JULY, 1950 RESEARCH BULLETIN 457

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

J. H. LONGWELL, *Director*

SOME FACTORS AFFECTING THE CAROTENOID AND VITAMIN A LEVELS IN THE BLOOD AND MILK OF DAIRY CATTLE

O. T. STALLCUP and H. A. HERMAN

(Publication authorized June 30, 1950)

COLUMBIA, MISSOURI

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INTRODUCTION

The physiological importance of vitamin A in dairy cattle has long been recognized. Vitamin A was first discovered in milk fat (McCollum and Davis, 1913, and Osborne and Mendel, 1913). The first severe pathological evidence of xerophthalmia in humans was found in the case of children fed fat-free milk (Bloch, 1924). If ruminants have access to sunshine they have need only for the provitamins A to meet all of their vitamin requirements in so far as diet is concerned. Hart (1940) believes that the demand for vitamin A in dairy cattle is perhaps tenfold the theoretical minimum need because much of it is destroyed in the rumen. Carotenoids and vitamin A are stored rapidly in the liver of dairy cattle and used as needed. Moore (1932) has estimated that a cow can store sufficient vitamin A to last about 75 days in so far as required for furnishing the amount normally secreted in the milk.

Vitamin A deficiency in dairy cattle is associated with constriction of the optic foramen which in turn constricts the optic nerve ultimately resulting in blindness. Vitamin A deficiency is also accompanied by syncope, likely produced by increased cranial pressure, papillary edema and nyctalopia; bleaching of the *tapetum lucidum* of the retina; degeneration of the germinal epithelium of the testis; changes in the pituitary; enteritis; kidney and liver lesions; and diarrhea. The synthesis of ascorbic acid appears to be dependent on vitamin A intake (Sutton *et al.*, 1942).

Numerous investigators have reported the fact that blood plasma carotenoid and vitamin A values are low in newborn calves and that, in order to survive, they must receive this vitamin from colostrum or some other source in relatively large quantities. Milk and butter are good sources of vitamin A and the significance of this fact in the nutrition of man and animals cannot be over-emphasized.

In view of the obvious importance of vitamin A, both from the standpoint of physiological significance and proper nutrition, it is important to understand as fully as possible the factors influencing the levels of this vitamin in the blood and the milk of dairy cattle. In this study an attempt has been made to determine the influence of certain nutritional, environmental, and physiological factors on vitamin A and carotenoid levels in the bovine.

REVIEW OF THE LITERATURE

Factors Influencing the Carotenoid and Vitamin A Levels in Milk Fat

The Influence of Carotenoid Levels in the Diet.—A preponderance of evidence is presented in the literature to the effect that the vitamin A potency of butterfat depends primarily on the carotene content of the cow's ration. Jenness and Palmer (1945) have published an excellent review of the literature on this subject. Atkeson *et al.* (1937) observed that raising the carotene content of the ration from 1 million to 6 million units daily resulted in practically no change in the carotenoid and vitamin A content of the butter. Fraps *et al.* (1934) observed that when lactating cows were restricted to 17,000 vitamin A units daily the vitamin A potency of the milk fat decreased from 38 to 16 units per gram in four weeks. Wilbur *et al.* (1940) likewise found that the vitamin A potency of the butter was associated with the carotene content of the ration. Sharp and Hand (1939) observed that the carotene content of summer milk was much higher than that of winter milk. The Bureau of Dairy Industry, U.S.D.A., (1947) has published a report on the analysis of 3500 samples of butter from 14 different states taken during the course of a year and found that winter butter averaged 12,000 I. U. of vitamin A per pound and summer butter averaged 18,000 I. U. per pound.

Wise *et al.* (1947) reported on the effects of a high vitamin A intake on the carotenoids and vitamin A in milk. They observed that the vitamin A concentration in milk was greatly increased but the carotenoid content was decreased. This finding is in accord with the results of many other investigators.

Hibbs *et al.* (1949) found that fluctuations in the carotenoid and vitamin A values of summer milk were related to the fluctuations in the carotenoid content of the pasture herbage consumed during three pasture seasons. The data indicate that a closer relation exists between pasture carotenoids and milk carotenoids than between pasture carotenoids and milk vitamin A. Moreover, they found that the fluctuations in milk carotenoids follow the changes in pasture carotenoids more closely when the carotenoid level in pasture is below 250 micrograms than when it is higher, indicating a maximum response at this level.

The Influence of Breed.—Baumann *et al.* (1934) analyzed the milk of different breeds and found that Guernsey milk was highest, Jersey and Brown Swiss milk intermediate, and Holstein and Ayrshire milk lowest in the carotene content. Similar differences between breeds have been reported by Wilbur *et al.* (1933). Burl and Peterson (1943) observed that butter made from the milk of Holsteins contained less carotene per unit but more vitamin A than butter made from Guernsey milk. Garret and Bosshardt (1944) found that milk fat of the Guernsey breed contained about three times as much carotene as the fat of the Holstein breed; on the other hand, the milk fat of Holsteins contained 60 per cent more vitamin A than that of the Guernsey. Sharp and Hand (1939) noted breed differences in the carotene content of milk

but observed that individual variations within the breed were much larger than the variations among average values for the different breeds. However, it has been shown that the total vitamin A activity in milk is of the same order of magnitude regardless of breed (Baumann *et al.*, 1934).

The Influence of the Individual and Stage of Lactation.—Numerous studies indicate that within breeds there are great individual differences in the carotenoid and vitamin A levels of milk fat (Gilliam *et al.*, 1936; Sharp and Hand, 1939; Burl and Peterson, 1943; Baumann *et al.*, 1934). von Winzenreid and Wanntorp (1948) analyzed the milk of 13 pairs of identical twins. The feeding of fresh greens and fresh hay raised the carotenoid and vitamin A values. The level of these constituents tended to become lower toward the end of lactation. It was concluded that vitamin A activity is strongly influenced by hereditary factors as is also the ratio of vitamin A to carotene. There appeared to be a genetically determined ceiling value to the conversion of carotene to vitamin A.

Vitamin A and carotenoids in colostrum have been the subject of numerous investigations. Parrish *et al.* (1949) have published an excellent review of this work. It has been observed that levels of vitamin A and carotenoids are generally high in the initial colostrum and colostric fat but decrease rapidly as the mammary secretions change to normal milk. Richter (1938) has shown that carotenoids and Parrish *et al.* (1949) have shown that both carotenoids and vitamin A follow a logarithmic trend during the early stages of transition from colostrum to normal milk.

Dann (1933) suggested that the calf received from twenty to fifty times as much vitamin A from colostrum as from an equal volume of whole milk. He found that the vitamin A potency of the colostrum from five heifers studied was double the average of that of nine older cows. Similar observations on differences between heifers and older cows have been made by Henry *et al.* (1940) and Hansen *et al.* (1946). A sevenfold variation in vitamin A potency of colostrum occurring in cows fed identical rations was also observed by Hansen *et al.* (1946). Stewart and McCallum (1938b) suggested that the length of the dry period prior to parturition significantly affected the colostrum vitamin A. Wide variations in the vitamin A content of colostrum have been reported by Dann (1933), Semb *et al.* (1934), Stewart and McCallum (1938a) (1938b), Henry *et al.* (1940), and Sutton and Kaeser (1946).

Henry *et al.* (1940) found that pasture increased the carotene potency of colostrum and Kramer *et al.* (1938) and Reed (1944) have reported that pasture increased both carotene and vitamin A. Recent investigations by Esh *et al.* (1948) and Spielman *et al.* (1947) prove that prepartal vitamin A supplements augment the vitamin A content of colostrum.

Parrish *et al.* (1949) in a comprehensive study of factors affecting the carotenoid and vitamin A content of colostrum found much difference in the potency of colostrum from cows of the same breed, lactation (first or later)

and diet. The carotenoid content of colostrum from Jerseys and Guernseys was higher than that from Holsteins and Ayrshires, but differences in vitamin A content were not marked. Concentrations of vitamin A but not carotenoids were higher in colostrum of first lactation cows than from cows in later lactations. Pasture increased the carotene and vitamin A potency of colostrum while high intakes of vitamin A concentrates prepartally increased vitamin A but decreased the carotenoid content. Neither number of lactation, breed, nor prepartal rations appeared to influence the rapid decrease of carotenoids and vitamin A which occurred during the first eight milkings following parturition.

Factors Influencing the Carotenoid and Vitamin A Levels in Blood Plasma

The Influence of Carotenoid Levels in the Diet.—The early work of Palmer and Eckles (1914) demonstrated that there was a positive relationship between the carotenoid content of the feed and the carotene content of the blood serum. This fact has since been confirmed by Gilliam and Ridi (1935), Semb *et al.* (1934), Moore (1936), and Sutton and Soldner (1945). Moore and Berry (1945) observed that plasma carotene and vitamin A values were higher for calves receiving lespedeza hay containing 50 micrograms per gram than in those receiving a No. 1 mixed clover and timothy hay which contained only 15 micrograms per gram.

Goss and Mead (1941) reported that the oral administration of crystalline carotene at levels of 500 and 1,000 mgm. daily was accompanied by a corresponding increase in blood plasma carotene of carotene-depleted cows. Intrajugular injections of crystalline carotene suspended in blood plasma and in cottonseed oil failed to bring about a significant increase in the blood plasma carotene.

Wise *et al.* (1947), Ross and Knodt (1948), and Eaton *et al.* (1947) have shown that the feeding of vitamin A concentrates is accompanied by increases in blood plasma vitamin A but the plasma carotenoid levels are decreased. Whiting *et al.* (1949) reported that the feeding of 5 oz. of cod liver oil daily increased the vitamin A but decreased the carotene content of milk fat and blood plasma.

The Influence of Breed.—According to Moore (1936) the blood plasma carotenoid values of different breeds kept under similar conditions of feeding ranked in increasing order as follows: Brown Swiss, Holstein, Ayrshire, Jersey and Guernsey. There was little difference in the values of the first three breeds named. Similar results have been reported by Sutton and Soldner (1945), and Moore and Berry (1944).

There is apparently a distinct difference in breeds with regard to the utilization of carotene to maintain normal blood levels. Boyer *et al.* (1942) observed that the daily intake of carotene required to maintain adequate blood plasma vitamin A was 75 mcg. per kg. for Holstein yearlings and 125 mcg. per kg. for Guernsey yearlings. Moreover, Moore *et al.* (1948) using spinal fluid pressure levels as a criterion for adequate vitamin A nutrition observed

that Guernsey calves require 34 mcg. of carotene per lb., Jerseys 32 mcg. per lb., and Holsteins and Ayrshires 30 mcg. per lb. body weight to maintain normal spinal fluid pressures.

Changes Associated with Parturition and Beginning Lactation.—In order to build a supply of vitamin A and carotenoids in the colostrum there are presumably changes in the permeability of the mammary gland a short time prior to parturition which permits a flow of these constituents out of the blood and into the gland in large amounts.

That there were decreases in blood plasma carotene associated with parturition was first observed by Gallup and Kuhlman (1941). Later Kuhlman and Gallup (1944) reported that in 63 cases there was an average decrease of 21 per cent in the plasma carotenoids during the period immediately preceding parturition. A lowering of both carotene and vitamin A levels associated with parturition has been reported by Sutton and Soldner (1943), Braun (1945), and Sutton *et al.* (1945). The latter investigators observed that some three weeks prepartum there was a gradual decline of these substances in the blood and minimal values were established shortly after parturition.

Placental Transmission and Fetal Storage of Carotene and Vitamin A.—The fact that colostrum contained large amounts of vitamin A was known long before the need of this substance by the new-born calf was fully realized. At birth the calf has very low levels of carotene and vitamin A in blood plasma as reported by Lundquist and Phillips (1943); Moore and Berry (1944) and Spielman *et al.* (1946). However, Moore and Berry (1944) observed that with the ingestion of colostrum there is a fivefold increase in the carotenoid and vitamin A values in the blood within 24 hours. Sutton and Kaeser (1946) and Kaeser and Sutton (1948) report similar increases. Spielman *et al.* (1946) by feeding rations ranging from low to high in carotene, and by carotene and vitamin A supplementation for 60 days prepartum, show that the prepartum diet of the cow may markedly influence the vitamin A concentration in the blood and liver of the new-born calf.

We thus have a very clear picture of the importance of high levels of carotene in the diet of the cow and the influences on the vitamin A potency of both normal and colostrum milk.

Losses of Carotenoids in Roughages During Storage

In view of the importance of carotene in the ration in order to insure adequate levels of vitamin A in blood and milk, it is necessary to feed this vitamin in generous amounts. Seventy to eighty per cent of the nutrients supplied to mature cattle and to cattle from six or eight months to maturity is furnished by roughages. It is apparent that the most feasible method of supplying carotene in the ration is through the production of high quality roughage in the form of pasture, hays and silage.

Virtanen (1936) found that carotene was highest in the rapidly growing plant and reached a maximum value before or at the beginning of flowering.

Fertilization of the soil increased the carotene content of plants. Similar results were reported by Douglas *et al.* (1933). Atkeson *et al.* (1937) showed that pasture grasses have the highest carotene value in early summer. The carotene content decreased during the hot months of midsummer but, in most cases, increased after the fall rains when new growth was resumed.

Russell and coworkers (1934) observed a loss of 80 per cent of the carotene in alfalfa during the first 24 hours of drying in the field. Similar results have been reported by Russell (1929), Bethke and Kick (1929), Smith and Briggs (1933), Guilbert (1935) and Vail *et al.* (1936).

Carotene is readily oxidized and is subject to photolysis (Guilbert, 1935). Hauge (1935) has shown that enzymes in the plant are capable of destroying carotene. Waugh *et al.* (1944) indicated destruction of carotene in hays due to enzymatic activity and that it is greater in alfalfa, red clover, and sweet clover than in the oat plant, Kentucky blue grass, and brome grass. Corn, soybeans, and lespedeza have intermediate enzyme activity.

Mills and Hart (1945) found that additional heating of dehydrated alfalfa was effective in increasing the stability of the carotene, probably due to heat inactivation of the enzyme carotene oxidase. The addition of 0.5 per cent hydroquinone, 0.1 per cent alpha-tocopherol, or a combination of both, did not bring about greater retention of carotene during storage. The addition of diphenylamine and pelleting and coating with wax were both effective in reducing losses in alfalfa. Sullman (1941) used alpha-tocopherol and Quackenbush *et al.* (1942) used a combination of hydroquinone and alpha-tocopherol to accomplish the stabilization of carotene in solution. However, there appear to be chemical substances in plants which inhibit the action of these anti-oxidants.

Very little information is available concerning the retention of carotene in lespedeza hay during storage. Moore and Berry (1945) reported the feeding of lespedeza hay of high carotene content to calves. Herman (1944) has reported that preliminary results indicate a high retention of carotene in lespedeza hay.

Fraps and Kemmerer (1937), Smith (1936), Taylor and Russell (1938), Wall (1940), and Wiseman *et al.* (1936) all found that the loss of carotene from alfalfa meal was fairly rapid during the summer and very slow during the winter. Guilbert (1935) found that a rise in temperature of 10°C. approximately doubled the loss of carotene. Russell *et al.* (1934) found no loss of carotene stored *in vacuo* at 0°C. Kane *et al.* (1937) found that carotene losses in alfalfa and red clover hays were low in winter and high during summer. Camburn *et al.* (1944) observed that after one year of storage alfalfa retained 21 per cent and red clover 28 per cent of their original carotene content.

Woodward and Shepherd (1936) (1938) found that hay stored with high moisture content lost practically all of its carotene, whereas hay stored with a low moisture content retained a fair amount of this substance. Chopped

hay loses much more of its carotene than does long hay.

Camburn *et al.* (1942) present carotene analyses on several different silages at the end of an eight months ensiling period. The retention of carotene varied from 18 to 78 per cent depending on the treatment used in preservation. Carotene preservation by ensiling was shown to be high by Taylor *et al.* (1940) and Stone *et al.* (1943). Woodward and Shepherd (1938) made a comparison of different methods of roughage preservation. First-cutting alfalfa in the fresh state contained 164 micrograms of carotene per gram dry basis, while "no-treatment silage", "acid silage", "molasses silage" and field-cured hay contained 175, 168, 112, and 16 micrograms of carotene per gram of dry matter respectively.

The Site of Conversion of Carotene to Vitamin A in Dairy Cattle

The fact that certain carotenoids are the mother substances of vitamin A synthesized in animals is well known. However, limited knowledge exists concerning the mechanism of the conversion of carotenoids to vitamin A particularly as it affects the site of conversion in the organism.

The early experiments of Moore (1929), since amply confirmed by numerous investigators, clearly established the appearance of vitamin A in the liver of rats following oral administration of carotene. It has been generally assumed that an enzyme "carotenase," in the liver, is responsible in the conversion of carotenoids to vitamin A but direct experimental evidence of a satisfactory character has not been fully advanced to substantiate this hypothesis.

The site of conversion of carotene to vitamin A in the rat was reported to be the liver by Olcott and McCann (1931) on the basis of *in vitro* experiments. Sexton (1944), Rea and Drummond (1932), and Ahmad (1931) were unable to confirm the work of Olcott and McCann. Negative results were reported on *in vitro* experiments using shark liver by Euler and Euler (1931), on cat liver by Drummond and McWalter (1933), Ahmad (1931), and Rea and Drummond (1932). Parienti and Ralli (1931) obtained one positive test out of four on dog livers, while Euler and Klussman (1932) reported positive conversion of carotene to vitamin A when cow liver was incubated *in vitro* with a carotene solution.

Wolff *et al.* (1930) removed a portion of the liver from rabbits which had been fed on a diet devoid of both carotene and vitamin A. Following injection of carotene into the blood stream the vitamin A content of the liver tissue showed an increase at the end of three days. Van den Bergh, Muller and Brockmeyer (1920) found that a collodial suspension of carotene injected into the jugular vein of rabbits was soon removed from the blood and, to a large extent, was stored by the liver. Wilson *et al.* (1937) reported positive results with rabbit livers undergoing anaerobic autolysis.

More recently Glover *et al.* (1947), Mattson *et al.* (1947), Wiese *et al.* (1947), McCord and Clausen (1948), and Krause and Pierce (1948) have

presented evidence demonstrating that the transformation of carotene to vitamin A in the rat occurs in the small intestine.

Goodwin and Gregory (1948) have presented evidence that the conversion of carotene to vitamin A occurs in the intestine in the case of sheep, goats, and rabbits. Klosterman *et al.* (1949) suggest that carotene is converted to vitamin A during digestion, and/or absorption, in sheep. Swick *et al.* (1949) have shown that carotene is converted to vitamin A in the intestinal wall of the pig.

Elliott (1949) reported an increase in the vitamin A value of the blood plasma of the intestinal wall and jugular circulation following the ingestion of carotene by dairy calves. He reported no rise in the vitamin A content of blood plasma of the calf following intravenous injections of high carotene cow plasma but reported that the vitamin A content of the liver increased following such injections.

If, as more recent data would lead us to believe, the intestinal wall is the site of conversion in the rat it is necessary to explain how carotene can be utilized as vitamin A following parenteral administration. Very limited utilization is indicated by the work of Sexton *et al.* (1946) and more effective utilization by Tomarelli *et al.* (1946). It is possible that, in the case of parenteral administration of carotene, it is excreted into the lumen of the intestine where it has been carried either by the bile or the blood. In the work of Sexton *et al.* (1946) it was found that following the intraperitoneal administration of carotene, 5.5 per cent could be recovered in the gastrointestinal tract and feces. The final step in such a hypothesis would be the re-absorption of carotene by the intestine at which time it would be converted to vitamin A.

There is apparently species variation in the metabolism of carotenoids. Zechmeister (1937) observed that carotenoids are absent from the blood and tissue fat of the rat and the pig, but may be detected in varying amounts in the chicken, cattle, horses, and men. Palmer (1928-1929) observed that carotenoids are absent from the liver of the pig. Jensen and With (1939) in an extensive study which included 33 mammals comprising 21 species, 41 birds of 36 different species, 4 reptiles from 2 species, and 8 different human specimens found wide differences in the carotene content of the liver. Few birds have carotene in their livers. Vipers, moles, bats, house mice, squirrels, hares, guinea pigs, dogs, ermine, otter, swine, and white-nosed grivet had no carotene in their livers. Man, roe deer, horses, foxes, badgers, and cats had carotene present in the liver in varying amounts.

The Influence of Temperature on the Carotenoid and Vitaman A Content of Blood Plasma and Milk Fat of Dairy Cattle

No references were found regarding the effect of temperature on either the milk or the blood plasma levels of carotene and vitamin A in dairy cattle. Aron *et al.* (1946) in a study of 92 human patients found that elevation of

the body temperature to 105.5° or 106.0°F. (rectal) by physically induced fever was followed by a depression of the plasma vitamin A and carotene. The extent of the depression was directly related to the duration of the fever. At the termination of fever the plasma vitamin A was almost at its lowest level. The restoration of the vitamin A level occurred by the second day after treatment and took place spontaneously without any special medication or dietary measures. The plasma carotene level showed a similar pattern to that of vitamin A. Similar results have been observed in febrile infectious diseases, especially pneumonia (Clausen and McCord, 1938; May *et al.*, 1940; Josephs, 1943). The depression of the plasma vitamin A is believed to be produced by the retention of vitamin A in the liver (Lindquist, 1937).

Turner (1949) has reported a decrease in the protein-bound iodine of the blood with increasing temperature. This would indicate that the thyroid secretion rate of the cow is decreased with increasing temperature. Hibbs and Krauss (1947) reported no change in blood plasma or milk carotenoids when "protomone" was fed. They observed frequent decreases in both blood and milk vitamin A of individual "protomone"-fed cows. Dyrendahl (1949) observed no difference in blood carotene and vitamin A values when a group of cows fed iodinated casein was compared with controls. Allen, Wise, and Jacobson (1948) found no evidence of increased conversion of carotene to vitamin A in calves fed 1.5 to 3.0 grams of iodinated protein per 100 pounds of body weight, nor was the conversion decreased when thiouracil was fed.

Early German workers reported an interesting relationship between vitamin A and carotene in the thyroidectomized goat (Von Fellenburg and Gruter, 1932; Fasold and Heidemann, 1933), which has recently been reviewed by Drill (1943). They report that thyroidectomy in the goat results in an impairment of the conversion of carotene to vitamin A, resulting in increased carotene content of the milk. Kaplansky and Balaba (1946) reported that both thyroglobulin and iodinated casein catalyzed the conversion of carotene to vitamin A.

Kon and coworkers (1944-1945) have not been able to repeat the older German work in that in their experiment both carotene and vitamin A in milk and blood remained unaffected by either thyroidectomy or thiourea feeding. Smith, Niedermeir, and Schultz (1948) reported that beta-carotene administration to thyroidectomized and thiouracil-treated goats resulted in a rise in the blood plasma vitamin A levels comparable to a control group. Moreover they reported that carotene was not present in sufficient quantities to be detectable by the Kimble method of analysis.

In rats several workers have reported significant effects of thyroxine on conversion of carotene to vitamin A (Johnson and Baumann, 1947; Kelley and Day, 1948; and Drill and Truant, 1947). However, Remington *et al.* (1942) and Wiese *et al.* (1947) have reported results to the contrary. More recently Cama *et al.* (1949) have shown that thyroxine does not effect con-

version in rats but rather is associated with the absorption of carotene in the intestine.

METHODS AND MATERIALS

The Determination of Carotenoids and Vitamin A in Blood Plasma

The method of Kimble (1939) was used for carotenoids and vitamin A. In all cases from 5.0 to 10.0 ml. of plasma from oxalated blood were used for the determinations.

The plasma was measured into a narrow-necked glass centrifuge tube of 50-ml. capacity. To this was added an equal volume of 95-per cent ethanol and 15 ml. of petroleum ether (B.P. 30°-60°C.). A cork stopper was then placed in the neck of the centrifuge tube and the contents mixed by end-over-end inversion for 10 minutes. The tubes were then centrifuged for 15 minutes at 1700 r.p.m. to aid in layering. Ten ml. of the supernatant petroleum ether was then pipetted into a colorimeter tube and the yellow color read with a filter of 440 mu. wave length against a petroleum ether blank. Readings were made by means of an Evelyn photoelectric macrocolorimeter. The galvanometer reading was recorded and the concentration of carotenoids determined by referring to a calibration curve, prepared by plotting the per cent transmission against concentration for a series of carotene solutions of known concentration. Figure 1 shows the calibration curve for the Evelyn photoelectric colorimeter. A crystalline carotene mixture of 90 per cent beta- and 10 per cent alpha-carotene (obtained from General Biochemicals Inc., Chagrin Falls, Ohio) dissolved in petroleum ether was used in preparation of this curve.

For the determination of vitamin A the solution used in the determination of carotenoids was evaporated under vacuum by placing the tubes in a water bath at 40° to 43°C. until all the solvent was removed. The residue was then dissolved in 1 ml. of chloroform (Mallinckrodt U. S. P. XIII). An antimony trichloride blank was placed in the colorimeter with a filter of 620 mu., and the galvanometer set at 100. The unknown tube with the chloroform extract was then placed in the colorimeter and 9 ml. of antimony trichloride solution (antimony trichloride 20 per cent in chloroform, weight in volume) was added rapidly down the side of the tube from a rapid-delivery pipette. The galvanometer reading at the maximum color was recorded and its corresponding density (read from the L-G table accompanying the instrument) recorded as L620.

The blue color produced by the antimony trichloride-vitamin A reaction (Carr-Price reaction) follows Beer's law; i.e., there is a constant relationship between density (L) and the amount of material in solution (Dann and Evelyn, 1938). A constant, then, can be evaluated by standardizing the colorimeter with vitamin A. In these studies purified vitamin A obtained from Eastman Kodak Company, Rochester, N. Y., was used. The constant determined for the Evelyn photoelectric colorimeter used was L620 $\times$ 13.8 = micrograms of vitamin A per 10.0 ml. of chloroform. For this calculation the

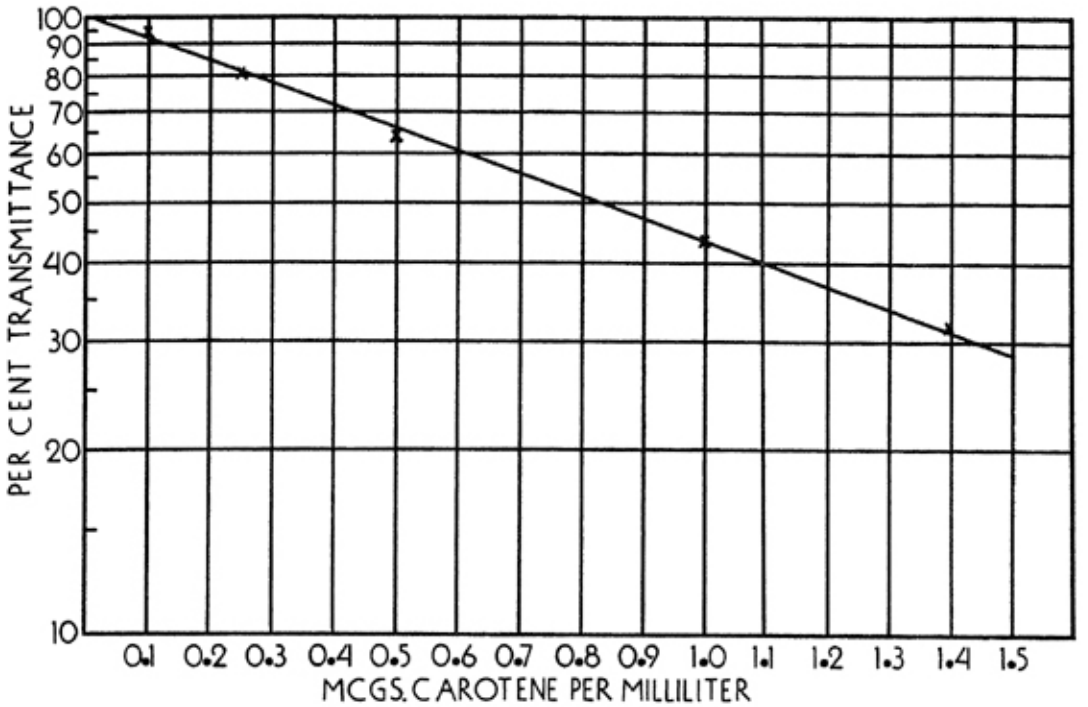

Fig. 1.—The standard curve used for carotene determination.

vitamin A reading was first corrected for the antimony trichloride reaction of the carotenoids present by using the correction factor of 0.14 (Dann and Evelyn, 1938). This value was checked and found to be applicable to the instrument used in this work by actually plotting the per cent transmission of blue color formed by the antimony trichloride-carotenoid reaction against the concentration of carotenoids in a series of solutions of known concentration. The calculation, then, for Vitamin A was (L620 — 0.14 L440) × 13.8 = micrograms of vitamin A per 10.0 ml. of unknown in chloroform.

The Determination of Carotenoids and Vitamin A in Milk

The method used for the analysis of milk was that of Boyer *et al.* (1944). The colorimeter used and the calculations for the determination of carotene and vitamin A were the same as those described for blood plasma.

The Determination of Carotenoids and Vitamin A in Liver and Intestine Tissues

These determinations were made by using the extraction procedure of Davies (1933). The calculations for carotenoids and vitamin A in the petroleum ether extract were made in the same manner as previously described for blood plasma.

This method was also used in the determination of carotenoids and vitamin A in intestine tissue except for one additional step; namely, the washing of the supernatant petroleum ether with an acidified alcoholic wash solution (1 ml. of hydrochloric acid to 100 ml. of 95 per cent ethanol and made to 1 liter) to reduce emulsions.

The Determination of Carotenoids in Plant Tissues

All plants, hays and silages were analyzed for carotenoids according to the method of Moore and Ely (1941). Green plants and silage were chopped fine for analysis. The hay was ground first in a hammer mill and then given a final grinding in a Wiley mill.

The Determination of Tocopherols in Plant Tissues

Plants were analyzed for tocopherol content by a modified Furter-Meyer procedure described by Wall and Kelley (1946). Dry or green plant materials were extracted with petroleum ether; chlorophylls and xanthophylls were separated from tocopherols by adsorption on a supercel-activated magnesia column. Carotenoids and tocopherolquinones present were destroyed by treatment with 85 per cent sulfuric acid. The tocopherols were then transferred to an ethanol solution, oxidized with nitric acid, and the insoluble lipoids removed by filtration.

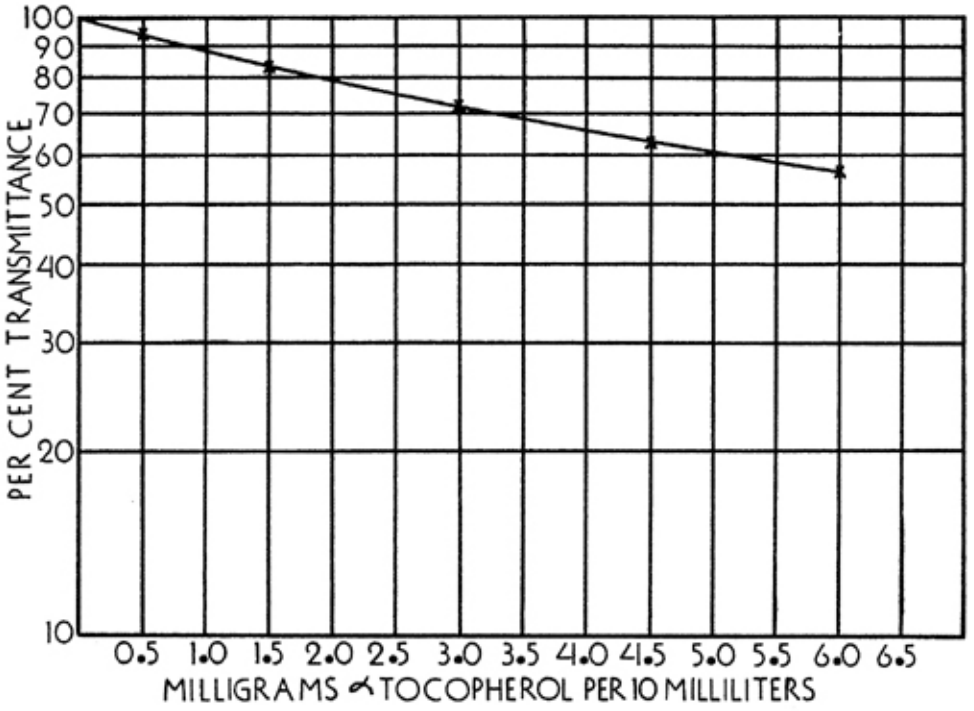

Fig. 2.—The standard curve used for tocopherol determination.

The reaction involving tocopherols and nitric acid gives rise to a product called a red hydroquinone which has maximum absorption at 480 mu. The percentage transmission of light of the unknown, as compared to a blank of ethanol and nitric acid, was measured by a Coleman Model 14 Universal Spectrophotometer at 480 mu. The concentration of tocopherol was determined by consulting a calibration curve, prepared by plotting the per cent transmission at 480 mu. against concentration for a series of solutions of known concentrations. Figure 2 shows the calibration curve used in this work. An alpha-tocopherol solution obtained from Nutritional Biochemicals Inc., Cleveland, Ohio, was used in preparation of this curve.

EXPERIMENTAL RESULTS

A Comparison of Lespedeza and Alfalfa Hays as Sources of Carotenoids for Dairy Cattle

Korean lespedeza is an important crop in Missouri with about nine million acres grown annually. Limited data reported in the literature indicate that the carotenoid content of lespedeza hay is higher than alfalfa hay. The purpose of this part of the investigation was: (a) to compare the carotenoid content of Korean lespedeza and alfalfa hay and (b) to determine if the higher carotenoid content of lespedeza, if present, can be utilized effectively by dairy cattle.

TABLE 1. - EXPERIMENTAL SOURCE OF CAROTENOIDS

Animal No.	Breed	Previous Source of Roughage	Source of Carotenoids	Amount of Hay Fed	Average Carotenoid Content of Hay
				(lbs./day)	(ug./g., dry basis)
508	Jersey	Pasture	Lespedeza hay	7	40.16
964	Jersey	Alfalfa hay	Alfalfa hay	7	8.09
513	Jersey	Pasture	Lespedeza hay	7	40.16
510	Jersey	Pasture	Alfalfa hay	7	8.09
156	Holstein	Alfalfa hay	Lespedeza hay	12	51.9
158	Holstein	Alfalfa hay	Alfalfa hay	12	13.46

Four pregnant Jersey heifers and two pregnant Holstein heifers were divided into two dietary groups approximately 8 weeks prior to calving in the case of the Jerseys and 12 weeks in the case of the Holsteins.

Each animal received a low carotenoid grain ration made up as follows:

Ground white corn	300 pounds
Ground oats	300 pounds
Wheat bran	300 pounds
Linseed oil meal (34%)	100 pounds
Salt	10 pounds
Steamed bone meal	10 pounds

The rate of feeding the grain mix was 7 pounds per day for the Jerseys and 10 pounds per day for the Holsteins. Each animal received 2 pounds of beet pulp (dry basis) per day as a source of roughage in addition to hay. The rate of feeding and the carotenoid content of the hays used in the trial are shown in Table 1. The Jerseys were placed on experiment in April, 1947. The hays fed were harvested and cured during the summer of 1946. Both the lespedeza and the alfalfa hay were sun-cured in the swath and baled in the field. The hay was then stored in a shed where it was protected from rain and sunlight. The Holstein heifers were placed on experiment in March

1948 and both the lespedeza and alfalfa hays were sun-cured in the swath and baled in the field during August 1947.

Three of the Jersey heifers were taken directly off pasture when placed on experiment. The remaining three, which included both Holsteins, had been fed under dry lot conditions. The roughage fed was alfalfa hay. This pre-experimental data is given in Table 1.

Weekly aliquot samples of the hay were collected by saving a center block from each bale of hay fed. These blocks of hay were placed in a burlap bag and, at weekly intervals, finely ground in a hammer mill. The ground hay was then mixed thoroughly and an aliquot sample taken for further grinding in a Wiley mill. The finely ground sample was then analyzed for carotenoids by the method of Moore and Ely (1941).

The animals were placed in individual stalls and bedded on shavings. They remained in the stalls except for a brief period each day when they were allowed to exercise in a dry lot. Each feeding of hay, grain, and beet pulp was weighed carefully. The amount of hay fed the animals was determined by observing the intake of the animals for 3 or 4 days prior to the beginning of each trial and then feeding slightly below the average intake to insure equal intake by animals of approximately equal size.

Blood plasma carotenoids and vitamin A analyses were made on two successive days at the beginning of the experiment and again at intervals during the prepartal and postpartal periods. The blood for analyses was drawn from the jugular vein. The plasma of newborn calves was analyzed for cartenoids and vitamin A before the ingestion of colostrum and again on the sixth day following birth. The sixth day was chosen due to the fact that usually the period of colostrum feeding had ended by this time. Blood plasma carotenoids and vitamin A were determined by the method of Kimble (1939).

The calves were kept in stalls separate from the dam so they could not nurse the cows. The cows were milked twice daily and the complete milking sampled for assay. The calves were fed portions of the first ten milkings at the rate of 10 per cent of the body weight. The carotenoid and vitamin A contents of the first 10 milkings were determined by the method of Boyer *et al.* (1944). In the case of the Jerseys a sample of milk was analyzed on the 60th day following calving since these animals were fed their respective hays for this period of time. After the colostral period grain feeding was based on milk production (1 pound of grain to 3 pounds of milk) and hay was fed at the rate of 2 pounds of hay per 100 pounds of body weight.

The blood plasma carotenoid and vitamin A values for the cows studied are shown in Table 2. The values for both carotenoids and vitamin A were higher in the case of animals 508, 513, and 510 due to their having been on pasture prior to being placed on experiment. The level of these substances in the blood of the other animals was low due to their previous dry lot ra-

TABLE 2. - MATERNAL BLOOD PLASMA CAROTENOIDS AND VITAMIN A

Animal No.	Source of Carotenoids	Prefreshening Data						Postfreshening Data		
		12 wk.	8 wk.	6 wk.	3 wk.	2 wk.	1 wk.	3 hrs.	1 wk.	4 wk.
		(ug./100 ml.)						(ug./100 ml.)		
		Blood-Plasma Carotenoids								
508-J*	Lespedeza		773.1	508.0	452.0			332.8		630.0
964-J	Alfalfa		168.6		151.2	165.2		72.3		130.0
513-J	Lespedeza		1304.0	574.0	416.4		536.0	298.0		445.1
510-J	Alfalfa		1332.0	321.4	188.6		192.8	141.0		194.4
156-H	Lespedeza	20.93	155.2		201.0		151.9	138.8	138.8	
158-H	Alfalfa	37.34	65.8		143.9		103.9	196.8	216.8	
		Blood-Plasma Vitamin A								
508-J	Lespedeza		42.70	33.12	31.3			17.39		29.30
964-J	Alfalfa		26.82		4.75	8.60		3.97		9.43
513-J	Lespedeza		61.30	10.35	11.20		30.17	9.94		23.71
510-J	Alfalfa		66.31	17.83	6.57		11.02	8.75		6.93
156-H	Lespedeza	4.10	32.04		11.80		25.55	18.59	17.97	
158-H	Alfalfa	6.33	28.77		22.34		20.05	10.31	13.93	

*The letters, J and H, refer to Jersey and Holstein breeds, respectively.

tions. There was a decline in the plasma carotenoid and vitamin A values in the Jerseys of both dietary groups from the eighth week prepartum to the time of parturition.

In the case of the Holstein heifers there was a rise in both plasma carotenoids and vitamin A from the twelfth week prepartum to the third week prepartum presumably due to a higher carotenoid content of the experimental rations as compared to their previous ration. This was followed by a decline in carotenoids and a rise in vitamin A at 1 week prepartum in the case of the heifer receiving lespedeza hay and a decline in both carotenoids and vitamin A in the heifer receiving alfalfa.

The differences between the plasma carotenoid and vitamin A values at 8 weeks and 2 weeks prepartum are a measure of the effects of the two dietary regimes. It is unlikely that the usual gestational decrease in plasma carotenoids and vitamin A was a factor during that period since the same observation has been reported (Kuhlman and Gallup, 1944; Semb *et al.*, 1934; and Sutton *et al.*, 1945) to occur often some two weeks prior to parturition.

Following parturition there was the usual decline in plasma vitamin A in the case of all animals, and a decline in plasma carotenoids in all cases save Holstein No. 158 in which a rise occurred—a phenomenon which occurs in a minority of cases (Kuhlman and Gallup, 1944).

It would appear from these data that the higher carotenoid content of the lespedeza hay was utilized by the animals as the plasma vitamin A was

maintained at a much higher level than in the animals receiving alfalfa hay. Likewise the plasma carotenoids were maintained at a higher level with the exception of one Holstein heifer during the postpartum period, during which time the plasma carotenoids of the heifer receiving alfalfa hay were higher.

Carotenoid and Vitamin A Content of Colostrum.—The carotenoids and vitamin A values for the first 10 milkings postpartum, and, in the case of the four Jerseys, the 120th milking, are presented in Tables 3 and 4 respectively. The output of vitamin A activity expressed as International Units of vitamin A per 100 ml. of colostrum is presented in Table 5. The carotenoid

TABLE 3. - THE CAROTENOID CONTENT OF COW'S COLOSTRUM AND MILK

Animal No.	Source of Roughage	Carotenoids at Successive Milkings										
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	120th
		(ug./100 ml.)										
508-J*	Lespedeza	115.05	146.70	103.20	65.70	29.57	30.19	45.23	33.04	30.81	36.03	60.12
964-J	Alfalfa	19.71	43.35	25.88	48.22	52.58	108.38	58.43	35.47	43.05	35.47	41.62
513-J	Lespedeza	315.00	285.30	99.90	73.43	71.93	51.38	45.60	52.20	56.25	31.78	71.25
510-J	Alfalfa	15.95	87.00	99.00	49.80	40.55	20.93	28.94	14.87	18.40	15.68	37.73
156-H	Lespedeza	48.21	66.90	44.10	77.48	40.88	36.03	17.03	20.08	20.08	16.05	
158-H	Alfalfa	27.70	36.03	45.60	52.58	33.07	20.63	12.02	24.99	50.55	22.94	

*The letters, J and H, refer to Jersey and Holstein breeds, respectively.

TABLE 4. - THE VITAMIN A CONTENT OF COW'S COLOSTRUM AND MILK

Animal No.	Source of Roughage	Vitamin A at Successive Milkings										
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	120th
		(ug./100 ml.)										
508-J*	Lespedeza	72.86	65.62	71.14	58.64	19.42	16.91	27.11	31.77	21.19	17.40	38.88
964-J	Alfalfa	19.21	39.85	18.61	16.17	15.01	38.39	15.98	5.09	13.99	13.08	13.42
513-J	Lespedeza	112.50	98.07	65.41	58.68	51.34	41.81	39.12	35.40	29.70	44.71	34.57
510-J	Alfalfa	14.59	23.38	84.75	47.71	46.59	26.23	22.70	17.16	46.98	19.58	24.03
156-H	Lespedeza	82.08	130.00	46.89	62.20	20.53	27.77	22.95	37.67	31.67	20.42	
158-H	Alfalfa	50.72	74.62	79.28	33.02	25.84	25.06	9.06	23.24	22.43	23.70	

*The letters, J and H, refer to Jersey and Holstein breeds, respectively.

values were converted to International Units by multiplying the micrograms of carotenoids by 0.6, a figure adopted at the International Vitamin Conference of 1934 and subsequently adopted for the United States Pharmacopoeia. To calculate the International Units of vitamin A *per se*, the micrograms of vitamin A were multiplied by 4.0, a figure used by Sutton *et al.* (1945) in similar computations. Then by combining the International Unit values of carotenoids and vitamin A, a figure was obtained which represents, perhaps approximately, the output of vitamin A activity.

An examination of these data reveals that the output of vitamin A activity as well as the carotenoid and vitamin A content of the colostrum of the cows receiving lespedeza hay was much higher than that from cows receiving alfalfa hay, reflecting a trend much the same as in the case of the blood picture of the cows.

One of the most interesting observations of the entire experiment was the vitamin A activity of the colostrum of animal No. 964. In view of the extremely low blood plasma carotenoid and vitamin A values, it is evident that there is a very efficient mechanism which utilizes the body stores of vitamin A for the nourishment of the young.

TABLE 5. - THE OUTPUT OF VITAMIN A ACTIVITY IN COW'S MILK AND COLOSTRUM
(In International Units)

Animal No.	Source of Roughage	Vitamin A Activity at Successive Milkings										
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	120th
		(I.U./100 ml.)										
508-J*	Lespedeza	360.5	350.5	346.5	274.0	95.4	85.8	135.6	146.9	103.3	91.2	191.59
964-J	Alfalfa	88.7	185.4	90.0	93.6	91.6	218.6	99.0	41.7	81.8	83.6	78.65
513-J	Lespedeza	639.0	563.5	321.5	278.8	248.5	198.1	183.8	172.9	152.6	197.9	181.03
510-J	Alfalfa	67.9	155.7	398.4	220.7	210.7	117.5	107.2	76.6	199.0	87.7	118.76
156-H	Lespedeza	357.3	560.1	214.0	295.3	106.7	132.7	102.0	162.7	138.7	91.3	
158-H	Alfalfa	219.5	320.1	344.5	163.6	123.2	112.6	43.4	107.9	120.1	108.6	

*The letters, J and H, refer to Jersey and Holstein breeds, respectively.

TABLE 6. - THE EFFECT OF MATERNAL DIET UPON THE PLASMA CAROTENOIDS AND VITAMIN A OF THE NEWBORN CALF

Number of Dam	Source of Roughage	Plasma of Newborn Calf		Plasma at 6 Days of Age	
		Carotenoids	Vitamin A	Carotenoids	Vitamin A
		(ug./100 ml.)		(ug./100 ml.)	
508	Lespedeza hay	5.56	5.26	44.95	15.32
513	Lespedeza hay	3.7	5.22	49.14	16.14
510	Alfalfa hay	0.8	2.4	23.07	4.53
156	Lespedeza hay	1.83	4.12	22.01	24.63
158	Alfalfa hay	1.60	3.40	14.55	9.98

Plasma Carotenoid and Vitamin A Values in Calves.—Five of the heifers on experiment gave birth to normal calves. The calf of animal No. 964 was stillborn. It is not unlikely that this was due to the low carotenoid diet in view of the low plasma values obtained. Proof of a definite nature, however, is lacking.

The plasma carotenoid and vitamin A analyses of the calves at birth, and on the sixth day after birth, are summarized in Table 6. Both the plasma carotenoid and vitamin A values were higher at birth, in the case of the calves whose dams received lespedeza hay as a source of carotenoids as compared to those whose dams were fed alfalfa hay.

Thus it would appear that associated with the higher carotenoid values of lespedeza hay are higher levels of carotenoids in the plasma of the dam, in the colostrum produced by the dam fed such hays, and likewise in the plasma of the calf receiving the colostrum from cows so fed.

A Comparison of Pasture and Dry Lot Feeding as Sources of Carotenoids for Dairy Cattle

The fact that the vitamin A activity of butterfat follows a definite seasonal trend has been well established. The highest values are obtained in summer when green forage is available. The concentration of carotene and vitamin A decreases progressively in roughages throughout the winter season. The degree of lowered butterfat values depends largely upon the carotenoid content of the roughage consumed.

During the pasture season of 1948 an investigation was begun to study the relative merits of pasture as a source of carotenoids as compared to dry lot feeding in which alfalfa hay was the principal source of roughage fed cattle in the Missouri Station dairy herd.

Experimental.—During the pasture season of 1948, three cows each of the Jersey, Guernsey, and Holstein-Friesian breeds on pasture were paired with three animals of like breed that were on Advanced Registry Test and maintained under dry lot conditions. All animals were paired for age and stage of lactation.

Both the pasture group and the dry lot group received a grain concentrate of the following composition:

Ground yellow corn	800 pounds
Ground oats	450 pounds
Wheat bran	400 pounds
Soybean oil meal	300 pounds
Steamed bone meal	25 pounds
Salt	30 pounds

This grain concentrate was fed on the basis of daily milk production. Usually 1 pound of grain to 3 pounds of milk was fed daily for Jerseys and Guernseys and 1 pound of grain to 4 pounds of milk in the case of Holsteins.

The animals in the dry lot were fed alfalfa hay *ad libitum* and in addition each cow, depending on body size, received from 3 to 4.5 pounds of dried beet pulp per day.

The animals on pasture received some alfalfa hay as a supplement during periods when the pasture was short. The pasture utilized included small

grains, principally barley, in early spring followed by a permanent pasture consisting of Kentucky blue grass, timothy, white clover, and Korean lespedeza. Toward the end of the experimental period the animals were pastured for a short time on small grains.

Just before the cows were turned on pasture in the spring of the year a sample of milk was collected from each of the cows in the two experimental groups. Subsequently, at approximate weekly intervals, throughout the pasture season, individual milk samples were collected and composited according to morning and evening production and analyzed for vitamin A and carotenoids. Milk carotenoids and vitamin A were determined by the method described by Boyer *et al.* (1944).

Results and Discussion.— The data showing the level of milk carotenoids and vitamin A during the barn feeding period prior to pasture and at weekly intervals during the pasture season for the pasture group and during the same period for the dry lot group are shown in Figure 3.

The animals in the pasture group were turned on barley pasture in April. There was a rapid increase in the carotenoid content of the milk following the beginning of pasture season, reaching a peak in mid May. This peak was followed by a leveling-off period. There was a decline in late September followed by a rise in October when the animals were placed on small grain pasture. The carotenoid content of the group fed in dry lot tended to remain fairly uniform throughout the period.

Milk vitamin A of the animals on pasture showed an initial rise and then tended to level off. There was a decline in September followed by a slight rise in October in the case of Holsteins and Jerseys but not in Guernseys. The vitamin A in the milk of the group fed under dry lot conditions was variable but did not show significant rises throughout the period.

The carotenoid content of the milk of the group utilizing pasture was significantly higher during practically all of the pasture period, remaining at a higher level in the case of all breeds.

The differences in the vitamin A content of the milk of the pasture and dry lot groups were not as marked as were the differences in carotenoid content. Hibbs *et al.* (1949) found that the vitamin A level in milk is subject to less fluctuation due to the intake of carotenoids from the forage than was carotenoids. There was little difference in the vitamin A content of the milk of the two groups in late September when the pasture was short due to dry weather. As a matter of fact, the vitamin A of the dry lot group was slightly higher for a period in the Holstein and Guernsey breeds. The fact that the vitamin A level in the milk is less subject to fluctuation due to the intake of carotenoids is attributed to the influence of liver storage of vitamin A and also to the influence of the conversion of carotene to vitamin A (Hibbs *et al.*, 1949). It is of interest in this connection that on numerous occasions the milk vitamin A was observed to increase at the same time

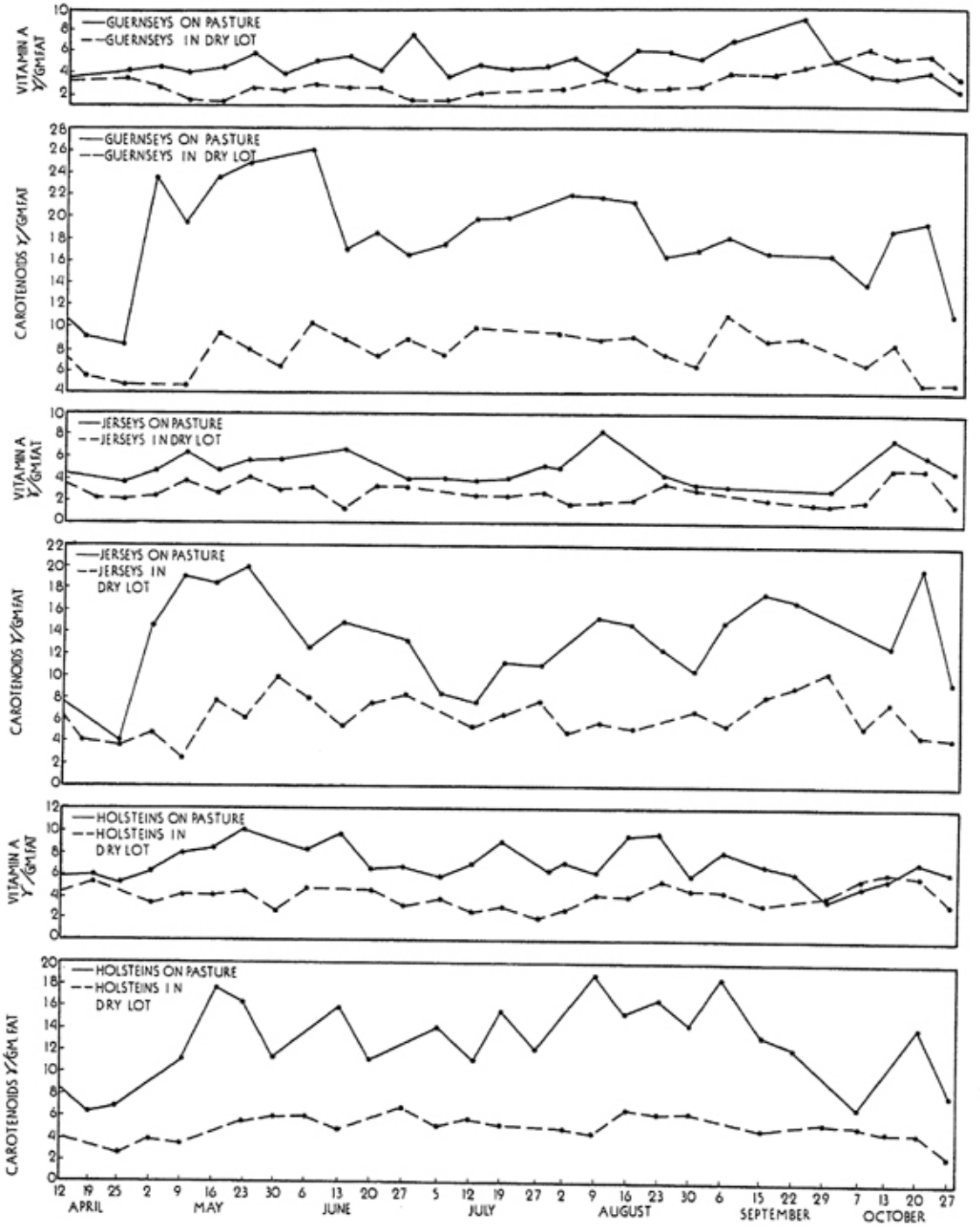

Fig. 3.—A comparison of the carotenoid and vitamin A content of the milk fat of cows on pasture with cows in dry lot.

the milk carotenoids decreased. This phenomenon has been observed in milk by Hibbs *et al.*, (1949) and in blood plasma by Braun (1945), Hibbs and Pounden (1948), and Sutton and Soldner (1945).

Breed differences in milk carotenoids were noted. The Guernsey had the highest, the Jerseys intermediate and the Holsteins the lowest values. The

data showed higher carotenoid content for Holstein milk than that reported by most investigators, a fact that is unexplained. The data for Jersey cows on pasture compare favorably with the work of Hibbs *et al.* (1949) and for the Guernseys with that of Sharp and Hand (1939).

These data seem to indicate clearly that (a) pasture is a better source of carotenoids than dry roughage such as hay provided there is sufficient rainfall and the pastures are managed so as to keep them growing rapidly, and (b) a closer relation exists between increased carotenoid content of the roughage and milk carotenoids than between roughage carotenoids and milk vitamin A.

Losses of Carotenoids and Tocopherols During Storage of Hays and Silage

Carotenoid losses in stored hays present a serious problem to the dairyman. Carotenoids (provitamins A), the forms in which vitamin A activity exists in plants, are unstable. These forms are readily oxidized, subject to photolysis, and destroyed by enzyme activity (Monroe *et al.*, 1946). Hays and silages which retain high percentages of their original carotenoid content during storage are of the greatest value in the nutrition of dairy cattle. This is especially true in early spring when vitamin A reserves of the animal are likely to be low and when green forage is not yet available.

In view of the fact that, in our previous work, Korean lespedeza hay was found to be a richer scurce of carotenoids than alfalfa hay, it was decided to collect data on losses of carotenoids in these two hays and in alfalfa silage during storage. It was also decided to analyze the hays and silage for tocopherol and lignin content in order to determine what influence, if any, these substances might have on the losses of carotenoids in hay during storage.

Experimental Results.—During the haying season of 1949 data were collected on two cuttings of clover hay, two cuttings of alfalfa hay, two lots of lespedeza hay, and one lot of alfalfa silage. At the time the forage was cut an aliquot sample was obtained by gathering individual plants over a large area of the field to be cut. The green plant material was chopped fine and analyzed for carotenoids by the method of Moore and Ely (1941). In the case of the hays cut later in the haying season the plants were analyzed for tocopherol content by the method of Wall and Kelley (1946).

All of the hays studied were sun-cured and baled in the field. The hays were analyzed for carotenoid content, per cent moisture, and, in some cases, for tocopherol content at the time they were placed in storage. The alfalfa silage was analyzed for carotenoid content and dry matter at the time it was ensiled. After six months in storage the hays and silage were analyzed for carotenoid, dry matter, and tocopherol content and the hays were analyzed for lignin content. The lignin content was determined by the method of Crampton and Maynard (1938). The dry matter content was determined according to the method of the Association of Official Agricultural Chemists (1945).

The first-cutting alfalfa hay was grown on river bottom land in Boone County, Missouri. An analysis of the green plants was not made. It was sun-cured and baled in the field. When placed in storage the hay was green in color, extra leafy, had fine pliable stems, but was high in moisture content. There was considerable heating of the hay in storage. This resulted in a browning of the hay and mold formed on the bottom bales in storage.

The alfalfa silage was made from first cutting alfalfa grown on the South Farms of the University of Missouri. The plants were in early bloom stage, leafy, and had fine stems. The alfalfa was mowed, windrowed with a side-delivery rake, and then chopped by a field ensilage cutter. Forty pounds of blackstrap molasses were added to each ton of plant material. When fed after six months storage in the silo the silage was green in color and very palatable. There was no spoilage except for a slight amount on the top layer.

Hay was made from second-cutting alfalfa grown on the University of Missouri South Farms. It was cut in full bloom, cured and baled in the field. The hay was green in color, of medium leafiness, and had medium-sized stems.

The first cutting of red clover likewise grown on the University of Missouri South Farms was harvested for hay. It was cut in full bloom due to inclement weather preventing earlier harvesting. The hay was exposed to two heavy rains while curing in the field. When stored as baled hay it was black in color, of medium leafiness, had large, stiff stems, and was of poor palatability.

The second crop of red clover on the same field was harvested in the early bloom stage. It was cured and baled in the field without rain and when stored it was green in color, leafy, had medium-sized stems, and was very palatable.

An early cutting of lespedeza was obtained on a plot at the University of Missouri South Farms in which lespedeza and oats were grown together. Due to inclement weather the oats were not removed and resulted in a retardation of the growth of the lespedeza. The plants were cut in the early bloom stage. When placed in storage the hay was of medium leafiness, dark green in color, and of high quality. A late cutting of lespedeza hay was made on September 3, 1949. This plot was adjacent to the one cut earlier. The plants had but little active growing tissue at the time of harvest and both blooms and seed were present. This hay, though green in color, had coarser, stiffer stems than the lespedeza cut earlier. There were fewer leaves, due to shattering, and it was typical of much of the late-cut lespedeza hay found in Missouri.

The losses of carotenoids during storage of the hays and silage studied are shown in Table 7.

The alfalfa silage had a higher carotenoid content at the end of six months storage than any of the hays studied. This is in accord with the findings of other investigators previously cited.

TABLE 7. - LOSSES OF CAROTENOIDS DURING STORAGE OF HAYS AND SILAGE

Roughage	Date Harvested	Carotenoid Content (Dry Basis)			
		Green Plant	When Stored	After 6 Mo. Storage	Loss in Storage
		(ug./g.)	(ug./g.)	(ug./g.)	(%)
Alfalfa silage	May 2, 1949	281.6	110.6	47.4	57.1
Alfalfa hay, 1st cutting	May 15, 1949		27.8	10.1	63.7
Alfalfa hay, 2nd cutting	June 30, 1949	172.3	27.6	13.9	49.6
Red clover hay, 1st cutting	June 28, 1949	233.3	12.1	7.0	42.1
Red clover hay, 2nd cutting	Aug. 4, 1949	233.7	34.9	8.0	77.1
Lespedeza hay, early cut	Aug. 2, 1949	232.9	63.5	35.1	44.7
Lespedeza hay, late cut	Sept. 3, 1949	105.6	50.2	23.9	52.4

Of the hays studied, early-cut Korean lespedeza hay had a much higher carotenoid content when placed in storage and at the end of the 6 months storage period than any of the other hays. Late-cut lespedeza hay though lower in carotenoid content than the early-cut lespedeza gave higher values than the alfalfa or red clover hays.

The alfalfa hays were slightly higher in carotenoid content than the clover hays after 6 months of storage. The growing plants of the second cutting contained less carotenoids than the first cutting presumably due to the fact that it was more mature when cut. The carotenoid content of the second-cutting hay in storage was slightly higher than in the first cutting. This may be attributed to the fact that there was less of the 6-month storage period during hot summer weather.

The red clover hays were very low in carotenoid content at the end of 6 months storage. The second cutting was much higher than the first cutting when placed in storage apparently due to the fact that the first was exposed to two rains while curing in the field.

From these results it is concluded that lespedeza hays maintain a much higher carotenoid content during storage than alfalfa or clover hays. Apparently these differences are due to variations in enzyme activity (Waugh *et al.*, 1944). Ensiling appears to be a more efficient method of conserving carotenoids than the sun-curing of hays.

The tocopherol content of the hays and silage studied is shown in Table 8. Tocopherol determination was not made on the green plants, hay, and silage at the time of storage in the case of alfalfa silage, first and second cuttings of alfalfa, and first-cutting red clover. These were analyzed after 6 months of storage. Complete analyses were made in the case of second-cutting clover and early- and late-cut Korean lespedeza hays.

The tocopherol analyses were made to determine whether or not they aid in stabilizing the carotenoids in the plant tissues; i.e., act as anti-oxidants as they do in the case of carotenoid solutions (Sullman, 1941).

TABLE 8. - THE TOCOPHEROL CONTENT OF SOME HAYS AND SILAGE

Roughage	Tocopherol Content		
	Green Plant	When Stored	After 6 Months of Storage
	(mgm./g., dry basis)		
Alfalfa silage			1.42
Alfalfa hay, 1st cutting			0.95
Alfalfa hay, 2nd cutting			0.84
Red Clover hay, 1st cutting			1.05
Red Clover hay, 2nd cutting	5.84	3.23	1.46
Lespedeza hay, early cut	3.02	1.83	0.65
Lespedeza hay, late cut	2.09	1.21	0.27

Red clover plants (green) and hays (dry) were found to be very high in tocopherols but retained little of their carotenoid content after 6 months of storage. The alfalfa hays were lower in tocopherol but higher in carotenoids as compared to red clover hay and were higher in tocopherols but lower in carotenoids as compared to the lespedeza hays. The lespedeza hays which were by far the highest in carotenoid content were the lowest, both in growing plants and in the stored hay, in tocopherol content.

From these data it would appear that tocopherols do not have a stabilizing effect on carotenoids in plant tissues. This finding is in accord with the observations of Mills and Hart (1945). The losses of tocopherols during storage appear to be of the same magnitude as that of carotenoids.

TABLE 9. - LIGNIN AND DRY MATTER CONTENT OF STORED HAYS

Kind of Hay	Dry Matter Content (%)			Lignin Content after 6 Mo. Storage (%, dry basis)
	Green Plant	When Stored	After 6 Mo. Storage	
Alfalfa hay, 1st cutting		80.1	94.4	11.12
Alfalfa hay, 2nd cutting	37.9	88.0	94.0	14.10
Red clover hay, 1st cutting	26.8	85.2	89.9	17.17
Red clover hay, 2nd cutting	21.4	87.1	94.0	13.37
Lespedeza hay, early cut	31.5	82.3	88.0	14.85
Lespedeza hay, late cut	34.2	85.4	89.9	18.55

The lignin and dry matter content of the hays studied is presented in Table 9. The highest lignin content was found in the late-cut lespedeza hay. The next highest lignin content was found in the first-cutting red clover hay, which was cut when in full bloom. The lignin content of early-cut lespedeza hay was slightly higher than that of second-cutting red clover and alfalfa hays. The first-cutting alfalfa hay contained the least amount of lignin of the hays studied. This hay was cut during the bud stage when the stems were soft and pliable. The lignin content of the lespedeza hays was slightly lower than that reported by Swanson and Herman (1943). The fact that

the hays used in the experiment being discussed were not above average in per cent of leaves may account for this difference since Swanson and Herman observed that the leaves were higher in lignin than the stems.

From these results one cannot make a positive statement as to whether or not lignin is a factor in the retention of carotenoids by hays during storage. In the case of the two lespedeza hays it would appear that lignin exerted no influence since the hay having the higher lignin content showed a higher per cent loss of carotenoids in storage. The same statement can be made in the case of the alfalfa hays. In the case of the red clover hays the one having the higher lignin content also had the highest retention of carotenoids during storage. This point should be the subject of further study.

In Vitro Studies on the Conversion of Carotene to Vitamin A in Dairy Calves

The effects of varying levels of carotenoids in the diet on the levels of carotenoids and vitamin A in the milk of dairy cattle are well known. However, limited knowledge exists concerning the physiological mechanism involved in the conversion of carotenoids to vitamin A. No attempt was made in this investigation to study all of the factors involved in such transformation. It was decided, however, that in view of the recent findings concerning the site of conversion in smaller animals that this phenomenon was worthy of study in dairy calves.

Euler and Klussman (1932) reported positive conversion of carotene to vitamin A when incubated *in vitro* with a carotene solution. Elliott (1949) submitted evidence that the intestine is a site of conversion of carotene to vitamin A.

It was decided that the conversion of carotene to vitamin A in dairy calves must be demonstrated more effectively by *in vitro* studies. In the intact animal, it is assumed that vitamin A is removed about as quickly as formed, especially in the case of synthesis in the intestinal mucosa.

Methods.—The calves used in this experiment were males of the Holstein-Friesian, Guernsey and Jersey breeds dropped in the Missouri Station herd. The calves were allowed to remain with the dam for 3 to 4 days and were permitted colostrum feeding as usual. At 4 days of age they were removed from the dam and received mixed herd milk (butterfat content 3.9 to 4 per cent) until 3 to 4 weeks of age. At 2 weeks of age the calves were given free access to a "calf-starter ration" low in carotene content and made up of:

White corn	400 pounds
Ground oats	300 pounds
Wheat bran	300 pounds
Linseed oil meal	100 pounds
Non-fat dry milk solids	60 pounds
Soluble blood flour	20 pounds
Irradiated yeast	1 pound

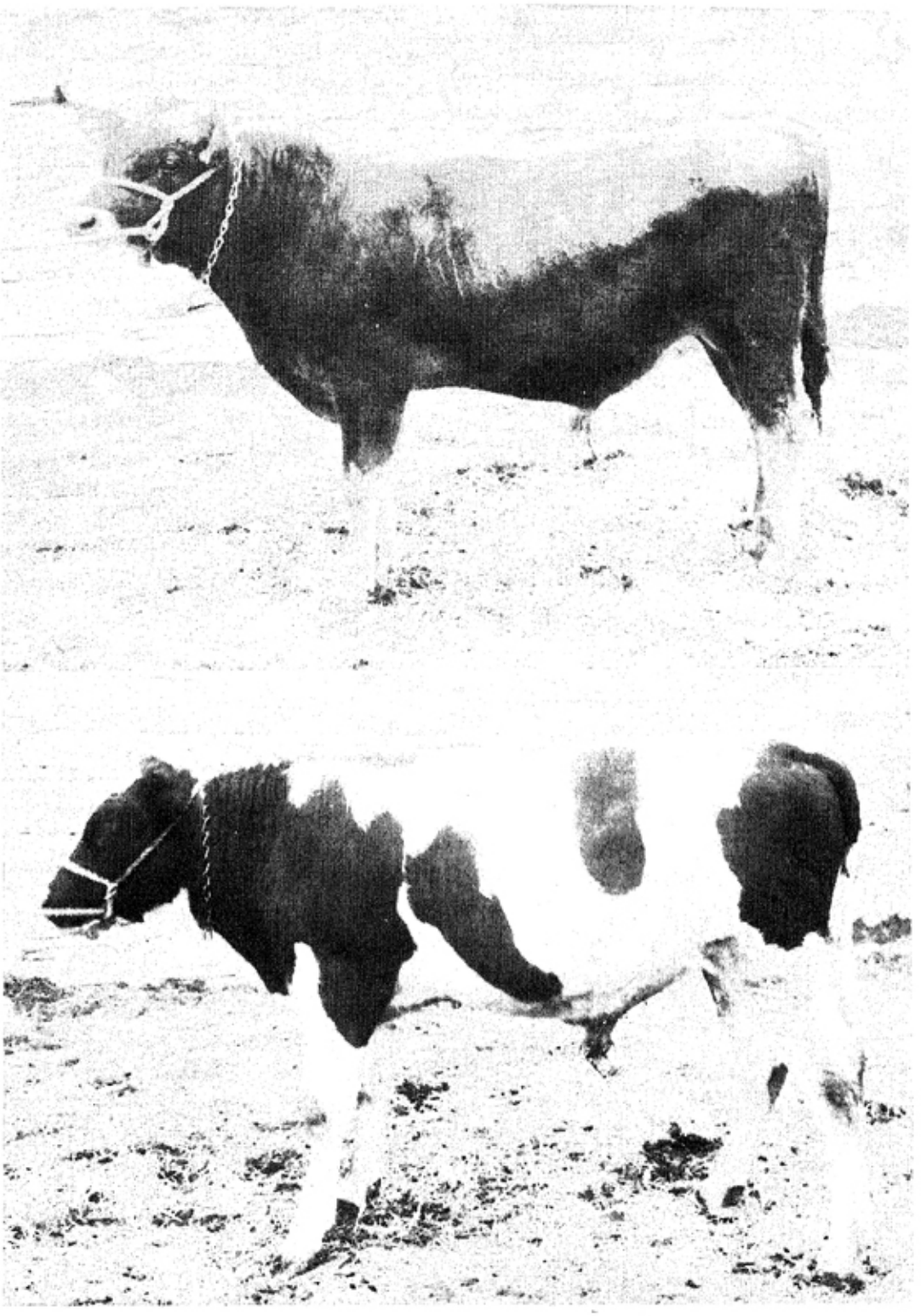

Fig. 4.—Animals No. 508 (above) and No. 189 (below).

Straw containing 1.87 γ of carotene per gram (dry basis) was fed *ad libitum* as the sole source of roughage. The animals were bedded with wood shavings in individual stalls. For a brief period each day they were allowed to exercise in a dry lot.

The calves were slaughtered when blood plasma vitamin A values reached lowered levels ranging from 5.16 to 14.5 γ per 100 ml. of plasma. Care was exercised to prevent the animals being depleted of vitamin A to the point that deficiency symptoms occurred.

In the case of animal 189 blindness occurred 3 days prior to slaughter. Microscopic sections of the cornea and uvula showed no keratinization of the epithelium. Figure 4 shows animals 508 and 189 immediately prior to slaughter. The age of slaughter and time required for depletion are shown in Table 10.

TABLE 10. - AGE AND THE TIME REQUIRED FOR DEPLETION OF VITAMIN A RESERVES OF CALVES USED IN IN VITRO EXPERIMENTS

No. of Animal	Breed	Age when Killed (days)	No. of Days in Depletion Period	Blood Plasma Vitamin A (γ/100 ml.)
200	Guernsey	156	38	6.49
130	Holstein	127	97	7.89
489	Guernsey	135	105	5.92
528	Jersey	156	126	9.07
504	Jersey	150	120	7.77
509	Jersey	116	86	5.16
119	Holstein	121	91	14.50
495	Guernsey	153	123	11.63
508	Jersey	226	196	9.2
189	Holstein	232	202	9.4

The animals were stunned by a blow on the head and the left carotid artery was severed to permit hemorrhage. The small intestine was removed immediately, ligated at both ends and placed in Ringer-Locke solution at a temperature of 38° C. Likewise, the liver was removed and placed in Ringer-Locke solution[1] at a temperature of 38° C.

The contents of the small intestine were flushed out three times with warm 0.9 per cent saline solution and the washings discarded. Two 3-foot sections then were taken from the mid-portion of the small intestine. A small amount of intestine was removed at the juncture of the two sections immediately after slaughter for analysis of carotene and vitamin A.

To one portion, 250 ml. of a colloidal carotene solutoin[2] were added and both ends ligated. This section was placed in a container of Ringer-Locke solution. The colloidal carotene solution was prepared by dissolving

[1]The Ringer-Locke solution employed had the following composition: NaCl, 0.9%; KCl, 0.042%; $CaCl_2$, 0.024%; $NaHCO_3$, 0.05%; $MgCl_2$, 0.02%; and glucose, 0.05%.

[2]The crystalline carotene used in this experiment was furnished by Valley Vitamins Inc., McAllen, Texas.

the carotene in acetone. The resulting solution then was mixed with water and the acetone evaporated under a vacuum.

The mixture containing the carotene was stabilized by adding 1 gram of Tween 80[3] to each 5 ml. of solution. The carotene concentration of the solution was varied. The levels used in each experiment are shown in Table 11. Ringer-Locke solution was placed inside the other section of intestine and it too was placed in a container of Ringer-Locke solution after having been ligated. The containers into which the two sections had been deposited were placed in a glass chamber in an atmosphere of nitrogen. They were incubated for 3 hours at 30° C. In some cases, a third section of intestine containing the carotene solution was incubated for 8 hours at 38° C., together with the corresponding section containing no carotene, which served as a control.

TABLE 11. - IN VITRO CONVERSION OF CAROTENE TO VITAMIN A IN THE INTESTINAL WALL OF THE DAIRY CALF

Calf No.	Intestine (Non-Incubated)		Intestine Incubated as Control		Intestine Incubated 3 Hrs. with Carotene		Intestine Incubated 8 Hrs. with Carotene		Concentration of Carotene Solution
	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	
	(γ/100 g.)		(γ/100 g.)		(γ/100 g.)		(γ/100 g.)		(γ/ml.)
200-G‡	23.54	16.16	----	----	96.57	7.26	-----	-----	226.8
489-G	----	----	62.28*	11.05	522.00	20.50	-----	-----	226.8
528-J	----	----	25.94*	5.80	848.00	41.75	-----	-----	226.8
504-J	154.65	40.05	126.50*	28.29	390.10	39.00	345.0	72.75	780.0
509-J	103.50	17.30	46.00+	----	228.3	45.60	125.8	29.10	780.0
119-H	68.77	21.30	59.5 +	28.0	1,108.0	114.20	615.0	506.80	780.0
495-G	63.20	9.73	55.88+	12.6	-----	-----	318.9	99.10	780.0
508-J	27.13	10.60	7.76+	3.81	-----	-----	76.45	26.22	780.0
189-H	5.47	7.62	7.33+	2.22	-----	-----	200.50	13.90	780.0

* Incubated 3 hours.
\+ Incubated 8 hours.
‡ The letters, G, J, and H, refer to the Guernsey, Jersey, and Holstein breeds respectively.

After incubation, the sections of intestine were removed from the chamber, the contents flushed out three times with 0.9 per cent saline and the washings analyzed for vitamin A. The intestines were minced in a food chopper and an aliquot taken for analysis. The intestinal wall was analyzed for carotene and vitamin A by the method of Davies (1932) for liver using the Carr-Price reaction for the determination of vitamin A.

The liver was finely ground by means of a food chopper. To 150 grams of minced liver, 100 ml. of a colloidal carotene solution and 250 ml. of a buffer solution[4] were added. The concentration of the carotene solution used was varied. Concentrations for each experiment are given in Table 12. The samples were incubated for 24 hours at 38° C. A control sample

[3]"Tween" 80 was obtained from the Atlas Powder Co., Wilmington, Delaware.

[4]The buffer solution was made up as follows: 39.5 ml. of 0.2 N NaOH, 50 ml. of 0.2 M acid potassium phosphate and this made up to 200 ml. with distilled water. The pH of this solution was 7.4.

TABLE 12. - IN VITRO CONVERSION OF CAROTENE TO VITAMIN A BY MINCED LIVER TISSUE

Calf No.	Liver (Unincubated)		Liver Incubated as Control		Liver Incubated 24 Hrs. with Carotene		Concentration of Carotene Solution
	Carotene	Vitamin A	Carotene	Vitamin A	Carotene	Vitamin A	
	(γ/150 g.)		(γ/150 g.)		(γ/150 g.)		(γ/ml.)
200-G*	374.76	77.70	-----	-----	46,903.0	638.75	560.00
130-H	616.50	186.60	389.50	143.52	46,962.0	641.35	560.00
489-G	623.25	211.50	550.40	232.47	50,040.0	1,145.40	560.00
528-J	460.35	156.45	456.0	113.57	18,200	686.55	226.80
504-J	131.25	156.30	63.35	61.48	8,920	296.70	127.60
509-J	348.45	464.55	306.75	357.08	9,512.5	1,195.43	127.60
119-H	302.5	266.25	268.0	232.50	8,680	416.76	127.60
495-G	478.5	483.90	246.95	190.44	8,762.5	560.62	127.60
508-J	47.44	17.08	59.53	8.45	3,450.0	141.45	-----
189-H	65.25	10.25	65.84	6.15	8,810.0	1,435.20	127.60

* The letters, G, H, and J, refer to the Guernsey, Holstein, and Jersey breeds, respectively.

composed of 150 grams of liver, 100 ml. of distilled water and 250 ml. of the buffer solution was incubated under the same conditions. This is essentially the same procedure outlined by Euler and Klussman (1932). Minced liver samples were analyzed for carotene and vitamin A immediately after slaughter by the method of Davies (1932).

Samples of blood plasma were incubated with a colloidal carotene solution for 24 hours at 37° C. A control sample containing no carotene was incubated under identical conditions. The carotene and vitamin A content of the blood plasma samples was determined by the method of Kimble (1939).

Results.—The results of the incubation of the small intestine of dairy calves with a carotene solution are shown in Table 11. Negative results were obtained with the intestine of calf No. 200 which was incubated for only 3 hours. In all other cases, there was an increase in the vitamin A content of the intestinal wall incubated with carotene above that present in the nonincubated intestine and above that present in control samples. There was no vitamin A in the material washed out of the intestine.

It is believed that the results obtained do not necessarily represent the rates of absorption and conversion as present in the intact animal since carotene is present in a different carrier. However, in spite of the difference in physiological conditions existing between *in vivo* and *in vitro* methods the conversion of carotene to vitamin A by the intestine was clearly demonstrated.

In all cases, there was conversion of carotene to vitamin A when livers from depleted calves were incubated with a carotene solution (Table 12). The fact that conversion in Guernseys appeared to be equal to or in some cases higher than in Jerseys and Holsteins is unexplained. It is generally believed that the conversion of carotene to vitamin A in Guernseys is less efficient in the living animal than in some of the other dairy breeds. It should be emphasized that in *in vitro* experiments caution is necessary in the application of results to the living organisms.

In no case was carotene converted to vitamin A by blood plasma. This is in agreement with the results of Elliott (1949) and Von Euler and Klussman (1932).

Summary.—Data obtained from the *in vitro* incubation of small intestines of dairy calves with a colloidal carotene solution indicate that the small intestine is a site of conversion of carotene to vitamin A.

The incubation of minced liver tissue with a colloidal carotene solution resulted in a conversion of carotene to vitamin A.

Conversion of carotene to vitamin A apparently is not a function of blood plasma.

The Influence of High Ambient Temperatures on the Carotenoid and Vitamin A Content of Blood Plasma and Milk Fat in Dairy Cattle

Climatic conditions in the United States are extremely variable, ranging from extreme cold in winter, through relatively mild temperatures in spring and autumn to very high temperatures in summer. Aron *et al.*, (1946) have reported a decrease in the blood plasma carotenoids and vitamin A in man associated with artificially induced high body temperature. In view of this report it was decided to measure the influence of temperature on the carotenoids and vitamin A content of the blood and milk of dairy cattle.

Fortunately, there was established on the campus of the University of Missouri a "climatic" or psychroenergetic laboratory for the purpose of studying the influence of climatic factors on dairy cattle. Analyses were made on the milk and blood plasma of dairy cattle maintained in this laboratory.

The climatic laboratory has been described in detail (McCalmont, J. R., 1946; Ragsdale, *et al.*, 1948). In brief the laboratory consists of two insulated test chambers 26 x 18 x 9 feet, each housing 6 cows. The temperature may be controlled, depending on the outside temperature, within the approximate range of 0° to 110° F. Humidity, air movement, light, and ventilation rate of each chamber may be varied independently of each other.

Milk samples were collected for analysis of carotenoids and vitamin A during the summer of 1948. The temperature of the "control" chamber was held between 50 and 60° F. for approximately five months. The temperature of the experimental chamber was increased from 50 to 105° F. by 5 to 10° F. intervals as shown in the lower section of Figure 5. Near the end of the temperature study the temperature of the experimental chamber was lowered from 105 to 60° F., and remained at the lower temperature to the end of the study. The relative humidity was kept between 60 and 70 per cent in both chambers.

Each cow of the experimental group had a similar "paired" mate in the control group. The pertinent facts concerning the cows on experiment have been published with the effects of high temperature on milk production

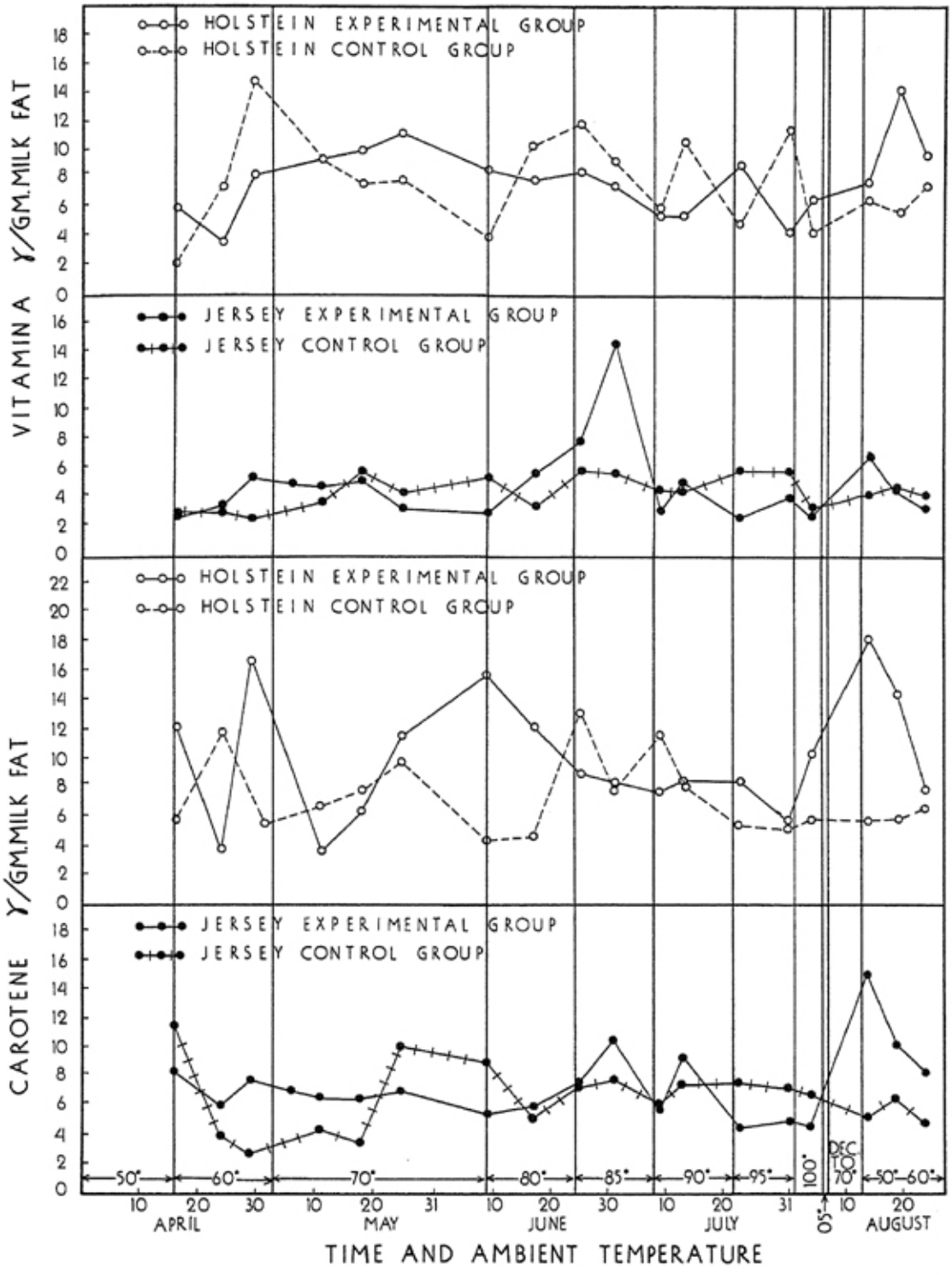

Fig. 5.—The carotenoid and vitamin A content of milk fat of cows maintained at environmental temperatures of 50 to 105° F.

and feed consumption by Ragsdale *et al.* (1948). Each group consisted of three lactating Jerseys, two lactating Holsteins, and one non-lactating Holstein.

Water was available in drinking cups. The animals were fed grain and beet pulp (2 pounds daily, dry basis) twice daily, and alfalfa hay *ad libitum*.

The grain mix fed had the following composition:

Ground yellow corn	800 pounds
Ground oats	400 pounds
Wheat bran	400 pounds
Soybean oil meal	300 pounds
Steamed bone meal	20 pounds
Salt	20 pounds

Beginning June 10, 1948, Nopco XX cod liver oil was added to the grain mix to the extent that each cow received 10,000 units of vitamin D and 75,000 units of vitamin A daily. The amount of grain fed daily was based on the previous week's milk production with changes made as early in a new period as production warranted. Jersey cows received 1 pound of grain per 3 pounds of milk and Holstein cows 1 pound of grain per 4 pounds of milk produced daily. The hay consumed by each animal was weighed daily.

Milk samples were collected at approximate weekly intervals throughout the trial. A composite sample was made by taking aliquot samples from night and morning milkings on the day samples were drawn. In most cases the samples were analyzed for carotenoids and vitamin A within a few hours after sampling. In some cases samples were stored at -16 to -20° C. for indefinite periods. The samples were analyzed for carotenoids and vitamin A by the method of Boyer *et al.* (1944).

The carotenoid and vitamin A content of the milk fat are plotted in Figure 5 as ug. per gram of fat. These data represent an average value for the 3 Jerseys in milk in both experimental and control groups and for the two Holsteins in milk in both groups. There was considerable variation in the carotenoid content of milk fat in both groups. About May 28 a better quality hay was furnished to the cows and as a result some of the cows increased their hay consumption and the carotenoid content of the milk became higher. There was a decline in feed consumption with increasing environmental temperature, reaching low levels at 105° F. When temperature was lowered the hay consumption increased. A parallel increase in the carotenoid content of milk fat was noted in both the Jersey and Holstein experimental groups. The vitamin A content of milk fat of the Holstein experimental group was increased markedly. The increase in the vitamin A content of the Jersey milk at this point was slight.

The correlations between the carotenoid and vitamin A content of milk fat and the daily hay consumption are graphically presented in Figure 6. The correlation coefficient, r, for carotenoids in milk fat and daily hay consumption was $+0.661$ ($P<.01$) and the correlation coefficient, r, for vitamin A in milk fat and daily hay consumption was $+0.678$ ($P<.01$) for the Holstein experimental group. There was a pronounced tendency for the carotenoids and vitamin A content of the milk to increase as hay consumption increased.

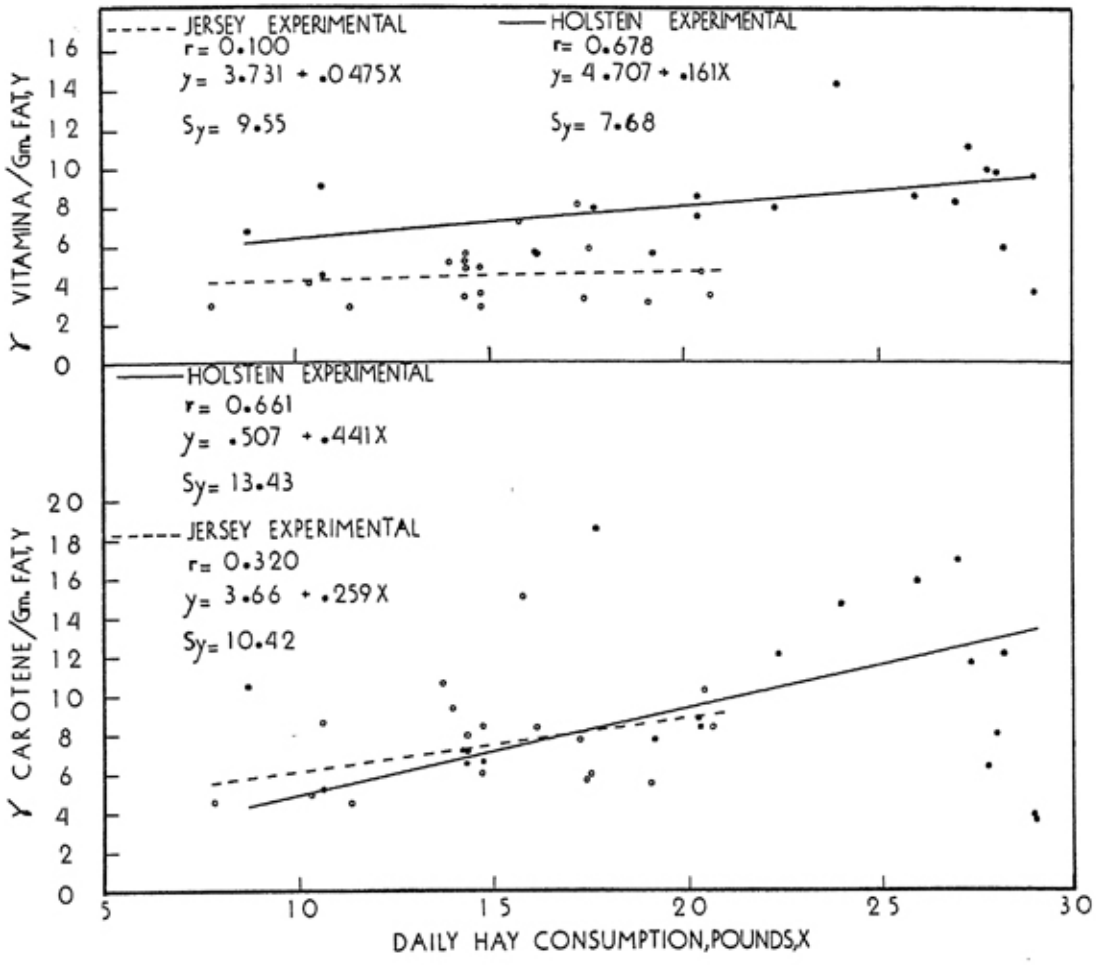

Fig. 6.—Carotene and vitamin A values of milk fat of cows maintained at environmental temperatures of 50 to 105° F. plotted against daily hay consumption. The equation for the average regression line, the coefficient of correlation, and the standard error of estimate are shown.

In the case of the Jersey experimental group the correlation coefficient, r, for the carotene content of milk fat and daily hay consumption was +0.320 and for vitamin A in milk fat and daily hay consumption +0.100. Although there was a tendency for the concentration of these substances in milk to follow hay consumption, it was not significant. This fact is believed to be due to the smaller depression in hay intake due to high ambient temperatures in the case of the Jerseys and the unexplained variations occurring in the carotenoid and vitamin A content of the milk fat during the course of the trial.

The correlations between vitamin A in milk fat and ambient temperature appeared to be nil as shown in Figure 7. There was a depression in the carotenoids with increasing temperature but again this is believed to be due to the effect of a lowered hay consumption associated with high temperature.

It is admitted that statistical analyses of these data are perhaps of little value due to the smaller number of animals. However, if the entire experi-

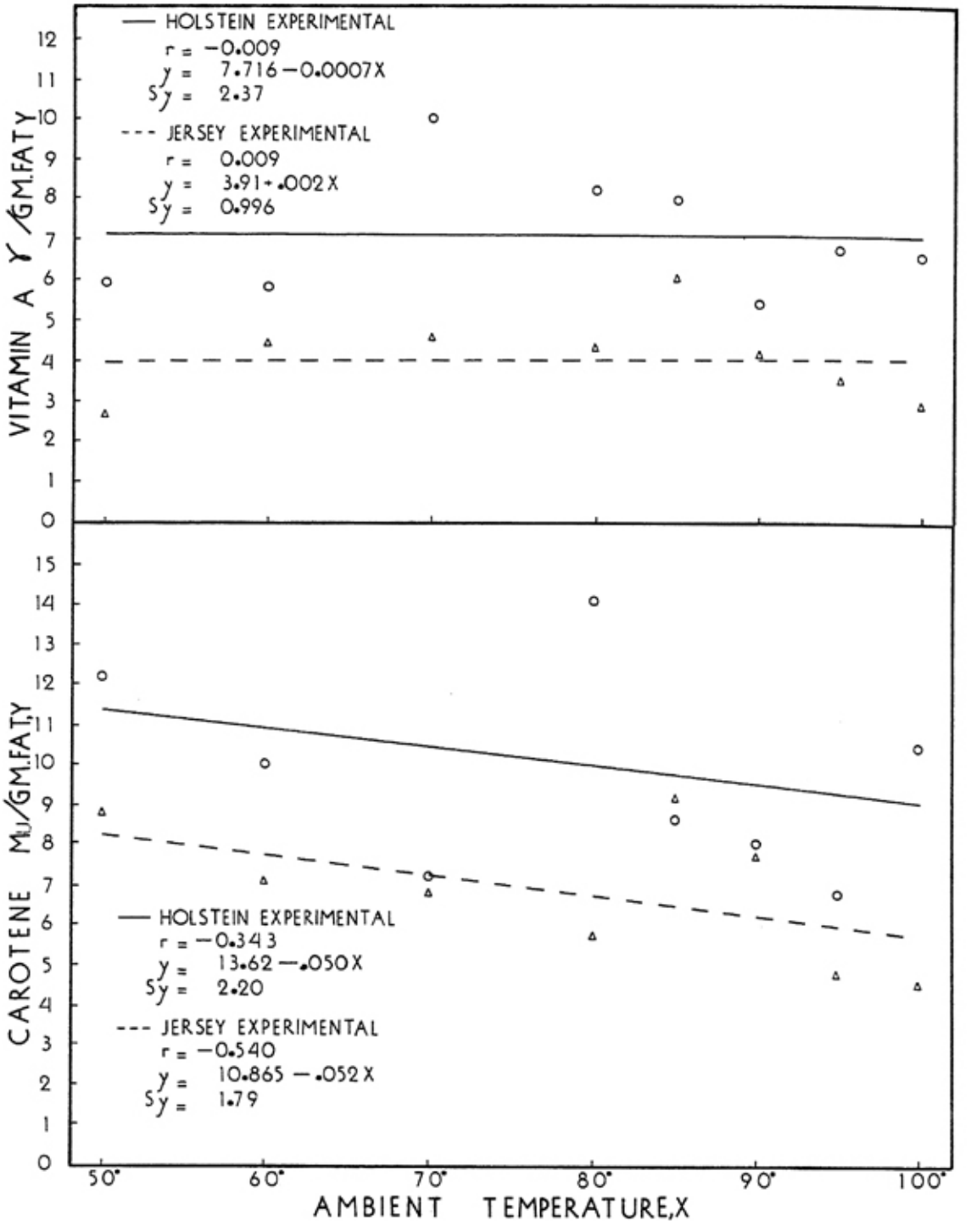

Fig. 7.—Carotene and vitamin A values of milk fat plotted against ambient temperature levels. The equation for the average regression line, the coefficient of correlation, and the standard error of estimate are shown.

mental period is considered as a unit there was no significant difference between experimental and control animals of either breed so far as the carotenoid and vitamin A content of milk fat are concerned.

In interpreting the values for carotenoids and vitamin A content of milk fat it must be kept in mind that vitamin A supplementation reduces the carotenoid content and increases the vitamin A content of milk fat. In these experiments vitamin A supplementation was made in equal amounts to animals of both groups.

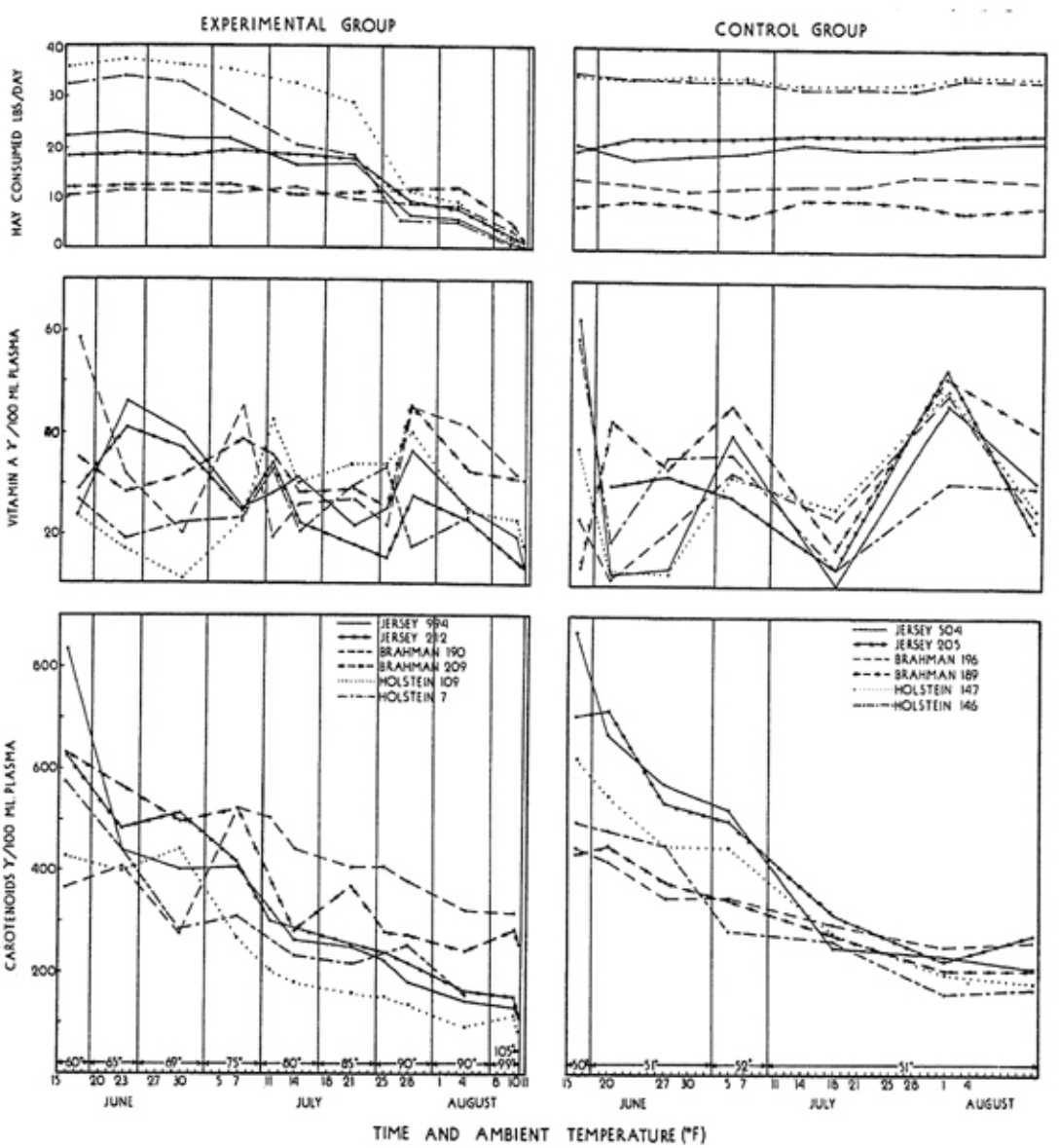

Fig. 8.—Blood plasma carotenoid and vitamin A values and daily hay consumption of cows maintained at environmental temperatures of 50 to 105° F.

The fact that the carotenoid content per gram of milk fat in Holsteins was greater than the carotenoid content per gram of fat in Jerseys may have been due to greater initial body stores, and/or a greater reduction in the volume of milk secreted as a result of high ambient temperature.

The blood plasma of cows used in the summer of 1949 trial in the climatic laboratory was analyzed for carotenoid and vitamin A content. During this trial both the experimental and control groups consisted of two lactating Jerseys, two lactating Brahman or Zebu cows, and two lactating Holsteins. Each cow in the experimental chamber had a "paired" mate in the control chamber. Prior to the experimental period both the experimental and control cows had been on pasture which accounts for the high initial levels of carotenoids and vitamin A in the blood plasma.

The animals were fed grain and roughage as described for the animals in the summer of 1948 trial. The temperature of the chamber used as control was held between 50 and 60° F.; the temperature of the experimental chamber was increased systematically from 50 to 105° F. at 5 to 10° F. intervals (Figure 8).

Blood samples were drawn from the jugular vein shortly after feeding and milking. The plasma carotenoids and vitamin A were determined by the method of Kimble (1939).

TABLE 13. - BLOOD PLASMA CAROTENOID LEVELS OF HOLSTEIN COWS

Ambient Temperature (°F.)	Experimental Group				Control Group (50°F)			
	No. of Samples per Cow	Mean Plasma Carotenoids	Standard Error of Mean	Standard Deviation of Mean	No. of Samples per Cow	Mean Plasma Carotenoids	Standard Error of Mean	Standard Deviation of Mean
		(ug./100 ml.)				(ug./100 ml.)		
60	1	497.8	±72.42	±102.11	1	557.1	±62.28	± 87.82
65	1	417.2	±22.94	± 32.34	1	512.0	±34.90	± 49.21
70	1	360.2	±80.03	±112.85	1	396.0	±49.35	± 69.58
75	1	286.2	±20.66	± 29.13	1	363.8	±81.65	±115.12
80	2	227.2	±25.83	± 51.66				
85	1	185.2	±29.28	± 41.29	1	267.8	± 6.22	± 8.77
90	2	191.3	±29.19	± 58.39				
95	1	121.9	±32.57	± 45.93	1	177.6	±20.01	± 28.21
100	1*	114.6						
105	1*	66.8						

* Sample taken from one cow.

The data on blood plasma carotenoids and vitamin A are presented in Tables 13 to 18 inclusive and in Figure 8. The carotenoid content of the blood plasma decreased in a similar fashion in the case of both experimental and control groups. This decline would be expected in view of the fact that the cows were taken off pasture and placed on hay and beet pulp as sources of roughage. No significant differences were observed between the plasma carotenoid values of experimental and control groups except at 75° F. in the case of the Brahman cows. The carotenoid levels of the experimental groups were slightly lower than their respective controls toward the end of the trial. This is presumably due to a lowered hay intake as shown in Figure 8.

The vitamin A content of blood plasma was variable in both experimental and control groups. No significant differences were noted between experimental and control groups.

TABLE 14. - BLOOD PLASMA VITAMIN A LEVELS OF HOLSTEIN COWS

Ambient Temperature (°F.)	Experimental Group				Control Group (50°F)			
	No. of Samples per Cow	Mean Plasma Vitamin A	Standard Error of Mean	Standard Deviation of Mean	No. of Samples per Cow	Mean Plasma Vitamin A	Standard Error of Mean	Standard Deviation of Mean
		(ug./100 ml.)				(ug./100 ml.)		
60	1	25.23	±1.50	± 2.11	1	47.71	±10.80	±15.23
65	1	17.89	±1.00	± 1.41	1	15.43	± 3.18	± 4.48
70	1	16.61	±5.59	± 7.88	1	23.68	±11.62	±16.39
75	1	22.85	±0.33	± 0.47	1	33.76	± 2.23	± 3.15
80	2	31.21	±4.58	± 9.16				
85	1	41.49	±7.78	±10.97	1	19.62	± 5.90	± 8.32
90	2	30.82	±4.76	± 9.52				
95	1	23.82	±0.50	± 0.82	1	39.79	± 9.07	±12.79
100	1*	22.63			1	27.85	± 2.19	± 3.08
105	1*	17.94						

* Sample taken from one cow.

TABLE 15. - BLOOD PLASMA CAROTENOID LEVELS OF JERSEY COWS

Ambient Temperature (°F.)	Experimental Group				Control Group (50°F)			
	No. of Samples per Cow	Mean Plasma Carotenoids	Standard Error of Mean	Standard Deviation of Mean	No. of Samples per Cow	Mean Plasma Carotenoids	Standard Error of Mean	Standard Deviation of Mean
		(ug./100 ml.)				(ug./100 ml.)		
60	1	728.0	±105.11	±148.25	1	784.2	±83.04	±117.09
65	1	458.6	± 18.65	± 26.30	1	690.4	±23.27	± 32.81
70	1	456.4	± 54.16	± 76.37	1	551.4	±18.66	± 26.31
75	1	411.0	± 5.42	± 7.64	1	508.6	±11.43	± 16.12
80	2	293.7	± 15.01	± 30.03				
85	1	250.2	± 1.00	± 1.41	1	281.6	±32.50	± 45.82
90	2	214.2	± 12.26	± 24.52				
95	1	151.1	± 9.93	± 14.00	1	228.4	± 4.82	± 6.79
100	1	140.5	± 10.53	± 14.85	1	243.2	±32.90	± 46.39
105	1	104.1	± 2.99	± 4.21				

TABLE 16. - BLOOD PLASMA VITAMIN A LEVELS OF JERSEY COWS

Ambient Temperature (°F.)	Experimental Group				Control Group (50°F.)			
	No. of Samples per Cow	Mean Plasma Vitamin A	Standard Error of Mean	Standard Deviation of Mean	No. of Samples per Cow	Mean Plasma Vitamin A	Standard Error of Mean	Standard Deviation of Mean
		(ug./100 ml.)				(ug./100 ml.)		
60	1	26.22	±2.44	±3.44	1	36.73	±5.59	± 7.88
65	1	43.47	±2.62	±3.70	1	20.98	±8.86	±12.49
70	1	37.94	±1.45	±2.04	1	22.52	±9.19	±12.96
75	1	24.73	±0.39	±0.55	1	33.96	±6.10	± 8.60
80	2	28.95	±2.61	±5.23				
85	1	14.89	±3.02	±4.26	1	11.95	±1.52	± 2.15
90	2	26.15	±4.28	±8.56				
95	1	23.85	±1.05	±1.48	1	49.81	±2.96	± 4.17
100	1	17.11	±2.38	±3.35	1	25.12	±4.13	± 5.83
105	1	12.70	±0.23	±0.32				

TABLE 17. - BLOOD PLASMA CAROTENOID LEVELS OF BRAHMAN COWS

Ambient Temperature (°F.)	Experimental Group				Control Group (50°F.)			
	No. of Samples per Cow	Mean Plasma Carotenoids	Standard Error of Mean	Standard Deviation of Mean	No. of Samples per Cow	Mean Plasma Carotenoids	Standard Error of Mean	Standard Deviation of Mean
		(ug./100 ml.)				(ug./100 ml.)		
60	1	495.2	±133.20	±187.81	1	437.7	± 6.07	± 8.56
65	1	482.4	± 80.24	±113.14	1	432.6	±16.25	±22.91
70	1	381.4	±109.73	±154.72	1	361.4	±16.65	±23.48
75**	1	520.0		0	1	344.6	± 2.21	± 3.11
80	2	402.8	± 46.99	± 93.98				
85	1	386.1	± 19.30	± 27.21	1	283.8	± 9.83	±13.86
90	2	331.7	± 34.58	± 69.16				
95	1	279.2	± 40.12	± 56.57	1	228.0	±23.27	±32.81
100	1	298.2	± 15.84	± 22.34	1	234.2	±27.48	±38.75
105	1*	233.2						

* Sample taken from one cow.
** Difference in means is significant $P < .02$.

TABLE 18. - BLOOD PLASMA VITAMIN A LEVELS OF BRAHMAN COWS

Ambient Temperature (°F.)	Experimental Group				Control Group (50°F.)			
	No. of Samples per Cow	Mean Plasma Vitamin A	Standard Error of Mean	Standard Deviation of Mean	No. of Samples per Cow	Mean Plasma Vitamin A	Standard Error of Mean	Standard Deviation of Mean
		(ug./100 ml.)				(ug./100 ml.)		
60	1	46.67	±11.82	±16.67	1	18.28	± 4.75	± 6.70
65	1	29.76	± 1.70	± 2.40	1	26.83	±15.67	±22.09
70	1	25.72	± 5.76	± 8.12	1	26.83	± 6.32	± 8.91
75	1	41.59	± 3.24	± 4.57	1	39.04	± 6.80	± 9.59
80	2	27.05	± 3.30	± 6.60				
85	1	27.85	± 1.02	± 1.44	1	20.43	± 2.99	± 4.21
90	2	35.16	± 6.93	±12.85				
95	1	36.64	± 4.37	± 6.16	1	49.84	± 1.60	± 2.26
100	1	31.14	± 0.38	± 0.54	1	32.44	± 8.94	±12.61
105	1*	30.47						

* Sample taken from one cow.

On June 17, 1949, the hay fed contained 11.07 ug./gm. (dry basis), and on August 4, 12.60 ug./gm. (dry basis). These analyses indicate that the carotenoid content of the hay was fairly uniform throughout the trial.

It would appear from these data that the blood plasma carotenoids and vitamin A content are not significantly influenced by high environmental temperature.

The Influence of Low Ambient Temperatures on the Carotenoid and Vitamin A Content of Blood Plasma and Milk Fat in Dairy Cattle

Data was collected on the carotenoid and vitamin A content of milk fat and blood plasma during the winter of 1948-49 trial in the climatic laboratory. During this period the temperature of the experimental chamber was (1) gradually lowered from 50 to 4° F.; (2) rapidly brought back to 50° F.; and (3) raised from 50 to 95° F. During the same period the temperature of the control chamber was held constant at the 50° F. level except for a two-week period when the temperature was held at 4° F. Following

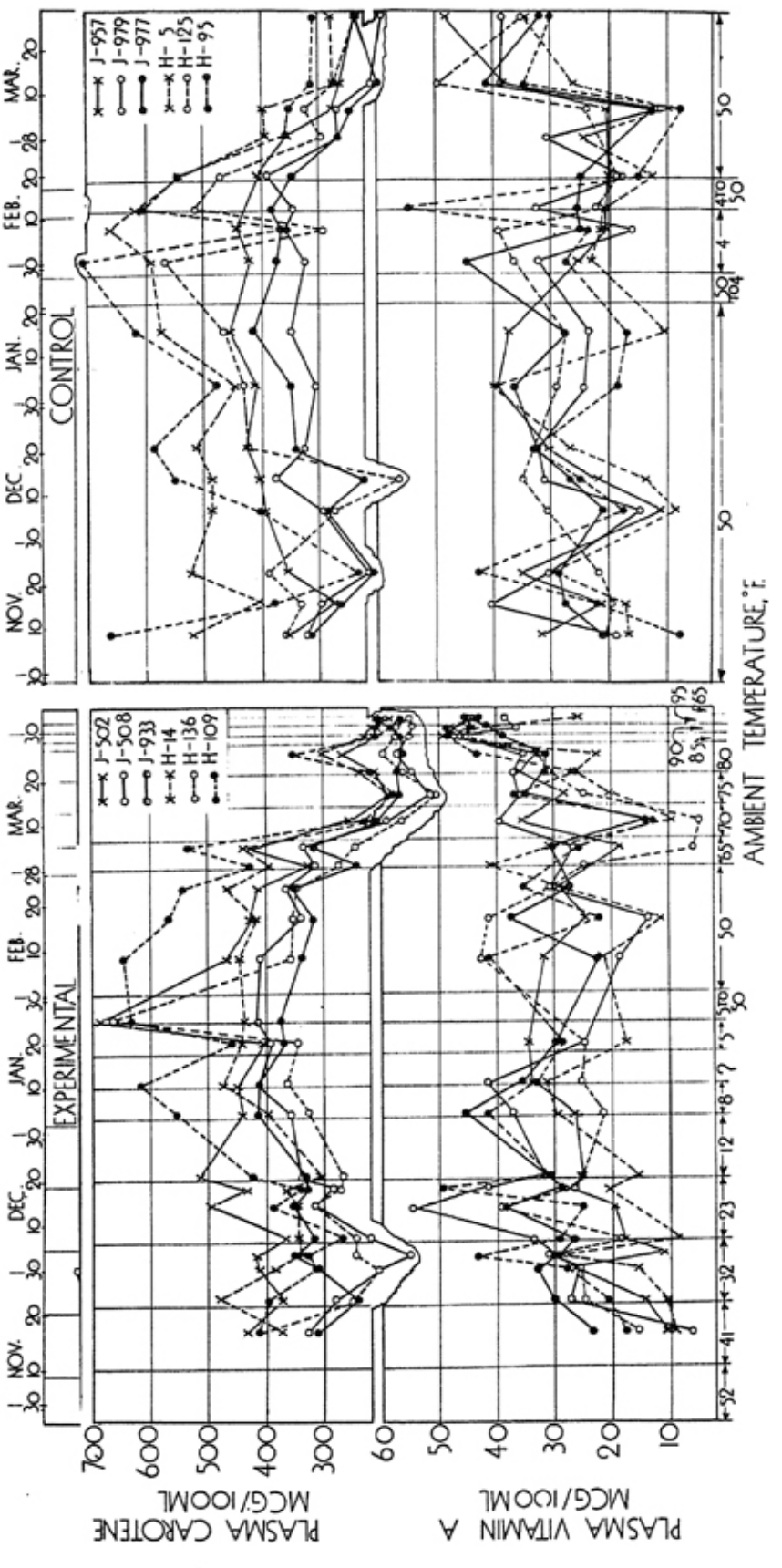

Fig. 9.—Blood plasma carotenoid and vitamin A values of cows maintained at environmental temperatures of 50 to 5° F. and 50 to 95° F.

this period the control chamber was increased to 50° F. The relative humidity ranged between 60 to 80 per cent in both chambers. The temperature calendar is shown graphically in Figures 6 and 7.

Each group of cows consisted of three lactating Jerseys, two lactating Holsteins, and one non-lactating, non-pregnant Holstein. The history of these cows has been published (Ragsdale *et al.*, 1949).

No change in the feeding arrangement nor in the handling of the cows was made over the previously described experiment. Alfalfa hay was fed *ad libitum*. The grain mix fed was the same as that reported for the summer of 1948 trial. Grain and beet pulp were fed twice daily.

Blood samples were collected at approximately weekly intervals. The dates of sampling are indicated in Figure 9. Hay consumption is shown in the upper segment of Figure 10. These data show a dramatic rise in the control cows during the brief exposure to 4° F. temperature. This increase in hay consumption was paralleled by a corresponding rise in the carotenoid content of the blood plasma. The experimental group did not show such an impressive rise in hay consumption but some increase was noted. The relatively greater values for hay consumption of the control group over the experimental group during March is, of course, associated with the rise in ambient temperature (60 to 95° F.) in the experimental chamber. The carotenoid content of the experimental and control groups tended to follow hay consumption. Vitamin A levels in blood plasma varied considerably with the individual cows. The data for carotenoids and vitamin A are presented in Figure 9 and Tables 19 to 22 inclusive. Significant differences existed in the carotenoid content of blood plasma between the experimental and the control groups when the experimental group was at 75° F. in the case of the Holsteins ($P<.05$) and 50 ($P<.05$), 70 ($P<.05$), and 75° F. ($P<.01$) in the case of the Jersey groups. Significant differences in the vitamin A content of blood plasma between experimental and control groups were observed when the Holsteins were maintained at 23° F. ($P<.05$) and at 75° F. ($P<.02$). In the case of the Jerseys significant differences were observed when the experimental group was kept at 50° F. ($P<.01$), at 85° F. ($P<.05$) and at 90°F ($P<.05$). In the case of the two last-named temperatures, a blood sample from the control group obtained when the experimental group was kept at 80° F. was used as a control. The use of this sample as a control may not be valid in view of the great variation in the group tendency to follow the pattern of the experimental group.

In this trial there was no consistent trend in blood plasma carotenoids and vitamin A that could be associated with decreasing ambient temperature.

The data on the carotenoid and vitamin A content of milk fat are shown in Figure 10.

The level of carotenoids and vitamin A in milk of the experimental and control groups tended to follow the level of hay consumption and there was

little difference between the two groups. Some variations were observed that are not explained by either the feed intake or the pattern of ambient temperature. There are doubtless factors other than those studied here that influence the levels of carotene and vitamin A in milk fat.

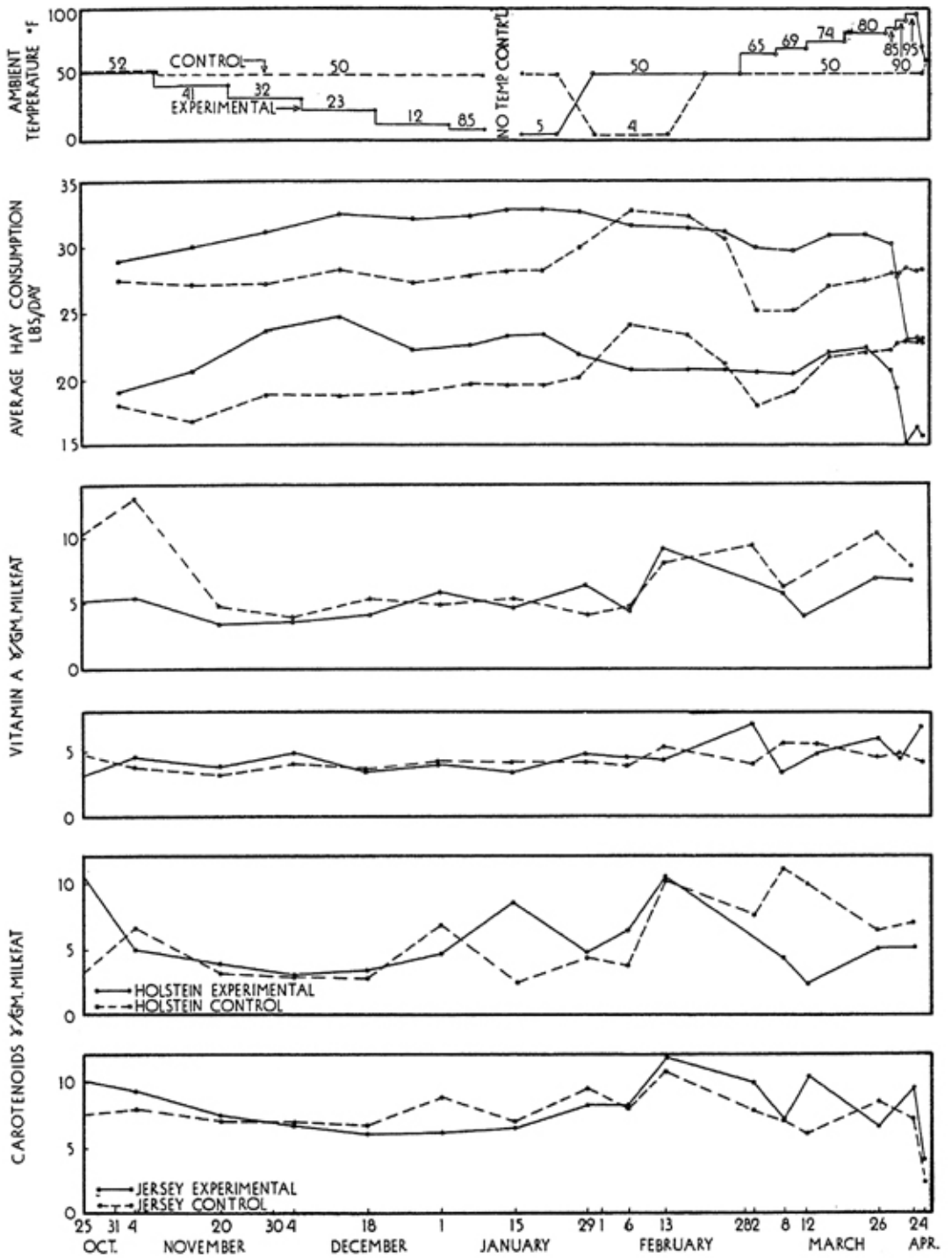

Fig. 10.—The carotenoid and vitamin A content of milk fat and daily hay consumption of cows maintained at environmental temperatures of 50 to 5° F. and 50 to 95° F.

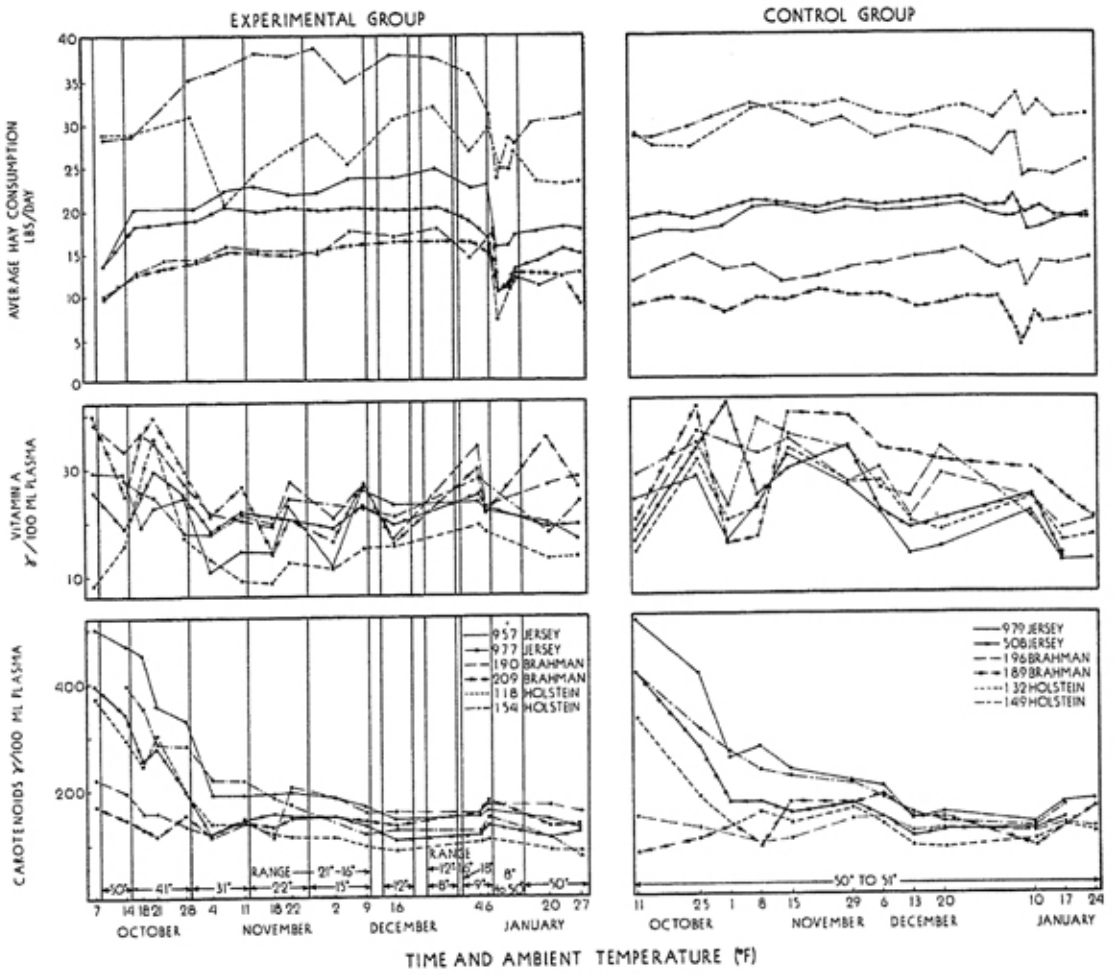

Fig. 11.—Blood plasma carotenoid and vitamin A levels and daily hay consumption of cows maintained at environmental temperatures of 50 to 8° F.

TABLE 19. - BLOOD PLASMA CAROTENOID LEVELS OF HOLSTEIN COWS

Ambient Temperature of Experimental Cows	Experimental Cows				Control Cows (50°F.)			
	No. of Samples per Cow	Mean Plasma Carotenoids	Standard Error of Mean	Standard Deviation of Mean	No. of Samples per Cow	Mean Plasma Carotenoids	Standard Error of Mean	Standard Deviation of Mean
		(ug./100 ml.)				(ug./100 ml.)		
41	2	326.2	±27.71	±67.88	2	304.6	±13.56	±33.21
32	3	305.0	±31.75	±95.25	3	260.0	±24.27	±72.80
23	3	376.3	±28.01	±84.03	3	333.7	±24.45	±73.34
12	1	405.5	±24.53	±42.43	1	365.0	±30.84	±53.36
5-8.5	3	433.0	±32.39	±97.16	3	391.0	±17.31	±52.11
					3*	378.8	±13.55	±38.34
50	4	359.7	±17.85	±61.76		384.0	± 7.11	±24.60
65	1	360.3	±34.04	±58.89	1	297.1	±30.14	±52.14
70	1	217.9	±18.95	±32.78	1	267.5	± 9.29	±16.07
75**	2	167.3	±12.88	±32.56	2	227.6	±13.06	±32.00
80	1	200.4	±33.09	±57.24	1	224.5	±16.09	±27.83
85	1	176.8	±19.11	±33.06				
90	1	179.9	±19.81	±34.27				
95	1	170.8	±14.39	±24.89				
Total	24							

* Control cows at 4° F.
** Difference in means is significant $P < .05$.

Blood plasma carotenoid and vitamin A values were determined for a second low temperature trial during the winter of 1949-50. The feeding and management of the cows was the same as that for previous trials. The animals consisted of two lactating Jerseys, two lactating Brahman, and two lactating Holsteins in each group. The Jersey and Holstein cows had been taken off pasture and placed on experiment. The Brahman cows had been on dry feed for an extended period of time. The temperature calendar is shown in the

TABLE 20. - BLOOD PLASMA VITAMIN A LEVELS OF HOLSTEIN COWS

Ambient Temperature of Experimental Cows	Experimental Cows				Control Cows (50°F.)			
	No. of Samples per Cow	Mean Plasma Vitamin A (ug./100 ml.)	Standard Error of Mean	Standard Deviation of Mean	No. of Samples per Cow	Mean Plasma Vitamin A (ug./100 ml.)	Standard Error of Mean	Standard Deviation of Mean
41	2	18.55	±3.94	± 9.67	2	27.04	±3.33	±8.17
32	3	25.81	±2.39	± 7.18	3	31.62	±0.95	±2.86
23†	3	32.03	±2.69	± 8.08	3	23.09	±2.31	±6.92
12	1	36.36	±5.51	± 9.53	1	31.96	±0.66	±1.15
5-8.5	2	33.34	±2.34	± 5.74	1	31.56	±2.89	±7.08
					3*	27.98	±3.10	±8.77
50	3	26.27	±2.38	± 7.15	3	20.97	±1.02	±3.05
65	1	25.48	±3.52	± 6.09	1	25.36	±3.23	±5.59
70	1	29.90	±7.99	±13.82	1	15.04	±2.68	±4.64
75‡	2	34.12	±1.42	± 3.47	2	39.80	±0.71	±1.73
80	1	33.41	±1.12	± 1.93	1	39.59	±4.77	±8.26
85	1	44.34	±2.87	± 4.97				
90	1	41.93	±3.19	± 5.52				
95	1	42.53	±2.12	± 3.67				
Total	22							

* Control cows at 4° F.
† Difference in means is significant $P < .05$.
‡ Difference in means is significant $P < .02$.

TABLE 21. - BLOOD PLASMA CAROTENOID LEVELS OF JERSEY COWS

Ambient Temperature of Experimental Cows	Experimental Cows				Control Cows (50°F.)			
	No. of Samples per Cow	Mean Plasma Carotenoids (ug./100 ml.)	Standard Error of Mean	Standard Deviation of Mean	No. of Samples per Cow	Mean Plasma Carotenoids (ug./100 ml.)	Standard Error of Mean	Standard Deviation of Mean
50†	4	436.2	±30.13	±104.26	4	518.0	± 9.82	± 33.98
41	2	385.1	±30.95	± 75.83	2	442.8	±52.54	±128.72
32	3	291.7	±31.33	± 63.99	3	381.0	±42.06	±126.19
23	3	336.0	±17.27	± 51.80	3	396.3	±52.37	±157.12
12	1	425.0	±66.63	±115.27	1	503.0	±41.58	± 71.94
5-8.5	3	491.3	±39.08	±117.23	3	520.0	±25.21	± 75.64
					3*	547.8	±45.64	±136.95
65	1	405.3	±85.51	±147.93	1	350.9	±28.57	± 49.43
70†	1	203.4	±20.54	± 35.54	1	358.5	±22.41	± 38.77
75‡	2	182.3	±18.46	± 45.52	2	287.2	± 9.24	± 22.65
80	1	250.9	±30.08	± 52.04	1	278.9	±20.73	± 35.87
85	1	204.5	±16.50	± 28.54				
90	1	190.9	±12.39	± 21.44				
95	1	182.1	±17.21	± 29.78				
Total	24							

* Control cows at 4° F.
† Difference in means is significant $P < .05$.
‡ Difference in means is significant $P < .01$.

TABLE 22. - BLOOD PLASMA VITAMIN A LEVELS OF JERSEY COWS

Ambient Temperature of Experimental Cows	Experimental Cows				Control Cows (50°F.)			
	No. of Samples per Cow	Mean Plasma Vitamin A	Standard Error of Mean	Standard Deviation of Mean	No. of Samples per Cow	Mean Plasma Vitamin A	Standard Error of Mean	Standard Deviation of Mean
		(ug./100 ml.)				(ug./100 ml.)		
50‡	4	31.08	±3.14	±10.41	4	15.51	±1.50	± 2.59
41	2	16.47	±2.41	± 5.91	2	17.46	±2.13	± 5.22
32	3	25.26	±3.38	±10.14	3	31.69	±3.17	± 9.52
23	3	29.22	±3.40	±10.19	3	22.82	±3.27	± 9.71
12	1	30.83	±5.88	±10.17	1	31.04	±2.06	± 3.57
5-8.5	2	26.16	±2.08	± 5.1	2	23.82	±4.32	±10.57
					3*	29.06	±2.99	± 8.97
65	1	20.58	±7.45	±12.88	1	19.57	±2.69	± 4.66
70	1	12.09	±1.49	± 2.58	1	13.76	±5.17	± 8.95
75	2	27.91	±1.98	± 4.86	2	36.97	±4.31	±10.57
80	1	32.92	±5.83	±10.08	1	33.54	±1.72	± 2.98
85†	1	47.93	±1.21	± 2.09				
90†	1	45.87	±1.47	± 2.54				
95	1	37.88	±3.66	± 6.33				
Total	23							

* Control cows at 4° F.
† Differences in means significant $P < .05$.
‡ Differences in means significant $P < .01$.

lower section of Figure 11. Hay consumption data and carotenoid and vitamin A data are presented in Figure 10.

There was a decline in blood plasma carotenoids as the trial progressed. This change appeared to be the same in both groups lending credence to the idea that the decline was the result of the animals consuming roughage of low carotenoid content following pasture. The vitamin A values do not appear to follow a rigid pattern. There was, however, a decline from the initial levels followed by a leveling off in the experimental group and a gradual decline in the control group.

In summary, it is indicated by these data that low ambient temperature has little appreciable effect on the carotenoid and vitamin A levels in blood plasma and milk fat, except as such temperatures affect the consumption of roughage. There did not appear to be increased conversion of carotenoids to vitamin A as a result of lowered temperature.

DISCUSSION

This investigation has succeeded in establishing some of the facts concerning factors influencing the carotenoid and vitamin A levels in blood plasma and milk fat of dairy cattle.

The well-known fact that the carotenoid content of the feed consumed markedly influences the levels of these substances in blood plasma and milk fat has again been substantiated. Korean lespedeza hay generally has a higher carotenoid content than alfalfa hay where conditions of harvesting and storage are comparable. When these hays were compared by feeding them in equal amounts to pregnant Jersey and Holstein heifers for 8 to 12 weeks

prepartum, the blood plasma and colostrum levels of carotenoids and vitamin A were higher in those animals fed Korean lespedeza hay. The blood plasma carotenoid and vitamin A values of the calves produced by cows fed on Korean lespedeza hay post-partum were higher at birth and at the end of the colostrum feeding period. It would therefore appear that the higher carotenoid content of Korean lespedeza hay is more effectively utilized by the animal than when alfalfa serves as a roughage. Pasture markedly increased the carotenoid content of milk fat over dry lot feeding conditions where alfalfa hay was the principal roughage. Grazing also resulted in an increase of the vitamin A content of milk fat, but not so striking as the increase in carotenoids.

A study of factors which may influence the losses of carotenoids in stored hays indicates that enzyme activity within the plant tissues (Waugh *et al.*, 1944) is perhaps the most important factor. Tocopherols do not appear to prevent the destruction of carotenoids in stored hays. From the limited number of hays studied it would appear that the lignin content of the hay does not markedly influence the retention of carotenoids by hay during storage. It does affect the digestibility of the hay (Swanson and Herman, 1943) which probably reduces the amount of carotenoids present that can be utilized by the animal.

Physiological factors in the intermediary metabolism of the animal which influence the levels of carotenoids and vitamin A in milk fat and blood plasma have been the subject of much discussion but facts of a definite nature are rather limited. It has been suggested that there is a genetically determined ceiling in the conversion of carotene to vitamin A (Winzenreid and Wanntorp, 1948). The differences between breeds in the carotenoid and vitamin A levels of blood and milk lend credence to this idea. That the vitamin A level in milk and blood is subject to less fluctuation due to the intake of carotenoids is attributed to the influence of the liver stores and to the influence of the conversion of carotenoids to vitamin A. It is of interest in this connection that, on numerous occasions during the course of the experiments in connection with pasture feeding the milk vitamin A was observed to increase at the same time the milk carotenoids decreased. This phenomenon has been observed before in milk (Hibbs *et al.*, 1949) and in studies on blood (Brown, 1945; Hibbs and Pounden, 1948; and Sutton and Soldner, 1945). This tendency, though temporary in nature, would lead us to believe that there are biochemical mechanisms perhaps of an enzyme or possibly of a hormonal nature, which influence the conversion of carotene to vitamin A. Much painstaking investigation is needed before a concept even approaching integrality can be reached accounting for some of the variations that have been observed in this connection.

From the study reported herein, it would appear that both the intestinal wall and the liver are sites of the conversion of carotene to vitamin A in dairy cattle. The more recent literature indicates that, in animals such as the goat,

sheep, pig, rat, and rabbits, the small intestine is the sole site of conversion. This work has a sound physiological basis since little or no carotenoids are found in the blood of these animals. Can it be that, in animals having carotenoids present in the blood stream, only a portion of these substances are converted in the intestine and the remainder converted by the liver? Carefully controlled *in vivo* experiments and perhaps the use of radioactive materials may give us a more clear understanding of this problem.

Neither high nor low temperature, *per se*, appear to have any appreciable influence on the carotenoid and vitamin A levels of blood plasma and milk fat, except as they may influence carotenoid intake. Vitamin A was influenced to a lesser degree than was carotenoid content by increasing or decreasing amounts of hay consumed by dairy cattle.

SUMMARY

1. The effect of dietary intake, the site of conversion of carotene to vitamin A, and the effect of environmental temperatures, on the carotenoid and vitamin A content of blood plasma and milk fat of dairy cattle have been investigated.
2. Alfalfa hay and Korean lespedeza hay were compared on the basis of carotenoid content. Pregnant heifers fed Korean lespedeza hay had higher vitamin A and carotenoid levels in the blood plasma and in the colostrum than those receiving alfalfa of similar quality. The calves of the cows fed Korean lespedeza hay had higher carotenoid and vitamin A blood plasma levels at birth and at the end of the colostral feeding period than calves from dams fed alfalfa hay.
3. The carotenoid and vitamin A content of milk fat was appreciably higher when cows were grazed on good pasture than when fed hay and silage under dry lot conditions.
4. Alfalfa silage, preserved by the addition of blackstrap molasses, retained a higher percentage of carotenoid materials after a six-month storage period than any of the hays studied. Korean lespedeza hay lost less carotenoids during storage than either alfalfa or red clover hays.
5. Tocopherols do not appear to prevent the destruction of carotenoids in hays. Limited data indicate also that lignin has no appreciable effect in preserving carotenoids in hays. Evidence has been presented to show that there are losses of tocopherols during the storage of hays.
6. *In vitro* studies involving the incubation of calf intestine with a colloidal carotene solution for 3- and 8-hour periods indicate that the intestinal wall is a site of the conversion of carotene to vitamin A in the bovine.
7. Minced liver tissue, obtained from vitamin A-depleted calves, incubated in a colloidal carotene solution was also found capable of converting carotene to vitamin A in the bovine.
8. The conversion of carotene to vitamin A does not appear to be a function of blood plasma.

9. Environmental temperature *per se* does not appear to markedly influence the carotenoid and vitamin A levels in milk fat and blood plasma. The variations in values observed were closely associated with the dietary intake which is influenced by environmental temperature.
10. High ambient temperatures, particularly those above 80-85° F., reduced the hay intake, which in turn was associated with a lowering of the carotenoids in the blood. When temperatures were reduced from 105 to 50° F. the hay intake was increased and an increase in the carotenoid, and to a lesser degree, the vitamin A content of blood plasma and milk fat resulted. Low ambient temperatures had no appreciable effect on either the carotenoid or vitamin A content of blood plasma and milk fat.

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