increased up to the fourth week on wheat germ oil, when nearly 70 per cent of the eggs hatched. As soon as the wheat germ oil was omitted from the ration, the hatchability of the eggs decreased, dropping to 3.4 per cent within two weeks after the wheat germ oil feeding was stopped. These experiments indicate definitely that poultry need vitamin E for successful reproduction.

Adamstone (1) made a histological study of the embryos which failed to develop in the eggs from the hens on the vitamin E-deficient ration from Card and associates' experiment. He showed that differentiation of tissue and growth of the embryo were slower than normal the first 24 hours, but rarely gave cause for malformation. The causes of the death of the embryos were several. Some died the first two days due to lack of development of the circulatory system. Only a few embryos lived after the fourth day, at which period a mesodermal cell proliferation, lethal ring, had developed and surrounded the embryos and caused asphyxia. In other embryos he found hemorrhages at different places. The hemorrhages apparently were caused by specific histiocytic mesenchyme cells with phagocytical function. The bleedings could also have caused the death of the embryo, partly because of the loss of blood and partly because of the marked disturbances of the metabolism which followed the loss of blood.

Barnum (16) showed a relation existed between the vitamin E-content of the ration and the vitamin E-content of the eggs. He substantiated Card and coworkers' findings. He also showed that eggs which had a low hatchability had a low vitamin E content. The addition of wheat germ
oil to vitamin E-free diets increased the vitamin E-content of the eggs to normal, while the addition of lettuce leaves did not. However, in view of the fact that only one lettuce head was fed to seven hens weekly as curative substance, this negative result can hardly be looked upon as being important. More recently Adamstone (3) has shown that chicks on vitamin E-free rations developed pathological changes in the cerebellum which resulted in ataxia and muscular disturbances. Concurrently with such disorders a degeneration of certain visceral tissues was noted followed by a marked proliferation of cells which appeared to be similar to certain types of malignant cell growth.

Adamstone and Card (4) demonstrated that a slowly developing testicular degeneration took place in the male fowl on vitamin E-free diets. They did not state whether such degeneration could be prevented or cured by feeding vitamin E-rich substances.

Ender (53) has substantiated Card's (29) and associates' (30,31,33) and Barnum's (16) findings. Eggs from hens, on a vitamin E-deficient ration, which contained the vitamins A, B and D, had a low fertility and hatchability.*

2. Sterility and wheat germ oil {vitamin E) therapy

No experimental work is available which demonstrates that deficiency of vitamin E in the ration or in the body is a limiting factor correlated

*For a recent review of data on the vitamin E requirement of poultry, see:

with breeding failures among farm animals. However, empirical therapy with wheat germ oil (vitamin E) has been reported to cure certain types of sterility in domesticated animals and man.

Aubel, Hughes and Lienhardt (11) reported that the addition of wheat germ to the ration of gilts resulted in the weaning of a greater number of pigs. The authors recognized, however, that the results might have been due to the increased vitamin E in the ration, as well as vitamin B. Also, the ration probably furnished adequate amounts of vitamin E without the addition of wheat germ meal. Dryerre (48) called attention to the fact that non-infectious abortion in sheep occurred to the extent of about 20 per cent in many parts of Scotland. He assumed that this condition was due to nutritional deficiencies. By administering a vitamin-A, -D and -E concentrate to these sheep a reduction in the abortion to about three per cent was noted. There was no information regarding the form and in what proportion the three vitamins were mixed and given.

Also, it has been reported that among cattle and swine good results with vitamin E therapy have been obtained in curing sterility. Vogt-Møller and Bay (202, 203) reported good results following administration of wheat germ oil to cows that suffered from non-infectious abortion. By intramuscular injection of 20 cc of wheat germ oil sterility was cured in 80 per cent of the cases observed.

Bay and Vogt-Møller (18) published results from continued studies on treatment of sterility in cows and breeding sows with wheat germ oil. Seventy apparently healthy but sterile cows, including those from a former experiment, in spite of repeated services, did not become pregnant. After
treatment in the manner as mentioned in the foregoing, pregnancy resulted
in 70 per cent of the cases. A similarly effective treatment with wheat
germ oil was noted in sterile sows. Tutt (194) recorded 25 cases of
sterility in cattle in which wheat germ oil was administered. Pregnancy
followed in 17 of the cases. The wheat germ oil was injected into the
gluteal muscles and the injection repeated in eight-day intervals. In
most cases three doses were given. The author limited the sterility
cases in which wheat germ oil treatment could be expected to give results
to those in which there was no clinical evidence of disease in the ova-
ries, Fallopian tubes, uterus or vagina. He thought the role of the
wheat germ oil was to preserve pregnancy in its earliest stages.

Jones and Ewalt (96) reported that injections of wheat germ oil in
amounts of 10 to 20 cc appeared to have a favorable influence in the case
of 15, indefinite results in the case of 23, and negative results with
five cows. These workers were of the opinion that more data were re-
quired before definite conclusions could be drawn on the value of vitamin
E injections for the completion of pregnancy in dairy cows.

A curative effect of wheat germ oil in women, who suffered from non-
lustic, habitual abortion, has been reported with good results. Vogt-
Möller (200) has treated idiopathic abortion in women with wheat germ oil.
The results were favorable. A greater number of similar cases later were
treated with similar good results. Twenty cases of habitual abortion
(each case two to six former abortions) were treated with wheat germ or
wheat germ oil during pregnancy, and in 17 cases the results were favorable
and 17 live children were born. Juhasz-Schaffer (102) has partly
substantiated Vogt-Moller's observations on the favorable influence of vitamin B preparations when administered during pregnancy to patients who suffer from habitual abortion. He has also extended his experiences by treating impotency in men and hypogalactosis in women and with apparently favorable results. The two latter claims, however, have not been verified. Vogt-Moller (201) has continued his studies on treatment of habitual abortion and recently has reported on 52 new cases treated with "Fertilan", a vitamin B preparation from wheat germ oil. In 38 of these cases pregnancy was successfully carried to term, or in about 75 per cent of the cases.

Other workers who have reported equally favorable results on wheat germ oil therapy are Watson (207), Watson and Tew (208), Currie (39) and Simse (174,175,176).

3. Growth function

The sensitivity of the germinal epithelium in the testes, the tissue of the developing embryo, and the newly born young to lack of vitamin B exhibit marked similarity. It is, therefore, an attractive supposition that vitamin B plays an important role and is needed by certain specific tissues in early growth where cell proliferation is rapid. Juhasz-Schaffer (99) has shown through tissue culture experiments that vitamin B stimulated growing fibroblasts to increased rate of growth. As negative control he used a culture to which there was added an autoclaved wheat germ oil. According to Juhasz-Schaffer this "vitamin B-free" culture possessed no growth stimulating influence per se since a control culture
without autoclaved wheat germ oil grew as well. In the tissue culture with active wheat germ oil, the growth increase in the first 24 hours was 48 per cent, the next day 120 per cent, and in the third day 150 per cent greater than in the controls. He concluded that vitamin E was the cause of this growth stimulus. Without judging the tissue culture experiment, one may pause to interpret one point of his results. Juhasz-Schäffer reasoned that vitamin E had been destroyed by autoclaving. That is hardly justifiable, as Evans and Burr (68) and Olcott (149) have shown that vitamin E in wheat germ oil can withstand distillation in vacuo at 250°C., heating for two hours at 170°C. under aeration, and autoclaving for two hours under 200 atmospheres pressure. Juhasz-Schäffer admitted, however, that besides vitamin E probably other factors were present in the wheat germ oil, especially vitamin E-complex. The latter, though, is hardly credible, since the wheat germ oil was obtained from the germ by extraction with ether.

Adamstone (2) has reported that the possible need of vitamin E by the cell was intimately associated with the nucleus of the cell during cell division and probably exerted an indirect controlling influence over it. Since the male germ cells exhibit the need for vitamin E and the female germ cells do not, and the former are in continuous mitosis, this difference might be looked upon as due solely to the linkage of the rate of tissue growth with the need of the developing fetus and the testes. However, Evans (58) has pointed out that the male germ cells were hardly produced at rates exceeding that of red blood cell formation, and noted no disturbance in hematopoiesis, when vitamin E was
absent in the diet of weanling rats. Furthermore, normal growth of the entire body apparently takes place in weanling rats restricted to vitamin 
E-free diets.

However, Evans (56) noticed that after being on experimental diet for seven to eight months his positive control animals grew better than his vitamin E-free animals. The administration of wheat germ oil to male rats, which had been on an avitaminosis E ration for eight months, caused a marked stimulation in growth. Evans showed that this increased growth and vigor were independent of the sex and also independent of endocrine growth stimuli from the gonads, since the same difference in growth appeared in castrated male rats on a vitamin E-free diet and on a vitamin E-free diet plus wheat germ oil. Evans pointed out that this failure of gain by the former appeared in animals that had been on vitamin E-free diets seven to eight months and at a time when there was very little growth. Vitamin E accordingly would appear not to have any influence on early growth but rather on late growth. Mason (125) showed that rats restricted to vitamin E-free rations were protected against testicular degeneration when they received a daily addition of five to 40 grams fresh lettuce or 1.6 gram dried lettuce. These animals grew more rapidly and became larger than the vitamin E-free animals without addition of lettuce. Mason's results might be interpreted to show that this early difference in gain could not be due to vitamin E but possibly to other factors present in lettuce. Later, Roscoe (167) showed that lettuce was rich in the growth factors of the vitamin B-complex. Blumberg (21) reported that vitamin E was necessary, not only for reproduction and later
growth stimulation, but also for normal early and middle growth and for maintenance of well-being, or that wheat germ oil carrying vitamin E contained an unrecognized fat-soluble growth factor. Emerson and Evans (49) and Martin (122), using the Blumberg (21) diet, showed that wheat germ oil contained, besides an antisterility effect, also a growth stimulating influence. Emerson and Evans (49) found that the Blumberg diet (21) gave typical resorption gestations in female rats. Growth and weight gains occurred at a steady rate for about 120 days on the same diet (21). The animals responded to the addition of wheat germ oil after the plateaued weight had been reached. The growth stimulating factor appeared to be in the non-saponifiable fraction of the oil, rather than in the fatty acid fraction. They concluded that defective growth in experiments involving vitamin E deficiency occurs only after the fourth month of life. Olcott and Mattill (155) concurrently arrived at the same result as Emerson and Evans (49) by using a "purified" vitamin E concentrate. They showed that addition of ethyl linolate or a cod liver oil concentrate did not improve the growth rate, but lard produced an acceleration in both males and females deprived of vitamin E. Apparently wheat germ oil stimulates growth after a plateaued weight has been reached on a vitamin E-low diet.

4. Endocrine functions

Several investigators, especially Verzar and coworkers (196) and Szarka (191), have suggested a relationship between vitamin E and the gonadotropic hormones of the anterior pituitary. Verzar (196) claimed that wheat germ oil concentrates containing vitamin E, like the
gonadotropic hormones, caused a hypertrophy of the uterus in infantile rats. The hair of males fed the vitamin E-deficient diet became soft, which was also the case with hypophysectomized rats. He concluded that lack of vitamin E disturbed the production of the hormone.

Barrie (17) found that when female rats were given only just enough vitamin E to permit reproduction, their young were stunted in growth and showed hypoplastic thyroids and soft skulls such as are seen in hypophysectomized animals. Degenerative changes were found in the pituitary glands of such young animals as well as in the pituitaries of adult rats maintained for long periods on diets deficient in vitamin E. From these findings he suggested that vitamin E was essential for the normal functioning of the pituitary gland, since failures of the normal reproductive process, of lactation and of the activity of the thyroid gland would indicate that secretion of the gonadotropic, galactotropic and thyrotropic hormones by the anterior lobe of the pituitary was impaired.

Olcott and Mattill (156), on the other hand, have run parallel tests with hebin (a gonad stimulating factor found in the anterior lobe of the pituitary and in urine of pregnant women) and a vitamin E concentrate (minimum dose eight to 10 mg.). The concentrates were injected into infantile female rats. The uteri of the hebin-treated animals were hypertrophied, while those of the control and vitamin E-injected animals were normal. One ovary of the control, one of a rat given vitamin E, and one of a hebin-treated animal were sectioned and examined. Only the ovary of the rat given hebin showed maturing Graafian follicles. Another series of 10 females were treated similarly and the time of opening of the vagina
and estrus was determined. The hebin-treated animals showed opening of the vagina in three to four days and were in estrus, while the vitamin E-treated and control animals required seven to 11 days before opening of the vagina occurred and remained in anestrus. Coupled with the knowledge that a lack of vitamin E in no way disturbs the ordinary sex cycle in the rat (68) such experiments forced Olcott and Mattill to indicate that vitamin E and the gonadotropic hormone of the pituitary play entirely different roles in the physiology of reproduction in the female. Recently Diakov and Krizenecky (42, 43) also reached the same conclusion.

Stein (134) and Müller and Müller (144) found no demonstrable changes occurring in the pituitaries of adult female rats kept for several months on a diet deficient in vitamin E, though such changes were seen in male rats. Similarly, no significant changes occurred in the differential blood count. The weights of the hypophyses or lobe volumes showed no changes as compared with controls. Saphir (169) was unable to demonstrate any estrogenic, gonadotropic or luteinizing effects by commercially prepared and biologically assayed wheat germ oil, when tested on immature female or normal and castrated adult female rats.

Singer (180) has investigated the thyroids of rats kept on similar deficient diets and found that they were consistently more hypoplastic than those of controls. The results suggested that the thyroid changes might be secondary to changes in the anterior pituitary caused by avitaminosis E. The usual appearance of the thyroid was restored by vitamin E extracts or wheat germ oil and partially by alkaline anterior pituitary extracts. Iodine administration for four or 10 days or even four weeks did not affect its.
histological appearance, nor did seven days of pregnancy. Barrie (17) noted hypoplasia of the thyroid in the litters born to rats partially deprived of vitamin E along with degenerative changes of the pituitary. These results suggest that modifications in the anterior pituitary due to avitaminosis E affect the histological appearance of the thyroid. However, since evidence is at hand as cited in the foregoing (184) that the pituitary histologically remains entirely normal in vitamin E-deficiency, it is most likely that the thyrotropic influence of this gland could not have been impaired. Besides, there undoubtedly is considerable variation in the thyroid of normal rats, suggestive of a cyclic secretion. Compared with the thyroid of the guinea pig, for example, that of the rat is apparently relatively hyperplastic. (Junkman and Schoeller (103)).

In 1934 Collip (37) formulated a general rule to the effect that the responsiveness of an animal to administration of glandular extracts may vary inversely with the hormone content or production of the animal’s gland, which may explain the hyperplasticity (Junkman and Schoeller, 103) of the normal rat’s thyroid. The pituitary of the rat is rich in thyrotropic substance so that its thyroid is active and its metabolic rate is accordingly high. Moreover, the rat is relatively insensitive to thyrotropic hormone injections, while its sensitivity is increased at least 10 times after hypophysectomy (37). It is conceivable that vitamin E may have a direct effect upon the secretory processes of the thyroid independently of the pituitary. However, a recent report by Telford, Emerson and Evans (192) does not substantiate such an assumption as these workers were unable to detect any histological abnormality of the thyroid
in the paralyzed suckling young rats of mothers deficient in vitamin E or in animals aged 22 months chronically deprived of vitamin E.

5. Other functions

Although avitaminosis E manifests itself primarily in sterility other anomalies in body functions have been accorded to it. These include paralysis, muscular dystrophy, iron assimilation, hemoglobin formation and tumor growth.

a. Relation to paralysis, nutritional muscular dystrophy and blood formation. A specific nutritional disease in the young of female rats on vitamin E-deficient diets was first described by Evans and Burr (71). On or about the 20th day of life the young animals developed a paralysis, especially of the hind quarters. Many of the afflicted rats died, some recovered completely, and a few lived but remained partially paralyzed through life. Mason (127) and Olcott and Mattill (156) confirmed these results, and tried to cure the paralysis with potent sources of vitamin E concentrate, but with negative results. Apparently vitamin E was needed by suckling young rats to assure proper development of the nervous system. Mason (127) emphasized the analogy between the enormous development of the central nervous system during the lactation period and the excessive cell proliferation which takes place in the testes and the developing fetus and pointed out that vitamin E possibly played an important role in the synthesis of the chromatin or in the preservation of the normal physico-chemical condition of the nuclear chromatin. These neurological disturbances in young rats from mothers on vitamin E-free diets are probably similar to those
observed by Ringsted (164) and Burr, Brown and Mosely (28) in adult rats. These workers noted that a distinct, slowly developing paralysis of the hind limbs appeared in adult rats, which were fed a diet complete except for the presence of vitamin E. Extra supplements of vitamin A, D and B-complex had no curative effect, nor was the condition improved by administering potent sources of vitamin E. Ringsted concluded that tissue changes characteristic of avitaminosis E are irreversible, and that when once started they cannot be stopped with curative methods, but continue unyielding through degeneration and necrosis. As reason for this view he cited the morphological and physico-chemical changes which take place in the fetal tissue in vitamin E-free fetus in the very early stage, sixth to eighth day, or the changes in the germinal epithelium in vitamin E-free testes, which, once in progress cannot be stopped by vitamin E therapy.

Goettsch (83) has noticed changes in the musculature on the femur and abdomen of guinea pigs and rabbits after feeding a ration treated with an ethereal solution of ferric chloride. The animals grew for one to two months, maintained constant weight for another month and rapidly declined in weight for two to three days and died. Concurrently with the stagnation in weight muscular atony and paralysis were noticed in the animals. On autopsy the voluntary muscles, particularly of the thigh and abdomen, were atrophied and pale and had lost irritability. The histological study which was turned over to Pappenheimer (160) disclosed a wax-like and hyaline

*For a recent summary on the study of this subject see:

necrosis of the myofibrils and marked proliferation of the nuclei of the muscle cells. The necrotic tissue became substituted and marked by fibroma and lipomatosis. The other organs apparently were not affected, the myocardium, the peripleural and central nervous system inclusive showed no pathological changes. Whether the muscular dystrophy producing diet was due to avitaminosis B or due to toxicity of the ferric chloride they did not commit themselves. More recently these same workers (161) have produced the same symptoms and lesions as just mentioned in adult rabbits, as well as in the offspring of a dystrophic animal on the same ration without treatment with ferric chloride. The ration, however, contained eight percent lard and one percent cod liver oil.

Nutritional muscular dystrophy in guinea pigs and rabbits was described later by Goettsch and Pappenheimer (84) as due to the toxic effect of cod liver oil. Woodward and McCay (210), Madson, McCay and Maynard (121) and Madsen (120) reported that lesions of the muscles appeared in herbivora fed synthetic diets. Olcott (152) observed that the paralysis in second generation young rats, in a very specific age group on a vitamin E-free diet resembled that observed by Rogers, Pappenheimer and Goettsch (166) and Victor (198) in guinea pigs and rabbits in that the lesions were primarily muscular and did not involve the nervous system. The histopathological condition of the muscles were also strikingly similar. Goettsch and Pappenheimer (84) showed that vitamin E concentrates alone did not prevent and could not cure the so-called nutritional muscular dystrophy in guinea pigs. Nevertheless, all of the effective foodstuffs which they investigated contained vitamin E. Morgulis et al (141,142)
reported that two factors were concerned in nutritional muscular dystrophy. One of these was supplied by wheat germ oil while the other was present in lettuce or in green alfalfa. Olcott (152), as well as Morgulis et al. (141, 142), assumed that the analogous pathological picture seen in rats might be caused by one of these factors possibly vitamin E. Olcott concluded that if this hypothesis were correct, it would furnish a possible explanation for results obtained by Madsen (120), who showed that the inclusion of cod liver oil in diets fed to herbivora markedly increased the incidence and severity of the disease. It has been postulated that the oxidative reactions, initiated by the aut-oxidation of cod liver oil, are destructive to vitamin E (38) and the diets containing cod liver oil possibly had much less of this vitamin than those from which cod liver oil was omitted.

Since the death of the embryo resulting from vitamin E deficiency was associated with failures of the circulatory system, several of the early investigators advanced the theory that vitamin E played a role in blood formation. Simmonds (177) and Simmonds, Becker and McCollum (178) linked vitamin E with iron assimilation. Hart, Steenbock, Elvehjem and Waddell (86) thought that vitamin E might be related to the non-iron complex in hemoglobin formation. However, Anderson (8) found no abnormalities in the chemical composition of the blood of rats suffering from vitamin E deficiency.

Hogan and Harshaw (91) found no evidence that the sterility due to vitamin E deficiency was caused by anemia, or that vitamin E was related to the formation of hemoglobin. In a later publication, Simmonds, Becker and McCollum (179) offered a different interpretation of their earlier
results, and concluded that there was no evidence that vitamin E had any role in iron assimilation. The opinion of most present-day investigators, including Evans (58), is that vitamin E is not concerned in the formation of hemoglobin.

b. Relation to tumor growth. Both hypovitaminosis E and hypervitaminosis E have been reported to be associated with the proliferation of certain types of cells. Evans (57) showed that spontaneous tumorous growths were formed in the uteri of about 60 per cent of the vitamin E deficient females when mated to vasectomized males, while only about four per cent of the females fed a normal, varied diet were so affected. Adamstone (2) noted a replacement of the normal tissue by a different type of cell growth in the development of chick embryos in the eggs of hens on a ferric chloride treated ration. He suggested that vitamin E is intimately associated with and probably exerts an indirect controlling influence over the cell nucleus during cell division. Recently (3) he reported on a brain disorder and malignant growth which occurred in the livers, hearts, and other organs of young chicks fed a similar ration.

Rowntree et al (168) reported that an excess of vitamin E in the form of crude wheat germ oil caused abdominal tumors in rats. The neoplasm produced by the ingestion of the oil, they termed malignant in nature and microscopically resembled sarcoma. It was readily transplantable and retained its malignancy through six successive transplantations. Subsequent work by Forrance et al (45) verified Rowntree's observations. By feeding increasing amounts of crude wheat germ oil the time required for the development of tumors was decreased. The tumors produced in 34 rats (100 per cent)
were successfully transplanted, both subcutaneously and intraperitoneally, into 260 rats through 15 successive tumor generations. The tumors were all defined as spindle-cell sarcoma. In rats bearing tumor implants no appreciable effect was observed in the implant itself following X-ray treatment, but in each secondary sarcoma nodules occurred. Sarcoma tumors were produced by intraperitoneal injections of saline extractions of heat-killed "fed" tumors.

Haddow and Russell (85) could not find that addition of wheat germ oil to the diet of mice had any influence on the number of tumors produced in mice by 3,4-benzopyrene. They did not state whether they used the same preparation of oil as was used by Rowntree et al. (163) or Dorrance et al. (45).

E. Occurrence of Vitamin E

1. Animals

Vitamin E occurs in a wide variety of natural foodstuffs. The vitamin is found both in the animal and plant kingdom. Evans and Burr (66, 67, 68) found that vitamin E was present, but never in very highly concentrated amounts, in a great variety of animal tissues such as musculature, subcutaneous fat and viscera. The latter included the pancreas, spleen, liver and heart, also the hypophysis and the placenta were found to contain vitamin E. However, the viscera were not as potent in this factor as the tissues. Its presence in milk fat and egg yolk was demonstrated also. They demonstrated further that vitamin E was present in the tissues of
rats reared upon natural foods but absent in those reared upon vitamin B-
deficient rations. Proof of the presence of vitamin B was given by the
direct detection of the vitamin in certain tissues of healthy animals by
feeding such tissues and thus curing females of proved sterility. The feeding
of similar tissues from animals reared without vitamin B would never in-
voke such cures. That storage occurred in the bodies of rats was further
indicated by the survival of fertility in females given appreciable amounts
of vitamin B and then shifted to a vitamin B-free regime; the length of
survival of fertility was strikingly correlated with the richness of vita-
min B in the initial regime. Animals reared on natural food diets, which
possessed entirely adequate though not abnormally high amounts of vitamin
B, could thus suffer the complete cessation of vitamin B-intake and yet
maintain their fertility for one or more litters. Vitamin B apparently
was used up in normal metabolic processes. Juhasz-Schaffer (100) demon-
strated that high doses of vitamin B administered per os to vitamin B-
depleted rats led to its presence in the feces. Evans (68) found that
when animals were not submitted to the drain of repeated pregnancies
their store of vitamin B was not conserved thereby, since it did not
persist longer than it did in their frequently bred sisters.

Clayton and Cummings (36) have reported on the vitamin B-potency
of dried meats. They found that the relative potency of the meats which
they investigated was in the following order: beef round, liver and kid-
ney. Their findings are in agreement with Evans' and Burr's earlier re-
results. Anderegg and Nelson (7) and Waddell, Steenbock and Hart (206)
showed that cow's whole milk contained vitamin B.
Cod liver oil is recognized as a poor source of vitamin E. Evans and Burr (68) and Sure (190) stated that cod liver oil was practically devoid of vitamin E, but in certain brands some vitamin E may occasionally be present as reported by the work of Nelson, Orbeck, Jones and Taylor (143), and of Simmonds, Becker and McCollum (179).

Hill and Burdett (89) and Burdett (27) reported that the royal jelly given the bee larvae, which develops into the queen bee, was richer in vitamin E than that given the worker bee, thus implying that bees add vitamin E to royal jelly, on which the queen larvae are reared, and withhold it from food of worker larvae. Schoorl (171) and Mason and Melampy (128) were unable to verify Hill and Burdett's findings. Evans et al (72) refuted the work of Burdett and Hill. Royal jelly, its fat-soluble fraction and pollen from the comb was fed at different levels to young female rats of proved sterility. No vitamin E activity was manifested in these substances, since all the rats resorbed their young. The quantities of royal jelly fed were far greater than previously reported by other workers.

2. Plants

In the plant kingdom seeds and green leaves are the best sources of vitamin E. Wheat germ and lettuce were early recognized by Evans and Burr (66, 67) as especially good sources of this vitamin, and ether extracts of these two substances are very potent. Sure (188, 189) reported that wheat germ, hemp seed, and yellow corn oil were good sources of vitamin E, that cottonseed-, olive-, soybean-, peanut oil, and oil of peach kernels were fair sources, but there was practically no vitamin E
in linseed oil, coconut oil and sesame oil. Evans (58) stated that vitamin E occurred in most, but not all, vegetable oils.

Most farm feeds contain vitamin E. Alfalfa was one of the substances mentioned in the early work by Evans and Bishop (61) as containing the X-substance and having the properties of curing dietary sterility. In an analysis of the constituents of a typical dairy cattle ration for vitamin E carried out by Hathaway and Davis (87), it was found that alfalfa, bran, shorts, white or yellow corn, linseed meal, cottonseed meal, hominy or kafir making up 20 to 25 per cent of an otherwise vitamin E-free ration furnished enough of the vitamin to allow reproduction in rats. However, beet pulp, corn gluten feed and corn gluten meal were almost deficient in vitamin E. When these latter substances made up 40 per cent of the ration, they did not furnish adequate amounts of vitamin E to allow reproduction in rats.

F. Factors Affecting the Presence of
Vitamin E in Natural Foodstuffs

1. Destructive agents

With the apparently wide distribution of vitamin E in natural foodstuffs, it does not seem likely that a deficiency of this food factor occurs in the usual diet. On the other hand, even though many of the ingredients commonly used in compounding rations may contain vitamin E, it does not necessarily hold that these rations will always supply sufficient vitamin E.
Under some conditions certain substances when mixed in a ration tend to destroy vitamin E. Decomposition products which are produced during rancidification of fats may prevent the normal physiological action of vitamin E in some obscure way (111,112,113). Treatment of a ration with one per cent by weight of ferric chloride in ether solution will destroy vitamin E (201). The specific manner in which vitamin E is destroyed in this process is not known.

It has been demonstrated that destruction of vitamin E does take place in natural foodstuffs. The destruction is considered to be due to certain oxidative conditions which are initiated in the incipiency of rancidity in fats and oils. The speed at which rancidity develops apparently depends largely upon the character of the fat since the unsaturated fats are more susceptible to the development of rancidity than the saturated ones (35, 38,131,139).

The question arises how important freshness of ingredients is in maintaining the vitamin E potency of milled feeds and roughages which make up the rations for farm animals. It is impossible to state at present what effect aging and rancidity have on the vitamin E content of these foodstuffs, since very little work has been done on this subject. Hathaway et al (83) found that artificially cured alfalfa hay was slightly superior to field cured alfalfa hay as a source of vitamin E. However, it is not intended to summarize here all the divergent opinions which have been presented concerning the potency of various foodstuffs in vitamin E, important as it may be in formulating rations containing adequate amounts of vitamin E. The potency of foodstuffs in vitamin E cannot yet be expressed
very accurately in units of the vitamin, since the technique for bioassaying is at best only roughly quantitative and frequently not more than qualitative.

At present it is only necessary to call attention to the fact that the early workers encountered many difficulties in composing vitamin E-free rations. The difficulties were not due only to the presence of vitamin E, but rather to the destruction of this factor, which took place in their rations at intervals depending upon the degree of oxidation or development of rancidity, a fact which was not then known by them.

Practically all of the vitamin E-deficient rations used in the early work leading to the discovery and recognition of the vitamin contained high percentages of fat. Those of Evans and coworkers and Mattill and associates contained from 10 to 24 per cent of lard. Mattill, Carmen and Clayton (135) noted that when the lard was removed from their ration, the rats reproduced normally. They thought, however, that the role of the lard was to increase the vitamin E requirements of the animal. Mattill and Clayton (136) found that the sterility of male rats on their high-fat milk diets could be prevented either by the addition of vitamin E or by a reduction in the amount of fat in the ration. Nelson, Jones, Heller, Parks and Fulmer (147) also noted that the removal of lard from the ration resulted in improvement in reproduction. They also found that rats failed to reproduce when cod liver oil was added to the feed mixture, but reproduced when the oil was fed separately.

Evans and Burr (68) noticed "initial fertility" in female rats, when mated to males after a period of two months on a vitamin E-free diet
(basal ration 51 (68)) in 75 per cent of the cases; and after four months feeding on the same ration 25 per cent of the cases exhibited "initial fertility". Since Evans and Burr at the same time could show a marked variation in "initial fertility", which apparently was dependent on the season, they thought that the varying vitamin E-content of the butterfat was the cause for the seasonal variations in "initial fertility". Removal of the butterfat reduced the "initial fertility" in rats to a small per cent, but a repetition of the experiments showed that a marked increase in "initial fertility" (46 per cent) took place in rats on a food mixture of like composition in which butterfat was substituted with lard (basal ration 88 (68)). Apparently the butterfat per se was not the cause for "initial fertility". On the basis of numerous experiments with different rations, Evans and Burr finally concluded that food mixtures with lard as the source of fat on the whole gave the least number of "initial fertilities", and that using the lard-containing ration resulted more quickly in sterility than feeding food mixtures which contained butterfat or vegetable oils.

They also adhered to the idea that lard contained substances which were antagonistic to vitamin E and presently called these substances "antivitamins". It was these substances or impurities in lard which caused the sterility and not the high lard per cent per se. They say: "We are hence driven to the conception of a very small proportion of an impurity in rendered lard, which when the latter is fed in very large amounts effectively opposes small amounts of E," and further "The conception of a deleterious influence merely of high fat is overthrown by our
extensive experience with proportions of milk fat in the diet; when 24 percent of milk fat is present in diets the animals show not only an "initial" but a persistent fertility." The same year Clayton (35) noted that the presence of unsaturated animal fats, in particular lard and cod liver oil, in the ration had a destructive action on vitamin E, and also that rations varied in their ability to protect vitamin E from destruction.

Mattill (131) showed that the destruction of vitamin E by the oxidative processes, which accompany the rancidification of fats and oils, could be accelerated by specific autoxidation catalysts such as ferrous sulphate, and Evans and Burr (69) concurrently came to the conclusion that the vitamin E-destroying substances increased with the degree of rancidity of fats, and that there was a relationship between the ration's vitamin E-destroying properties to its content of rancid fats. Further analysis by Evans and Burr (70) showed that the vitamin E-destroying properties were not caused by the formation of fatty acids. There was no relation between the fat's acid number and its vitamin E-destroying properties. Holm (92) noted that the administration of vitamin E in easily oxidizable oils was apt to lead to unreliable results in testing for vitamin E. An interesting early finding is that by Supplee and Dow (185), who reported that whole milk powder, stored for two years in hermetically sealed cans, had been rendered unfit for reproduction in rats. Even in the presence of carbon dioxide there was no marked improvement. Only when the air was deoxygenated and the milk powder had not been subjected to excessive oxidation after manufacture, was reproduction possible.
Investigations by Cummings and Mattill (38) and Mattill and Crawford (139) regarding the chemistry of rancidification and its importance in relation to the fat soluble vitamins A and E showed that the content of vitamin E in a fat containing food mixture depended on the oxidizability and autoxidizability of the fat, which again is dependent upon the number of double bonds in the fat, and the content of catalyst and antioxidants in processes of autoxidation. They did not explain whether the oxidizability or autoxidizability played the greater role in the oxidative destruction of vitamin E.

Evans and Burr (68) in the mean time had shown that vitamin E in wheat germ oil resisted aeration at high temperature (97°) for one hour, and Verzar (197) has substantiated this fact, while on the other hand Olcott (149) could show that aeration with oxygen, which contained 10 per cent ozone, quickly destroyed vitamin E. One may interpret these conditions as follows: it it probably not the oxidation with molecular oxygen as such which destroys vitamin E, but in the course of oxidation, processes of autoxidation are induced in the fats and oils by which peroxides and peroxide-like substances are formed, which in turn cause the destruction of vitamin E.

Some observations concerning the process of extraction, storing and destruction of wheat germ oil will support the viewpoint that Cummings and Mattill (38) observed that wheat germ oil, which gave positive peroxide reaction, had lost some of its antioxygenic and vitamin E effect. Vogt-Møller (199) showed that oil from wheat germ extracted with ether containing peroxide was only one-third to one-tenth as potent in vitamin E as
when peroxide free ether was used in the extraction. Evans and Burr (68) exposed wheat germ oil to ultraviolet light and found a marked destruction of vitamin E took place. However, they did not mention any formation of peroxides. Since wheat germ oil contains sterols, such as dihydro-sitosterol and three with sitosterol isomers, alpha, beta and gamma sitosterol (Anderson, Shriner and Burr (9)) and ergosterol and dihydro-ergosterol (Drummond, Singer and Macwalter (46)), and since these substances, when exposed to ultraviolet light may form peroxides, there is basis to suppose that the induced destruction here is caused also by the formation of peroxides and peroxide-like substances. In this connection it may be mentioned that Fredericia (79) emphasized the formation of peroxides as the possible cause for the destruction of vitamin A.

A revision of earlier experiments seems to indicate that vitamin E is more sensitive to certain types of oxidation (autoxidation) than vitamin A. Mattill, Carman and Clayton’s (135) vitamin E-free milk diet thus caused sterility, but no avitaminosis A. McCollum’s (117,118,119) “salt-opthalmia” was caused when a food mixture which contained ferrous sulphate was used, the latter salt being strongly catalytic in autoxidative processes (Karczag (104), Jones (97)). If three per cent wheat germ oil was added, no “salt ophthalmia” was noticed, but the animals remained sterile (Simmons, Becker and McCollum (178)). By using a ration which contained 15 per cent butterfat as the only source of vitamin A and E Lassen’s (114) and Ringsted’s (163) experiments on growth and fertility indicated a greater sensitiveness on the part of vitamin E to autoxidation than did vitamin A. The animals grew and thrived normally and exhibited no signs
of avitaminosis A. On the other hand, both male and female rats showed
typical signs of avitaminosis E. However, the latter may have been
caused by a smaller content of vitamin E due to seasonal variation and
storage of the butterfat, as the process of autoxidation is accelerated
during storage.

While the destruction of vitamin E has been most apparent in highly
purified diets containing only small amounts of the vitamin, some investiga­
tors have utilized the destructive substances in preparing vitamin E-
deficient rations from ingredients containing large amounts of vitamin E.
A method for the destruction of vitamin E in a ration composed of natural
and varied foodstuffs was first suggested by Waddell and Steenbock (204).
These investigators treated the ration with one per cent by weight of
ferric chloride, which had been dissolved previously in ether. The ether
was allowed to evaporate, leaving the ferric chloride in intimate contact
with the fat soluble material of the ration. This treatment completely
destroyed the vitamin E present in the ration. The recent experiments by
Waddell and Steenbock (205) indicate that the prooxidants (antivitamin,
Evans and Burr (70)) produced by treatment of rations with ferric chloride
in ether had a profound effect, not only in the ration but also in the
body of the rat on vitamin E. The addition of ferric chloride to the ra­
tion without the use of the fat solvent failed to destroy the vitamin E.

As a result of the observations cited in the previous pages, one may
assume that it is apparently the peroxides and peroxide-like substances
produced through oxidation and autoxidation which destroy vitamin E.
2. Preserving agents

Although it has been known for almost a century that certain plant tissues or plant products contain substances that act in a manner to prevent rancidity in fats (41), or to delay the drying of linseed oil (34), the exact nature of the chemical processes or the substances which prevent these changes have not been known. The increasing importance of antioxygenic materials in the chemistry and physiology of nutrition has been discussed by Cummings and Mattill (38), and Mattill (132), who have pointed out that the preservation of vitamins A and E, especially the latter, seemed to be dependent upon the presence of antioxidants.

Earlier McCollum and his coworkers (119) in their studies of "salt ophthalmias" noticed that protection of wheat germ and wheat germ oil were effective in preventing such ophthalmias. They concluded that vitamin E or wheat germ oil was concerned in some unknown manner in protecting vitamin A against destruction by the ferrous sulphate in the ration. Mattill (131) made further progress in the elucidation of this problem. He found that diets which induced sterility were very susceptible to oxidation, and that vegetable oils, especially wheat germ oil, delayed the autoxidation of fats and preserved vitamins A and E. He acted on the suggestion of Holm and his coworkers (93), that certain compounds present in oils and possessing hydroxy groups, were active in protecting fats against rancidity or oxidative changes. He found a correlation between the acetyl number of fats and the ease with which they are oxidized. From his data he was able to postulate the existence of a balance between unsaturated
fatty acids and the protective substances or antioxidants (139). Wheat
germ oil had a high acetyl value and, therefore, presumably was rich in
antioxidants, and it was to this it owed its stabilizing activity on vita­
mins A and E. Olcovich and Mattill (159), Olcott and Mattill (154,155)
have fractionated the unsaponifiable fractions of lipids of lettuce and
demonstrated that vitamin E and antioxidant are separate entities. They
stated that one hydroxyl group, phenolic in character, was present in the
antioxidant, and that the inhibiting action of this type of antioxidant re­
sided in the hydroxyl group. However, the mere presence of a hydroxyl

Olcott (150) tested a number of compounds for their antioxygenic
activity toward lard and found that pyrogallol, hydroquinone, pyrocatechol,
hydroxy-hydroquinone and apional all were excellent antioxidants, using the
oxygen absorption method for measuring the length of the induction period
(132). Later, French, Olcott and Mattill (60) studied the relationship of
the amount of inhibitor to the prolongation of the induction period, the
effect of the peroxide level on the induction period and an added inhibitor,
the influence of an inhibitor added to fats after the end of the induction
period, and related topics. As a result of these investigations they con­
cluded: "That the induction period by some natural antioxidants and several
phenolic compounds is proportional to the amounts used at the end of the induction period and the level of peroxide in lard or lard-cod liver oil mixtures is fairly uniform irrespective of the length of the induction period or of the original peroxide content.

"In the case of one natural inhibitor, \textsuperscript{1,24} isolated from the nonsaponifiable fraction of wheat germ oil, which was extensively studied, the effectiveness varied inversely with the amount of peroxides in the fat mixture, whether these had accumulated slowly or were added in the form of an oil of high peroxide content. Under such conditions there seemed to be a mutual destruction of antioxidant and active peroxides."

More recently, Olcott and Mattill (157) have attempted to classify the inhibitors or antioxidants into three groups: 1) acid-type inhibitors, 2) inhibiteds and hydroquinone, and 3) other phenolic inhibitors. They stated in general any type 1 inhibitor when used with any type 2 or 3 compound prolongs the induction period of fats and oils to a much greater extent than would be expected from a summation of the effects of each used alone. Lately Olcott and Emerson (153) have discussed the possible role of vitamin E itself as an antioxidant. They maintained that the alpha, beta and gamma-tocopherols, all of which possess vitamin E activity and their allophanates were effective antioxidants in lard. The degree of protection afforded by the tocopherols, however, was not proportional to their vitamin E activity, since the compounds were increasingly effective in regard to their antioxygenic activity in the order of alpha, beta and gamma, whereas in their vitamin E-activity beta-tocopherol had the least, alpha and gamma-tocopherol had the highest vitamin E potency.
From these important investigations one may reason that vitamin E is highly labile to chemical oxidations such as take place through the auto-oxidation of fats and oils. Further, vitamin E is probably destroyed in foodstuffs which become rancid naturally and destruction is accelerated when they are brought into contact with certain chemical compounds, which induce rancidity. However, its preservation in milled feeds and roughages, as used in the ration of our farm animals, is probably dependent on the balance of free peroxides or peroxide-like substances, oxidants and antioxidants, since when the amount of the latter is insufficient, auto-oxidation to which vitamin E is highly labile, is likely to occur.
III. EXPERIMENTAL PROCEDURE

A. Selection and Management of the Experimental Goats and the Maintenance Ration

1. Selection

Seven female goats of mixed origin and breeding were originally bought and placed on a ferric chloride treated experimental ration in the spring of 1933. The goats all showed evidence of being of a milking strain. Two exhibited characteristics of the Saanen breed, two showed definite Toggenburg markings, while the other three appeared to be of Nubian breeding. Four of the females had proved their fertility by giving birth to offspring within three months prior to the time they were placed on the experimental ration. The remaining three were young virgin does. These seven females and their progeny born during the experiment were the goats used in the trial. The history of these goats until July 1, 1935, was reported by Wilson (203). These goats were continued on trial and under observation for reproductive performance until November 5, 1937, when the work with the live goats was terminated.

All male goats used were reared during the experiment except two males of proved fertility and were restricted to the ferric chloride treated experimental ration. They were the progeny of the original seven purchased female goats.
2. Management

Certain precautions were taken to insure healthy goats at the start of the experiment. All appeared vigorous and healthy and when examined they reacted negatively to the tuberculin test, as well as to the agglutination test for contagious abortion. They were shorn and dipped just prior to starting the experiment (209). The procedure in caring for all goats throughout the four and one-half years of this experiment was designed to eliminate any chance of the animals receiving vitamin E by contamination. The goats were housed in pens located in an airy basement room of the Animal Chemistry and Nutrition Laboratory, which was well lighted and semi-artificially ventilated. The goats were kept in cages made of wire fencing stretched on wooden frames. The bottoms were made of three-fourths inch mesh number 9 gauge wire screen. Shavings were kept on the floor 18 inches below the screen bottoms to absorb the excrements. Cages were of such size as to allow several goats to run together. At kidding time each female was penned separately. The male goats kept for breeding purpose were removed from the laboratory at time of puberty to a separate building. They were confined there in pens similar to those used for the females. This was done because of the offensive odor which is associated with adult male goats. In case a goat was taken outside the laboratory for any reason, such as breeding, it was muzzled to prevent access to untreated feed.

The goats were observed for the occurrence of estrus in the sexual season, which appeared regularly between September and March. They usually remained in heat for one to two days. The does were always mated to proved
males unless the conditions of the experiment justified deviating from this plan. The fertility of the males was checked by obtaining samples of semen for microscopic examination to determine the motility of the spermatozoa. A complete breeding and birth record was kept on every goat, and any occurrence of estrus or failure to breed was recorded.

The goats were observed at all times during the gestation for any possible abnormalities. Weekly weights were taken of all the goats in this experiment from the time of birth until they were killed or terminated.

All the kids born to does on the experimental ration were allowed to suckle their mothers until they were weaned or killed. During the suckling period they had access to the experimental ration.

3. Vitamin E-deficient experimental ration fed to the goats

a. Composition of the goat ration. The goat basal ration (ration I, table 1) was made up of a finely ground grain mixture and chopped alfalfa hay. The grain mixture consisted of ingredients compounded in the proportions indicated and listed in table 1, ration Ia. The alfalfa hay, usually of fair quality, was chopped to approximately three-fourths inch lengths.

b. Treatment of the goat ration for the destruction of vitamin E. The hay (ration Ia, table 1) and the grain mixture (ration Ia, table 1) were treated with an ether solution of ferric chloride to destroy the vitamin E before feeding. The method of destruction of vitamin E in the ration was essentially that outlined by Maddell and Steenbock (204). Ferric chloride to the amount of one per cent of the ration was brought into intimate contact with the fat soluble material in the ration through the medium of an
Table 1. Composition of various rations fed to experimental animals.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Goat rations</th>
<th>Rat rations**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I: (lbs.):</td>
<td></td>
</tr>
<tr>
<td>Yellow corn (ground)</td>
<td>70.0</td>
<td>142.0: 142.0</td>
</tr>
<tr>
<td>Oats (ground)</td>
<td>60.0</td>
<td>100.0: 100.0</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>60.0</td>
<td>30.0: 30.0</td>
</tr>
<tr>
<td>Linseed oil meal</td>
<td>10.0</td>
<td>12.0: 12.0</td>
</tr>
<tr>
<td>Bone meal steamed</td>
<td>2.0</td>
<td>10.0: 10.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>2.0</td>
<td>4.0: 4.0</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>2.0</td>
<td>4.0: 4.0</td>
</tr>
<tr>
<td>Alfalfa hay (chopped)</td>
<td>100.0</td>
<td>6.0: 6.0</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>2.0</td>
<td>7.0: 7.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.0: 14.0</td>
</tr>
<tr>
<td>Skimmed milk powder</td>
<td>66.7</td>
<td>18.0: 18.0</td>
</tr>
<tr>
<td>Butterfat</td>
<td>2.0</td>
<td>4.0: 4.0</td>
</tr>
<tr>
<td>Crude casein</td>
<td>22.3</td>
<td>5.0: 5.0</td>
</tr>
<tr>
<td>Alfalfa meal</td>
<td></td>
<td>5.0: 5.0</td>
</tr>
<tr>
<td>Bone meal (steamed)</td>
<td>2.0</td>
<td>22.0: 22.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td></td>
<td>2.0: 2.0</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td></td>
<td>4.0: 4.0</td>
</tr>
<tr>
<td>Casein (alcohol extracted)</td>
<td>12.0: 12.0</td>
<td>40.0: 40.0</td>
</tr>
<tr>
<td>Corn starch</td>
<td>49.0: 49.0</td>
<td>71.0: 71.0</td>
</tr>
<tr>
<td>Lard (steam rendered)</td>
<td>2.0</td>
<td>22.0: 22.0</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>2.0</td>
<td>22.0: 22.0</td>
</tr>
<tr>
<td>Yeast</td>
<td>15.0: 15.0</td>
<td>4.0: 4.0</td>
</tr>
<tr>
<td>Salt mixture (Hawk &amp; Cuer)</td>
<td>10.0: 10.0</td>
<td>4.0: 4.0</td>
</tr>
</tbody>
</table>

*Ferric chloride treated goat and rat rations. Goat ration supplemented at feeding time with vitamin E-free yeast and cod liver oil, or β-carotene and irradiated yeast.

**All coarse ingredients ground finely.

***Part IIa checked previously with rats for resorption gestations.
Fig. 1. Apparatus used in treating the basal goat ration (ration I, table I) with ferric chloride and for the recovery of the ether.
ether solution. The ether was then evaporated off, leaving the ferric chloride in intimate contact with the fat soluble vitamins. The ferric chloride caused the destruction or inactivation of vitamin E present in the ration.

The grain mixture was treated in batches of approximately 35 to 40 pounds and the hay in 18 pound lots. Ferric chloride making up one per cent by weight of the feed to be treated was pulverized in a mortar and dissolved in ether. The ether solution of ferric chloride was then mixed with the feed and allowed to stand on the average for 12 hours. Enough of this solution was used to submerge the feed completely. After the feed had soaked for 12 hours steam was turned into the coil heating a water bath surrounding the vessel in which this ether-feed mixture was contained. The vessel consisted of a ten-gallon milk can containing a vertically fitted spiral one-fourth inch copper tubing with inlet and outlet at the top. The cover was fitted with an outlet for the ether. Further details of the apparatus used to recover the ether are shown in fig. 1. Heating was continued until no more ether distilled over. About eight to 12 hours were required to drive the ether out of the hay and the grain. The feed was then removed from the still and spread out in a shallow metal pan for six to 12 hours to allow the last of the ether to evaporate. The feed was stored in galvanized cans at room temperature until it was used. After a thorough mixing of the untreated feed, a sample was taken from each batch to test for the presence of vitamin E. The ferric chloride treated grain mixture and the hay constituted the experimental goat ration (ration II, table 1).

Wilson (209) had experienced no difficulties in inactivating the
vitamin E in the goat ration by the method just described. The sampled material from the treated goat ration usually was two or more weeks old at the time it was tested. The ration the goats received was always at least of that age and in most instances older, depending on the amount of accumulated treated feed, the goats always being fed the aged material.

Olcott and Mattill (156) had reported in 1934 that purified concentrates of vitamin E, even in the absence of an inhibitor, appeared to retain their activity for as long as four weeks at room temperature in a rancid fat mixture. These workers were unable at that time to reconcile these results with those of their previous researches.

On the basis of their report it was decided to make some tests of the experimental goat ration (ration II, table 1) to ascertain whether it contained any vitamin E immediately following treatment with ferric chloride.

The tests for the presence of vitamin E in ration II were carried out by feeding it to vitamin E-depleted rats according to the "cure" trial technique described by Evans and Burr (68). Since the possibility existed that vitamin E might have been destroyed more readily in the grain mixture than in the roughage, or vice versa, a modification of ration II was fed to the vitamin E-depleted rats in these trials. Another reason for modifying ration II before feeding it to the test rats was because of its high fiber content, which when present in excessive amounts is harmful to omnivora and not readily digested. The rat rations (ration VIII, IX and X) which are modifications of goat ration II are listed in table 1. Rations VIII, IX and X constitute also the rat rations as they were fed to vitamin E-depleted rats in carrying out the routine and other tests for vitamin E in
the experimental goat ration. Table 2 shows the effect of storage temperature and aging upon the content of vitamin E in the ferric chloride treated goat ration.

Six vitamin E-depleted female rats were fed 24-hour-old samples of the treated grain mixture and the treated hay. Five of these rats produced litters. One rat on the grain mixture resorbed her young. Forty-one vitamin E-depleted rats were fed weekly composited samples of treated grain mixture and hay. Thirty-one of these animals produced litters and 10 resorbed their young. Such accumulated weekly samples were kept and tested again when 60 to 90 days old. Seven rats were used. All these animals resorbed their young. Concurrently with these tests one to two-week-old samples from the treated goat ration were held at room temperature and at 50° C. in a drying room and tested. The tests carried out in these trials with 24 rats on these samples resulted in resorption gestations. It is evident from the results given in this table that vitamin E is not entirely inactivated under certain conditions at the completion of treatment with ferric chloride and recovery of the ether solvent. Aging of the treated material possibly through the indigenous development of rancidity which in turn may be accelerated by the effect of the ferric chloride apparently had a profound effect on the destruction of vitamin E. Inactivation of vitamin E apparently was complete in seven to 14 days or less.

On the basis of these data further precautions were taken for inactivation of any residual vitamin E in the treated goat ration. Following recovery of the solvent, each batch of ferric chloride impregnated feed was collected and stored at approximately 50° C. for two or more weeks to
Table 2. Effect of storage temperature and aging upon the content of vitamin B in the ferric chloride treated experimental goat ration.*

<table>
<thead>
<tr>
<th>Ration</th>
<th>Age of</th>
<th>Amount of</th>
<th>Average number of</th>
<th>Character of gestation</th>
<th>Storage temperature</th>
<th>Material tested</th>
<th>Source of matter</th>
<th>Age of</th>
<th>Re- of</th>
<th>Per- of</th>
<th>Total of</th>
<th>Sorption of</th>
<th>Young of</th>
<th>Per- of</th>
<th>Meting of</th>
<th>Litter</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIII</td>
<td>25°C.</td>
<td>1</td>
<td>83.0</td>
<td>3</td>
<td>27</td>
<td>22</td>
<td>49</td>
<td>1</td>
<td>2</td>
<td>13</td>
<td>6.5</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IX</td>
<td>Alfalfa IIa</td>
<td>ditto</td>
<td>1</td>
<td>22.3</td>
<td>3</td>
<td>13</td>
<td>22</td>
<td>35</td>
<td>0</td>
<td>3</td>
<td>14</td>
<td>4.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>Mixture of IIa and IIb</td>
<td>ditto</td>
<td>1-7</td>
<td>89.0</td>
<td>41</td>
<td>44</td>
<td>22</td>
<td>66</td>
<td>10</td>
<td>31</td>
<td>186</td>
<td>6.0</td>
<td></td>
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</tr>
<tr>
<td>IX</td>
<td>Alfalfa IIb</td>
<td>ditto</td>
<td>60-90</td>
<td>89.0</td>
<td>7</td>
<td>8</td>
<td>22</td>
<td>30</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIII</td>
<td>Grain mixture IIa</td>
<td>ditto</td>
<td>7-14</td>
<td>89.0</td>
<td>8</td>
<td>12</td>
<td>22</td>
<td>34</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
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<tr>
<td>IX</td>
<td>Alfalfa IIb</td>
<td>ditto</td>
<td>7-14</td>
<td>22.3</td>
<td>8</td>
<td>18</td>
<td>22</td>
<td>40</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>VIII</td>
<td>Grain mixture IIa</td>
<td>ditto</td>
<td>7-14</td>
<td>89.0</td>
<td>8</td>
<td>11</td>
<td>22</td>
<td>33</td>
<td>8</td>
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<td>0</td>
<td>0</td>
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<td></td>
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</tr>
<tr>
<td>IX</td>
<td>Alfalfa IIb</td>
<td>ditto</td>
<td>7-14</td>
<td>22.3</td>
<td>8</td>
<td>23</td>
<td>22</td>
<td>45</td>
<td>8</td>
<td>0</td>
<td>0</td>
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</table>

*The tests were carried out according to the "cure" trial technique.
**Rations ad. lib.
***Rats which died, nonbreeders, and those which showed pseudopregnancies, are not included.
accelerate the inactivation of vitamin E. In the early stages of this investigation the treated feed was held at room temperature instead of 50° C. for one or more months. All feed treated by either procedure soon acquired a rancid odor. The odor was most noticeable in the batches of treated grain mixture. This, however, did not appear to affect the palatability of the feed for the goats.

As a further precaution all the ferric chloride treated hay and grain mixture was biologically checked with rats for resorption gestations before being fed to the goats.

c. Feeding. The ferric chloride treated, aged and tested grain mixture and hay constituted the goat experimental ration and was fed throughout the experiment (ration II, table 1). The grain mixture (ration IIa) and the hay (ration IIb) were fed to the goats twice daily to the satisfaction of their appetite. This proportion represents the ration which was fed successfully to experimental goats in confinement by Steenbock, Hart, Hoppert and Black (183). The feeding utensils were galvanized iron feed pails. Tap water was kept before the goats at all times in galvanized iron pails. For a 24 hour period at weekly intervals the goats had access to water which contained .01 per cent potassium iodide. Block salt was kept in the pens at all times. The treated ration was supplemented by the feeding of cod liver oil at the rate of 10 cc per goat daily and by the addition of yeast to the extent of approximately one per cent of the goat ration. At times during the course of the experiment these supplements were substituted by beta-carotene, and irradiated yeast. These supplements were added as a precaution to insure an abundance of vitamin A, D and B-complex in the
ration. Though neither the yeast nor the cod liver oil were supposed to contain vitamin E all supplements were tested biologically with rats to insure their freedom from vitamin E before these materials were fed to the goats.

B. Biological Estimation of Vitamin E

1. Rats used for test purposes.

a. Source and breeding of test animals. All test animals were offspring from mothers which had been fed a stock ration of constant composition, a modification of Steenbock's stock ration for breeding animals.

Test animals have been used for the following experimental aim:
1) male rats for procuring histological material of normal testicles, as well as pathological material from testes of vitamin E-deficient animals; 2) female rats for qualitative biological tests of the goat ration and other materials for vitamin E; 3) numerous male and female rats for breeding purposes. The male and female stock colony breeding rats and certain males used in the collection of normal histological test material were fed the stock ration. Males and females which were used for biological tests received the vitamin E-free basal rations.

b. Housing, care and feeding. The rats were housed in suitable experimental cages and kept in a room equipped with thermostats to regulate temperature. The cages were wire mesh, about 10 x 12 x 22 inches in size placed in steel racks. Four to eight rats were kept in a cage. The cage floors were one-half inch mesh wire screens, three inches below which were
set pans of shavings to catch the excreta to prevent coprophagy. Stock colony breeding females were isolated on the 18th to 19th day of gestation and moved to cages with solid floors which were covered with shavings. Pregnant test animals were also isolated on the 18th to 19th day of gestation, but they were moved to cages with specially built floors which would allow any young born to drop through below immediately following birth, since it was important to determine whether any young were born or not and how many. These cages were eight x nine x nine inches in size.

The floor was slanting toward the center where there was a slit an inch in width. This precautionary measure was resorted to in order to prevent the test mothers from eating their young, since test rats which are on deficiency diets often turn to cannibalism. Pregnant test animals were weighed daily. Vaginal smears were run daily from the time of the beginning of the test period in order to determine the stages of estrus, time of conception, placental sign and the time for the culmination of a resorption or eventual pseudopregnancy. These manifestations will be dealt with more in detail in the succeeding pages. The vaginal smear also gave information about disturbances in the vaginal cycle, such as cessation of estrus, prolonged estrus, nymphomaniacs and pseudopregnancies, so that those animals could be discarded as they were of no use for breeding or test animals. From time to time all rats were bathed in an aqueous solution of "Sopex", an insecticide, to safeguard against vermin.

The rats were fed from tin cups fitted with partial covers to prevent wastage of feed. Feed was kept before the rats at all times, the cups being checked once daily. Distilled water was kept before the rats in bottles
fitted with gravity tips. Feed cups and shaving pans were washed and sterilized at weekly intervals. Drinking bottles were refilled daily and cleaned when dirty or at intervals of approximately two weeks.

2. Basal rations employed

a. Stock ration for breeding animals. The stock colony breeding animals were fed the laboratory stock ration which essentially is a modification of the Steenbock ration. The ingredients are listed in Table 1, ration III. This basal ration has been used in the Animal Chemistry and Nutrition Laboratory in procuring test animals for vitamin A, B and D experiments. It has been found to be equally well suited in obtaining test animals for vitamin E experiments.

b. Basal vitamin E-free rations used for depletion and test purposes. A basal ration for vitamin E tests must satisfy the two criteria of a suitable vitamin E-deficient ration. First it must be completely deficient in vitamin E; secondly it must not be quantitatively or qualitatively lacking in any other food constituent necessary for the production of living young, since failure to give birth to living young is considered as proof of the absence of vitamin E and the production of living young as proof of its presence.

There are three possible methods for compounding and obtaining vitamin E-free rations: 1) using ingredients which are low or devoid of vitamin E and contain one or more vitamin E-destroying substances; 2) destroying the vitamin E by using the Saddell and Steenbock technique; 3) use ingredients which are entirely devoid of vitamin E and which do not
contain any vitamin E-destroying substances.

The methods listed under 1) and 2) are well suited for composing depletion rations as one is mainly interested in depriving the test animals of their vitamin E reserves. However, rations which contain vitamin E-destroying substances offer some difficulties when they are used as basal rations in tests for vitamin E, especially when attempting to detect small amounts in a food substance. The destruction of vitamin E which takes place when certain foodstuffs are incorporated in the diet and in which autoxidative changes occur thereby producing vitamin E-destroying substances has been dealt with in detail in preceding paragraphs. The assumption was then made that it probably was the peroxide and peroxide-like substances produced through oxidation or autoxidation in certain fats and oils, especially unsaturated ones, which destroyed vitamin E; and that vitamin E also was destroyed in foodstuffs which became rancid naturally and destruction was accelerated perhaps through the catalytic effect of certain chemical compounds which induced rancidity. On the basis of these deliberations and whatever the nature of the vitamin E-destroying substances may be, the methods 1) and 2) have been employed in composing vitamin E-free depletion rations. To minimize the effect of vitamin E-destroying substances in the high fat basal vitamin E-free ration used for test purposes, a low fat basal ration was used which contained less of these inactivating substances. The vitamin E-free rations used were essentially modifications of Evans and Burr's high lard ration (basal ration 55, (68)). The ingredients making up these basal vitamin E-free rations are listed in table 1, rations V and VI.
Besides these two rations another vitamin E-free ration for depletion of adult rats was used (209) in the early part of the experiment, the ingredients of which are listed in Table 1, IV. Ration IV was treated with one per cent by weight of ferric chloride dissolved in ether to destroy the vitamin E present. The method of treatment and the procedure used was reviewed previously. The vitamin E-free rations V and VI have satisfied all expectations for depletion purposes. In the absence of prophylactic treatment with vitamin E, initial sterility as diagnosed by typical resorption gestations was observed in 100 per cent of the cases, the animals being on the depletion diet for 90 days before mating. It was suspected, however, that ration V might not prove to be very well suited for use as a basal ration, because of its high lard content, when testing for the presence of small amounts of vitamin E, since it has been reported that lard contains vitamin E-destroying substances (111,112,113). Before the experimental trials were begun in testing the goat materials for vitamin E, some preliminary work was instituted to ascertain the effect which such vitamin E-destroying substances perhaps present in the basal rations had upon the accuracy of measuring the vitamin E-content in certain food materials. Results of such tests made and employing ration V and VI as basal rations are shown in Table 3.

The data presented in Table 3 demonstrate that no vitamin E activity was manifested by normal goat milk and muscle tissue, nor cow milk, when these ingredients were fed as supplements to basal ration V, which contains 22 per cent lard. Only one litter was obtained from 13 rats. Conversely when similar ingredients were fed as supplements over
Table 3. Effect of percentage of lard in the ration upon the accuracy of measuring the vitamin E content in certain food materials.*

<table>
<thead>
<tr>
<th>Basal ration</th>
<th>Supplement and description**</th>
<th>Amount of supplement per rat daily</th>
<th>Character of gestation</th>
<th>Basal ration ad. lib.</th>
<th>Number of days receiving supplement</th>
<th>Number of rats</th>
<th>Total</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>High fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ration V, table 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ditto</td>
<td>Goat milk (composited fresh samples)</td>
<td>15 5 45 13 58 5 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ditto</td>
<td>Cow milk (composited fresh samples)</td>
<td>15 5 46 13 59 4 1 4 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ditto</td>
<td>Goat muscle tissue (composited frozen samples)</td>
<td>5 3 40 13 53 3 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low fat</td>
<td></td>
<td></td>
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<td>(ration VI, table 1)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ditto</td>
<td>Goat milk (composited fresh samples)</td>
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</tr>
<tr>
<td>ditto</td>
<td>Cow milk (composited fresh samples)</td>
<td>15 5 56 13 69 0 5 41 8.2</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>ditto</td>
<td>Goat muscle tissue (adults composited frozen samples)</td>
<td>5 3 23 13 36 0 3 16 5.3</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ditto</td>
<td>Goat muscle tissue (kids composited frozen samples)</td>
<td>5 3 29 13 42 0 3 23 7.7</td>
<td></td>
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</tbody>
</table>

*These tests were carried out according to the "cure" trial technique.
**Obtained from goats and cows fed natural, varied untreated foodstuffs.
***Basal ration ad. lib.
approximately the same period of time to basal ration VI which contained no
lard, 15 rats had normal litters at term. The negative control rats all
resorbed their young.

The results shown in table 3 are in agreement with those of workers
previously cited and with results obtained in trials in this experiment
(cf. tables 4 and 5) that lard and perhaps cod liver oil contain vitamin
D-destroying substances. Cod liver oil, however, is retained in ration VI,
since it is important to include certain unsaturated fatty acids in the
diet without which gestation is unsuccessful (Evans, Lepkovsky and Murphy
(75)) or testicular degeneration occurs (Evans, Lepkovsky and Murphy
(76)). The cod liver oil, however, may have a less profound effect than
that of the lard in vitamin D-destroying properties. In regard to the
consistency of the ration it was desirable to include some form of excip-
ient since a fat-free ration is dry and not very palatable. Therefore
cod liver oil was used as it supplied the vitamins A and D and at the
same time made the ration more cohesive. As a precaution against the
vitamin D-destroying properties of the oil when test materials were sup-
pplemented to the ration, the rations were made up twice weekly and kept
under refrigeration at 0° C.

3. Depletion of Vitamin E reserves of test rats

Vitamin E-free test animals may be secured either by depriving
adult fertile females of their reserves of vitamin E by placing them on
vitamin E-free rations or by subjecting weanling rats to such dietaries.
The time required for the depletion of body storage of this factor in
either case extends over a period from two to three months, depending on
the ration employed. Both procedures have been resorted to in this ex-
periment.

a. adult rats. In the early part of this experiment (209) female
rats of proved fertility were depleted of their storage of vitamin E by
feeding them a ration deficient in vitamin E (ration IV, table 1). After
two months on this ration their reproductive histories were followed un-
til they showed depletion of their body reserves of vitamin E as indi-
cated by the resorption of young.

b. weanling rats. The method used by Wilson (209) required an ex-
tended length of time for the depletion of vitamin E reserves in the body
of adult rats. In many cases the rats would show "initial fertility" for
a period as long as four months and by that time the rats would have gone
through as many as two or three gestations. Consequently senile sterility
was encountered in some cases. Another difficulty was that the ration
possibly contained sterilizing substances which prevented vitamin E from
exercising its physiological effect in vivo (111). Adult rats might go
through a pregnancy simulating a resorption gestation and still not be
depleted of their vitamin E reserves (113). Such rats would therefore
be useless as test animals.

On account of these hazards it was deemed advisable to abandon this
procedure in securing test animals. Since April, 1935, test animals
for use in testing materials for their antisterility effect have been
secured by placing weanling female rats approximately 21 days of age on
a vitamin E-free ration (ration V, table 1). The rats at weaning time
weighed 30 to 40 grams. The animals were kept on ration V for 90 days and then mated to normal males. After a resorption gestation they were used as test animals. It has been the usual practice, as far as one can gather from published work, to use test animals of this kind for biological estimations of vitamin A (e.g. animals that have undergone at least one resorption gestation) in order to make certain that they have been completely depleted of their vitamin A reserves.

Since no "initial fertility" was ever encountered by using this procedure it appeared that considerable time and expense might be saved without sacrificing accuracy by omitting altogether the process of submitting animals to one resorption on the ration cited above and administering the dose of test substance to animals immediately following the 90 days on the depletion ration, since there is a probability approaching certainty that without vitamin A they would undergo resorption gestations. Further, such animals would be of approximately the same age as if animals were mated following a depletion period of 60 days and then allowed to undergo a resorption gestation. Any possible deleterious effect of resorption gestation would thereby be avoided. This practice was therefore followed in some cases, as in testing the experimental goat rations for vitamin A. By using this procedure a test animal could be used for two resorption gestations on the ferric chloride treated goat ration and still not be older than if the usual technique was employed in procuring test animals. In a few instances test animals were used which had undergone two previous resorption gestations on the ferric chloride treated goat ration. The virgin rats which had been on the depletion ration for 90 days and which
were used as test animals along with rats which had undergone one or two resorption gestations are referred to in the following as "cures". The use of such older resorption gestation test animals may be criticised. Evans and Burr ((68) footnote p. 102) called attention to the fact that rats held upon "pure" diets for many months, the amount of vitamin B (in the form of wheat germ oil) required for "cures" might be higher; as much as four times above the minimum dose for successful gestation. They also emphasized the fact that they had encountered repeated instances in which a particular extraction of wheat germ oil was defective in its vitamin E-content for unexplained reasons. However, it has been demonstrated and previously cited that wheat germ oil loses its potency upon storage and that extraction of wheat germ by ether containing peroxide will give an oil which may be 1/3 to 1/10 less potent (199). In the case cited the aged animals may have received an oil of less potency. More recently Evans, Emerson and Eckert (72) have reported on the importance of using young vitamin E-deficient test animals. They quote: "We feel that the age of the test animals is highly important. Our experience has shown that in old animals no amount of wheat germ oil (high in vitamin E) will restore fertility. We employed, therefore, only female rats under six months of age. The rats were placed on the vitamin E-low diet at 21 days. The rats were placed with normal males after 60 days and observed for resorption gestations and then used for test animals." They show no experimental evidence, however, to substantiate their statement. On the basis of Evans and Burr's (68) earlier reports a number of test rats which had gone through one or more resorption gestations were fed
different sources of vitamin E in order to determine the response of vitamin E-deficient rats to vitamin E therapy following one or more resorption-gestations. Wheat germ oil and other natural foodstuffs were used as sources of this factor.

From the data presented in table 4 it will be noted that when vitamin E in the form of wheat germ oil was fed separately to vitamin E-depleted rats following zero to five resorption gestations all pregnancies resulted in litters at term. Conversely, when wheat germ oil was incorporated in ration V which contains high amounts of lard, all pregnancies terminated in resorptions or pseudopregnancies, excepting one case, rat 57-A, where one young was born dead. The test animals in the latter had undergone one to six previous resorption gestations. When vitamin E was administered in other forms than wheat germ oil, separately to basal ration V or replacing all the lard, or fed a ration made up entirely of natural, varied untreated foodstuffs all pregnancies culminated in the production of litters. The rats employed were vitamin E-deficient rats, which had undergone from zero to six resorption-gestations before they were subjected to vitamin E therapy. The two rats which had no previous resorptions had been on the basal ration V for 90 days. It is apparent from the data presented that neither the age of vitamin E-deficient test animals nor the number of resorption gestations had any influence in regard to response of vitamin
Table 4. Response of vitamin E-deficient rats to vitamin following one or more resorption gestations.

<table>
<thead>
<tr>
<th>Vitamin E-deficient basal ration</th>
<th>Amount of supplement fed</th>
<th>Number: Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source of vitamin E- supplement</td>
<td>As percent- Daily per-</td>
<td>Number of gest.</td>
</tr>
<tr>
<td>age of rat: ration (grams):</td>
<td>rat: of previous: previo</td>
<td>Number of rats: to post</td>
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<tr>
<td></td>
<td></td>
<td>used: sorp-: tive</td>
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<tr>
<td>Modified goat ration II (ration X, table 1)</td>
<td>heat germ oil</td>
<td>1</td>
</tr>
<tr>
<td>ditto</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>1</td>
</tr>
<tr>
<td>ditto</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>1</td>
</tr>
<tr>
<td>High fat (ration V, table 1)</td>
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<td>1</td>
</tr>
<tr>
<td>ditto</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>1</td>
</tr>
<tr>
<td>ditto</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>11</td>
</tr>
<tr>
<td>ditto</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>11</td>
</tr>
<tr>
<td>ditto</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>11</td>
</tr>
<tr>
<td>ditto</td>
<td>&quot; &quot; &quot; &quot;</td>
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<tr>
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<tr>
<td>ditto</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>11</td>
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<tr>
<td>Modified goat ration I (ration VII, table 1)</td>
<td>ad. lib.</td>
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<tr>
<td>ditto</td>
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<tr>
<td>ditto</td>
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</tr>
<tr>
<td>ditto</td>
<td>&quot; &quot;</td>
<td>1</td>
</tr>
</tbody>
</table>

*These tests were carried out according to the "cure" trial technique.

**heat germ oil incorporated in the basal ration replacing 11 per cent lard.

***Puratene*, a crude commercial preparation of carotene prepared from carrots, replacing all.

Several pseudopregnancies.

# One pregnancy, one litter with one young dead at birth.

Corpus luteum obtained from pregnant cows and fed as daily addition to the basal ration.
Historical examination of these rats revealed that no young would have been born (cf. table 5).

### Table: Effect of Vitamin B-Deficient Rats to Vitamin B Therapy

<table>
<thead>
<tr>
<th>Supplement Fed</th>
<th>Number of Days</th>
<th>Character of Gestation</th>
<th>Number of Days</th>
<th>Total</th>
<th>Average</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily per rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplement</td>
<td>Number of rats</td>
<td>Resorption Gestations</td>
<td>Number of Days</td>
<td>Total</td>
<td>Average</td>
</tr>
<tr>
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### Table: Results of Vitamin B-Deficient Rats to Vitamin B Therapy

<table>
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<th>Number of Days</th>
<th>Character of Gestation</th>
<th>Total</th>
<th>Average</th>
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Histological examination of these rats revealed that no young would have been born (cf. table 5).

### Notes:
- "ad lib." indicating ad libitum feeding.
- "trial technical" suggesting a specific technique used.
- "placing 11 per cent lard" indicating the addition of lard to the basal ration.
- "tene prepared from carrots" suggesting the use of carrots in the basal ration.
- "birth" indicating the daily addition to the basal ration.
E therapy,* but apparently rather is influenced by the basal ration employed. (cf. table 3). The negative response of the 10 rats which received the wheat germ oil incorporated in the basal ration containing lard strongly indicates that such is the fact. The vitamin E present in the wheat germ oil apparently had been destroyed by the incipient rancidity of the lard, or perhaps prevented from its action by the presence of sterilizing substances following rancidity. A subsequent thorough examination of the individual behavior of these 10 rats reveals that the latter may be the case.

The reproductive behavior of these individual rats along with a control was observed. Six of the rats receiving wheat germ oil and all the rats in the control were examined histologically. Results of the histological examination of the rats restricted to a ration containing lard and wheat germ oil are shown in table 5. As shown in the summary of table 5 histological examinations revealed that seven of the 15 rats were pregnant. The character of the corpora lutea and the uteri in the eight remaining animals indicated that they recently had been pregnant or at least were passing through a pregnancy simulating a resorption gestation or a pseudopregnancy. Implantation signs were detected in all the

*Recently Bacharach et al (14) have reported some evidence that resorption gestation animals had a higher vitamin E threshold compared with vitamin E-depleted virgin animals. A carefully preserved wheat germ oil concentrate was tested on resorption-gestation animals and virgin animals. He found that resorption-gestation rats required about six times as much vitamin E as virgin rats if they were to show the same fertility rate. This threshold ratio was the highest he encountered; it was more usually in the neighborhood of four or five to one.
Table 5. Results of histological examination of rats restricted to a ration containing lard and wheat germ oil.

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<thead>
<tr>
<th>Rat</th>
<th>Preg.</th>
<th>Degenera-</th>
<th>Weight: Sacrum-</th>
<th>Corpus</th>
<th>Fol.</th>
<th>Rat</th>
<th>Erythro-</th>
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High fat (ration V, table 1) plus wheat germ oil**

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<th>Degenera-</th>
<th>Weight: Sacrum-</th>
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High fat (ration V, table 1)

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<th>Fol.</th>
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Summary of histological examinations
### Summary of histological examinations

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<th>Number</th>
<th>Average</th>
<th>Number</th>
<th>Per cent</th>
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<td>High fat (ration V, table 1) plus wheat germ oil**</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>6</td>
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<tr>
<td>High fat (ration V, table 1)</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>4</td>
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**Diagnosed histologically and by the vaginal smear technique and weight curve.

**Wheat germ oil incorporated in the basal ration replacing 11 per cent lard.

***Eleven matings, no erythrocyte sign.

The histological examination revealed that the rat on high fat would have resorbed and the two on wheat germ oil also might have resorbed.
animals by the use of the vaginal smear technique before they were killed, and it showed that none had been in estrus since positive mating. The histological examination revealed that none had recently ovulated. No new corpora lutea were present and even those termed non-pregnant showed an increase in the size of the uteri, indicating a recent pregnancy or pseudopregnancy.

These findings are in agreement with the four rats which were continued on the wheat germ oil-lard ration. Three of these (57-A, 121-A and 148-A) went through 15 short resorption gestations or pseudopregnancies and two (57-A and 148-A) had normal resorption gestations (cf. tables 4 and 5). One rat (107-A) never showed implantation signs, however, although positive matings were ascertained 11 times. The 15 short resorption gestations or pseudopregnancies which occurred among the three rats (57-A, 121-A and 148-A) on the wheat germ oil-lard ration and whose reproductive behavior is shown in tables 4 and 5 are presented graphically in figure 2, curve I. Data for this graph were secured from the weights of those three rats while they were on ration V in which 11 per cent of the lard had been replaced by wheat germ oil. This pseudopregnancy or short resorption gestation curve is entirely in accord with the curve of 65 pseudopregnancies (curve II) which will be mentioned later (cf. fig. 3) and is presented here for comparative purposes. All the rats were old vitamin E-deficient animals which had undergone one or more previous resorptions. The curves run almost parallel until the 10th day, the plateau being reached the 10th to 11th day. The rather sharp decline in weight occurs on the 12th to 13th day.
Fig. 2. Comparison of average weight curves of rats during short resorption gestations and pseudopregnancies. Curve I: 15 short resorption gestations. Curve II: 65 pseudopregnancies.
and continues until the recurrence of estrus which in most cases fell on
the 15th day. The appearance of implantation signs was in close accord
in all these animals, occurring in most cases on the eighth to 10th day.

The significance of these findings may be interpreted thus: 1) it
is probably the vitamin E-destroying substances present in rancid lard
together with its sterilizing effect which affect the response of these
old vitamin E-deficient rats to vitamin E therapy, the sterilizing sub­
stances apparently decreased the implantation per cent and short re­
sorption gestations or pseudopregnancies were prevalent; 2) it is
apparently impossible to use the weight curve as a differential diag­
nostic criterion in distinguishing between short resorption gestations
and pseudopregnancies.

Kudrjasrov et al (111,112,113) have demonstrated that substances
in and isolated from rancid fat when fed per os or administered hypo­
dermically to normal rats will cause sterility in 100 per cent of the
cases. This sterility was characterized by normal ovarian function and
normal fertilization of ova, but the implantation per cent was markedly
reduced and in the cases where implantation succeeded, the development
was retarded early. The feti died and were resorbed during pregnancy
at about the eighth to ninth day of the gestation. These workers could

*Bacharach et al (14,15) recently have reported that animals which have
undergone a previous resorption gestation show a higher percentage of
failure of implantation (40 per cent) than virgin vitamin E-depleted
rats (11 per cent). He also noted that addition of vitamin E to the ra­
tion of either resorption-gestation rats or virgin E-depleted rats had
no significant effect on implantation rates. He suggested that the most
probable cause for reduced implantation rates of resorption-gestation
animals was that the process of resorption gestation itself produced
some permanent change in the animal's reproductive system.
at the same time show that this form of sterility was refractory to vitamin \( \mathcal{E} \) therapy.

On the basis of the data presented in this preliminary work it would seem justifiable to use virgin vitamin \( \mathcal{E} \)-depleted rats for test purposes, which had been restricted to avitaminosis \( \mathcal{E} \) rations for 90 days. The reasons for subjecting the animals to vitamin \( \mathcal{E} \)-free rations for that length of time (two to three weeks longer than is usual for test animals) were to avoid "initial fertility" and the time consuming procedure of proving their sterility.

Whatever the factors which affect the implantation rate and the response of vitamin \( \mathcal{E} \)-depleted rats to vitamin \( \mathcal{E} \) therapy may be, an attempt has been made to minimize them in the tests which were carried out for detecting vitamin \( \mathcal{E} \) in the various food materials all through this experiment. In most of the cases where the "cure" trial technique was employed the groups of rats were vitamin \( \mathcal{E} \)-depleted virgin rats and resorption gestation animals. Also, the "rear" trial technique has been employed. Thus the possible effect of resorption gestations on implantation rates and the higher requirement of vitamin \( \mathcal{E} \) by resorption gestation rats, which have recently been reported by Bacharach \((14)\), were minimized or excluded. The effect of sterilizing substances has also been avoided (cf. table 3, 4 & 5), since ration VI has been employed in the critical tests for the presence or absence of vitamin \( \mathcal{E} \) in certain materials used in this experiment.
Criteria used in the study of the physiology of reproduction during vitamin E deficiency and for the diagnosis of resorption gestations

a. Vaginal smear technique, weight curve and implantation sign.

When making biological tests for vitamin E it is necessary to determine the period in the estrus cycle at which the female will accept the male. Further it is important to determine if coitus, insemination and conception have taken place and whether a pregnancy or pseudopregnancy ensued.

For the diagnosis of these various incidents in the resorption gestation of rats on vitamin E-free diets one must resort to a rather complex technique. The vaginal smear technique as outlined by Long and Evans (115) and the weight curve are valuable guides.

The time of puberty is defined in many different ways. In this experiment the time of puberty in the female rat is understood to have taken place when the vaginal membrane disappeared spontaneously and vaginal smears could be taken. This took place at about the 40th to 60th day of life. Each vaginal smear was taken with a small glass rod having a burnished offset at one end. Just prior to taking each smear the glass rods were sterilized by boiling for 15 minutes in a beaker containing distilled water. The sterilized bent tip of a glass rod was then inserted into the vagina of the rat and followed by a gentle twist of the hand to obtain a smear of the epithelial cells of the endometrium. Shortly after coitus spermatozoa and later the vaginal plug may be obtained simultaneously with the vaginal smear. The smear thus obtained was placed on a glass slide in a drop of distilled water and immediately examined.
under the microscope. The epithelial cells, leucocytes or spermatozoa were easily distinguished in the field using darkened illumination. The stage of estrus, the finding of sperms or vaginal plug, as well as erythrocytes in the smear were noted, and recorded each day.

For the determination of the weight curve, the animals were weighed each day following the smear and all weights were recorded. This technique differed from that of Evans and Burr (68), who only weighed their animals at the time of positive mating and from the 20th to 25th day in and following the resorption gestation. All the rats on test in this experiment were weighed daily from the beginning of a test to the end of the gestation and five days thereafter. This procedure is important to distinguish between resorption gestations and pseudopregnancies. If pseudopregnancies occur the animals cease their gain in weight earlier than animals undergoing resorption gestations (cf. fig. 3) and this serves as a differential diagnostic guide.

According to Long and Evans (115) the time at which the female rat accepts coitus is in the beginning of estrus as determined by the vaginal smear. This, however, is not always true as rats in this experiment have been observed to accept the male in proestrus, as well as in metestrus. This is probably due to the fact that dissociation of the psychic estrus and vaginal estrus reaction occasionally takes place.

After coitus there is formed a sperma coagulum, "buchon vaginale" which occupies the upper space of the vagina and sends two prolongations into the cervical canals. It contains numerous spermatozoa in the upper part and in the prolongations. The sperma coagulum or vaginal plug
is expelled spontaneously, usually in a few hours but not longer than 24 hours, or it may be dissolved in the vagina. The spermatozoa can be observed in the vaginal plug or in the vaginal smear 12 to 24 hours after coitus. The presence of spermatozoa or vaginal plug is an assurance that insemination has taken place, but it reveals nothing about whether conception will ensue. The vaginal smear in the days following coitus, however, will give information about conception, as the animal remains in diestrus throughout gestation, which normally occupies 21 to 22 days. According to Long and Evans (115) there is an addition of erythrocytes to the smear on the 14th to 16th day following coitus. They describe this phenomenon as "placental sign", also called "erythrocyte" or implantation sign, and assume it owes its origin to the rupture of blood capillaries in the placenta. Evans and Burr (68) noticed the implantation sign occurring on the 13th to 15th day, Olcott and Mattill (156) on the 12th to 14th day. In this experiment it was detected on the 12th, 13th and 14th days and continued throughout the gestation in a great many resorption gestations. With the birth of young or at the close of the resorption gestation there is a ripening of ovarian follicles and the vagina reacts with a typical estrus period. Estrus following resorption gestation has been observed to occur from the 21st to the 25th day.

During normal pregnancy the weight of the female increases very little during the first eight to 10 days. From then on the gain in weight increases rapidly until shortly before the close of normal pregnancy. The birth of young results in a precipitous drop in weight and is then followed for a couple of days by a slight decrease after which
there is again a slight gain on the fourth to fifth day of the puerperium. According to Evans and Burr (68) the resorption gestation curve deviates from the normal on three points. There is a retardation in the gain in weight compared with the controls during the second half of pregnancy. The gain in weight ceases one day before gains stop in the controls and the normal steep drop typical of parturition in controls is substituted by a slow decrease, one to two grams daily the first four to five days of the puerperium. Evans and Burr, therefore, only weighed the animals from the 20th to 25th day.

Another physiological phenomenon in rats should be mentioned here; i.e., pseudopregnancy. By this is understood a condition resembling pregnancy in which the normal vaginal cycle is substituted by a shorter or longer diestrus period, while the uterine mucosa undergoes marked decidual proliferation. Local deciduomata or more or less diffuse decidual proliferations are formed in the uterine mucosa. This condition which is also known to occur in guinea pigs and rabbits occurs now and then in rats following sterile coitus and can be produced experimentally in different ways. Evans (57) observed that this condition occurred especially in vitamin B-free rats after sterile coitus in about 86 per cent of the cases, while in normal fed females it only occurred in three per cent of the cases. Pseudopregnancy is accompanied with a vaginal bleeding from the 10th or 11th day in the gestation. Clinically such bleeding cannot be distinguished from the usual vaginal bleeding which occurs later in normal pregnancy and resorption gestations. The duration of pseudopregnancies is reported to vary, the limits being from seven to 21 days.
and the average 14.5 days. Long and Evans found pseudopregnancies to last from eight to 23 days with an average of 14.53.

b. Differential diagnostic guides. In vitamin E tests it is important to distinguish between resorption gestations and pseudopregnancies or short resorption gestations, since the diagnoses of the gestations may reflect whether or not the animals are vitamin E-free, thereby conditioning the interpretation of an existent or subsequent test for vitamin E. The diagnosis is sometimes difficult. Evans (57) has called attention to an early vaginal bleeding in pseudopregnancy (10th to 11th day) as a valuable differential-diagnostic guide. In 65 pseudopregnancies in this experiment the bleeding was observed to occur on the eighth to 10th day in \( \frac{4}{5} \) cases, the other cases in four instances appearing earlier than the eighth and eight later than the 10th day of the pseudopregnancies. Since, however, vaginal bleeding in resorption gestations also can occur that early, even on the eighth to 10th day, this diagnostic guide is not always reliable. The weight curve based upon daily weighings is a much better criterion for the diagnosis. As a further guide it may be mentioned that the absence of vaginal bleeding occurs in pseudopregnancies and that late appearance of bleeding (13th to 20th day) never occurs in pseudopregnancies. Nor is an early recurrence of estrus a safe criterion for the diagnosis of a pseudopregnancy since estrus in rare cases may appear during the gestation, according to King (107), Donaldson (144) and Slonaker (181).

Figure 3 graphically presents a comparison of average weight curves of rats during normal pregnancy (Curve I), pseudopregnancy (Curve II) and resorption gestation (Curve III and IV). Data for these graphs were secured
Fig. 3. Comparison of average weight curves of rats during normal pregnancy, pseudopregnancy and resorption gestation. Curve I: 139 normal pregnancies; ration IV, V, VIII, IX and X, table 1, supplemented with vitamin E in the form of natural foodstuffs. Curve II: 65 pseudopregnancies; ration IV, V, VIII, IX and X, table 1. Curve III: 60 resorption gestations; ration IV, table 1. Curve IV: 59 resorption gestations; ration V, table 1.
from trials in this experiment. Curve II represents the average weights of rats during 65 pseudopregnancies while they were restricted to vitamin E-free rations (ration IV, V, VIII, IX and X, table 1). Curve III and Curve IV represent the average weights of 60 and 59 rats during their resorption gestations while restricted to vitamin E-free rations, ration IV and V, respectively. For comparison an average weight curve of 139 rats during their normal pregnancies is shown. All of these last rats had undergone a previous resorption gestation on vitamin E-free rations (ration IV, V, VIII, IX and X, table 1) and the normal pregnancies resulted from the administration of vitamin E in the form of natural foodstuffs.

The weights composing the pseudopregnancy curve were taken from older animals with one or more previous resorption gestations, since the phenomenon of pseudopregnancy is encountered mainly in older rather than virgin animals. The weights composing the normal gestation curve (Curve I) and the resorption gestation curve (Curve III) are average weights from older animals also. The weights composing the resorption gestation curve (Curve IV) of the 59 rats on ration V are all from virgin does with their first pregnancy.

From the graphs it will be seen that the curves run practically parallel until the eighth day of gestation. From the eighth day on, the normal gestation curve is much steeper and continues to rise until the 22nd day. The birth of young results in a precipitous drop in weight. The slopes in the resorption-gestation and the pseudo-pregnancy curves are not as great after the eighth day as the normal
pregnancy curve, and the following days they all reach a plateau which is followed by a gradual but marked decline. It will readily be seen that pseudopregnant rats plateau and decline in weight much earlier than in the case of resorbing rats, and this difference serves as a valuable differential-diagnostic criterion in distinguishing between the two pregnancies. The curves of the resorption gestations are in close accord with the anatomical findings encountered in pregnancies in avitaminosis A as reported by Evans and Burr (68) and Urner (195).

Evans and Burr noticed hypoplasia of the feti on the eighth day and a retardation in growth. On the 13th day the feti died. Urner called attention to the increased growth of the mammary tissue in the following days. The placenta also continued to grow but necrosis developed in it on the 16th to 18th day. From the 18th to 22nd day there was involution of the mamme. All these conditions resulted in a gradual but marked loss in weight.

Summarizing these deliberations and the results from the data presented it can be stated: the early implantation sign and the early recurrence of estrus are less dependable criteria for differentiating between pseudopregnancies and resorption gestations while in doubtful cases the weight curve is the safest criterion for use in diagnosing whether a pseudopregnancy* or a resorption gestation is at hand. All the test animals were therefore weighed every day during the test period.

*It should be mentioned here also that a pseudopregnancy may be confused with a short resorption gestation, a condition brought about when sterilizing substances are present in the ration (cf. fig. 2).
5. Methods used in the biological estimation of vitamin E.

No assay for vitamin E is possible in the absence of an international or other accepted unit and a standard preparation to be used in a test concomitant with that on the test substance. Comparative evaluation, however, as distinct from assays, can be carried out between any two sources of vitamin E tested simultaneously, in so far as we have knowledge of the relationship between dose and response and of the variation in effect shown by the animals used in the test. The response of vitamin E-deficient animals to the vitamin being of the "all or none" type has to be treated as a quantum since there is no possibility of measuring or assessing gradation in the outcome. In other words, it is necessary to take groups of adequate size and determine the percentage of positive responses given by all the members of each group. Obviously the biological estimation of vitamin E requires, therefore, a large number of animals and a great deal of time. Vitamin E-free female test rats must always be on hand, as well as males reared on normal nutritive diets and of proved fertility. The latter can be secured and ascertained by first mating them with normal females reared on standard rations. Rations which can be used for the depletion of test animals have been described previously.

In quantitative and qualitative biological determinations of vitamins one can choose between: 1) preventing appearance of the pathological symptoms, prophylactic method; and 2) induce the pathological symptom complexes to disappear, so-called curative method. Since testicular changes caused by avitaminosis E cannot be cured, only the prophylactic
method can be used on male rats. Evans and Burr (68) have outlined methods for biological determinations of vitamin E. They have used the curative as well as the prophylactic method in qualitative determinations.

a. Curative method. Female rats which have been depleted of their body reserves of vitamin E and have proven their sterility by going through a resorption gestation are termed and listed as test animals. Such rats are given the food material to be tested and mated to proved fertile males. If the resulting gestation permits normal litters at term the sterility induced by avitaminosis E has been cured and the food substance is considered to contain vitamin E. If resorption gestations ensue the food substance is considered to be devoid of vitamin E.

b. Prophylactic method

1. Females. Weanling female rats of uniform age and weight are placed on a vitamin E-free basal ration and the food substance to be tested is fed as a supplement. A negative control group simultaneously is carried along on the basal vitamin E-free ration. At the end of the normal depletion period all animals are mated to normal fertile males and the gestations diagnosed. The production or absence of litters in the test group will serve as a differential diagnostic criterion.

Evans and Burr (68) describe and criticize this method and caution against its use because of the phenomenon of "initial fertility". At the same time they call attention to the fact that the prophylactic method is a particularly sensitive instrument for detecting small traces of vitamin E in foods. However, when a detailed study of these workers' memoirs is undertaken, it is perplexing to note that they have used the
prophylactic and curative method alternatively. Only in a few of their experiments have they used the curative method. In most cases they began the administration of the vitamin E-containing substance a shorter or longer period before the test gestation. Also in their determination of the minimum amount of a daily effective dose they have resorted to this deviation (see table 55-58 (68)). The caution expressed by these workers was timely; however, in view of our broader knowledge to date in composing basal rations completely devoid of vitamin E the prophylactic method is safe. If basal rations which are free of this factor are employed and animals of uniform weight and age are selected "initial fertility" can be avoided entirely. Ringsted (165) has demonstrated that an avitaminosis E ration will cause initial sterility in 100 per cent of the cases (30 to 50 days on vitamin E-free ration), when the mothers of weanling rats are on a low but sufficient amount of vitamin E. His findings are in agreement with the experiences encountered in this experiment.

Therefore, it would seem justifiable to assume that the prophylactic method, "rear" trials, offers a more critical test for vitamin E than the cure method, "cure" trials, when attempting to detect traces of vitamin E in a food substance especially if the ration is made up entirely of the test material. Thus the rats would be induced to consume and add to their original stores of vitamin E, if it was present in the test substance. Furthermore, any effect of age would be eliminated as Evans ((68) page 4, footnote 1 and page 102, footnote 1) has mentioned that aged vitamin E-deficient animals require somewhat larger amounts of curative doses of wheat germ oil. *
*cf. also the footnote on page 97, this paper.
The basal ration employed may have a greater influence on the outcome of a test for vitamin E if sterilizing substances were present (cf. table 3, 4 & 5). An attempt has been made also to minimize these factors in the trials carried out in this experiment (cf. p. 102).

Summarizing these deliberations it may be assumed that when bio-tests for vitamin E employing the "reex" trial technique are negative, the material tested is extremely low in vitamin E if not entirely devoid of this factor.

1. Males. Ringsted (165) has demonstrated that testicular changes take place among weanling rats following a depletion period of 30 days. Using these facts, it is possible to use male rats for biological determination of vitamin E and obtain results in a short period of time, since avitaminosis E is associated with characteristic lesions in the germinal epithelium. These changes can be prevented by prophylactic administration of vitamin E-containing substances.

The practical application of this test has been outlined by him. A number of 30-day-old weanling male rats are placed on a vitamin E-free ration. A number are fed the food substance to be checked for vitamin E. The test material is administered as daily supplements. Simultaneously a negative control group is kept on the basal ration. After a period of 30 days the animals are killed and the testes are examined histologically. The production or absence of lesions in the test group serves as a differential diagnostic criterion.

g. Administering the test substance. In quantitative and qualitative tests for vitamin E the prophylactic as well as the curative method
can be employed. However, it must be remembered that only female rats can be used if one resorts to the curative method. When making quantitative tests employing either method the principle is to determine the least effective dose of a test substance which prevents the appearance of the pathological symptoms (prophylactic method); or induces the pathological symptom complexes to disappear (curative method). By using the curative method the choice can be made between determining the smallest effective single dose of a food substance which will permit a normal litter at term, or the smallest effective single dose administered during the 21 days of the gestation, and which will result in the birth of a normal litter. The latter method although more time consuming is preferable to the former and in many cases necessary when testing for small amounts of vitamin E in a food substance, since the daily consumption of a rat is limited. The tests for vitamin E in this experiment have been chosen to detect diminutive amounts or traces of the vitamin and consequently no attempt has been made to make quantitative determinations for this factor. All the tests have been qualitative estimations made on a comparative basis, and interest has concentrated on inducing the test animals to consume as much of the test material as possible. The vitamin E-depleted females were therefore fed throughout the gestation in most cases and also during a shorter or longer preliminary period before mating in "cure" and "rear" trials. This interval allowed a period during which any vitamin E in the ration could take effect. According to Evans and Burr (68) the latest day a curative dose of vitamin E can take effect and rescue a gestation is on the fifth day after
conception, and the critical period (i.e., the period during which its presence must be assured) extends approximately from day five to day 20 of the gestation.

The practical determination of vitamin E in the various food substances was carried out as follows: After the female rats had reached puberty and were depleted of their vitamin E reserves, daily vaginal smears were run to determine the stage in the estrus cycle. When the females were 70 to 90 days old, the animals which exhibited regular estrus were mated to normal males. When the resorption gestation was completed, the rat was ready for use as a test animal. In some instances virgin vitamin E-depleted female rats 90 days of age were used as test animals. Four to eight test rats of approximate uniform size and age made up the test group. Administration to the test animals of the food substance which was to be tested for vitamin E was started at once or as soon as insemination had been ascertained. Following each preliminary feeding period or when it was decided to mate the females, males of proved fertility were placed with the group of test rats. From the time the males were placed with the females, daily vaginal smears and weights were observed and recorded. The males remained with the females until positive mating had been ascertained for all the females in the group and smears typical of the diestrus period appeared. The males were then removed and returned to the stock colony. The male rats used were from the stock colony and received the normal rat ration (ration III, table 1) except during the mating period. At least two days before being placed in the mating cage the males were restricted to a vitamin E-free ration.
This practice was followed to minimize the possibility of the females' getting vitamin E from the feces of the males.

Positive mating was diagnosed by finding spermatozoa in the vaginal smear. If the vaginal smear on the fourth to fifth day of the gestation showed that the animal was in estrus again, feeding the test food was terminated and the rat disposed of, since it was useless as a test animal. A vaginal bleeding on the eighth to 10th day suggested that a pseudopregnancy might be suspected. However, this early bleeding also occurred in short resorption gestations. If the erythrocyte sign appeared on the 12th to 15th day, the animal was considered to be pregnant and the administration of the test substance was continued until and including the 21st day. The continued observation of the vaginal smear and the weight curve often gave a clue about the result as early as the 15th to 18th day, since the vaginal smear on account of the necrosis of the feti contains detritus and quite often animals going through a resorption gestation will continue to show blood in the smear through the resorption gestation from the 12th day. Continued observation of the pregnant animal until the close of the gestation (22nd day inclusive) determined positively whether a resorption or a normal pregnancy terminating with the birth of live young at term had occurred. The finding of young or a sudden drop in weight sufficient to indicate the birth of a litter on the 22nd or 23rd day was regarded as failure to resorb and a positive indication of presence of vitamin E. An early recurrence of estrus on the 14th to 15th day during the gestation (pseudopregnancy or short resorption gestation) was also regarded as failure to resorb but such animals
were discarded. Resorption of young was taken as evidence of the freedom of the ration from vitamin E. In doubtful cases of resorption gestations the animals were killed and an autopsy performed in order to ascertain other eventual morbid causes of resorption.

C. Procedure Used in Testing the Goat Rations for Vitamin E

1. Testing of materials treated with ferric chloride

Through the entire experimental period the experimental goat ration (ration II, table 1) was tested biologically for vitamin E before being fed to the goats. Vitamin E-depleted female rats were placed on modifications of ration II to be checked for resorption gestations. The composition of these modified rations (ration VIII, IX and X) as they were fed to the rats are listed in table 1. In the first part of the experiment the test animals used were adult rats which had been restricted to ration IV (table 1) and depleted of their vitamin E reserves as diagnosed by a resorption gestation (209). In the latter part of the experiment the test animals used were weanling female rats reared for 90 days on the basal depletion ration (ration V, table 1). These virgin vitamin E-depleted test rats were used in groups along with resorption gestation animals.

a. Preliminary tests. Before the experiment proper was begun, a number of preliminary observations were made on the methods to be used (209). This preliminary work was primarily to determine if the apparatus was adequate, if the treatment was effective in the destruction of
vitamin E and if the ration so treated was palatable to the goats.

The preliminary work covered the period of February 28, 1933, to July 1, 1933. During this time biological tests of the ferric chloride treated hay and the grain mixture were each made at intervals of approximately two weeks. Thus the experimental rats used for testing received the same treated batch of goat feed for about two weeks. The treated grain mixture and the treated hay were tested separately. The grain mixture (ration IIa, table 1) was very finely ground and fed as the sole ration (ration VIII) to the vitamin E-depleted female rats. The treated hay (ration IIb, table 1) was finely ground and 20 parts by weight were mixed with 80 parts of ferric chloride treated stock ration (ration IV, table 1).

In view of the possible harmful results of feeding a ration containing one per cent ferric chloride to the goats continuously a preliminary test was initiated to determine if such a high concentration of ferric chloride in the ration was necessary to destroy the vitamin present. The method of inactivating vitamin E here attempted was the same as has been outlined previously except that 0.25 per cent by weight of ferric chloride instead of one per cent was used in treating the feed. A sample of 15 pounds of ration Ia was treated by this method on September 17, 1933. This treated ration was then ground finely and fed to vitamin E-depleted female rats (209).

b. Routine tests of the experimental goat ration. As soon as the preliminary work was completed and the actual experimental trials begun, much more extended rat feeding tests were initiated. The continuous
treatment of the hay and grain mixture to supply a sufficient quantity for the consumption of the goats made an adequate check of the ration for vitamin E no small item. "Current" tests which are described below constitute the major portion of the test trials on the experimental goat ration in the first part of the experiment until July 1, 1935. In addition some monthly composited samples were tested for the presence of vitamin E.

1. "Current" tests. In the "current" tests the treated goat feed was given to the test rats at the same time as the goats were receiving the ration. Proportionate quantities of all batches of feed treated during an interval of two weeks or more, depending on the rate of treatment of the feed, were ground together and fed immediately to a group of test rats (ration X, table 1). Thus, while the goats were consuming the treated ration, the feed was being tested for vitamin E with rats. The group of test rats consisted of adult vitamin E-depleted females, which, following proof of resorption, were placed on the current goat ration. Once placed on the treated goat ration the test rats received only this ration until they died or were killed. As long as the rats showed estrus cycles they were placed with males at each heat period. The history of all matings and resulting gestations was traced by means of vaginal smears and weight curves (209).

II. Test of monthly composite samples. The "current" tests of the goat ration previously described were not begun until October, 1933. Prior to that time the tests of the goat ration for vitamin E were made on monthly composite samples of the treated feed. The "current" tests
and monthly composite tests were carried on together until June, 1934, when the monthly composite sample tests were discontinued in favor of the "current" tests. The "current" tests were more desirable since there was a shorter period between the treatment and the test of the feed. Any possible inactivation of the vitamin B by the ferric chloride between the time the goats received the ration and time the ration was tested thus would not be a factor under the "current" tests. However, the results of monthly composite sample tests are valuable for the period at the beginning of the experiment not covered by the "current" tests and as supporting evidence to the "current" tests.

The composite samples tested were made up of proportionate amounts of each batch of feed treated during the month. They consisted of three parts of ration IIIa and one part of ration IIb (table 1). The ingredients were thoroughly mixed and finely ground and fed to vitamin B-depleted female rats. The reproductive performance of the rats was observed while they were receiving the ration (209).

iii. Testing the goat ration for vitamin E following modification of feed treatment. Since it had been demonstrated that an occasional litter could be encountered in the treated goat ration immediately following removal of the ether solvent (cf. table 2), it was decided to take further precaution against the goats receiving any vitamin E. The changes in method of procedure after treating the goat ration have been outlined previously. The ferric chloride treated grain mixture and the hay were collected in batches of 200 pounds and 100 pounds, respectively. Composite samples from the hay and from the grain mixture were fed after
they had aged sufficiently to vitamin B-depleted rats. After positive mating to fertile males the gestation of each female was diagnosed by the vaginal smear technique and the weight curve. The samples of treated and aged grain mixture were fed as ration VIII (table 1), those of the treated hay, as a mixture of three parts of vitamin B-free grain mixture and one part of hay (ration IX, table 1). For each batch of feed tested, groups of four to six females were used. The rats received the ration for a preliminary period preceding mating and continued throughout the gestation. If the animals so fed resorbed their young, the batches of ration IIa and IIb were considered vitamin B-free and were then fed to the goats.

Measuring the efficacy of method employed in inactivating vitamin E in goat ration II. An attempt was made to ascertain whether the treated, aged and biologically tested experimental ration contained any appreciable amount of vitamin E. "Rear" trials employing weanling female rats were instituted since it was believed that this method offered a more critical test for detecting traces of the vitamin. Since the daily consumption of food material by a rat is limited an effort was made to concentrate any possible traces of the vitamin which might have remained in the treated ration. The concentrate would be equivalent to huge amounts of treated unextracted materials and when fed would induce the rats to consume large amounts of vitamin E in a short period of time.

1. "Rear" trials employing weanling female rats. A group of 21-day-old weanling female rats weighing from 30 to 40 grams were placed
and reared on ration X (table 1), a modification of goat ration II. The proportion of grain mixture to that of hay in ration X is approximately 3:1. This is the same ratio of grain mixture to that of hay as was used throughout the experiment when alfalfa was included in the ration for rats. The supplements of cod liver oil and yeast were included to protect against other avitaminoses in the growing rats. After they had received this mixture during a period of four to five months, they were mated to normal fertile males and their gestation diagnosed. Subsequently a number of these female rats reared on ration X were continued on this diet and fed a supplement of one cc of wheat germ oil per rat daily. This latter group served as a positive control.

ii. Test of concentrates prepared from the experimental goat ration. During the process of recovering the ether solvent and as the evaporation proceeded, the fat-soluble material was carried down with the ether. After the solvent had been completely recovered by evaporation, a fatty residue of the hay and of the grain mixture remained adhering to the bottom of the container. This fatty residue from the hay and grain mixture was collected, stored, and aged for the same length of time and under essentially the same conditions as have been outlined above. The residue from the hay and grain mixture was extracted with ether and a dark brown oil remained after the ether had been recovered. This oil was tested biologically for vitamin A in a "cure" trial. The oil replaced the lard (22 per cent) in basal ration V which was fed during a preliminary period of 37 days. The animals were then mated and the gestation diagnosed.
2. Test of untreated materials

a. Untreated supplements. Though cod liver oil and yeast are notably free from vitamin E, both substances were tested before being used. The yeast and cod liver oil were tested separately and incorporated in various basal vitamin E-free rations.

b. Testing of the untreated goat ration. From time to time groups of vitamin E-depleted female rats were placed on a modification of the untreated goat ration (ration VIII, table 1), mated and their gestation diagnosed. These rats served as positive controls. The animals were fed the material for approximately the minimum length of time the rats in the preliminary and routine tests received the treated material. An attempt was also made to determine the minimum amount of untreated feed required to rescue a gestation. Groups of vitamin E-depleted rats were placed on basal vitamin E-free ration VI and fed the untreated grain mixture (ration Ia, table 1) and the hay (ration Ib, table 1) as supplements. The levels fed to each rat were five and 12 grams respectively over a period of six days.

D. Procedure Used in Measuring the Effects of Prolonged Restriction of Animals to the Ferric Chloride Treated Experimental Ration

1. Reproduction

a. Goats. The reproductive performance of the male and female goats was observed throughout the experiment. Their fertility was measured by
various indices. The fertility of those males allowed to reach maturity was measured by the number of matings which produced offspring, or their virility was estimated by microscopic determination of the percentage of motility of their sperms. Also histological studies of the reproductive organs of the male goats were made. At the time they were killed the appearance of the germinal epithelium was judged both in the mature and in the immature animals. The microscopic appearance of the sperms in the epididymus was likewise judged. By comparing the various histological sections with the normal the relative fertility and virility of the animals could thus be obtained.

The fertility and fecundity of the female goats was compared with that of does at other stations reared under usual conditions. Their estrus periods, breeding history and the number of matings and gestations were observed and recorded. The sex ratio of the kids born to the does was compared with that obtained from does at other stations.

b. Rats. A number of weanling male and female rats were reared on a modification of the ferric chloride treated experimental goat ration. Their reproductive behavior was similarly studied. The "rear" trials with these rats served as a more critical test for possible traces of vitamin E in goat ration II and at the same time gave histological material for comparative studies with that of the goats under similar conditions.

The female group has been described previously. The male group consisting of 21-day-old weanling rats weighing from 30 to 40 grams was placed and reared on ration X (table 1). At sexual maturity the males
were observed for their reproductive behavior by mating them to normal virgin females which had been reared on ration III (table 1). Each male's potency was measured by mating him to several females and following the vaginal cycle on the latter by the vaginal smear technique. The females received the stock ration (ration III) except during the proestrus and estrus periods. The males received a commercial preparation of wheat germ meal to the extent of 15 per cent of the ration as a supplement to ration X after several matings failed. The sexual behavior of males and females was again observed as mentioned above. Finally the females were mated to normal males from the stock colony and the gestations diagnosed. The males which had been reared on the vitamin E-free ration were sacrificed at different intervals during this test and histological studies of the testes were made.

2. Growth and development of the goats

The goats were weighed at time of birth and at weekly intervals until they were killed or terminated. The birth weights of the kids, their gain in weight during the first 26 weeks and the weights of those which were allowed to reach maturity were compared with the weights of goats reared under usual conditions at other stations. Any abnormalities which occurred in the goats during the course of the experiment were noted and recorded. A photographic record of the goats in the various stages of development was also kept.
3. Determining the effect of ration on the vitamin E content of milk and certain body tissues

It has been amply demonstrated that vitamin E is present in a goodly variety of animal tissues (36,66,67). That storage of vitamin E in certain body tissues occurs is indicated by the survival of fertility in female rats given appreciable amounts of vitamin E and then shifted to an avitaminosis E regime. The length of survival of fertility is correlated with the richness of vitamin E in the original regime. Continued avitaminosis E, however, depletes the body stores of this factor. Notably the body tissues of the rat become depleted when they are restricted to rations devoid of this factor (68).

Failure to induce nutritional sterility among goats restricted to a ration which invariably produced sterility in female rats naturally suggested that either the vitamin reserves of goats are not readily decreased to the point of inducing reproductive abnormalities or vitamin E might possibly be synthesized by the goat. A study of the effect of prolonged avitaminosis E on the reduction of the amount of this factor occurring naturally in milk and certain tissues of goats was therefore undertaken. Comparative tests were made on the occurrence of this factor in the milk and tissues of goats under usual farm conditions and under experimentally induced avitaminosis E. Materials from beef cattle were also selected for a comparative study of their storage of vitamin E.

a. Source of materials for study. The materials studied in this trial were obtained from first and second generation male and female
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goats reared and restricted at all times to the vitamin E-deficient experimental ration (ration II, table 1). These materials included milk, butterfat, muscle and adipose tissue. The milk was obtained from lactating does shortly after parturition. The muscle and adipose tissue were secured from male and female goats killed at various ages ranging from 11 days to one and one-half years. Included in the former was muscle tissue from male kids 11 to 45 days of age. Table 6 shows the individual goats restricted to the ferric chloride treated experimental ration from which certain body tissues and secretions were obtained for vitamin E tests. (cf. Chart I).
Table 6. Goats restricted to the ferric chloride treated experimental ration from which certain body tissues and secretions were obtained for vitamin E tests

<table>
<thead>
<tr>
<th>Materials obtained for tests</th>
<th>Description of goats furnishing materials</th>
<th>Male number: Female number: Generation*: Gestation: offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk and butterfat</td>
<td>44-N, G1, 2nd</td>
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</tr>
<tr>
<td></td>
<td>44-P, G1, 2nd</td>
<td></td>
</tr>
<tr>
<td></td>
<td>44-S, G2, 1st</td>
<td></td>
</tr>
<tr>
<td></td>
<td>44-R, G1, 2nd</td>
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<tr>
<td></td>
<td>44-O, G2, 1st</td>
<td></td>
</tr>
<tr>
<td>Muscle tissue</td>
<td>H-1, G1, 1st</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N-1, G2, 1st</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N-2, G2, 1st</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F-1, G2, 1st</td>
<td></td>
</tr>
<tr>
<td></td>
<td>O-1, G2, 1st</td>
<td></td>
</tr>
<tr>
<td></td>
<td>O-2, G2, 1st</td>
<td></td>
</tr>
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<td>44-J, G1, 1st</td>
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<tr>
<td></td>
<td>44-P, G1, 2nd</td>
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</tr>
<tr>
<td></td>
<td>44-S, G2, 1st</td>
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<tr>
<td></td>
<td>44-O, G2, 1st</td>
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</tr>
<tr>
<td>Adipose tissue</td>
<td>E-1, G1, 1st</td>
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<td></td>
<td>H-1, G1, 1st</td>
<td></td>
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<td>N-1, G2, 1st</td>
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<td></td>
<td>N-3, G2, 1st</td>
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</tr>
<tr>
<td></td>
<td>G-1, G1, 2nd</td>
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<td></td>
<td>F-2, G2, 2nd</td>
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<td></td>
<td>K-2, G2, 1st</td>
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<td></td>
<td>D-2, G1, 2nd</td>
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</tr>
<tr>
<td></td>
<td>D-3, G1, 2nd</td>
<td></td>
</tr>
</tbody>
</table>

*G₀, G₁, and G₂ denote parental, first and second generations respectively.

The materials for comparative studies were obtained from goats reared under usual farm conditions, and included milk and muscle tissue. The milk was purchased from a farmer who kept goats. The muscle tissue was secured from adult goats and kids of approximately the same age as the
goats fed the vitamin E-deficient ration. The beef cheek muscle was obtained from cattle which were slaughtered at the Iowa State College Meat Laboratory.

b. Preparation and care of milk, butterfat and tissue samples. Careful precautions were taken to minimize fermentative or oxidative changes likely to occur in the test materials and in the basic ration before they were fed to the test rats. The basic ration was made up twice weekly and kept under refrigeration. The milk was fed fresh twice daily as an addition to the basic ration. Cream was separated every five days from accumulated samples of refrigerated milk and immediately churned into butter which was promptly melted on a steam water bath and centrifuged. The butter oil was then decanted into glass containers, sealed when cool and held at -15° C. for testing subsequently.

Immediately following the termination of each goat the muscle and adipose tissues were severed and separated. Then the tissues were ground finely, collected separately in jars and cooled at +1°C. After thorough cooling they were stored at -15° C. for testing later. The beef cheek muscle was handled in a similar manner.

c. Methods of feeding the test materials. The milk, butterfat, muscle and adipose tissue were tested by feeding these materials to groups of vitamin E-depleted rats according to the "cure" and "rear" procedures described by Evans and Burr (63). The rats received the materials as supplements to basal vitamin E-free ration VI (table 1). Concurrently positive control groups of rats were fed these materials plus one cc daily of biologically tested wheat germ oil. Two negative
control groups receiving only the basal vitamin E-free ration also were included for comparison. Following preliminary feeding periods ranging from six to 109 days the rats were mated and their gestations diagnosed by the vaginal smear technique and weight curve.

E. Anatomical and Histological Studies

Autopsies were made of all the goats used in this experiment at the time they died or were killed. Histological examinations of certain endocrine glands and tissues of these goats were undertaken and certain rats representative of certain test groups were also examined histologically. The histological examinations and the autopsies performed were carried out through the courtesy of the Iowa State College Veterinary Research Laboratory. Standard procedures of histological technique were used in preparing the sections. Certain results from these studies will be discussed and photomicrographs of the tissues and pictures of the organs from some of the animals used will be presented.
IV. RESULTS

A. Vitamin E Tests of the Ferric Chloride Treated and Untreated Goat Rations and Supplements

The data obtained from the vitamin E tests of the goat rations and the untreated supplements used in this experiment are summarized in table 7. Composite samples of the ferric chloride treated goat ration II and the untreated supplements were fed as modified rations to 350 vitamin E-depleted female rats which after positive matings resulted in pregnancies. All 350 pregnancies terminated in resorption gestations.

On the other hand when vitamin E-depleted female rats were fed the basal ferric chloride treated goat ration II supplemented with wheat germ oil or the untreated goat ration I was administered, normal pregnancies and litters were produced at term with a few exceptions. The duration of the preliminary period in which the animals received these materials ranged from three to 147 days. The total number of days during which the animals received the material varied from six to 169. Thus the animals were given every possible opportunity to receive any vitamin E present in the feed, to build up their body storage of this vitamin and make it available for the rescue of the gestation.

In testing the untreated goat ration I an attempt was made to ascertain the minimum amount required to rescue a gestation (cf. table 7). The
Table 7. Summary of vitamin E tests made on treated and untreated goat rations.

<table>
<thead>
<tr>
<th>Materials tested</th>
<th>Description of material</th>
<th>Amount of material or supplement fed</th>
<th>As percent of ration</th>
<th>Per rat daily (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain mixture IIa (VIII)</td>
<td>Grain mixture</td>
<td>39.0</td>
<td>20.0</td>
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</tr>
<tr>
<td>Alfalfa IIb (IV)</td>
<td>Alfalfa</td>
<td>ditto</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain mixture IIa* **</td>
<td>Grain mixture</td>
<td>89.0</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td>Mixture of IIa &amp; IIb (X)</td>
<td>&quot;Current&quot; samples</td>
<td>89.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>ditto (X)</td>
<td>Monthly composite samples</td>
<td>89.0</td>
<td>ditto</td>
<td></td>
</tr>
<tr>
<td>Grain mixture IIIa (VIII)</td>
<td>Aged samples</td>
<td>89.0</td>
<td>ditto</td>
<td></td>
</tr>
<tr>
<td>Alfalfa IIb (IX)</td>
<td>ditto</td>
<td>22.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixture of IIa &amp; IIb (X)</td>
<td>Mixture of grain &amp; hay</td>
<td>89.0</td>
<td>ditto</td>
<td></td>
</tr>
<tr>
<td>ditto plus wheat germ oil (X)</td>
<td>ditto</td>
<td>89.0</td>
<td></td>
<td></td>
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<tr>
<td>Ether extract of IIa &amp; IIb (V)</td>
<td>Aged mixture of grain &amp; hay</td>
<td>22.0</td>
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<tr>
<td>Cod liver oil (IV)</td>
<td>Squibb &amp; Son</td>
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<tr>
<td>ditto (IV)</td>
<td>Helgeng &amp; Lugtigher</td>
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</tr>
<tr>
<td>ditto (V)</td>
<td>ditto</td>
<td>5.0</td>
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<td>ditto (V)</td>
<td>ditto</td>
<td>24.0</td>
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<td>Yeast (IV)</td>
<td>Northwestern Yeast Co.</td>
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<tr>
<td>ditto (IV)</td>
<td>ditto</td>
<td>10.0</td>
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</tr>
<tr>
<td>Mixture of Ia &amp; Ib (VIII)</td>
<td>Mixture of grain &amp; hay</td>
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<tr>
<td>Grain mixture Ia (VI)</td>
<td>Grain mixture</td>
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</tr>
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<td>ditto</td>
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</tr>
<tr>
<td>Alfalfa IIb (VI)</td>
<td>Alfalfa</td>
<td>5.0</td>
<td></td>
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</tr>
<tr>
<td>ditto (VI)</td>
<td>ditto</td>
<td>12.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Rats which died, nonbreeders, and those which showed pseudopregnancies, are not included.
** Roman numerals in parentheses denote number of ration in table I used in making these tests.
*** The grain mixture in this test had been treated with 0.25 per cent of ferric chloride.
††† Includes 45 rats not tested for "initial fertility" following the 90 day depletion period.
‡‡‡‡ Each test rat consumed approximately 10 grams total supplemented ration daily. There were 3,500 grams of ferric chloride treated goat ration II.
Part of the cod liver oil in this test replaced all the lard in basal ration V.
vitamin E tests made on the ferric chloride treated goat ration and supplements.

<table>
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<th>Material or Supplement</th>
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<th>Number</th>
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<th>Average</th>
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<tr>
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<td>Per rat:</td>
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<td>Type of test:</td>
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<td>Number:</td>
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<td>Per rat:</td>
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<td>Per rat:</td>
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<td>Type of test:</td>
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<td>Per rat:</td>
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<td>Total:</td>
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</tr>
<tr>
<td>Average:</td>
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eliminations of goat ration II.

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<thead>
<tr>
<th>&quot;Cures&quot;</th>
<th>6</th>
<th>32</th>
<th>22</th>
<th>54</th>
<th>6</th>
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<td>&quot;</td>
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<td>33</td>
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Routine tests of goat ration II.

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<tr>
<th>&quot;Cures&quot;</th>
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<td>22</td>
<td>22</td>
<td>44</td>
<td>116</td>
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Methods employed in inactivating vitamin E in goat ration II.

<table>
<thead>
<tr>
<th>&quot;Rears&quot;</th>
<th>15</th>
<th>147</th>
<th>22</th>
<th>169</th>
<th>16</th>
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Tests of supplements

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<th>34</th>
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<td>22</td>
<td>49</td>
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<td>29</td>
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Tests of goat ration I.

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<th>15</th>
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<th>12</th>
<th>90</th>
<th>7.5</th>
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are not included.
in making these tests.
ferric chloride only.
by deplation period.
by deplation period.
daily. Therefore each rat must have consumed ether extract equivalent to that present in ration V.
total amount fed to each rat over a period of six days varied from five to 12 grams. Of 12 rats so fed, five resorbed their young, and seven had normal litters at term. The five rats which resorbed their young were killed on the 22nd day of the gestation period and autopsies were made. The rats had fully developed, but dead feti. The feti, however, were edematous and showed initial signs of disintegration. These post-mortem findings suggest the probability that the amount of feed material allowed per rat in these tests contained borderline amounts of vitamin E. However, it is significant to note that there was apparently no difference in the outcome, whether the rats consumed five or 12 grams of test material. Variability in individual rats in responding to vitamin E therapy may account for the variation in these results.

Rats kept exclusively on the untreated goat ration I for a total period of 28 days all produced litters at term. In the preliminary tests and the routine tests of the ferric chloride treated goat ration II the total number of days the rats received the test material was not less than 33, so the animals were allowed ample time to make use of any vitamin E which may have escaped inactivation. All these tests gave negative results for vitamin E.

The untreated supplements were incorporated in basal vitamin E-free rations, fed and tested as such (cf. table 7, tests of supplements). The percentage of material substituted in the basal rations for these rats was never lower than the corresponding percentage used in the modified rations of the experimental goat ration (cf. table 1 and table 7). The minimum length of time during which these supplements were fed to the rats covered
a period of 29 days. All these tests gave negative results.

Although the routine testing of the ferric chloride treated goat ration II for vitamin E gave remarkably uniform negative results, it was considered advisable to conduct a more critical analysis of the treated goat ration. Evans and Burr (68) and Evans et al (72) had reported that aged vitamin E-depleted rats might vary widely in their response to vitamin E therapy. Some females fail to respond to such therapy. Therefore, in order to measure the efficacy of the method of treating and testing the experimental ration, 16 weanling female rats were reared on the ferric chloride treated, aged and biologically tested goat ration II (cf. table 7). The animals were restricted to this ration for 169 days. All positive conceptions resulted in resorption gestations. Nine of the 16 animals subsequently were continued on this ration, but in addition they received one gram of biologically assayed wheat germ oil daily. These nine animals bore normal young at term. Furthermore, an attempt to concentrate possible traces of vitamin E in the ferric chloride treated and aged goat ration II was carried out. An ether extract of the aged fatty hay and grain residue, which has previously been described, was substituted in the basal low fat rat ration (cf. ration V, table 1). Six vitamin E-depleted rats were used in this trial. All animals resorbed their young (cf. table 7). Assuming that an adult rat will consume 8 to 10 grams of feed per day, the amount of concentrate each of these animals consumed over a period of 59 days was estimated to range from 100 to 130 grams. These quantities of concentrate correspond to approximately 2800 to 3500 grams of feed material of the ferric chloride treated goat
ration II.* This material was equivalent to approximately 12 to 15 times as much as the maximum amount of corresponding material fed of the untreated goat ration I or 300 to 700 times as much as the minimum amount which enabled the rescuing of a gestation. Even under these conditions it was not possible to obtain positive tests for vitamin E.

Resorption gestation curves of the rats employed in the routine tests for vitamin E in the ferric chloride treated experimental goat ration are presented graphically in figure 4. Data for these graphs were secured from the weights of 295 rats whose reproductive performances are recorded in table 7. A normal gestation curve is presented for comparison and includes the data from 139 rats which had undergone one or more previous resorption gestations on vitamin E-free and ferric chloride treated rations (cf. ration IV, V, VIII, IX and X, table 1) and whose normal or successful pregnancies** resulted from administering vitamin E as it is found in natural foodstuffs. The resorption gestation curves II, III, IV and V are in close agreement with those presented previously in figure 3. The curves in figure 4 are almost all parallel until the eighth day of the gestation. From this point the resorption gestation curves deviate quite markedly from the normal one, rising gradually until they reach a plateau.

*The goat grain mixture (ration IIa) contains 4.6 per cent fat; the alfalfa hay 2.0 per cent fat (Henry and Morrison). 100 pounds of the ferric chloride treated goat ration thus would contain: 4.6 x 2 plus 2 x 1 equals 11.2 equals 3.7 per cent fat. 100 grams of extracted oil therefore would be equivalent to: 100 x 100 which equals 2700 grams of feed.

**Includes 21 rats reared and depleted on ration X and which produced resorption gestations. Subsequently they were continued on this ration supplemented with wheat germ oil or they were fed a modification of the untreated goat ration (ration VII, table 1).
Fig. 4. The effect of feeding modifications of the ferric chloride treated goat ration upon the gestations of vitamin B-depleted rats and their subsequent response to administration of vitamin B in the form of various untreated foodstuffs. Curve I: 139 normal pregnancies; ration IV, V, VIII, IX and X supplemented with vitamin B in the form of various untreated foodstuffs. Curve II: 116 resorption gestations; ration IX. Curve III: 116 resorption gestations; ration VIII. Curve IV: 48 resorption gestations; ration X. Curve V: 15 resorption gestations; ration X.
or a peak varying from day 16 to day 19 of the gestation. Then there is a gradual decline until the recurrence of estrus which varied from day one to day four of the puerperium.

From the evidence presented in table 7 and the graphs in figure 4, it may reasonably be assumed that the ferric chloride treated experimental goat ration used under the conditions of this experiment was devoid of vitamin E as measured by biological tests with vitamin E-depleted rats.

B. Effect of Prolonged Restriction of Animals to the Vitamin E-deficient Experimental Goat Ration

1. Reproduction

a. Goats. The breeding history of all the goats used in this experiment is presented schematically in chart 1. During the four and one-half years of the experiment the goats have been continually restricted to a ration in which vitamin E had been effectively destroyed (cf. table 7). No unusual difficulty was experienced in expanding the original herd of seven goats to 48. A description of the matings of the different generations and the number of kids born in each case is presented in table 8. It is evident from chart 1 and table 8 that all types of matings tried were successful and resulted in pregnancies and the delivery of kids at term. Of the 48 goats depicted on the chart, 35 were born to mothers restricted to the experimental ration. Four of these kids, namely, H-2, H-3, H-3 and H-4-T were born dead. Post-mortem examination of these four dead kids, however, revealed no changes in the subcutaneous
Chart 1. Genealogy of goats restricted to the ferric chlorides-treated experimental ration.
LEGEND

○ Male
□ Female
• Offspring of known parentage
□ Unknown offspring
• Unknown parentage

1935

1936

1937
Table 8. Matings of goats restricted to the ferric chloride treated experimental ration

<table>
<thead>
<tr>
<th>Matings*</th>
<th>Number of kids born</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 $G_0$ females x 1 $G_0$ male</td>
<td>12 males and 5 females = 17</td>
</tr>
<tr>
<td>6 $G_0$ females x 3 $G_1$ males</td>
<td>6 males and 4 females = 10</td>
</tr>
<tr>
<td>2 $G_1$ females x 1 $G_0$ male</td>
<td>2 males = 2</td>
</tr>
<tr>
<td>2 $G_1$ females x 1 $G_1$ male</td>
<td>3 males and 1 female = 4</td>
</tr>
<tr>
<td>4 $G_1$ females x 1 $G_2$ male</td>
<td>5 males and 1 female = 6</td>
</tr>
<tr>
<td>1 $G_2$ female x 1 $G_2$ male</td>
<td>2 females = 2</td>
</tr>
</tbody>
</table>

28 males and 13 females = 41

* $G_0$, $G_1$, and $G_2$ denote parental, first and second generations, respectively.

*Human or animal tissues and muscles or any other changes except as noted below. These autopsies revealed without doubt that the animals were alive, at least up to a very few hours before parturition, and were perhaps alive when parturition occurred. Male kid H-2 was a twin and weighed 4.8 pounds at birth and apparently was entirely normally developed. The lungs were not inflated. A very small part of the lungs of male H-3 was inflated, indicating that this animal had breathed. This kid weighed 10.0 pounds at birth. Goat $44-N$, the mother of male kid H-3 died during her second gestation on the date she was due to kid. The kid was completely developed and appeared normal in every respect and weighed 8.3 pounds. There is a plausible explanation for the cause of the death of goat $44-N$. Subsequent to her first gestation an abomasal cannula had been inserted for the
purpose of obtaining digested food samples for vitamin E tests. She had carried the cannula for approximately nine months. During the latter part of her second pregnancy necrosis developed in the tissues adjacent to the cannula which resulted in gangrene. Infection spread to the subcutaneous tissue and an acute peritonitis ensued. The goat died from general toxemia. Female kid 44-T showed no inflation of the lungs. There was, however, some congestion, indicating that the circulation had been shut off during parturition and the kid had died of suffocation. This kid weighed 5.3 pounds.

1. Females

21. Periods of estrus. All the does reared to maturity and restricted to the vitamin E-deficient experimental ration exhibited estrus regularly each season between September and March. The does usually remained in heat from one to two days, which period agrees with that of goats reared under practical conditions (162). One doe, 44-L, at no time showed any signs of estrus. This abnormality will be discussed in detail later.

22. Fertility of the does. The fertility of the does restricted to the ferric chloride treated goat ration, as indicated by the number of services of the buck for each conception resulting in pregnancies, is summarized in table 9. Twenty-one conceptions have been secured from a possible 23. Nineteen conceptions were secured from a single service for each doe, two from the second service. One doe, 44-U, failed to conceive following two services. She was, however, not allowed to be bred again because of her inferior size. Thus, exactly 95 per cent of the does mated
Table 9. The number of services required per conception resulting in pregnancies among does restricted to the ferric chloride treated experimental ration

Number of pregnancies resulting from one service............... 19
Number of pregnancies resulting from two services............... 2

<table>
<thead>
<tr>
<th>Number of does bred once that did not conceive</th>
<th>Number of does bred twice that did not conceive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Total number of services........................................... 25
Total number of services required for the conceptions resulting in pregnancies.............................. 23
Total number of pregnancies......................................... 21
Average number of services per conception resulting in pregnancies........................................ 1.10

proved to be fertile. The average number of services for each of the 21 pregnancies was 1.10. Addington and Cunningham (5) found that the percentage of fertility among does reared on a practical ration in the herd at the New Mexico Experiment Station was about 95 per cent. The average number of services per conception among the does was 1.15 involving 144 pregnancies. Obviously then, the does reared on the vitamin E-deficient rations exhibited an average percentage of fertility and a lower average number of services per conception compared with the does reared under practical conditions.

c1. Fecundity of the does. Records are available for 21 parturitions among the does restricted to the vitamin E-deficient ration.
The does are grouped in two classes according to their gestation or age and the number of kids born to each doe in each case. Their summary is shown in table 10. The class of does over 18 months, however, consists only of does completing their second gestation and therefore can hardly be considered mature.

Table 10. Number of singles and twins born to does restricted to the ferric chloride treated experimental ration

<table>
<thead>
<tr>
<th></th>
<th>1 kid</th>
<th>2 kids</th>
<th>3 kids</th>
<th>Total</th>
<th>Average no. of kids per gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Does over 18 months of age at end of gestation period</strong></td>
<td>5</td>
<td>9</td>
<td>0</td>
<td>14</td>
<td>1.643</td>
</tr>
<tr>
<td><strong>Number of kids</strong></td>
<td>7</td>
<td>14</td>
<td>0</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td><strong>Yearlings under 18 months of age at end of gestation period</strong></td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>7</td>
<td>1.714</td>
</tr>
<tr>
<td><strong>Number of kids</strong></td>
<td>2</td>
<td>10</td>
<td>0</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><strong>Total no. of does</strong></td>
<td>7</td>
<td>14</td>
<td>0</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td><strong>Total no. of kids</strong></td>
<td>7</td>
<td>23</td>
<td>0</td>
<td>35</td>
<td>1.667</td>
</tr>
</tbody>
</table>

Of 14 does over 18 months of age at the time of parturition, five produced one kid and nine produced two. Thus a total of 23 kids resulted from the 14 pregnancies, and is equivalent to 164 for each 100 pregnancies. Of seven does under 18 months of age at parturition, two produced one kid and each of the remaining five produced two. This is a total of 12 kids for the seven does that kided under 18 months of age. Thus, these young does reproduced at a rate equivalent to 171 kids per 100 pregnancies. The 21
pregnancies resulted in 35 kids, which is equivalent to 167 kids per 100 does or a 167 per cent kid crop.

At the New Mexico Station (5) it was found that 144 pregnancies resulted in 286 kids. This is equivalent to 199 kids per 100 does, or a 199 per cent kid crop under practical conditions on a normal ration. Potts and Simmons (162) reported an average kidding rate for yearling Saanen does of 166 per 100, as compared with 168 per 100 for the Toggenburgs. These latter kidding rates are practically in agreement with those found in this experiment. The higher average kidding rate found at the New Mexico Station was undoubtedly due to the fact that there was a preponderance of mature does over yearlings among the 144 pregnancies, the ratio of the population of mature does to yearlings being 129:15. Their data show that mature does were more fecund than yearlings, 115 mature does having a kidding rate of 210 per 100 does, and yearlings 152 per 100 does taken from a population of 29. The fecundity of the does restricted to the vitamin S-deficient goat ration thus on the average was nearly that of does reared under practical conditions.

\textbf{d). Gestation period of the does.} The gestation periods of the does fed the experimental vitamin S-deficient ration were found to be in agreement with that of does reared under practical conditions. The average gestation period of the experimental does was 150 days for the 21 pregnancies and the range was between 146 and 153 days. At the New Mexico Station (5) the average length of the gestation was practically the same; namely, 149.92 days for 144 pregnancies within a range of 136 to 157 days. Potts and Simmons (162) stated that the average length of the
gestation for does of Saanen and Toggenburg breeding was 149 days, varying from 146 to 152 days. Howland (94) reported an average gestation period of 151 days for 262 does during a period of 23 years, and that the range was between 145 and 160 days.

**e. Proportion of male to female kids born.** There was a preponderance of male to female births among the does on the vitamin E-deficient experimental ration. Of the 35 kids born 24 were males and 11 females. This is equivalent to 215 males per 100 females. Howland (94) has presented data for 23 years records, in which he shows the birth of 304 buck kids and 219 doe kids, a proportion of 139 males to 100 females. The New Mexico Station (5) herd showed a preponderance of male over female births. Their records show the birth of 194 males to 169 females, which is equivalent to 115 males per 100 females. The proportion of male to female kids born to does on the vitamin E-deficient experimental ration was greater than those obtained by Howland or the New Mexico Station. The greater ratio which was obtained may not be of statistical significance since the population was small. Data indicate, however, that the preponderance of male to female births appears to be the rule among does under practical conditions (5,94). The does on the experimental ration did not reverse the order in this respect.

**f. Males.** Of the 24 males born and reared and at all times restricted to the vitamin E-deficient experimental ration, four were used for breeding. The remaining 20 males were not used for breeding but four of these bucks, however, were allowed to reach puberty; 11 were killed for experimental purposes, two were sold because of crowded
conditions, and three were dead at birth. The latter have been discussed previously and a plausible explanation made for the cause of their death, which might have occurred under practical conditions. Most of the male goats were ultimately killed and careful histological anatomical studies were made in each case of the reproductive organs in order to determine the status of their fertility. The fertility of these bucks as determined by the number of successful matings, microscopic determination of percentage of motility of the spermatozoa and the histological appearance of the seminiferous tubules or of the spermatozoa in the epididymis is summarized in table 11. It is obvious that with the bucks killed during infancy only the histological and morphological appearance of the seminiferous tubules would be of any value in determining whether or not they deviated from the normal (cf. fig. 10 and 11).

The fertility of the four bucks, D-1, E-1, H-1 and J-1, which were used for breeding purposes requires little further comment. Fifteen services performed by these four bucks resulted in 13 pregnancies and the delivery of 22 kids at term. (cf. chart 1). At the time they were killed microscopic sections of the testes showed a normal configuration and the epididymis contained an abundance of motile spermatozoa.

Of the other four bucks, E-2, R-1, F-1 and K-1, which were allowed to reach maturity but which were not used for breeding purposes, two, male E-2 and R-1, exhibited normal testes and epididymis similarly to the four service bucks mentioned above. (cf. fig. 5 to 9). Male K-1 was moribund killed at the age of 42 weeks. A few weeks prior to its termination the animal apparently had suffered from anorexia and cachexia.
Fig. 5. Testis of goat 3-2. The tubules show normal arrangement of the sperm forming cells in layers; spermatogenic cells next to the basement membrane, primary and secondary spermatocytes, spermatids and spermatozoa can be observed successively inward toward the lumen. Detritus from disintegrated protoplasm from developing spermatozoa can also be seen (X-200).

Fig. 6. Epididymis of goat 3-2. Abundant numbers of normal spermatozoa and secretions in the lumen (X-200).

*The geneticsology of the experimental male goats referred to in fig. 5, 6, 7, 8, 9 and 11 is illustrated in chart 1, page 139.
Fig. 7. Testis of goat R-1. Normal tubules, normal configuration ($\times$400).

Fig. 8. Epididymis of goat R-1. Dense masses of normal spermatozoa are present in the organ. Nothing abnormal found ($\times$100).

Fig. 9. Same as figure 8 but a higher magnification. To the left and below is the dense mass of spermatozoa (not much like the rats, cf. fig. 13 & 17) and to the right and above is the wall of the tubules. In the light area are masses of secretions from the wall which are normal but might be mistaken for spermatids. This photo was taken to show the secretion ($\times$400).
Fig. 10. Immature testis of 25-day-old goat kid, 44-100, reared on a normal ration under practical farm conditions. Large number of spermatogonial cells at the basement membrane. Very large cells centrally from the basement membrane are noticed. These are perhaps cells of Sertoli (X-400).

Fig. 11. Immature testis of 45-day-old goat kid, D-2, restricted to goat ration II. The section is similar in morphology to the section shown in fig. 10 (X-200).
Table 11. The fertility of male goats either reared on or born to does restricted to the ferric chloride treated experimental ration as measured by various indices.

<table>
<thead>
<tr>
<th>Indices of fertility</th>
<th>Age at time of disposal</th>
<th>Nature of disposal</th>
<th>Reasons for disposal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number: Microscopic matings: percentage productive organs:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>of: produc-: motility of: Seminif-: Epidid-: (Yr. - mo. - days):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male: ing: sperms: erous: ymis:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>off-: (estimated): tubules:</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Normal</th>
<th>1</th>
<th>2</th>
<th>29</th>
<th>Killed</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-1</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J-1</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-2</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-1</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F-1</td>
<td>Did not serve</td>
<td>Abnormal</td>
<td>Abnormal</td>
<td>9</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>E-1</td>
<td>ditto</td>
<td>Slightly abnormal</td>
<td>Normal</td>
<td>9</td>
<td>20</td>
<td>Moribund killed</td>
</tr>
<tr>
<td>B-1</td>
<td>3</td>
<td>1</td>
<td>Sold</td>
<td>Not needed for breeding purposes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-2</td>
<td>3</td>
<td>1</td>
<td></td>
<td>ditto</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-2</td>
<td>Normal</td>
<td></td>
<td>1</td>
<td>16</td>
<td>Killed</td>
<td></td>
</tr>
<tr>
<td>D-3</td>
<td></td>
<td></td>
<td>1</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-3</td>
<td></td>
<td></td>
<td>2</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F-2</td>
<td>Normal</td>
<td></td>
<td>1</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-1</td>
<td>Normal</td>
<td></td>
<td>1</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-2</td>
<td></td>
<td></td>
<td>1</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-1</td>
<td></td>
<td></td>
<td>0</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-1</td>
<td></td>
<td></td>
<td>0</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M-1</td>
<td></td>
<td></td>
<td>0</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-1</td>
<td></td>
<td></td>
<td>0</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-2</td>
<td></td>
<td></td>
<td>0</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-1</td>
<td></td>
<td></td>
<td>0</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Macroscopically, the reproductive organs appeared normal. Microscopically, it was noted that spermatogenesis had nearly ceased and there was some degeneration of the germinal epithelium and a moderate edema of the intertubular areas. The epididymis appeared normal with motile sperms. There is a possible explanation of the condition of this goat, however. Males K-1 and J-1 had been penned together in an unheated outdoor building during the winter of 1936 on account of crowded conditions in the main building and the offensive odor which is associated with male goats. Extremely cold weather prevailed during the month of January, and consequently the two animals suffered somewhat from the exposure to cold (10-30 below zero) and male K-1 was abused and also apparently injured by its penmate J-1. Possibly the slight abnormality of the testes may have been caused by abuse and exposure to excessively cold weather. Also, this goat appeared to have suffered somewhat from inanition due to the fact that he had been kept from eating by his penmate. This latter condition might have contributed to the slightly abnormal appearance of the testes.

The testes and epididymus of F-1 upon microscopic examination appeared abnormal. Spermatogenesis apparently never had occurred. The tubules were shrunken and showed only a few cells of Sertoli and apparently only a few spermatagonia remained. The epididymus contained no spermatozoa, only a little detritus in the tubules was observed. An occasional broken down cell was present which could not be identified. Aside from the histological appearance of the sex organs of this goat it was noted that the secondary sex organs, the seminal vesicles, prostate and bulbo-urethral glands were not developed beyond the infantile stage.
All other internal organs appeared normal except the thymus and thyroid glands which were enlarged. No explanation can be given for the condition of this buck.

The 11 bucks which were killed during infancy at the age of from 11 to 45 days were normally developed sexually as far as could be determined for males of their age and stage of development. The immature testes of these goats when examined microscopically showed the presence of a large number of spermatagonial cells at the basement membrane of the seminiferous tubules. Very large cells, perhaps Sertoli cells, centrally located from the basement membrane were noticed also. (cf. fig. 11). Male goats of approximately the same age and stage of development, which were reared under practical conditions, showed testes of similar configuration or identity in morphology (cf. fig. 10).

b. Rats

i. Females. The reproductive performances of 16 female rats reared on the ferric chloride treated goat ration II have been mentioned previously. The results are given in table 7.

ii. Males. The reproductive performance of a group of male rats which were reared to maturity on the ferric chloride treated goat ration is tabulated in table 12. After a period of 156 days on this ration five male rats of this group were mated to 44 normal virgin females selected at random from the stock colony. All matings proved unsuccessful and no pregnancies were obtained, nor were there ever any spermatozoa found in the vaginal smear although vaginal plugs were detected in a few instances, but even these were greatly reduced in number. The libidinosness of the
Table 1. The fertility of male rats reared on and restricted ration and their response to vitamin A therapy

<table>
<thead>
<tr>
<th>Rations fed</th>
<th>Number of days males</th>
<th>Number of days females</th>
<th>Number of days with vitamin A therapy</th>
<th>Number of days without vitamin A therapy</th>
</tr>
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"The wheat germ meal was purchased on the market and fed after the rats had been on ration X."

"The duration of the estrous cycles ranged from 4 to 11 days."

"Twelve rats were nonbreeders. Autopsies revealed that two had ovarian cysts and abscesses. Infertility in the remaining rats was not determined; it was, however, not due to any..."
reared on and restricted to the ferric chloride treated goat ration to vitamin therapy as measured by various indices.

<table>
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<th>No.</th>
<th>Sperm</th>
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<th>Histological appearance of male reproductive organs</th>
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rats had been on ration X for 197 days. The meal replaced 15 per cent of the ration.

\[ a \text{arion cysts and abscessed uteri, and one had an abscess in the peritoneal cavity. The cause of}\]
\[ \text{eover, not due to anatonical abnormalities.} \]
males apparently was not decreased as copulations always were observed when the female in estrus was placed with the male. The females were sexually normal as manifested by frequent estrus cycles during the period the trials were run. The duration of the cycles ranged from four to 11 days.

The males were killed at intervals and histological examinations of the testes and epididymis were performed. One male, number 3, table 12, was killed after it had been maintained on the vitamin D-deficient ration for 121 days. This rat showed an advanced testicular degeneration (cf. fig. 12) of the germinal epithelium but not as advanced as that observed in those rats which had been restricted for a longer time to the goat ration (cf. figs. 14, 15, 16 and 18). Sperm-forming cells were still present in the testicles of this rat but spermatids were being shed into the tubules and epididymis along with spermatozoa (cf. fig. 13). This condition is very abnormal and no doubt such sperms are abnormal since they rarely, if ever, are able to fertilize ova. The epididymis contained spermatids along with spermatozoa. The presence of goodly numbers of the former is conclusive evidence of advanced pathology.

The remaining five male rats remained sterile although they were allowed access to an allegedly rich source of vitamin D in the form of a commercial preparation of wheat germ oil meal during the latter part of the period they were restricted to the vitamin D-deficient ration (cf. table 12). At the time they were killed, microscopic examination of their reproductive organs showed complete destruction of the sperm-forming cells. (cf. figs. 14, 15, 16 and 18). The seminiferous tubules contained, aside from cells of Sertoli, a sort of mucus-like material. There was an apparent
increase of interstitial cells and in some instances an intertubular edema was noted. The epididymis contained only spermatids, masses of detritus and secretions together with a few immature sperms (cf. fig. 17).

2. Histological appearance of certain tissues in goats and rats

Photomicrographs of tissues from the reproductive organs of the male goats representing animals of the various gestations and generations which were born and reared on the ferric chloride treated goat ration are depicted in figures 5 to 11, inclusive, along with photomicrographs of similar tissues of the reproductive organs of male rats reared on the identical ration (cf. figures 12 to 18, inclusive). As will be noted, the histological findings support the results presented in tables 7, 11 and 12.

Figure 19 depicts a normal rat testicle, which is depicted for comparison. Figures 20 and 21 illustrate developmental sterility of male rats restricted to a basal vitamin E-free ration (ration V, table 1).

3. Growth and development of goats born, reared and restricted to the ferric chloride treated experimental ration.

a. Birth weight of kids. Table 13 shows the average birth weight (probably not true "birth weights" since a number of the kids may have nursed for a short time before they were weighed) of all kids which were born to does on the ferric chloride treated goat ration. The table also shows the birth weight of kids obtained at a German station (170) which were reared under practical conditions. The birth weights of the kids born on the vitamin E-deficient ration were nearly identical with those
Fig. 12. Testis of rat number 3. Sperm-forming cells are still present in this testicle. In the central part of the photomicrograph is a tubule running the width of the object and in which are many spermatids that have been shed into the tubule. No spermatozoa are seen. To the right and left completely degenerated tubules are present. This is an advanced pathological change (X-200).

Fig. 13. Epididymis of rat number 3. This photo shows the character of the cells that are being shed into the seminiferous tubules and collected in the epididymis. The dark lines are tails and other parts of the spermatozoa and with them it is not possible to see whether or not they are anatomically normal. The photomicrograph, however, was taken in an attempt to show the spermatids, their presence being conclusive evidence of advanced pathology. Note that many of the spermatids have two nuclei. It is not uncommon to observe those that have 4, 6 or 8 nuclei in conditions similar to this, but in this case the two nuclei were the most that were found. They seem to be unable to complete division and are large. As they become older they stain poorly and soon die. Poorly stained dead spermatids are also seen in the section (compare fig. 9 & 10) (X-400).

The sections depicted in fig. 12 to 18 inclusive were obtained from male rats reared and restricted to the ferric chloride treated goat ration II (cf. table 12). During the latter part of the period of which they were restricted to the vitamin E-deficient ration these rats received in addition a commercial preparation of wheat germ oil meal allegedly rich in vitamin E.
Fig. 14. Testis of rat number 7. The tubules contain only cells of Sertoli and a sort of mucus like material. There is an apparent increase of interstitial cells, this increase, however, is probably due to the shrunken tubules, so that the interstitial cells show up more clearly. Intertubular edema can be seen at one point (X-200).

Fig. 15. Same as fig. 14, but at a lower magnification. Much the same as the higher magnification but showing the edema more plainly in the intertubular areas. The medium dark staining amorphous material between the tubules is the edema (X-100).
Fig. 16. Testis of rat number 2. The condition here is the same as in fig. 14 and 15. Between the tubules note the interstitial cells and also an abundant amount of granular material which separates the tubules widely. This granular material is edema. It does not appear as a smooth, amorphous mass as in fig. 15 because of different fixation. Total destruction of sperm-forming cells (X-200).

Fig. 17. Epididymis of rat number 2. Spermatids and masses of detritus and secretions together with some spermatoscoes are present. Total sterility (X-200).
Fig. 18. Testis of rat number 4. Total destruction of sperm-forming cells, only cells of Sertoli remain. Intertubular edema (compare fig. 5, 7 & 19) (X-200).

Fig. 19. Testis of a normal rat reared on ration III (cf. table 1). In the tubules can be seen the sperm-forming cells arranged in layers. The lumen contains whorls of fully developed spermatozoa, some free and others with their heads seeking toward the basal membrane. Detritus from disintegrated protoplasm from developing spermatozoa can also be seen. The interstitial cells do not show up very well due to the fixation (X-200).
Fig. 20. Rat A reared on a basal vitamin D-free ration (cf. ration V, table 1). This section shows developmental sterility. The testicle is abnormal. Spermatids are present in the lumen of the tubules. However, there is little or no evidence of sperm-forming cell destruction and it still shows normal arrangement of the cells (X 200).

Fig. 21. Smear (unstained) from epididymis of a rat (mate to rat A, fig. 20) reared on ration V. The spermatozoa appear clumped together like tufts of hair. Such rats are sterile and the spermatozoa are unable to fertilize ova (X 100).
Table 13: Weights of goats reared on the ferric chloride treated goat ration II compared with those of goats reared under practical conditions on a ration of natural, varied untreated foodstuffs.

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<th>Females* (lbs.)</th>
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** Average weights of male and female goats reared at Iowa State College on the ferric chloride treated experimental ration.
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*: Female

Origin of data:
- Average weights of male and female goats reared at Iowa State College on the ferric chloride treated experimental ration.
- Male goats reared under practical conditions in Germany.
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<td>51.2 (7)</td>
<td>22.5</td>
<td>30.5</td>
<td>26.5</td>
<td>46.4 (18)</td>
<td>38.7 (3)</td>
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<tr>
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<td>65.6 (7)</td>
<td>67.7** (7)</td>
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<td>86.2*** (7)</td>
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<td>29</td>
<td>101.6 (2)</td>
<td>85.2** (6)</td>
<td>40.5</td>
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*Does not include weights of females 44-L, 44-U and 44-V.
**Figures in parentheses denote number of animals used in calculating.
***All females pregnant
+ Three females pregnant.
++ One female pregnant.
+++ Average weight during week prior to parturition.
+++ Average weight during week following parturition.
born under practical conditions. Birth weights obtained at the New Mexico Station (5) for 223 males averaged 6.7 pounds and for 212 females 6.0 pounds for kids born to grade and purebred does of Toggenburg breeding. However, some of these kids had suckled before they were weighed. Average weights for kids at the same station, which were known to have been weighed before nursing were for 43 males 6.1 pounds and 51 females 5.5 pounds on the average. These values are somewhat lower than those obtained for the male and female kids on the vitamin B-deficient ration and those at the German Station. The New Mexico Station showed, however, that by allowing kids to nurse before weighing, their weight would increase about 20 per cent over their actual birth weight, representing the consumption of about a pint of milk. Since it is not known definitely whether the weights of the vitamin B-deficient goat kids or the weights of the German goat kids are true "birth weight", allowance must be made for the higher values obtained. If this allowance is made it will readily be seen that vitamin B-deficiency had not affected the birth weight of the kids.

b. Weekly gain in weight of kids during the first 26 weeks. The weight of the male goats reared on the vitamin B-deficient goat ration at the close of 26 weeks ranged between 38.0 and 69.3 pounds with an average of 54.7. For the females the range was between 42.8 and 61.0 pounds with an average of 51.2 (excluding 44-L, 44-U and 44-V). The values obtained at the German station (170) showed for males a range between 32.3 and 55.4 pounds with an average of 38.7. The weights of the kids reared on the vitamin B-deficient goat ration at the close of the 26 weeks was on the average for the males and females approximately eight times as great as
the weight at birth. At the German Station the weight of the males was seven and the weight of the females six times as great as those at birth (cf. table 13).

The average weekly gain in weight of bucks and does reared on the ferric chloride treated experimental ration and the average gain in weight of male and female goats reared under practical conditions at the German Station (170) are shown graphically in figure 22. Curves for the three abnormal goats born and reared on the vitamin D-deficient ration are also shown. Figure 22 is a graphical presentation of the data presented in table 13. The weight curves are shown only until the goats reached 26 weeks of age since records were unavailable for comparison beyond that age. The gain in weight is most noticeable during the first eight weeks; from then on the slope of the weight curve is less for the females than for the males. The vitamin D-deficient animals apparently made more rapid gains (excluding 44-L, 44-U and 44-V) than the German animals reared under practical conditions but aside from this, the curves show the same general characteristics. The vitamin D-deficient goats, thus, on the average attained a greater weight than those born under natural conditions. The greater weight of the vitamin D-deficient goats may be accounted for, since they were crossbred goats of Saanen and Toggenburg breeding. Crossbred goats exceeded purebreds in gain in weight to a slight extent as reported by the German Station (170). Saanen goats exceeded Toggenburgs in weight also (162). The gain in weight of the vitamin D-deficient goats during the first 26 weeks of life may therefore on the average be
Effect of prolonged restriction of goats to the ferric chloride treated goat ration II upon their growth compared with the growth of goats reared under practical conditions on a ration of natural, varied untreated foodstuffs.

Curve I and II, male and female goats restricted to goat ration II, respectively. Curve III and IV, male and female goats restricted to a ration of natural, varied untreated foodstuffs. 44-U, 44-V and 44-L, growth curves of three female individuals restricted to goat ration II.
considered normal as compared to goats reared under practical conditions. Three single individuals, 44-L, 44-U and 44-V, however, apparently did not grow normally as compared to the other goats on the vitamin E-deficient ration, their retardation in growth as indicated by weight changes was noted as early as during their sixth to 12th week of life. The abnormality of these kids will be discussed in a subsequent paragraph.

c. Mature weight of goats. Potts and Simmons (162) reported that mature Saanen does reached an average weight of 120 pounds and mature Toggenburg 96. Brody (25) showed that individual goats reached 50 per cent of their mature weight in four to seven months. If the latter rule is applied to the vitamin E-deficient does, whose weight on the average at six months was 51.2 pounds, their mature weight would average between the weights reported by Potts and Simmons (162). Actually the female goats reared to sexual maturity on the vitamin E-deficient ration during the week following parturition in their first gestations ranged in weight between 69.0 and 109.5 pounds with an average of 83.0 (44-L, 44-U and 44-V excluded). Two male goats at 58 weeks of age attained an average weight of 101.6 pounds (cf. table 13).

d. Abnormality in the development of the three goats 44-L, 44-U and 44-V. Obviously there was a retardation in the development of the three female goat kids, 44-L, 44-U and 44-V, as early as in the sixth to 12th week of their life (cf. table 13 and fig. 22). At the end of the 26th week the growth retardation was most noticeable in 44-L and 44-V. At the time (40th to 58th week of age) these animals were killed 44-L, 44-U and 44-V weighed only 27.5, 40.5 and 30 pounds respectively or approximately
one-third to one-half of the average weight attained by the other female goats restricted to the vitamin E-deficient ration (cf. table 13). During the period of their retarded growth, the three animals had shown signs of general debility, anorexia and cachexia, and the hair coat of the animals had a rough appearance. Autopsies of these animals revealed certain lesions. Macroscopically, the internal organs appeared underdeveloped and there was an extensive pigmentation of the liver, adrenals and the submucosa of the duodenum. Goats 44-U and 44-V were somewhat emaciated, the skeletal muscles did not show the usual pink color but had a paler appearance and were flaccid or inelastic. The bones were soft and many articulating surfaces showed erosions. The shape of the head of 44-V was abnormal (ball-like) and the nose was short and small with upper part of the head large and round; the mandibles were greatly enlarged, the teeth badly worn and the incisor teeth were still primary. The proximal end of the left femur of 44-U had been fractured.

Histological examinations revealed there were lesions of the pancreas in both animals. The Islets of Langerhans could not be found in sections of 44-U. Sections of the pancreas of goat 44-V showed extensive necrosis; the central parts of the lobules were affected especially. In the case of this organ there was some similarity between the two goats, 44-U and 44-V. In the former there seemed to be destruction of the Islets of Langerhans while in the latter there was actual necrosis. The livers, aside from extensive pigmentation, showed a little fibrosis and slight fatty infiltration. The kidney of 44-V was nearly normal while that of 44-U showed a little glomerular nephritis. The ovaries of the two goats seemed
rather infantile and very little luteal tissue was detected in the sections. Estrus, however, had been observed in both animals prior to the time they were terminated. Goat 44-V was observed to be in estrus at the age of 30 weeks and again at 33; 44-U exhibited estrus at 33 and 36 weeks of age. The latter was mated to an outside male of proven fertility in both instances, but she failed to conceive.

Goat 44-L showed marked pathological changes of the thyroid. The gland was inactive and showed no colloid. The cells were columnar or nearly so, faint staining, containing clear spaces (vacuoles) near the base and the nuclei were superficial. The ovaries of this animal seemed normal although ovulation apparently had not taken place. There were many developing ova yet none seemed to have ruptured. Estrus in this goat had not been observed. The examination did not indicate anything as to the cause of the condition of these animals. The pigmentation which is thought to be due to the ration was extensive but other animals with as much pigmentation and restricted to the identical ration grew normally and reproduced. The apparent lack of the Islets of Langerhans in goat 44-U may or may not be of importance. They may have been present but the methods of preparation failed to show them. The condition of 44-U and 44-V cannot be explained by the other lesions mentioned. The condition of 44-L may be explained by the abnormal thyroid. From a study of the sections it appeared that the thyroid was almost, if not totally, without function. However, the thyroid dysfunction might be primary or secondary.

The abnormality of the goats may not necessarily be attributed to vitamin D-deficiency but may be attributed to inherited disturbances of
endocrine functions. Dwarfism caused by inbreeding has been known to occur among herds and flocks reared under usual farm conditions. The two goats $\lambda_1^U$ and $\lambda_1^V$ were inbred to the extent of 31 per cent and their abnormal growth may be attributed to this cause, since they were the produce of full brother and sister matings. The two animals, however, showed normal estrus. $\lambda_1^U$ was bred twice but did not conceive. A daily amount of 15 cc. of tested wheat germ oil was administered to $\lambda_1^U$ for a period of 28 days, but this treatment had no affect on either growth or reproduction. $\lambda_1^V$ died at the age of 40 weeks, and $\lambda_1^U$ was killed for examination at the age of 58. The probability exists that vitamin E-deficiency may have had some effect on these two animals. The more plausible explanation, however, is that their abnormalities were due to some other cause or causes, one being inbreeding, and because of this inbreeding, abnormalities in endocrine functions may have occurred, which affected their growth adversely. If their behavior is compared to goat $\lambda_1^-$, a second generation, second generation male offspring, which was restricted to the vitamin E-deficient ration for a longer period, it seems most likely that vitamin E-deficiency can be ruled out as the cause of the abnormality occurring in $\lambda_1^U$ and $\lambda_1^V$. The male goat $\lambda_1^-$ grew normally as previously discussed, and when sexually mature, this animal was killed and his reproductive organs examined. Smears from the epididymis showed motile spermatozoa and histological sections of the testes showed a normal configuration with active spermatogenesis taking place (cf. fig. 7, 8 and 9).

a. Photographic description of certain goats representing the generations in various stages of development. Figures 23 to 30 inclusive
depict two parental goats and their progeny in various stages of development while restricted to the ferric chloride treated experimental ration. Apparently, all the goats exhibited general health and vigor. However, the four goats (fig. 31 to 34 inclusive) which failed to develop normally do not appear as thrifty.

4. The efficacy of prolonged avitaminosis E among goats in decreasing the amount of vitamin E occurring naturally in milk and certain body tissues.

Results of the tests for vitamin E in materials obtained from goats restricted to the vitamin E-deficient goat ration II (cf. Table 6 and chart 1) and materials from goats reared on natural, varied, untreated foodstuffs are presented in Table 14. Comparative tests of muscle tissue from beef cattle are also listed. Materials from these sources were fed at different levels to 123 vitamin E-depleted female rats which after positive mating became pregnant. These pregnancies were diagnosed and measured by the vaginal smear technique and the weight curve which have been discussed previously in detail.

The data presented show that milk, butterfat, muscle and adipose tissue, which were obtained from goats restricted to the vitamin E-deficient experimental ration, when fed at various levels to 72 rats in "cure" and "rear" trials, resulted in resorption gestations. Conversely, when these same materials supplemented with wheat germ oil were administered to avitaminosis E-sterile rats normal litters ensued. Likewise, apportioning vitamin E-depleted rats milk or muscle tissue obtained from goats reared on untreated rations under usual farm conditions, or muscle
Fig. 23. Female $\text{♂} \text{♀}$-G*, a parental goat, and her second gestation offspring, male G-1 (white) and female $\text{♀} \text{♀}$-P.

Fig. 24. Female $\text{♀} \text{♀}$-P and male offspring, P-1. First and second generation offspring of $\text{♂} \text{♀}$-G.

*To visualize the development of the goats depicted in figures 23 to 34 refer to chart 1 on page 139 in which the genesiology of the experimental goats which were restricted to the ferric chloride treated experimental ration is illustrated.
Fig. 25. Female $44$-J and male offspring, J-1, and female, $44$-S (white). First and second generation offspring of $44$-C (cf. fig. 23). Male J-1 was used successfully as service buck during the third breeding season.

Fig. 26. Female $44$-S and two female offspring, $44$-U to the right, $44$-V to the left. The does are second and third generation offspring of $44$-C.
Fig. 25.

Fig. 26.
Fig. 27. Female 44-F, a parental goat and her second gestation offspring, female 44-X (white) and male F-2.

Fig. 28. Female 44-K and male offspring, K-1 and K-2. The goats are first and second generation offspring of 44-F.
Fig. 29. Female 44-R, second gestation offspring of 44-F (cf. fig. 27).

Fig. 30. Male B-1, second gestation offspring of 44-R.
Fig. 29.

Fig. 30.
Fig. 31. Male F-1, first gestation offspring of 4/4-F.

Fig. 32. Female 4/4-L, first gestation offspring of 4/4-D, a parental doe.
Fig. 33. Female 44-U, third generation offspring of 44-C (cf. fig. 23, 25 and 26).

Fig. 34. Female 44-V, full sister to 44-U above.
Table 14. The efficacy of prolonged avitaminc of vitamin E occurring naturally in foodstuff derived from goats reared on and reared on untreated rations.

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<tr>
<th>Materials tested</th>
<th>Source and description of material</th>
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<td><strong>Basal ration VI (table 1)</strong>**</td>
<td><strong>Basal vitamin E</strong></td>
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<td>ditto</td>
<td>From goats reared on and reared on untreated rations composite samples</td>
</tr>
<tr>
<td>Milk</td>
<td>Composited, fresh, daily samples</td>
</tr>
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<td>ditto plus butterfat***</td>
<td>ditto plus butterfat, accumulated as above</td>
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<tr>
<td>Milk**</td>
<td>Composited, fresh, daily samples</td>
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<tr>
<td>ditto plus butterfat***</td>
<td>ditto, accumulated as above</td>
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<td>Butterfat</td>
<td>Dried sample (adult male H-1)</td>
</tr>
<tr>
<td>ditto**</td>
<td>(adult female 44-J)</td>
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<tr>
<td>Muscle tissue**</td>
<td>Composited frozen samples (adults)</td>
</tr>
<tr>
<td>ditto**</td>
<td>&quot; &quot; (adults)</td>
</tr>
<tr>
<td>ditto**</td>
<td>&quot; &quot; (kids)</td>
</tr>
<tr>
<td>ditto**</td>
<td>&quot; &quot; (kids)</td>
</tr>
<tr>
<td>Body fat**</td>
<td>&quot; &quot; (adults &amp; kids, adipose)</td>
</tr>
<tr>
<td>Butterfat plus wheat germ oil</td>
<td>From accumulated, composited, fresh daily samples</td>
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<tr>
<td>Muscle tissue** plus wheat germ oil</td>
<td>Composited frozen samples (adults)</td>
</tr>
<tr>
<td>ditto**</td>
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<td>ditto**</td>
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<tr>
<td>Body fat**</td>
<td>&quot; &quot; (adults &amp; kids, adipose)</td>
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</tbody>
</table>

From goats reared on untreated rations composite samples |

| Milk | Composited, fresh, daily samples |
| " | ditto |
| Muscle tissue | Composited, frozen samples (adults) |
| ditto | " " (adults) |
| ditto | " " (kids) |
| ditto | " " (kids) |
| Muscle tissue | " " (adults & kids, adipose) |

From beef cattle reared on untreated rations composite samples |

| Muscle tissue | Composited frozen samples (cheek muscle) |
| ditto | ditto |

*Rats which died, nonbreeders, and those which showed pseudopregnancies, are not included.

**The materials tested were fed as supplements to or included in the basal ration.

***In the trials where the butterfat and body fat were incorporated in the low fat basal diet, the trials where the rats received milk ad. lib. as an addition to the basal ration were included.

**Dried or frozen muscle tissue replaced 50 per cent of the basal ration.
avitaminosis E among goats in decreasing the amount naturally in milk and certain body tissues.

<table>
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<tr>
<th>Amount of material or supplement</th>
<th>Type of supplement fed</th>
<th>Per cent of daily ration</th>
<th>Average number of days receiving ration</th>
<th>Character of gestation</th>
<th>Number of females used</th>
<th>Number of females positive mating</th>
<th>Total number of litters</th>
<th>Average number of young per litter</th>
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**Note:**

Fat basal ration, 22 per cent of the starch was replaced by each of these ingredients. Basal ration, they were refused access to water while the trials lasted.
tissue from beef cattle always rescued the gestations, resulting in the delivery of normal litters at term. On the other hand, 12 rats restricted to the basal vitamin E-free rations in "cure" and "rear" trials remained sterile or depleted themselves of their vitamin E reserves inside of 127 days.

The consumption per rat of each test material obtained from the goats restricted to the vitamin E-deficient experimental ration was greater in every instance than that eaten by the rats restricted to the material taken from goats reared under usual farm conditions. Thus the rats were given every opportunity to receive any vitamin E present in the food material being tested. The rearing of weanling female rats on the materials from the vitamin E-deficient goats failed to give positive tests for vitamin E, although the materials replaced amounts of the basal vitamin E-free diet ranging from 22 to 50 per cent (cf. table 14). These modified basal rations were fed for periods as long as 129 days. All the rats grew well and exhibited no signs of abnormality other than resorption gestations which occurred in all cases of vitamin E-deficiency. On the other hand, muscle tissue from normal goats fed in amounts of five grams daily per rat was sufficient to cure sterility in female rats. Similarly, daily doses of 15 cc of milk from normal goats when administered to each avitaminosis-E rat always resulted in the rescue of the gestation. Conversely, when six times (figured on the basis of butterfat) as much milk from the vitamin E-deficient goats was fed in "rear" trials, the rats remained sterile and underwent typical resorption gestations.

Rats from representative groups of animals presented in table 14 were
sacrificed at different intervals and gross anatomical examinations of their genitalia were undertaken to determine the exact status of their gestations. Figure 35 depicts a rat undergoing a typical resorption gestation while restricted to basal ration VI in which 50 per cent of the total ration had been replaced by muscle tissue obtained from adult goats reared on goat ration II (cf. table 14). Figure 37 depicts a rat which had been reared on the same ration as the one pictured in figure 35 and underwent a resorption gestation. Subsequently she was continued on basal ration VI but received in addition a daily amount of 20 grams of normal adult goat tissue. Figure 39 depicts a female rat showing the uterus in the virgin state and is presented for comparative purposes.

From the data presented in table 14 it is evident that milk, butterfat, muscle or adipose tissue obtained from goats restricted for one or more generations (cf. table 6 and chart 1) to a ration in which vitamin E had been effectively destroyed were very low if not entirely devoid of vitamin E, when the materials were fed in amounts as listed in table 14. Post-mortem findings of certain rats representative of certain test groups support this contention (cf. fig. 35 and 36). Conversely, biotests of similar ingredients from goats restricted to practical rations, when fed in much smaller amounts, always gave positive tests for this vitamin (cf. fig. 37 and 38). The occurrence of vitamin A in the tissues and milk of goats reared under usual farm conditions must thus be considered to be a usual phenomenon.
Fig. 35. Female rat 73-3B sacrificed on the 18th day of her gestation showing the uterus in situ containing seven resorbing feti: three in right and four in the left horn. At weaning the animal was placed on basal ration VI in which 50 per cent of the total ration had been replaced by muscle tissue obtained from adult goats restricted to the ferric chloride treated experimental ration (cf. table 14). The animal received an estimated amount of 615 grams of muscle tissue before it was killed. The tissue had been stored at -15°C, until it was incorporated in the diet and fed.

Other rats representative of test groups fed other materials obtained from the goats reared on the ferric chloride treated experimental ration and which were sacrificed showed an analogous picture.

Fig. 36. Excised uterus and ovaries of the rat shown in fig. 35. The enlargements of the uterine wall contain remnants of the dead feti and placentae which are undergoing necrosis. The necrotic tissue is ultimately resorbed or expelled in rats which undergo resorption gestations caused by avitaminosis A. The white area is mesovarial fat.
Fig. 37. Female rat 72-B sacrificed on the 21st day of her gestation showing the uterus in situ containing seven normally developed young; three in right, four in the left horn. This rat had been reared under the same conditions as rat 73-2B, fig. 35, and underwent a resorption gestation as determined by the vaginal smear technique and the weight curve after receiving an estimated amount of 635 grams of muscle tissue obtained from adult goats restricted to the ferric chloride treated goat ration II. Subsequently she was continued on basal ration VI but received in addition for a period of 20 days a daily amount of 20 grams of muscle tissue obtained from goats reared under usual farm conditions. The muscle tissue was stored under similar conditions as that mentioned under fig. 25 (cf. table 14).

Fig. 38. Excised uterus of rat shown in fig. 37 which contained seven normally developed live young.
Fig. 39. Female rat number 1000 sacrificed following a restriction period of 105 days to a vitamin D-free ration. The picture shows the uterus in situ in the virgin stage. The animal was taken from a group of weanling rats which had been reared and restricted to basal ration VI and whose littermate sisters underwent resorption gestations as diagnosed by the vaginal smear technique and weight curve (cf. table 14). The virgin uterus is shown here for comparative purposes and the photograph reveals a normal uterus and ovaries. No enlargement as shown in fig. 35 and 36 of a pregnant uterus in the resorbing stage during avitaminosis D.

Fig. 40. Excised uterus and ovaries of rat shown in fig. 39. The white masses are mesovarial fat.
V. DISCUSSION OF RESULTS

Four factors involved in an interpretation of the significance of the results of this experiment are: 1) the completeness of the destruction of vitamin E by the ferric chloride treatment of the experimental ration; 2) the adequacy of the experimental ration following the treatment with ferric chloride; 3) the efficacy of prolonged avitaminosis E in decreasing possible reserves of vitamin E in the tissue of the goats; 4) the reproductive performance of the goats on the vitamin E-deficient experimental ration. A discussion of these factors follows:

1) Administration of modifications of the ferric chloride treated experimental ration (cf. rations VIII, IX and X, table 1) and untreated accessory food factors (vitamins A, D and B-complex) to virgin vitamin E-depleted and resorption gestation female rats invariably resulted in nutritional sterility following mating as manifested by resorption gestations. The efficacy of method of treatment of the experimental ration with ferric chloride was demonstrated by the administration of an ether extract of goat ration II to vitamin E-depleted resorption gestation rats and by prolonged restriction of weanling male and female rats to a modification of the experimental ration (cf. ration X, table 1). Both methods of feeding resulted in nutritional sterility in both sexes (cf. table 7). The validity of the rat technique employed in tests for vitamin E has been amply demonstrated (68). Several modifications of the technique were
made as precautions against other factors (11,15,72,111,112,113) which might have influenced the interpretation of a given test. The results of biological tests of the ferric chloride treated experimental ration thus demonstrated that the goat ration was depleted of vitamin A as far as could be detected by using the rat as a test animal. A critical analysis of the results of the tests for vitamin A in the experimental ration established beyond any doubt that the ration was extremely deficient in this factor. The fact that the ether extract of the ferric chloride treated goat ration gave negative tests for vitamin A makes it a possibility almost approaching certainty that the ration was devoid of this factor. The ether extract was incorporated in the basal vitamin B-free ration in quantities estimated to be the equivalent of 2800 to 3500 grams of the ferric chloride treated feed per rat. This quantity was approximately 12 to 15 times as much as the maximum amount fed of the untreated ration and 300 to 700 times the minimum amount necessary for the rescue of a gestation. Therefore, it may be assumed that it is practically certain that the vitamin A content of the ferric chloride treated ration to which the experimental goats had been restricted was infinitesimally small.

2) It cannot be stated for certain what effect the ferric chloride treatment of the goat basal ration (cf. ration I, table 1) had upon other food constituents. No doubt other accessory food factors than vitamin A had been destroyed. The experimental ration, however, was supplemented at each feeding time with vitamins A and D and the B-complex. Thus the ration as far as known was adequate in this respect. That the ration was adequate in other respects was demonstrated by the fact that the rats and
goats reared and restricted to the experimental ration exhibited normal growth, except the three goats, \(44\)-L, \(44\)-U, and \(44\)-V. The effect of feeding the ration containing one per cent of ferric chloride over an extended period of time superficially had no effect on the animals. Histological examination of the goats, however, revealed an extensive pigmentation of the liver, kidney and the submucosa of the intestine. This pigmentation may have been due to the ferric chloride and may have affected the development of the three goats, \(44\)-L, \(44\)-U, and \(44\)-V. The majority of the goats, however, developed normally and exhibited no apparent effect because of the ferric chloride. No pigmentation was observed in the rats reared on the same ration.

It is possible that the ferric chloride may have caused resorption gestations in the rats and thus rendered the tests for vitamin E invalid. This, however, could not have been the case since administration of vitamin E in the form of the untreated ration I or ration II supplemented with wheat germ oil to resorption gestation rats which had been reared and restricted to the ferric chloride treated goat ration II cured the nutritional sterility and resulted in normal litters at term (cf. table 7). It may, therefore, be concluded that the experimental ration was adequate as far as it is now known except for the lack of vitamin E.

3) It is evident from chart 1 that the ability of the parental experimental goats to reproduce was not seriously impaired by the restriction to the vitamin E-deficient experimental ration during the first two breeding seasons (209). However, the possibility existed that the goats retained possible reserves of vitamin E in their tissues. Survival of
fertility in female rats given appreciable amounts of vitamin E and then shifted to an avitaminosis 3 regime is a known phenomenon and the length of survival of fertility is correlated with the richness of vitamin 3 in the original regimen, but continued avitaminosis 3 depletes the storage of this factor (68). Thus the storage of vitamin 3 in certain body tissues of the parental experimental goats might have accounted for their persistent fertility if the goat behaves analogous to the rat in this respect. Protracted restriction of weanling rats to an avitaminosis 3 ration, however, quickly results in the depletion of body stores of this accessory factor (68) especially when the mothers of the animals are on low but sufficient amounts of vitamin 3 to assure the birth of living young (165). Further it has been demonstrated that when weanling rats were reared on vitamin 3-free dietaries and when mature were not submitted to the drain of repeated pregnancies their store of vitamin 3 was not conserved thereby, since it did not persist longer than it did in their frequently bred sisters (68).

The observance of decreased body reserves of vitamin 3 in certain tissues and milk obtained from the progeny of the parental experimental goats was analogous to the conditions which occur in rats restricted to an avitaminosis 3 regimen (cf. table 14). That sterility was imposed in both male and female rats reared on the experimental ration has been demonstrated previously (cf. table 7 and 12).

It is evident from table 14 that muscle tissue and milk from goats normally under usual farm conditions contain vitamin 3 and thus must be considered to be a usual phenomenon. Conversely, similar ingredients from
The vitamin D-deficient goats apparently were very low in this factor, since when these ingredients were incorporated in the basal vitamin D-free ration and fed in much larger amounts to sterile female rats, the rats remained sterile and underwent typical resorption gestations. It is, therefore, very likely that the milk and tissues of the progeny of the parental experimental goats were practically devoid of vitamin D and that apparently the normal storage of this factor must have been minute or almost immeasurable. The decreased body reserves of vitamin D in certain tissues and milk of the male and female goats born, reared and restricted to the ferric chloride treated (avitaminosis-D) experimental ration thus must be considered to be due to lack of vitamin D intake.

4) The inhibitory effects imposed by the ferric chloride treated (avitaminosis D) ration invariably resulted in sterility in male and female rats. This phenomenon was not observed in the goats. Unlike rats, the reproductive behavior of male and female goats was unaffected, although both species were restricted continually to the identical ferric chloride treated (avitaminosis D) ration. The results of biological tests of the experimental ration demonstrated its deficiency of vitamin D (cf. table 7). The tests were distributed over the entire experimental period in such a manner that all the feed that the goats received was tested for vitamin D. The goats received no feed or supplements of feed during the experimental period unless it had given negative tests for vitamin D as manifested by resorption gestations of vitamin D-depleted female rats.

The fertility and fecundity of the experimental goats apparently was
not impaired, since no unusual difficulty was experienced in expanding the seven parental goats to 48 during the four and one-half years of the experiment. Of the 48 goats, 35 were born to mothers while restricted to the experimental vitamin B-deficient ration. The 21 gestations out of a possible 22 occurring among the 15 does in the course of four breeding seasons resulted in the birth of 31 live kids and of four others which were dead at birth but without any detectable abnormalities. Three kids failed to grow normally. No serious deficiencies were noted in the growth of the other kids reared for breeding purposes.

The possibility that vitamin B deficiency caused minor reproductive disturbances, though not entirely preventing the delivery of live kids, must also be considered in evaluating the results of the experiment. Only minor disturbances in the reproductive cycle of the parental goats were observed during the first two breeding seasons and these were of such a nature that they could easily have occurred under usual farm conditions (209). The four kids which were dead at birth have been discussed previously as have certain reproductive abnormalities which occurred to females 44-L, 44-U, 44-V and male F-1 during the second and fourth breeding seasons. It is thus seen that the reproductive disturbances occurring among the goats born, reared and restricted to the ferric chloride treated ration were very minor in comparison with the complete failure of the rats to reproduce when restricted to the identical ration. The attribution of such minor reproductive disturbances among the goats to lack of vitamin B cannot be made logically since factors other than vitamin B deficiency may have contributed to the reproductive abnormalities.
It has been shown that the possibility that the goats could have acquired vitamin E from the ration must have been minute, almost infinitesimally small. It has also been shown that the presence of vitamin E in the goat tissues as a source of this factor must have been negligible. If the goats required any vitamin E it was not possible to detect it in the ration or in the body tissues by using the now known and accepted methods of testing (i.e., biological tests with rats). If vitamin E is needed by the goat, the source can only be sought in a possible synthesis of this factor by certain tissues of this animal. It is well known that augmentation of certain physiological functions arises during pregnancy, resulting in the production of constituents essential to the success of each gestation and subsequent lactation. If synthesis does occur, the data presented indicate that this vitamin probably is produced only in amounts sufficient to meet the requirements of the developing fetus, since no vitamin E was detected in the milk or certain tissues of lactating experimental does shortly after parturition. Nor was the vitamin detected in the tissues of kids eleven days of age from dams which had been restricted since birth to a vitamin E-free ration (cf. table 14). It is impossible, however, to formulate a final statement as to whether the goat can synthesize vitamin E.

It is acknowledged that there are possible shortcomings in the experimental procedure. The full brother and sister mating in the third breeding season should have been avoided. From the standpoint of good breeding this particular procedure perhaps cannot be considered good practice although this is practiced in usual animal breeding. The ideal technique in the comparative studies of the vitamin E content of
the milk and tissue of the goats would have been the inclusion of positive controls. However, from the standpoint of practical farm animal husbandry, the results of the experiment are of importance. It requires a carefully planned procedure and it involves a long, tedious and complex laboratory technique to procure a vitamin D-deficient ration. If such a ration cannot cause reproductive failure among goats which are restricted to it, there is very little possibility that any ration normally fed to farm animals would be sufficiently low in vitamin D to cause any reproductive disturbances as is the case in the rat. Investigations have shown that vitamin D is present in most of the ingredients commonly used in compounding livestock rations. To be sure, there is no knowledge of the changes in vitamin D potency which may occur in milled feeds and roughages through processing, aging or rancidification. It is impossible to state at present how important freshness of ingredients is in maintaining the vitamin D potency of these feedstuffs.

It is popularly assumed, however, that deficiency of this vitamin occurs extensively in farm animals under normal farm conditions and that inhibitory effects of such deficiency are imposed on the developing embryo with sterility resulting from placental insufficiency, as is the case in the female rat when the latter is restricted to vitamin D-deficient rations. Successful empirical therapy with wheat germ oil in the treatment of certain types of temporary sterility in domestic animals has been reported (18,96,194,202,203). From the scant clinical evidence of such therapy it is surmised that wheat germ oil will offset certain breeding hazards and furnish the animals with an adequate amount of
vitamin Η required to insure breeding success in farm animals allegedly suffering from an inadequate supply of this vitamin. Such claims are contrary to the results obtained on goats in this experiment. The apparently good results obtained with wheat germ oil (vitamin Η) therapy by other workers (39,102,174,175,176,200,201,207,208) in certain types of habitual abortions is not claimed by all of them to be due altogether to vitamin Η. The majority of the workers acknowledge the fact that other factors may perhaps be involved and play a role in the treatment. Circumstantial evidence, however, points to the usefulness of vitamin Η in a proportion of cases in certain types of abortion although a logical cause-and-effect relationship is difficult to establish. In many cases other treatments were administered at the same time as the wheat germ oil treatment, such as better environmental conditions and improved diet. Caution should therefore be emphasized in drawing any premature conclusions from these treatments. Furthermore, many extremely different etiological factors seem to play a role in non-infectious abortion and sterility in animals and man such as malnutrition, disturbances of the endocrine function, anatomical disturbances of the genitalia and perhaps incompatibility of the gametes. The value of wheat germ oil (vitamin Η) therapy will therefore depend upon whether these conditions are excluded. A general gynecologic examination supplemented with a serological and hormonobiological investigation should be made before resorting to such therapy.

Finally it must be remembered that wheat germ oil is not identical with vitamin Η and that commercial grades of wheat germ oil are known to vary markedly in their vitamin Η potency (38,65,199) and they also contain
other biologically active substances such as carotinoids, lutein, hydroquinone, ergosterol, dihydroergosterol and sitosterols (9, 46). Also substances about whose biological activity very little is known such as beta-anthrone and squalene, are reported to be found in wheat germ oil (46).

Since these substances may play a direct or indirect part in the biological activity of wheat germ oil, any claim that the therapeutic value of this oil is due to vitamin A may be questioned until the vitamin is used and tested therapeutically in the form of pure natural product or as synthetic alpha-tocopherol.

While wheat germ oil (vitamin A) therapy does open up a possible field of research, such research should be carried out by means of detailed experimental work among the various species of our domesticated animals in order to establish proof that sterility can be induced experimentally on an avitaminosis A regime. The deficiency of this vitamin which could be experimentally produced in certain tissues and milk of these experimental goats and the fact that such deficiency and continued avitaminosis A had no inhibitory effects on their reproduction for several generations certainly do not lend support to the clinical evidence set forth by the workers just cited if the goat is used as a basis for comparison.

No scientific data exist to indicate to what extent vitamin A does actually affect fertility among our domesticated mammals. Nor are there any data available which demonstrate that deficiency of vitamin A in the ration or in the body is the limiting factor in breeding failures among farm animals. That an excess of vitamin A should serve to increase
fecundity is not supported by the work of Evans and Burr (65), quote: 
"The administration to sterile rats of foods or extracts of foods known to be twice or twenty times as rich in vitamin \( B \) as is required for the inception of reproduction does not improve the performance of the reproductive mechanism beyond normal limits."

It is acknowledged, however, that the variations in the reproductive behavior of the two different species of mammal, the rat and the goat, to avitaminosis \( B \), does not lend support to the idea that any conclusion can be reached as to how other species of mammals would react if restricted to an avitaminosis \( B \) ration. The production of final proof that any large mammal does or does not require vitamin \( B \) in its food presents a very hard problem owing to difficulties in providing vitamin \( B \)-free foodstuffs on an extensive scale, the large possible role of reserves in the body and the difficulty of studying specific physiological steps in reproduction as effectively as can be done for the rat. Whether the dietary vitamin \( B \) requirements of other farm animals are more comparable to those of the rat or negligible as in the case of the goat requires further study.
VI. SUMMARY

1. A technique was devised which was effective in producing an avitaminosis E regimen for smaller farm animals. The treatment of a standard ration composed of a variety of natural foodstuffs with one percent by weight of ferric chloride in ether solution was effective in inactivating vitamin E in the experimental ration. When the ferric chloride treated experimental ration or concentrates thereof was fed to avitaminosis E sterile rats, the rats remained sterile and underwent typical resorption gestations.

Conversely, administration of the untreated ration or the ferric chloride treated ration supplemented with wheat germ oil to resorption gestation female rats which had become depleted of their vitamin E reserves by restricting them to the avitaminosis E experimental ration cured the nutritional sterility and rescued the gestation resulting in normal litters at term.

2. It apparently was established that the experimental ration was adequate in all recognized food constituents except for the lack of vitamin E. Prolonged restriction of male and female rats to the experimental ration had no inhibitory effects other than insufficiency of vitamin E. The male and female goats reared to sexual maturity developed or reproduced normally except in the case of four goats. A possible explanation for the subnormal development of the four goats could not logically be
attributed to vitamin D-deficiency.

3. It was demonstrated that prolonged avitaminosis D was effective in decreasing the amount of vitamin D ordinarily present in the milk and certain body tissues of the experimental goats. Milk, butterfat, muscle or adipose tissue, obtained from goats restricted for one or more generations to a ration in which vitamin D had been effectively destroyed, when fed in large quantities to avitaminosis D sterile rats produced resorption gestations typical of an insufficiency of this factor. Conversely, much smaller quantities of similar materials obtained from goats reared under usual farm conditions gave positive tests for vitamin D. The substances tested from all sources were handled in the same manner, stored under essentially the same conditions for approximately the same length of time. The observance of decreased reserves of vitamin D in certain tissues and milk obtained from the experimental goats was analogous to the conditions which occur in rats restricted to an avitaminosis D regimen. On the other hand, the restriction of goats to an avitaminosis D ration for extended periods of time, which markedly decreased the quantity of vitamin D in their milk and certain of their body tissues apparently had no deleterious effect on their reproduction.

4. The results obtained demonstrate that goats do not require rations supplemented with vitamin D to insure satisfactory reproduction. Unlike rats, the reproductive behavior of male and female goats was unaffected during a period of four and one-half years, although both species were restricted continually to the identical avitaminosis D ration. No unusual difficulty was experienced in expanding the original herd of seven goats to forty-eight.
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