PART I. A STUDY OF SYSTEMS OF MANAGEMENT AND PROTEIN SUPPLEMENTS FOR RANGE HEREFORD COWS AND THE EFFECT OF EACH UPON CERTAIN BLOOD CONSTITUENTS OF THE COWS AND THEIR CALVES

PART II. A LIVER BIOPSY TECHNIQUE AND OBSERVATIONS OF ITS USE IN VITAMIN A STUDIES WITH BEEF CATTLE

By

WILLIAM J. VAN ARSDELL

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THESIS AND ABSTRACT APPROVED:

C R Whitehair
Thesis Adviser

A. E. Darlow
Faculty Representative

D. G. McFarlane
Dean of the Graduate School

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PART I. A STUDY OF SYSTEMS OF MANAGEMENT AND PROTEIN SUPPLEMENTS FOR RANGE HEREFORD COWS AND THE EFFECT OF EACH UPON CERTAIN BLOOD CONSTITUENTS OF THE COWS AND THEIR CALVES

INTRODUCTION

Extensive investigations have demonstrated the importance of proper nutrition of animals during gestion and lactation. Classical experiments have demonstrated that failure to provide an adequate dietary to the pregnant and lactating female has resulted in reproductive failures, abnormal or weak young and a decreased rate of growth of the offspring.

Many investigations have been reported concerning the nutritive requirements of the bovine not only for growth but also for reproduction and lactation. The most extensive studies have been those concerning cattle of dairy breeding. The high production of the dairy cow and the intensive systems of management followed by the dairy farmer have required that he be more cognizant of nutritional needs than the beef cattle producer. This does not mean that nutritional problems of beef cattle are not important but that investigators were aware at an earlier date of the need for the study of the nutritional requirements of the dairy cow.

Winter feed is a major problem for those maintaining a beef cow herd in Oklahoma. The grasses (Bluestem and associated grasses) in this section of Oklahoma are generally recognized as producing excellent summer but relatively poor winter feed. As these grasses mature their feeding value declines, so that by fall and winter they have only a fraction of their former nutritive value. The greatest losses are in levels of protein and phosphorus.

Ranchmen who maintain cow herds in the Bluestem area of Oklahoma are divided into two groups, namely--those who practice year-long grazing and the feeding of a protein supplement during the winter period and those who practice summer grazing and wintering on prairie hay plus a protein supplement. The protein supplement generally used is cottonseed cake.

Alfalfa is a crop which can be grown successfully in many sections of the state and contains approximately one-third as much protein per 100 pounds as

cottonseed cake. In addition, alfalfa hay properly cured is an excellent source of other nutrients, especially calcium, and carotene, the precursor of vitamin A. A considerable tonage of alfalfa hay is produced in Oklahoma, but a relatively small percentage is fed as a source of protein for wintering beef cattle. Much of the alfalfa hay produced is sold as a cash crop and either goes out of the state or is consumed by other farm animals.

The investigation reported herein was designed to study the above mentioned systems of management of a commercial cow herd adaptable to Oklahoma. In addition, nutritional studies involving two protein supplements (cottonseed cake and alfalfa hay) are presented together with fundamental information concerning certain blood constituents of Hereford cows and their calves.

REVIEW OF LITERATURE

The systems of beef production in this and in many other countries have undergone profound changes during the past decade. These changes have made it mandatory that the beef producer have a thorough knowledge of the nutrient requirements of his cattle.

Hart and Guilbert (1928) found that beef cows must put on weight in the late fall and early winter in order to be at "normal weight" by calving time in the spring.

Withcombe, et al. (1930) made an extensive study on the "deferred" breeding of beef cows. They found that heifers fed to the limit of their appetite were not only more expensive but failed to produce any more income than those fed more limited rations; only those cows fed a limited amount of cheap feeds showed a profit. The birth weight of the calves and the gain made by the calves from birth to weaning were found to increase with the age of the cow, at least, up to five years.

Lantow (1933) found that for wintering beef cattle one pound of cottonseed cake per head per day was more profitable than 2, 3, or 4 pounds and that the heavier feeding of cottonseed cake resulted in slower gains during the summer.

Vinke and Dickson (1933) reported that beef cows which just maintain their weight during the winter months actually lose at least sixty pounds per head because of a developing foetus; thus a winter loss in weight of sixty pounds means a loss of at least 120 pounds in condition. Such a loss by a cow in medium condition in the fall leaves a very thin cow in the spring. These workers also stated that although cows can be wintered on any kind of ration, meager rations cannot be continued for too long a period or into the calving season, without severe loss in weight of the cows or death of the calves.

In studies on limited vs. liberal amounts of feed for breeding cattle at the North Montana Branch Station (1936) it was found that the feed cost of "growing out" a range cow when fed a limited ration was $20.80 less than for those fed a full ration of alfalfa hay. While the cows on the limited ration weighed a little less, they were equal to the well-fed group in percentage calf crop and birth and weaning weight of calves.

Baker (1938) stated that in wintering yearling heifers, 10 percent more alfalfa hay than of western wheat grass was required in the production of similar winter gains. The use of wheat grass hay valued at the same price per ton as alfalfa would result, therefore, in lower winter feed costs.

Black, et al. (1938), studying beef cows wintered with and without a supplement of cottonseed cake, found that the weight losses of cows that received no supplement were significantly greater than weight losses of cows that received the supplement. However, the increased weight of calves at weaning time, from the supplement fed cows did not compensate for the increased winter feed costs.

Taylor (1942) found that 43 percent cottonseed cake was slightly more economical for wintering range cows on dry grass pasture than 41 percent soybean pellets.

Black, et al. (1943) stated that ranchmen have the objective in view to winter their breeding cows with a minimum outlay of feed and labor and still obtain a normal number of good calves.

Guilbert (1944) stated that any consideration of efficiency of beef production must begin with the cow herd, the percentage of calf crop, and the weaning weight of the calves.

Morrison (1946) has summarized much of the published work relative to the nutritive requirements of beef cattle and in addition presented some of the general factors that influence beef production. He stated that alfalfa hay

has no superior among roughages for beef cattle. He further stated that when even a reasonable part of the roughage consists of well cured alfalfa hay, there will be no deficiency in the quality of protein nor in the calcium and vitamin A in the ration.

Snapp (1946) as well as several other authors in the Yearbook of Agriculture (1942) have summarized a great deal of the work relative to systems of management, disease control and prevention and many other nutritional aspects of beef cattle production.

Ross, et al. (1947) reported a four year study of two systems of cow herd management. They found that grazing cows year long and supplementing the cured grass with cottonseed cake was more economical than grazing cows during the summer and feeding them prairie hay and cottonseed cake during the winter in a trap. They reported that there was no difference in the condition of the cows at the end of the experiment nor in the size of the calves at weaning.

Carotene and Vitamin A

The indispensable nature of vitamin A for the dairy calf was shown by Jones, Eckels, and Palmer (1926).

Baumann, et al. (1934) studied the influence of breed and diet of cows on the carotene and vitamin A content of butter. They found that 3.3 percent of the vitamin A ingested by cows fed a low carotene ration was secreted in the milk and for those fed a high carotene level, only 1.3 percent was secreted in the milk. Semb and others (1934) found that 8 percent of the plasma carotene was secreted in the milk daily.

Guilbert and co-workers (1934) found that calves from heifers fed a restricted intake of vitamin A developed diarrhea at two to eight days of age. The milk of the dams of these calves was found to be subnormal in its vitamin A content. No clinical sysmptoms were evident in the cows up to six months after parturition, but night blindness occurred in one of the calves.

Guilbert and Hart (1935) found the minimum daily carotene requirement for the bovine to be 26 to 33 mcg. per kg. of live weight, and they hypothesized that the vitamin A requirement was related to the body weight of the animal and not to the net energy requirement. They also stated that evidently the fetus was the first to suffer from a borderline carotene deficiency.

Converse and Meigs (1936) concluded that the vitamin A supplied by whole milk in rations for dairy calves was more valuable than the fat or energy supplied.

Guilbert, et al. (1936) showed that the minimum carotene requirement for all the species of farm animals that they studied was 25 to 30 mcg. per day per kilogram of body weight. The minimum vitamin A requirements were found to be 6 to 8 mcg. per day per kilogram of body weight.

Jones and Haag (1938) studying growth and reproduction in dairy heifers, obtained results which indicated that a comparatively low vitamin A ration resulted in serious disturbance to heifers when fed over a period of about six months, either preceded or followed by a pasture period.

Ward, et al. (1938) studying the carotene requirement of the dairy calf using varied rations, found that 12 to 14 mcg. per pound of body weight per day was sufficient to prevent vitamin A deficiency symptoms. They also found that dairy heifers fed a carotene deficient ration during the winter months showed no deficiency symptoms if they had been on good pasture during the summer months.

That calves need protective vitamin A during the first three to four months of life has been shown by Converse and Meigs (1939). They found that calves need carotene and vitamin A in amounts much larger than that of normal cattle from six months to two years of age. Moore (1939) studying calves maintained on low carotene rations until 40 to 90 days of age, found that the calves developed nyctalopia in from 48 to 73 days. Papillary edema also

developed in about the same period of time in these calves. An intake of 9 mcg. of carotene per pound of body weight was not sufficient to prevent nyctalopia or decrease papillary edema. An intake of 16 mcg. per pound of body weight was sufficient to maintain the plasma carotene at 0.2 mcg. per ml. and above in Holstein and Ayrshire calves. This intake was sufficient to prevent nyctalopia and maintain fair general health in the calves.

Guilbert, et al. (1940) reported that cows on a minimum vitamin A ration were able to produce live young, but that the calves were weak and soon died. Vitamin A supplementation at three to four times minimum requirement beginning the last month of pregnancy resulted in normal calves and the mothers supplied sufficient vitamin A in their milk for normal growth of the calves for at least three months following parturition.

Henry and others (1940) studying nine Shorthorn heifers that had access to good pasture before calving, found no increase in the secretion of vitamin A in the colostrum over normal milk, but an increase in the output of carotene was noted. Riggs (1940) stated that the accumulation of vitamin A in the body increased with age and was dependent on the character of the diet.

Kuhlman and Gallup (1940, 1941) reported that an average daily intake of from 40 to 45 mcg. daily per pound of body weight was about the minimum of carotene which would meet the requirements of Jersey cows for normal calving. Factors such as the health of the calf as well as the ability of the cow to begin normal lactation were taken into consideration.

Davis and Madsen (1941) studying cattle on restricted levels of carotene intake, found that the carotene and vitamin A content of blood plasma was dependent on the carotene intake and previous storage of these constituents.

Gallup and Kuhlman (1941) in an experiment with Jersey cows, found that plasma carotene values usually dropped immediately or soon after parturition

and that there was no consistent further change in these values during the first few weeks after parturition.

Moore (1941) found that mature dairy cows fed a vitamin A deficient ration failed to develop blindness due to constriction of the optic nerve such as has been reported in calves, but when the plasma carotene values were as low as 0.2 to 0.5 mcg. per ml. of blood, deficiency symptoms usually followed in a short time.

Boyer, et al. (1942) found that 10 or more mcg. of vitamin A per 100 ml. of plasma was necessary for adequate vitamin A nutrition of the growing dairy calf.

Kenner, et al. (1942) found the minimum carotene requirement of dairy calves maintained in an environment with the temperature ranging from 50 to 70 degrees Farenheit, to be approximately 12 mcg. per pound of body weight per day. Respiratory and bowel distrubances were more prevalent during periods of low blood vitamin A than when the level of plasma vitamin A was considerably higher.

Stewart and McCollum (1942) studying the effect of vitamin A enriched diets on the vitamin A content of the colostrum of dairy cows, failed to find a difference in the milk of control cows as compared to those fed the vitamin A rich concentrate.

Sutton and Soldner (1943) working with dairy cattle, reported that blood carotene and vitamin A levels remained at about constant levels up to about a week before calving. Just prior to calving, a decline in both plasma carotene and vitamin A were found to occur and a further drop was observed immediately after parturition. Kuhlman and Gallup (1944), studying carotene blood plasma levels of Jersey cows at parturition, reported changes similar to those found by Sutton and Soldner (1943).

Braun (1945) studying carotenoid and vitamin A levels in the blood of cattle, found a linear increase of plasma vitamin A as the carotenoid level increased. "The ratio of plasma vitamin A to plasma carotene at various carotene levels was found to decrease with increasing carotenoid levels".

A recent report of the committee of Animal Nutrition of the National Research Council (1945) states that 1.4 to 1.6 mg. of carotene per 100 lbs. live weight per day proved adequate for normal growth of beef cattle. At this intake, however, there was little or no storage to meet the exigencies of life. The recommended allowance for beef cattle was 5.5 mg. per 100 lbs. live weight per day. The minimum requirements of vitamin A for growth was established as 1000 I. U. daily for each 100 lbs. of live weight. For suckling calves, 6000 to 9000 I. U. were considered sufficient when milk was the sole source of this nutrient.

Lewis and Wilson (1945) in an experiment with dairy calves, obtained results which indicated that 32 U.S.P. units of vitamin A per kilogram of body weight was the minimum requirement for growth. The level required for optimum growth was found to be 64 U.S.P. units of vitamin A per kilogram of body weight. When both growth and liver storage were taken into consideration, the recommended daily intake of vitamin A for young calves was found to be about 250 U.S.P. units per kilogram of body weight.

Moore and Berry (1945) studying calves of the Holstein, Ayrshire, and Guernsey breeds from birth up to four months of age, found that the vitamin A content of the blood plasma varied from 7.2 to 14.0 mcg. per 100 ml.

Sutton and Soldner (1945) studying seasonal plasma carotene and vitamin A variations in the blood plasma of adult dairy cattle, found that the average monthly range of plasma vitamin A for all dairy breeds investigated ranged from 18 mcg. per 100 ml. of plasma in June, to 24 mcg. per 100 ml. of blood plasma in October.

Sutton, Warner and Soldner (1945) found that the maximum decrease in blood plasma carotene of lactating cows occurred one week following parturition. The maximum decrease in blood plasma vitamin A was reached three days after parturition.

Hansen, et al. (1946) found that the vitamin A of colostrum from barn fed dairy heifers in the first lactation was more than twice the vitamin A content of the colostrum from the same cows in the second lactation. Sevenfold variation in the colostral vitamin A potency occurred in these cows. The cows were fed identical rations and maintained under uniform conditions during two lactating periods. An increase in the blood plasma vitamin A concentration of the new born calf was observed following the ingestion of colostrum and the percentage increase tended to reflect the concentration of vitamin A present in the colostrum of their dams.

Hibbs and Krauss (1946) reported that regardless of the amount of vitamin A fed to dairy calves, the blood level seldom exceeded 25 mcg. per 100 ml. and that the decrease of blood vitamin A during the first few weeks after birth could largely be offset by feeding additional vitamin A.

Kaeser and Sutton (1946) studied the utilization of colostrum in calf feeding. They found that calves that received extra amounts of colostrum maintained higher levels of plasma vitamin A and carotene during the first four weeks after birth than those fed the lower amounts of colostrum. The calves also gained more rapidly and were superior in appearance at four weeks of age when fed extra colostrum.

Payne and Kingman (1946) reported that in order to support normal gestation, the carotene blood plasma level of first calf Hereford heifers must be considerably higher than that for aged Hereford cows. They also reported that at least 117 $\pm$ 7.21 mcg. per 100 ml. of blood plasma was necessary to support normal gestation in heifers. When a carotene level of 97.18 $\pm$ 7.68

mcg. per 100 ml. was found in range Hereford heifers retained placenta and nutritional abortion were observed. Aged Hereford cows with carotene blood levels as low as 82.88 ± 4.11 mcg. per 100 ml. showed no symptoms over a two year period which could be attributed to carotene or vitamin A deficiency.

Spielman, et al. (1946) studying carotene utilization by the newborn dairy calf, found that intestinal infection and scours resulted in reduced absorption and utilization of carotene.

Thomas, Spielman and Turk (1946) demonstrated that the concentration and total output of vitamin A and carotene in cows' colostrum was influenced by the ration fed during the two months immediately prior to parturition.

Frey and co-workers (1947) found that dietary vitamin A did not definitely increase the hepatic stores of carotene in Hereford steers. They concluded that the serum levels and the hepatic stores of vitamin A appeared to be controlled by different body mechanisms.

Glover and co-workers (1947) also found that rats, when given large doses of beta-carotene, converted a certain amount of it to vitamin A in the intestines. More than twenty international units of vitamin A were found in each of the three intestines of rats six hours after dosing with 5 to 15 mg. of beta-carotene. A very good summary of the site of conversion of carotene to vitamin A in the rat was published in Nutrition Reviews (1948).

Lemly, et al. (1947) found that the storage of vitamin A in the liver of the rat was in proportion to the intake of vitamin A. Glover, Goodwin, and Morton (1947) found that the plasma vitamin A level of rats was proportional to the total liver storage of vitamin A and consisted mainly of vitamin A esters. The plasma vitamin A levels were maintained near normal (35-40 mcg./100 ml.) even when liver storage approached exhaustion.

Maynard (1947) has summarized the requirements, physiological functions, and deficiency symptoms of vitamin A.

Parrish and co-workers (1947) reported that practically all of the vitamin A in both colostrum and milk was in the form of the vitamin A ester. Most of the fat soluble yellow pigment in colostrum and milk was found to be carotene.

Sutton, Warner and Kaeser (1947) found a rapid decline of carotene and vitamin A in colostrum and milk with each successive milking. Colostrum was approximately ten times as potent in carotene and six times as potent in vitamin A as normal milk. Levels closely approaching normal milk were reached at the end of the third day.

Wise and Atkeson (1947) found that vitamin A intake of the cow had no effect on total milk and fat production. High vitamin A intake increased the concentration of vitamin A in the milk fat, but tended to suppress the carotenoid content. Cows fed high vitamin A rations showed the same characteristic decline of plasma vitamin A at parturition as did non-supplemented cows but those receiving the additional vitamin A maintained higher levels at parturition than did the non-supplemented cows.

Ross, et al. (1948) using Holstein heifers, reported a blood plasma vitamin A level of from 6 to 8 mcg. per 100 ml. blood plasma to be the critical level for maintenance when gain in body weight was used as the standard for measurement.

Wise, et al. (1948) reporting on postnatal changes in the concentration of carotenoids and vitamin A in the blood serum of calves of their dairy herd, found that "there was a marked degree of variability in the concentration of carotenoids and of vitamin A in the blood serum of individuals of the group, but that the general trends in the levels of these constituents in each calf were similar". They concluded that the diet seemed to be the primary factor in determining the level of vitamin A and carotenoids in the blood serum and

that under their managerial practices vitamin A supplementation from the colostrum period to the hay period was needed for best performance of the calves.

Eaton, et al. (1949) studied plasma levels of carotene and vitamin A in calves from dams milked prepartum and in calves from dams milked postpartum. They found lower plasma carotene and vitamin A levels in calves from dams milked prepartum as compared to calves from dams milked postpartum only. The calves were observed for a period of one to four weeks after parturition. Four of the major breeds of dairy cattle were studied.

Eden (1949) published a summary of vitamin A deficiency in farm animals.

Elliott (1949) studied the site of absorption and conversion of carotene to vitamin A in the dairy calf. He presented evidence to support the view that carotene is converted to vitamin A in the intestinal wall. This had previously been shown to be the case in the rat.

Sellers and Eden (1949) found that calves with "white scours" had impaired absorption of vitamin A. Single and repeated oral doses of vitamin A produced a 300 to 500 % increase in plasma vitamin A levels four hours after administration to control animals. Little or no increase was obtained with scouring calves. Only traces of vitamin A were found in the faeces of control animals while in the scouring animals the bulk of the vitamin was lost in the faeces. The liver reserves of vitamin A in the scouring animals were only one-third those of the control animals.

Nezvesky, et al. (1950) studying the effect of vitamin A from prenatal storage and from ingestion of colostrum on the neonatal dairy calf, found that colostrum significantly increased plasma vitamin A from birth to five days of age and liver vitamin A at twenty-eight days of age. Prenatal storage elevated blood plasma A at birth significantly and contributed to greater liver storage of A at twenty-eight days of age.

Ronning and Knodt (1950) studying the blood plasma vitamin A and carotene of dairy calves treated with sulfonamides, found no detrimental effects of sulfonamides on the normal vitamin A and carotene metabolism of calves when the drugs were given as recommended.

Shaw (1950) reported studies on ketosis in dairy cattle. He found that ketosis could not be produced or blood glucose levels raised by feeding vitamin A supplement to cattle that were severely vitamin A deficient and on either a high or low energy ration.

Stallcup and Herman (1950) studied the in vitro conversion of carotene to vitamin A in dairy calves. In vitro incubation of small intestine of dairy calves with colloidal carotene solution indicated that the small intestine is a site of conversion of carotene to vitamin A. Incubation of minced liver tissue with a colloidal carotene solution also resulted in conversion of carotene to vitamin A. No conversion was found in the blood plasma.

Van Arsdell, et al. (1950) reported the effect of ration upon some constituents of blood and milk of Hereford cows and the blood of their calves. They found that plasma carotene levels were positively correlated with carotene intake. No consistent differences in plasma vitamin A could be attributed to ration. Both plasma carotene and vitamin A were found to decline at or shortly after parturition and then rise during a subsequent thirty day period. The plasma carotene and vitamin A levels of the calves were found to increase rapidly during the first few days of life and then to decrease to a level which was generally maintained throughout the first month of life.

Despite relatively low plasma levels of carotene and vitamin A (as low as 30 mcg. per 100 ml.) observed during this study all cows produced normal calves and raised them successfully. These authors suggested, as a result of this study, that seasonal declines of plasma vitamin A and carotene may occur for short periods of time without the appearance of clinical symptoms of avitaminosis.

Phosphorus

Eckles, et al. (1926) found a marked inhibition of oestrum in cows fed phosphorus deficient feeds. Cows in milk showed the most severe symptoms.

Eckles and Gullickson (1927) found that a phosphorus deficiency did not affect the digestibility of the ration but did affect the utilization of the nutrients after they had been digested.

The concentration of inorganic phosphates in the blood plasma of cattle as an aid in clinical diagnosis of a phosphorus deficiency even before physical symptoms became apparent was first reported by Palmer and Eckles (1927), and South African workers (1927, 1928).

Thieler, et al. (1927) reported that the phosphorus content of the blood of cattle receiving adequate phosphorus varied from 4 to 9 mg. per 100 ml. of whole blood with an average of 5.2 mg.

Green and Macaskill (1928) reported that the total inorganic plasma phosphorus of the blood of a newborn calf may be over twice that of its mother. For the first few days after birth, there was a tendency for the plasma phosphorus to increase. The level then decreased steadily and at ten weeks of age the difference between cow and calf was in some instances only 15 percent.

Theiler, Green and DuToit (1929) studied cattle grazing a phosphorus deficient pasture. They found that mineral supplemented cows showed increased fertility, superior development of calves, and reduced mortality as compared to unsupplemented cows.

Anderson, Gayley and Pratt (1930) studying the chemical composition of bovine blood, found that the average, maximum, and minimum level of inorganic plasma phosphorus for all animals studied was 4.46, 6.17, and 3.09 mg. per 100 ml. of plasma, respectively. The average for animals less than one month of age was 5.06 mg. per 100 ml. of plasma; for animals one to five months of

age, 5.54 mg. per 100 ml. of plasma; for animals six to ten months of age, 4.48 mg. per 100 ml. of plasma; and for animals one to nine years of age, 3.62 mg. per 100 ml. of plasma.

Palmer, et al. (1930) studying dairy cattle blood phosphorus variations, reported that a marked decrease in the inorganic phosphorus content of cows' blood occurred at or near the time of parturition. Most research workers agree that lactating cows and growing animals are most severely affected by a deficiency of available phosphorus in the ration.

Haag, Jones and Brandt (1932) indicated that a calcium-phosphorus ratio of 10.5 to 1 was no more detrimental than one of 7.6 to 1 for dairy cattle.

Huffman and others (1933) state that blood plasma phosphorus values lower than 4.0 mg. percent should be watched carefully, especially if the animal is less than one year of age. Greaves, Maynard and Reeder (1934) gave 5.0 mg. percent as the borderline value.

Huffman and co-workers (1933) found that the phosphorus requirement for milk production over and above that required for maintenance ranged from 0.5 to 0.7 grams of food phosphorus per pound of milk.

Greaves, et al. (1934) studying the influence of calcium and phosphorus intake upon bovine blood, found that phosphorus supplementation produced little if any effect on the blood calcium. Inorganic phosphorus in forty steers varied from 2.41 to 3.01 mg. per 100 ml. of plasma.

Fairbanks (1939) stated that the calcium-phosphorus ratio was of greatest importance when the vitamin D of the ration was inadequate.

Knox, Brenner and Watkins (1941) found that cows with blood plasma levels of 2.0 to 3.0 mg. percent in the winter and spring, and from 3.0 to 4.5 mg. percent in the summer were in excellent health.

Black and associates (1942) found that symptoms of a phosphorus deficiency developed in cattle grazing a phosphorus deficient range when the blood

phosphorus content was below 4.0 mg. per 100 ml. of whole blood. Bohstedt (1942) found that calves may tolerate a rather large proportion of calcium to phosphorus.

Black, et al. (1943) found that the phosphorus content of the forage increased during those months following heaviest rainfall.

Black and associates (1943) found that the feeding of 6.5 grams of phosphorus per cow per day to dry cows proved highly beneficial in southern Texas. For the two year period covered by this study, the control cows weaned a 58 percent calf crop whereas the supplement fed cows weaned an 81 percent calf crop. The difference in weaning weight per calf was 6 percent in favor of the supplemented group.

A report of the committee on Animal Nutrition of the National Research Council (1945) advised that cattle should be allowed free access to a phosphorus-rich mineral mixture if the forage should fall below 0.15 percent phosphorus on a dry-matter basis. This committee recommended a range of from 12 grams of phosphorus per head daily for wintering weaned calves, to 24 grams for cows nursing calves.

With many of our farm animals, the supply of phosphorus comes entirely from pasturage and hay which is often low in this element. Mitchell (1947) stated that if the content of pasturage or hay on a dry matter basis is 0.12 percent or less, the roughage will not provide adequate phosphorus for the bovine.

Tash and Jones (1947) found that the shorter grasses have a higher average phosphorus content than the taller grasses in the same pasture, if they are at approximately the same stage of development. They also found that palatable weeds contain more of this element than do grasses growing on the same soil.

Watkins and Knox (1948) published the results of several years work on inorganic blood phosphorus levels necessary for satisfactory production of range cattle. Breeding cows that received calcium and phosphorus supplements continuously gave good production results. The results varying from 2.11 mg. during the winter to a high of 5.37 mg. during the summer and fall months. These workers noted an average of 3.53 mg. per 100 ml. of plasma for all determinations made. Even though the cows had good production records the blood plasma inorganic phosphorus was definitely lower than that which has been considered to be a critical level by most workers.

Nelson, et al. (1951) reported reproduction and lactation studies of range Hereford cows fed various levels of phosphorus. On the basis of data obtained, they concluded that a total daily intake of 12.5 grams of phosphorus per head is as effective as an intake of 19.2 grams for pregnant beef cows during the winter. They also obtained data which indicated that range Hereford cows allowed access to native grass pasture during the summer, and wintered on prairie hay and a protein supplement which provided a daily phosphorus intake of approximately 10 grams per head daily, had a phosphorus intake adequate to meet the requirement for reproduction and lactation.

EXPERIMENTAL

Objectives:

1. To test two systems of management, one a system of grazing year-long and the other, grazing for a part of the year and feeding prairie hay during the winter months.
2. To determine the value of alfalfa hay as compared to cottonseed cake when fed as a protein supplement during the winter months.
3. To study the effect of the systems of management and winter supplements fed upon certain blood constituents of the blood of cows and calves.

EXPERIMENT I: Systems of Management

Procedure:

In the spring of 1942, two groups of twenty head each of good, grade Hereford cows were placed on experiment initiating a long-term study of the two systems of management mentioned above. One group of cows was grazed year-long on 10 acres of Bluestem grass and 2.25 acres of old cultivated fields per cow. Cottonseed cake (2.57 pounds per cow daily) was fed as the protein supplement during the five winter months. The second group of cows was grazed for seven months on seven acres of Bluestem grass plus 1.6 acres of old cultivated fields. Cottonseed cake (1.33 pounds per cow daily) and prairie hay (20.95 pounds per cow daily) were fed in a five acre trap during the five winter months. The acreage of old fields included land which at one time had been under cultivation but which had been allowed to go back to grass. These fields had been out of cultivation for approximately twelve years but the grass and productivity was not comparable to that found in native pastures.

The cows under each system of management had free access to salt and a mineral mixture consisting of equal parts salt, ground limestone and bone meal. The older and poorer producing cows were culled from year to year and replacements added. Every effort was made to keep the two groups as much alike as possible as to age, breeding and conformation. Good purebred bulls were used. The breeding and producing efficiency of the herd was maintained at a very high level throughout the experiment.

The results of four years work (spring 1942 to fall 1946) were reported by Ross, et al. (1947).

> "It was found that there was no difference in the condition of the cows at the end of the experiment nor in the size of calf at weaning. The most economical method was to graze the cows year-long and supplement the cured winter grasses with cottonseed cake."

Beginning with the 1946-1947 season the pattern of the experiment was changed, with the objectives being those as mentioned above.

The study reported herein is a summary of four years data (Fall 1946 to Fall 1950). All values reported are four year averages unless otherwise specified.

Forty grade Hereford cows were divided equally into two lots of 20 cows each. The average age of these cows was four years. All cows had been pasture bred and upon examination were thought to be pregnant. The cows were started on the experimental rations November 20, 1946, at the Lake Carl Blackwell experimental range. This range is located approximately thirteen miles west of Stillwater, Oklahoma, on the north side of Lake Carl Blackwell.

The cows of lot 1 grazed a pasture, each year, providing about 12.25 acres of native grass per cow. Of this acreage approximately 2.25 acres had been cultivated for many years but about 1936 was taken out of cultivation and allowed to go back to grass. At the time this experiment was started very little of the better species of grass could be found on the previously cultivated acres.

The cows remained on this pasture during the entire year. Salt and/or a mineral mixture were available at all times. During the five winter months (November to April) the cows of lot 1 were fed alfalfa hay as a protein supplement.

The cows of lot 2 were wintered in a four acre trap and fed alfalfa and prairie hay. Each year during the seven summer months (April to November) the cows of lot 2 were grazed in a native grass pasture providing approximately 8.6 acres of native grass pasture, 1.6 acres of which consisted of land previously cultivated.

The alfalfa hay fed to the cows of lot 2 was fed at a level to provide approximately the same crude protein intake as 1.3 pounds of cottonseed cake which had been found to satisfactorily supplement prairie hay (Ross, et al. 1947). The alfalfa hay fed to the cows of lot 1 was fed at a level to provide the same crude protein level as 3.0 pounds of cottonseed cake which Ross, et al. (1947) had found to satisfactorily supplement dry cured grass for wintering pregnant cows.

Each year the cows were pasture-bred to registered Hereford bulls. The bulls were placed with the cows on May 1 and removed September 1. Each two week period the bulls were rotated between the lots as insurance against poor conception due to sterility or poor breeding performance of an individual bull. The same breeding procedure had been followed the season before the experiment was started.

Blood samples were collected by venous puncture when the cows were placed on experiment and at approximately monthly intervals thereafter. With the exception of the winter months of 1946-47 at which time only 10 cows of each lot were bled, during the winter months and at the bleeding date in April when the cows were turned to pasture all of the cows were bled. During the summer months only 10 cows of each lot were bled. The blood was collected in citrated

tubes. It was kept under refrigeration until aliquots were taken for the various chemical determinations. The chemical determinations made and the methods employed were: Plasma inorganic phosphorus, Youngburg and Youngburg, (1930) and plasma carotene and vitamin A, Kimble, (1939).

All of the calves that had been borne by the April bleeding date were bled on that date. Only those calves from the cows bled during the summer months were bled after April. The blood samples from the calves were taken on the same date that the cows were bled. The calves were not bled after August of each year.

All of the calves were dehorned at approximately 2 months of age, and the bull calves castrated at 1 month of age. All calves were vaccinated for blackleg.

The following records were maintained:

1. Gain or loss in weight of the cows.
2. Birth weight of the calves.
3. Weaning weight of the calves.
4. Percentage calf crop weaned.
5. Records of all feeds fed.
6. Yearly feed costs.
7. Blood data.

The prairie hay fed in this experiment was grown in the same general area where the pastures were located.

All supplemental feeds fed were analyzed by A. O. A. C. methods (1940). In addition, grass samples were collected periodically and analyzed chemically. The samples which were largely Big Blue Stem, Little Blue Stem, Indian and Switch grasses, were collected from approximately the same area in each of the pastures each time. The grass was cut one to two inches above the ground and collected in paper bags.

The data was analyzed statistically by the methods of Snedecor (1946).

RESULTS AND DISCUSSION

Production Record:

Pertinent data relative to weights and feeds fed are shown in Table I.

The cows of lot 1 grazed year-long and fed 8.17 pounds of alfalfa hay daily during the winter months, lost an average of 15 pounds per cow to time of calving. At the end of the summer phase, however, they were 39 pounds heavier than at the beginning of the winter phase. The cows of lot 2, grazed seven months of the year and fed 4.71 pounds of alfalfa hay and 15.95 pounds of prairie hay per head daily during the winter months, lost 6 pounds per cow to time of calving. The cows of this lot were 16 pounds lighter at the end of the summer grazing season than at the beginning of the winter period. This difference of 55 pounds in average yearly gain for the four years was significant at the 1 percent level of probability.

The cows of lot 1 weaned a 92 percent calf crop as compared to an 89 percent calf crop for the cows of lot 2. During the year 1947-48, six calves were borne dead or died shortly after birth in lot 2. These calves were borne during extremely cold weather; however, no calves were lost in lot 1, thus the cold weather was not considered entirely responsible for the death of the calves in lot 2.

The calves of lot 1 averaged 5 pounds heavier (75 as compared to 70) per calf at birth than the calves of lot 2. This difference was significant at the 1 percent level of probability.

The calves of lot 1 averaged 445 pounds at weaning as compared to 414 pounds for the calves of lot 2. This difference was significant at the 1 percent level of probability.

The average feed cost per cow for the four years for lot 1 was $35.17 as compared to $40.49 for those of lot 2. This did not include labor costs.

Table I. SUMMARY OF PRODUCTION DATA: AVERAGE OF FOUR SEASONS, (1946-47, 1947-48, 1948-49, 1949-50).

	Lot 1 (a) Grazed Year-long Fed Alfalfa Hay	Lot 2 Grazed 7 months Fed Prairie and Alfalfa Hay
Average weight per cow (lbs)		
Beginning winter period	1001	975
Before calving	986	969
At end of summer period	1040	959
Gain for the four years	39 **	-16
Average birth weight per calf (b)	75 **	70
Average weaning weight per calf (b)	445 **	414
Calf crop weaned (percent)	92	89
Winter rations		
Prairie hay	(c)	15.95
Native grass pasture	12.25 acres	—
Alfalfa hay	8.17 lbs.	4.71 lbs.
Mineral mixture (d)	free choice	free choice
Summer rations		
Native grass pasture	12.25 acres	8.6 acres
Mineral mixture	free choice	free choice
Total cost	$35.17	$40.49

(a) One cow died during 1948-49 season.

(b) Weights corrected for age, sex, season, and age of dam.

(c) Prairie hay fed only when the ground was covered with snow for an extended period.

(d) 1-1-1 (salt, bone meal, and ground limestone) during 1946-47 and summer of 1950 seasons.
3-1 (3 salt to 1 bone meal) during 1947-48 season.
2-1 (2 salt to 1 bone meal) during 1948-49, and winter of 1949-50 seasons.

** Statistically significant at the 1% level of probability.

The only difference in treatment during the summer between the two lots of cows was the grazing acreage, but a vegatitive survey conducted during and at the conclusion of each year indicated that both lots of cows were provided ample amount of summer herbage.

The system of grazing cows year-long in native grass pastures and supplementing the winter grass with approximately 8 pounds of alfalfa hay was a better system of management than grazing for 7 months and wintering in a trap with prairie hay and supplementing with approximately 4.7 pounds of alfalfa hay daily per cow. The average yearly feed cost was $5.32 less for the year-long grazed cows and in addition the cows gained more during the year than the trap fed cows of lot 2. Significant differences (1% level of probability) in favor of the system of grazing year-long were found in average gain for the four years and average birth and weaning weight of the calves.

Feed Analysis:

The averaged analyses of the feeds fed are presented in Table II. It should be noted that at only the May sampling date did the grass contain adequate phosphorus (0.126 %) as prescribed by Mitchell (1947). He stated that if the content of pasture on a dry matter basis is 0.12 percent or less, the roughage will not provide adequate phosphorus for the bovine. The National Research Council (1945) advised that cattle should be allowed free access to a phosphorus-rich mineral mixture if the forage falls below 0.15 percent phosphorus on a dry matter basis.

The National Research Council (1945) reports that dry range forage containing less than 8 percent crude protein is deficient in protein for all classes of cattle. In this study, the crude protein content (at the dates samples were taken) of the grasses ranged from 2.53 (Nov.) to 9.68 percent (May).

Blood Composition, Cows:

The analyses for the various constituents of the cows' blood are shown in Table III. A graphic picture of these various constituents is presented in Fig. 1.

Table II. FEED ANALYSES: FOUR-YEAR AVERAGE

	Percent dry matter	Percent composition of dry matter							
		Ash	Prot.	Fat	Fiber	N.F.E.	Ca	P	Carotene (a)
Alfalfa hay	93.34	7.95	15.79	2.57	30.08	42.08	1.34	.19	24
Prairie hay	94.13	7.50	4.20	2.16	34.78	50.83	.47	.06	13
Bone meal	96.08	91.70					32.73	15.30	
Ground limestone	99.67						36.18		
Grass (b)									
November (c)	82.39	5.01	2.53	1.74	40.02	50.40	.253	.046	14
January (c)	94.85	5.92	2.57	1.57	40.86	48.74	.309	.039	Trace
May	52.29	6.39	9.68	2.40	32.02	49.08	.308	.126	407
August	54.71	6.21	5.06	2.23	35.42	50.66	.346	.078	112
October (c)	63.09	5.18	3.23	1.62	37.24	52.24	.244	.048	16

(a) Parts per million.

(b) Averages, by species, of the four predominant grasses: Big Bluestem, Little Bluestem, Indian, and Switch.

(c) Three years only.

Phosphorus: The cows of lot 1 were found to have normal plasma inorganic phosphorus values throughout most of the year. They were higher in this constituent at every bleeding date than the cows of lot 2. Statistical analysis of the differences between lots showed a significance at the 1 percent level of probability for every month except November and September. The cows of lot 2 were found to have relatively low blood plasma phosphorus levels during most of the year, despite the fact that they had access, free choice, to a phosphorus rich mineral mixture. The blood data suggested that the phosphorus nutrition of the lot 2 cows may not have been optimal.

The average plasma inorganic phosphorus values of both lots were found to follow similar trends from one bleeding date to the next. The phosphorus values averaged 3.8 mg. per 100 ml. of blood plasma for lot 1 as compared to 2.98 mg. per 100 ml. for the cows of lot 2 during the winter period.

The fact that the phosphorus consumption for both lots (approximately 12 and 9 grams per cow daily for lots 1 and 2, respectively) was barely within the minimum range suggested by the National Research Council (1945) might partly explain the low blood plasma phosphorus levels that were found. The plasma phosphorus values observed were, in general, lower than those values reported by Black and Associates (1942) who described symptoms of a phosphorus deficiency that developed in cattle when the blood phosphorus content was below 4 mg. per 100 ml. of whole blood. The values were also lower than those reported by Huffman and Others (1933) and Greaves et al., (1934). However, the values were in good agreement with those reported by Knox, Brenner and Watkins (1941) and Watkins and Knox (1948) of the New Mexico Station. These workers reported values of 2.11 mg. per 100 ml. of blood plasma during the winter months to a high of 5.37 mg. per 100 ml. of plasma during the summer months. They noted an average of 3.53 mg. per 100 ml. of blood plasma on all determinations made over a four year period.

Table III. BLOOD CONSTITUENTS OF COWS BY LOTS, FOUR-YEAR AVERAGE.
(Units per 100 ml.)

	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May (a)	June	July	Aug.	Sept.	Oct.
						Phosphorus (mg.)						
Lot 1	3.4	4.1**	3.9**	3.5**	4.2**	4.0**	5.4**	4.2**	3.2**	4.0**	4.0	3.7**
Lot 2	3.3	3.1	3.0	2.7	2.8	3.3	3.7	3.5	2.5	3.1	3.6	2.9
						Carotene (mcg.)						
Lot 1	359 (b)	196	155	153	145	528**	1097	1088	808	950	575	443
Lot 2	405	182	150	153	117	307	1055	1035	816	978	621	462
						Vitamin A (mcg.)						
Lot 1	28.2 (b)	29.9**	30.2**	24.0	24.0**	18.0	29.4*	25.1*	32.5	44.9*	35.9*	27.9
Lot 2	27.5 (b)	28.0**	26.8	23.6	19.7	21.6**	24.6	21.5	29.4	38.0	31.7	25.2

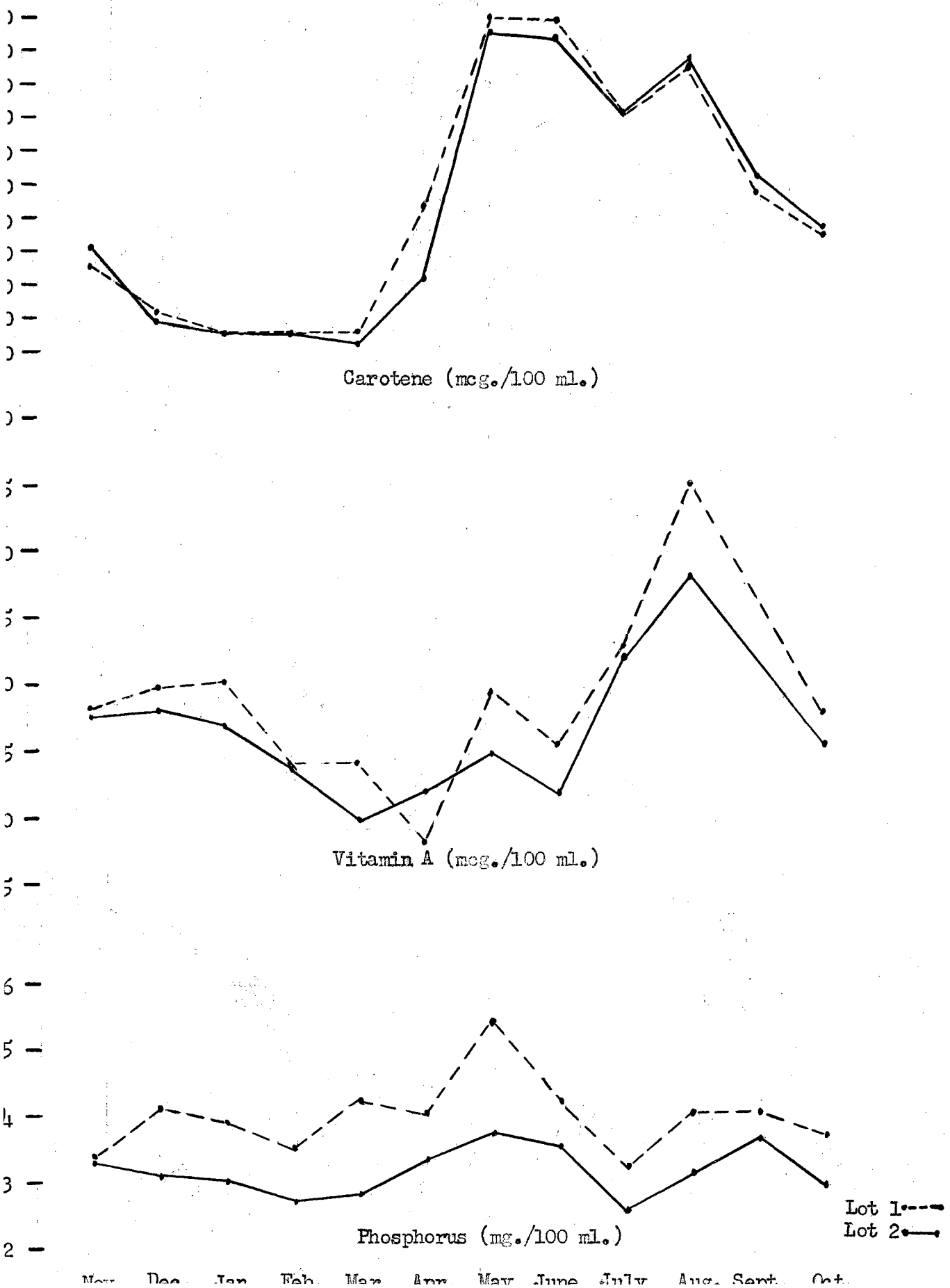
(a) Two years only.

(b) Three years only

* Statistically significant at the 5% level of Probability.

** Statistically significant at the 1% level of Probability.

FIGURE 1


Carotene (mcg./100 ml.)
Vitamin A (mcg./100 ml.)
Phosphorus (mg./100 ml.)
Lot 1
Lot 2
Nov. Dec. Jan. Feb. Mar. Apr. May June July Aug. Sept. Oct.

Carotene: Definite seasonal trends in plasma carotene were found in both lots of cows; however, there was no consistant pattern of differences between the two lots at the bleeding dates. A significant difference at the 1 percent level of probability was found at the April bleeding date. The lowest plasma carotene levels were found to occur during the month of March (145 mcg. for lot 1 and 117 mcg. per 100 ml. of plasma for lot 2). It is assumed that this was due, in part, to the fact that many of the cows were calving and starting lactation during this month and the carotene content of the dry forage was low. Davis and Madsen (1941) and Sutton and Soldner (1945) found marked fluctuation in blood carotene levels due to differences of carotene intake as affected by season of the year.

Gallup and Kuhlman (1941), and Sutton and Soldner (1943), Kuhlman and Gallup (1944), and Sutton and Co-workers (1945) observed a drop in plasma carotene levels a few days before parturition and a further drop immediately following parturition in dairy cattle. Van Arsdell et al., (1950) obtained similar results with Hereford cows.

Although the average carotene levels of both lots remained within the accepted normal range at all bleeding dates, during the winter of 1948 (March bleeding date) the level for lot 2 dropped as low as 85 mcg. per 100 ml. of plasma. Payne and Kingman (1945) found that aged Hereford cows with carotene levels as low as 82.88 $\pm$ 4.11 mcg. per 100 ml. of plasma showed no symptoms of deficiency over a two year period. However, a carotene level of 97.18 $\pm$ 7.68 mcg. per 100 ml. of plasma produced retained placenta and nutritional abortion in range Hereford heifers. No carotene deficiency symptoms were observed in any of the cows of this study. This observation agrees with that reported by Van Arsdell, et al. (1950). They found that aged Hereford cows were able to produce calves normally when subjected to short periods of deficient carotene intake.

Vitamin A: The plasma vitamin A values of the cows fluctuated with season and ration. The cows of lot 1 were higher in this plasma constituent than those of lot 2 at every bleeding date except April. Significant differences were found at the 1 percent level of probability at the January and March bleeding dates and at the 5 percent level at the May, June, August and September bleeding dates. A significant difference at the 1 percent level of probability was found at the April bleeding date in favor of lot 2. Davis and Madsen (1941) studying cattle on restricted levels of carotene intake, found that the vitamin A content of the blood plasma was dependent on the carotene intake and previous storage of these factors. Sutton and Soldner (1945) found that the average monthly range of plasma vitamin A values for both lots were found to be lowest during the months of March and April. Approximately half of the cows of each lot had already calved and were lactating by the March bleeding date. Sutton and Soldner (1943) and Kuhlman and Gallup (1944), studying dairy cow, found that plasma vitamin A dropped at or immediately after parturition. A further drop was noted immediately after parturition. Van Arsdell, et al. (1950) obtained similar results with Hereford cows.

Calcium and Ascorbic Acid: Plasma calcium and ascorbic acid determinations were made during the first two years of the experiment. The plasma calcium was found to remain rather constant and within the accepted normal range (9 to 13 mg. %) regardless of treatment. The plasma ascorbic acid values were found to vary widely within lots and between lots. The average values fell in the range accepted as normal (300 to 400 mcg. %) for the bovine. After two years it was felt that no data was being derived by determining plasma calcium or ascorbic acid, thus the determinations were discontinued.

Blood Composition, Calves:

The analyses for the various constituents of the calves' blood are shown in Table IV.

Phosphorus: The average inorganic plasma phosphorus content of the calves of both lots was about the same at each bleeding date. On the first bleeding date (March) the calves were found to have average values of 8.1 and 8.0 mg. per 100 ml. of blood plasma for lots 1 and 2 respectively. These values decreased gradually, with fluctuation, until they were 7.3 and 6.8 mg. per 100 ml. of blood plasma for lots 1 and 2, respectively, at the last date the calves were bled (August).

Carotene and Vitamin A: The blood plasma carotene values of both lots of calves were quite low at the initial bleeding (March), but increased rapidly up to the July bleeding date. Significant differences at the 5 percent level of probability were found at the April and August bleeding dates. The lot 1 plasma values were found to be slightly higher at each bleeding than those of lot 2.

Although differences were found in plasma vitamin A levels between the two lots, a definite trend was not indicated.

Table IV. BLOOD CONSTITUENTS OF CALVES BY LOTS, FOUR-YEAR AVERAGE.
(Units per 100 ml. plasma)

	March	April	May (a)	June	July	August
			Phosphorus (mg.)			
Lot 1	8.1	7.9	8.5	8.1	7.6	7.3
Lot 2	8.0	7.9	8.0	8.2	7.3	6.8
			Carotene (mcg.)			
Lot 1	16.4	129*	394	543	739	629*
Lot 2	11.8	77	390	518	723	549
			Vitamin A (mcg.)			
Lot 1	18.1	14.9	20.4	21.2	34.0	26.7
Lot 2	16.9	12.8	23.6	20.7	36.3	26.2

(a) Three years only.

* Significant at the 5% level of probability.

EXPERIMENT II: Protein Supplements

Procedure:

The study reported herein is a summary of three years data (Fall 1947 to Fall 1950). All values reported are three year averages unless otherwise specified.

Two groups of grade Hereford cows, 20 in each group, were used in this study. The cows of each lot were grazed year-long in native grass pastures containing 12.25 acres per head. Approximately 2.25 acres of the 12.25 acres consisted of old cultivated fields allowed to go back to grass. This acreage provided relatively poor forage. The winter period started in late October or early November and terminated in early April for each of the years of this study. During the winter period, the cows of lot 1 were fed alfalfa hay as the protein supplement and the average consumption was 8.19 pounds per head per day. The cows of lot 2 were fed cottonseed cake and consumed an average of 2.57 pounds per head per day. The supplements were fed every other day. The total daily intake of crude protein supplied by the supplements during the winter months was approximately the same for the two groups. The cows of each lot had access, free choice, to salt and/or a mineral mixture containing phosphorus.

The records kept, general location, methods of handling, and chemical determinations made were identical to those reported for experiment I.

RESULTS AND DISCUSSION

Production Record:

Pertinent data relative to weights and feeds fed are shown in Table V.

Production of the cows of both lots was considered satisfactory.

The cows of lot 2, fed 2.57 pounds of cottonseed cake during the winter months, lost 11 pounds per cow to time of calving as compared to a loss of 20 pounds for the same period for the cows of lot 1, fed 8.19 pounds of alfalfa hay.

The cows of lot 1, however, gained 21 pounds more per cow during the year than the cows of lot 2. The cows of lot 2 weaned a 97 percent calf crop as compared to a 90 percent calf crop for the cows of lot 1. The average weaning weight per calf for lots 1 and 2 was 439 and 445, respectively.

The total yearly feed cost per cow for those fed cottonseed cake during the winter was $40.92 compared to $35.27 for those fed alfalfa hay. Labor costs were not included.

The production records of these two lots of cows demonstrated that approximately 8 pounds of alfalfa hay satisfactorily replaced 2¼ pounds of cottonseed cake as a protein supplement for commercial cows grazing dry native grass pastures. No statistically significant differences were found in average gain in weight per cow, average birth weight per calf, or average weaning weight per calf for the three years covered by this study.

Feed Analysis:

The averaged analyses of the feeds fed are presented in Table VI.

The phosphorus content of the grasses ranged from 0.034 percent in January to 0.114 percent in May. At each date the grass samples were taken, they were found to be lower than the value (0.12%) Mitchell (1947) reported to be minimal for the bovine.

Table V. SUMMARY OF PRODUCTION DATA: AVERAGE OF THREE SEASONS.
(1947-48, 1948-49, 1949-50.)

	Lot 1 (a) Grazed Year-long Fed Alfalfa Hay	Lot 2 (b) Grazed Year-long Fed C.S.C.
Average weight per cow (lbs)		
Beginning winter period	1012	1005
Before calving	992	994
At end of summer period	1074	1046
Gain for three years	62	41
Average birth weight per calf (c)	75	75
Average weaning weight per calf (c)	439	445
Percent calf crop weaned	90	97
Rations fed, winter		
Prairie hay	(d)	(d)
Native grass pasture	12.25 acres	12.25 acres
Alfalfa hay	8.19 lbs	———
Cottonseed cake	———	2.57 lbs.
Mineral mixture (e)	free choice	free choice
Rations fed, summer		
Native grass pasture	12.25 acres	12.25 acres
Mineral mixture (e)	free choice	free choice
Total feed cost (dollars)	$35.27	$40.92

(a) One cow died during 1948-49 season.
(b) Two cows died, one during 1947-48 season and one during 1948-49 season.
(c) Weights corrected of age, sex, season and age of dam.
(d) Prairie hay fed only when snow covered the ground for an extended period.
(e) 3 parts salt to 1 part bone meal during 1947-48 season.
2 parts salt to 1 part bone meal during 1948-49 and winter of 1949-50 seasons.
1-1-1 fed during summer of 1950.

Table VI. FEED ANALYSES: Three-Year Average.

	Percent Dry Matter	Percent composition or dry matter							
		Ash	Prot.	Fat	Fiber	N.F.E.	Ca.	P.	Carotene*
Cottonseed cake	93.15	6.08	43.74	5.23	11.90	37.92	.236	.971	—
Alfalfa hay	93.45	7.95	15.64	2.65	29.84	42.44	1.300	.182	22
Bone meal	95.66	91.70					32.91	15.38	
Ground limestone	99.67						36.18		
Grass**									
November	82.39	5.01	2.53	1.74	40.02	50.40	.253	.046	14
January***	93.37	5.74	2.52	1.70	40.42	49.30	.283	.034	Trace
May	37.12	6.48	8.40	2.39	32.13	50.20	.289	.114	401
August	41.90	5.99	5.27	2.20	36.82	49.35	.284	.084	112
October	53.09	5.18	3.23	1.62	37.24	52.44	.244	.048	16

*Parts per million.
**Averages, by species, of the four predominant grasses: Big Bluestem, Little Bluestem, Indian, and Switch.
***Two years only.

The crude protein content (dry matter basis) of the grass samples ranged from 2.52 percent in January to 8.40 percent in May. The grasses were deficient in crude protein at each sampling date except the May date when compared to that level stated by the National Research Council (1945). They stated that dry grasses containing less than 8 percent crude protein is deficient in protein for all classes of cattle.

Blood Composition, Cows:

The analyses for the various constituents of the cows' blood are shown in Table VII. A graphic picture of these various constituents is presented in Figure 2.

Phosphorus: The blood plasma inorganic phosphorus content of the cows of both lots was considered satisfactory for normal production, but at each winter bleeding date the phosphorus level was highest in the plasma of the cows receiving cottonseed cake. The phosphorus content of this supplement compared to alfalfa hay was probably responsible for the higher blood plasma phosphorus levels. Significant differences at the 1 percent level of probability were found in favor of lot 2 at each of the winter bleeding dates and at the April, July and September bleeding dates of the summer months.

The average phosphorus level during the winter months was 3.8 and 5.5 mg. per 100 ml. of plasma for lots 1 and 2, respectively. The plasma phosphorus values were, in general, lower than those values reported by Black and Associates (1942) who described symptoms of a phosphorus deficiency that developed in cattle when the blood phosphorus content was below 4 mg. per 100 ml. of whole blood. The values were in agreement with those reported by Watkins and Knox (1948). These workers reported values of 2.11 mg. percent during the winter months to a high of 5.37 mg. percent during the summer months.

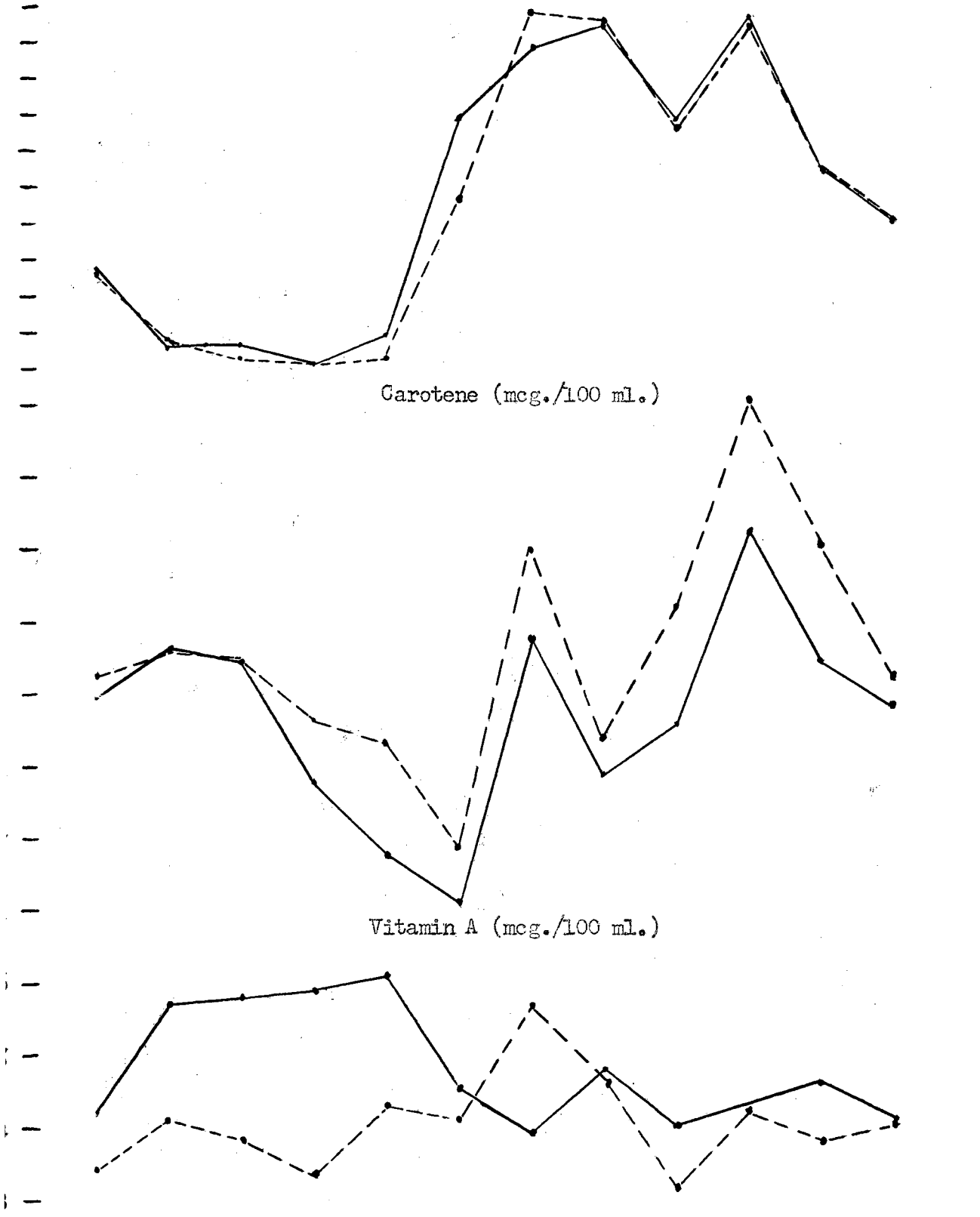
Carotene: Considerable seasonal variation of plasma carotene was observed in this study. The lots followed essentially the same trend. The

Table VII. BLOOD CONSTITUENTS OF COWS BY LOTS: THREE-YEAR AVERAGE.
(Units per 100 ml. plasma)

	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May (a)	June	July	Aug.	Sept.	Oct.
							Phosphorus (mg)					
Lot 1	3.4	4.1	3.8	3.3	4.3	4.1	5.7**	4.7	3.1	4.2	3.8	4.0
Lot 2	4.2**	5.7**	5.8**	5.9**	6.1**	4.5**	3.4	4.8	4.0**	4.3	4.6**	4.1
							Carotene (mcg)					
Lot 1	359	153	124	115	127	571	1088	1066	770	1056	650	519
Lot 2	371	160	163**	117	190**	797**	991	1046	794	1079	654	509
							Vitamin A (mcg)					
Lot 1	28.2	29.9	29.4	25.2**	23.5**	16.3**	37.0*	23.8	33.0**	47.2**	37.4**	28.1
Lot 2	26.9	30.1	29.2	20.7	15.9	12.5	30.9	21.1	24.8	38.2	29.3	26.2

(a) One year only
* Significant at 5% level of probability.
** Significant at 1% level of probability.

FIGURE 2


Carotene (mcg./100 ml.)
Vitamin A (mcg./100 ml.)
Phosphorus (mg./100 ml.)
Lot 1
Lot 2
Nov. Dec. Jan. Feb. Mar. Apr. May June July Aug. Sept. Oct.

The carotene levels of both lots remained within the accepted normal range throughout the year.

The lowest blood plasma carotene levels in both lots were found to occur during the late winter months (115 and 117 mcg percent for lots 1 and 2, respectively at the February bleeding date). This was probably due, in part, to the fact that the carotene content of the dry forage was low and that the cows began lactating during this period. Significant differences at the 1 percent level were found in favor of lot 2 at the January, March, and April bleeding dates. It might be expected that the plasma carotene of the cows fed alfalfa hay would be higher than those fed cottonseed cake, because of the greater carotene content of the alfalfa hay as compared to cottonseed cake. This was not the case, however. A possible explanation for this finding was the fact that the cows receiving alfalfa hay did not graze as much as those receiving only the cottonseed cake. The latter were presumably eating larger amounts of green winter grasses found in each of the pastures during most of the winter. Davis and Madsen (1941) and Van Arsdell, et al. (1950) found marked fluctuation in blood carotene levels due to differences of carotene intake. Several other workers studying dairy cattle have made the same observation.

Vitamin A: The average vitamin A values for both lots were within the accepted normal range. The values were, in general, lowest during the months of March and April. Most of the cows of each lot were in beginning lactation during this time. Sutton and Soldner (1943) and Kuhlman and Gallup (1944) studying dairy cows found that plasma vitamin A values dropped at or immediately before parturition. A further drop was found immediately after parturition. Van Arsdell, et al. (1950) found similar results with Hereford cows.

The cows of lot 1 were found to be higher in plasma vitamin A at every bleeding date (except December) than the cows of lot 2. Significant differences

at the 1 percent level of probability were found at the February, March, April, July, August, and September bleeding dates in favor of lot 1.

Blood Composition, Calves:

The analyses for the various constituents of the calves's blood are shown in Table VIII.

Phosphorus: The average inorganic blood plasma phosphorus content of the calves from each lot varied somewhat during the summer months. The calves of lot 2 were slightly higher in this blood constituent at four of the six bleeding dates. However, a significant difference was found only at the June bleeding date. The values of both lots ranged from 7.5 to 9.7 mg. per 100 ml. of plasma.

Carotene and Vitamin A: The plasma carotene content of the calves of lot 1 was higher at every bleeding date than that of the calves of lot 2; however, the calves of lot 2 had higher levels of vitamin A at every bleeding (except the initial bleeding) than the calves of lot 1. These differences, though relatively consistant, were not great and were not believed to indicate differences in the nutritional status of the animals. Significance at the 1 percent level was found in plasma vitamin A in favor of lot 2 at the August bleeding date.

Table VIII. BLOOD CONSTITUENTS OF CALVES, BY LOTS: THREE-YEAR AVERAGE
(Units per 100 ml. plasma)

	March	April	May (a)	June	July	August
			Phosphorus (mg)			
Lot 1	7.8	7.9	8.9	8.6	7.8	7.5
Lot 2	7.6	8.4	9.0	9.7**	7.7	7.8
			Carotene (mcg)			
Lot 1	13.5	161	426	542	745	647
Lot 2	9.6	127	409	539	708	627
			Vitamin A (mcg)			
Lot 1	17.4	15.9	22.2	20.0	37.5	25.2
Lot 2	16.8	16.2	24.8	22.6	40.8	30.5**

(a) Two years only
** Significant at 1% level of probability.

SUMMARY

1. Under the conditions of this study, the system of grazing cows year-long and feeding 8.17 pounds of alfalfa hay daily during the winter months was a more practical and economical method of handling commercial range Hereford cows than the system of grazing during the seven summer months and feeding approximately 4.7 pounds of alfalfa and 16 pounds of prairie hay in a small trap during the five winter months. The average yearly feed cost of the cows grazed year-long and fed alfalfa hay during the winter was less than the feed cost of cows which were grazed only seven months and fed alfalfa hay and prairie hay in a small trap during the winter. In addition, the cows grazed year-long were heavier at the end of the year and produced more calves that were heavier at both birth and weaning than were the calves from the cows wintered in the trap.

2. Statistically significant differences were obtained between lots in plasma inorganic phosphorus levels during most of the year. The cows fed prairie and alfalfa hay in a trap during the winter had the lowest levels at each bleeding date. Although typical phosphorus deficiency symptoms were not observed, the plasma phosphorus levels were low in this lot and may have had some affect on both the cows and the calves produced.

3. Seasonal variation in the plasma carotene and vitamin A levels were observed. The lowest plasma carotene levels in both lots were found at the March bleeding date. This bleeding date was near the average parturition date, suggesting the direct correlation between lactation and decrease in plasma carotene level. Significant differences in plasma vitamin A were found in favor of the cows grazed year-long at two of the winter and four of the summer bleeding dates.

4. The average inorganic blood plasma phosphorus content of the calves of both lots was about the same at each bleeding date.

5. The blood plasma carotene content of both lots of calves was quite low at the initial bleeding (March), but increased rapidly up to the July bleeding date. The calves from the cows grazed year-long had plasma values that were slightly higher at each bleeding date than those from the cows grazed only 7 months. Although differences were found in plasma vitamin A levels between lots, a definite trend was not indicated.

6. The results of the protein supplement study showed that approximately 8 pounds of alfalfa hay satisfactorily replaced 2¼ pounds of cottonseed cake as a protein supplement for commercial cows grazing dry native grass pastures.

7. The blood plasma inorganic phosphorus content of the cows of both lots was considered satisfactory for normal production, but at each winter bleeding date the phosphorus level was significantly higher in the plasma of the cows receiving cottonseed cake.

8. Again, as under systems of management, seasonal variation in plasma carotene and vitamin A levels was observed. The lowest blood plasma carotene levels in both lots were found to occur during the late winter months. Statistically significant differences were found in plasma vitamin A at 7 of the 12 bleeding dates in favor of lot 1 (alfalfa lot).

9. The calves of lot 2 (cottonseed cake lot) were slightly higher in plasma inorganic phosphorus, at four of the six bleeding dates, than the calves of lot 1.

10. The plasma carotene content of the calves of lot 1 was higher at every bleeding date than that of the calves of lot 2; however, the calves of lot 2 had higher levels of vitamin A at every bleeding (except the initial bleeding) than the calves of lot 1.

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PART II. A LIVER BIOPSY TECHNIQUE AND OBSERVATIONS OF ITS USE IN VITAMIN A STUDIES WITH BEEF CATTLE

INTRODUCTION

In studies with range Hereford cows at this station, carotene and vitamin A levels of blood plasma have been observed for several years. Seasonal changes in these two blood plasma constituents have been noted. In some cases extremely low values have been found although no apparent deficiency symptoms were present. The validity of these values as estimates of the vitamin A liver storage in the animal is not known.

Many investigations have been conducted on the metabolism of carotene and vitamin A, using the rat as an experimental animal. Such problems as the conversion, absorption, storage and distribution of carotene and vitamin A in the body of the rat have been extensively studied. Only a few such studies have been reported in beef cattle. This seems reasonable since the rat is relatively an inexpensive experimental animal and large numbers of them may be sacrificed for liver studies. Such is not the case with beef cattle which partially explains why only a few studies on the liver metabolism of vitamin A have been conducted.

Recently, vitamin A studies in beef cattle involving the liver have been attempted where the animal was not sacrificed. The technique used to obtain liver samples has been partial hepatectomy or aspiration biopsy. The study reported herein was initiated to study certain aspects of carotene and vitamin A metabolism in beef cattle using a liver biopsy technique developed for this purpose.

REVIEW OF LITERATURE

The Metabolism of Carotene and Vitamin A

Vitamin A was one of the first vitamins recognized as an essential dietary substance. Factors influencing the storage of vitamin A in the body and its distribution in the liver and blood have been the subject of many investigation. Research on the absorption of carotene and vitamin A and the relationship of the level of dietary intake to liver and blood levels has given information on the metabolism of these nutrients.

Conversion of Carotene to Vitamin A:

The fact that carotene is converted to vitamin A in the animal body has been known for several years. Steenbock (1919) conceived the brilliant idea, on the basis of such experimental data as was then available, that the vitamin A effect of foods was associated with the yellow pigment in these foods. He prepared carotene and tested it on the rat for its vitamin A activity. From his results he concluded that carotene afforded the same protection to rats as substances that contained vitamin A.

The liver as a site of conversion:

The liver has for a long time been considered as the main site of metabolic activity and it seemed natural that Moore (1931) was correct in assuming that the liver was the site of conversion of carotene to vitamin A. He found that rats receiving a diet rich in carotene, for three months, contained little carotene and high amounts of vitamin A in their liver. Olcott and McCann (1931) found a slight deflection could be detected in the absorption spectrum at 328 mu when rat liver slices were incubated with a carotene solution for thirty-six hours. From this result they concluded that conversion of carotene takes place in the liver.

The intestine as a site of conversion:

(1) Rat Studies

During recent years several investigators have questioned the liver as the site of conversion of carotene to vitamin A. Sexton, et al. (1946) demonstrated that intravenously administered carotene was deposited in the liver of rats and that it was present 46 days after injection. The rats died from vitamin A deficiency although sufficient carotene was found in the liver to maintain life. These authors interpreted their results as evidence that carotene was converted to vitamin A at some site in the body other than in the liver.

Glover, et al. and Wiese, et al. (1947) studied the "in vitro" conversion of carotene in vitamin A depleted rats. These authors obtained similar results indicating that conversion of carotene to vitamin A takes place in the intestine of the rat.

Further evidence of the conversion of carotene to vitamin A in the intestine was reported by Mattson, et al. (1947). They give oral doses of partially purified beta-carotene solution to vitamin A depleted rats. They found that during the first 3 hours after dosing, the amounts of vitamin A in the intestinal wall invariably exceeded that in the liver.

Probably the most convincing evidence supporting the intestine as the site of conversion of carotene to vitamin A has been that of Krause and Pierce (1948). These workers completely cut off the hepatic circulation and then give carotene orally to normal rats. Their results indicated that the hepatic circulation was not necessary for conversion of carotene to vitamin A. These authors did not, however, rule out the liver as a possible auxillary site of conversion of carotene to vitamin A.

Mattson (1948) conducted a qualitative test for vitamin A in 10 vitamin A deficient rats given a carotene supplement 4 hours previous to the test. This experiment was conducted because the "Carr-Price" reaction, used by many of the previous investigators for vitamin A determinations, is not specific for vitamin A. His results indicated that the material isolated from the intestines of the rats was vitamin A.

Thompson and others (1949) obtained further evidence for the conversion of carotene to vitamin A in the intestine by dispersing carotene in water by the use of acetone and then feeding it as a colloid mixed with a fat free diet to vitamin A deficient rats. They found little or no carotene in the portal or systemic blood; however, vitamin A ester appeared in increased amounts in the blood within 2 hours after carotene had been fed. It appeared exclusively in the ester form within 75 minutes in the lymph from ducts draining the mesenteric lymphatics.

Thompson, Ganguly and Kon (1949) reported similar results to those reported by Thompson and others (1949) while studying vitamin A deficient rats given beta-carotene by mouth.

Reviews of the above mentioned works are presented in recent volumes of Nutrition Reviews (1948, 1949).

(2) Large Animal Studies

Probably the most complete work on the large animal to date, has been that of the English workers, Goodwin and Gregory (1948). These workers studied the conversion of carotene to vitamin A in rabbits, goats and sheep. Rabbits were used to check the efficiency of the conversion mechanism. To study the problem of conversion in the sheep and goat, they used a fistulae of the thoracic duct and an abomasal cannula. In goats, increases in vitamin A levels in the lymph were obtained after feeding carotene, proving that vitamin A was formed from carotene in the gut wall and was transported to the liver via the lymph. Similar results were obtained with sheep.

Elliot (1949) studied the site of absorption and conversion of carotene to vitamin A in the dairy calf. The exact levels of carotene fed were not reported. Blood samples were taken at various sites along the intestinal tract and significant increases of vitamin A were found following intakes of carotene concentrate. This investigator also injected blood plasma high in carotene,

intravenously, into dairy calves. The liver values of vitamin A did not increase in Guernsey calves but did increase in Holstein calves. The results indicated conversion could take place in tissues other than the intestines.

Klosterman, et al. (1949) injected a group of 75 pound vitamin A deficient lambs with crystalline carotene suspended in water. Upon injection into the blood stream the carotene was removed very rapidly; however, no vitamin A increase in the blood or liver was found. Lambs given carotene by mouth or allowed green grass for a short period showed an increase of vitamin A in the blood serum.

Thompson, et al. (1947) and Thompson, Ganguly and Kon (1949) studied the intestine as a site of conversion of carotene to vitamin A in the pig. They found that pigs given 600 mg. of beta-carotene in arachis oil 3 to 7 hours before slaughter had increased vitamin A ester in the blood plasma. Vitamin A was also found in the mesenteric lymphatics and in the intestinal wall in quantities much larger than in control pigs.

Swick, et al. (1949) found that weanling pigs depleted of vitamin A had a marked increase in the concentration of vitamin A in the mesenteric lymph and a smaller rise in vitamin A content of the blood plasma and intestinal wall, when large amounts of carotene or vitamin A were administered 6 to 7 hours before slaughter. These results as well as those of Thompson, et al. were taken as evidence that carotene is converted to vitamin A in the intestinal wall of the pig.

Stallcup and Herman (1950) studied the " in vitro" conversion of carotene to vitamin A in the dairy calf. They obtained results which indicated the small intestine converted carotene to vitamin A; however, minced liver slices resulted in an even better conversion.

Absorption:

Ahmad (1931) reported that only 10 percent of the carotene fed in a fat

free diet was absorbed. Absorption was nearly complete, however, when fed in a diet containing 10 percent fat.

Zechmeister and Tuzson (1934) noted that about 75 percent of the carotene and 40 percent of the xanthophylls fed horses were present in the feces.

Kemmerer and Fraps (1938) studied the digestibility of carotene by rats and chicks and found that the percentage of carotene digested apparently depended on the quantity fed, carrier, and kind of animal.

Ericksen and Hoeygaard (1941) found that absorption of carotene depended upon the source and method of preparation of the carotene containing food.

Kruela (1947) reported that, in man, the excretion of carotene on a fat-free diet amounted to about 90 percent when finely grated carrots were consumed and to about 30 to 35 percent when dissolved in oil.

Eden and Sellers (1948) studied the absorption of vitamin A in the bullock. Eleven bullocks weighing 150-250 kg. were given halibut liver oil emulsified in reconstituted separated milk. They were unable to obtain conclusive evidence that vitamin A was carried from the intestine by the portal blood; however, the lymph appeared to be the main pathway by which vitamin A reached the general circulation.

Jacobson, et al. (1948) fed milk, into which a vitamin A supplement had been homogenized, to a series of paired, dairy calves, alternately by nipple pail and by stomach tube introduced into the rumino-reticular cavity. In a second series of calves, administration of the vitamin supplement by capsule was compared with nipple pail feeding of the homogenized product. The results indicated that the rate of absorption, as measured by the concentration of the carotene and vitamin A in the blood plasma, was more rapid when these substances passed directly into the abomasum than when they entered the rumino-reticular cavity. The absorption rate following administration of carotene in capsules indicated passage into the rumino-reticular cavity before entering the abomasum.

Eden and Sellers (1949) studying the absorption of vitamin A, found that four hours after the oral administration of vitamin A ester almost complete hydrolysis had occured in the intestinal lumen of some bovine, whereas in others hydrolysis was only partial. Estimates of vitamin A in the intestinal mucosa showed that 75 percent of the absorbed vitamin A was in the ester form. The same was true when vitamin A alchol was fed. The results indicated that the intestinal wall is capable of esterifying free vitamin A alchol, but no evidence was obtained that vitamin A was absorbed in its unaltered form.

Sellers and Eden (1949) studied the effect of "white scours" on the absorption of vitamin A by calves. Determination of the concentration of vitamin A in plasma, feces and liver of control and scouring calves indicated that scouring caused impaired absorption of the vitamin. Single and repeated oral doses of vitamin A showed a 300 to 500 percent increase in plasma level four hours after administration in control animals, while little or no increase was obtained with scouring calves. Only traces of vitamin A were found in feces of controls, while in the scouring animals the bulk of the vitamin was lost in the feces. The liver reserves of vitamin A in the scouring animals were only one-third those in the controls.

Crowley and Allen (1950) studied the effect of the kind of "carrier" and method of dispersion on the absorption of carotene by young dairy calves. They found a difference in the absorption of carotene when it was dispersed in different oils. Excretion data was not considered reliable, but an estimated average of 2/3 of the carotene fed was found in the feces.

Storage and Mobilization: (1) Rat Studies

Baumann, et al. (1934) found that 95 percent of the vitamin A stored in rats was found in the liver, providing body stores of vitamin A were adequate.

Davies and Moore (1934) studied the distribution of vitamin A in the normal and hypervitaminotic rat and found that hypervitaminosis A did not depend on the

absolute amount of "A" present in the organism but on the ingestion of the vitamin A at a greater rate than at which it could be stored by the liver or eliminated by the organism. Carotene was found to be more slowly absorbed than vitamin A.

The findings of McCoord and Luce-Clausen (1934) indicated that as long as there was a fair amount of vitamin A in the liver, there was little relationship between the blood level of vitamin A and its concentration in the liver.

Horton, et al. (1941) found that rat blood and liver levels of vitamin A declined, when the subjects were fed a diet deficient in vitamin A, in a parallel manner. This indicates a relationship between blood level of vitamin A and concentration in the liver when the liver stores drop below a critical level.

Grey and Cowley (1942) found that when vitamin A was fed rats in moderate amounts over a long period of time, the vitamin A stored in the liver was gradually transformed from its combination with several fatty acids to union with only one, possibly palmitic acid.

Josephs (1942) (1939) conducted studies on rats which furnished information on the relationship between blood and liver levels of vitamin A. Weanling rats were placed on a basal vitamin A deficient diet; one group was fed 2 to 5 I. U. of vitamin A daily; another, 20 to 100 I. U. and a third 1000 I. U. or more daily. Some of the animals in each group were killed 24 hours after the last dose of vitamin A was fed, and the content of vitamin A in the serum and liver determined. Others were killed at varying intervals up to about 40 days after discontinuance of the vitamin administration. He found the vitamin A tended to be established, at a level of 0.5 to 1.0 units per cc. of serum, and that the level could be forced higher by an excessive administration which taxed the ability of tissues to remove it from the blood, but that the level in the blood would fall rapidly when the excessive administration was

discontinued. He considered the serum vitamin A levels of rats fed on maintenance and moderate amounts as "essentially the same." Josephs also stated that "blood lipids acted as a vehicle for vitamin A, holding it in the blood so that for a time it may tend to accumulate."

Lewis, et al. (1942) reported that high vitamin A blood levels resulting from ingestion of large doses of vitamin A were maintained over a long period after discontinuance of excessive intake. They suggested that, "There may well be a degree of liver saturation beyond which the regulatory mechanisms for control of blood level of vitamin A is no longer effective."

Popper and Brenner (1942) studied changes in the histologic distribution of vitamin A in rat liver in an attempt to learn the role of the Kupffer cells in hypervitaminosis A and during vitamin A deficiency. They found the Kupffer cells were high in vitamin A fluorescence during the hypervitaminosis stage, while the liver cells showed but moderate fluorescence localized in lipoid droplets. Beginning with the second week of a deficiency diet the vitamin A fluorescence in the Kupffer cells commenced to diminish. They concluded, that, "Kupffer cells destroy the excess in times of hypervitaminosis and do not discharge the excess into the blood. During depletion the Kupffer cells appear to distribute the residual vitamin A."

Popper and Chinn (1942) found that fatty livers appeared in 2 to 10 days in rats fed a low choline, low methionine, high cystine diet, supplemented with carotene and the crystalline vitamins. Under these conditions, the total vitamin A content of the livers decreased despite liberal carotene feeding.

Popper, Steigmann, and Dyniewicz (1942) found that rats intoxicated with carbon tetrachloride showed an accumulation of vitamin A fluorescence in the liver lobules. Proliferation of the connective tissue also occurred. The fluorescence in the central fatty areas became stronger but disappeared from liver and Kupffer cells in the uninvolved peripheral areas. When true liver

cirrhosis condition occurred, vitamin A fluorescence was found in only a few of the original centers and in the surrounding histiocytes. Rats receiving a high carbohydrate diet showed more vitamin A fluorescence in the normal Kupffer cells and liver cells than did rats on a high protein or high fat diet. Their findings indicated that during experimental liver damage, there was actually a shift of A from the unchanged liver areas to the central fatty areas.

Josephs (1944) studying hypervitaminosis A and carotenemia in rats, found evidence of existance of a mechanism to maintain a constant level of vitamin A in blood but such was not true for carotene. Large doses of vitamin A were toxic but such was not the case for carotene.

Glover, et al. (1947) studied blood vitamin A levels and liver stores in rats. They demonstrated that plasma vitamin A levels of rats were proportional to concentration of vitamin A alchol in the liver, but not proportional to the total liver stores of vitamin A which consisted mainly of esters. The plasma vitamin A levels were maintained near normal (35 to 40 I. U./100cc) even when liver stores approached exhaustion.

Davies and Moore (1948) studied the quantative aspects of vitamin A storage in the rat and found three factors appeared to lower the percentage storage of vitamin A: (1) At low doses about 100 I U. was lost, or applied to other purposes, before storage in the liver commenced. The proportion 'lost', decreased as the dose was raised until a toxic level was reached. (2) With toxic overdoses a high proportion of the dose disappeared. (3) With prolonged massive dosing, without obvious toxic symptoms, the percentage of vitamin A stored was found to be low when a high concentration was attained in the liver. These workers inferred that in the human subject and domestic animals actual saturation of the liver with vitamin A presumably never occurred under ordinary conditions of nutrition.

Guerrant (1949) studied the influence of age and vitamin A intake on the storage of vitamin A in the liver of the rat and found that livers of normal newly-born rats contained very little vitamin A. It more than doubled during the first seven days and then remained constant for the rest of the nursing period. The values increased rapidly during periods of rapid growth and reached a maximum at 170 days of age. The amounts stored in the livers of depleted rats depended upon the intake. With vitamin A intakes in excess of that required for optimum growth, liver stores increased in proportion to intake up to 17,600 U.S.P. units.

Krause (1949) found that an inverse relationship existed between blood and liver levels of vitamin A in normal male and female rats and in those depleted females whose liver content ranged as low and 600 I. U./total liver. When the total liver content fell below 600 I. U. in males there was a parallelism between blood and liver levels.

Sobel and Rosenberg (1950) found that the vitamin A stores of suckling rats whose dams were given vitamin A in an aqueous dispersion were about 4 times as great as the stores of rats whose dams received the same amount of vitamin A dissolved in oil. They concluded that orally ingested vitamin A in an aqueous dispersion was more effectively transferred to milk and stored in the suckling than vitamin A in an oily solution.

Vavich and Kemmerer (1950), studying factors that influence the utilization of carotene for storage of vitamin A, found that the size of the rat used as an experimental animal markedly influenced the utilization of carotene. When 60 mcg. of beta-carotene equivalent was fed, the combined liver and kidney storage of vitamin A was higher in three groups of rats averaging 44 to 50 grams than it was in three groups of rats averaging 99 to 103 grams. The amount of basal diet consumed had no effect on the vitamin storage.

(2) Large Animal Studies

Guilbert and Hart (1935) found 93% of the body stores of vitamin A in the liver of cattle. The principal stores of carotene were found in the body fat.

In a clinical study, Meyer, et al. (1942) made a comparison of liver vitamin A stores and plasma levels in humans. Liver biopsies were taken during laparatomies in 34 patients. Blood was withdrawn before, during, and immediately after the operation. They found no distinct parallelism between plasma vitamin A values and liver stores. However, when the plasma value was above 80 I. U. per 100 ml. the liver vitamin A was never low. On the other hand, a low plasma value was not necessarily correlated with low liver storage. The authors suggested, that, "the plasma level of vitamin A reflected largely the recent intake and that a liver-plasma balance was not rapidly established during a period of depletion." If such is the case, plasma vitamin A would indicate the degree of storage only after a long control period.

Braun (1945) studied carotenoid and vitamin A levels in cattle. Carotenoid and vitamin A values were obtained from liver samples of cows maintained on four dietary groups with different carotenoid and vitamin A intake. The vitamin A levels of the liver were significantly different among three of the four dietary groups. Results obtained from the livers of animals which had been maintained on a vitamin A enriched diet 6 to 8 months previously indicated a rather lasting effect on vitamin A storage of a relatively short period of vitamin A feeding. A relationship between carotenoid and vitamin A levels in the liver were found to exist similar to the vitamin A/carotene ratio observed in the blood. Changes in the ratio with changing carotenoid levels were found and the authors stated that this was probably due to the organism tending to maintain a constant vitamin A store. A tendency towards a direct relationship between vitamin A stores and the vitamin A level of the blood was found to exist only when the former fell below normal levels.

Frey and Jensen (1946) (1947) showed that a rapid decrease in hepatic stores of vitamin A and carotene occurred in cattle on a fattening ration. The rate of depletion of initially large hepatic reserves of vitamin A and carotene was given for 140 steers over an experimental period of 166 days. The rate of depletion of the hepatic reserves of vitamin A and carotene decreased as the liver reserves of the two constituents decreased. Hepatic reserves of vitamin A were found to be more readily depleted than were hepatic reserves of carotene. Increasing values were found for the ratio Vitamin A/carotene in the liver with increasing carotene reserves of the liver. The hepatic reserves of carotene were maintained in direct proportion to the carotene intake. An increasing rate in loss of hepatic reserves of vitamin A occurred with decreasing carotene intake.

Frey, Jensen and Connell (1947) maintained 8 month old Hereford steers on a carotene-free basal ration containing daily vitamin A supplement levels of 0, 25, 100, 200 and 500 I. U. per pound of body weight. Serum levels of vitamin A and carotene were determined at 0, 27, 83, 159 and 277 days, and hepatic stores at 0, 166 and 280 days. Dietary vitamin A was found not to exert a sparing action on hepatic stores of carotene. Blood stores of carotene were depleted sooner than were hepatic stores. Hepatic stores of vitamin A increased in practically a linear relationship with intake throughout the range of vitamin A supplement fed. Serum levels increased rapidly up to an intake of 100 I. U. of vitamin A per pound of body weight daily. An intake of 100 I. U. of vitamin A per pound of body weight daily maintained nearly maximum serum levels of vitamin A in cattle under the conditions of their experiment. Serum levels and hepatic stores of vitamin A appeared to be controlled by different body mechanisms.

Spielman, et al. (1948) conducted studies to determine whether or not the vitamin A stored prenatally was utilized by the new born dairy calf. At birth,

calves from vitamin A-supplemented cows had higher plasma vitamin A levels, which increased with age, whereas no such increase was noted in calves from non-supplemented cows. At the end of 10 days the vitamin A remaining in the livers of calves from vitamin A-supplemented cows was higher than the amount in livers of calves from non-supplemented cows.

Hibbs and Pounden (1949), studying liver storage of carotenoids and vitamin A of dairy calves, found that rumen inoculations did not effect blood or liver vitamin A levels although a marked difference of rumen microorganisms resulted from inoculation and variation in feed.

Jacobson, et al. (1949) studied the effect of vitamin A and carotene intake on depletion time of young dairy calves. Calves were fed different quantities of vitamin A for the first 90 days of age then were given a vitamin A-deficient ration to determine the time required to deplete their stores. Stores were considered depleted when the blood plasma vitamin A values reached 4.0 mcg. per 100 ml. of plasma. Calves permitted to run with their dams on pasture required 4 months on the deficient ration to deplete their stores. Calves fed a limited whole milk ration with hay of above average quality required only 2 to 4 weeks. Fifty thousand I. U. of vitamin A for 50 or more days, in addition to the vitamin A received in the feed, was necessary in order to maintain stores and blood levels equal to those of calves reared with their dams on pasture.

Kachmar, et al. (1950) measured the relation between low tocopherol intake and utilization of vitamin A and carotene by dairy cattle. Liver samples were taken by a simple biosy technique. Increase in blood plasma vitamin A, decrease in blood plasma carotene, and the liver storage of vitamin A were essentially the same for vitamin A supplemented and non-supplemented groups.

Nezvesky, et al. (1950) studied the effect of vitamin A from prenatal storage and from ingestion of colostrum on the neonatal dairy calf. Their

data indicated that colostrum significantly increased plasma vitamin A from birth to 5 days of age and liver vitamin A at 28 days of age in calves from dams fed 1,000,000 U. S. P. of vitamin A daily for 30 days before the calculated date of parturition. Prenatal storage elevated blood plasma A at birth significantly and contributed to greater liver storage at 28 days of age.

EXPERIMENTAL

Objectives:

1. To perfect a liver biopsy technique to be used in beef cattle.

2. To study the sources of error in obtaining representative liver samples with such a technique.

3. To determine the liver and blood plasma relationships of carotene and vitamin A using the liver biopsy technique to collect the liver samples.

4. To study metabolism of carotene in steers given carotene concentrate by gelatin capsule.

Procedure:

The liver biopsy technique used in vitamin A studies:

The materials and methods were as follows: The steer (or cow) was held in a box stantion. No other means of restraint were used. The hair was clipped-off the right mid-section of the steer (or cow). An area (approximately 4 square inches) over the 12th and 13th ribs, 9 inches from the midline of the backbone was shaved clean. This area was anesthetized with 50 cc. of 3 percent procaine hydrochloride solution. After a 20 minute period an incision approximately 1½ inches long was made between the 12th and 13th ribs 10 inches from the midline of the backbone. A trocar and cannula 1 inch in diamenter and 6 inches in length was inserted into the incision. The instrument was rotated and pushed through the musculature until the liver surface was reached. The point of the trocar was made rather blunt so as to push or tear the muscles apart rather than cut them. After the liver surface was reached, the trocar was removed and the cannula left in place. A flashlight with a light bulb extended (on flexible neck) 6 inches from the main body was used in order to examine the liver surface (see Figure 1 for picture of above explanations). The instrument used to obtain liver samples by gelatin capsule.

FIGURE 1 Examination of the Liver Surface

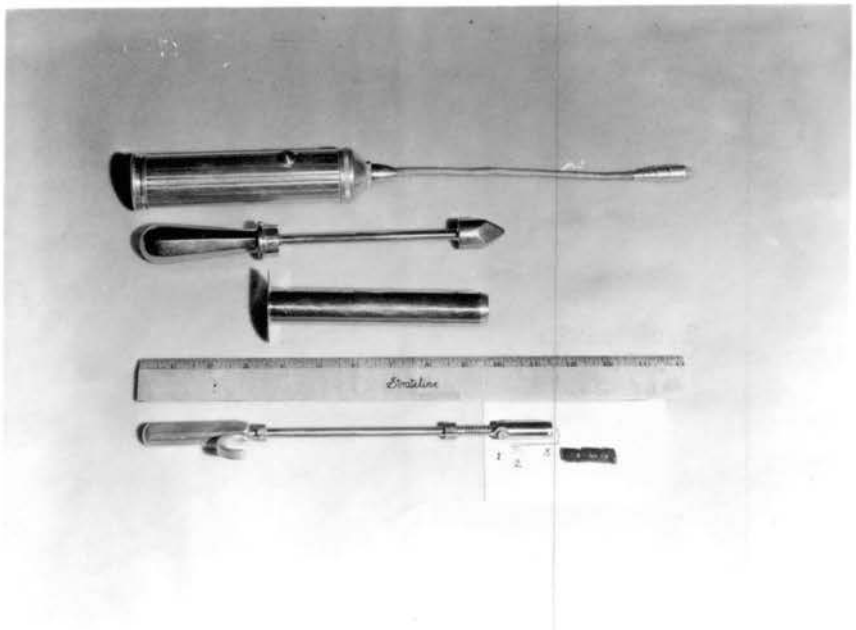
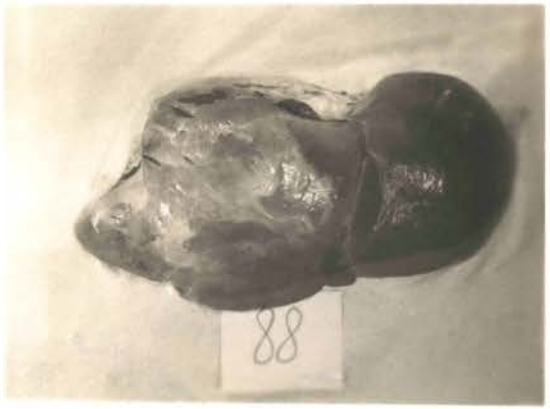
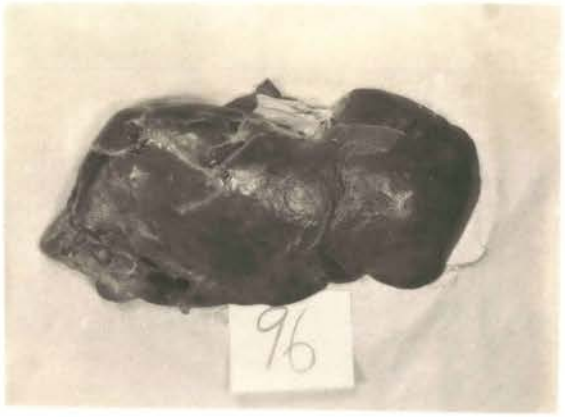


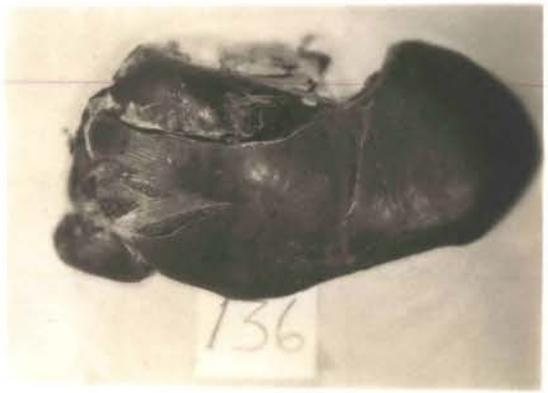
FIGURE 2 Biopsy Equiptment and Sample of Liver

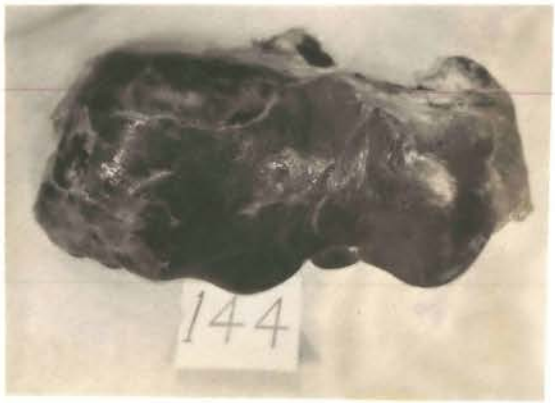
FIGURE 3

Livers of Animals From Which Twelve Biopsy Samples Had Been Taken









is shown in Figure 2. With this instrument, a 1 to 2 gram sample of liver (see Figure 2) was obtained by boring into the liver and then pulling the "trigger" of the "gun". The "trigger" was attached to a small piece of piana wire which in turn entered a hole in the side of the "gun" bore and looped around the inside of it in such a manner that when the "trigger" was pulled the piana wire was pulled through the hole in the bore and by so doing cut off the sample of liver (see insetsl, 2, and 3, Figure 2).

After the sample was taken, the cannula was removed. The incision was sutured and an antiseptic applied. The operation was conducted under as near aseptic conditions as possible.

Figure 3 shows four livers from which samples were taken at approximately monthly intervals for 12 consecutive months. No damage other than mild adhesions and a little scar tissue was observed. Over 100 biopsy samples were taken using the technique described above. No apparent ill effects on the animals were observed.

EXPERIMENT I

Sources of Error:

A. Variation of vitamin A in the liver:

A source of error of this technique examined was that of the homogeniety of the vitamin A in the liver. In order to evaluate data obtained using the liver biopsy technique, some idea of the homogeneity of vitamin A stores in the liver was required.

Four livers were obtained from freshly slaughtered cattle. Samples were obtained in duplicate from six locations. The locations were as follows:

(1). In central part of dorsal lobe.

(2). Two inches medial to lateral border and 1½ inches ventral to the

dorsal border of the dorsal lobe.

(3). Two inches medial to the lateral border at junction of the lower and middle 1/3 of the dorsal lobe.

(4). In central part of caudal lobe.

(5). Three inches medial from lateral border of anterior edge of the ventral lobe.

(6). Six inches medial from the lateral border of the anterior edge of the ventral lobe.

Approximately one gram samples were taken.

The samples were weighed rapidly on a percision balance to prevent loss of moisture and any possible vitamin A destruction. They were then placed in digestion flasks containing 5 ml. of purified alcohol and analyzed by the method of Gallup and Hoefer (1946).

B. Regression of liver weight on body weight:

This study was conducted to determine the regression of liver weight on body weight of steers. If the variation of the liver weight and body weight followed a regression pattern, the weight of the liver could be estimated from the live weight of the steer. Also the total vitamin A stores of the liver could be estimated from a biopsy sample and thereby interesting additional information about the vitamin A nutrition of an animal might be established.

The data used for this study was obtained at the slaughter house from steers that were previously in a feed-lot experiment at this station. Data was collected for two years (1950-51). The calculations were based on 42 and 46 steers for the years 1950 and 1951, respectively.

Results and Discussion:

Sources of Error:

The results of the study on homogeniety of vitamin A stored in the liver are shown in Table 1.

The variation among locations (analyzed by the method of Snedecor, 1946) was not significant (at 5 percent level). The F value, however, did approach significance (2.48 as compared to 2.90 required for significance at 5 percent level).

A highly significant difference among livers was found. This, of course, was expected since the animals from which the livers came were on different carotene intakes.

A highly significant difference was found for interaction (liver times location). This means that the different locations in separate livers failed to follow the same pattern of vitamin A content. One location that was above the average in vitamin A content in one liver was not necessarily above the average in another liver. The fact that the interaction mean square (36.13) was highly significant caused it to be used for the error term in testing the difference due to location.

The mean square for error was small (4.38). This indicated that the error in the chemical analysis for vitamin A was small and that there was little variation between duplicate samples. The percent range and average percent variation from the average vitamin A content of the livers were as follows:

Liver No.	Range	Average
1.	4 to 21	14
2.	7 to 40	17
3.	4 to 18	8
4.	2 to 43	15
Average	4 to 30	15

The minimum deviation in any one liver from the average vitamin A content was 2 percent as compared to a maximum of 43 percent. The average minimum and

TABLE 1

Results of Liver (Vitamin A) Analysis
(wet basis - mcg. per gram)

Location		1	2	3	4	5	6
Liver							
1	a	16.97	15.27	10.78	13.36	15.26	10.21
	b	17.80	17.44	12.40	14.53	15.46	12.58
2	a	9.07	12.55	7.93	6.32	7.75	5.75
	b	7.61	10.24	9.16	6.26	7.34	8.02
3	a	78.06	75.13	58.74	58.74	64.81	63.85
	b	78.28	77.12	67.97	54.16	61.03	58.03
4	a	11.83	14.71	11.04	6.52	16.22	13.49
	b	13.17	15.10	6.76	7.33	16.03	14.70

a = sample 1; b = sample 2

Analysis of Variance

Source	D. F.	S. S.	M. S.
Total	47	28,304.61	
Subclass	23	28,199.54	
Liver	3	27,208.95	9,070.00**
Location	5	448.73	89.75
Liver X Location	15	541.96	36.13**
Error	24	105.07	4.38

** Significant at 1% level
Location F = $\frac{89.75}{36.13}$ = 2.48 (2.90 for Sig. at 5% level)

maximum deviation from the average value was 4 and 30 percent, respectively. The average deviation from the average analysis of vitamin A content was 15 percent. Thus it may be concluded that a liver sample taken from these livers by the biopsy technique, on the average, would not vary over 15 percent from the average content of vitamin A in mcg. per gram of liver. However, on the average, a maximum error of 30 percent could occur.

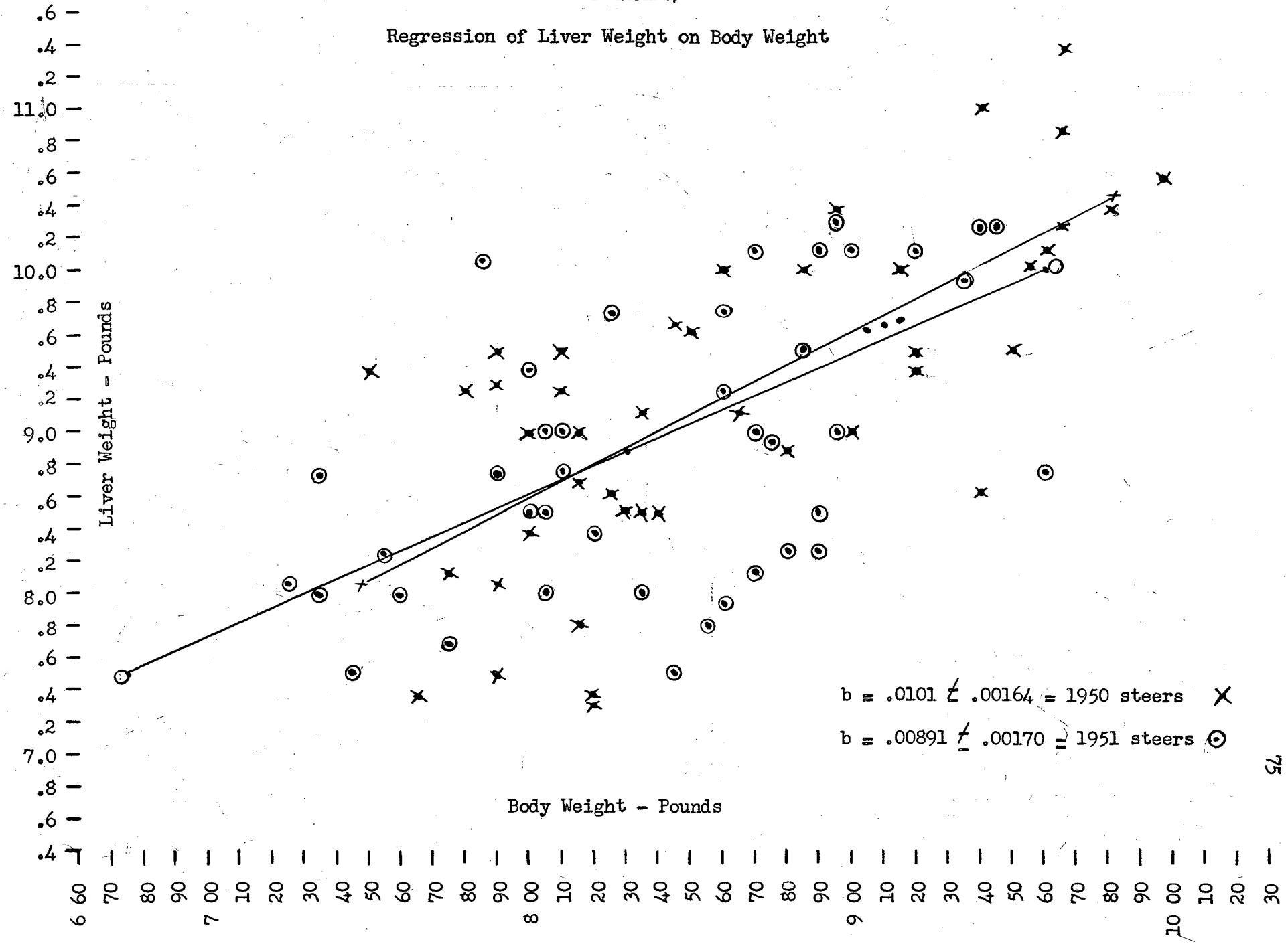
The regression of liver weight on body weight is shown by Figure 4.

The regression coefficient (b) and standard deviation from regression ($s_{x.y}$) was found to be 0.0101 (b) $\pm$ 0.00164 ($s_{x.y}$) and 0.00891 (b) $\pm$ 0.00170 ($s_{x.y}$) for the years 1950, '51, respectively. This is interpreted to mean that for each 100 pounds change in body weight of these steers the liver would change 1.01 $\pm$ 0.164 and 0.891 $\pm$ 0.170 pounds for the years 1950, '51, respectively. The fact that the regression coefficients are so near the same indicates that there was little difference in regression between the years 1950 and 1951. The standard deviations from regression were nearly identical for the two years indicating a nearly constant standard deviation from regression.

The average liver weight was found to be 1.067 percent of the body weight. The range from the average when compared with the average for each year was $\pm$ 0.004 percent.

FIGURE 4

Regression of Liver Weight on Body Weight



EXPERIMENT II. Vitamin A and Carotene Metabolism Studies in Cattle Using the Biopsy Technique

Procedure:

A. Two mature, dry Hereford cows that had been previously fed 2.5 pounds of cottonseed cake daily and grazed on dry native grass pasture were placed on a carotene deficient ration on January 15, 1950. Two yearling steers that had previously been used for digestion work were added to the experiment on February 16, 1950. The four animals were fed 15 pounds of cottonseed cake daily. Wheat straw and a mineral mixture containing two parts salt and one part steamed bonemeal were fed ad libitum. The animals were maintained on this ration throughout the experiment. The straw was found to contain about 1 ppm. of carotene.

The initial liver samples were taken on June 5, 1950. Samples were taken at approximately monthly intervals thereafter. The technique for sampling was mentioned previously. As soon as the sample of liver was taken it was wrapped in cellophane and placed in a small container and kept under dry ice. The samples were then placed in a deep freeze and analyzed for vitamin A as soon as possible. Blood samples were collected at the same time liver samples were taken, in citrated tubes, from the jugular vein and handled in the same manner as the liver samples. The liver samples were analyzed for carotene and vitamin A by the method of Gallup and Hoefer (1946). The blood plasma was analyzed for carotene and vitamin A by the method of Kimble (1939).

A carotene concentrate (furnished by Valley Vitamins Inc.) was mixed with mazola oil in a waring blender and given to the animals in gelatin capsules. The capsules were administered every other day. The calculations were made for each level of intake on the basis of weights taken the same day the liver samples were taken. Thirty micrograms of carotene per kilogram of body weight (as reported by Guilbert and Hart, 1935) was used at the minimum requirement in

computing the different levels of carotene intake.

B. On October 19, 1950, four weanling Hereford heifer calves were added to the experiment. They were from 8 to 9 months of age and were brought in off grass pasture where they had been "running " with their dams. They were placed on a ration of 1 pound cottonseed cake and 1½ pounds oats. Wheat straw and a mineral mixture were fed ad libitum. The heifers were fed this carotene deficient ration during the remainder of the experiment. The initial liver and blood samples were taken on November 6, 1950. Samples were taken at approximately monthly intervals thereafter. The method of handling the samples and chemical determinations made were identical to those as mentioned previously.

Results and Discussion:

A. The results of the liver and blood analyses of the cows and steers are summarized in Tables 2 and 3. The details of the carotene and vitamin A analyses of livers of the animals while on different levels of intake were as follows:

Steer 88— The carotene content dropped from 1.04 (1st 3 mo.,av.) to 1.00 mcg. per gram (2nd 3 mo.,av.) when 2 times daily minimum requirement was given. This indicated that two times minimum requirement when given as carotene concentrate in gelatin capsule was not sufficient to maintain liver stores of carotene. The carotene level increased from 1.10 (1st 3 mo., av.) to 1.58 mcg. per gram (2nd 3 mo.,av.) when 16 times minimum requirement was given. This result indicated that the rate of increase in liver carotene was relatively slow when the carotene intake was increased from 2 to 16 times minimum requirement.

The vitamin A levels of the liver followed the same pattern as the carotene levels. They decreased from 5.20 (1st 3 mo.,av.) to 3.30 (2nd 3 mo.,av.) mcg. per gram of liver on 2 times minimum requirement. They increased from 5.64 to 14.59 mcg. per gram when 16 times minimum requirement was fed.

Steer 96— The carotene content increased from 0.55 (0 times min. req.) to 0.81 (8 times min. req.) mcg. per gram. When 16 times minimum requirement was

TABLE 2

A Summary of Liver and Blood Carotene and Vitamin A Analyses of Cows and Steers on Different Levels of Carotene Intake

	Liver (mcg./gram)				Blood (mcg./100 ml.)			
	Level of Carotene Concentrate Given by Capsule times (X) Minimum Requirement							
***	2X		16X		2X		16X	
	1st 3 mo.	2nd 3 mo.	1st 3 mo.	2nd 3 mo.	1st 3 mo.	2nd 3 mo.	1st 3 mo.	2nd 3 mo.
88- Carotene**	1.04	1.00	1.10	1.58	44	47	102	121
Carotene / Vitamin A	0.20	0.30	0.20	0.10	2.28	3.11	5.50	3.34
Vitamin A	5.20	3.30	5.64	14.59	19.30	15.11	18.55	36.18
	OX-2 mo.	8X- 4 mo.	16X-1st 3 mo.*	16X-2nd 3 mo.	OX-2 mo.	8X-4 mo.	16X-1st 3 mo.	16X-2nd 3 mo.
96- Carotene	0.55	0.81	1.02	1.21	30	40	57	104
Carotene / Vitamin A	0.31	0.31	0.25	0 19	2.14	2.26	2.69	3.30
Vitamin A	1.80	2.58	4.05	6.30	14.00	17.72	21.19	31.56
	2X 1st 3 mo.	2X 2nd 3 mo.	2X 3rd 3 mo.	2X 4th 3 mo.	2X 1st 3 mo.	2X 2nd 3 mo.	2X 3rd 3 mo.	2X 4th 3 mo.
136- Carotene	2.00	1.74	1.77	1.63	36	35	35	43
Carotene / Vitamin A	0.01	0.02	0.04	0.03	1.95	1.90	1.76	2.17
Vitamin A	139.50	75.79	48.29	56.73	18.43	18.38	18.76	19.82
	OX-2mo.	8X-1st 4 mo.	8X-3mo foll.	2nd-3mo foll.	OX-2 mo.	8X-1st 4 mo.	8X-3 mo foll.	8X-2nd 3 mo. foll.
144- Carotene	1.90	1.61	1.57	1.28	24	38	38	46
Carotene / Vitamin A	0.05	0.06	0.15	0.11	1.26	1.76	2.75	2.01
Vitamin A	41.1	24.66	10.22	11.63	19.00	21.62	13.79	22.92

* 3 mo. indicates a value obtained by averaging three samples taken at approximately monthly intervals.

** $\frac{\text{Carotene}}{\text{Vitamin A}}$ = ratio of carotene to vitamin A.

*** Animal Number.

given, the level increased from 1.02 to 1.21 mcg. per gram. This indicates that the rate of increase in liver carotene was slow when the carotene intake was increased from 8 to 16 times minimum requirement. The vitamin A content again followed the same pattern as the carotene content. At 0, 8, 16 (1st) and 16 (2nd) times minimum carotene requirement, the observed vitamin A levels were 1.80, 2.58, 4.05 and 6.30 mcg. per gram, respectively.

Cow 136— The average carotene content was found to decrease from 2.00 (1st 3 mo., av.) to 1.63 (4th 3 mo. av.) mcg. per gram at 2 times the minimum requirement. The vitamin A content decreased from 139.50 to 56.73 mcg. per gram during the same period of time. This indicates, as in Steer 88, that carotene concentrate at 2 times minimum requirement was not sufficient to maintain liver carotene and vitamin A values.

Cow— The average carotene content decreased from 1.90 (0 times min. req.) to 1.28 (8 times min. req.) mcg. per gram even though this cow had been getting 8 times minimum requirement of carotene daily for 7 months previous to the period at which the average value of 1.28 was obtained. The vitamin A values followed essentially the same pattern. This indicated that 8 times minimum daily carotene requirement failed to maintain liver carotene and vitamin A stores.

In general, the carotene and vitamin A analyses of the blood plasma of the animals while on different levels of carotene intake may be summarized as follows:

The carotene and vitamin A levels of the steers' plasma increased with increased carotene intake. A similar "lag" was found to exist between the first and second 3 months at 16 times minimum daily carotene requirement (as in the liver) regardless of whether the intake followed 2 or 8 times minimum daily requirement supplementation.

Cow 136 maintained rather constant carotene and vitamin A blood plasma levels at 2 times minimum daily carotene requirement. The plasma carotene content varied from 33 to 43 mcg. per 100 ml. while the plasma vitamin A varied from 18.38

to 19.82 mcg. per 100 ml. Cow 144 increased in plasma carotene content (24 mcg/100 ml. at 0 times min. req.) to (38, 38 and 46 mcg/ 100 ml. at 8 times min. req.). In general, cow 144's plasma vitamin A content tended to remain constant.

These results indicate that intakes of carotene concentrate when administered at 2 and 8 times the minimum requirement failed to maintain liver carotene and vitamin A levels. The blood plasma carotene and vitamin A appeared to be maintained relatively constant at these levels of intake at the expense of the liver.

The ratios of the liver and blood constituents of the cows and steers while on different levels of carotene intake were as follows:

In general, the carotene/ vitamin A ratio of the samples taken from the animals livers varied inversely with the carotene content of the livers (3 exceptions). This result indicated that the increase or decrease of vitamin A in the liver was more rapid then the increase or decrease of carotene in the liver. The carotene/ vitamin A ratio of the blood plasma tended to vary concurrently with carotene level of the blood plasma.

At carotene intake levels of 2 and 8 times minimum requirement, the carotene liver/ carotene blood plasma ratio varied concurrently with level of carotene in the liver. However, going from 8 to 16 times minimum requirement, the ratio varied inversely with level of carotene in the liver.

The vitamin A liver/ vitamin A blood, ratio, varied concurrently with vitamin A level of the liver (1 exception). This variation was no in direct proportion. However, this indicates that after several months on a particular intake level high enough to increase liver vitamin A stores, some degree of correlation could be expected between liver and blood plasma vitamin A.

TABLE 3

Ratio of Liver Constituents to Blood Constituents (Cows and Steers)
Level of Carotene Concentrate Given by Capsule Times (X) Minimum Req.

Animal No.	2X		16X	
88	1st 3 mo.	2nd 3 mo.	1st 3 mo.	2nd 3 mo.
Carotene liver/Carotene blood	0.024	0.021	0.011	0.013
Vitamin A liver/Vitamin A blood	0.269	0.218	0.304	0.403
96	0X-2 mo.	8X-4 mo.	16X-1st 3 mo.	16X-2nd 3 mo
Carotene liver/Carotene blood	0.018	0.020	0.018	0.012
Vitamin A liver/Vitamin A blood	0.128	0.146	0.191	0.200
136	2X-1st 3 mo.	2X-2nd 3 mo.	2X-3rd 3 mo.	2X-4th 3 mo.
Carotene liver/Carotene blood	0.056	0.050	0.054	0.038
Vitamin A liver/Vitamin A blood	7.57	4.12	2.57	2.86
144	0X-2 mo.	8X-1st 4 mo.	8X-3mo. foll.	8X-2nd 3 mo.foll.
Carotene liver/Carotene blood	0.079	0.042	0.041	0.028
Vitamin A liver/Vitamin A blood	2.16	1.14	0.74	0.51

B. Weanling heifers while on a carotene deficient ration:

A summary of the liver and blood data collected from these heifers is presented in Tables 4 and 5.

The average carotene content of the liver samples dropped 52, 11, and 29 percent in order of succeeding 2 months averages. The vitamin A content as measured by analysis of the samples dropped 30, 36, and 39 percent at the same periods of measurement.

The plasma carotene levels dropped 55 percent (1st 2 mo. to 2nd 2 mo.) and then remained practically constant. The vitamin A content of the plasma tended to remain constant at each 2 month average.

These results again (as in part A) indicate that plasma carotene and vitamin A tend to remain constant at the expense of the liver stores.

The ratios of the liver and blood constituents of the heifers while on a carotene deficient ration were as follows:

Aside from the initial decrease in carotene liver/ vitamin A liver ratio, there appeared to be an increase in the ratio with a decrease in carotene content of the livers of these heifers. This result agrees with that found in part A.

The carotene blood/ vitamin A blood ratio decreased from 2.16 to 1.03 (between 1st and 2nd 2 month av.) and then tended to remain constant.

As the carotene content of the liver decreased, the carotene liver/ carotene blood ratio decreased. This fact occurred because the carotene level of the blood tended to remain constant. Also, as the vitamin A content of the liver decreased, the vitamin A liver/ vitamin A blood ratio decreased. This decrease was due to the tendency of the blood plasma vitamin A to remain constant while the liver decreased in vitamin A content.

TABLE 4

SUMMARY OF LIVER AND BLOOD ANALYSES

(Liver in mcg./gram - Dry Weight Basis, Blood in mcg./100 ml.)

HEIFERS ON VITAMIN A DEFICIENT RATION

Animal No	Liver				Blood			
	1st 2 mo*	2nd 2 mo	3rd 2 mo	4th 2 mo	1st 2 mo	2nd 2 mo	3rd 2 mo	4th 2 mo
	Carotene				Carotene			
11	8.5	4.0	3.8	1.9	42	19	25	18
15	12.6	5.5	4.2	3.2	58	24	26	27
18	11.3	4.2	3.9	2.2	68	28	26	31
30	9.6	4.6	4.6	2.5	62	28	32	28
Average	10.5	4.6	4.1	2.9	56	25	27	26
	Vitamin A				Vitamin A			
11	311.48	188.83	116.74	51.33	34.44	26.55	39.40	22.20
15	439.88	373.87	217.16	133.61	19.31	20.02	18.60	26.90
18	243.48	142.60	81.34	21.46	26.14	23.53	21.00	18.62
30	461.21	317.22	237.58	133.59	23.70	26.55	29.15	24.90
Average	364.01	255.63	163.20	85.00	25.90	24.16	27.04	23.16

* 2 mo. indicates an average of two samples taken at approximately monthly intervals.

TABLE 5

Summary of Ratios
Heifers on Carotene Deficient Ration

Average Values

2 month period	1st	2nd	3rd	4th
Carotene Liver/Vitamin A Liver	0.029	0.018	0.025	0.034
Carotene Blood/Vitamin A Blood	2.16	1.03	1.00	1.12
Carotene Liver/Carotene Blood	0.188	0.184	0.152	0.112
Vitamin A Liver/Vitamin A Blood	14.05	10.58	6.04	3.67

EXPERIMENT III Carotene Metabolism Study

Procedure:

Three 8 month old Hereford steers were placed (11-6-50) on a ration of 1 pound of cottonseed cake, and 1½ pounds of whole oats each. Straw and a mineral mixture (2 parts salt to 1 part steamed bonemeal) were fed ad libitum; however, records of the amount of straw eaten were kept. The feeds were analyzed for carotene by A. O. A. C. Methods (1945). The amounts of carotene in the feeds were: Straw- 1.26 mcg./gram, whole oats- 0.67 mcg./ gram, and cottonseed cake- 0.285 mcg./gram. The daily carotene intake from the ration was computed and added to that given by capsule.

Liver samples were taken (3-15-51), and the steers were then placed in metabolism stalls (3-19-51). The ration fed was the same as previously mentioned. After a 5 day period of adjustment, samples of feces were collected for three days. The feces samples were analyzed for carotene by a method adopted from the alcoholic KOH extraction method of the A. O. A. C. (1945).

The amount of carotene excreted daily (dry matter basis) during the basal adjustment period was as follows: Steer 1, 932.51 mcg/gram; Steer 2, 653.40 mcg/gram; Steer 3, 926. 28 mcg/gram. These values were subtracted from the daily carotene excretion values in computing the percent carotene excreted. The carotene concentrate (prepared as described in Experiment I) was administered in gelatin capsule. The rate of supplementation was as follows:

Trial Steer	1	2	3	4	5
1	4 x m.r.	8 x m.r.	32 x m.r.	16 x m.r.	200 x m.r.
2	8 x m.r.	32 x m.r.	16 x m.r.	4 x m.r.	200 x m.r.
3	32 x m.r.	16 x m.r.	4 x m.r.	8 x m.r.	200 x m.r.

x m.r. = amount times minimum requirement

The steers were weighed at the beginning of each preliminary period and that weight used in calculating the amount of carotene concentrate at a particular specified intake. A 10 day preliminary on each carotene intake was observed.

Three 3 day collections were made. The feces were analyzed at the end of each 3 day collection to avoid excess loss due to destruction by oxidation. The values used for the calculations reported herein were calculatedfrom an average of these three day collection periods.

Results and Discussion:

The results of the carotene metabolism study are presented in Tables 6, 7, 8, and 9.

The liver content of carotene and vitamin A (mcg/gram) tended to vary concurrently with carotene intake and the order in which a particular intake was given. Some evidence of overlapping of the liver carotene and vitamin A levels was present from one trial to the following trial. The blood plasma carotene and vitamin A levels also appeared to overlap from one trial to the next.

Ratios: The carotene liver/ carotene blood ratio appeared to be dependent upon the order in which a particular level of carotene concentrate was given and not consistant with increase or decrease of carotene content of the liver. However, when 4, 8, or 16 times minimum daily carotene requirement was followed by 200 times minimum requirement, the ratio, in each case, decreased with an increase in carotene level of the liver. This was interpreted to mean that the carotene of the blood increased more rapidly than the carotene of the liver.

No consistant pattern of variation was found for the vitamin A/ liver vitamin A blood ratio. This ratio appeared to be dependent upon and affected by the order in which a particular carotene level was given.

The carotene blood/ vitamin A blood and carotene liver/ vitamin A liver ratios appeared to be confounded by the order in which a particular level of carotene concentrate was given. No consistant pattern of variation was found.

The carotene liver/ carotene intake and vitamin A liver/ carotene intake ratios were found to vary inversely with the carotene intake level (1 exception).

TABLE 6
SUMMARY OF LIVER AND BLOOD CAROTENE AND VITAMIN A ANALYSES OF METABOLISM STEERS

		Liver (mcg./gram, dry matter basis)						Blood (mcg./100 ml.)					
**	TRIAL		1	2	3	4	5		1	2	3	4	5
	Level fed X * Min. Req.	I	4X	8X	32X	16X	200X	I	4X	8X	32X	16X	200X
1.	Carotene	2.93	1.94	3.36	8.20	6.32	22.69	36	41	58	159	114	463
	Vitamin A	22.23	14.40	28.97	45.58	37.70	169.62	18.92	11.06	19.83	42.08	28.49	51.05
	Level fed X Min. Req.		8X	32X	16X	4X	200X	I	8X	32X	16X	4X	200X
2.	Carotene	3.16	2.11	6.32	6.10	4.28	17.17	16	46	90	148	53	408
	Vitamin A	62.58	45.05	82.71	107.45	45.35	159.31	13.79	15.33	13.55	38.09	26.27	52.98
	Level fed X Min. Req.		32X	16X	4X	8X	200X	I	32X	16X	4X	8X	200X
3.	Carotene	2.19	5.02	4.83	4.00	5.49	11.69	18	39	116	76	65	309
	Vitamin A	50.80	60.37	74.50	59.14	39.52	82.25	13.00	6.60	22.68	22.44	16.40	50.97

I - Initial sample

* Level of carotene concentrate given in relation to minimum requirement.
** Steer Number.

TABLE 7
SUMMARY OF RATIOS - Metabolism Steers
Blood and Liver

TRIAL	I	1	2	3	4	5	I	1	2	3	4	5	I	1	2	3	4	5
Intake * X Min. Req.	0	4X	8X	32X	16X	200X	0	8X	32X	16X	4X	200X	0	32X	16X	4X	8X	200X
	Steer 1						Steer 2						Steer 3					
CL/CB	0.081	0.048	0.059	0.052	0.055	0.049	0.200	0.045	0.070	0.041	0.081	0.042	0.120	0.130	0.042	0.053	0.084	0.037
VAL/VAB	1.18	1.30	1.46	1.08	1.32	3.32	4.54	2.94	6.10	2.82	1.73	3.01	3.91	9.15	3.28	2.64	2.41	1.61
CL/VAL	0.131	0.135	0.116	0.180	0.168	0.134	0.050	0.047	0.076	0.057	0.094	0.108	0.043	0.083	0.065	0.068	0.139	0.142
CB/VAB	1.90	3.69	2.92	3.77	4.00	9.09	1.16	3.03	6.62	3.79	2.02	7.69	1.38	5.92	5.10	3.39	3.97	6.06
CL/CI		0.51^{-3}	0.49^{-3}	0.32^{-3}	0.46^{-3}	0.13^{-3}		0.28^{-3}	0.22^{-3}	0.42^{-3}	0.11^{-2}	0.09^{-3}		0.18^{-3}	0.35^{-3}	0.10^{-2}	0.74^{-3}	0.67^{-4}
VAL/CI		0.39^{-2}	0.42^{-2}	0.18^{-2}	0.28^{-2}	0.10^{-2}		0.61^{-2}	0.28^{-2}	0.73^{-2}	0.10^{-1}	0.87^{-3}		0.22^{-2}	0.55^{-2}	0.15^{-1}	0.54^{-2}	0.47^{-3}

I_o Ratio of initial samples.
* Level of carotene concentrate given times minimum requirement.

CL - Carotene Liver
CB - Carotene Blood
VAL - Vitamin A liver
VAB - Vitamin A Blood
CI - Carotene Intake

Excretion data: When the excretion data was subjected to statistical analysis (Snedecor, 1946), significance was found at the five percent level. When the 200 times minimum requirement data was omitted there was no significant difference found. Steer number 3 was found to excrete 51.05 percent of his carotene intake at a level of 4 times minimum requirement.

The carotene liver/ percent carotene excreted and carotene blood/ percent carotene excreted ratios varied concurrently with carotene intake (except in 3 cases, each of which were found when the steers were changed from an intake of 32 to 16 times minimum carotene requirement. This data indicates that as the carotene intake increased the percent carotene excreted decreased and visa-versa. Similar results were obtained with the vitamin A liver/ percent carotene excreted and vitamin A blood/ percent carotene excreted ratios. This may be interpreted to mean that as the level of carotene intake increased the amount of carotene converted to vitamin A increased and thereby less carotene was excreted. This fact may also be helpful in explaining the increase in carotene liver/ percent carotene excreted and carotene blood/ percent carotene excreted ratios.

TABLE 8

Percent Carotene Excreted
At Different Intakes of Carotene
Concentrate

	1	2	3	4	5
Intake * Level X Min. Req.	4X	8X	32X	16X	200X
Steer 1	41.45	46.68	40.38	37.05	30.71
Intake Level X Min. Req	8X	32X	16X	4X	200X
Steer 2	35.98	38.96	32.88	41.51	31.86
Intake Level X Min. Req	32X	16X	4X	8X	200X
Steer 3	33.26	34.05	51.05	28.14	31.54

* Level of carotene concentrate given by capsule in relation to minimum requirement.

TABLE 9
SUMMARY OF RATIOS - Metabolism Steers

TRIAL	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Level Carotene * Intake	4X	8X	32X	16X	200X	8X	32X	16X	4X	200X	32X	16X	4X	8X	200X
	Steer 1					Steer 2					Steer 3				
CL/PCE	0.047	0.072	0.20	0.17	0.74	0.059	0.16	0.19	0.10	0.54	0.15	0.14	0.08	0.19	0.37
CB/PCE	0.99	1.24	3.94	3.08	15.08	1.28	2.31	4.51	1.28	12.8.	1.17	3.41	1.49	2.31	9.80
VAL/PCE	0.35	0.62	1.13	1.02	5.52	1.25	2.12	3.27	1.09	5.00	1.18	2.07	1.16	1.40	2.61
VAB/PCE	0.27	0.42	1.04	0.77	1.66	0.43	0.35	1.16	0.63	1.66	0.20	0.67	0.44	0.58	1.62

* As given in gelatin capsule times minimum requirement.
CL - Carotene Liver
CB - Carotene Blood
VAL - Vitamin A Liver
VAB - Vitamin A Blood
PCE - Percent Carotene Excreted

SUMMARY

A liver biopsy technique was perfected for use in a series of carotene and vitamin A studies with beef cattle. Some sources of error using the biopsy technique in reporting total vitamin A stores in the liver were examined.

The average deviation, from the average vitamin A content, of a sample taken at one of six locations on four separate livers was found to be 15 percent. Thus, it may be concluded that a liver sample taken from these livers by the biopsy technique, on the average, would not vary over 15 percent from the actual content of vitamin A (mcg. per gram of liver). An average maximum error of 30 percent, however, was found to occur.

The change in liver weight for each 100 pounds change in body weight was found to be 1.01 and 0.89 pounds on 42 and 46 steers, respectively. The standard deviation from regression for each 100 pounds change was 0.164 and 0.170, respectively. On the average, the liver was found to be 1.067percent of the body weight.

Increases in liver carotene were relatively slow when the carotene intake was increased from 2 to 16 times minimum carotene requirement.

These studies indicated that intakes of carotene concentrate when administered in gelatin capsule at 2 and 8 times minimum requirement, failed to maintain liver carotene and vitamin A levels. When intakes of carotene were given that failed to maintain liver stores, the blood plasma carotene and vitamin A appeared to remain constant at the expense of the vitamin A in the liver.

Increases in vitamin A in the liver were found to be more rapid than carotene.

At carotene intake levels of 2 and 8 times minimum carotene requirement, the carotene liver/ carotene blood plasma ratio was found to vary concurrently with level of carotene in the liver. At changes of from 8 to 16 times minimum carotene requirement, the ratio varied inversely.

The vitamin A liver/ vitamin A blood plasma ratio increased with increasing vitamin A levels of the liver. This variation was not in direct proportion. However, this fact indicated that when increasingly high levels of carotene concentrate were fed, some degree of correlation was present between liver and blood plasma vitamin A.

The ratios, carotene liver/ percent carotene excreted and carotene blood/ percent carotene excreted, varied concurrently with carotene intake (except in 3 cases, each of which were found when the steers were changed from an intake of 32 to 16 times minimum carotene requirement).

The ratios, vitamin A liver/ percent carotene excreted and vitamin A blood/ percent carotene excreted, followed the same general pattern of variation. These data indicated that as the carotene intake increased, the percent carotene excreted decreased and visa-versa, and/or that as the level of carotene intake increased the amount of carotene converted to vitamin A increased and thereby less carotene was excreted.

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TYPIST PAGE

THESIS TITLE: Part I. A STUDY OF SYSTEMS OF MANAGEMENT AND PROTEIN SUPPLEMENTS FOR RANGE HEREFORD COWS AND THE EFFECT OF EACH UPON CERTAIN BLOOD CONSTITUENTS OF THE COWS AND THEIR CALVES.

Part II. A LIVER BIOPSY TECHNIQUE AND OBSERVATIONS OF ITS USE IN VITAMIN A STUDIES WITH BEEF CATTLE.

NAME OF AUTHOR: William J. Van Arsdell

THESIS ADVISER: Dr. C. K. Whitehair

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NAME OF TYPIST: Mrs. Bill J. Tidmore