Supplementation with Vitamins A and D of Beef Cattle Finishing Rations Containing Various Levels of Carotene from Corn Silage and Corn Grain

Harold Richard King

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SUPPLEMENTATION WITH VITAMINS A AND D OF BEEF CATTLE
FINISHING RATIONS CONTAINING VARIOUS LEVELS OF
CAROTENE FROM CORN SILAGE AND CORN GRAIN

BY

HAROLD RICHARD KING

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in
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State University

1970
SUPPLEMENTATION WITH VITAMINS A AND D OF BEEF CATTLE FINISHING RATIONS CONTAINING VARIOUS LEVELS OF CAROTENE FROM CORN SILAGE AND CORN GRAIN

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.
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HRK
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INTRODUCTION

Corn silage and corn grain are two of the most widely used feed ingredients in beef cattle rations in the United States. Corn can be grown under a wide variety of conditions with a high yield of digestible nutrients per acre whether the crop is harvested as grain or as silage. Numerous experiments have shown that economical beef production can be obtained from nearly any combination of corn grain and corn silage when the rations are properly supplemented with protein, minerals and vitamin A.

Rations composed largely of corn grain often will not supply sufficient carotene to meet the vitamin A requirements of fattening cattle over long feeding periods. On the other hand, rations containing large amounts of properly harvested and stored corn silage may supply more than adequate amounts of carotene for fattening cattle without a supplementary source of vitamin A. However, large losses of carotene in corn silage may occur during silage formation and during storage resulting in levels inadequate for optimum performance of growing and finishing beef cattle. Recent reports in the literature have also indicated that corn silage may contain an unidentified factor, other than nitrate or nitrite, which interferes with the conversion of carotene to vitamin A or increases destruction of carotene and vitamin A in the digestive tract. Animals fed silage apparently adequate in carotene to meet their vitamin A requirements have been reported to suffer from what appeared to be a vitamin A deficiency.
In addition to these reports, other workers have noted that the feeding of certain feeds containing large amounts of carotene have resulted in rickets in some species. The administration of vitamin D with these feeds overcomes the rachitic symptoms and normal growth ensues. Although the mechanism is not clearly understood, carotene has been identified as a factor which inhibits the activity of vitamin D.

Corn silage harvested and stored under proper conditions may contain a high level of carotene. At the same time, it may be low in vitamin D and the utilization of vitamin D may be impaired by excessive carotene. Under such conditions, a probability exists that supplementation of the ration with vitamin D would result in improved performance.

Since corn silage is used to such a great extent in bovine rations, it is important that more information be obtained concerning the possible benefits of supplementing rations containing corn silage with vitamins A and D. The purpose of this experiment was to determine the need for vitamin A and vitamin D supplements with rations composed of various levels of corn silage and corn grain when fed to cattle. Early investigations with vitamin D demonstrated that the reduced intensity of sunlight during the winter months resulted in decreased cutaneous formation of vitamin D and that dark skinned animals synthesized less vitamin D than light skinned animals under given conditions of light intensity. Thus this experiment was conducted with Angus and Angus x Hereford crossbred steers and, to the extent possible,
under seasonal conditions considered to be optimum for the demonstration of any beneficial effect of supplementary vitamin D on performance.
REVIEW OF LITERATURE

Vitamin A

Functions of Vitamin A and the Effects of a Deficiency

All animals require vitamin A for proper vision, growth, reproduction and maintenance of good health. When inadequate amounts are supplied in the diet, various signs of a deficiency develop as described by West and Todd (1961), Embry and Kortan (1961), Maynard and Loosli (1962), Weichenthal (1962), Ewing (1963) and Hansen (1963).

The classical symptom of vitamin A deficiency is night blindness. According to Guilbert and Hart (1934) and Guilbert, Miller and Hughes (1937), this is the first sign of vitamin A deficiency and the last to disappear during recovery following adequate supplementation. Apparently other needs have a greater priority for the vitamin and are met first.

Perhaps the single most important function of vitamin A is concerned with the maintenance of the health and integrity of the epithelial tissue. The effects of a vitamin A deficiency upon the epithelial tissue and organs throughout the body have been reported by several workers (Wolbach and Howe, 1925, 1928; Aberle, 1934; Hetler, 1934; Madsen et al., 1948; Byers, Jones and Bone, 1956). The healthy epithelium, which is involved with secretion and absorption throughout the body and which provides a continuous barrier against the entrance of microorganisms into the body, is replaced by a stratified, keratinizing epithelium which is incapable of fulfilling its normal roles.
Some of the nonspecific symptoms which can be directly related to the effect of a deficiency of vitamin A on the epithelial tissue in the bovine include watering eyes, diarrhea, nasal discharge, enteritis and intestinal inflammation, failure of implantation, abortion of the fetus, sterility of males and females, a roughened hair coat and a dry scaly skin. Although some of these symptoms are evident, the rate of growth may continue at a nearly normal level until feed consumption is reduced.

Secondary diseases may accompany a vitamin A deficiency because the epithelial tissue no longer provides an effective barrier to the entrance of infectious organisms into the body and the resistance of the animal is thus lowered. Pneumonia and other respiratory diseases are encountered more frequently in the deficient animal. The cloudy appearance and subsequent ulceration of the cornea of the eye, condition which result in partial or complete blindness but which are not directly related to night blindness, may be the result of bacterial activity. In the event that a secondary disease becomes established it should be treated as a separate problem. However, administration of vitamin A at adequate levels will bring about repair of the epithelium and thus enable the animal to become more resistant to further infection.

Vitamin A plays an essential role in successful reproduction. It is concerned in the maintenance of the health of the reproductive organs of both the male and the female, a function related to the maintenance of healthy epithelial tissue. Evidence of damage resulting from a deficiency of vitamin A may be observed long after the gross symptoms of
the deficiency have disappeared. Although bulls may regain their fertility following correction of the deficiency (Hodgson et al., 1946; Erb et al., 1947), the germinal epithelium may exhibit some evidence of damage for as long as 20 months subsequent to the beginning of carotene administration following a serious deficiency (Madsen et al., 1948).

Guilbert and Hart (1934) reported that vitamin A deficient heifers commonly aborted or bore dead offspring at term. Live calves from deficient heifers were small, weak and rapidly showed other signs of vitamin A deficiency, the first of which was generally diarrhea. Colostrum from the deficient dams contained no detectable amounts of vitamin A.

Vitamin A plays an essential role in the maintenance of a healthy epithelial lining in the mammary gland. This is necessary for the continued production of appreciable quantities of milk. In addition, vitamin A is secreted in colostrum and milk. The newborn calf, having no appreciable body stores of vitamin A under normal conditions, must acquire sufficient amounts from the colostrum and milk or receive a supplementary source to prevent the occurrence of deficiency symptoms.

Another function of vitamin A is to permit the establishment of conditions necessary for normal development of bones and teeth. Mellanby (1947) demonstrated the alteration of bone shape which may occur during growth under conditions of vitamin A deficiency. Vitamin A apparently exerts its influence upon the osteoblasts and osteoclasts.
of the developing bone. Wolbach and Howe (1933) showed that poor development of the ameloblasts and odontoblasts during a deficiency of vitamin A resulted in the absence or malformation of the dentin of the teeth. Dye, Bateman and Porter (1945) reported that sections of incisors from deficient rats showed irregularities in dentin development and irregular pulpal outlines.

A defect in bone development, an indirect result of vitamin A deficiency, has been noted in the literature. This defect, manifested as permanent blindness in young and growing cattle, has been reported by Kuhlman, Gallup and Weaver (1936) and others. Moore (1939a) postulated that this type of blindness was due to the constriction and subsequent degeneration of the optic nerve resulting from defective development of the optic foramen during growth. This abnormal development appeared to be caused by increased intracranial pressure. Later work by Moore and Sykes (1940, 1941) demonstrated the validity of this hypothesis while the work of Millen and Dickson (1957) has provided further support.

Dehority et al. (1960) and Eaton, Rousseau and Lucas (1964a) have noted that the increase in cerebrospinal fluid pressure appears to be the first measurable symptom of a vitamin A deficiency in the bovine. The cause of the increased cerebrospinal fluid pressure appears to be a decreased rate of absorption of the cerebrospinal fluid as shown by the reports of Bitman et al. (1962a, 1962b) and Okamoto et al. (1962). According to Dehority et al. (1960), no important changes in the
composition of the cerebrospinal fluid occur as a result of a vitamin A deficiency.

A deficiency of vitamin A also results in an edematous appearance of the brisket, legs and joints in cattle. This condition, known as anascara, was at first thought to be related to changes in the concentrations of the blood plasma proteins. Madsen et al. (1947) and Erwin, Elam and Dyer (1957) reported slight increases in the globulin fractions and decreases in albumin concentrations. However, Madsen et al. (1947) stated that changes in the colloid osmotic pressure of the blood did not appear to be the only cause of this condition. Dehority et al. (1960) reported no consistent trends in the distribution or concentrations of blood serum albumins or globulins when a vitamin A deficiency was induced in calves.

Anascara may, however, be related to or result from numerous other physiological changes. Byers et al. (1956) reported a thickening of the adrenal capsule and degeneration of the glomerulosa in the kidneys of mature cattle and Junega, Murthy and Ganguly (1966) observed that a deficiency of vitamin A in rats apparently had a marked deleterious effect upon the synthesis of corticosterone and deoxycorticosterone in rats. Woelfel et al. (1963) reported that the urine of vitamin A deficient calves contained significantly increased quantities of phosphorus while the total daily outputs of sodium and chloride were reduced. Potassium appeared to be unaffected. These various changes, while probably not in themselves primary causes of anascara, may contribute to its appearance.
Another symptom of vitamin A deficiency in cattle has been noted in the literature. Deficient cattle may become clumsy, incoordinated and spastic with the rear quarters being most affected. Convulsions, sometimes ending in death, are not uncommon in severely deficient cattle. These symptoms may be caused by degenerative lesions of the nerves, a condition observed in deficient rats by Aberle (1934).

Vitamin A has thus been shown to have several functions in the maintenance of good health in the bovine. The mechanisms through which some of these functions are performed are as yet unknown. It is quite clear, however, that the failure to provide adequate vitamin A intake in the bovine can have far-reaching and serious consequences.

Methods Used to Determine Vitamin A Requirements and Status

Although it is vitamin A which is required by animals, carotene can be converted to vitamin A in the animal body and thus may be used to satisfy the vitamin A requirement. Requirements are therefore stated in terms of either vitamin A or carotene. Vitamin A requirements are given in terms of the international unit (IU) or the United States Pharmacopeia (USP) unit, each of which is equal to the activity of 0.30 μg of pure crystalline vitamin A alcohol.

The amount of carotene needed to satisfy vitamin A requirements is commonly stated in terms of milligrams, international units or United States Pharmacopeia units. The international unit and the United States Pharmacopeia unit are each equal to the activity of 0.6 μg of beta-carotene, which is equal to the activity of 0.30 μg of vitamin A alcohol as determined by rat assay techniques. However, the
vitamin A value of carotene for the ruminant is not the same as for the rat and may be influenced by several factors.

**Growth and Deficiency Symptoms.** Rate of growth of test animals and the appearance of deficiency symptoms are often used as the criteria by which the adequacy of intake of a nutritional factor is judged. This method must be used with caution, however, because in many instances relatively large body stores of the nutritional factor being investigated may be present. Intakes which appear to be sufficient to provide for optimum growth and avoidance of deficiency symptoms during short experimental periods may be insufficient over a long time.

Guilbert and Hart (1934) studied the effects of liver stores upon the length of time required to deplete steers of vitamin A. The first symptom of a deficiency in one steer was lacrimation followed 23 days later by night blindness. Appetite remained good until the steer developed diarrhea. Weight gain was not affected until feed intake declined. Guilbert et al. (1937) later described the use of the night blindness test in determining the vitamin A requirements of cattle. It was noted that the minimum amount of carotene or vitamin A necessary to prevent the occurrence of night blindness appeared to represent the true physiological minimum requirement. Animals fed at these levels over long periods of time made excellent gains. However, liver storage of the vitamin was meager. The appearance of night blindness was also reported by Riggs (1940) to be the first observable symptom indicative of a vitamin A deficiency in feeder cattle and calves.
The inadequacy of using growth rate as the only measure of the vitamin A status of animals has been shown by several workers. Guilbeil and Hart (1935) reported that suboptimum levels of carotene intake had been achieved which allowed nearly normal growth in the presence of persistent night blindness. Boyer et al. (1942) noted that, when blood plasma vitamin A levels of calves decreased rapidly from adequate to clearly inadequate values, decreases in growth rate sometimes did not appear for as long as 30 days. Low rates of growth occurred at vitamin A levels which resulted in marked gross deficiency symptoms. Growth of calves partially depleted of vitamin A during a 16-week period was not affected although feed consumption tended to be decreased during the final stages of the period (Grifo et al., 1960a).

It would thus appear that the use of growth by itself is not a good means by which to evaluate the adequacy of carotene or vitamin A intake during short experimental periods. However, intakes of carotene or vitamin A sufficient to prevent the occurrence of deficiency signs and to provide for optimum gains over long periods should be considered adequate under the conditions imposed.

**Concentration of Carotene and Vitamin A in the Blood Plasma.** Use has been made in many instances of the concentration of vitamin A or carotene in the blood plasma as an indicator of the vitamin A status of cattle. There is, however, some disagreement among researchers as to what constitutes the minimum acceptable level of plasma vitamin A or carotene indicative of adequate vitamin A nutrition.
Ellmore and Shaw (1954) considered 10 µg/100 ml to represent a safe level of vitamin A in the blood plasma of calves about 3 months of age. Levels of 10 to 12 µg/100 ml were found to be adequate in dairy calves while levels of 7 to 8 µg/100 ml were considered borderline and lower levels resulted in the appearance of gross deficiency symptoms (Boyer et al., 1942). Concentrations of 15.6 µg/100 ml of vitamin A in the blood plasma of calves were reported by Eaton, Rousseau and Norton (1961) to result in the maintenance of constant cerebrospinal fluid pressure.

Plasma vitamin A concentrations necessary to prevent deficiency signs and result in optimum weight gains have generally been reported to be higher for fattening cattle than for young calves. Beeson et al. (1961) reported that levels of plasma vitamin A of 11 and 16 µg/100 ml appeared to be associated with reduced rates of gain in fattening cattle. A level of 15 µg/100 ml was reported by Pope, Baker and MacVicar (1961) and Kohlmeier and Burroughs (1964) to be inadequate for optimum feedlot gains. Concentrations from 15 to 25 µg/100 ml resulted in variable performance in finishing cattle while levels of 25 µg/100 ml or more indicated adequate vitamin A nutrition (Kohlmeier and Burroughs, 1964). On the other hand, Smith et al. (1961) and Jordan et al. (1963) reported the presence of apparent deficiency symptoms in finishing cattle fed corn silage when the vitamin A concentration in the plasma of these cattle was about 26 µg/100 ml.

Madsen et al. (1947) noted that normal blood plasma protein patterns were associated with plasma vitamin A levels of about
23 µg/100 ml in steers while abnormal patterns were associated with levels of 7 and 8 µg/100 ml. Later work indicated that the chances of producing a normal living calf were poor when the blood plasma vitamin A level of the dam was below 18 µg/100 ml at or near the end of the gestation period (Madsen and Davis, 1949).

Blood plasma carotene concentrations have also been used as an indicator of the adequacy of carotene intake and of vitamin A status. Moore (1939b) reported that nyctalopia and pappillary edema developed in calves when their plasma carotene concentration fell below about 13 µg/100 ml. However, plasma carotene concentrations below 40 µg/100 ml have been reported to be indicative of a vitamin A deficiency in beef calves (Pope et al., 1961) while levels of 50 to 70 µg/100 ml in Holstein calves and 110 to 140 µg/100 ml in Guernsey calves have been considered minimum for the prevention of deficiency symptoms (Boyer et al., 1942).

A series of blood analyses over a period of time may be used to determine depletion or repletion rates and may give some indication of whether carotene or vitamin A intake is inadequate or greatly in excess of requirements. However, the possible presence of relatively large body stores of vitamin A requires the observation of certain precautions when this method is utilized in determining the vitamin A or carotene requirements of cattle. Through the utilization of body stores, plasma vitamin A concentrations may be maintained well above levels considered to indicate a deficiency for considerable periods of time even though intakes are entirely inadequate. For this reason, cattle are usually
partially or wholly depleted of their body stores of vitamin A before any attempt is made at determining their carotene or vitamin A requirements.

It appears that vitamin A deficiency symptoms in cattle are usually associated with plasma vitamin A concentrations of about 16 µg/100 ml or less. Depending upon the rapidity with which concentrations are reduced, lower levels may or may not be accompanied immediately by gross deficiency symptoms. Newborn or other young calves may, however, have blood plasma vitamin A concentrations of considerably less than 16 µg/100 ml and may not exhibit the classical symptoms of a vitamin A deficiency. Because of the wide variations noted in blood carotene levels indicating adequate vitamin A nutrition and because of the many factors which influence carotene utilization, the level of plasma carotene does not appear to be as sensitive as plasma vitamin A concentration when used as an indicator of vitamin A status.

**Relationship Between Dietary, Blood and Liver Concentrations of Carotene and Vitamin A.** Since the early recognition that the liver may store large quantities of vitamin A which could be utilized during periods of intake insufficient to meet requirements, investigations have been carried out to determine the relationships between dietary, blood plasma and liver levels of vitamin A and carotene. Several reports have indicated mathematically definable relationships between these variables.

Almquist (1952) stated that a survey of the literature revealed that in several animal species the plasma vitamin A concentration, in
micrograms per 100 ml, is linearly related to the logarithm of the dietary vitamin A concentration. The author also concluded that a linear relationship existed between the plasma concentration of vitamin A and the logarithm of the liver concentration of vitamin A. An arithmetic relationship between increasingly higher levels of vitamin A intake and liver vitamin A stores in calves was indicated in a study reported by Rousseau et al. (1956a), whereas a study by Braun (1945) indicated that the only time vitamin A stores in the liver were accurately reflected by plasma vitamin A concentrations was when both values were low and rapid depletion was occurring. In other studies, mathematically definable relationships between the plasma and liver concentrations of carotene and vitamin A appeared to exist only when the test animals were on a strictly controlled dietary regime or when their body stores of carotene and vitamin A were at critical levels (Diven et al., 1960).

Studies have also been conducted in which carotene furnished the vitamin A activity in the ration. Thomas and Moore (1952) reported that the carotene intake of calves was linearly related to liver storage of both carotene and vitamin A and to plasma carotene concentrations. On a given constant intake of carotene, plasma carotene concentrations appeared to be a better indicator than plasma vitamin A of carotene intake. Plasma vitamin A concentrations were directly related to carotene intake until intake reached a level of about four times the minimum requirement.
Other investigations have not been able to establish exact relationships between carotene and vitamin A intake and the resulting levels of carotene and vitamin A in the blood plasma or liver. In one such study, no relationship between plasma vitamin A and liver vitamin A concentrations in steers could be established, except that the highest level of vitamin A supplementation, 2.5 million IU per head daily, resulted in the highest plasma and liver concentrations of the vitamin (Hale et al., 1961a). Similar results have been reported with calves fed up to 1024 IU of vitamin A per kilogram of body weight per day (Lewis and Wilson, 1945). Thomas, Jacobson and Moore (1952) concluded that certain dietary regimes or changes in the diet may make even plasma vitamin A concentration an unreliable indicator of vitamin A intake. In other studies, arithmetic and logarithmic correlations failed to establish a relationship between blood plasma and liver vitamin A concentrations under conditions of uncontrolled carotene intake in cows (Ralston and Dyer, 1959).

It thus appears that the level of vitamin A in the blood plasma is not by itself a reliable indicator of the total amounts of hepatic stores of the vitamin except under conditions of near or complete depletion. Under these conditions, low plasma concentrations of vitamin A reflect low levels of liver vitamin A stores and may be accompanied by apparent deficiency symptoms.

Carotene concentration in the plasma may vary considerably due to the level of carotene intake. Low carotene intake may be reflected by the level of carotene and vitamin A in the blood plasma and liver
under strictly controlled conditions. However, higher levels of intake may not be accurately indicated by plasma or liver concentrations of vitamin A.

**Changes in Cerebrospinal Fluid Pressure.** One of the first measurable symptoms of a deficiency of vitamin A in the bovine appears to be a change in the cerebrospinal fluid pressure. This criterion has been used at times to establish the minimum vitamin A or carotene requirements of cattle.

Moore and Sykes (1940) reported that a deficiency of vitamin A in the diet of the young bovine produced an increased cerebrospinal fluid pressure which was later accompanied by visual symptoms of a deficiency. On a return to a normal diet, deficiency symptoms disappeared and the cerebrospinal fluid pressure slowly returned to normal. Further work (Moore and Sykes, 1941) indicated that in terminal stages of a vitamin A deficiency the cerebrospinal fluid pressure may increase to as much as 1060 mm of saline. Normal pressures in nondeficient animals ranged from 75 to 120 mm of saline. The change in cerebrospinal fluid pressure was later used to determine the carotene requirements of dairy calves (Moore et al., 1948a). It was reported that the method was sufficiently sensitive to determine differences in carotene intake of as little as 2 ug/lb of body weight under the proper conditions.

Factors other than hypovitaminotic A conditions have been reported to affect cerebrospinal fluid pressure. Increasing age up to about 90 days from birth has been reported to result in slightly
increased pressures (Calhoun et al., 1965), whereas depressions in the cerebrospinal fluid pressure may be caused by the administration of very high levels of vitamin A (Grey et al., 1964).

Although the use of cerebrospinal fluid pressure as an indicator of the presence or absence of vitamin A deficiency symptoms appears to offer excellent sensitivity, the method does have certain limitations. Experimental animals must be restrained in order to prevent damage to the nervous system during the determination. Only limited numbers of animals can be used in such work because of possible limitations in time, equipment and assistance. In addition, once the determination has been made, the results indicate only that a deficiency is or is not present and do not indicate the extent of body reserves of the vitamin or the sufficiency of the vitamin A or carotene intake.

Factors Which Affect Carotene and Vitamin A Requirements

Several factors have been mentioned in the literature as having an influence upon the carotene and vitamin A requirements of the bovine. It is important that the extent of these influences be understood in order that adequate intakes of vitamin A or carotene may be provided.

Breed, Sex and Age. Breed has been investigated as one of the factors which may influence carotene and vitamin A requirements. Darlow et al. (1949) and Pope et al. (1961) reported no important or consistent differences between the apparent carotene and vitamin A requirements of Angus, Herefords and Shorthorns. Significantly higher concentrations of plasma carotene and vitamin A were found by Erwin (1960) for Brahman cows.
than for Angus cows grazing the same alfalfa pasture, but the author attributed the differences to differences in carotene intake.

Work done with cattle of the dairy breeds has, however, revealed differences in the amount of carotene needed to satisfy vitamin A requirements. Holstein and Ayrshire calves appear to require about 30 µg of carotene per pound of live weight to satisfy their vitamin A need (Moore, Berry and Sykes, 1943), while Jersey and Guernsey calves require about 32 and 34 µg/lb of live weight, respectively (Moore et al., 1948a). The results of other investigations (Boyer et al., 1942) indicate that 75 µg of carotene per kilogram of body weight is needed to maintain plasma vitamin A levels of 8 to 10 µg/100 ml in Holstein calves while an intake of 125 µg is needed by Guernsey calves. However, the vitamin A requirements of Guernsey and Holstein calves appear to be quite similar (Boyer et al., 1942; Lewis and Wilson, 1945). Guilbert et al. (1937) and Guilbert, Howell and Hart (1940) make no distinction between breeds in stating the minimum carotene and vitamin A requirements of cattle.

It appears that the vitamin A requirements of cattle of the different breeds are quite similar. Small differences may exist in the amount of carotene necessary to satisfy these requirements, however, especially as applies to the dairy breeds. Such differences as have been noted are probably of little practical significance.

The effect of the sex of an animal on carotene and vitamin A requirements has not been studied in a great number of experiments. Guilbert et al. (1940) noted no differences between the amounts of
carotene needed per unit of body weight to meet the vitamin A requirements of steers and heifers, but they did report that the amount of carotene required appeared to be increased as a result of reproduction and lactation. In other reports, Guilbert et al. (1937) and Guilbert and Loosli (1951) make no distinction between cattle of different sexes concerning the amount of carotene needed to satisfy their vitamin A requirements. Bentley and Morgan (1946) stated that the sex of rats had no influence upon the storage of vitamin A when carotene was fed.

Maintenance and growth requirements for vitamin A and carotene do not appear to be affected by the sex of an animal. Reproduction and lactation are of much greater importance.

The determination of the effect of age upon the carotene and vitamin A requirements of cattle is somewhat complicated by the amount of vitamin A storage. Guilbert and Hart (1935) reported that the minimum amount of carotene needed to meet the vitamin A requirement of cattle ranging in age from 7 months to 4 years (130 to 500 kg of body weight) was about 29 µg/kg of body weight per day. Variations were noted between individuals, with requirements ranging from 26 to 33 µg/kg of body weight. These workers noted that vitamin A requirements and the amount of carotene needed to meet these requirements appeared to be proportional to body weight rather than to age. Guilbert et al. (1937) also noted that vitamin A requirements appeared to be proportional to body weight and made no distinction concerning carotene and vitamin A requirements between cattle of different ages.
On the other hand, age has been reported to influence the length of time required to deplete range cattle of vitamin A stores (Riggs, 1940). In this study, younger animals were depleted in a shorter period of time than older animals after being removed from pasture, but the largest variation in time required for depletion occurred with the older animals. Thus it was concluded that differences in depletion time were more closely related to the total amount of vitamin A stores than to age.

Arnich and Morgan (1954) studied the conversion of carotene to vitamin A in rats of various weights and ages. They concluded that body weight and growth, rather than age or basal metabolic rate, govern the utilization of vitamin A.

It thus appears that age, other than as reflected through weight and growth rate, has little direct influence upon the carotene and vitamin A requirements of the animal.

Environmental Temperatures. Environmental temperatures have been investigated as one of the factors which possibly influence the carotene and vitamin A requirements of animals.

Early investigational work in this area indicated that cold temperature stress shortened the survival time of depleted rats (Aberle, 1934). Later work indicated that cold environmental temperatures shortened both the vitamin A depletion time and the survival time of depleted rats (Ershoff, 1950). In other reports (Ershoff, 1952; Sundaresan, Winters and Therriault, 1967), it was shown that rats possessed an increased vitamin A requirement under cold environmental
conditions. Environmental temperatures of 35° C, however, may not have an adverse effect on the utilization of vitamin A by rats (Anderson, Hubbert and Roubicek, 1964).

Possible effects of temperature stress upon the apparent vitamin A status of the bovine have also been reported. Extreme temperatures, either hot or cold, have been reported to intensify apparent vitamin A deficiency symptoms in feeder cattle (Jones et al., 1943; Page, Erwin and Roubicek, 1958a). Perry et al. (1962) also observed that certain of the vitamin A deficiency symptoms were more noticeable during the hot summer months. Differences in feed consumption, and thus in carotene intake, were greatest during periods of hot weather. This factor appeared to the authors to be at least partly responsible for the apparent increased severity of the deficiency symptoms.

Investigations have been carried out to determine the extent to which heat stress influences the utilization of carotene by cattle. Page, Erwin and Nelms (1959) fed limited amounts of carotene to two groups of calves, one of which was kept in a shaded area while the other was exposed to direct sunlight. Liver analyses indicated that higher ambient temperatures resulted in decreased concentrations of vitamin A in the liver, suggesting an increase in requirements or a decrease in the efficiency with which carotene was converted to vitamin A. Stallcup and Ragsdale (1949), on the other hand, could not demonstrate a change in the efficiency of carotene utilization in dairy cows exposed to a heat stress. These investigators maintained one group of Holstein and Jersey cows at temperatures of about 50° F
while a similar group was placed in a psychroenergetic chamber in which the temperature was raised from 50° to 105° F over a 5-month period. Cod liver oil furnished vitamin A to the rations and alfalfa hay was fed ad libitum. The data indicated that the carotenoid and vitamin A contents of the milk fat appeared to be more closely dependent upon feed intake than upon the temperature under which the cows were kept. Decreased feed consumption and total carotene and vitamin A secretion accompanied the higher temperatures. The greatest change in total carotenoid and vitamin A secretions occurred as the result of increased feed consumption following the removal of the cattle from the chamber.

It thus appears that the temperature under which an animal is maintained may, under certain conditions, either directly or indirectly influence the vitamin A status and perhaps the vitamin A requirements. Whether the effect is mediated through the influence of temperature on thyroid secretion rate and consequently upon metabolic rate as some workers have suggested, or whether variations in feed consumption or other mechanisms are more important is open to some debate. Regardless, it is clear that vitamin A deficient animals are more susceptible to the effects of stress and demonstrate greater discomfort under heat or cold stress than do nondeficient animals. Deficiency symptoms appear to be intensified under temperature stress conditions.

**Effect of Previous Carotene Intake and Vitamin A Status.** A deficiency of vitamin A causes certain deleterious changes in the epithelial lining of the intestinal tract. Since this lining, or closely associated tissues, appears to be of primary importance in the
conversion of carotene to vitamin A (Olson, 1960; Pope et al., 1961),
it would follow that an existing deficiency of vitamin A may conse-
quently interfere with the conversion of orally administered carotene
to vitamin A. Under such conditions amounts of carotene considered
adequate under normal conditions could be inadequate. On the other han-
the efficiency of utilization of carotene may also be affected by
previous levels of intake in excess of requirements (Guilbert and
Loosli, 1951; Page et al., 1958b). Such an effect, however, would be o:
considerably less practical importance than the decreased efficiency of
utilization resulting from a deficiency.

Studies have shown that a vitamin A deficiency can have an
adverse influence upon carotene utilization. Partially depleted calves
were used in a changeover experimental design to study the effect of
partial depletion upon the subsequent utilization of carotene (Grifo
et al., 1960a). Based upon blood plasma and liver vitamin A concen-
trations, calves fed 12 µg of carotene per pound of body weight daily
for a 16-week period subsequently utilized intakes of 60 and 240 µg
about 0.6 and 0.8 times, respectively, as efficiently as calves fed 48
µg of carotene per pound of body weight daily during the preliminary
period. Reducing the length of the preliminary period to 4 weeks
slightly increased the subsequent efficiency of carotene utilization.
Vitamin A deficiency in sheep has also been observed to impair the
efficiency of carotene conversion (Erwin, Varnell and Page, 1959).

Studies with rats given doses of labeled beta-carotene have shown
that the rate of conversion and the efficiency of utilization depends to
some extent upon the amount of carotene administered (Olson, 1960). Cattle also apparently utilize carotene less efficiently as intake increases above minimum requirements. This decrease in efficiency has been noted in steers (Erwin et al., 1957; Page et al., 1958b) and in calves (Guilbert and Loosli, 1951; Rousseau et al., 1958). On the other hand, Pope et al. (1961) noted that the apparent digestibility of carotene appeared to be about 40% over a range of intake of 4 to 200 times maintenance requirements. However, it was also observed that the digestibility of the carotene appeared to decrease as the trial progressed.

The efficiency with which vitamin A is utilized as the daily intake is increased above minimum requirements may also be reduced as compared to the utilization obtained at intakes near maintenance requirements. Early experimental work with rats revealed that the body does not store quantitatively the entire surplus of vitamin A received in the diet (Sherman and Cammack, 1926). Storage appeared to be a relatively rapid process in early stages but became slower as maximum storage levels were approached. Other workers (Lewis and Wilson, 1945; Frey, Jensen and Connell, 1947) have also noted nonlinear increases in liver stores of vitamin A in calves as intake was increased above maintenance requirements.

There are several factors which may influence the apparent storage of vitamin A. First, many organs and tissues of the body other than the liver are known to store vitamin A, although the amounts are generally small. Most studies have involved only the concentrations
of vitamin A in the liver as the criterion by which efficiency of carotene or vitamin A utilization is studied. Thus, in a previously depleted animal, even though the true efficiency of utilization may not have been impaired, the unmeasured amount of vitamin A required to replete the stores of vitamin A in organs throughout the body before appreciable liver storage occurred would cause the apparent utilization of carotene or vitamin A, as measured by blood and liver values only, to be lower than the apparent efficiency of utilization of an animal whose stores had not been previously depleted. In a similar manner, the extent to which depletion had occurred may also influence the apparent efficiency of utilization. These effects would be especially noticeable in short experimental periods. On the other hand, the apparent utilization of high intakes of carotene and vitamin A by animals possessing high levels of blood and liver vitamin A would also be lowered, probably due to the increased amounts of the vitamin which are destroyed in the body as stores are increased.

Energy Intake. Although beef cattle rations normally contain at least a small portion of roughage, there has been an increasing tendency to feed rations containing very high proportions of concentrates to finishing cattle. Under conditions of drought or other severe climatic conditions, it also sometimes becomes necessary to feed rations containing high proportions of concentrates to beef cows as a maintenance ration. Thus it becomes necessary to understand the effect of the energy content of the ration and of energy intake by the animal upon carotene and vitamin A requirements.
Many reports in the literature deal with the influence of the energy content of the ration upon the absorption, storage and conversion of carotene to vitamin A and with the influence of the energy intake upon the vitamin A status of the animal. Such studies should be carried out by making adjustments in total feed intake or by adjusting the concentrate to roughage ratio in the ration under strictly controlled conditions. In many instances, however, animal or vegetable fats have been used to adjust the energy levels of the rations or the energy intake, thus possibly confounding the results obtained.

Richardson et al. (1965) reported an experiment in which vitamin was administered with rations which contained either 7 or 14 lb of low quality corn silage and a full feed of sorghum grain. The level of silage in the ration appeared to make no difference in the final liver carotene and vitamin A concentrations of the cattle. In another study (Rousseau et al., 1954), the effect of two levels of intake of a vitamin A depletion ration on the rate of depletion of dairy calves was studied. Plasma carotenoids decreased more rapidly than did vitamin A concentrations. Plasma vitamin A concentrations decreased at an essentially linear rate with time. However, neither plasma vitamin A nor plasma carotenoids were significantly affected by the level of intake of the depletion ration.

Other investigations have shown an apparent influence of dietary energy intake on vitamin A status. Willey et al. (1952) fed steers rations containing 2.9 or 7.5% fat, each of which was fed with two levels of net energy, either 58 or 64 therm per 100 lb of feed.
Carotene intake was about 382 mg per head daily in each of the treatment groups. Steers fed the higher fat rations had considerably higher plasma levels of vitamin A and nearly twice the plasma carotene concentrations as those fed the rations containing only 2.9% fat. Increasing the energy level of the ration while maintaining a constant fat content decreased plasma vitamin A concentration but had little effect on plasma carotene. The feeding of high energy rations has also been observed to result in significantly larger depletions of liver vitamin A stores in steers (Hale, Hubbert and Taylor, 1961b; Erwin, Gordon and Algeo, 1963).

Conflicting reports regarding the effect of dietary energy upon the vitamin A status of cattle make an accurate assessment of the situation somewhat difficult. There are many factors to consider. Gain over considerable periods of time may be different and thus total vitamin A requirements will differ. Additionally, energy levels of rations may be adjusted through changes in the concentrate to roughage ratio in the ration or by altering the fat content of the ration. The latter method may tend to confound results through changes in the intestinal absorption of carotene and vitamin A or, if the added fat is rancid, may result in increased destruction of carotene and vitamin A before the ration is fed. The former method, adjustment of the concentrate to roughage ratio of the ration, may result in changes in rumen pH and perhaps a difference in the amount of carotene and vitamin A destroyed in the rumen. Differences in particle size in the rations could bring about similar changes in addition to causing differences in the rate of passage of feed through the digestive tract.
It appears that the energy intake of an animal may, through influencing the rate of gain, affect the total vitamin A requirement of that animal. However, the contention that energy intake will directly influence the vitamin A status of an animal is not incontrovertibly supported, although the possibility of such an effect under certain conditions definitely appears to exist.

Protein. Protein appears to be closely associated with carotene and vitamin A in several instances, the more notable of which are the absorption and conversion of carotene to vitamin A in the lining of the intestine (Olson, 1960), the transport of carotene and vitamin A in the blood plasma (Erwin et al., 1959; Garbers, Gillman and Peisach, 1960) and the formation, with isomers of vitamin A, of the visual pigments in the retina of the eye. In view of the widespread interrelationship which appears to exist between protein and the vitamin, it is possible that the vitamin A status of an animal could be influenced to some extent by the amount and quality of protein consumed.

The quality of dietary protein has been shown to influence the conversion of carotene to vitamin A. Carotene was fed to rats in diets containing 0, 9 or 18% casein, 18% zein or 18% gluten (Berger et al., 1962). Each of the diets containing protein was fed with and without supplementary amino acids. It was reported that the efficiency of conversion of carotene to vitamin A, as measured by vitamin A storage in the liver and kidneys, was increased as the level of protein increased or as the quality of the protein was improved. Similar results were reported by Rechcigl et al. (1962). These investigators
also reported that rats on a vitamin A depletion diet and fed proteins of inferior biological value could not mobilize existing liver vitamin A stores as well as rats fed casein. Suboptimum protein intakes have also been observed to result in reduced efficiency of carotene conversion in chicks (Nir and Ascarelli, 1967) and in pigs (Friend et al., 1961). On the other hand, pigs fed diets containing only 8% protein have been observed to utilize dietary carotene for liver vitamin A storage more efficiently than pigs fed diets containing 16% protein (Eaton, Boucher and Shaw, 1964b). However, carotene was not as effective in maintaining plasma vitamin A concentrations in the pigs fed the lower level of protein. Friend et al. (1961) also reported that doses of carotene given to pigs fed diets containing 6% protein failed to result in increased plasma vitamin A concentrations and that serum and liver vitamin A concentrations were reduced by a deficiency of protein even when supplemental vitamin A was administered.

Effects of protein deficiency upon the carotene and vitamin A metabolism of ruminants have also been investigated. Anderson et al. (1962) reported that feeding diets containing 5.9% protein to sheep apparently reduced their ability to store intraruminally injected vitamin A to one-half that of sheep fed a diet containing 10.4% protein. The efficiency of conversion of carotene to vitamin A in the sheep fed the lower level of protein did not appear to be affected as determined by liver vitamin A storage, although serum vitamin A levels were lower than in sheep fed the higher protein diets. On the other hand, protein intakes insufficient to maintain a satisfactory concentration of
hemoglobin in the blood of calves had no apparent effect on the concentration of vitamin A or carotene in the blood plasma (Rousseau et al., 1954). Steers fed diets containing 6% protein did not have a significantly slower rate of liver vitamin A turnover than steers fed 12% protein diets, although a tendency toward slower rates was exhibited (Hayes, Mitchell and Little, 1968).

Levels of protein approaching those considered to be optimum do not appear to greatly influence carotene and vitamin A metabolism. Weichenthal (1962) reported that increasing the protein content of the diet of finishing cattle from 10.5 to 11.6% had no apparent effect on the vitamin A and carotene concentrations in the blood plasma and liver tissue of these cattle.

High dietary protein levels may, in some instances, have an undesirable effect on carotene and vitamin A metabolism. Stoewsand and Scott (1964a, 1964b) reported that feeding diets containing up to 33.3% crude protein to chicks caused increased mobilization of vitamin A from liver stores. Feeding diets containing as much as 36% protein to rats, however, did not affect their vitamin A utilization (Dye et al., 1945). On the other hand, increasing the protein content of diets fed to finishing cattle from 11 to 19% has been reported to cause an increase in the rate with which liver vitamin A stores become depleted (Erwin et al., 1963).

Certain feed ingredients may also have an influence upon the vitamin A status of an animal. Soybeans have been reported to affect carotene and vitamin A metabolism. Levels of 30% raw or cooked soybeans
in the diet of calves have been shown to reduce the efficiency of conversion of carotene to vitamin A (Ellmore and Shaw, 1954) and to lower the concentration of vitamin A in the plasma and liver of calves (Ellmore et al., 1948; Ellmore and Shaw, 1954). The amount of carotene required to satisfy vitamin A requirements appeared to be increased by this level of soybeans. Feeding 1 g of iodinated casein per 100 lb of body weight daily did not prevent the depression of plasma vitamin A concentrations caused by feeding soybeans (Ellmore and Shaw, 1954).

Soybean meal has also been reported to reduce carotene and vitamin A utilization in sheep as indicated by blood and liver concentrations of carotene and vitamin A (Gallup et al., 1951).

The apparent effect of soybeans upon carotene utilization may be due to a portion of the protein itself. Ewing (1963) states that certain legumes, particularly soybeans, contain an enzyme known as carotene oxidase which readily destroys carotenes and xanthophylls and probably also destroys vitamin A. The enzyme appears to have a temperature optimum of about 43°C and to be more active at pH 4 to 5 than at higher pH ranges (Walsh and Hauge, 1954). It is therefore possible when raw or insufficiently cooked soybeans are present in the diet that large amounts of carotene and vitamin A may be destroyed by this enzyme.

Insufficient levels of protein in the diet and, for monogastric animals at least, poor quality dietary protein appears to have some influence upon the utilization, transport and storage of carotene and vitamin A. Evidence seems to indicate that a reduced concentration of proteins associated with carotene and vitamin A in the blood is one of
the primary factors involved in this mechanism. Excess protein in the diet may also influence vitamin A status, although the mechanism through which this effect is mediated is unclear.

**Nitrate and Nitrite.** The widespread use of commercial nitrogen fertilizers has increased the probability of higher concentrations of nitrates and other nonprotein nitrogenous compounds in plant tissues used for feed.

Diets containing nitrate have been reported to have various deleterious effects on vitamin A metabolism. The addition of 1% of potassium nitrate to rations fed to fattening steers resulted in an apparent, but not statistically significant, increase in the rate with which liver vitamin A stores were reduced during depletion (Hale et al., 1961b). The presence of nitrate at a level equivalent to 1% potassium nitrate on a dry basis in diets fed to lambs caused a highly significant depression in both plasma and liver vitamin A concentrations (Hatfield et al., 1961). Goodrich, Emerick and Embry (1964) observed that the addition of 3% sodium nitrate to diets fed to lambs resulted in reduced concentrations of vitamin A in liver tissue whether the diet contained carotene alone or with supplemental vitamin A. However, the addition of nitrate to these diets had no effect on the plasma vitamin A concentration of the sheep.

Other investigations have failed to reveal an influence of nitrate upon the metabolism of carotene and vitamin A. The addition of 3% nitrate to a pig diet appeared to have no influence upon the concentration of vitamin A in the liver and blood plasma, although gains were
reduced (Hutaglung et al., 1968). Carotene utilization in these pigs did not appear to be altered as compared to that of control pigs. Other investigators working with cattle (Zimmerman et al., 1962; Wallace, Raleigh and Weswig, 1964; Davison, 1965) and with sheep (Smith et al., 1962; Davison, 1965) have not observed an effect of dietary nitrate upon the various indices of vitamin A status used in their respective investigations.

Several investigations have shown that nitrite in the diet appears to exert a much more serious effect than nitrate. O'Dell et al. (1960) reported that 0.3% potassium nitrite in the diet of rats resulted in suppression of growth and increased the rapidity with which liver stores of vitamin A were depleted. Emerick and Olson (1962) demonstrated that the feeding of nitrite to rats resulted in a reduction of the amount of vitamin A stored in the liver when the vitamin was administered orally but not when it was administered subcutaneously. This suggested that nitrite exerted its effect upon vitamin A utilization through some mechanism within the gastrointestinal tract. Later work at the South Dakota station (Hoar, Embry and Emerick, 1968) has indicated that nitrate, which is reduced to nitrite in the rumen, may also exert its principle effect on the utilization of dietary carotene or vitamin A prior to the storage of vitamin A in the liver. In this experiment, lambs fed 2.5% sodium nitrate with supplemental carotene or vitamin A during the repletion phase of a depletion-repletion study had significantly lower plasma vitamin A concentrations and lower concentrations of liver vitamin A than sheep fed no nitrate. The effects of nitrate during
vitamin A repletion were more pronounced than those observed during vitamin A depletion. The data indicated that a reduction in the amount of dietary vitamin A activity reaching hepatic stores, possibly a result of destruction of dietary vitamin A or carotene, is of greater importance than an accelerated depletion of existing vitamin A stores in affecting the vitamin A status of nitrate-fed sheep.

Nitrite has been shown to have deleterious effects on the metabolism of carotene and vitamin A in other species also. Diets containing 0.3% nitrite significantly reduced blood serum concentrations of vitamin A in pigs and tended to reduce liver stores of the vitamin (Hutaglung et al., 1968). The effect was demonstrated when either carotene or vitamin A was present in the ration. Flynn, Garner and Pfander (1961) observed that the liver vitamin A concentrations in sheep fed nitrite were lower than those in control sheep. The diets fed in this experiment were supplemented with vitamin A.

The factors associated with the apparent destruction of carotene and vitamin A by nitrate and nitrite have been investigated. The in vitro destruction by potassium nitrite of carotene and vitamin A in aqueous dispersions has been studied (Pugh et al., 1962). Destruction of carotene was greatest from pH 1 to 3 followed by an abrupt decrease to a lesser rate of destruction at pH 5 to 7. Vitamin A was destroyed quite rapidly by potassium nitrite in solution at pH 2. Emerick, Embry and Olson (1963) reported that nitrite appeared to exert little effect on carotene when the pH of the medium was 6.25 or above, but destruction increased with decreases in the pH. Nitrate did not appear to cause
destruction of beta-carotene under a variety of conditions. When incubated in-vitro with rumen fluid at pH's approaching neutrality, nitrate did not cause a significant loss of carotene. Similar observations were made by Davison and Seo (1963). Roberts and Sell (1963) observed the in-vitro and in-vivo destruction of vitamin A by nitrite in sheep rumen and abomasal fluid and fluid from the crop, ventriculus and intestine of chickens. They reported that rapid destruction of vitamin A resulted from the presence of nitrites at pH 4 or lower in abomasal and ventricular fluids. Little destruction was observed when the pH was 6 or above or when nitrite was absent.

It has been shown that nitrate and nitrite are capable of influencing the vitamin A status of animals. There is some evidence to indicate that this effect is mediated through the thyroid depressing effect of nitrate. However, at least part of the effect of nitrate and nitrite appears to be exerted through destruction of carotene and vitamin A before it can be absorbed from the gastrointestinal tract. This effect is exhibited in ruminants as well as nonruminants, though it may be greatly reduced due to the rapid reduction and absorption of nitrite from the rumen. Under conditions of high nitrate concentrations, the destruction of carotene and vitamin A probably assumes a secondary importance, since under these conditions the life of the animal is probably in jeopardy.

Other Factors. There have been several factors of apparently minor importance reported in the literature to have some influence upon the metabolism of carotene or vitamin A.
Phosphorus appears to be necessary for the conversion of carotene to vitamin A in cattle (Ross and Gallup, 1949; Thomas, Gallup and Whitehair, 1953; Gallup et al., 1953; Pope et al., 1961) and in sheep (Gallup et al., 1953; Pope et al., 1961). The mobilization of vitamin A from liver storage sites has also been reported to be influenced by a deficiency of phosphorus in cattle (Thomas et al., 1953) and in sheep (Gallup et al., 1953). It is apparent that a phosphorus supplement should be provided to animals consuming a low phosphorus diet, especially in times of drought or extended dry periods when the possibility of a vitamin A deficiency already exists.

Antioxidants, because of their protective properties, have been tested to determine their effects on carotene and vitamin A metabolism. Tocopherols added to the diet of rats (Bentley and Morgan, 1946), sheep (Cline, Hatfield and Garrigus, 1963) and cows (Espe, Bolin and Bolin, 1948) did not result in any significant improvement in liver vitamin A storage from dietary sources of carotene. Synthetic antioxidants included at low levels in the diets fed to hens (Nakaue et al., 1966), calves (Rousseau et al., 1956b; Pirchner, Allen and Jacobsen, 1957) and to fattening cattle (Nelson, Diaz and Catron, 1962; Erwin et al., 1963) have demonstrated beneficial effects on vitamin A or carotene utilization. Feeding large amounts of an antioxidant may interfere with the utilization of dietary vitamin A (Pirchner et al., 1957). Antioxidants may, under certain conditions, provide for increased efficiency of utilization of carotene and vitamin A by preventing a portion of the destruction which appears to occur in the digestive tract.
Investigations concerning the effects of thyroid-active agents on vitamin A metabolism indicated that the efficiency of conversion of carotene to vitamin A was closely related to thyroid activity (Johnson and Baumann, 1947a, 1948). This view was supported by others (Cama and Goodwin, 1949; Cama et al., 1957). Thyroid-active agents have also been shown to influence the conversion of carotene to vitamin A in cattle (Chanda et al., 1951; Burroughs et al., 1961a, 1961b) and goats (Chanda et al., 1951). However, other investigations with rats (Arnich and Morgan, 1954; Worker, 1956; Anderson et al., 1964; Ascarelli et al., 1964), lambs (Cline et al., 1963) and calves (Allen, Wise and Jacobson, 1948) have failed to reveal a close association between thyroid activity and the conversion of carotene to vitamin A. The results of administering thyroid-active agents appear to be quite variable and the mechanisms through which their effects are exerted are unclear.

Adrenalin and other adrenal gland hormones have also been reported to influence vitamin A and carotene metabolism in rats (Young and Wald, 1940; Klopp, Tabor and Danish, 1951; Clark and Colburn, 1955), although the response to injected adrenalin has been observed to vary considerably between individuals (McGillivray, 1961) or to be entirely absent (Goodwin and Wilson, 1949). Adrenalin injections have been reported to have no effect upon the blood plasma carotene or vitamin A concentrations in sheep and calves (Varnell and Erwin, 1959). The effects on vitamin A status of administering adrenal hormones, or of causing their release through stresses applied to the animal, appear to
be quite variable and the physiological mechanisms involved are not apparent.

The effects of diethylstilbestrol (DES) on carotene and vitamin A metabolism in cattle have been investigated (Erwin, Dyer and Ensminger, 1956; Dyer, Ensminger and Blue, 1957; Bohman and Wade, 1958; Mitchell et al., 1960; Perry et al., 1962). Generally, DES appears to exert a favorable effect upon the concentration of carotene and vitamin A in the blood plasma and liver tissue. This effect is not usually of statistical significance and is probably the result of slightly increased feed intake. The administration of DES to vitamin A deficient animals has been reported to increase the apparent severity of deficiency symptoms (Perry et al., 1962). However, growth stimulants such as DES probably do not influence the vitamin A status or requirements of the bovine except as they may affect feed consumption, body weight and growth.

Several studies have been made concerning the effects of adding antibiotics such as chlortetracycline (Erwin et al., 1956; Bohman and Wade, 1958; Perry et al., 1962; Roy et al., 1964) and oxytetracycline (Dyer et al., 1957) to the diet of cattle. These experiments have shown that antibiotics may, under certain conditions, provide for more efficient utilization of dietary carotene and vitamin A. As with growth stimulants, antibiotics may influence carotene or vitamin A requirements through influencing feed consumption, body weight and growth.
The addition of a small amount of fat or lipid material to the diet of rats (Thorbjarnarson and Drummond, 1938; Bring, Ricard and Zaehringer, 1965) and cattle (Raper, 1950; Willey et al., 1952; Erwin et al., 1956) has been reported to exert favorable effects on the absorption and conversion of carotene. However, the effect may not be demonstrated with certain diets (Dyer et al., 1957; Bohman, Wade and Torrell, 1959) and the addition of relatively large amounts of fat may have a distinctly deleterious effect on vitamin A metabolism in cattle (Bohman and Wade, 1958). It appears that the net effect of the addition of fat to a given diet depends partially upon the level at which the fat is added to the diet and partially upon the characteristics of the fat as well as those of the remainder of the diet.

Lipotropic agents, because of their ability to mobilize hepatic fat under certain conditions, have been used in investigations to determine their effects on carotene absorption and vitamin A metabolism. Lecithin has been reported to promote the absorption of carotene by rats (Slanetz and Scharf, 1945) and of vitamin A by calves (Roy et al., 1961). However, other investigations with sows and sheep (Eaton et al., 1948) and calves (Davis, Elliott and Lassiter, 1956) have failed to demonstrate this effect. Choline fed to rats (Popper and Chinn, 1942; Bentley and Morgan, 1946) and betaine fed to chicks (Edwards, Dunahoo and Fuller, 1958) failed to result in improvements in carotene utilization. Clayton and Baumann (1944) concluded after extensive tests that the hepatic storage of vitamin A appeared to be relatively independent of other biochemical processes taking place in the liver.
It would appear that the results obtained with a given lipotropic agent would depend to some extent upon the specific agent and the amount given, the nature of the diet with which it was administered, and the conditions under which it was administered. Lipotropic agents, except as they may influence the absorption of carotene or vitamin A as in the case of lecithin, probably have little effect upon the vitamin A status of an animal.

Sources and Methods of Furnishing Vitamin A and Carotene

The principal source of vitamin A activity for cattle is the carotene present in certain feeds. Diets containing a large proportion of leafy, green-colored roughage fed fresh or harvested and preserved in a manner to maintain a high content of nutrients will generally furnish sufficient carotene to satisfy the vitamin A needs of cattle. However, diets containing meager amounts of roughage or mature and weathered ones may not contain carotene in sufficient amounts and a supplemental source of vitamin A or carotene will be needed.

Natural sources of the vitamin include milk and fish liver oil, neither of which is generally fed to mature cattle. The supply of these materials may be limited and expensive and the vitamin A in these products is not well protected from destruction by oxidation. Consequently, use is more often made of synthetic vitamin A. Vitamin A from this source is available in several kinds of carriers designed for various methods of administration and to improve stability of the vitamin.
Because of the number of different forms in which carotene and vitamin A products are available, and because of the different means by which carotene and vitamin A may be administered, it is important to understand the influence of these factors upon the vitamin A nutrition of animals.

Relative Vitamin A Value of Different Carotenoids As Compared to Beta-Carotene. The vitamin A activity of plant tissues is due to the presence of carotene which is composed of a mixture of various carotenoid compounds existing as different isomers and stereoochemical forms. The relative biological value of several of the isomers of carotene, as determined by liver and kidney storage of vitamin A in depleted weanling rats, was investigated by Johnson and Baumann (1947a). The relative activities of various forms, with a value of 100 assigned to all-trans beta-carotene, were reported as follows: neo-beta-carotene B, 48; neo-beta-carotene U, 33; and all-trans alpha-carotene, 25. Milas (1954) reported the following values for some of the carotenoids, again with a value of 100 assigned to beta-carotene: alpha-carotene, 53; gamma-carotene, 27; cryptoxanthin, 57; and alphanin, 50. Values for other forms have been reported (Karrer and Jucker, 1950; Deuel et al., 1945).

The most prevalent isomer of carotene, both in quantity and distribution, is beta-carotene (Morrison, 1959; Maynard and Loosli, 1962) and it is this form which accounts for practically all the vitamin A potency of forage crops (Ewing, 1963). Thus for practical purposes, the mixed carotenes from various forages should be of nearly equal value
on a weight basis. It should be noted, however, that differences in the digestibility of the different forages and other factors which influence the absorption and conversion of carotene may cause the carotene from certain sources to be utilized less efficiently than that from other sources.

**Vitamin A Activity of Carotene As Compared to Vitamin A.**

According to present standards 0.3 µg of vitamin A alcohol or 0.6 µg of beta-carotene is equal to 1 international unit (IU) of vitamin A. On this basis, 1 mg of pure crystalline beta-carotene has a biopotency equal to that of 1667 IU of vitamin A. This value is derived from investigations with rats, however, and other species are not as efficient as the rat in converting carotene to vitamin A. In addition, the carotene in feedstuffs is a mixture of heterogeneous carotenoid compounds and would necessarily have somewhat lesser biopotency than pure beta-carotene.

The source of carotene may influence its relative value. Hoeffer and Gallup (1947) compared the value of carotene furnished by alfalfa meal with that furnished by a commercial carrot oil concentrate using blood plasma vitamin A concentrations and liver storage of vitamin A in lambs as the bases for comparison. It was reported that the carotene furnished in alfalfa meal was nearly twice as potent as an equal weight of carotene from the carrot oil concentrate. Similar results were obtained for the two products when fed to milk cows (Bullis et al., 1958). In other experiments, forage species appeared to have an effect upon the value of carotene for maintaining plasma vitamin A
concentrations in horses (Fonnesbeck and Symons, 1967). Carotene from alfalfa hay was reported to be more effective in maintaining plasma vitamin A concentrations than that from any other hay tested. Carotene from canarygrass hay was more effective than that from any other grass hay used. Other reports, however, have indicated that when carotene is furnished by roughages in the diet the source of carotene has little or no effect on the efficiency with which it is utilized (Cullison and Ward, 1965; Miller et al., 1967). Differences in apparent utilization of carotene from different sources may be due primarily to differences in the digestibility of the feedstuffs or to other characteristics of the diet which might influence carotene absorption and conversion.

The level of carotene intake may have an influence upon its comparative vitamin A value. Guilbert et al. (1940) stated that at levels of carotene intake meeting the minimum requirements of cattle the ratio of efficiency of vitamin A to carotene on a weight basis was six to one. However, the ratio was about 10 to 1 at levels of intake required to obtain significant liver storage of vitamin A. They concluded that the ratio of the relative efficiencies with which vitamin A and carotene are utilized widens with increasing levels of intake. Similar results have been reported by Rousseau et al. (1956a) and by Grifo et al. (1960b) using blood plasma vitamin A concentrations of calves as the basis for the comparison.

Carotene furnished by alfalfa meal was compared with vitamin A from fish liver oil in experiments by Hoeffer and Gallup (1947). When vitamin A and carotene intakes of 50 IU/kg of body weight were
administered, lambs fed the fish liver oil maintained a liver vitamin A storage approximately three times greater than that of the lambs fed the alfalfa meal. When an intake of 500 IU from each compound was administered, the difference in liver vitamin A storage was approximately six times greater in favor of the fish oil. It thus appears that as the level of carotene intake is increased the efficiency with which it is utilized decreases and its value compared to that of vitamin A decreases.

Several investigators have attempted to determine the vitamin A equivalent value of carotene for cattle from a comparison of responses obtained in feeding trials. Such comparisons should be made at levels of carotene intake not greatly in excess of minimum requirements in order to have meaningful application. Embry et al. (1962) fed dehydrated alfalfa meal as a source of carotene to vitamin A depleted steers. On the basis of weight gains and liver vitamin A storage, 2.5 mg of carotene appeared to be slightly more valuable than 1000 IU of vitamin A palmitate. In later work at the South Dakota station (Hansen, 1963), dehydrated alfalfa was again used to provide carotene in diets fed to vitamin A depleted steers. Based on plasma vitamin A values, 1 mg of carotene from dehydrated alfalfa appeared to be about equal to 500 IU of vitamin A. Record, Beeson and Smith (1963) reported that 1 mg of beta-carotene appeared to be about equal to 400 IU of vitamin A when fed to steers. Carotene in other experiments (Grifo et al., 1960b) appeared to have a value equivalent to about 425 IU of vitamin A per milligram for calves when blood plasma vitamin A concentrations were used as the basis for the comparison.
The National Research Council (1963) considers that for beef cattle 1 mg of carotene from a feedstuff is equivalent to 400 IU of vitamin A. This factor was selected after careful consideration of much of the available literature and assigns to carotene a value of about one-eighth that of an equal weight of vitamin A alcohol. This value appears to be a justifiable approximation of the average relative vitamin A value of carotene when levels of intake do not greatly exceed minimum requirements.

Effect of Carrier and Method of Administration. Various carrier materials are used in the preparation of carotene and vitamin A supplements to obtain products of desirable vitamin concentration and to provide these supplements with characteristics suitable for different methods of administration. It is important to understand the effect of carrier and mode of administration upon the efficiency with which these supplements are utilized.

The digestibility or solubility of the carrier employed may influence the availability of orally administered carotene or vitamin A. Crowley and Allen (1950), using blood carotene and vitamin A concentrations as indicators, administered carotene in different carriers to partially depleted dairy calves. They reported that corn oil, soybean oil, goat butter and cow butter were of nearly equal value as carriers, but the availability of carotene from the mineral oil carrier was very low. Dutcher et al. (1934) reported that rats could not utilize supplemental carotene when it was added to the ration in a mineral oil solution nor was vitamin A from butterfat utilized when dissolved in
minerol oil. However, carotene dissolved in cottonseed oil has been reported to be as available as carotene from alfalfa for the maintenance of blood plasma vitamin A and carotene levels in calves (Boyer et al., 1942).

Administration of vitamin A in aqueous dispersion by capsule to vitamin A depleted dairy calves promoted more rapid absorption into the blood than when the vitamin was prepared in an oil solution and administered (Blake, Jacobson and Allen, 1950). Similar results were reported by Wise et al. (1949, 1958). Administration of vitamin A palmitate in gelatin beadlets to vitamin A depleted steers resulted in higher blood plasma concentrations of vitamin A and in greater amounts of liver vitamin A storage 1 week after administration than when the vitamin was dissolved in corn oil or was furnished by fish liver oil (Sherman et al., 1958). Dispersing the vitamin in water with Tween 80 did not improve results over those obtained by administering the vitamin in gelatin beadlets.

The particle size of the carotene or vitamin A administered in the supplement may also have an effect upon availability. Homogenization of carotene into a goat milk diet fed to partially depleted calves resulted in much higher plasma carotene concentrations that those obtained by simply stirring the supplement into the milk (Crowley and Allen, 1950). However, plasma vitamin A concentrations were apparently not affected by treatment. Luther, Goett and Cragwell (1952) reported that particles of vitamin A 2 microns or less in diameter dispersed in gelatin beadlets brought about 69% greater liver storage of vitamin A in
rats than was obtained with an equal dose of the vitamin administered in an oil solution. Improvement in liver storage over the oil solution was only 24% when the particle size of the vitamin A in the beadlets was increased to 5 microns. With a particle size of 20 microns, the resulting liver storage was approximately the same as that obtained with the vitamin A in the oil solution.

Often, the simplest means of administering a vitamin A supplement is oral administration. However, the oral administration of supplements to young calves by some methods may result in losses due, apparently, to factors associated with the rumen and reticulum. Ingestion of vitamin A with a milk diet fed by means of a bucket-type nipple feeder resulted in more rapid absorption and higher concentration of plasma vitamin A than when the vitamin was administered separately by stomach tube (Wise et al., 1958). Similar results were obtained in earlier work in which carotene from carrot oil was administered by these means (Wise et al., 1949). Administration of vitamin A to vitamin A depleted calves by nipple feeder has been shown to result in more efficient liver storage of vitamin A than when the vitamin was administered by capsule (Blake et al., 1950). However, methods which allow the administration of vitamin A or carotene into the gastrointestinal tract beyond the stomach may result in inefficient utilization of these compounds. Barrick, Andrews and Bullard (1948) used blood plasma carotene and vitamin A concentrations to study carotene and vitamin A utilization in feeder lambs. Vitamin A administered via fistulas of the colon or caecum resulted in no marked changes in plasma vitamin A concentration.
Administration of the vitamin by means of a fistula of the small intestine resulted in rapidly increased plasma vitamin A concentrations. However, oral administration or administration by means of a rumen fistula resulted in longer lasting and higher concentrations of vitamin A in the plasma than when the vitamin was administered into the small intestine. Results obtained with carotene followed the same pattern as those obtained with vitamin A.

Other routes for the administration of carotene and vitamin A have been investigated. Varnell and Erwin (1960), using carotene and vitamin A concentrations in the blood of lambs as indicators, reported that intramuscular and intraperitoneal injections of vitamin A gave greater and more rapid responses than intraruminal or subcutaneous injections. The intramuscular injection appeared to be slightly superior to the intraperitoneal injection and subcutaneous injection was superior to intraruminal injection in increasing plasma vitamin A concentrations. When carotene was used, intramuscular and subcutaneous injections were superior to intraperitoneal and intraruminal injections in raising plasma carotene concentration. However, only the intramuscular and intraperitoneal injections of carotene brought about increases in plasma vitamin A concentration.

In addition to various routes for the administration of carotene and vitamin A different time schedules may be used. The vitamin may be given as one large dose or administered in smaller doses at intervals over a period of time. However, a single large dose may not be as efficiently utilized over a long period as would the same total amount
of vitamin A administered in smaller daily doses. A single intra-muscular injection of 3,360,000 IU of vitamin A palmitate in polysorbate at the beginning of a 56-day feeding trial resulted in significantly higher concentrations of liver vitamin A at the end of the trial than did feeding the same total amount at the rate of 60,000 IU per head daily during the trial (Smith et al., 1964). In a 210-day trial, however, feeding 20,000 IU of the vitamin per steer daily resulted in higher final liver and blood plasma concentrations of vitamin A than were obtained with initial injections of 1 million or 6 million IU (Perry et al., 1967). Steers given 1 million IU in a single initial dose were nearly depleted of vitamin A at the end of the period. Those given 6 million IU had about the same plasma and liver vitamin A concentrations as steers fed 20,000 IU per head daily. In a second 210-day trial, an initial intramuscular injection of 4 million IU of vitamin A resulted in slightly higher final liver vitamin A but not serum vitamin A concentrations than the daily feeding of 20,000 IU per head.

Carotene and Vitamin A Requirements of Cattle

It has been demonstrated repeatedly that the vitamin A requirement of the bovine can be satisfied with either the preformed vitamin or with carotene even though the actual requirement appears to be for the vitamin itself. Many rations contain adequate amounts of carotene and no supplementation is required. However, when the total amount of vitamin A activity in the ration is inadequate, supplementation of the ration with either carotene or vitamin A becomes necessary. In some instances, both vitamin A and a source of carotene are added. Thus
it is not uncommon for diets to contain both carotene and preformed vitamin A. It is for this reason that feeding standards commonly list requirements both in terms of vitamin A and in terms of the amount of carotene needed to satisfy the requirement.

**Carotene and Vitamin A Requirements for Maintenance, Growth and Fattening.** Guilbert and Hart (1935) used depleted cattle ranging in weight from 130 to 500 kg in a recovery type of experiment to determine the amount of carotene required to cure vitamin A deficiency symptoms and to provide for normal weight gains. The minimum requirement was established by repeatedly increasing or decreasing the intake of alfalfa hay or dehydrated alfalfa until night blindness and other symptoms disappeared or reappeared. Suboptimum levels of intake were achieved which permitted nearly normal growth, yet night blindness persisted. These investigators reported that the minimum carotene intake which prevented deficiency symptoms and promoted normal weight gains on an otherwise adequate diet was 29 μg/kg of live weight. In a later experiment in which vitamin A from cod liver oil and carotene from dehydrated alfalfa meal were fed to depleted mature cows (Guilbert et al., 1937), it was observed that vitamin A requirements were 6.7 to 8.4 μg/kg of body weight daily. The carotene requirements observed in this experiment were in agreement with those established earlier by Guilbert and Hart (1935). These investigations were repeated at a later date (Guilbert et al., 1940) and the carotene and vitamin A requirements for the prevention of night blindness were found to be of about the same magnitude as reported earlier. It was noted that these levels, in all
cases, provided for normal growth and general well-being but permitted little or no storage of vitamin A.

Nyctalopia and pappillary edema were used as indicators by Moore (1939b) to determine the carotene requirements of previously depleted Holstein and Ayrshire calves. An intake of 9 µg of carotene per pound of body weight was not sufficient to prevent the appearance of these symptoms, both of which developed when plasma carotene concentrations fell below 0.13 µg/ml. An intake of 16 µg/lb of body weight daily prevented vitamin A deficiency symptoms and maintained plasma carotene levels at 0.2 µg or more per milliliter. This level of intake also provided for a fair state of general health.

Lewis and Wilson (1945) used vitamin A depleted male and female grade Holstein calves in their investigations to determine vitamin A requirements. Vitamin A was administered daily in capsules at one of six different levels ranging from 32 to 1024 USP units/kg of body weight during an 8-month period. Night blindness and suboptimum rates of growth resulted from administering 32 units while a level of 64 USP units/kg of body weight provided for maximum gains and prevention of night blindness.

Holstein and Guernsey calves, depleted of vitamin A reserves, were used by Boyer et al. (1942) to determine carotene and vitamin A requirements. Vitamin A was furnished from shark liver oil and carotene was administered in crystalline form or furnished by alfalfa hay. Optimum gains over a 6-month period were achieved when the blood plasma level of vitamin A was maintained at 10 µg/100 ml or more. Intakes of
20, 40 or 60 µg of carotene per kilogram of body weight daily were insufficient as were intakes of less than 18 µg of vitamin A per kilogram of body weight daily. Carotene intakes of 75 µg/kg of body weight daily promoted optimum rates of gain.

In a study conducted by Keener et al. (1942), the amount of carotene required to satisfy the vitamin A requirements of previously depleted Holstein and Guernsey calves was reported to be about 27 µg/lb of body weight daily. Alfalfa meal was used as the source of carotene and was administered in gelatin capsules. Ophthalmoscopic examination of the eyes, histopathological examination of selected tissues and the general appearance of the calves were used as indicators in this experiment.

Carotene administered at levels of 16, 24 or 32 µg/lb of body weight daily was insufficient to prevent the appearance of vitamin A deficiency symptoms in a study conducted by Dehority et al. (1960). However, the previously depleted male Holstein calves used in this study showed no deficiency symptoms when an intake of 40 µg of carotene per pound of body weight was administered daily. Changes in the cerebrospinal fluid pressure and in the composition of the blood serum, cerebrospinal fluid and aqueous humor of the eye were used as criteria.

Other investigators have supplemented rations with various levels of carotene or vitamin A to determine the vitamin A and carotene requirements of finishing cattle. Many of these investigations were conducted with cattle which were not depleted, or were only partially depleted, of their vitamin A stores. In much of this work, vitamin A
requirements could not have been accurately determined because the
exact vitamin A status of the animals was not determined at any time
during the trials.

Partially depleted steers averaging 880 lb initially were used
in a 169-day experiment in which vitamin A was added to a low carotene,
high concentrate finishing ration (Nelson et al., 1962). The vitamin
was administered at levels of 0, 5,000, 10,000 or 20,000 IU per head
daily. The most rapid daily weight gains were obtained with cattle fed
10,000 IU daily. In an experiment conducted by Beeson et al. (1962),
the administration of vitamin A at 12,550 IU per head daily with a low
carotene finishing diet fed to nondepleted steers resulted in a signifi­
cant improvement in daily gains as compared to gains obtained in control
cattle. Increasing the amount of vitamin A to 25,000 IU per head daily
resulted in a slight additional improvement in weight gains. No improve­
ment in rate of gain was obtained when vitamin A was added to rations
containing 50,000 IU of carotene from dehydrated alfalfa. Kohlmeier and
Burroughs (1964) reported that the administration of 6,000 IU of vitamin
A per head daily promoted optimum gains in yearling steers fed a ration
composed of 70% cracked corn, 20% ground corn cobs and 10% supplement.
The steers used in this experiment were not previously depleted of their
vitamin A stores.

Other investigations have involved steers which have been depleted
of their vitamin A reserves. Jones et al. (1943) used night blindness,
body weight gain and general health as indicators in experiments to
determine the carotene and vitamin A requirements of previously depleted
feeder cattle. It was reported that carotene intakes of 800 to 1,250 µg/100 lb of body weight daily resulted in satisfactory gains but the cattle demonstrated a lack of normal well-being. Levels of 1,500 µg were inadequate to control night blindness; however, levels of 2,000 µg/100 lb of body weight daily provided for normal well-being, normal weight gains and a satisfactory degree of night vision. Intakes of 2,000 IU of vitamin A from cod liver oil appeared to result in maintenance of satisfactory condition and optimum weight gains while levels of 1,000 or 1,500 IU resulted in inferior performance.

Embry et al. (1962) depleted steers to average plasma vitamin A levels of 4.8 to 7.5 µg/100 ml. The rations of rolled barley plus protein and mineral supplements were supplemented to furnish up to 5,000 IU of vitamin A per 100 lb of body weight. Carotene, furnished from dehydrated alfalfa, was also fed at 2.5 mg/100 lb of body weight alone or with 1,000 or 2,000 IU of vitamin A. Optimum rates of gain were obtained with cattle fed 2,000 IU of vitamin A per 100 lb of body weight daily. On the basis of weight gains, 2.5 mg of carotene appeared to be about equal to 1,000 IU of vitamin A. In other experiments conducted at the South Dakota station (Hansen, 1963) yearling Hereford steers previously depleted of their vitamin A reserves were fed 500, 1,000, 2,000 or 4,000 IU of vitamin A per 100 lb of body weight daily with rolled barley rations. Optimum rates of gain were achieved with 2,000 IU of vitamin A per 100 lb of body weight. In this experiment carotene, from dehydrated alfalfa, fed at the rate of 6 mg/100 lb of body weight daily promoted more rapid gains than did 1, 2 or 4 mg. However,
on the basis of plasma vitamin A values 1 mg of carotene appeared to be about equal to 500 IU of vitamin A. Other workers (Perry et al., 1962, 1967) have reported that maximum rates of weight gain were achieved by feeding 20,000 IU of vitamin A per head daily to fattening cattle when the cattle were fed low carotene finishing rations.

Values reported for the minimum vitamin A requirements of cattle for maintenance, growth and fattening range from the equivalent of about 1,033 to about 3,300 IU or more per 100 lb of body weight daily. In some instances, however, the lower values have not provided for optimum rates of gain under all conditions and thus should probably be considered as being more nearly the minimum requirement for maintenance under optimum conditions. The N.R.C. (1963) lists vitamin A requirements for the bovine as being about 1,580 to 2,300 IU of vitamin A per 100 lb of body weight daily. These levels are considered adequate for normal health, growth and finishing under usual conditions but do not include allowances for stress or other factors which may increase the apparent vitamin requirement.

Values reported as representing the minimum carotene requirements of the bovine for maintenance, growth and fattening range from about 1 mg to nearly 6 mg of carotene per 100 lb of body weight daily. Several reports have indicated that intakes of from 1.3 to 1.7 mg of carotene per 100 lb of weight are sufficient for maintenance and, under optimum conditions, a fair rate of growth. On the other hand, intakes of about 3 mg or more per 100 lb of body weight daily generally appear to insure normal health and an optimum rate of growth except under adverse
conditions. The N.R.C. (1963) lists carotene requirements for normal growth of steers and heifers as being about 4 to 5.75 mg of carotene per 100 lb of body weight daily. These values have been calculated from the vitamin A requirements. An older, but equally well accepted set of feeding standards, those of Morrison (1959), state recommended allowances rather than minimum requirements. The recommended values include a reasonable safety margin and thus are slightly higher than those of the N.R.C. (1963). Morrison (1959) recommends practical allowances of 5.5 to 6.5 mg of carotene per 100 lb of body weight daily for growing and finishing cattle.

Because vitamin A is often used to supplement various diets which already contain carotene, it is advantageous to know the relationship which exists between vitamin A and carotene. This permits the total vitamin A equivalent value of the carotene in the ration to be estimated and required amounts of supplemental vitamin A supplied accordingly. While the vitamin A equivalent value of carotene may show a considerable range depending upon level of carotene intake as previously discussed, a value of 400 IU of vitamin A activity per milligram of carotene (N.R.C., 1963) appears to be a reasonable approximation of the value of carotene under most conditions.

Carotene and Vitamin A Requirements for Storage. The liver is the primary site of storage of vitamin A in the body. The amount of vitamin A stored in the liver generally increases when carotene or vitamin A intake is in excess of requirements. Large stores can thus be accumulated over a period of time. When intake is insufficient to
satisfy daily requirements, vitamin A is withdrawn from the liver at a rate proportional to the dietary deficiency. Consequently, large stores of vitamin A may be sufficient to sustain the animal over a considerable period of time if the dietary deficiency is small.

The value of different sources of carotene and vitamin A in meeting the vitamin A requirements of the bovine is often estimated by comparing the levels of storage obtained by administration of the different sources over a period of time, preferably to animals having no initial stores, or stores of only small magnitude. Liver samples, upon which these comparisons are based, are obtained either at slaughter or through the use of biopsy techniques and are subjected to analysis to determine the concentration of vitamin A present.

The level of vitamin A storage in the liver at any given time is the result of the dynamic equilibrium existing between factors promoting storage and those causing withdrawal from storage. The amount of vitamin A available for storage will be influenced by the level of carotene or vitamin A intake, factors which affect the utilization of carotene and vitamin A and the amount of vitamin A utilized to meet daily requirements. The depletion of liver vitamin A stores, on the other hand, may be affected by environmental and dietary factors, reproductive status, lactation and growth. In addition, the amount of vitamin A lost from storage may be higher when storage levels are high than when storage levels are low. Guilbert and Hart (1935) and Frey et al. (1947) observed that the rate of withdrawal of vitamin A from hepatic stores in cattle appeared to decrease as depletion advanced.
In other work (Page et al., 1958b), it was observed that heifers in a depletion trial lost a similar percentage of their hepatic vitamin A stores over a given time period regardless of the initial amount of storage. These workers concluded that higher levels of vitamin A are retained less tenaciously than lower levels in the liver. Such observations may account in part for the observations of other workers (Sherman and Cammack, 1926; Lewis and Wilson, 1945) that liver vitamin A stores do not appear to increase in a linear relationship with increased intakes of carotene or vitamin A.

In order for considerable amounts of vitamin A to accumulate in the liver during a short period, carotene or vitamin A intake must exceed the daily requirement by a relatively large amount. Guilbert et al. (1940) observed that in cattle appreciable storage of vitamin A would occur during a 90-day period if vitamin A intake was about three times the minimum requirement or if carotene intake was about five times the minimum requirement. An intake of eight times the minimum carotene requirement was necessary in order to secure appreciable storage of vitamin A in the livers of lambs during a similar period (Gallup et al., 1951). Heaney and Thomas (1956) reported that liver storage of vitamin A in depleted steers was initiated by the administration of 50,000 IU of vitamin A per head for 10 days but not when the administration period was only 5 days in length.

Liver stores of vitamin A do not need to be great in order to obtain optimum growth rates in the bovine. Guilbert et al. (1937) obtained excellent growth rates at levels of carotene and vitamin A
administration which permitted only meager storage levels of the vitamin. Finishing steers with liver vitamin A concentrations of less than 2 µg/g of tissue have been observed to perform excellently for many months, as have steers with essentially no liver vitamin A reserves (Kohlmeier and Burroughs, 1964) and calves with less than 1 µg of the vitamin per gram of liver tissue (Miller et al., 1967).

The maximum concentration of vitamin A which may be stored by the liver in cattle does not appear to have been established. However, Guilbert and Hart (1934) observed that the liver tissue of mature cows raised under favorable conditions had a concentration of vitamin A approximating that of high potency cod liver oil. Hale et al. (1961a) observed a concentration of 5,000 µg of vitamin A per gram of tissue in livers of steers fed 2.5 million IU of the vitamin per head daily for 168 days. It would thus appear that the concentration of vitamin A in the liver tissue of cattle may reach very high levels under some conditions.

Several workers have observed the relationship between carotene or vitamin A administration over long periods and the resulting amount of vitamin A stored in liver tissue. Many of the studies have involved animals which were not previously depleted of vitamin A stores. However, the use of previously depleted animals likely results in a better estimate of requirements for storage than would the use of nondepleted animals.

In a review of work conducted at the Oklahoma station (Pope et al., 1961) a series of 160- to 170-day trials with nondepleted
weanling steer calves was summarized. In one trial, average intakes of 3.78, 20.2 or 32.2 mg of carotene per head daily from alfalfa resulted in final concentrations of 0.9, 2.2 and 3.3 μg of vitamin A per gram of dry liver, respectively. In subsequent similar trials, daily carotene intakes of up to 68 mg per head resulted in average liver vitamin A stores of no more than 8.2 μg of vitamin A per gram of dry tissue while intakes up to 124 mg per head resulted in average liver vitamin A levels of not more than 16.4 μg/g of dry tissue. Page et al. (1958b) observed that an intake of about 7.5 mg of carotene per 100 lb of body weight daily maintained liver stores of about 17 μg of vitamin A per gram of tissue in heifers for 110 days. Nondepleted dairy bull calves, 91 to 155 days of age, were fed 97 μg of carotene from corn silage per kilogram of body weight daily in an experiment reported by Miller et al. (1967). This level of carotene resulted in a final hepatic vitamin A concentration of 0.91 μg/g of tissue.

Kohlmeier and Burroughs (1964) conducted two trials in which nondepleted yearling steers were fed a finishing ration with different levels of supplemental vitamin A. The administration of 6,000, 12,000, 18,000 or 36,000 IU of vitamin A per head daily resulted in final average liver vitamin A concentrations of 2.0, 5.8, 13.7 and 18.0 μg/g of tissue, respectively. The length of the trial was not stated, although as indicated by weight gains it apparently exceeded the time required to deplete the control steers of their vitamin A reserves. In work conducted by Perry et al. (1962), vitamin A was administered in a low carotene finishing ration at the rate of 0, 2,000, 4,000, 8,000, 16,000
or 32,000 IU per head daily to nondepleted steers. Liver vitamin A concentrations at the end of the 182-day trial were 1.23, 1.71, 2.45, 4.62, 12.9 and 52.0 μg/g of tissue for the cattle in these treatment groups, respectively. Fattening steers fed 72,000 IU of vitamin A per head daily for 112 days did not maintain average initial liver stores of 73.4 μg of the vitamin per gram of liver (Roberts and Phillips, 1963). However, Frey et al. (1947) observed that the administration of 200 IU of vitamin A per pound of body weight daily was sufficient to maintain initial hepatic vitamin A concentrations of 47 μg/g of tissue for a period of 280 days in nondepleted steers.

Work at the South Dakota station has involved finishing steers depleted of their vitamin A reserves. Embry et al. (1962) fed low carotene diets supplemented to provide 1,000, 2,000, 3,000, 4,000 or 5,000 IU of vitamin A per 100 lb of body weight daily to steers. These levels of supplemental vitamin A resulted in liver vitamin A stores of 0.92, 1.17, 2.67, 6.07 and 4.62 μg/g of liver, respectively. The experimental period was 202 to 204 days. In other work (Hansen, 1963) depleted yearling Hereford steers were fed barley rations supplemented to furnish 500, 1,000, 2,000 or 4,000 IU of vitamin A or 1, 2, 4 or 6 mg of carotene from dehydrated alfalfa per 100 lb of body weight. The length of the supplementation period was 133 to 134 days. Final liver vitamin A concentrations were 0.29, 1.11, 2.05, 8.55, 0.19, 0.47, 1.68 and 2.54 μg/g of tissue for the cattle in the above treatment groups, respectively.
It appears that the storage of vitamin A by liver tissue in the bovine begins at approximately the same level of carotene or vitamin A intake as that which satisfies minimum daily requirements. Stores at this level of intake will not be great, even over extended periods. When intake is increased to a given level above minimum requirements, the amount of vitamin A stored may increase but probably not in a linear manner. This may be due to the increasing amounts of vitamin A destroyed in the body as storage level increases and to the additional amount of vitamin A needed to meet daily requirements for maintenance and growth as body size increases.

Vitamin D

Functions of Vitamin D and the Effects of a Deficiency

Vitamin D is the name given to a group of related compounds, a number of which have been shown to possess antirachitic activity. These compounds are related to the perhydrophenanthrenecyclopentane ring system and thus also to the naturally occurring sterols. Only two forms of the vitamin are of major importance at present. Vitamin D₂, also known as calciferol, is produced in plants by irradiation of ergosterol. The other, vitamin D₃ or cholecalciferol, is produced in animals when 7-dehydrocholesterol is exposed to ultraviolet light. Both forms are relatively stable in comparison to carotene and vitamin A in the presence of heat and light.

Vitamin D is required by many species of animals and is necessary for growth, reproduction and lactation and normal calcification of bones
and teeth. Vitamin D promotes absorption of calcium and phosphorus from the intestine and maintenance of normal calcium and phosphorus levels in the blood plasma. When adequate amounts of the vitamin are unavailable, various deficiency symptoms develop. Morrison (1959), Maynard and Loosli (1962), West and Todd (1961) and Ewing (1963) have reviewed much of the literature pertaining to the effect of a vitamin D deficiency in various animal species. Forbes (1967) has discussed many of the factors relating to vitamin D nutrition with emphasis on vitamin D in human nutrition.

A deficiency of vitamin D results in grossly subnormal mineral retention in deficient animals. This condition is referred to as rickets when present in young growing animals and is characterized in part by the appearance of soft, misshaped or deformed bones which result from a lack of normal calcification. In the adult animal, a deficiency of vitamin D results in demineralization of previously well-calcified bones, which causes the adult bone to become weak and brittle. This condition is known as osteomalacia.

Rickets is the classical disease related to a deficiency of vitamin D in the growing bovine as well as in the young of other species. The symptoms of rickets in the young bovine have been described in detail by Bechtel, Hallman and Huffman (1935), Bechtel et al. (1936a), Thomas (1952) and Thomas and Moore (1951). The first symptom is generally a decrease in the concentration of calcium and inorganic phosphorus in the blood plasma. This may be accompanied by an increase in plasma alkaline phosphatase activity and may be followed by
irritability, anorexia, weakness and retardation or cessation of growth. The knees, hocks and pastern joints become swollen, stiff and tender and the forelegs may bow either to the side or front. The back becomes humped and the animal shows stiffness and difficulty in walking. Posterior paralysis may occur as the result of fractured vertebrae. Rapid, labored breathing, tetany and convulsions are frequently observed during the latter stages of the disease. Postmortem examination may reveal an accumulation of viscous orange-yellow bile in the gall bladder, excess synovial fluid in some joints, joint erosion and, occasionally, enteritis.

The symptoms of vitamin D deficiency in the adult bovine have been described in detail by Wallis (1938, 1946). In addition to many of the symptoms of rickets observed in the immature animal, a deficiency of vitamin D in the adult animal may result in fracture of the pelvis or femur bones and a decrease in milk production and reproductive efficiency. Deficient cows exhibit fewer or no heat periods and calves born to deficient cows may be weak or dead and will have poorly calcified bones.

One of the most important functions of vitamin D is its role in the absorption and utilization of dietary calcium and phosphorus. Many of the symptoms of a deficiency of vitamin D are associated with changes in the calcium and phosphorus content of the different tissues of the body and with the metabolism of these minerals. These changes are brought about by a lack of absorption or retention of calcium and phosphorus. The administration of vitamin D to deficient animals
results in decreased fecal and urinary excretion of calcium and phosphorus (Sjollema, 1923), increased concentration of calcium and inorganic phosphorus in the blood (Steenbock et al., 1923; Wallis, 1937) and increased bone ash (Bechdel, Hilston and Guerrant, 1937; Bechdel et al., 1938; Thomas and Moore, 1951) with the end result being a large positive calcium and phosphorus balance and rapid remission of deficiency symptoms (Wallis, 1938).

The mechanism through which vitamin D influences the absorption of these elements from the lumen of the intestine is not wholly apparent. However, in the case of calcium, recent evidence appears to indicate that vitamin D promotes the formation of a proteinaceous carrier material in the mucosal lining of the intestine (Wasserman and Taylor, 1966) and that this material is in turn responsible for the transport of calcium from the intestine (Norman, 1965).

Vitamin D has been shown to be involved in metabolic processes other than those concerned directly with the absorption of calcium and phosphorus. Steenbock and Herting (1955) observed that vitamin D appeared to be necessary for the growth of the soft tissues of the body as well as for mineralization of bone. The vitamin has been related to citrate and isocitrate oxidation in kidney mitochondria (DeLuca and Steenbock, 1957; DeLuca, Gran and Steenbock, 1957a; DeLuca et al., 1957b), citrate metabolism in bone tissue (DeLuca et al., 1961) and the metabolism of pyruvates in epiphyseal cartilage from rats (Tulpule and Patwardhan, 1954). Vitamin D has also been related to thiamine phosphorylation in normal human blood cells (Raiha and
Forsander, 1952) and to phosphorylation and the electron transport system in palatal mucosa of rats (Mendelsohn, 1962).

**Methods Used to Determine Vitamin D Requirements**

A review of the literature reveals several means by which the vitamin D requirement of a given animal or species has been determined. The methods utilized generally make use of changes in the general health, appearance or growth rate of the animal, or of changes in blood constituents, bone characteristics or of some combination of these factors.

**Growth and Deficiency Symptoms.** Because rickets is not easily produced in the ruminant, several conditions must be strictly observed in vitamin D studies. The experimental animals must be kept away from direct and reflected sunlight, preferably in totally darkened stalls. The feed and bedding must contain no vitamin D activity. Depletion may require a period of several months even under these conditions. The diet must be palatable and nutritionally adequate except for vitamin D because the most florid rickets can apparently be produced only in the presence of a fair rate of growth (Duckworth, Godden and Thomson, 1943; Bechtel et al., 1935).

The rate of growth and appearance of deficiency symptoms have often been used as means of determining the adequacy of a given intake of vitamin D. However, such procedures should be used only under very carefully controlled conditions. Body stores of vitamin D may be of such magnitude in some instances that intakes which appear to be sufficient
for short periods of time may be entirely insufficient over a much longer period.

Retardation or cessation of growth in young animals has been reported by several workers to be one of the first symptoms of a vitamin D deficiency (Rupel, Bohstedt and Hart, 1933; Huffman and Duncan, 1935a; Thomas, 1952). However, other studies (Bechtel et al., 1936a; Bechdel et al., 1938) have indicated that growth is not affected until the appetite becomes retarded during the latter stages of the disease or until the animal has difficulty in moving about. Bechtel et al. (1936a) stated that retardation and cessation of growth were the final stages in the progress of vitamin D deficiency. It thus appears that the use of growth by itself as an indicator of the sufficiency of vitamin D intake is not a valid practice.

The appearance of visible deficiency symptoms was used as an indicator of the sufficiency of vitamin D intake in some of the earlier studies and has been reported to be among the first indications of a deficiency of vitamin D (Bechdel et al., 1938; Thomas and Moore, 1951). However, visible symptoms of vitamin D deficiency may not appear, as has been noted to occur in the absence of growth (Bechtel et al., 1935; Duckworth et al., 1943). The appearance of visible symptoms of a deficiency should be used with some other indicator for the detection of rickets.

Changes in Blood Constituents. Vitamin D is known to be essential for the absorption of calcium from the diet and for the maintenance of normal plasma calcium concentrations. Apparently the first discernible
change brought about in an animal by a deficiency of vitamin D is a decrease in the concentration of calcium in the blood plasma (Bechtel et al., 1935, 1936a; Duckworth et al., 1943; Moore et al., 1948b). Bechtel et al. (1935) stated that changes in the bones were preceded by decreases in the calcium and inorganic phosphorus levels of the blood. Because of the apparent sensitivity involved and the ease with which the determinations can be made, the concentration of calcium in the blood plasma is generally used as an indicator of the onset of vitamin D deficiency symptoms.

Vitamin D is also apparently essential for the absorption of phosphorus and maintenance of normal concentrations of inorganic phosphorus in the blood plasma. However, the initial response of plasma inorganic phosphorus is uncertain since inorganic phosphorus levels have been reported to decrease (Colovos et al., 1951; Thomas, 1952), remain unchanged (Kuhlman and Gallup, 1939) or demonstrate a tendency to rise (Moore et al., 1948b) at the onset of a vitamin D deficiency. Generally, however, the concentration of inorganic phosphorus will decline as the deficiency progresses. It appears that the inorganic phosphorus level of the blood plasma should be used with other indicators to detect the presence of a deficiency of vitamin D.

Because it is closely associated with bone metabolism, the level of alkaline phosphatase activity in the blood plasma is often used as an indicator of the presence of rickets. Several studies have indicated that alkaline phosphatase activity increases greatly in the presence of a vitamin D deficiency (Kuhlman and Gallup, 1939; Moore et al., 1948b;
Colovos et al., 1951; Thomas and Moore, 1951) and that this increase occurs at about the same time that changes in the calcium and phosphorus levels of the blood occur (Thomas and Moore, 1951; Thomas, Okamoto and Moore, 1954). However, the level of alkaline phosphatase activity in the blood can be increased by any factor, such as a bone fracture, which causes the rapid deposition of bone. On the other hand, osteoclastic activity can also result in increased levels of alkaline phosphatase activity in the blood (Guyton, 1961). The presence of high levels of phosphatase activity would not, by itself, indicate whether rapid deposition or absorption of bone salt was occurring. Therefore, alkaline phosphatase activity probably should not be used alone as an indicator of a deficiency of vitamin D.

**Calcium and Phosphorus Balance.** Calcium and phosphorus balance studies have been used to investigate vitamin D requirements. This method is based upon the response of deficient animals to vitamin D as indicated by changes in the retention of dietary calcium and phosphorus. A vitamin D deficiency in dairy calves has been shown to decrease the apparent digestibility of the mineral matter in the ration (Colovos et al., 1951), and deficient calves have been reported to demonstrate negative or very small positive calcium balances (Wallis, Palmer and Gullickson, 1935). Normal calcium and phosphorus balances can only be restored through the administration of sufficient vitamin D (Wallis et al., 1935; Wallis, 1938).

Balance studies are limited somewhat in their application because only small numbers of animals may be used and because great amounts of
labor are involved. They are also generally short term studies and consequently may not be as sensitive as other indicators.

**Bone Mineralization.** Vitamin D is necessary for normal calcification of growing bone and for the maintenance of normal levels of mineral in adult bone. Various tests have been devised to determine the progress or state of bone mineralization. Bone ash (McCann and Barnett, 1922; Huffman and Duncan, 1935a), x-ray photos of bones (McCann and Barnett, 1922; Thomas, 1952) and the line test (McCullum et al., 1922; Thomas et al., 1954) or modifications of these tests have all been used to study bone calcification with success. Vitamin D assay work commonly involves one or more of these indices.

Thomas et al. (1954) reported that values for plasma calcium, serum phosphatase and the width of the ulnar epiphyseal cartilage have approximately the same value for the detection of rickets. A preferred method, according to these authors, would be the observation of two of these indicators at different times and the expression of the values as a rate of change per unit of time. Roentgenograms, the line test and the ash content of the eighth rib also appear to be sensitive indices (Huffman and Duncan, 1935b). The state of calcification of the costochondral junction at the ventral end of the eighth rib appears to be a more sensitive index than the calcification of the humerus, femur, metacarpus or metatarsus to the sufficiency of vitamin D intake in calves (Bechtel et al., 1936a).

Several methods have been used to study the vitamin D requirements of various types and species of animals. The indicators utilized
in these studies range from the appearance of gross deficiency symptoms to minute changes brought about in the calcification of bones. No single indicator appears to be sufficiently reliable and sensitive to warrant its sole use under all conditions as a detector of rickets in vitamin D studies. However, intakes of vitamin D sufficient to provide for optimum rates of gain and to prevent the occurrence of deficiency symptoms should be considered sufficient under the conditions imposed. Other indices may be used in addition if greater sensitivity is desired.

Factors Affecting Vitamin D Requirements

The physiological requirements of the bovine for vitamin D are normally satisfied with vitamin D from two separate sources. One source is the vitamin D provided in the feed while the other is the vitamin D which is synthesized in the skin of the animal when it is exposed to sunlight or other forms of ultraviolet radiation. In the present discussion, therefore, the term "requirement" means "dietary requirement" unless otherwise specified.

The literature concerning factors which may influence the vitamin D requirement of ruminants is somewhat limited in volume. It is quite probable that the lack of a number of serious problems with vitamin D nutrition in practice and the number of difficulties involved in vitamin D assay work have had some influence in this situation.

Breed, Age, Sex, Reproduction and Lactation. Insofar as can be determined, the effect of breed upon the vitamin D requirement of the bovine has not been subjected directly to investigation. However, it
has been observed that light skinned animals appear to be less subject
to rickets than animals having dark skins, apparently because the
lighter skin and overlying hair permits the penetration of a larger
amount of ultraviolet radiation into the underlying tissues (Morrison,
1959). Thus the dietary requirement of dark skinned animals for vitamin
D would be slightly higher than that of light skinned animals kept under
the same conditions provided that a portion, but not all, of the
physiological requirement for vitamin D was met by exposure to ultra­
violet radiation. The physiological requirements, however, may not be
different.

Dairy calves of the Holstein, Brown Swiss and Jersey breeds have
been used in studies to determine vitamin D requirements (Huffman and
Duncan, 1935a; Moore et al., 1948b; Thomas, 1952; Swanson, Carpenter and
Thomas, 1962). Although the effect of breed of the calves was not the
subject of these investigations, no apparent differences in vitamin D
requirements due to breed were noted.

On the basis of the sparse information available, it appears
likely that the physiological requirements of the bovine for vitamin D
are not greatly influenced by the breed of the animal.

The sex of an animal probably has little influence upon its
vitamin D requirement for growth and maintenance. Calves of both sexes
have been used in experiments to determine the vitamin D requirements and
no mention has been made of differences due to the sex of the calves
(Bechtel, 1936b; Duncan and Huffman, 1936; Thomas et al., 1954). More
likely, factors which are associated with the sex of the animal, such as lactation, have a greater effect.

An adequate supply of vitamin D appears to be necessary to enable the bovine to reproduce normally. Wallis (1938, 1946) observed that deficient cows demonstrated few or no estrus periods and gave birth to weak or dead calves. Wallis (1946) stated that the development of the fetus apparently created an additional demand on the vitamin D available to the dam, particularly during the last trimester of pregnancy when the greatest development of the fetus was occurring. However, the extra amount of vitamin D required could not be measured.

It is not likely that the fetus will absorb appreciable amounts of the vitamin, since the administration of relatively large doses of vitamin D to pregnant cows does not significantly affect the concentration of vitamin D in the plasma and liver of newborn calves (Eaton et al., 1947a). The vitamin apparently does not pass through the placental membranes easily. However, vitamin D is found in small quantities in the plasma and liver of newborn calves before they have received colostrum and is normally secreted in the colostrum in appreciable amounts. Both factors would tend to draw upon the vitamin D available to the cow. Thus it appears that the physiological requirements of the dam for vitamin D may be increased slightly by reproduction.

Lactation apparently causes a drain upon the amount of vitamin D available to a cow because of the vitamin secreted in the milk. Butterfat contains a variable amount of vitamin D, the amount
being roughly proportional to the amount of the vitamin available to
the cow (Wallis, 1946). Heavy production results in a heavy drain on
the vitamin D reserves stored in the cow's body. Cows used in experi-
ments at the South Dakota station (Wallis, 1946) were fed diets con-
taining as little vitamin D as possible and kept indoors. Those
producing 60 to 65 lb of milk daily became depleted of their vitamin D
stores in about 4 months. Cows giving 25 to 40 lb usually became
depleted in 6 to 8 months. In one case, a dry cow was kept under
these conditions for 20 months before even mild symptoms of a
vitamin D deficiency became apparent. In other tests, cows fed
19,000 IU of vitamin D daily secreted about 1.5 to 1.75% of the daily
dose of the vitamin in their milk during the first part of the
lactation period. The rate of secretion of vitamin D declined to
about 0.5 to 0.75% of the daily intake toward the end of lactation.
Jerseys produced smaller quantities of milk and butterfat than did
Holsteins, but the concentration of vitamin D in the butterfat of the
milk from Jerseys was considerably higher than that in the butterfat
of the milk from Holsteins.

Hess et al. (1932) reported that 2 to 3% of the dietary
vitamin D is secreted in milk. These workers also stated that the
vitamin D potency of butterfat is inversely proportional to the
percent butterfat in the milk and nearly directly proportional to the
amount of the vitamin fed in the experiment. Other investigations
have also shown that the amount of vitamin D secreted in cow's milk
is directly related to the total amount of the vitamin available to
the cow (Steenbock et al., 1925a; Hart et al., 1930; Wallis and Olson, 1936). However, the cows used in these trials received vitamin D in excess of their daily requirements, and this may have had an influence upon the secretion rate.

The rate of secretion of vitamin D in the milk may approach zero as the amount of the vitamin available in excess of physiological requirements declines. Butterfat from previously depleted cows being fed about 5,000 IU of vitamin D per head daily contained so little vitamin D that it would not initiate healing of rickets in rats when fed at the highest level they would consume (Wallis and Olson, 1936). Butterfat obtained in April from cows kept under normal herd management conditions had a very low content of vitamin D.

Evidence presently available does not strongly support nor invalidate the conclusion that the cow will secrete vitamin D in her milk even though the amount available to her is not sufficient to meet her requirements. It seems likely, however, that the physiological requirement of the bovine would tend to be increased by lactation.

Although it appears that no studies have been conducted to determine directly the effect of age upon the vitamin D requirement of the bovine, some indication of the extent of this relationship could perhaps be obtained by comparing the vitamin D requirements reported for cattle of different ages. If gross differences occur, such a comparison should indicate their presence.

The vitamin D requirement of calves from birth up to about 7 months of age has been reported to be as low as 35 to 45 USP units per
100 lb of body weight (Huffman and Duncan, 1935b) and as high as 300 IU or more per 100 lb of body weight (Bechdel et al., 1937, 1938; Moore et al., 1948b). Most reports have indicated that requirements for cattle in this age group fall in the range from 150 to 300 IU per 100 lb of body weight. On the other hand, cattle weighing about 700 lb have maintained positive calcium and phosphorus balances with vitamin D intakes of about 450 IU per head daily (Wallis et al., 1935).

In later work (Wallis, 1946) it was observed that cows fed 1,000 or 2,000 IU of vitamin D daily did not recover from rickets while the administration of 3,000 IU daily allowed another cow to recover her health and to produce a fair amount of milk. The latter level of administration appeared to be close to the minimum physiological requirement.

There appears to be large variations between the reported requirements of calves used in the different experiments cited above. These variations may be due to experimental error or to actual differences resulting from other factors. That such variation may not be peculiar to the bovine is indicated by reports that changes in the sensitivity of rats used in vitamin D assay work have given rise to an apparent fluctuation of 300% or more in the apparent potency of vitamin D standard preparations (Bourdillon, Bruce and Webster, 1932; Tourtellotte and Bacon, 1935). Huffman and Duncan (1935a) also stated that the results of their experiments with calves indicated that rather large individual variation in vitamin D requirements existed. It would appear, however, that there is not sufficient evidence to
conclude that age, other than through possible effects on growth and body weight, has an effect on the vitamin D requirement of the bovine.

**Dietary Protein.** There is evidence which indicates that vitamin D is closely associated with the formation of protein in the lining of the intestine (Wasserman and Taylor, 1966) and that vitamin D is transported in the blood in combination with a protein or protein-like material (Chen and Lane, 1965). An active metabolite of vitamin D has been reported to be bound to a protein carrier in pig blood (Blunt, DeLuca and Schnoes, 1968). Other investigations have shown that vitamin D is necessary for normal, efficient protein metabolism (Colovos et al., 1951; Steenbock and Horting, 1955; Harrill and Girfford, 1965). These reports indicate that a definite relationship exists between vitamin D and protein metabolism.

Other reports have indicated that specific kinds of protein may have some effect on the utilization of vitamin D in poultry. Thompson (1968) studied the rachitogenic effects of isolated C-1 soy protein added to the diet of turkey pouls. The protein constituted 42 or 44% of the diet and caused the appearance of ricketsic symptoms in pouls even when vitamin D3 was administered at four times the N.R.C. (1960) recommended levels. The rachitogenic effect of isolated soy protein has also been demonstrated by others (Carlson, McGinnis and Jensen, 1964a; Carlson et al., 1964b; Jensen and Mraz, 1966). Jensen and Mraz (1966) have presented evidence that this effect is due to an influence of the protein upon the absorption of calcium and phosphorus.
Baby pigs fed diets containing isolated soy protein excreted excessive amounts of fecal calcium, phosphorus and magnesium (Miller et al., 1965a). These effects were not entirely overcome by the administration of vitamin D at levels up to two and one-half times the amounts observed to be sufficient in diets containing casein as the protein source. Jensen and Mraz (1966) stated that the rachitogenic effect of isolated soybean protein is probably a general phenomenon among monogastric animals.

A deficiency of vitamin D in calves has been reported to reduce the retention and utilization of absorbed dietary protein (Colovos et al., 1951) and to produce muscular atrophy (Bechtel et al., 1936a). It is thus apparent that protein metabolism in the ruminant can be affected by a deficiency of vitamin D. On the other hand, the extent to which a deficiency of protein will influence vitamin D requirements cannot be determined from the literature presently available.

Minerals. Since the demonstration by McCollum et al. (1922) that cod liver oil contained two vitamins, one of which cured xerophthalmia and one of which cured rickets, many investigations have been conducted concerning vitamin D and mineral interrelationships.

The rat appears to be somewhat unique in that it can maintain a satisfactory calcium and phosphorus balance in the absence of a source of vitamin D provided that the levels of calcium and phosphorus in the ration are adequate and that the ratio of calcium to phosphorus is between 1:1 and 2:1. Healing of rachitic bones will proceed under these conditions if the weight of the rat is maintained or decreased
(West and Todd, 1961). On the other hand, rickets can be induced in the rat if a diet containing an upset calcium to phosphorus ratio is fed, even in the presence of vitamin D. Rickets can be induced with a diet containing a high calcium to phosphorus ratio, a very low calcium to phosphorus ratio, or with a normal calcium to phosphorus ratio when the levels of these minerals are low (Brown et al., 1932; Shohl and Wolbach, 1936; Feaster et al., 1953). Normal calcium concentrations can be maintained in the blood serum of rats when diets containing only 0.018% calcium and 0.016% phosphorus are fed (Steenbock and Herting, 1955). However, vitamin D is required in order to maintain plasma inorganic phosphorus levels and a normal rate of growth under these conditions.

Ruminants, however, appear to require vitamin D regardless of the balance or level of calcium and phosphorus in the ration. Ricketastic conditions have been observed numerous times in cattle fed diets containing adequate amounts and optimum ratios of the minerals (Wallis et al., 1935; Bechtel et al., 1935; Bechdel et al., 1937, 1938).

Increasing or adjusting the ratios of calcium and phosphorus in the diet does not appear to have much effect on the vitamin D requirement of the bovine. Using twice the normal levels of calcium and phosphorus, and in a ratio of two parts of calcium to one of phosphorus, will not prevent or alleviate ricketastic symptoms in milk cows (Wallis, 1946). Increasing the amount of calcium in the diet by the addition of about 2% ground limestone had a definite but limited
alleviating action on the development of rickets in calves (Thomas, 1952) and adding calcium carbonate to the diet of dairy calves promoted only a temporary increase in blood plasma calcium concentrations (Wallis et al., 1935). Calcium intakes of 40 to 50 g per head daily would not promote adequate mineral retention in calves in the latter study, and adding phosphorus to a ration low in phosphorus but adequate in calcium increased the severity of rickets. In other studies (Theiler, 1934), calves fed a ration containing a low level of phosphorus developed rickets despite the presence of a large supply of vitamin D in the form of radiant energy.

Vitamin D has been noted to have an influence upon the utilization of zinc in rats (Becker and Hoekstra, 1966) and pigs (Whiting and Bazeau, 1958) and magnesium in rats (Heintzer and Steenbock, 1955) and pigs (Miller et al., 1964, 1965b). In other trials, the addition of magnesium carbonate to the diet of rickettic calves appeared to cause a temporary remission of rickettic symptoms (Huffman and Duncan, 1935c). In a second experiment, magnesium oxide or magnesium carbonate appeared to have a vitamin D sparing action but did not prevent the occurrence of rickets. Calcium and phosphorus utilization appeared to be increased temporarily by the addition of magnesium to the ration.

Vitamin D appears to be required by the ruminant regardless of the mineral level of the diet or of the ratios which may exist between those minerals. Imbalances existing between calcium and phosphorus in the diet can be tolerated reasonably well provided that adequate
vitamin D is available. There is, however, insufficient evidence to indicate that the vitamin D requirement of the ruminant can be greatly influenced either by the levels of or ratios between the various mineral elements in the diet.

Sunshine and Ultraviolet Radiation. Early investigations with rats (McCann and Barnett, 1922; Steenbock and Nelson, 1923; Steenbock and Black, 1924) and with a goat (Steenbock et al., 1925b) demonstrated that the vitamin D requirement of these animals could be satisfied by administration of sufficient cod liver oil or by exposing them to sunlight or ultraviolet radiation. At about the same time, other investigations (Hess and Weinstock, 1924; Steenbock and Black, 1924; Steenbock et al., 1925a; Hess and Weinstock, 1925a) demonstrated that antirachitic activity could be imparted to various inert substances by exposing them to ultraviolet radiation. Hess, Weinstock and Helman (1925) reported that phytosterol, lanolin and cholesterol each possessed antirachitic activity when irradiated. Cholesterol was found to be activated when irradiated at wavelengths which had been found to be effective in preventing rickets by direct irradiation of animals (Hess and Weinstock, 1925b).

Hess et al. (1925) took note of the large amount of cholesterol in animal skin and suggested that the activation of this cholesterol might be the mechanism through which solar radiation prevented rickets in animals. Subsequent work (Hess and Weinstock, 1925b) demonstrated that irradiation of human skin or calf skin imparted antirachitic activity to the skin.
Investigations have since shown that the vitamin D requirement of the bovine can be satisfied by exposure of the animal to sunlight or to ultraviolet light. Exposure of calves to ultraviolet light for only 15 minutes per day is sufficient to prevent or cure rickets in calves fed a rachitogenic diet (Bechdel, Landsburg and Hill, 1933). Rickets can only be produced in calves when the calves are kept from exposure to direct or reflected sunshine (Kuhlman and Gallup, 1939).

Several factors have been shown to have an influence upon the effectiveness of sunshine in promoting the formation of vitamin D in animal skin. Basically, the extent to which any given factor will influence this process depends upon the ability of the factor to reduce the length of time of exposure or the intensity of sunshine reaching the skin during the period of the exposure.

Seasonal variation in the intensity of sunlight apparently causes a related variation in the amount of vitamin D formed in the skin and subsequently in the calcium and inorganic phosphorus concentrations in the blood of nonricketic calves (Duncan and Huffman, 1936). In these studies calcium showed a tendency to drop during January and then to increase to maximum concentrations in April while phosphorus and magnesium declined during January and February and reached maximum concentrations during July and August. In other studies (Wallis, 1939), sunshine in late August, September or mid-November cured rickets in cows but was not as effective as sunshine during summer or late spring. Later investigations (Eaton et al., 1947a) demonstrated that the level of vitamin D in the blood plasma
of cows may be two to three times higher in the fall than in the spring and that colostrum from cows is appreciably richer in vitamin D activity in the fall than in the spring.

The thickness of the hair or wool coat, as well as its color, can also apparently influence the amount of vitamin D formed in the skin. Quarterman, Dalgarno and Adam (1964a) reported that shearing resulted in an increase in the amount of vitamin D in the blood plasma of sheep.

The amount of sunshine to which an animal is exposed can greatly influence the amount of dietary vitamin D required by the animal. As little as 15 minutes of exposure to direct sunlight can be sufficient to provide the entire amount of the vitamin required, thus obviating the need for dietary vitamin D. Additional sunshine will promote storage of vitamin D in the body.

Factors such as the intensity of the sunshine, shade from trees, sheds or cloud cover and the amount of hair or wool covering the skin, as well as the color of the skin and hair, influence the effectiveness of sunshine in providing vitamin D.

Carotene and Vitamin A. It was recognized at an early date that the amount of carotene present in forages was generally related to the green color of the material. Similarly, the vitamin D activity of a forage was generally thought to be directly related to the length of time that the forage had been cured with direct exposure to sunlight. However, the theory was not always borne out by results of investigations as can be demonstrated by a comparison of the work of
Steenobck *et al.* (1925b), Hart *et al.* (1929), Newlander (1948), Moore *et al.* (1948b), Newlander, Jones and Foote (1950), Newlander and Riddell (1952) and Wallis, Kennedy and Fishman (1958). Wallis, Smith and Fishman (1949) and Wallis *et al.* (1958) stated that the results of their studies emphasized that wide and unpredictable variations occurred in the vitamin D content of roughages. Factors other than exposure to sunlight appeared to have some influence upon the net biological vitamin D activity of a given sample of forage.

Ewer (1950) reported problems with rickets in lambs. Common green feeds appeared to be the causative factor even though chemical analysis of the feeds indicated that sufficient calcium and phosphorus was present and the ratio of these minerals in the feeds was satisfactory. Green oat hay produced rickets in lambs and guinea pigs, but the effect could be overcome with the simultaneous administration of vitamin D. The factor responsible for the ricketic symptoms was not identified.

In subsequent work, the rachitogenic factor of green oats was found to be soluble in chloroform (Grant, 1951) and in ether and petroleum ether (Weits, 1952; Grant, 1953). The dried extract could reduce the effect of a given dose of vitamin D from 20 to 50% when given in sufficient quantities (Weits, 1952).

The rachitogenic material was identified as carotene by Grant (1953), who isolated the factor by chromatographic separation of a petroleum ether extract of dried green oat hay. Increasing the dose of carotene given to rats from zero to 100 mg per rat per day
decreased the ash content of the dry, fat-free femur about 5%. Crystalline vitamin A was also found to exert a similar effect on bone ash. In other experiments, 50 IU of vitamin A demonstrated about the same deleterious effects on bone calcification as 33 ug of carotene when fed to albino rats (Grant and O'Hara, 1957). The main effect in these and in other studies (Weits, 1952) seemed to be a depression of the calcifying process of the bone, rather than an inhibition of mineral absorption. It was suggested that the direct effect was a depression of phosphorus utilization (Grant and O'Hara, 1957).

Xanthophylls, unlike carotene and vitamin A, apparently do not exhibit a rachitogenic influence (Weits, 1954). Processes such as ensiling, which destroy some carotene in a feedstuff, may destroy the rachitogenic activity of the feedstuff, resulting in an apparent increase in the antirachitic activity (Keener, 1954).

Different animal species are apparently not equally influenced by the rachitogenic effect of carotene and vitamin A. Rats appear to be less sensitive than sheep (Weits, 1954), whereas guinea pigs appear to be affected to about the same extent as sheep (Ewer, 1950). The vitamin D requirements of the pig do not appear to be increased when carotene instead of vitamin A is used to furnish the required amounts of vitamin A activity in the ration (Hendricks et al., 1967).

The extent to which carotene or vitamin A will interfere with the activity of functions of vitamin D in the bovine cannot be determined from the literature presently available. Even though species differences apparently exist, the fact that sheep, which are
also ruminants, are highly susceptible to the rachitogenic effect of carotene and vitamin A makes it imperative that more work be done to clarify the extent of this problem.

**Vitamin D Requirements**

As with many other factors relating to vitamin D in the nutrition of the ruminant, there is relatively little information concerning the vitamin D requirement of the bovine. Most of the investigational work concerned with defining these requirements has been conducted with dairy calves and thus may not be strictly applicable to cattle of beef breeds. In addition, very little work has been done with mature cattle. It is generally assumed, however, that under practical conditions such cattle will be provided sufficient vitamin D from exposure to sunlight or from dietary roughages.

There are three principal means by which the vitamin D requirement of cattle may be met. The animal may be exposed to sunlight, the diet may furnish adequate vitamin D from plant sources or vitamin D supplements may be added to the diet or administered by some other means.

Exposure to sunlight is quite effective in causing the formation of vitamin D. The length of the exposure period does not need to be great (Steenbock *et al.*, 1925a; Bechdel *et al.*, 1933). As little as 15 to 30 minutes exposure to sunlight each day appears to be sufficient, depending to some extent upon the season, the type of animal involved and other factors.
Roughages such as hay or silage can be used to meet the vitamin D requirements of cattle. The amount of vitamin D in a given roughage varies considerably depending upon the type of roughage, its condition and the method used in harvesting it (Newlander, 1948; Newlander and Riddell, 1952; Wallis et al., 1958), but even small quantities can provide the entire amount of vitamin D required by calves (Bechtel et al., 1936b; Huffman and Duncan, 1935a). Cereal grains, on the other hand, contain little or no vitamin D activity (Morrison, 1959).

Supplementary sources of vitamin D, such as cod liver oil, irradiated yeast, irradiated ergosterol and activated animal sterols, are available for use. However, the administration of such supplements generally requires extra time and expense.

Both vitamin D$_2$ and vitamin D$_3$ are commonly used in nutrition. However, the species of animal involved may determine the form of the vitamin to be used. Chickens commonly require from 30 to 50 times as many units of D$_2$ as of D$_3$ to satisfy their requirements (Remp and Marshall, 1938) and the D$_3$ form is thus preferred in poultry nutrition. On the other hand, the results of experiments in which both vitamin D$_2$ and vitamin D$_3$ have been used to meet the requirements of the bovine have shown that the two forms appear to be equally effective for cattle (Bechdel et al., 1937, 1938; Wallis, 1946). In addition, Morrison (1959) states that vitamin D$_2$ and D$_3$ have approximately the same value for all four-footed animals and for human beings, and Maynard and Loosli (1962) state that the two forms have the same antirachitic value for the rat, dog, pig, calf and man.
It thus appears that the vitamin D requirements of the bovine can be met, when required, by supplementing the diet with either vitamin D$_2$ or vitamin D$_3$ on a unit for unit basis. For this reason, feeding standards list the vitamin D requirement of the bovine simply in terms of the units of activity needed without referring to the form of the vitamin used to provide the activity. One unit of vitamin D activity, either the United States Pharmacopeia or the international unit, is defined as the activity of 0.025 µg of vitamin D$_3$ contained in the United States Pharmacopeia reference standard.

Maintenance, Growth and Reproduction. Newborn calves were fed a basal rachitogenic ration supplemented with skim milk or with whole milk to supply different levels of vitamin D in a study to determine the vitamin D requirements of calves (Huffman and Duncan, 1935b). Roentgenograms, histological studies and the ash content of the eighth rib were used as indicators in the study. Results indicated that 35 to 45 USP units of vitamin D from milk per 100 lb of body weight were sufficient to meet the vitamin D requirements of these calves from birth to 5 months of age.

Calves about 10 days of age were used in other studies (Thomas and Moore, 1951). Alfalfa hay was used as the source of vitamin D in these studies and was added to a rachitogenic ration to provide different vitamin D intakes. The calcium, inorganic phosphorus and alkaline phosphatase levels of the blood, x-ray photos of bone, ash content of bone and postmortem examination of bones were used as indices in this 7-month trial. Calves fed the basal ration
demonstrated ricketic symptoms in 2 to 3 months. Calves fed 144 IU of vitamin D per 100 lb of body weight daily appeared to be getting sufficient vitamin D with very few exceptions. None of the calves fed 218 IU or more per 100 lb of body weight showed symptoms of avitaminosis D. In later studies (Thomas, 1952) in which a similar experimental design was used, calves fed only 150 IU of vitamin D per 100 lb of body weight daily demonstrated no signs of rickets. Earlier studies were conducted by these investigators (Moore et al., 1948b), but the lowest vitamin D intake was 256 IU per 100 lb of body weight daily. The authors in this instance concluded that the recommended levels of 300 IU per 100 lb of body weight daily were apparently sufficient for normal growth and maintenance of health.

Holstein calves were housed indoors and fed a basal rachitogenic ration supplemented with activated yeast or cod liver oil to furnish from 100 to 500 IU of vitamin D activity per 100 lb of body weight daily (Bechdel et al., 1937). Rickets were observed after 4 to 5 months in some calves. Based upon blood calcium and inorganic phosphorus levels and x-ray photos of the ulna, the authors concluded that the average minimum vitamin D requirement for calves from birth to 6 months of age is 300 IU per 100 lb of body weight daily. Similar results were obtained in other studies (Bechdel et al., 1938).

Slightly higher levels of vitamin D were recommended by Guilbert and Loosli (1951). They recommended that cattle should be fed 400 IU of vitamin D per 100 lb of body weight from birth until
they reached 30% of their mature weight. These levels were listed as suggested allowances rather than minimum requirements.

Cattle weighing about 700 lb were used in experiments conducted by Wallis et al. (1935). Prairie hay was included in the basal ration and provided a vitamin D intake of about 135 Steenbock units (445 IU) per head daily. This amount of vitamin D appeared to be sufficient to protect the cattle from rickets.

Yearling cattle were fed corn silage as a source of vitamin D in an experiment conducted by Bechtel et al. (1936b). It was reported that a vitamin D intake of about 123 IU per 100 lb of body weight daily was sufficient to prevent symptoms of rickets in these cattle and to provide for normal reproduction.

Wallis (1946) reported that intakes of 1,000 or 2,000 IU of vitamin D per head daily were not sufficient to alleviate symptoms of a vitamin D deficiency in dairy cows, but intakes of 3,000 IU per day in one cow resulted in the disappearance of gross deficiency symptoms. Another cow appeared to produce slightly better when given 6,000 IU of the vitamin per day. It was concluded that 5,000 to 6,000 IU of vitamin D per head daily appeared to be the minimum amount required to maintain a reasonable degree of health, milk producing ability and reproductive efficiency in dairy cows.

The N.R.C. (1963) states that the vitamin D requirements of beef cattle are estimated at about 300 IU per 100 lb of body weight daily. In view of the reports in the literature cited above, this
level is probably fully adequate for growth and maintenance and may be sufficient to provide for normal reproduction as well.

Storage. In contrast to vitamin A, the main site of storage of vitamin D appears to be the blood rather than the liver in the pig (Quarterman et al., 1964b) and in the sheep (Quarterman et al., 1964a). The data in other reports (Guerrant et al., 1938; Eaton et al., 1947a, 1947b) indicate that the total amount of vitamin D in the blood of cattle is greater than the respective value for vitamin D in liver tissue.

Vitamin D, like vitamin A, appears to be stored during periods when intake exceeds requirements and utilized as necessary to meet requirements when intake is insufficient. Stores of sufficient magnitude to meet requirements for considerable periods can apparently be accumulated. Several experiments have shown that appreciable lengths of time—ranging up to about 20 months for mature, non-lactating cows (Wallis, 1946)—may be required for depletion. It should be noted that newborn animals are nearly deficient of vitamin D and depend upon the relatively high content of vitamin D in the colostrum for an initial supply of the vitamin (N.R.C., 1966).

Concentrations of vitamin D in the blood may not be exceptionally high under usual conditions. The blood of normal cows may contain 5 to 6 IU of vitamin D per cubic centimeter during the summer, whereas normal concentrations during the winter may range between 1 and 4 units (Wallis, 1946). In these studies, levels of 0.20 to 0.25 IU/cc were normally associated with borderline vitamin D deficiency symptoms.
while mild symptoms of a deficiency were exhibited when the levels dropped to 0.15 to 0.20 IU/cc.

Attempting to increase the blood plasma and liver tissue vitamin D concentrations in calves by supplementation of the dam prior to parturition is probably not the most effective means of achieving this goal. Supplementation of cows with vitamin D at the rate of 100,000 IU per head daily for 8 weeks prepardum did not result in a statistically significant improvement in the concentration of vitamin D in the plasma or liver tissue of calves, although blood plasma concentration of the vitamin showed an appreciable response to the supplementation (Eaton et al., 1947a). On the other hand, administering 50,000 IU of vitamin D to calves with the first feeding of colostrum resulted in considerable advantage in plasma concentration of vitamin D in the supplemented calves at 42 days of age (Eaton et al., 1947b). Calves not fed the supplement had about 55 IU of vitamin D per 100 ml of plasma, whereas the level was about 90 IU per 100 ml in the supplemented calves. However, the authors concluded that the total storage of the administered dose appeared to be small.

The efficiency of storage of a given amount of vitamin D may be small in older cattle also. Wallis et al. (1935) depleted 700 lb yearlings of vitamin D stores and then administered 15,000 Steenbock units (about 50,000 IU) of vitamin D per head daily for a period of 2.5 months. Sufficient amounts of the vitamin were stored during the supplementation period to protect the cattle against rickets for an additional 3.5 months following the end of the supplementation period.
Apparent efficiency of utilization of the total amount of vitamin D administered appears to be rather low.

Evidence currently available does not permit definite conclusions to be drawn concerning many of the aspects of vitamin D storage. Much additional work needs to be done with respect to the effect of various different factors on the storage and utilization of vitamin D by the bovine.
METHODS AND PROCEDURE

The objectives of this experiment were to determine whether supplemental vitamin A would be of benefit when added to rations containing various amounts of corn silage and furnishing different amounts of carotene and to determine the need for supplemental vitamin D under conditions of the experiment.

One hundred sixty Angus and Angus x Hereford crossbred steers were purchased in late January for use in the experiment. After arrival, and until the experiment was started, they were fed a daily ration consisting of about 4 lb of alfalfa-brome hay, a full feed of corn silage and 2 lb of protein supplement. For the first 4 weeks, the supplement was formulated to furnish 10,000 IU of vitamin A and 350 mg of chlortetracycline per head daily.

Several of the calves were suffering from ringworm and pinkeye when they arrived. Treatment for these conditions was begun at once and most of the cases had cleared up by the time the experiment was started. One steer died of pneumonia during the pre-experimental period and thus only 159 were started on experiment.

The experimental design used was a 2 x 2 x 4 factorial in which two levels of vitamin A and two levels of vitamin D were fed with each of four levels of corn silage. Vitamin treatments consisted of control, 20,000 IU of vitamin A, 10,000 IU of vitamin D and a combination of 20,000 IU of vitamin A and 10,000 IU of vitamin D per head daily. The vitamin treatments were administered in a soybean meal-base supplement which contained 5 mg of diethylstilbestrol per
pound and was fed at the rate of 2 lb per head daily. Levels of corn silage with which each of the vitamin treatments were administered were 5 lb, 15 lb, 30 lb and a full feed per head daily. Coarsely rolled corn grain was full-fed with each of the three restricted levels of silage.

The corn silage used in this experiment was made from crops raised in two different years. The silage fed during the first 178 days of the experiment was made from the corn crop of the previous year. The corn was well-eared but had been subjected to a killing frost prior to ensiling. It contained about 0.36 mg of carotene per pound as fed on a 62.7% moisture basis. The silage fed during the remainder of the experiment was from the current corn crop and contained about 2.94 mg of carotene per pound as fed on a 65.1% moisture basis.

The corn grain fed averaged about 0.25 mg of carotene per pound with a moisture content of 13.2%. No analysis was made to determine the carotene content of the protein supplement since solvent extracted soybean meal was the primary ingredient.

The experiment was initiated in mid-March. Initial filled weights were taken for use in allotting the cattle and to follow periodic performance during the experiment. Initial shrunk weights were taken the following day after the animals had been held overnight without feed and water. The cattle were then allotted on the basis of breed and filled weight into 16 pens of 10 head each with the exception of one pen which contained only 9 head.
The cattle in all pens were started on trial with 30 lb of corn silage and 2 lb of the appropriate protein supplement per head daily. Corn silage was increased to appetite for those cattle which were to receive the full feed level during the trial. It was decreased 2 lb per head daily to the appropriate level for cattle which were fed the 5 and 15 lb levels. Corn grain fed to the cattle in each lot receiving the restricted levels of corn silage was started at 0.5 lb per head daily and increased by 0.5 lb per head daily until only small amounts of the grain were left in the bunks at the time of the next feeding with the silage offered being consumed. Thereafter, the grain was offered in amounts to maintain feed before the cattle at all times. All rations were fed once daily in fence-line bunks.

Trace-mineralized salt and dicalcium phosphate were fed free choice. Water was supplied to the cattle in each of the unsheltered, paved lots by a circulating watering system.

Daily records were made of the feed offered each lot of steers. When a steer was lost or removed from a lot, the total feed consumption for the lot was corrected on the basis of the gain made by that animal using the formula suggested by Garrett, Meyer and Lofgreen (1959).

Individual filled weights were obtained at 28-day intervals during the experiment, at 238 days immediately prior to the marketing of the cattle fed the 5 lb level of corn silage and immediately prior to the marketing of the cattle fed the other respective levels of corn silage.
Blood samples were taken for carotene and vitamin A analyses 5 days prior to the beginning of the experiment, at 117 days after the beginning of the experiment, at 178 days immediately prior to the change of silage, at 238 days immediately prior to the marketing of the cattle fed the 5 lb level of corn silage and as the cattle fed the other levels of silage were marketed. The samples, about 40 ml in volume, were obtained by jugular venipuncture and were collected in individual tubes in which 0.5 ml of a saturated solution of sodium citrate had been placed to prevent coagulation. Immediately after the samples were drawn they were placed in an ice chest and cooled. The samples were then transported to the laboratory, centrifuged and the plasma was removed and frozen until analyzed. Blood plasma carotene and vitamin A concentrations were determined on the individual samples in accordance with the method described by Koehn and Sherman (1940).

Samples of the corn grain and corn silage used in this experiment were collected at weekly intervals. Samples were composited every 4 weeks and analyzed for moisture, protein and carotene (A.O.A.C., 1960).

The cattle fed a given level of silage were marketed as a group when the average weight of those fed the vitamin A and vitamin A plus D supplements was about 1075 lb. The experimental period for each group of cattle was terminated by removing the supplement containing diethylstilbestrol 3 days prior to marketing and substituting an equal amount of soybean meal as the protein supplement for the remainder of the period. The final filled weights and final blood samples were
taken the day prior to marketing. Feed and water were left before the
cattle overnight and they were shipped to market the next morning.
Individual weights taken at market after the cattle had been shipped
about 75 miles served as final shrunk weights.

Liver samples were taken at the time of slaughter for carotene
and vitamin A analyses. The samples were taken from the visceral
surface and caudate lobe of each liver and consisted of approximately
0.75 lb of tissue. Immediately after the samples were removed from
each liver they were placed in individual plastic bags, labeled and
cooled with dry ice. The samples were then taken to the laboratory
and frozen until such time as they could be analyzed. Liver carotene
and vitamin A analyses were done in accordance with the method described
by Johnson and Baumann (1947b).

Carcass data were taken from the scribed carcasses 48 hours after
the cattle were slaughtered. The data included hot carcass weight,
carcass grade, marbling and carcass conformation. Loin eye area and
fat thickness over the loin eye were determined from acetate tracings
made on the left side of the ribbed carcasses at the twelfth rib.
Carcass grading was done by a U.S.D.A. grader and grades were recorded
to the nearest one-third of a grade.

Because of losses which occurred throughout the duration of the
trial, several of the lots contained less than the original number at
time of marketing. Since no pattern could be established between
treatment groups for these losses, the number of cattle in each lot was
reduced on a random basis to 8 head, the least number of cattle in any
one lot at the termination of the trial. Feed data for each lot were
reduced by an amount assuming average intakes for each of the steers
removed in this process. Thus only the data for 8 head in each lot
were used in the statistical analysis and are presented in this thesis.

The data were analyzed using the analysis of variance for
factorial design and Duncan's new multiple range test, each as
described by Steel and Torrie (1960).
RESULTS AND DISCUSSION

During this experiment, differences in feedlot performance were obtained which were attributable to the different silages fed. The old crop silage fed during the first 178 days of the experiment contained very little carotene. During this time, vitamin A deficiency symptoms were observed in some of the cattle which were not supplemented with vitamin A. The new crop silage which contained carotene in appreciably larger amounts and was fed after 178 days corrected the vitamin A deficiency symptoms and caused distinct changes in the performance of the cattle. Because these differences were attributable to the different silages rather than to the vitamin treatments, feedlot performance data are presented in two parts. The first part, Phase I, includes data obtained during the first 170 days of the experiment and reflects performance of the cattle to the end of the weigh period nearest the date on which the new crop silage was first fed. The second part, Phase II, includes data obtained after the first 170 days of the experiment. The data obtained during each of the phases are presented and discussed on the basis of the various vitamin treatments within a given level of silage. Comparisons were not made on the basis of roughage level in the diet since results were influenced by vitamin A supplementation and such comparisons were not the primary objective of this thesis.
Phase I

Carotene Intake and the Occurrence of Vitamin A Deficiency Symptoms. Based on the average carotene content of the corn grain and the old crop corn silage fed during this phase of the experiment and the amounts of feed consumed by cattle in the different silage level groups, the average daily total carotene intakes of cattle fed the 5 lb, 15 lb, 30 lb and full feed level of corn silage were about 5.5, 8.1, 11.6 and 13.2 mg per head daily, respectively. Based upon the average weight of the cattle in each of these groups for this period, the daily carotene intake averaged about 0.8, 1.2, 1.7 and 2.0 mg per 100 lb of body weight, respectively.

Several investigators have shown that carotene intakes of slightly less than 1.7 mg/100 lb of body weight are sufficient for growth (Guilbert and Hart, 1935; Guilbert et al., 1937; Moore, 1939b). However, others (Boyer et al., 1942; Keener et al., 1942; Dehoriy et al., 1960) have demonstrated that levels of intake up to 3 mg/100 lb of body weight daily may be insufficient. Observations made during this phase of the experiment tend to support the latter reports.

Vitamin A deficiency symptoms first became evident about 143 days after the experiment was initiated in cattle fed 5 lb of corn silage with vitamin D supplement. The first gross symptom observed was watering of the eyes in one steer. About 21 days later, three of the steers in this lot were showing rather severe vitamin A deficiency symptoms including edematous legs, watering eyes, elevated body temperature and, in one steer, scours. Symptoms rapidly became even
more severe within the next week and other steers in this lot began to show mild deficiency symptoms. To correct this condition, on the 171st day of the experiment all of the steers in this lot were given a bolus containing 100,000 IU of vitamin A and one steer, showing especially severe symptoms, was given 500,000 IU at this time.

Two cattle in the lot fed 15 lb of silage with control supplement showed evidence of deficiency symptoms about 175 days after the experiment started. One, showing only mild symptoms, was given a bolus containing 100,000 IU of vitamin A and the other, because of the severity of his symptoms, was given an intramuscular injection of 500,000 IU of vitamin A. None of the other cattle in this lot were given vitamin A.

Blood Plasma Carotene and Vitamin A Concentrations. The concentrations of carotene and vitamin A in the blood plasma of the cattle receiving the various vitamin and silage treatments are shown in table 1. The over-all initial concentrations of blood plasma carotene and vitamin A, which are not shown in table 1, averaged about 229 and 37 μg/100 ml of plasma, respectively.

Blood samples were obtained from all the cattle 117 days after the experiment started. At this time, blood plasma vitamin A in the cattle not supplemented with vitamin A had declined considerably from the initial values. Carotene values had declined considerably in all groups. Cattle fed a full feed or 30 lb of corn silage with no vitamin A supplement still had plasma vitamin A values slightly above 19 μg/100 ml. These levels would be considered adequate to prevent
<table>
<thead>
<tr>
<th>Treatment</th>
<th>117 days Carotene</th>
<th>117 days Vit. A</th>
<th>178 days Carotene</th>
<th>178 days Vit. A</th>
<th>238 days Carotene</th>
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<sup>a</sup> Table 1. Blood Plasma Carotene and Vitamin A (µg/100 ml)
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<th>178 days</th>
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**a** All statistical comparisons are made within dates and within silage levels.

**b** Significantly different (P < .05) from concentration obtained with no vitamin A.

**c** Significantly different (P < .01) from concentration obtained with no vitamin A.

**d** Significantly different (P < .05) from concentration obtained with no vitamin D.
the occurrence of vitamin A deficiency symptoms but are within the 15 to 25 μg/100 ml range considered by Kohlmeier and Burroughs (1964) to be associated with variable performance in finishing cattle. Other workers have reported apparent vitamin A deficiency symptoms in the presence of plasma vitamin A concentrations as high as 26 μg/100 ml (Smith et al., 1961; Jordan et al., 1963). However, no gross deficiency symptoms were observed in cattle fed a full feed or 30 lb of corn silage in the present study.

With each of these levels of corn silage, the administration of vitamin A supplement resulted in significantly higher (P < .01) concentrations of plasma vitamin A and significantly lower (P < .05) concentrations of plasma carotene.

Cattle fed the 5 lb and 15 lb levels of corn silage with no vitamin A supplement had average plasma vitamin A concentrations of about 13 and 14 μg/100 ml, respectively, after 117 days on experiment. Values of this magnitude are considered to indicate inadequate vitamin A nutrition in cattle of the size in this experiment (Eaton et al., 1961; Madsen et al., 1947). Mild deficiency symptoms did not become apparent until about 26 days after the 117-day samples were obtained.

The administration of vitamin A with 5 lb and 15 lb of the low carotene corn silage resulted in significantly higher (P < .01) concentrations of plasma vitamin A at 117 days. However, with these levels of silage, it did not have the significant effect upon plasma carotene concentration that was obtained with the two highest levels.
The administration of vitamin D resulted in significantly higher (P < .05) plasma carotene concentrations at the 117-day sampling with the 5 lb level of corn silage. Plasma vitamin A level was significantly lower (P < .05) at this time for the full-fed silage group supplemented with the vitamin.

Only the cattle fed no vitamin A supplement with the various levels of silage were bled on the 178th day of the experiment. By this time, plasma vitamin A concentrations in cattle from all the sampled groups had declined from values observed on the 117th day and were all well within the range considered to indicate the presence of vitamin A deficiency. However, visible symptoms of vitamin A deficiency had been observed only in cattle fed 5 lb of corn silage with vitamin D supplement and in cattle fed 15 lb of silage with the control supplement. Deficiency symptoms probably would have occurred in the cattle in all lots not supplemented with vitamin A had feeding of the old crop silage continued.

The supplemental vitamin A administered on the 171st day of the experiment to cattle in the lot fed 5 lb of silage with vitamin D supplement appears to have had very little influence on the blood plasma vitamin A concentrations observed in these cattle on the 178th day of the experiment.

Weight Gains. Average daily gains, feed consumption and feed efficiency for Phase I are shown in table 2. Cattle offered a full feed of corn silage gained about 2 lb per head daily. A small, but statistically insignificant, improvement in rate of gain was achieved
# TABLE 2. PHASE I INITIAL AND FINAL WEIGHTS, AVERAGE DAILY GAINS, AVERAGE DAILY RATIONS AND FEED EFFICIENCY

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial filled weight (lb)</th>
<th>Filled wt. 170 days (lb)</th>
<th>Filled avg da. gain (lb)</th>
<th>Avg daily ration (lb)</th>
<th>Feed efficiency (Feed/100 lb gain, lb)</th>
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<tbody>
<tr>
<td></td>
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<td></td>
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<td>Corn - silage</td>
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<tr>
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<td></td>
<td>Corn - silage</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Corn - silage</td>
<td>Total</td>
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<td>1.9</td>
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<td>818</td>
<td>1.98</td>
<td>36.2</td>
<td>1.9</td>
</tr>
<tr>
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<th>Filled avg da. (lb)</th>
<th>Avg daily ration (lb)</th>
<th>Feed efficiency (Feed/100 lb gain, lb)</th>
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<td>12.8 27.6</td>
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<td>13.6 28.5</td>
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<td>13.6 28.5</td>
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<tr>
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</tr>
<tr>
<td>Control</td>
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<td>880</td>
<td>2.25</td>
<td>5.9</td>
<td>15.4 20.3</td>
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<td>6.0</td>
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<td>874</td>
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<td>5.9</td>
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<td>16.3 22.3</td>
</tr>
<tr>
<td>Avg no vit. A</td>
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<td>877</td>
<td>2.20</td>
<td>5.9</td>
<td>14.4 20.3</td>
</tr>
<tr>
<td>Avg with vit. A</td>
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<td>951</td>
<td>2.62c</td>
<td>6.0</td>
<td>16.1 22.1b</td>
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<td>15.1 21.1</td>
</tr>
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<td>Avg with vit. D</td>
<td>501</td>
<td>910</td>
<td>2.40</td>
<td>5.9</td>
<td>15.4 21.3</td>
</tr>
</tbody>
</table>

* Statistical comparisons are made only within silage levels.*
* b Statistically different (P < .05) from value obtained with no vitamin A.*
* c Statistically different (P < .01) from value obtained with no vitamin A.*
* d Statistically different (P < .05) from value obtained with no vitamin D.*
by the addition of vitamin A to the high silage rations. The absence of any apparent effect of vitamin A supplementation on rate of gain indicates that the low blood plasma vitamin A concentrations at 178 days probably had not been present for an appreciable length of time. Other investigations (Guilbert and Hart, 1935) have shown that nearly normal growth rates may be obtained even in the presence of deficiency symptoms and that in some instances (Boyer et al., 1942) growth rates may not be affected for as long as 30 days after the observance of clearly deficient plasma vitamin A concentrations.

Vitamin D, alone or in combination with vitamin A, did not appear to affect rates of gain when added to rations containing a full feed of corn silage.

When 30 lb of corn silage was fed with a full feed of corn grain, the addition of vitamin A or vitamin D, alone or in combination, did not improve rates of gain over those achieved with the control supplement. Rates of gain of the cattle fed 30 lb of silage with no vitamin A supplement had apparently not been affected by vitamin A deficiency at this time as indicated by low levels of plasma vitamin A (table 1).

The addition of vitamin A to rations containing 15 lb of corn silage and a full feed of corn grain resulted in apparent benefits in rates of gain. Over-all, supplementing rations containing 15 lb of the old crop silage with vitamin A resulted in significantly higher (P < 0.05) rates of gain than those obtained when no vitamin A supplement was fed. The vitamin A deficiency noted in the cattle fed 15 lb of
silage with no vitamin A supplement had apparently progressed to the point of reducing rates of gain.

The addition of vitamin D to rations containing 15 lb of corn silage did not improve the over-all average rate of gain of cattle fed these rations during Phase I. Cattle fed no vitamin D gained significantly more (P < .05) than cattle fed the vitamin supplement. There does not appear to be a good explanation for this effect.

Cattle fed rations containing 5 lb of corn silage with no vitamin A supplement were the first and most seriously affected by vitamin A deficiency. Gains made by cattle not supplemented with vitamin A appear to have been slightly reduced as early as about 120 days after the experiment was initiated and were further reduced as the deficiency progressed. Thus at the termination of Phase I, the over-all average rates of gain of cattle fed rations containing 5 lb of corn silage supplemented with vitamin A were significantly higher (P < .01) than gains made by cattle fed no vitamin A with this level of silage.

The addition of vitamin D to the rations containing 5 lb of silage did not appear to have any significant influence on rate of gain during Phase I.

Feed Consumption. Cattle fed the 5 lb level of corn silage actually consumed an average of about 5.9 lb of silage during this phase due to the manner in which the amount of silage offered daily was adjusted to the 5 lb level at the beginning of the experiment. Due to feed refusals, cattle offered the 15 and 30 lb levels of
silage daily consumed only about 14.9 and 28.6 lb, respectively, on the average. Overall average daily silage consumption in the full feed lots was about 37.0 lb.

In general, cattle fed vitamin A with the different levels of silage during Phase I tended to consume slightly more feed than did cattle not supplemented with the vitamin. Differences between these groups in total feed consumption tended to be greater as the level of silage in the ration was decreased. However, only with the 5 lb level of silage did the addition of vitamin A to the ration result in a significant (P < .05) difference in average daily feed consumption. The vitamin A deficiency apparent in some of the cattle fed 5 lb of silage with no vitamin A supplement had apparently progressed sufficiently to significantly reduce appetites in these cattle.

The addition of vitamin D to rations containing the various levels of corn silage resulted in small and inconsistent differences in average daily feed consumption. Only when fed with rations containing 5 lb of corn silage did the vitamin tend to increase daily feed consumption. When vitamin D was fed with rations containing a full feed of corn silage, the average daily feed consumption was significantly lower (P < .05) than that obtained when no vitamin D was fed. There appears to be no logical explanation for this effect.

**Feed Efficiency.** No significant differences in feed efficiency due to the different vitamin treatments were observed. However, the addition of vitamin A to rations containing the different levels of silage tended to result in slight improvements in feed efficiency.
except with the 30 lb level of silage. Differences in feed efficiency between cattle supplemented and not supplemented with vitamin A were greatest, although not statistically different, when the vitamin was fed with 5 or 15 lb of silage. Other workers have indicated that the feed efficiency of vitamin A deficient cattle may not be significantly influenced until the deficiency becomes quite severe (Guilbert and Hart, 1934; Boyer et al., 1942; Hansen, 1963).

The addition of vitamin D to rations containing the different levels of corn silage did not result in any large or significant differences in feed efficiency. However, over-all feed efficiency with the different levels of silage tended to be better in the absence of supplementary vitamin D. No sound explanation appears to be evident for this observation.

**Phase II**

**Carotene Intake and Remission of Vitamin A Deficiency Symptoms.**

Average final feedlot weights and average daily feed consumption of the cattle for Phase II are shown in table 3. Average total carotene intakes for cattle fed the 5 lb, 15 lb, 30 lb and full feed levels of corn silage during Phase II were about 17.5, 42.7, 82.1 and 138.2 mg per head daily, respectively. Based on the average weights for Phase II of the cattle in the respective groups, carotene intakes averaged about 1.8, 4.3, 8.5 and 14.5 mg/100 lb of body weight daily. The lowest level of carotene intake achieved during this period, 1.8 mg/100 lb of body weight daily, was about 0.2 mg/100 lb of body weight less than the highest level of intake which was attained during
**TABLE 3. PHASE II FINAL FILLED WEIGHTS, AVERAGE DAILY GAINS AND FEED EFFICIENCYa**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Final filled weight (lb)</th>
<th>Filled avg da. gain (lb)</th>
<th>Avg daily ration (lb)</th>
<th>Feed efficiency (Feed/100 lb gain, lb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Corn silage</td>
<td>Concentrate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>1096</td>
<td>1.46</td>
<td>48.7</td>
<td>2.1</td>
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<tr>
<td><strong>Vitamin A</strong></td>
<td>1094</td>
<td>1.39</td>
<td>47.5</td>
<td>2.0</td>
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<td>46.3</td>
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<tr>
<td><strong>Vitamin A plus D</strong></td>
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<td>1.33</td>
<td>44.6</td>
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<tr>
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<td>2.0</td>
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<td>Avg with vit. D</td>
<td>1050</td>
<td>1.35</td>
<td>45.5</td>
<td>2.0</td>
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</table>

30 lb corn silage, 132 days

<p>| Control                   | 1099                     | 1.47                     | 27.3                  | 11.8        | 39.1           | 1856        | 803        | 2659  |
| Vitamin A                 | 1064                     | 1.40                     | 27.0                  | 11.6        | 38.6           | 1926        | 824        | 2750  |
| Vitamin D                 | 1060                     | 1.45                     | 27.0                  | 11.8        | 38.8           | 1858        | 813        | 2671  |
| Vitamin A plus D          | 1027                     | 1.09                     | 27.1                  | 11.3        | 38.4           | 2480        | 1031       | 3511  |
| Avg no vit. A             | 1079                     | 1.46                     | 27.1                  | 11.8        | 38.9           | 1857        | 808        | 2665  |
| Avg with vit. A           | 1045                     | 1.24b                    | 27.1                  | 11.4        | 38.5           | 2203        | 927        | 3130  |
| Avg no vit. D             | 1081                     | 1.43                     | 27.1                  | 11.7        | 38.8           | 1891        | 813        | 2705  |
| Avg with vit. D           | 1044                     | 1.27                     | 27.1                  | 11.5        | 38.6           | 2169        | 922        | 3091  |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Final filled weight (lb)</th>
<th>Filled avg da. gain (lb)</th>
<th>Avg daily ration (lb)</th>
<th>Feed efficiency (Feed/100 lb gain, lb)</th>
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<tbody>
<tr>
<td></td>
<td></td>
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<td>Corn silage</td>
<td>Concentrate</td>
</tr>
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<td>4.8</td>
<td>20.5</td>
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</tbody>
</table>

a Statistical comparisons are made within silage levels.
b Significantly different (P < .05) from value obtained when no vitamin A supplement was fed.
c Significantly different (P < .05) from value obtained when no vitamin D supplement was fed.
d Significantly different (P < .01) from value obtained when no vitamin D supplement was fed.
Phase I with the full feed level of silage. Cattle consuming only 2.0 mg of carotene per 100 lb of body weight daily during Phase I had become nearly depleted of their body stores of vitamin A, as indicated by low blood plasma concentrations of the vitamin observed on the 178th day of the experiment, but had not demonstrated visible deficiency symptoms at that time.

During Phase II, daily carotene intakes of 8.5 and 14.5 mg/100 lb of body weight, achieved with the 30 lb and full feed levels of corn silage, respectively, were considerably in excess of the carotene intake in Phase I where no visible symptoms were observed.

Cattle fed 15 lb of the new crop silage supplemented with no vitamin A and consuming an average of about 4.3 mg of carotene per 100 lb of body weight daily made rapid recoveries from the deficiency noted earlier. Carotene intake of this magnitude is generally considered sufficient to promote normal growth rates in cattle of the weights involved (Keener et al., 1942; Dehority et al., 1960; Pope et al., 1961).

Cattle fed the 5 lb level of new crop corn silage and consuming an average of only about 1.8 mg of carotene per 100 lb of body weight daily also recovered from the vitamin A deficiency. The recovery period in these cattle was longer than that observed in the cattle fed 15 lb of silage with an average daily intake of 4.3 mg of carotene per 100 lb of body weight. Deficiency signs had also been present for a longer period of time and had been more severe in cattle fed the 5 lb level of corn silage.
carotene and vitamin A concentrations of 229 and 37 µg/100 ml, respectively.

Cattle fed 15 lb of the new crop corn silage supplemented with no vitamin A had plasma carotene concentrations of about 177 µg/100 ml which were lower than initial values, but plasma vitamin A concentrations of about 31 µg/100 ml present in these cattle at 238 days approximated initial plasma vitamin A concentrations.

With each of the three highest levels of corn silage, the plasma vitamin A concentrations at 238 days were of a magnitude commonly considered indicative of adequate vitamin A nutrition.

At 238 days, only 60 days after feeding of the new crop silage had begun, cattle fed 5 lb of silage supplemented with no vitamin A had plasma carotene and vitamin A concentrations of about 112 and 23 µg/100 ml, respectively. The magnitude of the increase in plasma vitamin A concentrations which had taken place since the cattle were bled on the 178th day of the experiment and the remission of deficiency symptoms noted previously indicates that this level of carotene intake was sufficient to meet the vitamin A requirements of these cattle.

The administration of vitamin A supplement with 15 lb, 30 lb or a full feed of corn silage during Phase II had no significant effect on plasma vitamin A concentrations at 238 days but tended to reduce plasma carotene concentrations with the two higher levels of silage. When vitamin A was fed with 5 lb of the new crop corn silage, plasma vitamin A concentrations were significantly higher (P < .01) than when no vitamin A supplement was fed. Blood plasma carotene
concentrations did not appear to be influenced greatly by the addition of vitamin A to rations containing this level of silage.

Plasma carotene and vitamin A concentrations in blood samples obtained immediately prior to market from cattle fed 15 or 30 lb of silage with no vitamin A supplement were slightly lower than the concentrations observed at 238 days. Cattle fed a full feed of silage maintained plasma vitamin A concentrations equal to those observed at 238 days. Plasma carotene concentrations showed a tendency to increase between the 238-day and the premarket sampling dates in cattle fed the highest level of silage.

No statistical analyses were performed on these data because of the lack of practical significance in the differences involved. It appears, however, that supplemental vitamin A had an appreciable influence on plasma vitamin A concentrations in the premarket blood samples only when added to rations containing 15 lb of corn silage.

**Weight Gains.** Average daily gains, feed consumption and feed efficiency values for Phase II are shown in table 3. Average daily gains of cattle fed the full feed of corn silage were not benefited by the addition of vitamin A or vitamin D to the ration during Phase II, and the combination of vitamin A with vitamin D resulted in the least weight gain obtained with this level of the silage. Overall average daily gains were not significantly influenced by the presence of either supplemental vitamin A or vitamin D in the ration.

Feeding supplemental vitamin A or vitamin D with rations containing 30 lb of corn silage did not improve rates of gain over those
obtained with the control supplement. The combination of vitamin A with vitamin D appeared to result in a depression in rates of gain, but the presence in this lot of a foundered steer, which made little net gain during this period, accounts for much of the observed difference. Over-all, cattle fed no vitamin A supplement gained significantly faster \((P < .05)\) than cattle fed vitamin A with this level of silage. However, the practical significance of this difference, and of the apparent difference in rates of gain between cattle supplemented and not supplemented with vitamin D, is in doubt in view of the above circumstance.

During Phase II, the cattle fed 15 lb of silage with no vitamin A supplement recovered from the deficiency previously noted and appeared to make small compensatory gains. Consequently, average daily weight gains for Phase II were not improved by the addition of supplementary vitamin A to the ration. Supplementary vitamin D was also of no benefit when added to rations containing 15 lb of silage during this period.

Feeding vitamin A with 5 lb of corn silage appeared to improve average daily weight gains during Phase II of the experiment. However, the over-all average daily gains of cattle not supplemented with the vitamin during this time were influenced to a considerable degree by the relatively slow rates of gain caused by the presence of vitamin A deficiency at the beginning of Phase II. Gains made by cattle fed no vitamin A supplement with 5 lb of silage were fully as rapid during the last 3 to 4 weeks of the experiment as gains made during the same
period by cattle fed vitamin A with this level of silage. Over-all, the addition of vitamin A during Phase II to rations containing 5 lb of corn silage resulted in no significant improvement in rate of gain.

Average daily weight gains made by cattle fed rations containing 5 lb of corn silage supplemented with vitamin D during Phase II were significantly higher (P < .05) than daily weight gains made by cattle not supplemented with the vitamin. Large compensatory gains appear to have been made by the cattle fed this level of silage with vitamin D supplement.

**Feed Consumption.** During Phase II of the experiment, average daily feed consumption was not significantly improved by the addition of vitamin A to any of the rations except those containing only 5 lb of corn silage. Cattle fed 5 lb of silage with vitamin A supplement consumed significantly more feed (P < .05) than those fed no vitamin A supplement with this level of silage. However, the cattle fed no vitamin A supplement consumed considerably less feed at the beginning of Phase II while vitamin A deficiency symptoms were present, but daily feed consumption at the termination of this period in these lots was fully equal to that in lots fed the vitamin A supplement.

Cattle fed a full feed of corn silage with no vitamin A supplement consumed significantly more feed (P < .05) than those fed this level of silage with vitamin A. However, slight differences in average weight may have accounted for a portion of this difference.

The administration of vitamin D with rations containing the various levels of corn silage did not improve feed consumption during
Phase II. To the contrary, cattle fed 15 lb of silage with no vitamin D consumed significantly more ($P < .05$) feed than cattle fed this level of silage with supplemental vitamin D, while cattle fed a full feed of corn silage consumed significantly more feed ($P < .01$) when not supplemented with the vitamin. In each case, however, cattle not supplemented with the vitamin were somewhat heavier at the termination of the experiment than those which were fed supplemental vitamin D.

**Feed Efficiency.** Feed efficiency was not consistently nor significantly influenced by any of the vitamin treatments administered with the different levels of silage during Phase II. Generally, however, cattle which gained weight the most rapidly during this period tended to utilize their feed most efficiently.

**Concentrations of Carotene and Vitamin A in Liver Tissue.** The concentrations of carotene and vitamin A present at market time in the livers of cattle from the various treatment groups are shown in table 4. At the time of slaughter the cattle in lots fed 5 lb, 15 lb, 30 lb and a full feed of corn silage had consumed an average of 1.8, 4.3, 8.5 and 14.5 mg of carotene per 100 lb of body weight daily, respectively, for periods of 60, 81, 123 and 158 days. Liver vitamin A stores at the beginning of the feeding of the new crop corn silage, although not determined directly, were probably very nearly depleted in cattle not fed vitamin A with the various levels of silage. The highest plasma vitamin A concentration observed at the 178-day
<table>
<thead>
<tr>
<th>Level of silage</th>
<th>Full feed</th>
<th>30 lb</th>
<th>15 lb</th>
<th>5 lb</th>
</tr>
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<td>8.45</td>
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<td>2.87</td>
<td>26.91</td>
</tr>
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<td>Vitamin D</td>
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<td>14.92</td>
<td>3.21</td>
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<tr>
<td>Vitamin A plus D</td>
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<td>7.68</td>
</tr>
<tr>
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<td>37.08b</td>
<td>2.93</td>
<td>24.24b</td>
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<td>Avg with vit. D</td>
<td>4.86</td>
<td>26.87</td>
<td>3.10</td>
<td>14.24</td>
</tr>
</tbody>
</table>

*a* Statistical comparisons are made only within silage level.

*b* Significantly different (P < .01) from concentration observed when no vitamin A was fed with this level of silage.
bleeding in any of the cattle not supplemented with vitamin A was about 12.7 µg/100 ml of plasma. Other workers have shown that the plasma vitamin A values shown in table 1 represent low or nearly depleted liver stores of vitamin A (Braun, 1945; Diven et al., 1960; Pope et al., 1961).

In the present study, cattle fed a full feed of silage with no supplemental vitamin A had liver vitamin A concentrations of about 14.5 µg/g of fresh tissue. Similar levels were observed in cattle fed 15 lb of silage with 20,000 IU of supplemental vitamin A per head daily.

Feeding 30 lb of the higher carotene silage with no vitamin A supplement for a period of 123 days resulted in liver vitamin A concentrations of about 7.7 µg/g of tissue. In other work done with depleted steers (Hansen, 1963) the administration of 4,000 IU of vitamin A per 100 lb of body weight daily for 133 days resulted in liver vitamin A stores of about 8.5 µg/g of tissue.

Steers fed 15 lb of corn silage with no vitamin A supplement and consuming about 4.3 mg of carotene per 100 lb of body weight daily for the last 81 days of the experiment had liver vitamin A concentrations of about 2.2 µg/g of tissue. Similar levels of storage were obtained in other experiments by feeding previously depleted steers 3,000 IU of vitamin A per 100 lb of body weight daily for 202 days (Embry et al., 1962). While these levels of carotene and vitamin A appear ample to correct vitamin A deficiency signs and to
promote normal growth, they allow little, if any, increase in liver storage.

Cattle fed 5 lb of the new crop corn silage with no vitamin A supplement had liver vitamin A concentrations of about 0.96 µg/g of tissue. Such levels of storage have been associated with poor rates of gain and the presence of deficiency symptoms (Pope et al., 1961). Other workers have concluded that the presence in the liver of less than 2 µg of vitamin A per gram of tissue is not sufficient evidence to indicate inadequate vitamin A nutrition (Kohlmeier and Burroughs, 1964). Observations made in this experiment tend to support the latter conclusions and that plasma levels are more indicative of the current status of vitamin A nutrition than is liver vitamin A.

Feeding vitamin A with 15 lb, 30 lb or a full feed of corn silage resulted in higher (P < .01) concentrations of vitamin A in liver tissue at market time. When supplemental vitamin A was fed with 5 lb of corn silage, liver vitamin A concentrations tended to be higher, but this level of vitamin A did not appear to result in any appreciable liver storage.

Liver carotene concentrations were lower (P < .01) when cattle fed a full feed of corn silage were supplemented with vitamin A than when no vitamin A supplement was fed. The administration of supplemental vitamin A with rations containing 5 lb, 15 lb or 30 lb of corn silage had no significant effect on liver carotene concentrations at market time.
Feeding supplemental vitamin D with the various levels of corn silage had no consistent or significant effect on liver carotene or vitamin A concentrations.

**Carcass Characteristics.** The carcass data are shown in table 5. Statistically significant differences between values are indicated. Generally, differences in carcass traits appeared to be related more to rate of gain and length of feeding period than to vitamin treatments. Differences in the level of roughage in the diet resulted in slight differences in dressing percent, but similarity in market weights resulted in about the same degree of finish and quality in the carcasses of cattle fed the different levels of silage.
### TABLE 5. CARCASS CHARACTERISTICS

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<tr>
<th>Treatment</th>
<th>Full feed</th>
<th>30 lb</th>
<th>15 lb</th>
<th>5 lb</th>
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<tr>
<td><strong>Dressing percent</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>59.99</td>
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<td>61.51</td>
<td>62.61&lt;sup&gt;g&lt;/sup&gt;</td>
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<td>20.18</td>
<td>20.37</td>
<td>20.68</td>
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<td>0.57</td>
<td>0.74</td>
<td>0.70</td>
<td>0.82</td>
</tr>
</tbody>
</table>

a Based on cold carcass weight and market weight.

b Carcasses graded to one-third of a grade, and scores based on average good = 17, average choice = 20 and average prime = 23.

c Numerical values for degree of marbling: devoid, 1; moderate, 7; extremely abundant, 12.

d Significantly different (P < .05) from value obtained with no supplemental vitamin A with this level of silage.

e Significantly different (P < .01) from value obtained with no supplemental vitamin A with this level of silage.
SUMMARY

One hundred fifty-nine yearling Angus and Angus x Hereford crossbred steers were used in an experiment utilizing a $2 \times 2 \times 4$ factorial design to determine the need for supplementation with vitamins A and D of rations containing various amounts of corn silage and corn grain. The levels of corn silage used were 5 lb, 15 lb, 30 lb and a full feed per head daily. A full feed of rolled corn grain was fed with each of the restricted levels of silage. The vitamin treatments fed with each level of corn silage consisted of control, 20,000 IU of vitamin A, 10,000 IU of vitamin D and a combination of 20,000 IU of vitamin A plus 10,000 IU of vitamin D. The vitamin treatments were administered daily in 2 lb of a soybean meal-base protein supplement which contained 5 mg of DES per pound in addition to the appropriate vitamin treatment.

Corn silage from two different crops was used in this experiment. The silage fed the first 178 days of the trial was harvested after frost and contained about 0.36 mg of carotene per pound as fed on a 62.7% moisture basis. The silage fed during the remainder of the experiment contained about 2.94 mg of carotene per pound on a 65.1% moisture basis.

The average carotene intakes during the first 178 days of the experiment for the cattle fed the 5 lb, 15 lb, 30 lb and full feed levels of corn silage were about 0.8, 1.2, 1.7 and 2.0 mg/100 lb. of body weight daily, respectively. Vitamin A deficiency symptoms were observed in cattle fed the 5 lb and 15 lb levels of corn silage with
no vitamin A supplement after about 143 and 175 days on experiment, respectively. After 178 days average blood plasma levels of vitamin A were in ranges associated with the appearance of deficiency symptoms in all lots of cattle not fed vitamin A. However, visible deficiency symptoms were not observed in the cattle fed the 30 lb and full feed levels of corn silage.

Rations containing 5 lb, 15 lb, 30 lb and a full feed of new crop corn silage furnished average daily carotene intakes of about 1.8, 4.3, 8.5 and 14.5 mg/100 lb of body weight, respectively. Vitamin A deficiency symptoms previously noted in the lots of cattle fed the 5 and 15 lb levels of silage with no vitamin A regressed. Blood plasma vitamin A concentrations at subsequent sampling dates in cattle fed no vitamin A with the various levels of silage indicated the vitamin A requirements of these cattle were being satisfied with carotene present in the rations.

During the first 170 days of the experiment, the administration of vitamin A with 5 lb and 15 lb of corn silage resulted in improved rates of gain.

Feed consumption appeared to be improved with all levels of silage when vitamin A was added to the ration.

During the last phase of the experiment no significant benefit in rate of gain or feed consumption was obtained by supplementing rations containing 15 lb or more of the higher carotene silage with vitamin A.
Differences in carcass traits appeared to be affected more by rate of gain and length of feeding period than by vitamin treatments. Supplemental vitamin D appeared to be of no practical benefit under the conditions of this experiment. It would appear that a carotene intake up to 14.5 mg/100 lb of body weight daily with 20,000 IU of supplemental vitamin A per head daily does not influence vitamin D requirements.
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