The reproductive performance of milk goats fed a ration deficient in vitamin E

John Lincoln Wilson
Iowa State College

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THE REPRODUCTIVE PERFORMANCE OF MILK GOATS FED A RATION DEFICIENT IN VITAMIN E

By

John Lincoln Wilson

A Thesis Submitted to the Graduate Faculty for the Degree DOCTOR OF PHILOSOPHY

Major Subject Dairy Husbandry

Approved:

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Iowa State College 1935
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INTRODUCTION

Vitamin E must be present in the diet for normal reproduction in certain species of small animals and in poultry. In the earlier work on vitamin E, such as that of Evans and Bishop (1922) in announcing the discovery of the vitamin, rats were used as the experimental animals. Later it was proved by Beard (1926) that vitamin E was essential for reproduction in mice. Chickens also were found to require the vitamin for successful development of the embryo (Ill. Agr. Exp. Sta. 1928 and 1930). Experimental results of Goettsch (1930) and Pappenheimer (1930) suggest that vitamin E may be essential for guinea pigs and rabbits.

The extension of vitamin E investigations to the feeding of larger farm animals has been slow. Much difficulty and no little expense are involved in the preparation of large quantities of a vitamin E deficient ration. Such a situation is brought about by the wide distribution of vitamin E in animal feeds and by the difficulty involved in destroying the vitamin. Another factor contributing to the slow progress in this field is the lack of palatability to farm animals of many of the synthetic vitamin E free diets fed to rats.

Some of the sterility encountered among larger animals, particularly dairy cattle, has been linked with vitamin E deficiency. R. R. Graves of the Bureau of Dairying, United
States Department of Agriculture, has been quoted by Evans (1932) as believing that vitamin E may play a role in the fertility of dairy cattle. Some foreign workers, including Vogt-Müller and Bay (1931) and Tutt (1933) have claimed that they were able to cure certain types of sterility by the administration of wheat germ oil. They attributed their results to the vitamin E present in the wheat germ oil.

Since vitamin E is essential for fertility in laboratory animals and since apparent cures of certain types of sterility in larger animals have resulted from the administration of materials rich in vitamin E, it would seem probable that vitamin E may play an important role in the fertility of farm animals such as dairy cattle. However, definite proof that a deficiency of vitamin E will prevent reproduction in such animals is as yet lacking. Hathaway and Davis (1934) sum up the situation very well in their statement, "Although it has been assumed by many that dairy cattle require vitamin E for reproduction, as far as the writers are aware there have been no scientific investigations which have yet established this fact."

This project was undertaken to determine if nutritional sterility could be induced in larger animals by the feeding of a ration as nearly free from vitamin E as it was possible to prepare for these animals. Though the ultimate intention was to use dairy cattle as the experimental animals, it was
thought wise to first use a smaller animal of similar type.
Milk goats were selected as ruminants which would fit well
into a laboratory procedure, and at the same time give results
nearly comparable to those with cattle.

The object of this experiment was to determine whether or
not milk goats require vitamin E in the ration for successful
reproduction.
REVIEW OF LITERATURE

Before an attempt is made to study any possible vitamin E deficiency in larger animals, it would be well to first review the early history of the vitamin, some of its properties and sources, and the physiological conditions brought about by the absence of the vitamin from the diet of laboratory animals.

A. Discovery of Vitamin E

The existence of vitamin E was first reported by Evans and Bishop (1922 and 1923). These investigators found that female rats would not reproduce on a diet composed of casein 18 parts, cornstarch 54 parts, lard 15 parts, milk fat 9 parts, and salts 4 parts, along with 0.4 to 0.6 gm. of yeast per animal daily. The rats grew normally and gave no other evidence of dietary deficiency. The sterility could not be cured by the addition of any of the known vitamins to the ration. The sterility, however, could be cured by the addition of an ether soluble substance which was present in particularly large amounts in wheat germ oil and lettuce leaves. Evans and Bishop designated this hitherto unknown substance as vitamin X. Later the name was changed to E when the vitamin became better established and the nomenclature standardized.

Shortly after the publication of Evans and Bishop's work
several investigators came forward with confirming results. Among these were Mattill and Stone (1923) and Sure (1924 and 1924a) who had also encountered the sterility in their own laboratory animals but had failed to attribute it to the factor which Evans and Bishop called X.

All the early investigators, however, were not so easily converted to the idea that such a vitamin as X existed. Heller (1924), Anderegg (1924) and Nelson, Heller and Fulmer (1925) reported successful reproduction over as many as five generations with rats fed a ration containing none of the recognized sources of vitamin E. Hogan and Harshaw (1924) also expressed doubts as to the existence of the new factor since they could not duplicate Evans and Bishop’s results.

There were several reasons for the varied viewpoints of the early investigators. Storage of vitamin E in the body which resulted in the occurrence of one or two litters or "initial fertility" among females raised on a vitamin E free diet was probably a factor which delayed the recognition of vitamin E. The occurrence of small quantities of vitamin E in substances thought not to contain any also contributed. For example, Nelson, Ohrbech, Jones and Taylor (1928) reported that the occurrence of vitamin E in certain brands of cod liver oil had resulted in their conclusion that vitamin E was not necessary for reproduction. Kennedy (1927) attributed the divergence of opinion of early workers to a variation in the
vitamin E content of butterfat used in the ration.

More recent evidence, including that of Evans and Burr (1927a and 1927b), Clayton (1927) and Cummings and Mattill (1931) would indicate that the destruction of vitamin E in the ration by fats was a major cause for the difference in results. Hogan and Marshaw (1926) attributed their failure to duplicate Evans' results to the use of crisco instead of lard in their diets. Anderag and Nelson (1925) had noted the decomposition of the ration in storage when cod liver oil was mixed in the ration, and Nelson, Jones, Heller, Parks and Fulmer (1926) recognized the influence of high amounts of fat in the ration in causing sterility. These workers, however, did not at that time realize the role of fats in the destruction of vitamin E, and did not attribute failure in reproduction on such a ration to lack of vitamin E.

When more experimental evidence accumulated the nature of vitamin E became more completely understood and even the more hesitant investigators recognized the existence of this vitamin and its necessity for reproduction. Evans and Burr (1927) 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 (1931) and Evans (1932).
B. Physical and Chemical Properties of Vitamin E

The exact chemical composition of vitamin E is as yet unknown since the vitamin has not been isolated. However, concentrated fractions of vitamin E prepared from wheat germ oil, lettuce and cottonseed oil have been studied extensively to determine the chemical and physical nature of the vitamin.

Vitamin E was found to be insoluble in water, but readily soluble in ether, alcohol, and similar fat solvents. When a fat containing vitamin E was saponified the vitamin E separated out in the non-saponifiable portion. Evans and Burr (1925a and 1927) outlined a procedure taking advantage of these properties for the concentration of the vitamin E contained in wheat germ oil. The oil was passed through a series of processes involving saponification, solution in various solvents, crystallization, and finally vacuum distillation. The active portion was concentrated in a fraction making up only three-tenths of one per cent of the original volume. Olcott and Mattill (1931) have used a similar process for the preparation of a vitamin E concentrate from lettuce.

Vitamin E as present in the concentrated fraction of wheat germ oil was very stable to heat, light and laboratory manipulation according to Evans and Burr (1925a and 1927). In the dry state vitamin E has been found to be stable to temperatures up to 250° C. Ordinary light had no apparent effect on vitamin
other substance which occurred in combination with vitamin E, but were not associated with the development or absorption of vitamin E. However, Oootto (1977) and Kefftli (1973) have associated these in other work on vitamin E, Rizzo and Decker (1977, 1978) have also been able to prepare a compound with vitamin E, and Rizzo and Rizzo (1977) have associated these in the development of vitamin E, and Rizzo and Rizzo (1977) have associated these in the development of vitamin E.
C. Occurrence and Destruction of Vitamin E

Vitamin E occurs in a wide variety of natural foodstuffs. The amount of vitamin E present in a ration is primarily dependent on two factors: (1) the amount of vitamin E contained in the individual constituents going to make up the ration, and (2) the presence of substances which tend to destroy any vitamin E in the rest of the ration.

The best sources of vitamin E are seeds and green leaves. Wheat germ oil and lettuce were early recognized by Evans and Burr (1925) as especially good sources of the vitamin. Vitamin E occurs in most but not all vegetable oils (Evans 1932). Sure (1924a and 1925a) reported that wheat germ oil, hemp seed oil, and yellow corn oil were good sources of vitamin E, that cottonseed oil, olive oil, soy bean oil, peanut oil and oil of peach kernel were fair sources, but that there was practically no vitamin E in linseed oil, coconut oil and sesame oil. Olcott (1934) was able to prepare a very active vitamin E concentrate from cottonseed oil.

The occurrence of vitamin E is not confined to vegetable sources alone. Some vitamin E is found in animal musculature and fat, and a little in viscera according to Evans and Burr (1925a). Clayton and Cummings (1930) found that dried meats contained some but not large quantities of vitamin E. Anderegg and Nelson (1926) and Waddell, Steenbock and Hart (1931) both stated that milk
contained vitamin E. Cod liver oil is generally recognized as a poor source of vitamin E. Evans and Burr (1927) and Sure (1927) stated that cod liver oil was practically devoid of vitamin E, but in certain brands some vitamin E may occasionally be present as evidenced by the work of Nelson, Ohrbach, Jones and Taylor (1928) and of Simmonds, Becker and McCallum (1928).

Most farm animal feeds contain vitamin E. In an analysis of the constituents of a typical dairy cattle ration for vitamin E carried out by Hathaway and Davis (1934) it was found that alfalfa, bran, shorts, white or yellow corn, linseed oil meal, cottonseed meal, hominy feed 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 meal and corn gluten feed up to 40 per cent of the ration did not furnish adequate amounts of vitamin E. Alfalfa leaves were mentioned as a good source of vitamin E by Evans and Bishop (1922). Hathaway, Davis and Graves (1932) found that artificially cured alfalfa hay was slightly superior to field cured alfalfa hay as a source of vitamin E.

Even though some of the ingredients that go to make up a diet may contain vitamin E, it does not necessarily hold that the ration will supply sufficient amounts of the vitamin. Certain substances when mixed in a ration tend to destroy vitamin E in other ingredients, especially if vitamin E is present in only small amounts. Among the substances destructive
to vitamin E are certain fats, particularly unsaturated ones and those in which rancidity changes are taking place. Ferric chloride when brought into intimate contact with the fat soluble material also will bring about the destruction of vitamin E in the ration.

Practically all of the vitamin E deficient diets 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 25 per cent lard. Mattill, Carmen and Clayton (1924) 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 (1925) 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 (1926) also noted that the removal of lard from the ration resulted in improvement in reproduction.

Evans and Burr (1927a) found that quantities of vitamin E sufficient to permit reproduction if added to diets containing 8 per cent lard were inadequate to insure reproduction if the diet contained 15 per cent lard. In further studies these authors (1927b) found that fats varied in their destructive influence on vitamin E, and that the destructive influence was
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\& (1957) noted that the destruction &n
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-15-
of natural and varied foodstuffs was first suggested by Waddell and Steenbock (1926). These men treated the ration with one per cent by weight of ferric chloride which had been previously dissolved in ether. The ether was allowed to evaporate leaving the ferric chloride in intimate contact with the lipid constituents of the ration. This treatment completely destroyed the vitamin E present in the ration. Waddell and Steenbock (1931) stated that they believed the deactivation of vitamin E was due to the production of a substance actively opposed to vitamin E for which they accepted Evans and Burr's (1927b) designation "antivitamin". The addition of ferric chloride to the ration without the use of the fat solvent failed to destroy the vitamin E. A number of other investigators, particularly Card (1929) and Agemstone (1931) in their work with poultry have used the ferric chloride treatment for obtaining a vitamin E deficient ration.

D. Physiological Conditions in Rats Arising from Lack of Vitamin E in the Ration

The most notable physiological disturbance occurring among rats fed a diet lacking in vitamin E is failure in reproduction. In the female the sterility accompanying vitamin E deficiency is characterized by failure in the placental functions. Ovulation and fertilization occur normally and implantation of the embryo takes place. The fetus, however, is
retarded in development, death of the embryo ensues, and the products of conception are resorbed. Ovulation recurs a few days after resorption and the normal estrus cycle is resumed. Administration of vitamin E prior to or within five days after fertilization will allow normal development of the fetus. Any 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 required for fertility will not increase fecundity.

The intra-uterine changes occurring in sterility caused from lack of vitamin E are discussed by Evans and Burr (1927). Though accompanied by some leakage of blood, implantation took place without much disturbance. The retarded development of the fetus was noticed at the end of eight days. The subnormality occurred in the embryo and fetal part of the placenta. The yolk sac was under-developed and the hemopoietic organs were abnormal. Between the twelfth and seventeenth days the fetus died due, probably, to lack of oxygen following the breakdown of the circulatory system. In the process of resorption a marked breakdown of the placenta occurred on about the twentieth day of gestation. The histological picture of the uterine changes accompanying the resorption has been given by Urner (1931).

Vitamin E is necessary for fertility in the male rat as well as in the female. The conclusion by Mattill and Carmen
(1923) that the lack of some unknown dietary essential was the cause of degeneration of the testes of rats on a ration high in fat first associated lack of vitamin E with sterility in males. Mattill, Carmen and Clayton (1924) found that the testicular degeneration could be prevented by the addition of vitamin E. Mattill and Clayton (1925) reported that sterility in males on their high fat milk diets could be prevented by the addition of vitamin E or by a decrease in the amount of fat in the ration.

The development of the sterility in males on a ration lacking in vitamin E has been described by Evans (1925) and Evans and Burr (1927). Male rats raised from weaning on a vitamin E free diet became sterile in about four months. The stages of loss of fertility were (1) normal abundance of sperm but the sperm had lost their fertilizing power; (2) loss of sperm from the vaginal plug; (3) loss of power to form the vaginal plug; and (4) loss of all sex interest. The sterility was characterized by changes and even loss of the seminiferous epithelium. After sterility had developed only about one-fifth of the males could be cured by the feeding of a ration rich in vitamin E.

Mason (1925, 1926, 1929, and 1930) has discussed in detail the histological changes which accompanied the development of sterility in males on the diet low in vitamin E. The testes of such rats underwent a slow progressive degeneration of the germinal epithelium. The germ cells degenerated in the inverse
order of their formation. Usually the degeneration began 50
to 75 days after the rats were placed on the vitamin E free
ration and became nearly complete after 100 to 150 days on
the ration.

It will be noted that the sterility caused by lack of vita-
min E was quite different in the two sexes. In the female the
sterility was of placental origin and the germ cells did not
appear to be directly affected. The sterility was also of
temporary nature and was readily curable by the administration
of a good source of vitamin E. In males, on the other hand,
the sterility occurred as the result of destruction of the germ
cells, and regeneration of the germinal epithelium could be
brought about in only a small per cent of the cases.

Although vitamin E is concerned primarily with reproduc-
tion, some attention has been accorded the vitamin in relation
to other physiological processes. The body functions to which
vitamin E has been thought to be related include growth; iron
assimilation and hemoglobin formation; hormone action, particu-
larly that of the anterior pituitary; and tumor growth.

Evans (1928) reported the results of experiments in which
rats on high vitamin E diets showed greater growth and more
vigor than those on low vitamin E rations. The growth differ-
ences were more apparent in the later stages of life. Mason
(1929) suggested a relation between vitamin E and the increased
growth rate and greater growth capacity of male rats fed
lettuce. It has also been suggested (Blumberg 1935) that vitamin E may have more effect on growth during early and middle life than has been previously supposed, though this author notes that another unknown fat soluble factor might be responsible.

Since the death of the embryo resulting from vitamin E deficiency was associated with failures of the circulatory system, several of the early investigators conceived the idea that vitamin E played a role in blood formation. Simmonds (1925) and Simmonds, Becker and McCollum (1927) advanced the hypothesis that vitamin E was linked with iron assimilation. Hart, Steenbock, Elvehjem and Waddell (1925) thought that vitamin E might be related to the non-iron complex in hemoglobin formation. However, Anderson (1926) found no abnormalities in the chemical composition of the blood of rats suffering from vitamin E deficiency. Hogan and Harshaw (1926) 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 article Simmonds, Becker, and McCollum (1928) 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 (1932), is that vitamin E is not concerned in the formation of hemoglobin.

In the last four or five years it has been suggested by
several workers that vitamin E might be associated with endocrine secretions of the anterior pituitary. Leitch (1933) indicated that extracts of the anterior pituitary could replace vitamin E in a deficient diet and that the anterior pituitary ceased to be active in animals fed a vitamin E deficient diet. However, other investigators have not confirmed these findings. Evans (1932) stated that proof of any relationship between the vitamin and the anterior pituitary was lacking. Diakow and Krizenecky (1933) found that vitamin E failed to give the stimulation to sexual development that is brought about by anterior pituitary hormone preparations. They also found that the injections of extracts from the anterior pituitary would not induce fertility in females on a ration free from vitamin E. Olson and Mattill (1934) also concluded that there was no relationship between vitamin E and the sex hormone of the anterior pituitary. Further work on the relationship of the vitamin and the anterior pituitary has been recently reported by Stein (1935). No histological change in the hypophysis was apparent in vitamin E deficient females. He also found that there was no significant change in the size of the hypophysis of vitamin E depleted females when they were placed on a "cure" ration containing vitamin E.

Lack of vitamin E in the diet has been found to be associated with the proliferation of certain types of cells. Evans (1928a) found that spontaneous tumorous growths were formed in
the uteri of about sixty per cent of the females on a vitamin E deficient ration which were mated to vasectomized males, while only about four per cent of the females on a normal varied diet were so affected. Adamstone (1934) 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 suggests that vitamin E is intimately associated with, and probably exerts an indirect controlling influence over the cell nucleus during cell division.

E. Vitamin E Experiments With Animals Other Than Rats

Only a limited amount of experimental work on vitamin E has been attempted with animals other than the rat and mouse. Goettsh (1930) and Pappenheimer (1930) attempted to feed guinea pigs and rabbits on a ferric chloride treated ration. An emaciation of the voluntary muscles soon developed followed by paralysis. Death ensued in all cases before there was a chance to test the reproductive performance of the animals. Bertolini (as quoted by Evans, 1932) noted that vitamin E was probably necessary for reproduction in rabbits. Aubel, Hughes and Lienhardt (1930) found that the addition of wheat germ to the ration of gilts resulted in the weaning of a greater number of pigs. The work was not carefully enough controlled, however, to determine whether vitamin E had any influence on the results.
A study of the vitamin E requirements of poultry has been carried on by Gard and his associates (Ill. Agr. Exp. Sta. 1926, 1930 and 1931). Eight weeks old pullets were placed on a ration which had been treated with ferric chloride to destroy the vitamin E. At the age of one year they were mated to normal cocks. Though a large percentage of the eggs was 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 cubic centimeter 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 (1931) made a histological study of the embryos which failed to develop in the eggs from the hens on the vitamin E deficient ration. He noted a critical period at about the fourth day of incubation beyond which few embryos survived. The development of the general structures and organs was retarded but apparently otherwise not much changed from normal. The cause of the death of the embryo was a failure of metabolic and gas exchange arising from circulatory failures. The break-
Severely infected wheat germ, by treatment of the germ with a suitable antibiotic, was found to have been obtained by several investigators. Vogt-Weiler and others have been carried out on wheat germ of certain types by the formation of the antibiotic, followed by treatment and determination of the course of growth.

Though no experimental work has been carried out in which

\[ \text{reaction} \]

In reverse order of their formation as was true in the male

veririon, the pollen, on the other hand, were observed to degenerate

showed a marked variation in the stage of degeneration of the

male and female sex. The histological examination of the tissues

separate of the females have been of some years, but on the other hand, some of the

on the female, although no broad variation of the sex was still

on the male, the marked variation in the stage of degeneration, to develop then in the female, at the end of the first year

symptoms of degeneration, and in the male, were much slower.

Degeneration of the tissues of the male occurred a few months

oranges were found, but with no abnormalities of the liver and hemoprotein.

in the absence of evidence, in the antibiotic and determined reaction,

such conditions are needed. These with the conditons of the

formation of the antibiotic, antibiotics are formed from the antibiotic system being the formation from hemoprotein.

Taking system at the time of the formation, from hemoprotein
The cases so treated were restricted to ones in which estrus was occurring regularly and in which no known pathological lesions were present. The cows had previously failed to respond to ovarian treatments. The amount of wheat germ oil injected ranged from 10 to 30 cubic centimeters. Seventeen of the twenty cows treated with wheat germ oil became pregnant.

Tutt (1933) records 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 estrus occurred normally and regularly and in which there was no clinical evidence of disease in the ovaries, Fallopian tubes, uterus or vagina. He thought the role of the wheat germ oil was to preserve pregnancy in its earliest stages.

The conclusion drawn by these authors that vitamin E was the factor in wheat germ oil which cured the sterility does not, on critical analysis, appear to be very definitely established. In the case of Tutt's work other treatments were administered at the same time as the wheat germ oil treatment in all but two of the cases in which pregnancy resulted. Under such conditions it would certainly not be safe to attribute the results to only the injection of the wheat germ oil. Further-
more neither of the investigators gave any proof that vitamin E, rather than some other constituent of the wheat germ oil, was the curative factor. While these experiments open up a possible field, they need to be supported by detailed work to determine if sterility such as that cured by the wheat germ oil injections can be produced by the feeding of a ration deficient in vitamin E.
A. Vitamin E Deficient Experimental Ration Fed to the Goats

1. Composition of the goat ration

The ration fed to the goats in this experiment consisted of alfalfa hay and a grain mixture. Before they were fed, both the hay and the grain were treated with an ether solution of ferric chloride to destroy the vitamin E. The grain mixture contained:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground corn</td>
<td>35</td>
</tr>
<tr>
<td>Ground oats</td>
<td>30</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>30</td>
</tr>
<tr>
<td>Linseed oil meal</td>
<td>5</td>
</tr>
<tr>
<td>Bone meal</td>
<td>1</td>
</tr>
<tr>
<td>Salt</td>
<td>1</td>
</tr>
</tbody>
</table>

Before treatment with ferric chloride the alfalfa hay was chopped into approximately one inch lengths and the grain mixture was reground. Three parts of grain to each part of hay were fed until August, 1934, when the ratio was changed to two parts of grain and one part of hay. This latter proportion represents the ration which was fed successfully to experimental goats in confinement by Steenbock, Hart, Hoppert and Black (1925).

The treated ration was supplemented by the feeding of cod liver oil at the rate of 10 cubic centimeters per goat daily and by the addition of yeast to the extent of 1 per cent of the goat ration. These supplements were added as a precaution to insure an abundance of vitamins A, B, D and G in the ration. Though neither the cod liver oil nor the yeast was treated with
ferric chloride, both were tested to insure their freedom from vitamin E before they were fed to the goats.

2. **Treatment of the goat ration for the destruction of vitamin E**

The method of destruction of vitamin E in the ration was essentially that outlined by Waddell and Steenbock (1923). Ferric chloride to the amount of 1 per cent of the ration was brought into contact with the fat soluble material in the ration through the medium of an 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 deactivation of vitamin E present in the ration.

Grain was treated in batches of 50 pounds and the hay in 16 pound lots. Ferric chloride making up 1 per cent by weight of the feed to be treated (227 grams for the grain and 72 grams for the hay) was finely pulverized in a mortar and dissolved in approximately 50 pounds of ether. The ether solution of ferric chloride was then mixed with the feed and allowed to stand one to two hours. Enough ether was used to cover the feed with the ferric chloride solution. After the feed had soaked one to two hours steam was turned into the coil heating a glycerine bath surrounding the vessel in which the ether-feed mixture was contained. Heating was continued until no more ether
distilled over. About 12 hours were required to drive the ether out of the hay and about 18 hours for the grain. The feed was then removed from the still and spread out in a shallow metal pan for 6 to 12 hours to allow the last of the ether to evaporate. The feed was stored in galvanized cans at room temperature until used. It was nearly always consumed within ten days following treatment. After a thorough mixing of the treated feed, a sample was taken from each batch to test for the presence of vitamin E.

In August 1934 a slight modification was made in the apparatus used to treat the feed. The same method was used but the feed was treated in smaller lots, there being 30 pounds of grain or 15 pounds of hay handled at one treatment. The feed was allowed to soak in the ether solution of ferric chloride for twelve hours instead of only two or three. This greater length of time was thought advisable to insure penetration of the solution into any larger particles of feed that might be present. With the new apparatus a shorter time was required to drive the ether out of the feed, the operation being accomplished in about eight hours with grain and twelve to fifteen hours with hay. The methods of airing, sampling and storing of the feed were the same as previously outlined.
B. Method of Testing the Goat Ration for the Presence of Vitamin E

Samples of the treated goat feed were tested for vitamin E by feeding to rats. The methods outlined by Evans and Burr (1927) were followed. Female rats which had just completed a resorption on a vitamin E deficient ration were placed on the goat ration to be tested, bred and the result of the mating determined.

1. Rats used for test purposes

The rats used in the experiment came chiefly from the stock colony of the Animal Chemistry and Nutrition Laboratory. These rats were a strain of mixed white and hooded rats descended from stock secured from the Steenbock colony. A number of the test rats were from the colony of the Foods and Nutrition Laboratory, these being white rats originating from Wistar stock. A few rats were also secured from the colonies of Dr. Waters of the Poultry Department and Dr. Lambert of the Genetics Department.

The rats were housed in the experimental cages of the Animal Chemistry and Nutrition Laboratory. These cages were wire mesh about eight by twenty inches in size set in steel frames. Three to five rats were kept in a cage. Cage floors were 3/8 inch mesh wire screens, below which were set pans of shavings to catch the excreta. The rats were fed in tin cups
<table>
<thead>
<tr>
<th>Time</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Dr. check</td>
</tr>
<tr>
<td>4</td>
<td>Alert on meet</td>
</tr>
<tr>
<td>10</td>
<td>Drug cause</td>
</tr>
<tr>
<td>15</td>
<td>Butter take</td>
</tr>
<tr>
<td>30</td>
<td>Tinseed oil meet</td>
</tr>
<tr>
<td>60</td>
<td>Bitter mix powder</td>
</tr>
<tr>
<td>120</td>
<td>Tetter smear</td>
</tr>
</tbody>
</table>

The laboratory rat ration concentration of:

- The need or weight end.
- The method of measurement was determined by the variance of the present test.
- The ration of the variance of the test was necessary to determine the variance of the laboratory observation.

Only remove all proved reactive as shown by the previous

2. Determination of the variance & reserves of test rate

Immediately on one month.

- Bottles were verified daily and cleaned at the beginning of each daily.
- Blank pans were verified at the end of each daily.
- Read one and end of
- Read one and end of
- Read one and end of
- Read one and end of
- Read one and end of
- Read one and end of
- Read one and end of
- Read one and end of
- Read one and end of
Treatments of the rat ration for destruction of vitamin E were essentially the same as for the goat ration. Ferric chloride amounting to 1 per cent by weight of the ration to be treated was pulverized in a mortar and dissolved in an amount of ether which would completely saturate and cover the feed to be treated. The ether solution of ferric chloride was then mixed thoroughly with the rat ration and allowed to stand for 10 to 30 minutes. The ether was then allowed to evaporate from the mixture leaving the ferric chloride in the ration. The mixture was stirred occasionally during the evaporation process. The evaporation was carried out in a shallow vessel at room temperature or at a temperature of approximately 130 degrees F. in a drying room. Beginning in August 1934 the evaporation of ether was carried out from a closed container set in hot water. The purpose of this modification was to enable the recovery of the ether used in the treatment.

The rat ration treated by the method outlined above for the destruction of vitamin E will be referred to in this paper as "ferric chloride treated rat ration".

After a period of one to two months on the ferric chloride treated rat ration the female rats were placed with the males and their reproductive performance carefully followed by means of weights and vaginal smears* taken daily. As soon as re-

*Vaginal smears as outlined by Long and Evans (1922) were used to follow the estrus cycle of the rats.
sorption of the young typical of vitamin E deficiency occurred
the females were moved to the group being used to test the goat
ration for the presence of vitamin E.

3. Testing of the goat ration for vitamin E

After an initial resorption to prove their freedom from
body reserves of vitamin E, the test rats were placed on the
ration to be tested. The ration to be tested consisted of the
same ration the goats received or of material included in the
good ration mixed with ferric chloride treated rat ration. The
exact composition of each ration tested is given in the section
on results.

The vitamin E depleted females were kept for a preliminary
period of five days on the ration being tested before they were
mated. This interval allowed a period of at least ten days
(five days before mating and five days after) during which any
vitamin E in the ration could take effect. In two or three
instances this period was decreased slightly, but in no case
was the critical period on a test ration less than five days.
In most cases it was much longer than ten days.

After the preliminary interval on the goat ration the
rats to be used as test animals were placed with males of
proved breeding ability at each proestrus period. The time
for placing with the male was determined by daily vaginal smears.
The female was allowed to remain with the male until smears typical of the diestrus period appeared, usually two days, and then removed.

The male rats used were from the stock colony and received the normal rat ration except during the time they were used for mating. At least two days before being put in the mating cages the males were placed on the ferric chloride treated rat ration. This practice was followed to eliminate the possibility of the females getting vitamin E from the feces of the male. The males were not kept continuously in the mating cages on the ferric chloride treated rat ration, but were returned to the stock colony after about two weeks. Placing of the females in the male cage proved more satisfactory from the standpoint of securing a positive mating than did placing the male in the female cage.

Positive mating was diagnosed by the finding of sperm in the vaginal smear. Implantation of the fetus was recognized by the occurrence of the "placental sign" which is the appearance of blood in the smear about the thirteenth day after mating. After implantation the results of the gestation were determined by checking the daily weights and by examination of the rat for any indications of birth of young. Vaginal smears were also continued to indicate any recurrence of estrus. Increase in weight of the female after the appearance of the placental sign up to the 15th to 20th days of gestation followed by a gradual
decrease in weight was regarded as a resorption. The finding of young, or a sudden precipitous drop in weight sufficient to indicate the birth of a litter on the 22nd or 23rd day was regarded as failure to resorb. Resorption of young gave evidence of the freedom of the ration from vitamin E.

C. Goats Used in the Experiment

1. Females

Seven female goats of mixed origin and breeding were placed on the ferric chloride treated experimental ration. The goats all showed evidences of being of 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 living young within three months prior to the time they were placed on the experimental ration. The other three were female kids which had not yet been bred. These seven females and the offspring produced by them while on the experiment constituted all the goats used in the trial.

The goats were healthy and vigorous. All reacted negatively to the tuberculin test and to the agglutination test for contagious abortion. Before being taken to the experimental pens to be placed on the treated experimental ration, all goats
were dehorned, clipped and washed with creosote dip.

The four female goats of proved fertility (44-B, 44-D, 44-E, and 44-F) were placed on the vitamin E deficient experimental ration July 2, 1933. The ration as previously indicated was made up of a ferric chloride treated grain mixture and ferric chloride treated alfalfa hay supplemented with cod liver oil and yeast. On August 21, 1933, the three female kids (44-G, 44-H, and 44-I) were placed on the same ration.

2. Males

The male goat used during the first breeding season (fall and winter 1933-34) was a white male of proved fertility. The male received a varied ration of natural foodstuffs. During the second breeding season (fall, 1934) male kids which had been raised on the vitamin E deficient ration were used to breed the experimental females.

3. Housing and care of the goats

The methods of caring for the goats used in the experiment were designed to eliminate as far as possible any chance of the animals receiving vitamin E. The goats were housed at the Animal Chemistry and Nutrition Laboratory in a room as free from dust and other sources of contamination as was possible to secure. The goats were kept in cages made of wire fencing
To prevent access to unopened seed.

the reservoir for any reason, such as breeding, it was

the gate was taken out of

fore the gate at all times. Therefore, keep the

water with the apparatus sterilized. Keep the gate

watered because they demanded more feed than could be

when they were treated. During this period they were some-

rationed they would consume, except during June and July, 1934.

watered from feed pellets. The gate was then fed at the

watered between and fed to maintain growth of the

Takes daily the gate were fed a mixture of a mixture of 75% silage

Kidder time each female was penned separately.

of such size as to allow several cages to run together. A

below the screen bottom to absorb the excrement. Gates were

framed wood screen. Shovels were kept on the floor. 12 inches

the bottom were made of 3/4 inch mesh No. 9

wooden frame. In the latter part of the experiment

extended on wooden frame.
RESULTS

A. Tests of the Goat Ration for Vitamin E

1. Preliminary studies

Before the experiment proper was begun a number of preliminary observations were made on the methods to be used. 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 to July 1, 1933. During this time ferric chloride treatments of hay and of grain 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 and treated hay were tested separately in the initial work. The grain was very finely ground and fed as the sole ration to the vitamin E depleted female rats. The hay was finely ground and 20 parts by weight were mixed with 80 parts of ferric chloride treated rat ration. Testing of the rations was carried out by methods previously outlined.

Four vitamin E depleted female rats were placed on the treated goat grain on February 29, 1933. The test rats were kept continuously on the treated ration being remated as soon
as the result of each previous mating became known. The results of each gestation* resulting from matings while the rats were on the treated goat ration are shown in table I.

**TABLE I**

REPRODUCTIVE HISTORY OF RATS IN PRELIMINARY TESTS WITH FERRIC CHLORIDE TREATED GRAIN PREPARED FOR GOATS

<table>
<thead>
<tr>
<th>Rat number</th>
<th>Date of breeding</th>
<th>Result of gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>March 2, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>15</td>
<td>March 5, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>19</td>
<td>March 6, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>2</td>
<td>March 10, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>19</td>
<td>May 9, 1933</td>
<td>Litter**</td>
</tr>
<tr>
<td>15</td>
<td>May 15, 1933</td>
<td>Litter**</td>
</tr>
<tr>
<td>1</td>
<td>May 21, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>2</td>
<td>May 27, 1933</td>
<td>Fetus resorbed</td>
</tr>
</tbody>
</table>

**No. 15 and 19 escaped from the experimental cages and were on the floor of the laboratory over night on April 25, 1933, thus having access to feed other than the treated ration.

On March 18, 1933, seven vitamin E depleted females were placed on a ration 20 per cent of which consisted of the treated alfalfa hay being tested and 80 per cent of which was ferric chloride treated rat ration. Results of the gestations occurring on this ration are given in table II.

In view of 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

*Gestation is here interpreted as a positive mating followed by the placental sign as definite proof of implantation.
TABLE II

REPRODUCTIVE HISTORY OF RATS ON RATION OF 20 PER CENT FERRIC CHLORIDE TREATED ALFALFA HAY AND 80 PER CENT FERRIC CHLORIDE TREATED RAT RATION

<table>
<thead>
<tr>
<th>Rat number</th>
<th>Date of breeding</th>
<th>Result of gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>March 31, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>10</td>
<td>April 4, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>5</td>
<td>April 4, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>9</td>
<td>April 16, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>10</td>
<td>May 6, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>9</td>
<td>May 23, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>20</td>
<td>June 5, 1933</td>
<td>Fetus resorbed</td>
</tr>
</tbody>
</table>

Rats No. 3, 8, and 22 failed to become pregnant so are not listed above.

The concentration of ferric chloride in the ration was necessary to destroy the vitamin E present. The method of deactivating 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 1.0 per cent was used in treating the feed. A sample batch of 15 pounds of goat grain was treated by this method on September 17, 1933. This treated ration was then ground finely and fed to E-depleted female rats, four individuals being placed on the ration September 22, 1933. The reproductive performance of the rats was followed by the methods already outlined. Results of the gestations are shown in table III.

The results of all the tests carried out in the preliminary trials are given in table IV.
TABLE III
RESULTS OF GESTATIONS OF RATS RECEIVING 0.25 PER CENT FERRIC CHLORIDE TREATED GOAT RATION

<table>
<thead>
<tr>
<th>Rat number</th>
<th>Date of breeding</th>
<th>Result of gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>September 27, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>9</td>
<td>October 3, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>4</td>
<td>October 8, 1933</td>
<td>Fetus resorbed</td>
</tr>
</tbody>
</table>

The fourth rat, No. 5, placed on the ration failed to conceive.

TABLE IV
RESULTS OF PRELIMINARY TESTS FOR VITAMIN E IN GOAT FEEDS TREATED WITH FERRIC CHLORIDE

<table>
<thead>
<tr>
<th>Feed</th>
<th>No. of rats</th>
<th>No. of gestations</th>
<th>No. of resorptions</th>
<th>No. of litters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain treated with 1.0 per cent ferric chloride</td>
<td>4</td>
<td>8</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Hay treated with 1.0 per cent ferric chloride</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Grain treated with 0.25 per cent ferric chloride</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>18</td>
<td>16</td>
<td>2</td>
</tr>
</tbody>
</table>
The results of the preliminary investigations indicated that the treatment with ferric chloride dissolved in ether was effective in bringing about the destruction of vitamin E in both the grain and the hay used as goat feed. Where the feed had been treated with one per cent of ferric chloride six resorptions occurred on the grain ration and seven on the hay ration. Furthermore where the grain was later treated with only twenty-five hundredths of one per cent of ferric chloride the three test gestations all resulted in resorptions. The two litters which occurred in the preliminary tests came from rats which had not been confined to the ration tested, and hence should not be considered in the results. Several of the rats completed two consecutive resorptions on the treated ration.

The preliminary results further indicated that the treated ration would be satisfactory from the standpoint of the goats. The goats ate the ration readily and showed no apparent ill effects from the consumption of it. Although the treatment with the smaller per cent of ferric chloride seemed effective in destroying the vitamin E in the ration, it was decided to continue to treat the feed with one per cent by weight of the ferric chloride to afford a margin of safety.

2. Routine tests of the treated 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 hay and grain 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 treated goat ration. In addition some monthly composite sample trials were run and the untreated supplementary constituents of the ration were tested for the presence of vitamin E.

a. "Current tests" of ferric chloride treated goat ration for vitamin E.

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 a week or less, depending on the rate of treatment of the feed, were ground together and fed immediately to a group of test rats. 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 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 rat received only this ration until it died or was 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 followed by means of vaginal smears and weight curves.

The results of all "current tests" for the presence of vitamin E in the ferric chloride treated goat ration are shown in table V.

b. Test of monthly composite feed samples for vitamin E.

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 E 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.
TABLE V

REPRODUCTIVE PERFORMANCE OF RATS USED IN "CURRENT TESTS" OF FERRIC CHLORIDE TREATED GOAT RATION

<table>
<thead>
<tr>
<th>Rat number</th>
<th>Date placed on ration</th>
<th>Date bred</th>
<th>Result of gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>31*</td>
<td>October 13, 1933</td>
<td>October 23, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>29</td>
<td>October 13, 1933</td>
<td>October 26, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>33</td>
<td>October 18, 1933</td>
<td>November 1, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>31b</td>
<td>December 30, 1933</td>
<td>January 4, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>56</td>
<td>December 26, 1933</td>
<td>January 10, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>72</td>
<td>December 27, 1933</td>
<td>February 7, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>72b</td>
<td>February 11, 1934</td>
<td>February 13, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>31c</td>
<td>February 15, 1934</td>
<td>March 5, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>72c</td>
<td>March 11, 1934</td>
<td>March 30, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>56c</td>
<td>March 25, 1934</td>
<td>April 3, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>101</td>
<td>April 16, 1934</td>
<td>April 21, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>72e</td>
<td>May 7, 1934</td>
<td>May 29, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>55</td>
<td>May 13, 1934</td>
<td>June 9, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>75</td>
<td>June 29, 1934</td>
<td>July 23, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>55b</td>
<td>July 18, 1934</td>
<td>August 1, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>93</td>
<td>August 1, 1934</td>
<td>August 22, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>94</td>
<td>September 14, 1934</td>
<td>October 4, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>82</td>
<td>October 1, 1934</td>
<td>October 7, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>101d</td>
<td>October 11, 1934</td>
<td>October 11, 1934</td>
<td>Fetus resorbed</td>
</tr>
</tbody>
</table>

*The letters b, c, etc. attached to the number of the rat indicate gestation 2, gestation 3, etc. after being placed on the ration.

**The figure in parenthesis indicates the number of young found.
TABLE V continued

<table>
<thead>
<tr>
<th>Rat number</th>
<th>Date placed on ration</th>
<th>Date bred</th>
<th>Result of gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>94b</td>
<td>October 8, 1934</td>
<td>October 15, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>112</td>
<td>October 17, 1934</td>
<td>October 17, 1934</td>
<td>Litter (1)</td>
</tr>
<tr>
<td>55e</td>
<td>October 25, 1934</td>
<td>October 26, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>121</td>
<td>September 21, 1934</td>
<td>October 31, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>120</td>
<td>September 27, 1934</td>
<td>October 26, 1934</td>
<td>Litter (1)</td>
</tr>
<tr>
<td>116b</td>
<td>November 16, 1934</td>
<td>November 30, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>121b</td>
<td>December 5, 1934</td>
<td>December 9, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>116c</td>
<td>December 24, 1934</td>
<td>December 28, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>110</td>
<td>January 4, 1935</td>
<td>January 13, 1935</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>94c</td>
<td>February 2, 1935</td>
<td>April 1, 1935</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>121d</td>
<td>April 4, 1935</td>
<td>April 18, 1935</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>250b</td>
<td>April 24, 1935</td>
<td>April 24, 1935</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>158</td>
<td>April 26, 1935</td>
<td>April 26, 1935</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>150</td>
<td>April 28, 1935</td>
<td>May 2, 1935</td>
<td>Litter*</td>
</tr>
<tr>
<td>258</td>
<td>May 6, 1935</td>
<td>May 6, 1935</td>
<td>Litter (?)</td>
</tr>
</tbody>
</table>

Summary

<table>
<thead>
<tr>
<th>No. of rats on ration</th>
<th>No. of gestations</th>
<th>No. of resorptions</th>
<th>No. of litters</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>54</td>
<td>48</td>
<td>6</td>
</tr>
</tbody>
</table>

*Weight changes indicate a probable litter but no young were found.

They consisted of three parts ferric chloride treated goat grain and one part of ferric chloride treated alfalfa hay. The ingredients were thoroughly mixed and finely ground and fed to vitamin E depleted female rats. The reproductive performance
of the rats while on the ration was observed. Results of the
tests of the monthly composite samples are given in tables VI
and VII.

### TABLE VI

**SUMMARY OF REPRODUCTIVE PERFORMANCE OF RATS IN TESTS OF MONTHLY
COMPOSITE SAMPLES OF FERRIC CHLORIDE TREATED GOAT RATION**

<table>
<thead>
<tr>
<th>Month of treatment of feed</th>
<th>No. of rats on ration</th>
<th>No. of gestations</th>
<th>No. of resorptions</th>
<th>No. of litters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1933</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>August</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>September</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>October</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>November</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>December</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1934</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>February</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>March</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>April</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>May</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Total 38* 14 14 0

*Six rats were used on two tests and on on three tests.
**TABLE VII**

HISTORY OF INDIVIDUAL RATS USED IN TESTS OF MONTHLY COMPOSITE SAMPLES OF FERRIC CHLORIDE TREATED GOAT RATION

<table>
<thead>
<tr>
<th>Date of feed treatment</th>
<th>Rat No.</th>
<th>Placed on ration</th>
<th>Removed from ration</th>
<th>Date bred</th>
<th>Result of mating</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 1933</td>
<td>38</td>
<td>Oct. 28, 1933</td>
<td>Dec. 9, 1933</td>
<td>Nov. 4, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>Oct. 28, 1933</td>
<td>Dec. 9, 1933</td>
<td>Nov. 5, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>Oct. 28, 1933</td>
<td>Dec. 9, 1933</td>
<td>Nov. 5, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>Oct. 29, 1933</td>
<td>Dec. 9, 1933</td>
<td>Failed to breed</td>
<td></td>
</tr>
<tr>
<td>August 1933</td>
<td>35</td>
<td>Oct. 17, 1933</td>
<td>Dec. 9, 1933</td>
<td>Oct. 28, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>Oct. 17, 1933</td>
<td>Dec. 9, 1933</td>
<td>Oct. 28, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>Oct. 17, 1933</td>
<td>Dec. 9, 1933</td>
<td>Oct. 28, 1933</td>
<td>Implantation occurred but resorption was not definite</td>
</tr>
<tr>
<td>September 1933</td>
<td>56</td>
<td>Nov. 11, 1933</td>
<td>Dec. 26, 1933</td>
<td>Nov. 27, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>Nov. 11, 1933</td>
<td>Jan. 15, 1934</td>
<td>Failed to breed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>Nov. 11, 1933</td>
<td>Jan. 14, 1934</td>
<td>Failed to breed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>Dec. 21, 1933</td>
<td>Jan. 16, 1934</td>
<td>Failed to breed</td>
<td></td>
</tr>
<tr>
<td>October 1933</td>
<td>61</td>
<td>Nov. 14, 1933</td>
<td>Feb. 3, 1934</td>
<td>Nov. 19, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>Nov. 14, 1933</td>
<td>Dec. 11, 1933</td>
<td>Failed to breed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>Nov. 14, 1933</td>
<td>Feb. 3, 1934</td>
<td>Failed to breed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>Dec. 7, 1933</td>
<td>Feb. 3, 1934</td>
<td>Failed to breed</td>
<td></td>
</tr>
<tr>
<td>November 1933</td>
<td>22</td>
<td>Dec. 3, 1933</td>
<td>Dec. 30, 1933</td>
<td>Failed to breed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>Dec. 9, 1933</td>
<td>Feb. 5, 1934</td>
<td>Failed to breed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>Dec. 7, 1933</td>
<td>Feb. 5, 1934</td>
<td>Failed to breed</td>
<td></td>
</tr>
<tr>
<td>December 1933</td>
<td>42</td>
<td>May 20, 1934</td>
<td>June 24, 1934</td>
<td>May 31, 1934</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>May 23, 1934</td>
<td>July 23, 1934</td>
<td>Failed to breed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>87</td>
<td>May 20, 1934</td>
<td>Aug. 23, 1934</td>
<td>Was bred but implantation did not occur</td>
<td></td>
</tr>
<tr>
<td></td>
<td>97</td>
<td>May 20, 1934</td>
<td>Aug. 1, 1934</td>
<td>Was bred but implantation did not occur</td>
<td></td>
</tr>
<tr>
<td></td>
<td>92</td>
<td>July 15, 1934</td>
<td>Aug. 11, 1934</td>
<td>July 20, 1934</td>
<td>Implantation sign appeared on July 29, but weight did not change sufficiently to prove a resorption.</td>
</tr>
<tr>
<td>Date</td>
<td>Tons Prepared to Feed</td>
<td>Date</td>
<td>Tons Prepared to Feed</td>
<td>Date</td>
<td>Tons Prepared to Feed</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------</td>
<td>------------</td>
<td>-----------------------</td>
<td>------------</td>
<td>-----------------------</td>
</tr>
</tbody>
</table>

This table contains data related to tons prepared to feed. The data is recorded over several dates from June 24, 1974, to August 31, 1974. The numbers range from 45 to 105 tons prepared to feed each day.
3. Test of untreated supplements in control ration.

a. Cod liver oil.

The cod liver oil used in the ration was tested for vitamin E. Five parts of cod liver oil were mixed with 95 parts of ferric chloride treated rat ration. The cod liver oil was mixed with the ration twice each week to prevent the development of rancidity. Each of the two brands of cod liver oil used during the experiment was tested separately. Vitamin E depleted females were placed on the ration containing the cod liver oil and their subsequent reproductive performance followed. The results of the gestations on the two cod liver oil rations are given in tables VIII, IX, and X.

Test of Squibbs cod liver oil

On August 3, 1933, eight vitamin E depleted females were placed on the ration consisting of 5 per cent Squibbs cod liver oil and 95 per cent ferric chloride treated rat ration. Results are given in table VIII.

Test of Ealsingh and Lughtigheir cod liver oil

On December 24, 1933, female rats No. 32 and No. 33 were

---

*Specifications of cod liver oil from Ealsingh and Lughtigheir: 100 per cent pure Norwegian poultry cod liver oil, guaranteed to contain 500 units vitamin A and 250 units vitamin D per gram, with a maximum free fatty acid content of 1.4 per cent. Steam refined, filtered and non-freezing. Amber.*
<table>
<thead>
<tr>
<th>Date of Resorption</th>
<th>Result of Gestation</th>
<th>Rate No.</th>
<th>Date Fed</th>
<th>Result of Gestation</th>
<th>Rate No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>January 10', 1934</td>
<td>1</td>
<td>12</td>
<td>January 9', 1934</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>January 4', 1934</td>
<td>23</td>
<td>23</td>
<td>January 4', 1934</td>
<td>23</td>
<td>23</td>
</tr>
</tbody>
</table>

**TABLE IX**

GROWTH HORMONE ADDITION PACE 5 PER CENT EXTENSION AND REDUCTION IN PERCENTAGE OF ORTHORADIAL CAST

The data presented in Table IX illustrate the results of the experiment on the growth hormone addition pace 5 per cent extension and reduction in percentage of orthoradial cast. The results show a consistent increase in growth hormone addition rate, with a corresponding decrease in the rate of orthoradial cast. The data are tabulated in the following manner:

<table>
<thead>
<tr>
<th>Date of Resorption</th>
<th>Result of Gestation</th>
<th>Rate No.</th>
<th>Date Fed</th>
<th>Result of Gestation</th>
<th>Rate No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>August 27', 1933</td>
<td>9</td>
<td>9</td>
<td>August 18', 1933</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>August 12', 1933</td>
<td>4</td>
<td>4</td>
<td>August 12', 1933</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>August 11', 1933</td>
<td>10</td>
<td>10</td>
<td>August 9', 1933</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

**TABLE XI**

GROWTH HORMONE ADDITION PACE 5 PER CENT SQUARES GOD LAYER OIL

The data presented in Table XI illustrate the results of the experiment on the growth hormone addition pace 5 per cent squares God layer oil. The results show a consistent increase in growth hormone addition rate, with a corresponding decrease in the rate of squares. The data are tabulated in the following manner:

<table>
<thead>
<tr>
<th>Date of Resorption</th>
<th>Result of Gestation</th>
<th>Rate No.</th>
<th>Date Fed</th>
<th>Result of Gestation</th>
<th>Rate No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>August 27', 1933</td>
<td>9</td>
<td>9</td>
<td>August 18', 1933</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>August 12', 1933</td>
<td>4</td>
<td>4</td>
<td>August 12', 1933</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>August 11', 1933</td>
<td>10</td>
<td>10</td>
<td>August 9', 1933</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>
TABLE X

SUMMARY OF RESULTS OF GESTATIONS WHEN 5 PER CENT OF COD LIVER OIL WAS FED WITH A VITAMIN E FREE RATION

<table>
<thead>
<tr>
<th>Oil</th>
<th>No. of rats</th>
<th>No. of gestations</th>
<th>No. of resorptions</th>
<th>No. of litters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squibbs</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>E &amp; L</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>8</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

Every gestation occurring among the rats fed the ration of 5 per cent cod liver oil and 95 per cent ferric chloride treated ration terminated in a resorption. This indicates that the cod liver oil in the goat ration contained no measurable amount of vitamin E.

b. Yeast.*

Though yeast is notably free from vitamin E, that fed in this experiment was tested before use. A ration was made up containing 10 per cent yeast and 90 per cent ferric chloride treated rat ration. Female rats depleted of vitamin E were placed on the ration and their subsequent reproductive performance determined.

On September 15, 1933, rats Nos. 1, 10, and 19 were placed on the ration. On September 27, 1933, rats Nos. 22 and 25 were placed on the ration.

*Northwestern powdered yeast foam tablets.
added. The rats were taken off the ration November 10, 1933 except No. 10 which died September 26, 1933 and No. 25 which was removed October 26, 1933. Results of gestations on the ration are given in table XI.

TABLE XI

REPRODUCTIVE PERFORMANCE OF RATS FED 10 PER CENT YEAST TOGETHER WITH 90 PER CENT FERRIC CHLORIDE TREATED RAT RATION

<table>
<thead>
<tr>
<th>Rat number</th>
<th>Date bred</th>
<th>Result of gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>September 21, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>19</td>
<td>September 21, 1933</td>
<td>Fetus resorbed</td>
</tr>
<tr>
<td>19</td>
<td>October 28, 1933</td>
<td>Fetus resorbed</td>
</tr>
</tbody>
</table>

Summary

<table>
<thead>
<tr>
<th>Rats</th>
<th>Gestations</th>
<th>Resorptions</th>
<th>Litters</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Rat No. 10 died before being bred and rats No. 22 and No. 25 failed to breed

4. Summary of rat tests on the goat ration

In table XII are brought together all the tests made with rats to determine if the goat ration contained vitamin E.

It will be noted that among the 100 rats which were actually used for the experimental testing of the goat feed 97 gestations occurred of which 89 resulted in resorptions. Of the eight litters two are explained by accidental access of the rats to other feed and should not be considered in the results. The
TABLE XIX
SUMMARY OF ALL TESTS FOR THE PRESENCE OF VITAMIN E IN
THE GOAT RATION

<table>
<thead>
<tr>
<th>Kind of test</th>
<th>No. of rats</th>
<th>No. of gestations</th>
<th>No. of resorptions</th>
<th>No. of litters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preliminary tests of treated goat feed</td>
<td>15</td>
<td>18</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Current tests of treated goat ration</td>
<td>32</td>
<td>54</td>
<td>48</td>
<td>6</td>
</tr>
<tr>
<td>Monthly composite tests of treated goat ration</td>
<td>38</td>
<td>14</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>12</td>
<td>8</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Yeast</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total all tests</td>
<td>100</td>
<td>97</td>
<td>89</td>
<td>8</td>
</tr>
</tbody>
</table>

circumstances surrounding the birth of the other six litters are taken up in detail under discussion of results.

B. Reproduction of the Goats on the Ferric Chloride Treated Ration

Five of the seven female goats originally placed on the treated ration were bred between December 24, 1933 and January 9, 1934. Approximately six months had elapsed between the time the goats were placed on the experimental ration and the
date they were bred. The five gestations resulted in the birth of nine kids, eight of which were alive and healthy. Each of the five females 44-D, 44-E, 44-F, 44-G and 44-H delivered one or more live offspring. One of the twin kids of 44-H was born dead although examination by Dr. McNutt of the Veterinary Research Department indicated that the kid was completely developed and apparently normal.

Two of the seven original female goats did not reproduce during the first year. Goat 44-B died on September 8, 1933, before any test of her reproductive ability could be made. A detailed post mortem examination by Dr. McNutt failed to reveal the cause of her death. However, the apparent health of all the other goats on the same ration would indicate that lack of vitamin E was probably not the cause. The other female not producing offspring, 44-I, failed to come in heat the first year. The second year, however, she was bred and delivered live offspring.

The six female goats were continued on the ferric chloride treated experimental ration for a second year. All six were bred between October 16, 1934 and November 11, 1934. Five of the females, 44-D, 44-E, 44-F, 44-G, and 44-I, delivered four pairs of twins and one single, all alive and healthy. The sixth female, 44-H, encountered trouble during gestation which involved a possible abortion. She was bred again later in the season and delivered a dead kid. A more detailed de-
scription of the case is given later under discussion of results.

Three of the eight kids born in the spring of 1934 were females. These female offspring were raised on the ferric chloride treated experimental ration supplemented only by the milk from their mothers which also received the treated ration. Two of these female kids, 44-J and 44-K, grew well and were bred on the 14th and 17th of November 1934. They completed successful gestation periods which culminated in the birth of two sets of healthy kids on April 14 and 16, 1935. The third female kid, 44-L, did not grow well from birth though her male twin was apparently normal and healthy in every way. She did not come in heat during the breeding season and was finally killed and posted on February 28, 1935. The examination after death revealed the presence of several abscesses and a marked underdevelopment of the thyroid glands.

Table XIII gives a complete description of the reproductive performance of the female goats which received the vitamin E-deficient ration. A detailed history of each goat is given in the appendix.


<table>
<thead>
<tr>
<th>Female goat</th>
<th>Date placed on experimental ration</th>
<th>First year reproduction</th>
<th>Second year reproduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Date</td>
<td>Date of parturition</td>
<td>Date of off-bred parturition</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. of off-spring</td>
<td>No. of off-spring</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>44-B</th>
<th>July 2, 1933</th>
<th>Died Sept. 8, 1933</th>
<th>Never bred</th>
</tr>
</thead>
<tbody>
<tr>
<td>44-D</td>
<td>July 2, 1933</td>
<td>Jan. 9, June 7, 1934</td>
<td>D-1♂</td>
</tr>
<tr>
<td></td>
<td>Sep. 20, 1933</td>
<td>1934</td>
<td>44-D♀</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1934</td>
<td>1935</td>
</tr>
<tr>
<td>44-E</td>
<td>July 2, 1933</td>
<td>Dec. 27, May 26, 1934</td>
<td>E-1♂</td>
</tr>
<tr>
<td></td>
<td>Sep. 20, 1933</td>
<td>1934</td>
<td>44-E♀</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1934</td>
<td>1935</td>
</tr>
<tr>
<td>44-F</td>
<td>July 2, 1933</td>
<td>Jan. 2, June 3, 1934</td>
<td>F-1♂</td>
</tr>
<tr>
<td></td>
<td>Sep. 20, 1933</td>
<td>1934</td>
<td>44-F♀</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1934</td>
<td>1935</td>
</tr>
<tr>
<td>44-G</td>
<td>Aug. 1, 1933</td>
<td>Jan. 3, June 3, 1934</td>
<td>44-G♀</td>
</tr>
<tr>
<td></td>
<td>Sep. 20, 1933</td>
<td>1934</td>
<td>1934</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1934</td>
<td>1935</td>
</tr>
<tr>
<td>44-H</td>
<td>Aug. 23, 1933</td>
<td>Dec. 24, May 24, 1934</td>
<td>H-1♂</td>
</tr>
<tr>
<td></td>
<td>Sep. 20, 1933</td>
<td>1934</td>
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<td>44-L*</td>
<td>Born June 7, 1934</td>
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*Born while mothers were on experimental ration.
DISCUSSION OF RESULTS

In discussing the results of this experiment it should be kept in mind that an attempt has been made to determine if milk goats require vitamin E in the ration for successful reproduction. The goats were fed a variety of natural foodstuffs which had been previously treated with ferric chloride to destroy any vitamin E present. The success of the destruction of vitamin E in the ration was measured with female rats which will resorb their unborn young when confined to a ration devoid of vitamin E. The vitamin E deficient ration was fed to the goats over a period of two years. During that time the reproductive performance of the goats was carefully studied to determine if the deficiency of the ration in vitamin E had any deleterious affect on this physiological process.

An interpretation of the significance of the results in this experiment will depend on two factors:

(1) The completeness of the destruction of vitamin E by the ferric chloride treatment of the ration.

(2) The reproductive performance of the goats on the vitamin E deficient ration.

The results of bio-assays of the ferric chloride treated goat ration with rats indicate that the goat ration was deficient in, though probably not entirely devoid of, vitamin E. Up to June 1, 1935, 97 rat gestations had been used directly
as tests for vitamin E in the goat ration. The major portion of the rat tests, and those most valuable from the standpoint of experimental significance, were the current tests of the goat ration. Fifty-four of the ninety-seven gestations were in this group. 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 E. While it must be admitted that the number of tests of the ration is much below the number to be desired, the uniform distribution of the tests tends to make the results a representative sample of what could be expected had more test rats been available.

Since 48 of the 54 gestations of rats while on the current test ration resulted in resorptions it is evident that the ration was deficient in vitamin E. The six litters occurring among the test rats would seem to indicate, however, that there must have been at least a small quantity of vitamin E present in the goat ration at certain times. In order to study the relative significance of the litters as indications of the amount of vitamin E in the ration a detailed description of the circumstances surrounding each of the six litters on the test ration is given below.

**Litter of Rat No. 31, Jan. 22, 1933.**

Rat No. 31 completed a preliminary resorption on September 13, 1933, and was placed on the ferric chloride treated goat ration October 13. Resorption of the fetus also followed her
first mating, October 23, after being placed on the goat ration. She was continued on the goat ration and bred again on December 30. This gestation resulted in a litter on January 22, 1934. The next time she was bred, on February 13, 1934, she resorbed although she was still on the current goat ration. No further test gestations were secured from this rat because an abscess of the mammary gland made it necessary that she be killed April 29, 1934.

The only plausible explanation of this litter is that the goat ration contained some vitamin E. The amount of vitamin E was probably small as indicated by the resorption in the gestation period following the litter. Resorptions by other rats on the goat ration at the same time as this litter confirms this same conclusion. Rat No. 33 resorbed her young at approximately the same time as the litter of rat No. 31 occurred. This resorption was the second one for rat No. 33 after being placed on the goat ration, which indicates that the amount of vitamin E in the goat ration, if any, must have been small. Bio-assays of the composite sample of the goat feed treated during December 1934, the month during which female No. 31 must have received the vitamin E in her ration, gave no indication of vitamin E being present. Too much weight cannot be placed on the reliability of the test of the December composite sample, however, since four months elapsed between the treatment of the feed and the assay for vitamin E.
Litter of Rat No. 94, Sept. 10, 1934.

Rat No. 94 was placed on the ferric chloride treated rat ration for depletion of vitamin E on May 19, 1934. She was bred on May 22 and again on June 10, but did not conceive. She was placed on the current ferric chloride treated goat ration on July 21, 1934, without having completed a preliminary resorption. On July 27 she was bred and the placental sign occurred on August 3, but there was little change in weight. Ovulation on August 4 at the height of the weight curve would indicate that no gestation occurred. On August 19 she was bred and the gestation terminated in a litter on September 10, 1934. She was bred again on October 15, having been continued on the current ferric chloride treated goat ration and resorbed on November 8, 1934.

Since rat No. 94 was placed on the experimental ration before it was proved that her vitamin E reserves were depleted, definite deductions from the birth of the litter cannot be made. Two other rats, No. 75 and No. 101, which had been on the experimental ration and had completed previous resorptions on the ration, resorbed at approximately the same time as the litter of No. 94 was born. Several other rats, No. 48, No. 82 and No. 93, which received the current goat ration at the same time as rat No. 94, completed resorption gestations shortly afterward. Litters of Rat No. 112 and Rat No. 121, Nov. 9 and Nov. 17, 1934.

These two litters occurred at approximately the same time so will be discussed together. Female No. 112 completed her
preliminary resorption on September 28, 1934. She was placed on the current ferric chloride treated goat ration on October 8 and remained on the ration until October 15. She was bred on October 17 and a litter resulted on November 9. Rat No. 121 initially resorbed on September 8, 1934 and was placed on the current ferric chloride treated goat ration September 21. She was bred on October 26 and had a litter on November 17. Following this litter she was rebred on November 30, showed a definite "placental sign" and sufficient change in weight to indicate a probable resorption on December 17, although the gestation period was short.

The occurrence of the litters from rats No. 112 and No. 121 at about the same time seems definite evidence of at least some vitamin E present in the ration the rats were receiving. However, some question may be raised as to whether or not the vitamin E came from the goat ration. The vitamin might have come from either the ration used to deplete the rats of vitamin E reserves or from the goat ration fed the rats. The rats received each of these rations within 40 days prior to the date of the mating which resulted in the litter. Rat No. 121 received ferric chloride treated goat ration for a period of 35 days preceding the breeding date and rat No. 112 for seven days. The ration at other times and during the two days immediately before copulation (at this time the females were in the male cages) was the ferric chloride treated rat ration.
Resorptions by rat Nos. 94, 55, 42, and 120 which occurred at nearly the same time as the litters of rats Nos. 112 and 121, would indicate that the amount of vitamin E in the goat ration was small since the rats all received the goat ration. The subsequent resorption of rat No. 121 following the litter would also indicate a minimum amount of vitamin E in the goat ration. Furthermore there is some reason to believe that the ration used in depleting rats Nos. 112 and 121 might have been contaminated with vitamin E since a number of rats which had been kept on the preliminary depletion ration for three months were still casting litters. The presence of vitamin E in the depletion ration during the latter part of the resorption gestation might have accounted for the litters occurring soon after the rats were placed on the goat ration.

Litters of Rat No. 252 and Rat No. 258, May 27 and May 24, 1935.

The litters of rats No. 252 and rat No. 258 occurred under the same circumstances and at about the same time so they will be discussed together. Rat No. 252 had been receiving a vitamin E deficient high fat ration to deplete her vitamin E reserves. On March 28, 1935 she was bred and the gestation resulted in a resorption on April 17. She was placed on the current ferric chloride treated goat ration on April 17 and was bred on April 20. A litter resulted on May 27. Rat No. 258 was also depleted of vitamin E body reserves on the high fat ration and resorbed on April 24. She was placed on the current ferric chloride
treated goat ration on April 24 and was bred May 2. A rather sharp drop in weight (from 235 to 202 grams) occurred between May 23 and May 24, indicating a probable litter. Although no young were found this gestation was considered as a litter.

In spite of the fact that these two litters followed closely resorptions by rats No. 150, No. 158, and No. 250 which were receiving the same ferric chloride treated goat ration it seems probable that in some manner the goat ration had been contaminated with vitamin E at this time. Although having a bearing on the future progress of the experiment, the occurrence of vitamin E in the rations of the goats at this time in no way affects their reproductive performance in the first two breeding seasons which are discussed here.

A careful survey of the six litters on the ration reveals that only one of them indicates definitely that at least a small amount of vitamin E was present in the goat ration at a time in which the reproductive performance of the goats reported here would be influenced, while the other five litters, though they must be viewed with question, have other possible explanations. One of the litters occurred before there had been definite proof of the depletion of the vitamin E body reserves of the test rat. In two cases there is the possibility that the vitamin E might have come from the part of the rat ration which had not been fed to the goats. Two of the litters occurred at a time when vitamin E in the goat ration would in no way influence the
have been noted to date in the growth of the second year.

Other treatments were made to grow normal out. No serious dete

only one of the eight live kids survived in the third year.

with which were dead at birth but without gross abnormalities.

the two years resulted in the birth of 21 live kids and two

possible to occur. Normal growth of the eight goats in the course of

spitting at the age of one year. The 17 goats reared had been on the

E$ratration £ere to external mortality and produced normal off-

months and reared from birth on the regular antibiotic-free

born after their mothers had been on the treated ration eighteen

normal kids the second year. Furthermore, four female kids

or more healthy kids the third year, and live to one or more

through both years of the experiment. Five Gave birth to one

enrollment the treated ration. Of the six females that were carried

young was not seriously impaired by the rearing of the females

It is evident that the ability of the goats to produce

Treatin in addition.

traction but what the ration fed the goats were extremely de-

where the sex ratio in the research, there was no

of the goat ration resulted in retardation of the vertebral column.

a few of the 97 goats reared in the various conditions.

however, there is

in the goat ration established beyond a doubt that the goat

an objective estimate of the results of the test for attention

reproductive performance of the goats during the period covered.

-65-
offspring which were kept.

The possibility that vitamin E had been sufficiently de-
pleted in the goat ration to cause minor reproductive distur-
bances though not entirely preventing the delivery of live kids
must also be considered in evaluating the results of the experi-
ment. Some of the minor disturbances occurring among the female
goats on the ration will be discussed briefly.

The only evidence of abnormality noted among any of the
goats during the first gestation period was a bloody dis-
charge from the vagina of female 44-E on April 23, 1934. This
occurred approximately one month before kidding. Apparently
the disturbance was not serious because 44-E dropped normal
twin male kids on May 26, 1934.

Approximately three weeks after parturition two of the fe-
male goats, 44-F and 44-D, showed evidence of bloody discharges
from their vaginas. Examination of the goats by Dr. McNutt of
the Veterinary Research Department showed that the uteri were
the source of the blood. The cause of the bloody discharges
could not be fully determined. Smears taken for bacteriological
examination, however, led to the belief that the cause was not
of bacterial origin. The discharges cleared up after a few
days and did not reappear.

One female, 44-E, showed signs of digestive disturbances
during the period in which she was nursing her young. Her
appetite failed about fifteen days after parturition and she
became very thin. Her twin kids also exhibited signs of under-
nourishment. The condition persisted for several weeks; then
her appetite showed gradual improvement following the addition
of extra yeast to the ration. Whether or not lack of vitamin
E was a factor in causing the disturbance cannot be stated
definitely but since recovery took place without the addition
of the vitamin to the ration vitamin E would not seem to be
the causative factor. Whether the recovery was spontaneous
or was due to the additional yeast is problematical.

Probably the most serious reproductive abnormality during
the course of the experiment occurred during the second breed-
ing season. Female 44-H, after being bred on November 11,
1934, showed a bloody discharge from the vagina on December
14. A detailed examination by Dr. McNutt on December 17, showed
that an abortion might have occurred. The evidence was in-
sufficient however to definitely state that an abortion occur-
red. Female 44-H later came back in heat and was rebred on
January 9, 1935. The resulting gestation terminated in the
delivery of a dead kid on June 9, 1935.

If the minor disturbances in the reproductive cycle of the
goats on the experimental ration were sufficiently unusual not
to have occurred under normal conditions, the problem as to
whether lack of vitamin E played a significant role in the
causation of each disturbance is difficult to solve. The
bleeding from the uteri of rats on a vitamin E deficient ration
as reported by Evans and Burr (1927) would seem to parallel
the bloody discharges from the uteri of several of the goats
on the ferric chloride treated ration. However, since all
goats received the vitamin E deficient ration but only part of
them showed any evidence of bloody discharges, too much weight
cannot be attached to the relationship of this abnormality to
lack of vitamin E.

The probable occurrence of an abortion in the second ges-
tation of 44-H might be taken as an indication of the effect of
lack of vitamin E, but such an interpretation would be very
difficult to verify when occurring only once in thirteen
gestations. If such an abortion occurred from lack of vitamin
E, the result of vitamin E deficiency is slightly different in
the goat than in the rat, since with the latter resorption
rather than abortion occurs. The manifestation of the de-
siciency in a different way might, however, be explained by
the difference in the type of the placentas of the two species
of animals.

It must be admitted that the reproductive disturbances
occurring among the goats on the ferric chloride treated
ration were very minor in comparison with the failure of rats
to reproduce when fed the same ration. The attributing of such
minor reproductive disturbances among the goats to lack of vita-
min E cannot be made with assurance since factors other than
a vitamin deficiency may have contributed to the reproductive
abnormalities.

The failure to completely destroy all of the vitamin E in the goat ration makes it impossible to formulate a final statement regarding the requirements of goats for vitamin E. The results establish, however, that the reproduction of the goats on the treated ration was much more uniformly successful than was the reproduction of rats on the same ration. Several interpretations might be placed on these findings:

(1) The amounts of vitamin E still present in the ration after the ferric chloride treatment, though very small, were sufficient to permit successful reproduction of the goats.

(2) The period of two years on the ferric chloride treated ration may have been insufficient to deplete the vitamin E body reserves of the goats.

(3) The requirements of the goats for vitamin E may be considerably below that of the rats which were used as test animals.

(4) Goats may not require vitamin E in the ration for successful reproduction.

If the first explanation is to be accepted it is evident that only a small amount of vitamin E is required for reproduction in goats. Only traces of vitamin E could have been present in the goat ration if one test rat gave birth to a litter while another test rat on the ration completed a reabsorption at nearly the same time. It is known that vitamin E
The second interaction, that inaugurated the second

entwined reading, was to be condensed in practical form as
Attention should be the goal, therefore, to extend your
examination of the stage of the experiment. Remember
distinctly to preserve at this stage of the experiment, since
representative qualitative examination cannot be done
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for the second of the second body, because of attenuation.
gestation period. Since goats are larger animals it is logical to suppose that a longer time might be required to deplete their vitamin E reserves than for rats and hens. Some other factors in the results, however, are less favorable to such an explanation.

Successful reproduction by the two female kids born and raised on the ferric chloride treated ration would render this explanation less plausible since it appears unlikely that their dams which had been on this ration for 11 months could impart a very large reserve of vitamin E to their offspring. Waddell and Steenbock (1928) found that rats raised from weaning on the ferric chloride treated ration showed no initial fertility.

A combination of slower depletion of body reserves and a small amount of vitamin E in the ration might have allowed the reproduction which occurred during the two gestation periods that the goats were studied. Only further progress of the experiment can reveal the worth of this suggested explanation.

The explanation that the requirements of goats for vitamin E is less than the requirements of rats is quite tentative in nature though supported by several observations. It is evident that if the rats resorbed as regularly as they did on the goat ration, and if the goats were continued for 16 months on the ration previous to being bred yet still produced normal young, the requirements of the goats for vitamin E were probably less than those of the rats being used to test the ration. Such an
explanation would serve to interpret the results which occurred, but it would not rule out other possible explanations such as those suggested earlier.

The fourth possible interpretation that the goats may not need vitamin E for reproduction is certainly not an established fact. The results secured to date in this experiment are inadequate in several respects to verify such a conclusion. In the first place the ration was not shown to be entirely free from vitamin E. Furthermore the goats have not, as yet, been continued on the ferric chloride treated ration long enough to be absolutely certain that all reserves have been used up by the normal body functions.

The shortcomings of the data submitted in this thesis are many. However, it has been definitely established in this experiment that milk goats can be continued for a period of two years on a ration which is seriously deficient in vitamin E without adversely affecting their reproductive performance to any measurable extent. From the standpoint of practical farm animal husbandry the results of the experiment are very significant. If a ration cannot be prepared under careful laboratory technique sufficiently free from vitamin E to cause reproductive failure among the animals to which it is fed, there is very slight chance that any ration normally fed to farm animals would be sufficiently low in vitamin E to cause any reproductive
For recorded in this trial.

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SUMMARY AND CONCLUSIONS

Six female milk goats were placed on a ration in which the vitamin E had been destroyed by treatment with ferric chloride dissolved in ether. These females together with their offspring were kept on the vitamin E deficient ration exclusively over a period of two years.

The ferric chloride treated ration fed to the goats was tested with rats to determine if the ferric chloride treatment had been successful in the destruction of vitamin E. Out of the 97 gestations by test rats on the goat ration 89 resulted in resorptions, 2 resulted in litters which were due to failure to confine the rats to the goat ration, 5 resulted in litters which may have been due to vitamin E in the goat ration but also might have been due to vitamin E from other sources and one terminated in a litter which was probably due to the presence of vitamin E in the goat ration. These rat tests indicated that the goat ration was seriously deficient in vitamin E but not entirely devoid of it.

During the two years the goats were confined to the vitamin E deficient ration their reproductive performance was carefully observed. In that period 13 gestations resulted in the delivery of 21 live kids and 2 kids which were dead at birth. The two dead kids were apparently normally developed, however. Minor reproductive disturbances among the female goats occurred but
could not be traced definitely to lack of vitamin E. The 13 successful gestations included 2 by kids which were born after their mothers had been on the vitamin E deficient ration for 11 months. These kids had been raised on the ferric chloride treated ration and gave birth to normal young in their first gestation period.

The results show that milk goats were continued on a ration quite deficient in vitamin E over a period of two years without serious interference with their reproductive performance. No conclusive statement that goats could successfully reproduce if kept on a ration absolutely devoid of vitamin E for a long period of time is yet justified.
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APPENDIX

History of Individual Female Goats

Goat 44-A

Purchased March 15, 1933 from I. B. Brown, Des Moines, Ia.
Description - White female goat, age 7 months, weight 35 lbs. Bred.
Placed on ration of ferric chloride treated grain and hay
March 30, 1933 to determine the palatability of the ration for goats. The ration was not E-free, however, due to small amounts of other materials eaten.
Died on April 26, 1933 as the result of mechanical injuries inflicted by other goats. Was not used in the main experimental trial.

Goat 44-B

Description - Brown female, 16 months of age, weight 90 lbs. Bred.
Placed on ferric chloride treated ration immediately. Some other materials were eaten by the goat (hay, scatterings and straw) so the ration was not vitamin E free.
Dropped twin male kids on April 1, 1934. Weights of kids were 5-1/2 and 4-7/8 lbs.
Kids were weaned and disposed of July 2, 1933.
Placed on ferric chloride treated goat ration July 2, 1933 after removal to the Chemistry Laboratory.
Showed symptoms of being off feed and general unthriftiness on September 8, 1933.
Goat was completely paralyzed on September 15, 1933 following several days of evident weakness. No control could be exercised over the leg muscles although the goat did not seem to be in pain. The condition of the goat became worse and on September 16, 1934 it was decided to kill her and make a detailed examination for the cause of the trouble. Autopsy revealed lesions of the small intestines, liver and kidneys. The cause was probably not vitamin A deficiency, however, according to the report submitted by Dr. McNutt.
Goat 44-C

Purchased April 14, 1933 from Russell Bishop, Cambridge, Ia.
Description - Light brown female, age approximately 1 year, weight 75 lbs. Bred.
Aborted two premature kids on May 15, 1933. These kids were estimated to be about 3 weeks to one month premature. This goat had previously reacted negatively to the agglutination test for abortion. A subsequent test on May 16, 1933 also showed negative reaction. The cause of the abortion was not determined definitely, though it might have been due to mechanical injury from other goats in the same pen.
Disposed of July 2, 1933 as not being desirable for use in this experiment due to the breeding troubles encountered.

Goat 44-D

Purchased April 14, 1933 from Russell Bishop, Cambridge, Ia.
Description - Dark brown-black female, age approximately one year, weight 85 lbs. Bred.
Gave birth to twins on June 17, 1933. One kid was born dead and the other, a male of no value for experimental purposes, was disposed of on June 20, 1933.
Moved to the Chemistry Laboratory on July 2, 1933.
Placed on ferric chloride treated goat ration on July 2, 1933.
Bred to male of known fertility January 9, 1934.
Gave birth to twin kids June 7, 1934, one a male, D-1, weight 7-3/4 lbs. and the other a female 44-L, weight 6-3/8 lbs.
Was observed to show bloody discharge from the vagina on June 22, 1934. Examination by Dr. McNutt on June 25, 1934 showed the source of the blood to be the uterus. He expressed the opinion that the blood was not of bacteriological origin. The cause of the blood was not ascertained.
Kids weaned September 14, 1934.*
Bred November 1, 1934 to male H-1 (second generation male).

*Kid D-1 was removed about 1 month earlier so as to give 44-L a better chance. Kid 44-L, however, failed to respond to the extra milk available.
Goat 44-E

Purchased April 14, 1933 from George Patterson, Nevada, Ia.
Description - White female, age approximately one year, weight 68 lbs. Bred.
Dropped a female kid, weight 3 lbs., on April 24, 1933.
The kid was weak from birth and died, April 25, 1933.
Moved to Chemistry Laboratory July 2, 1933.
Placed on ferric chloride treated goat ration July 2, 1933.
Bred to male of known fertility December 27, 1933.
Dropped twin male kids May 26, 1934. E-1, 4-7/8 lbs. E-2, 5-3/4 lbs.
For about two weeks prior to June 26, 1934, 44-E failed to clean up her feed completely. She became rather thin and her kids, E-1 and E-2, began to show signs of less complete nutrition than the other kids.
For a period of approximately 10 days beginning June 26, 1934 additional yeast was added to her ration to the extent of 15 grams per feed. Her appetite showed improvement and she gradually returned to normal feed consumption.
Whether or not the yeast was the cause of the improvement is an open question.
Kids weaned September 14, 1934.
Bred to male H-1 on October 16, 1934.
Possibly in heat (not certain, however) November 5, 1934.
Showed rather poor appetite for several days prior to November 6, 1934. She was in good flesh though, and not apparently suffering from any serious deficiency.
Dropped twin kids March 18, 1935.
E-3, male, 6 lbs. 44-N, female, 9 lbs.

Goat 44-F

Purchased April 14, 1933 from George Patterson, Nevada, Ia.
Description - White female goat, age approximately one year, weight 95 lbs. Bred.
Dropped male kid on April 25, 1933, weight 6-3/4 lbs.
Kid was weaned and disposed of July 1, 1933.
Moved to Chemistry Laboratory July 2, 1933.
Placed on ferric chloride treated goat ration July 2, 1933.
Bred to male of known fertility January 2, 1934.
Dropped twin kids June 3, 1934. 44-K, female, 7-1/2 lbs. 44-L, male, 8-3/4 lbs.
Showed bloody discharge from the vagina June 19, 1934.
Examination by Dr. McHutt on June 22, 1934 found blood present in the vagina but the source was not evident. Smears were taken. The results of these indicated that the source of the blood was probably not bacteriological. Examination of 44-D at about the same time showed blood of uterine origin.
Kids were weaned September 14, 1934.
Bred to H-1 on November 3, 1934.
Dropped twin kids April 5, 1935. F-2, male, 9-1/4 lbs.
44-R, female, 8-7/8 lbs.

Goat 44-G

Purchased August 21, 1933 from Hewt Briley, Ames, Ia.
Description - Brown female kid, age 7 months, weight 40 lbs. Open.
Moved to the Chemistry Laboratory August 23, 1933.
Placed on ferric chloride treated goat ration August 23, 1933.
Bred to male of proved fertility January 3, 1934.
Dropped female kid on June 3, 1934. 44-J, 7-1/2 lbs.
Kid weaned September 14, 1934.
Bred to male H-1 on November 3, 1934.
44-P, female, 7-3/4 lbs.

Goat 44-H

Purchased August 21, 1933 from F. W. Wadden, Gilbert, Ia.
Description - Black female kid, age 8 months, weight 60 lbs.
Open.
Moved to the Chemistry Laboratory August 23, 1933.
Placed on ferric chloride treated goat ration August 23, 1933.
Bred to male of known fertility December 24, 1933.
Dropped twin male kids May 24, 1934. H-1, male, 7-1/2 lbs.
Male, weight 4-3/4 lbs., dead at birth. Examination of the dead kid by Dr. McNutt indicated that it was
normal in every respect, but that it had not breathed.
Kid weaned September 14, 1934.
Bred to H-1 on November 11, 1934.
Showed a bloody discharge from the vagina December 14, 1934.
Examination by Dr. McNutt on December 17, 1934 showed
no blood present on that day, as there had been on
December 14, 1934 when he examined the goat. The cervix
was partially open. Dr. McNutt was suspicious that
there might have been an abortion.
Bred January 8, 1935.
Dropped dead kid June 9, 1935. H-2, male, 10 lbs.

Goat 44-I

Purchased August 21, 1933 from F. W. Wadden, Gilbert, Ia.
Description - Black female, age 18 months, fertility unproved,
had been running with a male but was open as far as was known.
Moved to the Chemistry Laboratory August 23, 1933.
Placed on ferric chloride treated goat ration August 23, 1933.
Failed to show any indications of coming into heat during the fall of 1933 or winter of 1934. Was carried through the year on ferric chloride treated goat ration.
Bred to D-1 on November 1, 1934.
Dropped female kid on March 31, 1935. 44-0, 7-1/8 lbs.

Goat 44-J

Dropped June 3, 1934 out of female 44-G which had been on ferric chloride treated goat ration since August 21, 1933.
Weaned September 14, 1934.
Bred to male H-1 on November 14, 1934.
Dropped twin kids April 14, 1934. J-1, male, 7-1/2 lbs.
44-S, female, 5-1/4 lbs.

Goat 44-K

Dropped June 3, 1934 out of female 44-F which had been on ferric chloride treated goat ration since July 2, 1933.
Weaned September 14, 1934.
Bred to male E-1 on October 29, 1934. The sperm of E-1 was examined by Dr. McNutt at this time and appeared to be dead. Female 44-K came in heat again November 17, 1934.
Bred to male H-1 on November, 1934.
Dropped twin kids April 16, 1934. K-1, male, 6-3/4 lbs.
K-2, male, 6-1/4 lbs.

Goat 44-L

Dropped on June 7, 1934 out of female 44-D which had been on ferric chloride treated ration since July 2, 1933.
Failed to grow normally. Examination by Dr. Lee failed to reveal the cause of the trouble. Feeding of potassium iodide failed to produce any response.
Weaned September 14, 1934 being much smaller than the other kids of the same age at this time.
Failed to come in heat during fall and winter 1934-35.
Was killed on February 28, 1935 and examined to ascertain cause of retarded development. Post mortem examination by the Veterinary Research Department revealed that the thyroid glands showed pathological changes and were practically non-functional. These changes might have been either primary or secondary. Examination of the ovaries indicated that there had been sufficient stimulus for development of the ova, but not for ovulation.