THE INFLUENCE OF CAROTENE LEVELS IN DAIRY CATTLE RATIONS ON REPRODUCTIVE BEHAVIOR, FEED UTILIZATION AND OTHER PHYSIOLOGICAL PROCESSES

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DEDICATED TO

MY WIFE

LOIS ANN

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INTRODUCTION

"Yea, the hind also calved in the field, and forsook it, because there was no grass."

Jeremiah 14:5

Carotene, the vitamin A precursor, has been proven to be essential in the nutrition of the bovine. The level of carotene necessary in the ration varies depending on the function and stress under which the animal is operating. The physiological function measured determines to a great extent the amount of carotene required. The carotene intake needed by the ruminant for normal reproduction has not been definitely agreed upon by investigators. Differences in conditions under which the various investigations have been conducted, particularly the duration of experiments, appear to have influenced the conclusions drawn by the investigators with respect to the amount of carotene required. Most investigations have been confined to a study of the carotene requirements for reproduction in the bovine over short periods of one gestation or less. Little attention, if any, has been devoted to a study of the minimum requirements of carotene over an extended period, such as a lifetime or at least through growth and two or three gestations and lactations. Likewise, very little attention has been given to the influence of carotene levels during prenatal life on the subsequent growth and reproduction of the individual.

In most of the investigations dealing with the influence of carotene on reproduction, rations definitely deficient in carotene

have been utilized to produce abnormal reproductive conditions. Few investigations have dealt with a suboptimum carotene intake sufficient to prevent external symptoms such as nightblindness.

The purpose of this study is to show the influence of suboptimum carotene levels during prenatal life and growth on subsequent reproduction, feed utilization and other physiological phenomena of the individual.

No attempt has been made to study all physiological processes and a strictly deficient ration has not been utilized.

The investigation, although primarily of a fundamental nature, may have practical implications. Since long time exposure to a carotene deficiency does influence reproduction and growth, short time exposure to a suboptimum level, particularly in the early growing period, may have an adverse effect on the future reproductive and other physiological abilities of the individual.

REVIEW OF LITERATURE

The necessity of an adequate amount of vitamin A in the diet of animals has been known for nearly forty years. The metabolism and function(s) of vitamin A and its precursors, the carotenes, have been a field of constant study by investigators in the fields of small and large animal research and in the fields of human medicine and nutrition since the discovery of the vitamin in 1913.

HISTORICAL

The early investigations into the physiology and nutritional ramifications of vitamin A were conducted using small laboratory animals. It was not until 1926, when Jones, Eckles and Palmer (42, p.130) reported on the role of vitamin A in the nutrition of dairy calves, that any systematic study with large domestic animals was made. Earlier reports, however, had been made with reference to particular difficulties of raising normal calves from cows that had been maintained on a variety of types and qualities of hays. In 1924 Hart, et al. (32, p.315) in reviewing the observations made on the nutritive value of the wheat plant, reported the difficulty in obtaining normal calves. Huffman (41, p.3) at Michigan in 1928 reported calves going into convulsions when on a diet excluding hay and concluded hay contained some factor(s) necessary for normal growth. Converse and Meigs (13, p.141) reported in 1937 some of the conditions the Beltsville Station of the U.S.D.A. encountered with their cattle raised on timothy or alfalfa hay. In 1935 Moore and associates (64, p.533) at

Michigan reported on blindness in cattle which was associated with constriction of the optic nerve at the optic foramen. They believed this blindness to be the result of a deficiency of a factor(s) in the diet. Some of these investigations reported similar symptoms that were attributed to deficiencies in the roughage, but not at the time directly associated with carotene and/or vitamin A.

Jones, Haag and Brandt (44, p.35) in 1934 found that cod-liver oil, when added to a carotene-deficient diet, improved reproduction over the deficient group. They believed the cod-liver oil of considerable value in improving fetal development and the strength of the calves. Milk production by the carotene deficient group was only 50 per cent of normal; whereas when cod-liver oil was added to the deficient ration, milk production approached normal.

GROWTH

In 1911 Hart and co-workers (33, p.180) reported that Holstein heifers maintained on a sole diet of the wheat plant did not sustain growth. They failed to come into heat and pathological conditions of blindness and emaciation were observed.

Jones, Eckles and Palmer (42, p.136) found that calves consuming less than 40 per cent wheat straw in their diet did not make satisfactory growth. Wheat straw was considered, at that time, to be a fair source of vitamin A, probably because of harvesting methods of the time which allowed the plant to be quite green when cut.

Bechdel, Honeywell and Dutcher (2, p.20) fed five heifers a ration deficient in vitamin A and, together with usual reproductive

troubles, they produced edema in the front legs, declining appetite, and increased respiratory rate. Ward and Bechdel (99, p.115) in a study of carotene and vitamin A for growing calves found wide variations in the amount of vitamin A necessary to prevent avitaminosis. Similar variations were found in the amount of carotene required, depending on the source. They found the minimum carotene required for growing calves was 23 micrograms per kilogram of body weight per day in order that no vitamin A deficiency symptoms developed. Jones and Haag (43, p.632) found 25 micrograms per kilogram of body weight sufficient for growing Jersey bulls and to maintain the blood at 0.30 p.p.m. carotene. Hilton and co-workers (38, p.631) found 7,500 I.U. of vitamin A daily sufficient for satisfactory growth, while 30,000 I.U. were required for normal reproduction.

Ritzman and wo-workers at New Hampshire (74, p.28) found that calves receiving 500 I.U. carotene per 100 pounds of body weight gained only one-half the weight of those calves receiving adequate carotene, even though some of the calves on the low level consumed more food. Hodgson and associates (39, p.669) feeding to dairy bulls a hay containing only two p.p.m. carotene plus a carotene supplement, found the animals grew only at 77 per cent of normal and wondered what effect this impaired growth had on the subsequent breeding ability of the bulls. Converse and Meigs (13, p.142) feeding low quality timothy hay found 1.5 milligrams of carotene per 100 pounds of body weight necessary for normal growth. Guilbert and Hart (28, p.417) found 26-33 micrograms of carotene per kilogram of body weight required for non-lactating beef cattle to maintain body weight. Guilbert, Miller and

Hughes (30, p.549) found practically normal gains over long periods of time have been made by animals receiving vitamin A or carotene in amounts that did not permit normal vision in semi-darkness. They give the minimum intake of carotene as six to eight micrograms per kilogram of body weight, but suggest for practical purposes that the intake be five to ten times this amount.

Lamming, et al. (53, p.230) working with rabbits and studying the effect of incipient vitamin A deficiency on reproduction, found a difference of four grams in the weight of the fetus between controls and deficient animals. The National Research Council (68, p.4) recommends for growing dairy cattle six milligrams of carotene per 100 pounds of body weight.

REPRODUCTION

By far the greatest effort in the study of vitamin A and carotene has been directed to investigations of the influence of the vitamin and its precursors on reproduction. The early reports published in the second decade of the century, while not specifically mentioning vitamin A or carotene, did in the main mention the effects of a deficiency of vitamin A and/or carotene. Many of the basic studies connected with vitamin A and reproduction were carried out using small laboratory animals.

Mason (56, p.315) working with rats, found that vitamin A deficiency caused serious placental damage together with fetal tissue damage, but that the placental tissue damage was much more severe than the fetal tissue damage. He also found that the decidual tissue

damage was much more severe when the rats were only vitamin A-deficient than when they were vitamin A-deficient, but supplied with vitamin E. This tissue damage is thus different than when rats are supplied with vitamin A, but are vitamin E-deficient. In vitamin E deficiency tissue damage is well confined to the fetal tissues. Mason (56, p.321) suggests that fetal resorption and the prolongation of gestation and of parturition due to vitamin A deficiency does not cause irreparable injury to the female reproductive tract. In his work with the rats Mason (56, pp.330-335) did not find the rhythm of estrus directly altered, nor evidence of corpora lutea persisting in the vitamin A-deficient animals. He is of the opinion that death of the fetus is probably due to decreased food supply, because of the damage to the placental tissue and to the uterine wall tissue (56, p.338).

Wilson and co-workers (103, p.195) found they could prevent ocular defects, diaphragmatic hernia and renal anomalies in the young, pregnant rats on a vitamin A-deficient diet by supplying large doses of vitamin A on the 10th or 11th day of pregnancy. On the other hand they found cardiac and lung abnormalities increased by late dosage with vitamin A. They concluded (103, p.215) that vitamin A-deficiency causes malformations during the period of active organ formation and not while the primordia are still undifferentiated.

Lamming and associates (52, pp.217-225) working with rabbits, were able to recover 93.4 per cent of the eggs ovulated in the controls, but only 78.7 per cent in the vitamin A-deficient rabbits. By making serial sections of the ovary they were able to establish that ovulation was complete, thus indicating early degeneration of the ova. Further

investigation revealed that 40 hours post-coitus 88.3 per cent of the eggs in the controls were cleaving normally, but only 75.4 per cent in the deficient animals. At four days post-coitus 97 per cent of the eggs recovered appeared in normal blastocysts compared with only 66.7 per cent in the deficient animals.

In a subsequent paper Lamming, et al. (53, pp.227-239) found a significant difference in the number of normal fetuses favoring the control animals. The placenta of the deficient animals showed a mottled appearance with gross indications of a reduced vascularity. With these observations Lamming (53, p.237) suggests that vitamin A deficiency may result in the degeneration of the placenta which in turn limits the supply of endogenous progesterone, thus causing abortion and resorption of the fetus late in gestation. They observed that vitamin deficiency reduced the number of females accepting the males. Phillips and Bohstedt (73, pp.209-219) found that rabbits did not develop constriction of the optic foramen on a bovine blindness-producing ration.

Hart and Guilbert in 1933 (35, pp.18-25) report on a severe vitamin A deficiency in a range herd where the cows aborted and gave birth to weak, blind and dead calves. The cattle had been carried for nine months on dry pastures and grain stubble. In a later publication Guilbert and Hart (28, p.417) state the minimum intake of carotene necessary for normal reproduction in the cow is approximately 30 micrograms of carotene per kilogram of body weight. Hilton and associates (38, p.632) state 30,000 I.U. of vitamin A are required daily for normal reproduction. Davis and Madsen (15, pp.135-146) report weak, blind

and dead calves when the cows were receiving 45 micrograms of carotene per kilogram of body weight, but at 60 micrograms the calves were normal. Kuhlman and Gallup (51, p.688) found that the minimum carotene intake for normal reproduction was between 40-45 micrograms per pound with first calf heifers requiring an additional amount to take care of growth, maintenance and reproduction. At 20-39 micrograms per pound, 1.99 services per conception were required; while at 60-99 micrograms, 1.15 services were necessary. Payne and Kingman (72, p.53) found that first calf Hereford heifers required a considerably higher blood carotene level than aged cows for normal reproduction. When the blood plasma level was 1.17± 0.71 p.p.m., no clinical symptoms of vitamin A deficiency were evident; but when the blood level dropped to 0.97 ± 0.07 p.p.m., deficiency symptoms developed. On the other hand with aged cows no symptoms were observed when the blood carotene level was 0.83± 0.04 p.p.m.

Ronning, et al. (75, p.52) and Moore, et al. (66, pp.533-538) found significant breed differences in the carotene requirement necessary to prevent reproductive difficulties. Ronning (75, p.52) states the minimum safe level for reproduction in Guernseys to be 90 micrograms per pound of body weight and this level should be maintained throughout gestation to insure no difficulties. Moore (66, p.533) found Holstein calves required only 30 micrograms per pound; while Guernseys required 34 micrograms per pound. Ronning and associates, in their latest paper published in 1953 (75, pp.52-56) draw a comparison in the variability in the requirements of carotene for reproduction as presented by various investigators. Guilbert, et al. (29, p.91-103)

give 26-33 micrograms per kilogram as the minimum for successful reproduction with 130-165 micrograms the last month of gestation. Converse and Meigs (13, p.144) report 176-264 micrograms per kilogram the last months of gestation. Davis and Madsen (15, p.135-146) obtained satisfactory reproduction with 132 micrograms of carotene per kilogram of body weight; while Kuhlman and Gallup (51, pp.688-689) found 99 micrograms satisfactory for reproduction with Jersey cows. The National Research Council (68, pp.1-30) recommends six milligrams of carotene per 100 pounds of body weight and nine milligrams per 100 pounds the last three months of gestation.

Jones, Haag and Dougherty (45, p.689) found no relation between so-called low-quality sperm and rations fed. In 1940 Sutton and associates (90, p.274) reported yearling dairy bulls on a low-carotene diet showed constriction of the optic nerve and degeneration of the germinal epithelium of the testes. Jones and Haag (43, pp.632-633) found blindness and death in young Jersey bulls with blood plasma values of 0.3 p.p.m. carotene. Five and 20 micrograms of carotene per kilogram of body weight did not prevent blindness. Twenty-five micrograms per kilogram of body weight was sufficient for normal growth and to maintain blood plasma carotene at 0.3 p.p.m. When 35 micrograms of carotene per kilogram of body weight were fed, the bulls grew normally but one animal went blind. Blood carotene was 0.46 p.p.m. Fertility was maintained 35 micrograms, but at 25 micrograms one animal did not settle a cow. Erb and co-workers (20, p.769) reporting on the use of the testicle biopsy technique in vitamin A studies, fed a low-carotene diet of beet pulp and grain. The bulls developed blindness, loss of

co-ordination and gastro-intestinal disturbances. The testicle biopsy showed degeneration of the seminiferous tubules and almost complete disappearance of sperm. The quality of the semen declined until a vitamin A supplement was given. When the supplement was increased from 60,000 I.U. of vitamin A to 150,000 I.U. daily, semen quality improved. A second biopsy showed the beginning of repair to the testicles.

Hodgson, et al, (39, p.669) found that bulls receiving two p.p.m. carotene in their hay failed to serve at the expected age of 10-12 months. When the carotene intake was raised to ten p.p.m., the bulls began breeding. They found gross vitamin A deficiency symptoms appearing before impairment of breeding efficiency. The semen of the deficient bulls was found to be low in concentration, high in abnormal forms, high in pH and with low livability. Erb and associates in 1947 (21, p.687) found complete blindness in bulls receiving 2000 I.U. of vitamin A per day. After five months of therapy with 100,000 I.U. per day, only moderate sperm production was found. They also found that moderate prepubertal vitamin A deficiency did not completely inhibit sperm production. On the other hand fertility and reproductive capacity appeared to be seriously impaired. Bratten, et al. (8, p.779) likewise, found gross vitamin A-deficiency symptoms appearing before any appreciable influence on the semen quality was evident. They did find, however, degeneration of the seminiferous tubules of the testicles.

LACTATION

That the feed of the cow has a direct bearing on the carotene and vitamin A content of the milk fat has been well established (40, pp.513-528; 14, pp.1-12; 23, pp.1-21; 24, pp.2-26) Other workers have shown that the amount of carotene in the feed required for lactation does not exceed the amount for normal reproduction (51, p.689; 50, p.522; 104, p.279). Vitamin A or carotene far in excess of that required for normal reproduction did not increase total milk and fat production (50, p.522; 104, p.282).

Hauge and associates (36, p.63) found that dairy cows can utilize carotene in alfalfa hay as readily as carotene from carrot oil for the production of butterfat of high vitamin A potency. Parrish and co-workers (71, pp.551-559) with swine found crystalline carotene only one-half to two-thirds as potent as a vitamin A concentrate. Semb and associates (81, p.701) in studying the carotene and vitamin A content of colostrum state a normal Holstein cow secretes in her milk only 0.8 per cent daily of the carotene contained in her blood.

BLOOD CAROTENE AND VITAMIN A

Blood carotene and vitamin A values have been used for some time as a measure of carotene nutrition in livestock. Of late some doubts have arisen as to the correlation between blood values and the true carotene and vitamin A nutritional level.

In 1942 Boyer, et al. (6, p.233) stated that blood plasma vitamin A level was a more delicate measure of the state of vitamin nutrition of the dairy calf than either growth or blood carotene levels.

They found that the blood carotene levels necessary to maintain adequate blood vitamin A were 0.5-0.7 p.p.m. for Holsteins and 1.1-1.4 p.p.m. for Guernseys. Spielman, et al. (86, p.717) found that the diet of the dam had no significant effect on the plasma carotene of calves. Payne and Kingman (72, p.53) found that unless the blood carotene of range heifers was 1.17 p.p.m., clinical symptoms of vitamin A deficiency would develop; that of aged cows must be above 0.83 p.p.m. to prevent symptoms. Wise, et al. (104, p.279) found excess vitamin A increased the plasma level of vitamin A but decreased the carotene level. They also found that feeding vitamin A did not prevent the decline in plasma vitamin A prior to parturition. Shaw, Moore and Sykes (82, p.566) found that the feeding of raw soybean meal to calves gave lower plasma values than with calves not receiving the soybeans. Two control calves had values of 9.60 and 9.32 micrograms/100 milliliters; while two calves on raw soybean meal had values of 5.64 and 6.60. These results are in agreement with Squibb, et al. (87, p.421) who also found soybean products depressed blood carotene values.

Ronning, et al. (75, p.54) found wide variations in blood carotene values. Five animals with an average daily carotene intake of 70 micrograms per pound of body weight maintained blood plasma carotene values ranging from 200-945 micrograms per 100 milliliters.

Thomas and Moore (93, p.687) caution that plasma vitamin A values should be used with some reservations as regards an indicator of vitamin A intake and storage.

Weswig (100) showed that Jersey and Holstein cattle raised on a normal ration of alfalfa hay, grain, grass silage and irrigated

pasture had blood values for Jerseys ranging from 4.8-12.0 p.p.m. of carotene and 0.28-0.94 p.p.m. of vitamin A. With Holstein cows the blood carotene ranged from 6.3-14.3 p.p.m. and the vitamin A from 0.34-1.03 p.p.m.

CAROTENE AND VITAMIN A LIVER VALUES AND STORAGE

Even though the liver has long been recognized as the main storage organ in the body of vitamin A, the liver values have been obtained only with slaughter specimens. Therefore in studies of carotene utilization and metabolism, the liver has been of little use to the investigator. The development of the liver biopsy technique for cattle as outlined by Seghetti (80, pp.9-11), Whitehair (101, pp.285-287) and more recently by Bone (4) allows a number of treatments and the course of one treatment to be followed without sacrificing the animal.

Guilbert and Hart (28, p.417) found that from 67-93 per cent of the storage of carotene and vitamin A in the animal is found in the liver. Hodgson (39, p.669) found when bulls were fed a low carotene hay containing two p.p.m. of carotene that liver values approximated 0.09 p.p.m. of carotene and 0.05 p.p.m. of vitamin A. Bratton, et al. (8, p.779) found the liver value of low carotene bulls to be 40 micrograms per gram of fresh liver with control bulls at 450 micrograms.

Baker and associates (1, pp.591-574) using the liver biopsy technique in a study of placental and mammary transfer of vitamin A and carotene found the following:

	Micrograms Vit A per Gram Fresh Liver		
	6 months prepartum	Parturition	3 months post partum
1. no supplement	68.9	5.4	1.7
2. 330 micrograms carotene per lb. during lactation	70.7	9.4	18.9
3. 60 micrograms carotene per lb. during gestation	122.6	20.6	7.4
4. 330 micrograms carotene per lb. lactation and gestation	92.2	14.3	23.4
1. calves--no supplement		4.2	1.9
2. calves--330 micrograms carotene to dam during lactation		3.4	14.2
3. calves--60 micrograms carotene to dam during gestation		2.6	2.5
4. calves--330 micrograms carotene to dam during lactation and gestation		3.0	22.3

Elliot (18, p.771) found that when Guernsey calves were injected intravenously with high-carotene plasma, the liver vitamin A values did not rise. Eaton and co-workers (16, p.764) were able to increase the liver storage of vitamin A in calves by feeding supplemental vitamin A to the dams, and the calves were subsequently able to utilize this fetal storage of vitamin A. Spielman, et al. (86, p.717) relate the liver storage of carotene and vitamin A of the new born calf to the prepartum diet of the dam. Hibbs and Krauss (37, p.115) found no linear

correlation between plasma vitamin A and liver storage when storage is high, but at low levels of liver storage plasma vitamin A may be used as an index of liver storage. When the calf is suffering from scours, plasma and liver vitamin A values are low (37, p.117). Wise and associates (104, p.279) were able to increase the liver values of vitamin A of cows on a normal ration, but did not find any evidence of a correlation between the vitamin A levels in the blood and the liver.

Shaw, Moore and Sykes (82, p.566) found that not only did the feeding of soybeans depress the blood plasma vitamin A values, but also the liver vitamin A level was depressed. Two calves receiving raw soybean meal had liver values of 0.72 and 2.75 micrograms of vitamin A per gram of fresh liver; while two calves not getting soybean meal had values of 9.07 and 9.22 micrograms per gram of liver.

ABSORPTION, CONVERSION AND UTILIZATION OF CAROTENE AND/OR VITAMIN A

The site of absorption of vitamin A and the location of absorption of carotene and its conversion to vitamin A has long been of concern to investigators. Until recently it was thought that the liver was the site of conversion of carotene to vitamin A. Recently it has been shown that the greatest portion of the carotene in the diet is converted to vitamin A in the wall of the small intestine (27, p.65; 102, p.75). By using vitamin A deficient rats Mattson (57, p.67) was able to show when carotene was administered, vitamin A first appeared in the intestinal wall. No increase in the yellow pigment was found in the liver; while there were increased levels in the intestinal wall. Wiese (102, p.75) by incubating slices of small intestine tissue with

carotene was able to show an increase in the vitamin A level of the tissue.

Elliot (18, p.711) by taking blood samples from different positions along the intestinal tract of live dairy calves, found the plasma vitamin A increasing as he progressed down the tract. He also found that although the carotene increased in the liver when carotene was fed, the vitamin A value of the liver increased to a greater degree. Niedermeier, et al. (69, p.714) checked the absorption at the abomassum and the small and large intestines after massive doses of vitamin A were fed. Increases in the blood plasma vitamin A values were in the neighborhood of 250,000 I.U. When the vitamin A was injected, there was a ten microgram per cent increase in the abomassum, a 20-50 microgram per cent increase in the small intestine and a 14 microgram per cent increase in the large intestine. The peak was reached within ten hours after injection. This work was confirmed by Ronning and Knodt (77, pp.283-287). They found the most active absorption of vitamin A in the upper two-thirds of the small intestine. The low concentration of carotene in the middle third of the small intestine indicated absorption taking place most readily in this area (77, p.283).

Stallcup and Herman (88, p.237) using excised portions of the small intestine were able to show conversion of carotene to vitamin A by minced liver tissue, but that conversion was not a function of blood plasma.

Certain dietary factors occuring in natural feedstuffs have been proven to influence the efficiency of carotene utilization. Soybeans, whether in the raw state or in the form of the oil, have been shown to

depress blood carotene values and lower the liver storage (82, p.566; 87, p.421). Burns and associates (10, p.347) were able to show as little as 0.08 per cent of mineral oil in the diet of the rat diminishes the utilization of beta carotene. Smaller percentages of the mineral oil evidence the same but not significant effect. The influence is greater on beta carotene utilization than on vitamin A. Increasing the dietary fat from five to ten per cent did not improve the utilization of vitamin A or carotene. Esh, et al. (22, p.461) found that when lecithin was fed with vitamin A, the absorption of vitamin A was enhanced. The liver storage of calves was increased when their dams were fed lecithin and vitamin A. Te-Ch'in Chou and Marlatt (12, p.305) found carotene utilization in the diet of Chinese people was increased by substances such as tocopherols, lecithin and ascorbic acid which apparently protect carotene from oxidation.

Burns and co-workers (2, p.341) found no difference when 1.0 milligram of alpha tocopherol was fed daily with 1.0 microgram of vitamin A and 1.0 microgram of beta carotene, but did find that 2.0 milligrams of tocopherol dimished the efficiency of utilization of beta carotene. They also found that the fat content of the diet improved the utilization of vitamin and beta carotene. Koehn (49, p.337) found 1.0 milligram daily per rat of alpha tocopherol allowed quantitative conversion of beta carotene to vitamin A. These investigations lead to the conclusion that certain dietary factors affect the release or utilization of vitamin A stored in the body.

Thomas, et al. (94, pp.372-378) found that in phosphorus deficient cows the average plasma carotene levels were higher than in those

fed adequate phosphorus, but plasma vitamin A appeared to be unaffected. The milk from the phosphorus-deficient cows contained more carotene, but less vitamin A than that from the cows fed adequate phosphorus. Liver values of both groups were normal with more than 200 micrograms per gram of vitamin A and about ten micrograms of carotene per gram of dry liver.

Ellenburger and associates (17, pp.1-84) found when studying the effect of feeding different grades of hay and cod-liver oil to calves, that the vitamin-fed groups made greater daily gains and used less total digestible nutrients. Per pounds of gain the vitamin-fed group required 0.34 pounds less total digestible nutrients. Ritzman, et al. (74, pp.1-28) when studying the influence of vitamin A on the utilization of energy and protein by calves, found that those calves receiving only 500 I.U. of carotene per pound of body weight gained only half as much as those receiving adequate carotene. After ten weeks the utilization of protein was 35.6 per cent less than normal in one calf and 13.6 per cent in another. Digestion, absorption and efficiency of energy metabolism was each depressed five to six per cent by the low carotene intake. In measuring the influence of carotene and/or vitamin A on feed utilization it should be remembered that when the food intake is restricted the basal metabolism is sometimes significantly increased.

HISTOLOGICAL

Histological and pathological changes in the organism as a result of carotene and vitamin A deficiency have been reported mainly in connection with the male. Moore (61, pp.893-902) describes the use

of the opthalmascope in diagnosing carotene deficiency in cattle. Viewing the eye of a carotene-deficient animal with a opthalmascope one may see a mottled appearance of the tapetum lucedum or tapetum nigrum. Frequently one will see papilledema as evidenced by a cottony white appearance at the nerve head. Moore (61, p.894) states the mottling of the tapetum lucidum is seen only in the more mature animal.

Numerous workers have reported cystic pituitaries and degenerative changes in the testicles of animals on a vitamin A-deficient diet (55, p.669); Erb, et al. (20, p.769); Erb, et al. (21, p.687); Hodgson, et al. (39, p.669). Thorp (95, pp.27-31) reported that calves fed 6.7 micrograms of carotene per calf showed degenerative and inflammatory changes in the kidney, but these changes were not consistent. He also showed degeneration of the seminiferous tubules of the testicle. Hodgson and co-workers (39, p.669) found their bulls receiving only two p.p.m. carotene in their hay all showed greater or less development of cysts in the pituitary with some showing only ten per cent normal tissue in the anterior pituitary. Erb and co-workers (20, p.769; 21, p.687) showed degenerative changes in the seminiferous tubules and cystic pituitaries. Histological changes were observed in both the anterior pituitary and in the adrenal glands of the bulls.

Jungherr and associates (46, pp.666-675) found consistent changes in the anterior pituitary and a decrease in chromatic cells. The thryoid gland showed mild hyperplasia. They state that the marked hyperplastic condition of the epithelium of the parotid gland lends itself to specific morphologic diagnosis of vitamin A deficiency in the bovine.

With the exception of the pituitary gland little has been reported of the histological changes in the female as a result of carotene and/or vitamin A deficiency.

RESPIRATION

Oxygen consumption is a function of spermatozoa metabolism. Numerous investigators have endeavored to utilize the amount of oxygen consumed by sperm as a measure of the fertilizing and livability of the semen (19, pp.1-24; 27, pp.265-270). Ghosh and associates (27, p.269) found no significant correlation between respiratory metabolism and the fertilizing ability. The respiration level approximated 9.0 microliters of oxygen per billion sperm. They found a negative correlation between the microliters of oxygen consumed and the per cent motility, significant at the five per cent level. Walton and Edwards (98, p.254) indicate that spermatozoa with a high initial respiration rate retain good motility for a longer period than spermatozoa with a low initial respiration rate. Ely and co-workers (19, p.20) found that semen with large numbers of abnormal spermatozoa had lower oxygen consumption per billion living spermatozoa than normal semen.

PROCEDURE

It was the purpose of the study to investigate the minimal level of carotene required by male and female dairy cattle for normal reproduction. The study was to begin with the prenatal existence of the individual. The minimum level of carotene, referred to throughout the remainder of the paper as the "low carotene ration", was that amount based on previous investigations (28, p.417; 34, p.264; 43, p.632) thought necessary to prevent any external symptoms of carotene deficiency in the offspring. The level of carotene selected was 50 micrograms per kilogram of body weight. Because of difficulty in maintaining the original stock, it was found necessary to first raise this level to 90 micrograms per kilogram of body weight and finally to 130 micrograms per kilogram.

In general the procedure was to select a group of cows so that they would calve in March or April. This meant the cows were maintained in the barn on the low carotene ration for periods ranging from 2.8 to 7.9 months. Six Holsteins and nine Jerseys were maintained on the low carotene ration with two of the Jerseys being maintained for a second gestation. Table I shows the pertinent data with respect to these first generation low carotene females.

While these cows were in the barn, they were maintained on a low carotene ration of grain and hay supplying 50 micrograms of carotene per kilogram of body weight. The calves, when born, were allowed to nurse their dams for three days and then were fed their dams' milk for eight weeks at the rate of eight per cent of their body weight. After the milk feeding period, the calves were maintained on a grain mix and

TABLE I

LOW CAROTENE ANIMALS USED IN STUDY

Animal (no.)	Breed	Gestation (days)	Animal on Low Carotene Ration (months)	Sex of Calf	Experimental Animal (no.)	Disposition and Ration of Calf
			First Generation Low Carotene Females			
M-2	Jersey	290	6.5	female	255	Low carotene ration of 50 ug./kg.
W-3	"	273	4.0	"	251	" " "
221	"	275	7.0	male	$221B_1$	" " "
221	"	273	7.9	female	267	" " "
216	"	277	3.5	female	250	" " "
200	"	273	2.8	male	$200B_1$	" " "
218	"	279	7.0	"	218B	" " "
218	"	274	7.0	female	266	" " "
M-1	"	277	4.0	"	252	" " "
Dreamer	"	279	4.0	"	253	" " "
484	Holstein	272	5.0	male	$484B_1$ twins	" " "
				female	552	
490	"	273	5.0	"	553	" " "
496	"	280	5.0	male	$496B_2$	" " "
500	"	283	6.0	"	500B	" " "
503	"	280	3.3	"	$503B_2$	" " "
526	"	276	0	female	557 *	" " "
			Second Generation Low Carotene Females			
250	Jersey	260	53	female	G-17	Low carotene hay supplemented to 390 ug./kg.
252	"	273	28	male	$252B_1$	Low carotene ration of 50 ug./kg.
253	"	281	43	female	G-12	" " 130 "
266	"	?	23	"	G-6	" " 50 "
266	"	281	37	"	G-16	Low carotene hay supplemented to 390 ug./kg.
267	"	275	36	male	$267B_1$	" " " "
557	Holstein	?	42	female	G-13	Low carotene ration of 130 ug./kg.
557	"	?	54	male	$557B_1$	" " "

*526, dam of 557, on normal ration during gestation

a low carotene hay. Supplements of either alfalfa hay, alfalfa leaf meal or carrot oil were fed in addition to the low carotene hay to supply the daily carotene intake. The description and analyses of the hays and supplements used throughout the experiment are given in Table II.

A group of six Holstein and six Jersey cows on a normal ration were selected as controls. The calves from these cows were also maintained on a normal ration. The eyes of the calves born to the low carotene females were examined with an opthalmascope, designed for humans, to help in diagnosing blindness.

Jersey heifers were placed on the breeding list at 15 months of age and Holstein heifers at 17 months. Most of the breeding was done by artificial insemination and the semen was no older than 72 hours. In a few cases in an attempt to get the cows to conceive even though no estrus was evident the cows were bred artificially every day for a period of two to three months.

Semen was collected from the Jersey bulls beginning at about eight months of age and from the Holstein bulls at ten months and once per week thereafter. All semen was collected with an artificial vagina using recognized procedures of handling and processing semen. Semen quality tests run were total spermatozoa, per cent abnormal spermatozoa, per cent live spermatozoa, raw and diluted semen motility. Livability was determined by determination of motility at seven days. The differential stain used for the determination of live spermatozoa was a Swanson's and Bearden's (92, pp.981-987) modification of Blom's stain (3, pp.176-178). At intervals oxygen consumption of the semen

TABLE II

FEED ANALYSIS
(air dry basis)

Date			Carotene (p.p.m.)	Crude Protein (percent)
1948-49	Alta fescue refuse straw--low carotene hay		1.0	1.95
	Artificially dried alfalfa hay			
	Carotene supplement	1948	100.4	
		1949	52.0	12.45
	Rye grass straw--low carotene hay		3.54	2.68
1950	Rye grass straw--low carotene hay		3.54	2.68
	Alfalfa hay third cutting--carotene supplement		71.4	15.55
1951	Alta fescue refuse straw--low carotene hay		9.7	6.14
	Alfalfa hay, second cutting--carotene supplement		59.3	12.02
	Alfalfa hay--March, 1952		45.2	
	Silage		28.7 (D.B.)*	
1952	Oat hay (July, 1952)--low carotene hay		3.4	4.36
	Alta fescue refuse straw--low carotene hay		1.4	1.77
	Alfalfa hay--carotene supplement		39.6	13.57
	Alfalfa leaf meal--carotene supplement		107.5	17.25
	Oat hay--low carotene hay (Nov., 1952)		2.6	3.91
	Grass hay--low carotene hay (Jan., 1953)		4.7	5.52
	Rye grass hay--low carotene hay (Jan., 1953)		2.3	6.49
	Silage		108.0 (D.B.)	
1953	Alfalfa and oat grass--low carotene hay		3.8	11.65
	Alfalfa leaf meal--carotene supplement		44.3	
	Alfalfa hay		32.6	
	Carrot oil--carotene supplement		6160.0	
1954	Alfalfa and oat grass--low carotene hay		2.4	
	Silage		97.0 (D.B.)	

* D.B.--dry basis

was determined using a Warburg respirometer.

Fertility of the bulls was determined by artificial insemination of a limited number of cows in cooperating herds, consisting of one to twenty-five cows and maintained under a variety of conditions of management. In calculating the breeding efficiency of any bull no attention was paid to the condition of the cow. In some cases this caused bias against the bull, since some cows were bred up to eight times to as many as four different bulls, indicating the difficulty in conception lay with the cow. Nevertheless, these services were charged against the bulls.

Bulls on the low carotene ration were maintained at the same level of carotene intake as the females and when the females were changed the bulls also were changed.

All chemical analyses were conducted by the Department of Agricultural Chemistry of Oregon State College. The analyses of feeds for carotene were carried out using the modified A.O.A.C. method (96, pp. 219-224). Results are reported in parts per million (p.p.m.) on the air dry matter basis (five per cent moisture). Blood analyses for carotene and vitamin A were run on the blood plasma using a modification of Kimbles' method (47, pp1055-1065).

Liver analyses for carotene and/or vitamin A were run on either slaughter or biopsy specimens. In the case of the slaughter specimen, any amount of liver was available for analysis. In the case of the biopsy specimen, a biopsy punch eight inches long and one-quarter inch inside diameter was used in securing the samples. An incision three-quarters of an inch long between the eleventh and twelfth ribs and

about twelve inches below the spinal column on the right side was made. The punch was thrust into the liver about five inches and a sample of liver weighing between 350 to 2000 milligrams was secured. Analyses of the samples for carotene and vitamin A were made according to the method of Whitehair, et al. (101, pp.285-287).

Analyses of the milk for carotene and vitamin A were made by separating the accumulated milk from at least two and in most cases three milkings. Butter was made from the cream and the butterfat was secured from the butter. Carotene and vitamin A were then determined on the butterfat according to the method of the United States Department of Agriculture (97, pp.1-10).

Histological analyses were made of biopsy specimens of the testicles of all bulls on the low carotene diet and of a representative number of the bulls on the control ration according to the method of Byers (11, pp.1165-1171). When the animals were slaughtered, histological analyses of specimens of the pituitary gland, thyroid, adrenal gland and the ovary or testicles were made. In all circumstances tissues were fixed in Bouin's solution. Staining by Harris' hematoxylin was a standard practice.

Metabolism of semen and of certain tissues was measured by oxygen consumption through the use of the Warburg respirometor. Semen and tissues were brought to the laboratory in a substrate of Ringer's-phosphate solution and respiration was measured within one hour of collection.

In an attempt to measure the efficiency of feed utilization use of automatic collecting and excreta-separating digestion stalls was

made. Animals fed the low carotene ration for more than three years were compared with animals on a control ration. The animals were subjected to a preliminary period of three weeks and a digestion trial of five days. Two trials were run; one where the cows were fed alfalfa-grass silage and the second where the cows were fed the low carotene hay.

RESULTS

ANIMALS USED AND THEIR FEEDING

The animals used in the experiment are listed in Table I, page 23. Of particular significance in this study is the level of carotene intake and the period of low carotene feeding which varied considerably during the gestation periods of the different cows. Cow 513 aborted one month early and the dead calf, when autopsied, showed no clinical symptoms. The herd has for years been brucellosis free. Cow 484 gave birth to twins, a bull and a heifer. The bull later died from an accident and the heifer, kept for more than one year, was diagnosed as a freemartin.

The calves were allowed to nurse their dams for the first three days after birth. Milk from cows on a normal ration was then fed twice per day at the rate of eight to ten per cent of the body weight daily and a high quality calf meal was placed before the calves when they were ten days to two weeks of age. The low carotene hay was placed before the calves at approximately two weeks of age and the carotene supplement was mixed with an amount of calf meal that would be consumed to insure the calves were obtaining their daily carotene allotment.

Throughout the study the ration fed the experimental, low-carotene animals consisted of a good grain mix, low carotene hay and a carotene supplement that consisted of either good, green alfalfa hay, alfalfa leaf meal or carrot oil. Table II, page 25, gives the feeds used for the low carotene group with the carotene analyses and, in

many cases, the crude protein value.

The original 17 cows and the calves of these cows received 50 micrograms of carotene per kilogram of body weight. The low carotene roughage fed in 1948 was alta fescue straw and the supplement, artificially dried alfalfa hay. In 1949 the low carotene roughage was rye grass straw, in 1950 rye grass straw and in 1951 fescue straw and grass hay. During 1952 oat hay, alta fescue straw, grass hay and rye grass hay, and in 1953 poor quality, bleached alfalfa, oat-grass hay served as the low carotene roughage. High quality alfalfa hay or alfalfa leaf meal was used as the carotene supplement until carrot oil was substituted for the alfalfa leaf meal in June, 1953.

Grain was fed at the rate of three pounds per day for Jerseys and four pounds for Holsteins until the females calved when the allowance was increased. The bulls were fed three to four pounds daily until about two years of age when the Jerseys were fed six pounds and the Holsteins, eight pounds.

Inasmuch as the low carotene hay was frequently low in protein and unpalatable, grain was fed to the lactating cows in excess.

In September, 1951, when the majority of the second generation low carotene females had calved for the first time and a low percentage of normal calves was obtained, their daily carotene intake was raised to 90 micrograms per kilogram of body weight. Calves that were normal after being carried in dam at 50 micrograms per kilogram of body weight were also given 90 micrograms of carotene. The increased intake of carotene was continued through the second gestation of the majority of the cows. As with the lower carotene intake during the first gestation,

very few calves were normal at birth with a carotene intake level of 90 micrograms per kilogram of body weight. The intake of carotene was thus raised in October, 1952, to 130 micrograms per kilogram of body weight--equivalent to 60 milligrams per 1000 pounds of body weight. A higher percentage of normal calves was obtained at this level of carotene feeding. The difficulty of obtaining normal calves from low carotene animals and existing conditions necessitated the changing of the feeding schedule in some cases.

In the second lactation Jersey 250 was continued on the low carotene hay, but was given 260 micrograms of carotene per kilogram of body weight in the form of alfalfa leaf meal.

Jersey cows 252, 253, 266 and 267 were placed on a normal ration of irrigated pasture and grain according to milk production after their second calvings. Jersey 267 was retained on the normal ration in her second lactation for six months and then returned to the low carotene hay and grain for one month. She was then given, intravenously, 50,000 I.U. of vitamin A per day for one month, returned to the low carotene hay for one month and then injected with 250,000 I.U. of vitamin A per day for one month.

After over three years on the low carotene ration, Holstein cow 553 developed severe edema of the rear legs, so following calving the first time she was placed on a normal ration. After her second calving, she was returned to the low carotene ration of 130 micrograms of carotene per kilogram of body weight.

Holstein 557 was given the low carotene hay, grain and sufficient carrot oil to supply 390 micrograms of carotene per kilogram of

body weight when she calved for the second time.

G-6 was fed 50 micrograms of carotene per kilogram of body weight as soon as she began eating hay and grain. At five months of age, G-6 was changed to 90 and at 18 months to 130 micrograms per kilogram of body weight. G-12 and G-13 were fed 130 micrograms of carotene per kilogram of body weight from the time they began eating grain and hay. Bull $252B_1$ received for the first three months 50 micrograms of carotene per kilogram and then was changed with the rest of the animals. Bull $557B_1$ received 130 micrograms per kilogram of body weight.

After they began eating grain and hay, G-16, G-17 and $267B_1$ were fed the low quality alfalfa, oat-grass hay and grain, but were given sufficient carrot oil so that their carotene intake was 390 micrograms per kilogram of body weight. These animals were fed in this manner to check the influence of the palatability of the ration on the problems being studied.

The carotene intake of the low carotene group of bulls was changed at the same time as the females.

The normal group of females were fed good quality alfalfa hay, grain, grass silage and irrigated pasture in season and the normal bulls, alfalfa hay and grain. Carotene values of the alfalfa hay and silage are given in Table II, page 25.

GROWTH

All of the second and third generation low carotene females were appreciably below normal weight at birth as shown in Table III. Even though the animals were fed all the roughage they would consume

TABLE III

WEIGHT COMPARED TO NORMAL

Animal Number	Per Cent of Normal by Months												
	Birth	2	4	6	8	10	12	14	16	18	20	22	24
Low Carotene Jersey Females													
250	76	72	76	63	74	83	82	86	88	97	99	109	107
251	83	95	74	76	79	88	88	97	96	98	100	105	103
252	85	92	62	56	69	77	82	86	87	90	101	107	109
253	92	71	67	68	76	83	93	86	86	87	91	103	102
255	74	80	88	100	88	82	95	95	95				
266	76	82	83	87	89	93	90	95	98	95	96	97	93
267	81	76	77	79	85	87	91	97	96	94	97	95	91
G-12	83	88	67	94	86	90	95	96	99				
G-16	74	99	96	72	77								
G-17	78	81	86	67	67								
Control Jersey Females													
244	113	116	110	115	101	102	101	104	101	108		102	101
245	91	65	107	103	100	101	103	105		109	113		107
246	113	117	102	103	106		110	108	101	102	103	103	
247	98	96	82	67	59	76	84	93	95	100	92	99	
248	89	100	95	91	87	96	103	101	98	100	98		102
249	89	98	82	67	72	75	89	90	98	99			
Low Carotene Holstein Females													
553	81	66	78	98	99	106	104	103	100	94	100	100	
557	94	93	90	77	89	101	102	101	97	90	98	97	
G-6	83	84	85	83	87	78	84	93	98	99	107	102	
G-13	77	91	60	68			89	88	84				
Control Holstein Females													
543	101	80	80	85	101	107	101	102	104	103	108		101
544	108	97	94	96	94	92	96	99	99	98		102	
545	101	100	97	98	88	92	94	97	96	98		100	
546	90	72	71	73	74	78	80		89	98		96	95
547	85	87	78	70	76	72	76	77		91		90	97
548	104	87	78	82	79		85	89	91	92	99		96

TABLE III (continued)

Animal Number	Per Cent of Normal by Months												
	Birth	2	4	6	8	10	12	14	16	18	20	22	24
Low Carotene Jersey Males													
200 B1	68		86		87								106
218 B	77	76	93	102	99	91	100	98	100				
221 B	87	83	86	100	97	96	101	100	102	104	103	107	109
252 B1	60	62	70	89	88	91	90	87	93	85	79	85	92
267 B1	73	94	87	89	67								
Control Jersey Males													
A29 B	77	65	74	67	74	80	87	85		109			
200 B2	68	72	73					97					92
212 B3	60	68	65	56	75		86	87					92
264 B2	90	76	59	62	74		86	87					
Low Carotene Holstein Males													
496 B	100	92	81	84	87	93	90	86	85				
500 B	116	100	92	87	89	96	93	91	87	87	91	91	90
503	94	80	87	90	92	94	95	90	89	86	87	85	89
557 B1	98	100											
Control Holstein Males													
493 B2	81		81	83		92			84				93
493 B2	91		92	87		96			87				95
506 B2	93		86	90		94			89				

plus an excess of a grain mix of about 12 per cent protein, growth as measured by weight did not proceed at a normal rate until the cattle reached about 20 months of age at which time they about reached or surpassed the normal values. Plates I and II show two bulls on the low carotene ration at three and four years of age. Plates III and IV show two of the low carotene cows at four years of age. Although usually the control females were heavier at birth and approached normal weights through the first two years to a greater extent than did the low carotene animals, there were many that were below normal for a good part of their growing period. When the low carotene cows 252, 253, 266, 267 and 553 were returned to a normal ration, they were observed to lose weight rapidly. They required about two weeks to become accustomed to the normal ration, particularly pasture.

The incidence of disease was low with the exception of a severe outbreak of scours, first thought to be due to the ration, but later diagnosed as an infection that spread through the entire herd. This scouring condition occured when the second generation low carotene animals were from four to eight months of age. The two third generation Jersey heifers, G-16 and G-17 and third generation bull $267B_1$, did not grow any faster up to eight months of age even though obtaining 390 micrograms of carotene per kilogram of body weight.

Two Jerseys, numbers 251 and 255 and Holstein bull, 500B went blind at 16-18 months of age when receiving 50 micrograms of carotene per kilogram of body weight. This was surprising since Guilbert and Hart (28, pp.417-426) had recommended 30 micrograms of carotene per kilogram of body weight and Jones and Haag (43, pp.632-633) found at 35

PLATE I

Low carotene Jersey bull 221B at three years of age

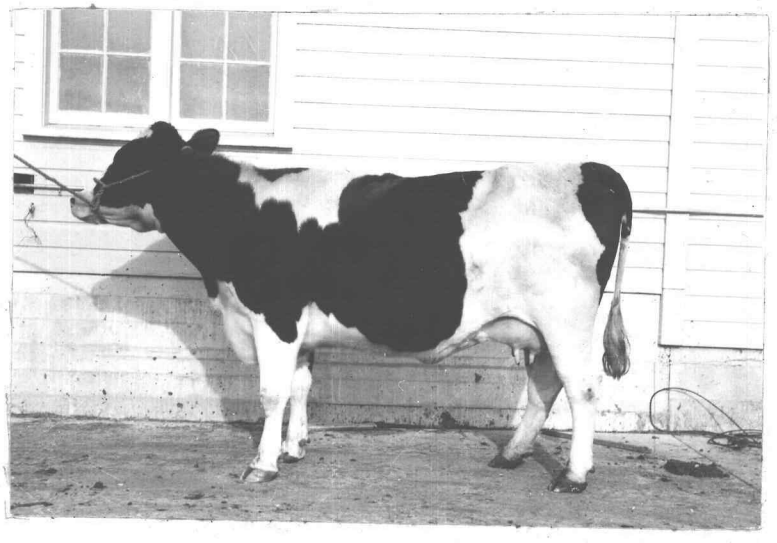
PLATE II

Low carotene Holstein bull 500B at four years of age

PLATE III

Low carotene Jersey cow 251 at four years of age

PLATE IV



Low carotene Holstein cow 553 at four years of age

micrograms of carotene per kilogram of body weight one animal going blind and the other remaining normal.

BLOOD CAROTENE AND VITAMIN A DETERMINATIONS

During the first two years of the experiment, blood carotene was the only determination made. Beginning at the time when the ration was changed to 90 micrograms of carotene per kilogram of body weight, both blood carotene and vitamin A analyses were made at frequent intervals in the low carotene group. The results are reported in Table IV. Also given are some values for normal Holstein and Jersey animals of a comparable age. The blood data show an apparent increase up to about one and one-half years in carotene and vitamin A values as the age of the animals advances. It would appear that good quality alfalfa hay fed ad libitum to the control animals is not satisfactory in maintaining blood carotene and vitamin A values near those of cows which receive alfalfa hay, grass silage and irrigated pasture.

In the case of animals $267B_1$, G-16 and G-17 which received 390 micrograms of carotene per kilogram of body weight from the time they were able to consume grain, the blood carotene and vitamin A values are not appreciably different from animals G-6, G-12 and G-13 which received only 130 micrograms of carotene per kilogram of body weight.

The blood carotene and vitamin A values for the low carotene animals at three and one half to four and one-half years of age when the cattle were receiving 130 micrograms per kilogram of body weight are not appreciably different from the values when the animals were receiving 90 micrograms per kilogram of body weight at approximately

TABLE IV

AVERAGE BLOOD CAROTENE (C-TE) AND VITAMIN A VALUES (P.P.M.)

Animal No.	0-6 months C-te	Vit.A	6 mo.-1½ yr. C-te	Vit.A	1½-2½ yr. C-te	Vit.A	2½-3½ yr. C-te	Vit.A	3½-4½ yr. C-te	Vit.A	4½-5½ yr. C-te	Vit.A
					Low Carotene Animals							
200 B1	1.39		0.17		1.26	0.25	0.62	0.23	0.66	0.20		
218 B			0.60		0.55							
221 B1			0.42		0.71		1.70	0.23	0.69	0.17		
252 B1	0.46	0.14	0.80	0.19	0.61	0.17	1.77	0.25				
267 B1	0.29	0.08a	1.42	0.29a								
496 B	0.17		0.71									
500 B	1.09		0.54		1.21	0.20	0.77	0.17	0.48	0.11		
503 B	0.48		0.91	0.26	0.54	0.14	0.33	0.10				
250			0.20		0.98	0.21	0.79	0.17	1.17	0.19	3.63	0.26b
251			0.18		1.16	0.19	0.77	0.18	1.18	0.21	1.30	0.19
252			0.29		1.58	0.20	0.58	0.15	3.70	0.27c		
253			0.38		1.03	0.13	0.92	0.18				
255			0.11		1.06	0.18	1.02	0.19				
266			0.67	0.24	1.18	0.25	0.75	0.19	4.63	0.38c		
267			0.48	0.16	0.77	0.19	0.60	0.18	2.89	0.56c		
G-12	0.10	0.04	0.91	0.24								
G-16	1.20	0.29a										
G-17	0.89	0.26a										
G-6	0.32	0.06	0.37	0.17								
G-13	0.14	0.08	0.42	0.16								
553			0.04		1.08	0.30	0.46	0.24	9.62	0.29c	2.05	0.28
557			0.09		0.80	0.22	0.69	0.21	1.49	0.17	3.84	0.32a

a. 390 mg/kg body weight from carrot oil b. 260 mg/kg body weight from alfalfa leaf meal c. normal ration

TABLE IV (continued)

Animal No.	0-6 months C-te	Vit.A	6 mo.-1½ yr. C-te	Vit.A	1½-2½ yr. C-te	Vit.A	2½-3½ yr. C-te	Vit.A	3½-4½ yr. C-te	Vit.A	4½-5½ yr. C-te	Vit.A
					Control Animals							
29 B							5.89	0.48	5.52	0.43	2.38	0.22d
200 B2			2.72		3.71	0.31	3.40	0.38	3.40	0.30		
212 B3			7.30		2.01	0.39	4.16	0.48				
264 B2	4.0		4.68	0.44	4.81	0.35	3.28	0.27				
493 B2	0.36		1.41	0.37	2.50	0.31	2.24	0.21				
493 B3	0.44		1.63	0.40	2.62	0.38	1.09	0.15d				
506 B2	5.0		2.85	0.65								
244					6.25		6.46	0.82				
245					6.71		10.11	1.06	9.82	0.84	9.60	0.31
246					6.78	1.05			9.94	0.74		
247					6.10	0.75			10.06	0.67		
248					5.46		8.90	1.33	9.29	0.59	13.12	0.84
249			7.61		7.80	1.10						
543					5.58		9.50	0.94	9.55	0.53	13.10	0.23
544					4.69		8.60	0.58	6.18	0.49		
545					6.21							
546					4.65		9.16	0.79	10.04	0.69	11.42	0.27
547			3.35		4.99	0.45	9.41	0.68	6.21	0.61	10.85	0.25
548			5.29		8.59	0.81	7.78	0.54	2.60			

d. 130 mg carotene per kg body weight

two and one-half years of age. It would seem, therefore, that as the animals were maintained on the low carotene ration, the blood vitamin A values were not increased by the increased intake of carotene indicating the possibility of some mechanism of conversion of carotene to vitamin A had been interfered with by the continued exposure to the low carotene ration.

BREEDING PERFORMANCE OF FEMALES

The females were placed on the breeding list at approximately 15 months of age. Table V gives detailed data on the breeding performance of those animals of breeding age together with data on the control animals. Those animals giving birth to a calf in less than 265 days gestation were arbitrarily considered to have aborted.

Estrous cycles were very erratic in length. Cow 557 showed only one heat period for her first gestation and in her second gestation did not show heat at all. Cow 251 in her third gestation failed to show heat periods; thus both she and cow 557 were bred every day for a period of about two months to which treatment they conceived. Cow 553 was bred 11 times with estrous cycles ranging from 15 to 181 days. Contrasted to this very poor reproductive behavior were cows 266 and 267 who in their first gestation conceived to one breeding and cows 250 and 252 which in their second gestation required only two services. The two services required by cow 250 in her second gestation was a great contrast to the 11 services required in her first gestation with estrous cycles varying from 21 to 183 days. Cow 250 had two gestations each of which was less than 265 days duration. In the first gestation

TABLE V

BREEDING PERFORMANCE

Animal	Age First Bred	Times Bred	Calving to Breeding	Length of Estrous	Length of Gestation	Age at Calving	Sex of Calf	Calf	Condition, Disposition and Weight in lbs. of Calf
no.	yr.mo.	no.	days	days	days	yr. mo.		no.	
Second Generation Low Carotene Females									
250	1 6	11		21-183	233	2 3	M	$250B_1$	Weak, abnormal, autopsied, 26
		2	84	81	260	4 5	F	G-17	Normal--low carotene, 42
		4	55	24-68	Open--slaughtered as non-breeder				
251	1 4	6		20-60	261	2 6	M	$251B_1$	Dead, normal, postmortem, 35
		3	32	23-24	278	3 6	M	$251B_2$	Weak, abnormal, autopsied, 33
		?	107	70	?	4 9	M	$251B_3$	6-8wks.premature,autopsied 1 day, 22
		2	62	38	open				
252	1 3	6		20-38	272	2 4	M	$252B_1$	Normal, low carotene, 36
		2	55	22	267	3 3	F	G-8	Blind,weak,autopsied at 5 days, 36
		2	163	143	Open--slaughtered as non-breeder				
253	1 4	2		25	277	2 2	M	$253B_1$	Weak,blind,abnormal front legs, 44 autopsied 2 days
		7	88	6-44	281	3 7	F	G-12	Normal, low carotene, 45
255	1 1	6		24-68	204	2 2	M	$255B_1$	Dead, postmortem
		4	67	24-160	Open--slaughtered as non-breeder				
266	? ?	?			?	1 11	F	G-6	Normal, low carotene, 60
		2	149	51	281	3 1	F	G-16	Normal,low carotene,390 micrograms,40
267	1 2	1			260	1 11	F	G-5	Weak,blind,abnormal front legs, 42 autopsied at 1 week
		2	40	67	275	3	M	$267B_1$	Normal,low carotene,390 micrograms,44
553	1 3	11		15-181	278	3 5	M	$553B_1$	Dead,strangulation,postmortem, 93
		3	56	24-64	276	4 7	M	$553B_2$	Dead,strangulation,postmortem, 91
557	1 3	1		No heat	?	3 6	F	G-13	Normal, low carotene, 70
				No heat	?	4 6	M	$557B_1$	Normal, low carotene, 85
Third Generation Low Carotene Females									
G-6	1 9	1			Open				
G-12	1 4	1			Pregnant				
G-13	1 3	1			Pregnant				

TABLE V (Continued)

Animal	Age First Bred		Times Bred	Calving to Breeding	Length of Estrous	Length of Gestation	Age at Calving		Sex of Calf	Calf	Condition, Disposition and Weight in lbs. of Calf
no.	yr.	mo.	no.	days	days	days	yr.	mo.		no.	
						Control Females					
244	1	3	13		25-110	Persistent corpus lutea					Slaughtered as non-breeder
245	1	3	3		20-86	275	2	3	F	281	Normal ration, 42
			9	167	21-93	274	4	8	F	307	Normal ration, 42
			4	152	12-32						
246	1	3	3		19-65	281	2	3	F	278	Normal ration, 36
			6	109	23-96	282	4		M	$246B_1$	Normal ration, 53
			2	115	25	283	5	1	M	$246B_2$	Normal ration, 50
			4	50	19-46						
247	1	2	3		12-19	268	2		M	$247B_1$	Normal ration, 32
			1	87		277	3		M	$247B_2$	Normal ration, 39
			1	99	73	279	4		F	306	Normal ration, 41
			1	49	23	282	4	11	M	$247B_3$	Normal ration, 42
			3	51	25-47						
248	1	2	2		19	277	2		F	282	Normal ration, 44
			1	146	100	272	3	2	F	295	Normal ration, 46
			4	127	20-62	278	4	7	F	318	Normal ration, 44
			7	83	20-41						
249	1	2	5		20-273	Cystic ovaries					Slaughtered as non-breeder
543	1	6	4		5-29	280	2	3	F	575	Normal ration, 78
			4	89	24-57	286	3	7	M	$543B_1$	Normal ration, 109
			1	69		277	4	6	F	207	Normal ration, 92
			4	123	23-120						
544	1	3	7		2-20	289	2	2	M	$544B_1$	Normal ration, 97
			13	66	20-46	280	4	1	M	$544B_2$	Normal ration, 83
			6	46	24-41						
545	1	4	8		21-32	Pregnant but sold because of abnormal uterus					

TABLE V (Continued)

Animal	Age First Bred		Times Bred	Calving to Breeding	Length of Estrous	Length of Gestation	Age at Calving		Sex of Calf	Calf	Condition, Disposition and Weight in lbs. of Calf
no.	yr.	mo.	no.	days	days	days	yr.	mo.		no.	
546	1	3	2		21	286	2	2	M	$546B_1$	Normal ration, 90
			1	84		281	3	2	M	$546B_2$	Normal ration, 97
			1	73		285	4	1	F	603	Normal ration, 92
			4	58	22-47	286	5	4	F	628	Normal ration, 89
547	1	4	3		19-118	284	2	6	M	$547B_1$	Normal ration, 92
			5	154	25-47	285	4	1	M	$547B_2$	Normal ration, 95
			2	126	25	275	5	3	F	627	Normal ration, 89
			1	47							
548	1	3	1			277	2		M	$548B_1$	Normal ration, 86
			2	92	22	282	3		M	$548B_2$	Normal ration, 83
			4	91	21-67	279	4	6	M	$548B_3$	Normal ration, 90
			2	175	20						

of 233 days she produced a weak, abnormal dead calf weighing only 26 pounds. During the second gestation of 260 days she produced a normal 42 pound, healthy calf, G-17, that was placed on the low carotene ration and given sufficient carrot oil to supply 390 micrograms of carotene per kilogram of body weight. Cow 250 was slaughtered as a non-breeder after being bred four times over a period of eight months and not conceiving.

Cow 251 produced three calves, two weak and abnormal that were autopsied shortly after birth and the other born dead. The first gestation dead calf weighing 35 pounds was delivered after 261 days gestation. The abnormal calf of her second gestation was carried 278 days and although the days of the third gestation were not known, the condition of the calf at birth indicated it was six to eight weeks premature. Although the calf was alive at birth, it died within a matter of minutes and was autopsied.

Cow 252 after 272 days gestation gave birth to a bull calf, $252B_1$, weighing 36 pounds that was placed on the low carotene ration of 50 micrograms of carotene per kilogram of body weight. Her second gestation, although not strictly considered an abortion at 267 days, did result in a blind, weak calf that was autopsied after five days. Cow 252 was slaughtered as a non-breeder on a normal ration after showing only two heat periods in 11 months since calving. After a 279 days gestation, cow 253 gave birth to a weak, blind calf with abnormal front legs and the calf was autopsied at two days. Her second gestation was 281 days and the calf, G-12, was normal at birth. After her second calving, cow 253 was placed on a normal ration but contracted

acute mastitis and was slaughtered. Cow 255 aborted a dead calf at 214 days and after four breedings with two of the heat periods 160 days apart was sold as a non-breeder.

Jersey cow 266 for her first gestation was accidently bred by a Holstein bull and gave birth to a crossbred calf, G-6, weighing 60 pounds. G-6 was originally placed on the low carotene ration of 50 micrograms of carotene per kilogram of body weight. In her second gestation, cow 266 was bred only twice and after 281 days gave birth to a normal calf, G-16, weighing 40 pounds which was placed on the low carotene ration supplemented with carrot oil so that her carotene intake was 390 micrograms of carotene per kilogram of body weight. Cow 267 in her first gestation of 260 days gave birth to a weak, blind calf weighing 42 pounds with abnormal front legs that was autopsied at one week of age. Her second calf, $267B_1$, weighing 44 pounds was born normal after 275 days gestation. The calf was placed on the low carotene ration and given a carrot oil supplement to supply 390 micrograms of carotene per kilogram of body weight.

Holstein cow 553 after successive gestations of 278 and 276 days gave birth each time to a dead calf that on post mortem gave every indication of having strangled at birth. Both gestations of cow 557 were of unknown duration, but both calves born were healthy and normal. As noted in Table I, page 23, cow 557 was from a dam fed a normal ration.

Third generation heifer, G-6, failed to show estrus until one year and nine months of age. Since that breeding to which she did not conceive, she has not again during three months to-date shown estrous.

Heifers G-12 and G-13 were bred only once at 15 months of age and conceived to one service.

The small population of randomly selected control cows presents a high percentage of difficult breeders. Jersey cows 244 and 249 did not conceive with 244 continually showing a persistent corpus luteum. At two years of age the right ovary with the corpus luteum was removed believing it might improve the chances of the cow becoming pregnant. She was sold, however, as a non-breeder at 37 months of age after 13 services. A similar situation existed with low carotene cow 557 whose left ovary was removed. She became pregnant at 33 months of age to every day breeding for three months. Jersey cow 249 continually had cystic ovaries. After having the ovaries broken by rectal massage, the cysts returned in a short time. She was disposed of at 32 months of age.

All gestations in the control group of cows were of normal length with all calves being born alive and normal. Nine animals in the low carotene group had 18 pregnancies, while 12 cows in the control group had 28 pregnancies. Using a Chi square analysis, this difference is not statistically significant. The difference of eight normal calves being born to the low carotene group compared with the 27 normal calves born to the control group is highly significant.

POST MORTEM FINDINGS

Whenever possible, post-mortems were conducted on calves born dead and autopsies were performed on those animals that gave every indication of not living.

Calf 250B_1, Plate V, born blind and abnormal could not stand up. The right eye was cloudy with corneal deficiency, protrusion of Descomets membrane and aqueous. Opthalmascope observations indicated slight papilledema and constriction of nerve heads. Slight doming of the vault and slight prognathia of lower jaw was shown and sucking was accomplished only with difficulty. All glands and organs were normal except that the brain showed slight hydrocephalus in the left lateral ventricle. The left hemisphere of the cerebrum was slightly larger and the optic nerve constricted at the optic foramen. The hydrocephalic condition is shown in Plate VI.

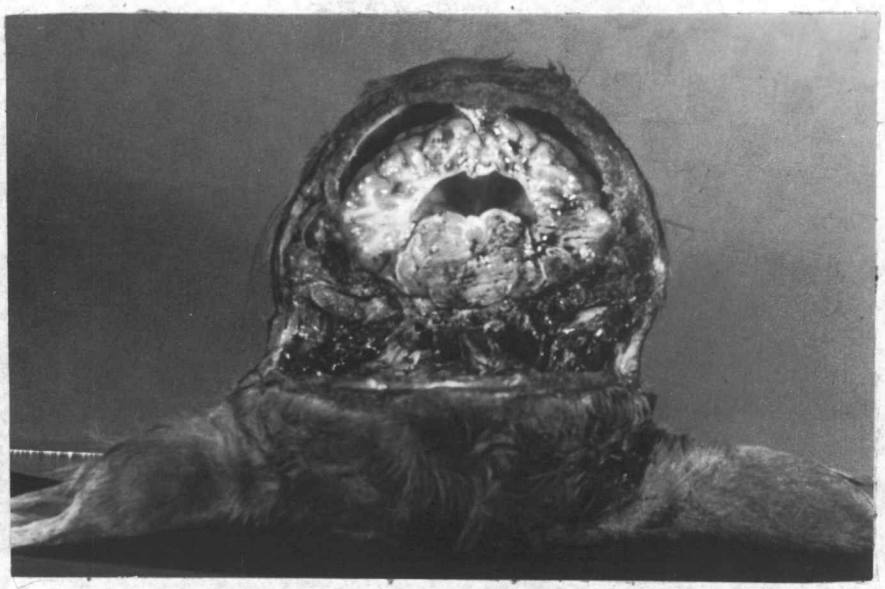
Cow 251 had three calves either born dead or born so weak that they were autopsied shortly after birth. Only one of the three was full term, the other two being abortions of three weeks and six to eight weeks early. Calf 251B_1, on autopsy, showed no clinical symptoms of any disorder. Calf 251B_2 was unable to lie on its sternun and continually flopped on its side. The left eye, when viewed with the opthalmascope, showed the tapetum lucidum mottled while the right eye, although appearing more normal, did show slight papilledema. The optic nerves showed constriction. All glands and organs appeared normal with the exception of the rumen which showed hemorrhagic areas on the outer surface. The pituitary gland appeared normal but small. Calf 251B_3 aborted six to eight weeks prematurely, appeared normal in all respects. There was no indication of constriction of the optic foramen and degeneration of the optic nerve.

The second calf of cow 252, number G-8 showed every indication of suffering from poor nutrition. The calf never stood and sucked

PLATE V

Low carotene calf 250B_1 showing domed skull and flexed forelegs

PLATE VI



Cross section of skull of calf G-8
showing internal hydrocephalus and distortion of skull

with difficulty due to a dropped or paralyzed lower jaw. Both eyes showed clouding and erosion of the cornea with protrusion of Descomets membrane; see Plate VII. A domed vault suggested a hydrocephalic condition. The thryoid gland appeared large and the pituitary small with an apparent fluid-filled cavity on the anterior ventral side. The brain was marked with internal hydrocephalus involving all chambers, but particularly the lateral ventricle and foramen of Munro. The optic nerves were constricted.

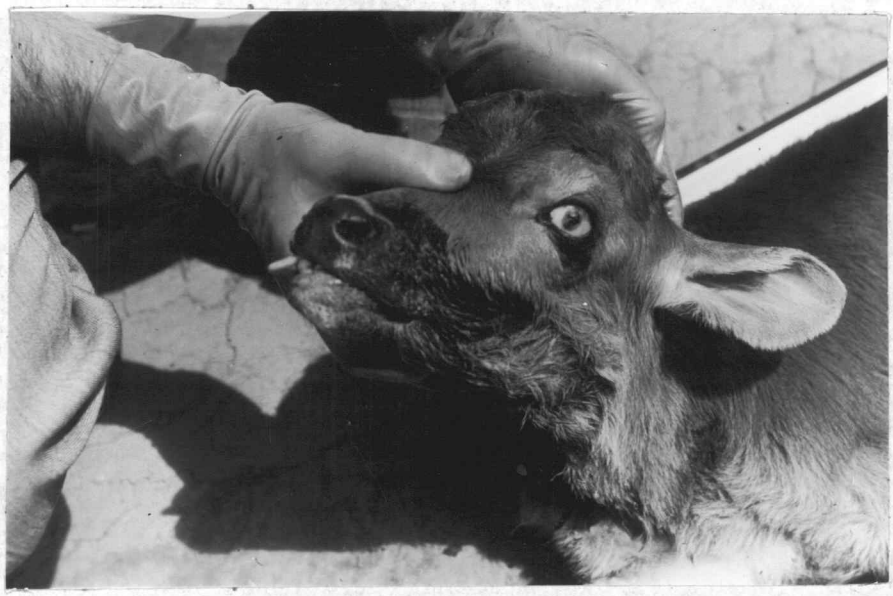
The full term calf of 253 was autopsied at two days of age. The pastern of the front legs were unable to straighten out and the left front leg had a twist at the ankle. Opthalmoscope examination of the eyes showed papilledema in an advanced state. All organs and glands appeared normal except the pituitary was small weighing one-half gram. The optic nerves were constricted at the optic foramen.

The 204 day aborted calf of cow 255 on post-mortem showed no clinical symptoms of any disorder.

Cow 267 aborted a live calf at 260 days which was weak and abnormal. The calf continually threw its head back over the spinal column, very similar to lethal spasms. The calf was unable to stand, the hocks were at 25 per cent greater than normal angle. An opthalmascope examination of the eyes showed restricted blood vessels and nerve fibers with evidence of papilledema. There was evidence of mottling of the tapetum lucidum. Autopsy at seven days showed constriction of the optic nerves at the optic foramen with all other glands and organs appearing normal.

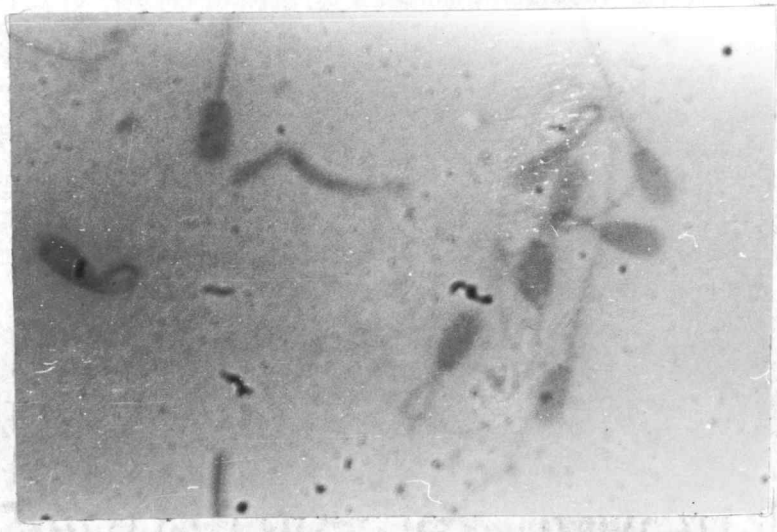
The two dead calves of cow 553 both were diagnosed as having

PLATE VII



Head of low carotene calf G-8
showing domed skull and corneal opacity

PLATE VIII



Semen of low carotene Holstein bull 500B
showing typical curled tails

died of strangulation during parturition. Calf 553B_1 showed constriction of the optic foramen and degeneration of the optic nerves.

LACTATION

Actual milk and fat production records of the low carotene and the control cows are given in Table VI. Figures 1, 2, 3 and 4 show the lactation curves of the low carotene cows compared with a normal curve.

Milk production of the low carotene group of cows was only from 25 to 50 per cent of what was expected and was little better when some of the cows were returned to a normal ration. As can be seen from Table VI and Figures 1, 2, 3 and 4, the lack of persistency of lactation was very noticeable with the cows on the low carotene ration.

Cow 250 in her second lactation was fed the low carotene hay but was supplied with sufficient alfalfa leaf meal to make her carotene intake 260 micrograms per kilogram of body weight. In spite of this increased carotene intake, milk production was 1000 pounds less than when she received only 90 micrograms of carotene per kilogram of body weight. A carotene intake of 260 micrograms per kilogram of body weight is equivalent to approximately 118 milligrams per 1000 pounds of body weight which is above the National Research Council recommendation (68, pp.1-30).

The blind cow, 251, in her first lactation produced the highest any of the Jerseys on the low carotene ration. Her second lactation was considerably below the first, but she had only a short dry period. In the first four months of her third and present lactation when she is receiving 130 micrograms of carotene per kilogram of body weight, as

TABLE VI

MILK AND BUTTERFAT PRODUCTION(Actual)

Cow (no.)	Age at Calving (yr.mo.)		Milk (lbs.)	Fat (lbs.)	Days	Ration
				Low Carotene Cows		
250	2	3	2,501.4	157.1	249	Low carotene
	4	5	1,571.4	116.9	125	Low carotene supplemented to 260 ug/kg bf body weight with alfalfa meal
251	2	6	3,970.7	266.7	330	Low carotene
	3	6	2,587.8	166.0	180	Low carotene
	4	9	2,072.7	141.2	112*	Low carotene
252	2	4	2,645.4	171.0	244	Low carotene
	3	3	3,123.2	178.0	212	Normal
253	2	2	3,251.6	202.6	289	Low carotene
	3	7				Sold--acute mastitis
255			Not milked			Aborted after 204 days gestation
266	1	11	1,186.5	64	114	Low carotene
	3	1	1,577.1	98.2	122	Normal
267	1	11	1,488.2	72.1	119	Low carotene
	3		4,355.4	296.4	271*	Normal first six months
553	3	5	6,186.7	258.7	340	Normal
	4	7	3,992.9	136.4	133*	Low carotene
557	3	6	8,256.9	359.1	330	Low carotene
	4	6	3,409.9	154.2	108*	Low carotene supplemented to 390 ug/kg of body weight with carrot oil

* Incomplete lactation

TABLE VI (Continued)

Cow (no.)	Age at Calving (yr.mo.)		Milk (lbs.)	Fat (lbs.)	Days	Ration
				Control Cows		
245	2	3	8,583	534	305	Normal
	4	8	8,404	593	305	Normal
246	2	3	6,271	386	305	Normal
	4	0	5,536	346	305	Normal
247	2	0	6,399	352	305	Normal
	3	0	6,073	355	274	Normal
	4	0	7,204	403	301	Normal
248	2	0	6,381	380	305	Normal
	3	2	6,818	412	383	Normal
	4	7	4,619	291	214*	Normal
543	2	3	12,745	496	305	Normal
	3	7	9,119	353	303	Normal
	4	6	10,929	447	305	Normal
544	2	2	12,161	481	305	Normal
	4	1	15,307	614	305	Normal
546	2	1	11,043	419	305	Normal
	3	2	7,210	259	238	Normal
	4	2	10,327	379	259	Normal
547	2	6	10,854	430	305	Normal
	4	1	13,017	535	305	Normal
548	2	0	13,788	557	305	Normal
	3	0	13,760	524	305	Normal

* Incomplete lactation

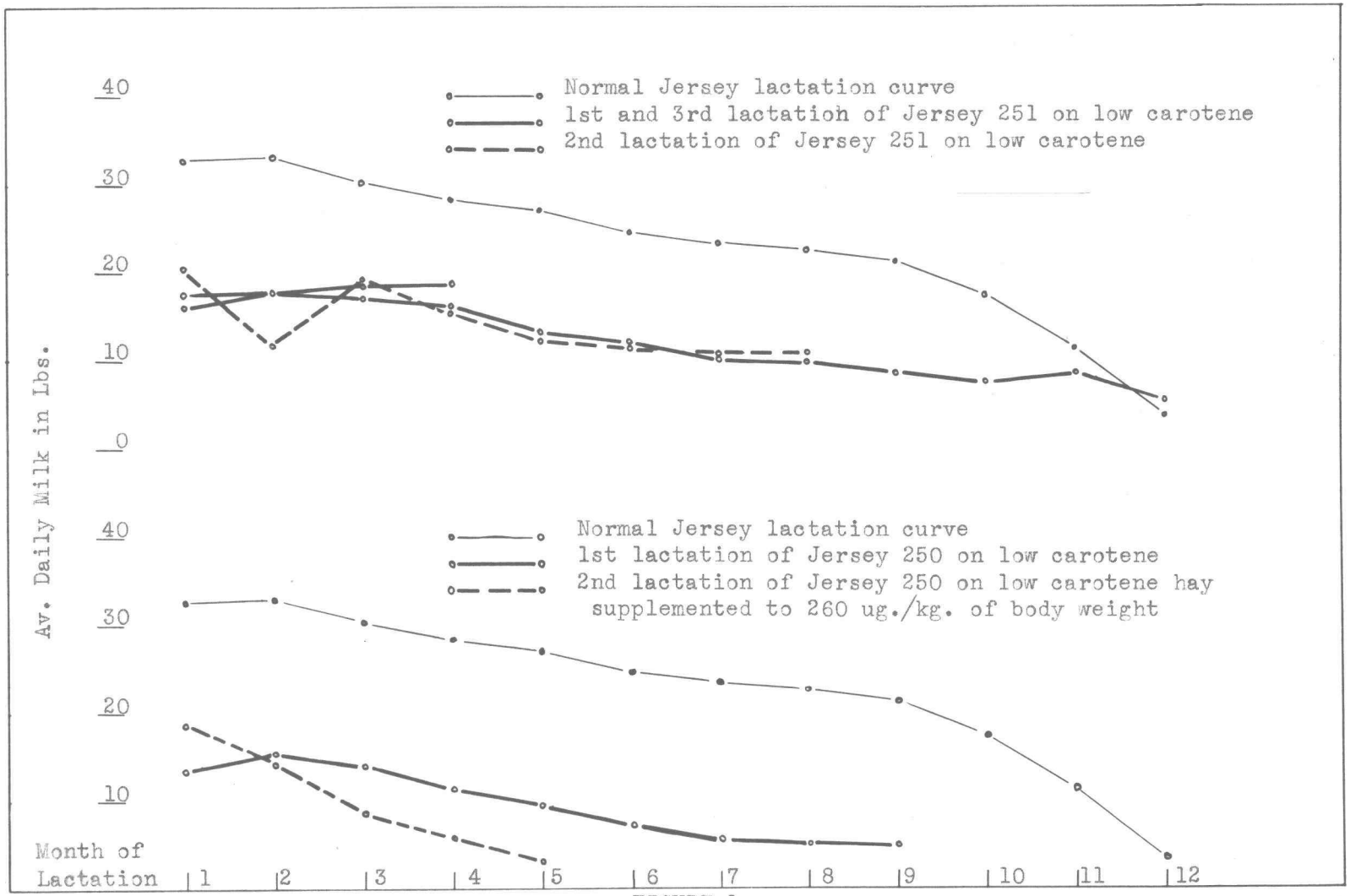

Normal Jersey lactation curve
1st and 3rd lactation of Jersey 251 on low carotene
2nd lactation of Jersey 251 on low carotene
Normal Jersey lactation curve
1st lactation of Jersey 250 on low carotene
2nd lactation of Jersey 250 on low carotene hay supplemented to 260 ug./kg. of body weight
Av. Daily Milk in Lbs.
40
30
20
10
0
40
30
20
10
Month of Lactation
1
2
3
4
5
6
7
8
9
10
11
12

FIGURE 1

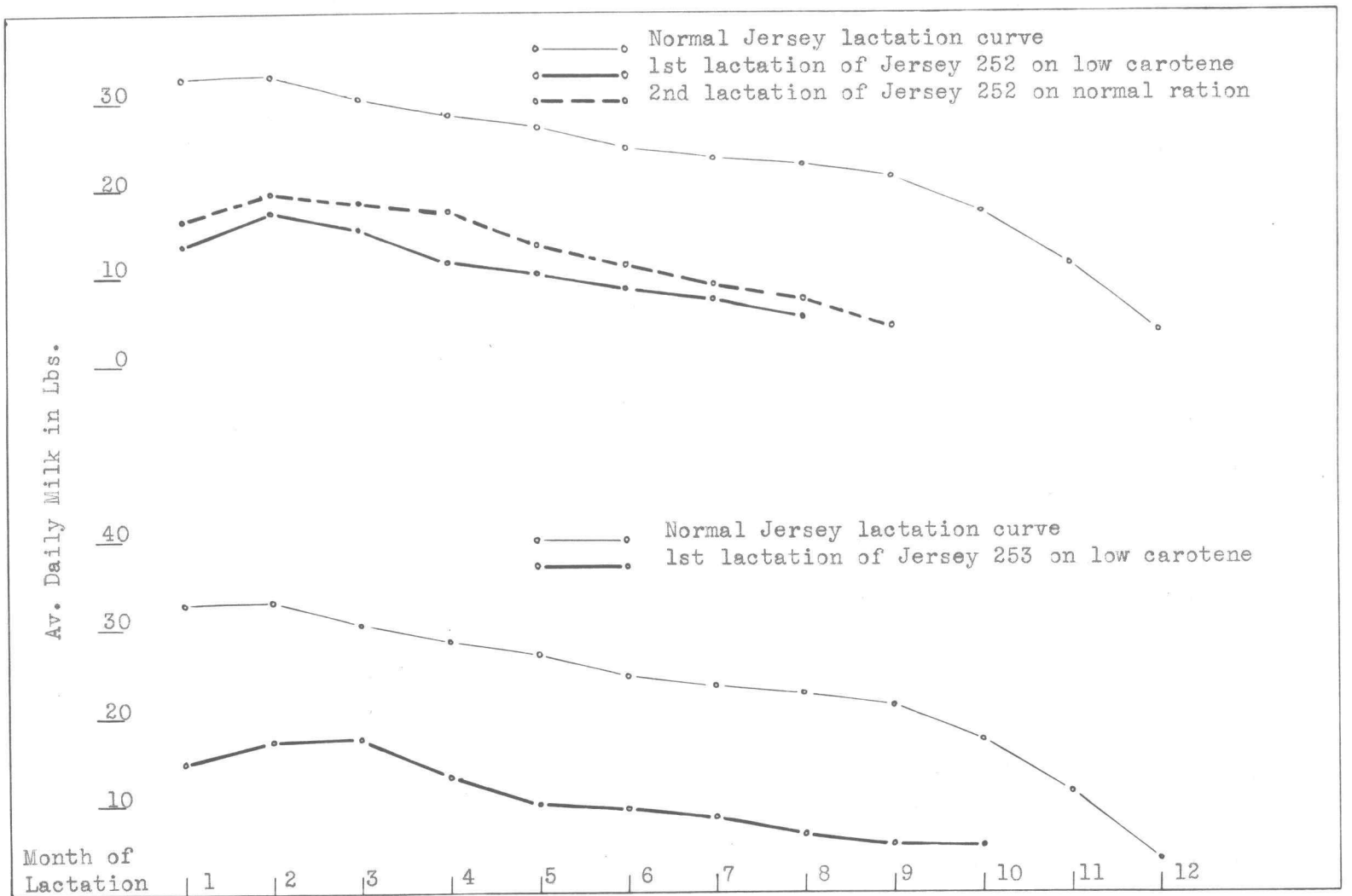

Normal Jersey lactation curve
1st lactation of Jersey 252 on low carotene
2nd lactation of Jersey 252 on normal ration
Normal Jersey lactation curve
1st lactation of Jersey 253 on low carotene
Av. Daily Milk in Lbs.
30
20
10
0
40
30
20
10
Month of Lactation
1
2
3
4
5
6
7
8
9
10
11
12

FIGURE 2

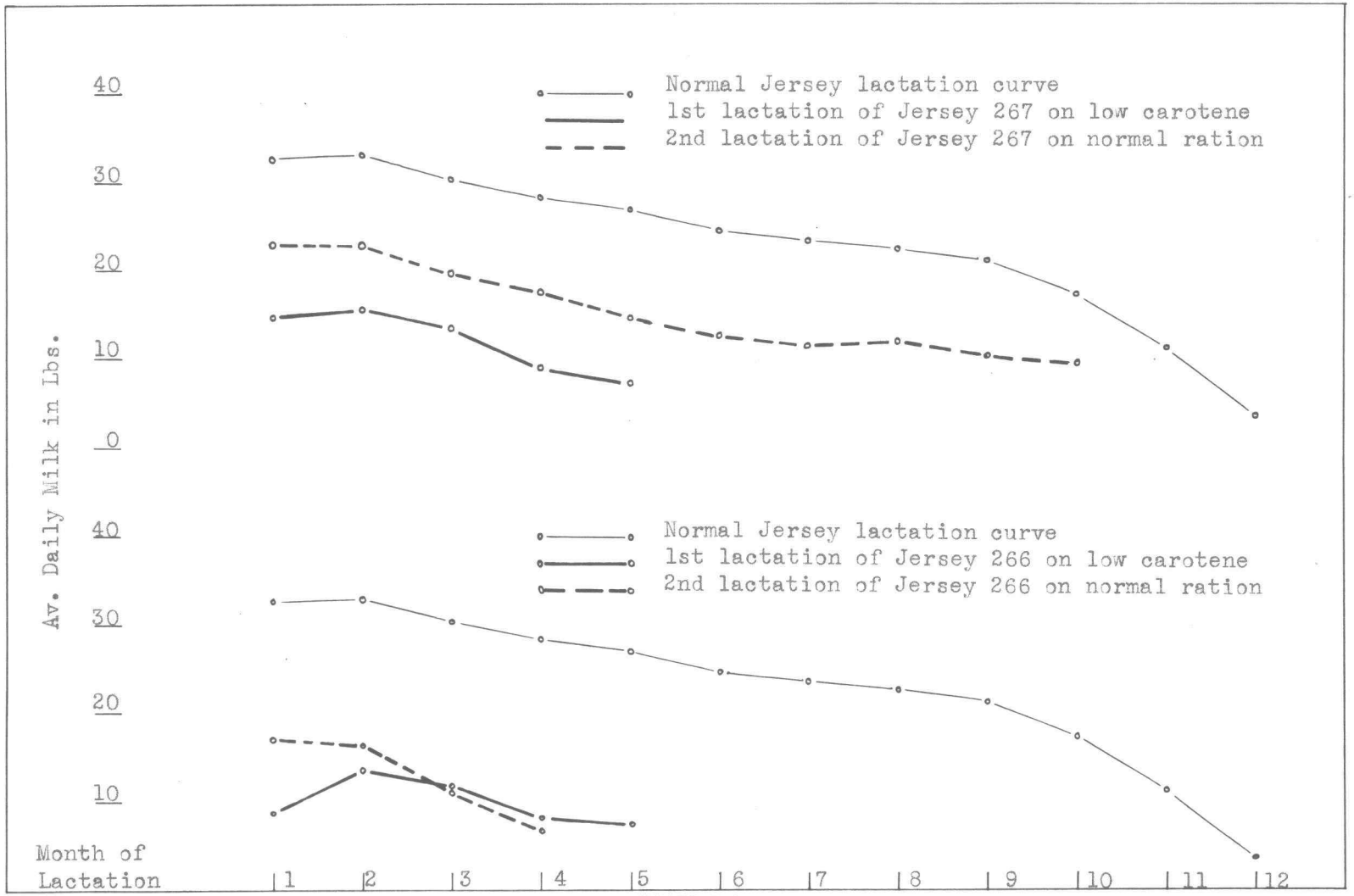

Normal Jersey lactation curve
1st lactation of Jersey 267 on low carotene
2nd lactation of Jersey 267 on normal ration
Normal Jersey lactation curve
1st lactation of Jersey 266 on low carotene
2nd lactation of Jersey 266 on normal ration
Av. Daily Milk in Lbs.
40
30
20
10
0
40
30
20
10
Month of Lactation
1
2
3
4
5
6
7
8
9
10
11
12

FIGURE 3

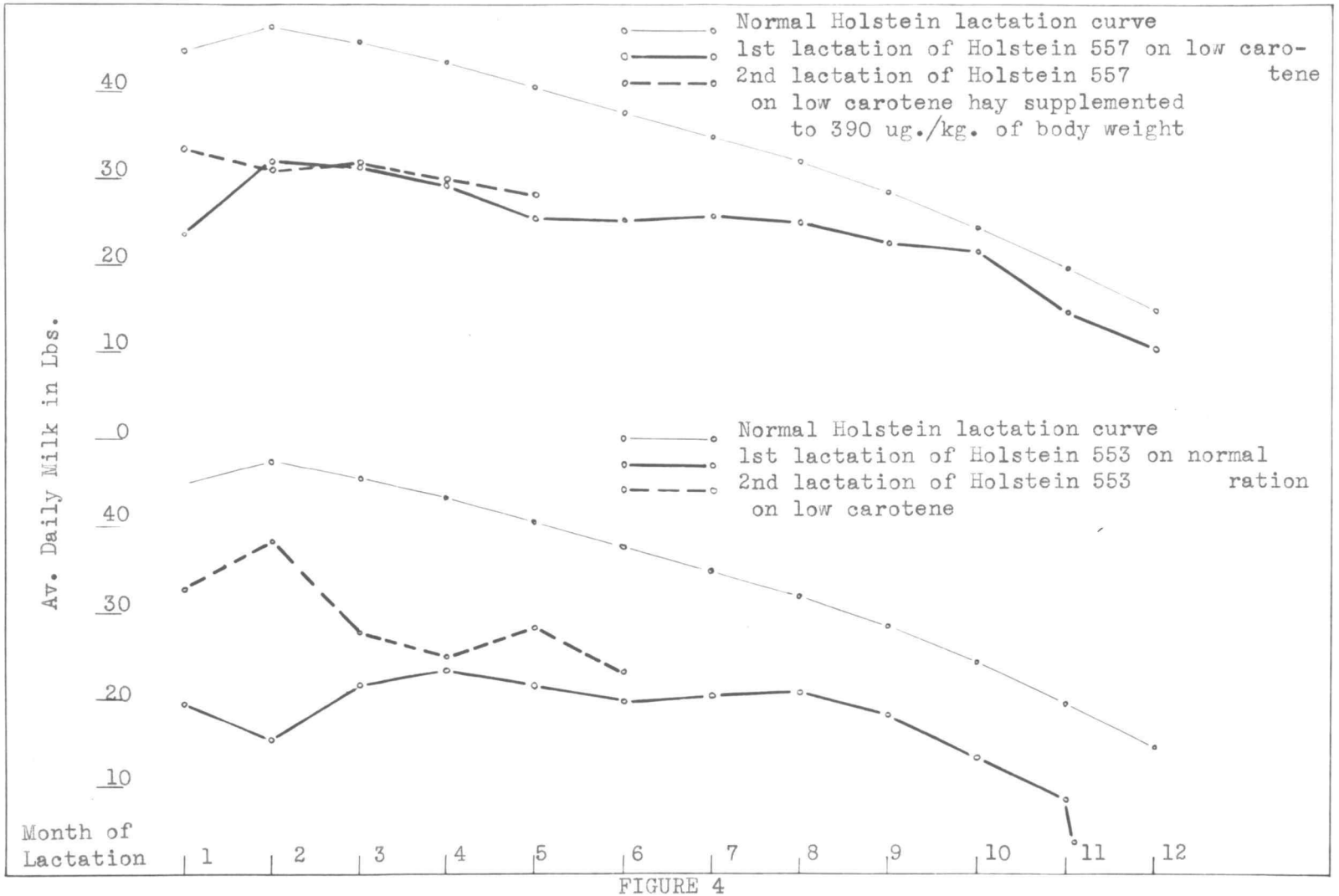

Normal Holstein lactation curve
1st lactation of Holstein 557 on low carotene
2nd lactation of Holstein 557 on low carotene hay supplemented to 390 ug./kg. of body weight
Normal Holstein lactation curve
1st lactation of Holstein 553 on normal ration
2nd lactation of Holstein 553 on low carotene
Av. Daily Milk in Lbs.
40
30
20
10
0
40
30
20
10
Month of Lactation
1
2
3
4
5
6
7
8
9
10
11
12

FIGURE 4

contrasted to her first lactation on 90 micrograms per kilogram of body weight, her milk production, as shown in Figure 1, is not any higher than during the previous lactations.

The first lactation of cow 252 was average for the low carotene cows and after her second calving, whe was placed on a normal ration of lush, irrigated pasture and grain. During this time she produced about 500 pounds more milk in about 30 less days. However, her milk production of 3,123 pounds in 212 days is only about 50 per cent of the expected.

Jersey cows 266 and 267 both calving at one year and 11 months of age on the low carotene ration produced respectively only 64 and 72 pounds of fat in the first lactation. This production is not even 25 per cent of the expected. In their second lactation on a normal ration beginning with the lush spring pasture, cow 266 produced only 98 pounds of fat, while cow 267 did considerably better, producing 296 pounds in 271 days.

Holstein cow 553 began her first lactation on a normal ration after three years and five months on the low carotene ration. For a Holstein cow, milk production of 6,186 pounds and fat production of 258 pounds in 340 days is low. In her second lactation cow 553 is milking at a higher level even though receiving the low carotene ration of 130 micrograms of carotene per kilogram of body weight.

Cow 557, whose dam did not receive a low carotene ration, had a fair record of 8,256 pounds of milk and 359 pounds of fat in 330 days on the low carotene diet of 130 micrograms per kilogram of body weight. When receiving 390 micrograms of carotene per kilogram of body weight,

however, her milk production in the second lactation for the first four months is no higher as shown in Figure 4.

Two factors were thought possibly responsible for the low milk production. The first was the level of carotene intake and the second the unpalatability of the ration. By placing cow 250 on a carotene intake of 260 micrograms of carotene per kilogram of body weight and cow 557 on an intake of 390 micrograms with no marked improvement in milk production, the factor of the level of carotene intake appears to have been eliminated. The returning of cows 252, 266, 267 and 553 to a normal ration of irrigated pasture or grass silage and alfalfa hay and grain with no marked improvement in milk and fat production, with the exception of cow 267, seems to have eliminated the unpalatability of the ration as a factor in the abnormally low milk production. As was the case of the blood carotene and vitamin A values failing to rise with the increased carotene intake, there appears some factor(s) of metabolism or utilization of the feed nutrients that has been interfered with as a result of the low carotene ration to the extent that the animals are unable to respond in milk and fat production to an adequate diet.

The milk and fat production of the control cows are well within the expected for Jersey and Holstein cows in the Oregon Agricultural Experiment Station herd.

BLOOD, LIVER AND MILK FAT ANALYSES

In an effort to further study the phenomena of the low carotene cows failing to respond in milk and fat production to the normal ration,

analyses of the blood, liver and milk fat were made when the cows were subjected to a variety of treatments.

Table VII presents liver analyses and some blood analyses of a group of animals under a variety of carotene intakes, while Table VIII presents the data for blood, liver and milk fat analyses together with the calculated vitamin A potency of the butter produced for a group of cows on a normal ration and of a group of low carotene cows receiving different amounts of carotene and vitamin A.

Table VII shows considerable variation in the liver values of cows on a normal ration. Cow 259 was slaughtered when on pasture and her liver had 108 micrograms of vitamin A per gram of fresh liver; while heifer 296 was being fed alfalfa hay and had a liver value of 34.8 micrograms of vitamin A per gram of fresh liver.

G-16 and G-17 receiving 390 micrograms of carotene per kilogram of body weight had liver values from biopsy specimens of about 13 micrograms of vitamin A per gram of fresh liver; whereas G-6, G-12 and G-13 receiving 130 micrograms of carotene per kilogram of body weight had liver values of about 10.5 micrograms of vitamin A per gram of fresh liver. The data again suggests that some factor(s) may be inhibiting the metabolism and/or utilization and storage of vitamin A in the low carotene animals, or it may be that 390 micrograms of carotene per kilogram of body weight is not sufficient carotene intake to maintain the liver values at nearer normal values.

Cow 266 on a normal ration of irrigated pasture, grass silage and alfalfa hay for six months had a liver value of 78 micrograms of vitamin A per gram of fresh liver and was not able to maintain this

TABLE VII

BLOOD AND LIVER ANALYSIS OF SOME NORMAL AND LOW CAROTENE ANIMALS

Animal	Date	Age	Ration	Blood		Liver		Remarks
				Carotene	Vit. A	Carotene	Vit. A	
no.		yr.mo.		p.p.m.		p.p.m.		
252	4-22-53	4 2	L.C.[a]--3 years, normal 1 year			0.99	52.6	Slaughter specimen
G-8		birth	Dam 252 on L.C.			-.35	-.20	" "
G-5		birth	Dam 267 on L.C.			-.75	-.26	" "
G-6	11-13-53	1 7	L.C.[a]			0.39	12.15	Biopsy specimen
	3- 8-54	1 11	L.C.	1.94	0.41	1.42	14.60	" "
	3-29-54	1 11	L.C.	1.25	0.19	0.65	10.95	" "
G-12	1-28-54	1 4	L.C.	1.37	0.38	1.95	12.1	" "
	3- 8-54	1 5	L.C.	1.56	0.27	2.44	9.80	" "
	3-29-54	1 6	L.C.	1.73	0.15	1.74	9.85	" "
G-16	1-25-54	7	L.C.[b]	1.56	0.34	3.18	13.70	" "
	3-22-54	9	L.C.	2.16	0.28	0.90	11.92	" "
G-17	1-28-54	7	L.C.	1.25	0.32	2.18	15.40	" "
	3-22-54	9	L.C.	1.18	0.22	0.28	13.20	" "
250	12-11-53	4 10	L.C. supplemented to 260 ug/kg			1.12	21.0	" "
	1-28-54	4 11	Grass silage only	2.56	0.23	14.88	26.4	" "
	3- 8-54	5 1	Grass silage only	6.90	0.38	6.64	40.3	" "
	3-10-54	5 1	Grass silage only			9.45	55.8	Slaughter specimen 8 hours postmortem
266	12-11-53	3 6	L.C.[a] normal last 6 months			8.50	78.2	Biopsy specimen
	1-28-54	3 8	Grass silage only	2.04	0.31	15.0	55.5	" "
	3-15-54	3 10	Grass silage only	7.22	0.45	7.61	10.35	" "
G-13	1-28-54	1 3	L.C.[a]	2.04	0.37	5.12	7.95	" "
	3- 8-54	1 4	L.C.	1.94	0.32	0.61	10.72	" "
	3-29-54	1 5	L.C.	1.37	0.18	0.21	12.55	" "
259	8-18-53	3 8	Normal			9.80	108.0	" "
295	3-29-54	2 1	Normal	3.07	0.24	4.40	40.9	Biopsy specimen
296	3-29-54	2 1	Normal	5.75	0.28	8.10	34.8	" "
605	3-29-54	1 3	Normal	1.94	0.10	1.42	48.7	" "

a. Low carotene ration supplying 130 micrograms carotene per kg of body weight

b. Low carotene hay + carrot oil to supply 390 micrograms carotene per kg of body weight

TABLE VII (Continued)

Animal no.	Date	Age yr.mo.	Ration	Blood Carotene p.p.m.	Blood Vit. A p.p.m.	Liver Carotene p.p.m.	Liver Vit. A p.p.m.	Remarks
242B$_3$	2-24-54	1	L.C.[a] milk	0.10	0.08	0.16	11.0	Biopsy specimen
	3-29-54	2	L.C. milk	0.65	0.13	0.17	3.87	" "
250B$_1$		birth	Dam 250 on L.C.[a]			-.55	-.25	Slaughter specimen 6 wks premature
251B$_2$		birth	Dam 251 on L.C.			-.33	-.26	Slaughter specimen
251B$_3$		birth	Dam 251 on L.C.			0	7.6	Aborted 6wks early
252B$_1$	11- 2-53	2 6	L.C.			0.92	17.3	Biopsy specimen
	1-28-54	2 8	L.C.	1.87	0.32	5.85	14.0	Biopsy specimen
	3-15-54	2 10	L.C.	1.68	0.19	1.50	14.2	" "
	3-17-54		L.C.			1.23	12.45	Slaughter specimen
200B$_1$	8-24-53	3 8	L.C.			1.03	9.05	" "
221B	4-28-53	3 11	L.C.			1.35	5.83	" "
523B$_5$	2-24-54	1	L.C. milk	0.03	0.03	0	4.05	Biopsy specimen
	3-19-54	2	L.C. Milk	0.03	0.02	0	1.30	" "
557B$_1$	11-13-53	3 days	L.C.			0	2.33	" "
	3-22-54	4	L.C.	0.14	0.10	0.27	0.96	" "
553B$_1$		birth	Dam 553 on L.C.			-.25	-.15	Slaughter specimen
503B	9- 2-53	3 9	L.C.			0.31	8.55	" "
TA1B$_1$	9- 1-53	3 days	Normal			0	17.30	Biopsy specimen
222B	9- 1-53	3 days	Normal			0	11.15	" "
283B$_1$	9- 1-53	3 days	Normal			0	11.80	" "
284B$_1$	9- 1-53	3 days	Normal			0	19.85	" "
29B	2-15-54	5 2	Normal L.C. last 6 months	3.64	0.26	8.58	15.45	Slaughter specimen
200B$_2$	1-28-54	3 1	Normal	3.84	0.26	16.0	33.1	Biopsy specimen
	3-22-54	3 4	Normal	3.67	0.28	6.77	27.60	" "
493B2	8-18-53	3	Normal			2.80	27.3	Slaughter specimen
493B$_3$	2-10-54	3 6	Normal L.C. last 6 months	2.52	0.27	3.87	26.9	" "

a. Low carotene ration supplying 130 micrograms of carotene per kilogram of body weight

TABLE VIII

BLOOD, LIVER AND MILK FAT ANALYSES OF SOME NORMAL AND LOW CAROTENE COWS

Animal	Date	Ration	Blood		Liver		Milk Fat		Vit A Potency Butter
			Carotene	Vit A	Carotene	Vit A	Carotene	Vit A	
			p.p.m.		p.p.m.		p.p.m.		I.U. per pound
			Low Carotene Cows						
251	11-18-53	L.C.[a]	1.35	0.06	1.57	9.15	0.60	1.58	2,663
	12-18-53	L.C.	1.75	0.23	1.05	8.91	0.60	2.00	3,267
	1-28-54	L.C.+50,000 I.U. Vit A/day	1.61	0.33	3.45	11.5	0.40	2.56	3,958
	2-24-54	L.C.	0.96	0.21	0.65	6.52	0.20	1.82	2,765
	3-22-54	L.C.+250,000 I.U. Vit A/day	0.67	0.29	0.70	7.25	0.10	2.18	3,224
267	11-28-53	Normal	7.20	1.04	13.60	70.60	4.40	3.96	8,410
	12-18-53	L.C.	2.66	0.53	5.00	67.5	0.80	3.34	5,332
	1-28-54	L.C.+50,000 I.U. Vit A/day	1.11	0.37	9.45	43.8	0.80	3.22	5,160
	2-24-54	L.C.	0.60	0.33	1.63	25.5	1.00	3.62	5,861
	3-22-54	L.C.+250,000 I.U. Vit A/day	0.24	0.23	0.96	21.8	0.20	3.04	4,533
553	12-18-53	Normal	2.86	0.31	1.25	112.5	0.40	5.28	7,910
	2-15-54	L.C.	1.85	0.30	1.41	44.7	0.40	4.24	6,398
	3-15-54	L.C.	1.44	0.25	0.63	63.5	1.00	5.60	8,737
557	11-13-53	L.C.[b]	-	-	1.38	15.15	-	-	-
	12-18-53	"	3.10	0.26	1.25	24.0	1.00	3.16	5,193
	2-15-54	"	4.80	0.31	6.50	40.6	1.00	3.32	5,425
	3-15-54	"	3.64	0.41	3.48	34.9	1.00	4.56	7,225
			Controls						
224	11-18-53	Normal	6.28	0.48	16.20	201.0	3.80	6.82	12,203
	12-18-53	L.C.[a]	2.33	0.29	7.04	182.0	1.40	4.52	7,411
	1-28-54	L.C.+50,000 I.U. Vit A/day	2.50	0.31	17.55	141.0	-	-	-
	2-24-54	L.C.	1.32	0.20	4.45	120.0	-	-	-
	3-22-54	L.C.+250,000 I.U. Vit A/day	0.65	0.21	3.58	92.7	-	-	-
218	3-8-54	Normal	8.18	0.40	12.20	134.1	7.60	6.64	14,239
	3-29-54	Normal	7.75	0.15	8.60	149.0	8.00	7.40	14,375
577	3-29-54	Alfalfa hay only	5.65	0.22	5.32	52.7	1.40	5.56	8,919
533	3-29-54	Grass silage only	8.35	0.12	9.17	162.0	2.60	7.78	12,869

a--Low carotene ration of 130 micrograms of carotene per kg/body weight

b--Low Carotene hay plus carrot oil to give 390 mgper kg/body weight

value in the liver when placed on a sole diet of grass silage supplying her with a daily intake of approximately 200 milligrams of carotene per day. On the other hand, cow 250 who had been receiving 260 micrograms of carotene per kilogram of body weight had a liver value of 21 micrograms of vitamin A per gram of fresh liver and when fed grass silage only for three months had a liver value of 55.8 micrograms of vitamin A per gram of fresh liver.

Table VII which gives some blood and liver values of bull calves suggests that the low carotene ration supplying 130 micrograms of carotene per kilogram of body weight to the dam is not satisfactory in maintaining the vitamin A of the liver of the calf near the level of normal calves biopsied at three days of age. Normal calves show liver values of approximately 14 micrograms of vitamin A per gram of fresh liver, while a calf from a low carotene cow had a liver value of 2.33 micrograms at three days. Control bulls of two to five years of age being fed good quality alfalfa hay had low liver values of approximately 29 micrograms of vitamin A per gram of fresh liver, while low carotene bulls of a comparable age receiving 130 micrograms of carotene per kilogram of body weight had liver values of approximately 11 micrograms per gram of fresh liver. These results would appear to suggest the inadequacy of good quality alfalfa hay in maintaining liver vitamin A at the more normal values of animals receiving grass silage or irrigated pasture.

The data presented in Table VIII suggests a number of interesting phenomena. When cow 251, raised and maintained on the low carotene ration, was injected intravenously every five days with the equivalent

of 50,000 I.U. of vitamin A per day for a month and later with 250,000 I.U. of vitamin A per day, the liver stores of vitamin or the milk-fat potency of vitamin A were not significantly increased. Cow 267 that had been on a normal ration with a liver value of 70 micrograms of vitamin A per gram of fresh liver and a butter value of 8,410 I.U. per pound was not able to maintain these values when receiving the equivalent of 50,000 I.U. or 250,000 I.U. of vitamin A per day. After a month of receiving the equivalent of 250,000 I.U. of vitamin A per day, the butter of 267 had dropped to a vitamin A potency of 4,533 I.U. A similar phenomenon existed with cow 224 that had been raised and maintained on a normal ration.

These data suggest that 250,000 I.U. of vitamin A per day injected every five days is not sufficient to maintain the liver values of vitamin A when the liver values at the beginning of the injections are in the vicinity of 70 micrograms of vitamin A per gram of fresh liver or higher.

Cow 553 which had been on a normal ration but raised to her first calving on the low carotene ration was not able to maintain her liver vitamin A value when consuming 130 micrograms of carotene per kilogram of body weight, but did maintain her milk fat values indicating that the cow was drawing on her liver stores of vitamin A to maintain the milk fat potency. Cow 557, beginning her second lactation on 390 micrograms of carotene per kilogram of body weight after completing her first lactation on 130 micrograms of carotene per kilogram, was able to slowly raise her liver value of vitamin A from a low of 15.15 micrograms of vitamin A per gram of fresh liver to 34.9 micrograms and

her butter vitamin A potency from 5,193 I.U. per pound to 7,225 I.U. of vitamin A per pound.

Cow 577, raised and maintained on a normal ration but for approximately eight weeks prior to the liver and milk fat analyses, fed a sole diet of alfalfa hay and grain had a liver value of 52.7 micrograms of vitamin A per gram of fresh liver and a butter potency of 8,919 I.U. of vitamin A per pound; while cow 533, raised on a normal ration but for the eight weeks prior to the analyses, fed only grass silage and grain had a liver value of 162 micrograms of vitamin A per gram of fresh liver and a butter potency of 12,869 I.U. per pound indicating once more the apparent inadequacy of good quality alfalfa hay in maintaining the vitamin A liver value of dairy cattle at a high level.

The vitamin A potency of the butter of the normal cows is average for Oregon for the month of March; while that of the low carotene cows is considerably below average and even the butterfat of cow 557 receiving 390 micrograms of carotene per kilogram of body weight is a low potency butterfat.

Comparing cows 267 and 224 at the first liver analysis lends support to the thought that blood carotene and vitamin A values must be used with considerable caution as a measure of the vitamin A status of the animal. Cow 267 had blood values of 7.2 p.p.m. carotene and 1.04 p.p.m. vitamin A, yet the liver showed only 13.6 p.p.m. carotene and 70.6 p.p.m. of vitamin A; while cow 224 had blood values of 6.28 p.p.m. carotene and 0.48 p.p.m. vitamin A, but had liver values of 16.2 p.p.m. carotene and 201.0 p.p.m. vitamin A.

SEXUAL PERFORMANCE AND SEMEN QUALITY

It was observed when the low carotene bulls were approaching puberty, they had the desire and ability to mount either a live cow or the dummy cow but were not able to get an erection of the penis. They performed in this manner for a period of about six weeks. The first ejaculate was obtained from the low carotene bulls from four to eight weeks later than from the control bulls. A consistent characteristic of the low carotene bulls was the delay in ejaculating. A mount of either a live cow or the dummy cow would be made in a reasonable time and an erection produced. After insertion of the penis in the artificial vagina, considerable time would elapse before ejaculation. Frequently the bull would rest on the cow with his penis in the vagina and there would be no activity. This performance might be repeated several times before the ejaculation would be produced. Bulls $200B_1$, 221B and $252B_1$ were particularly guilty of this characteristic performance. On the other hand bull 500B did not show this behavior until he was four years old. In spite of this bull being blind his sexual libido was until he reached four years of age excellent.

All bulls on the low carotene ration as they approached three years of age began to lose libido and before bulls $200B_1$ and $252B_1$ were two and one-half years of age, they refused to mount the dummy cow. On the other hand bull $200B_2$ in the controls never would ejaculate on a live cow, but would mount and ejaculate on the dummy cow. A number of the bulls would operate on either the live or dummy cow. It was observed, however, that the quantity and quality of the semen was

generally superior when it was collected off the live cow.

Bull 503B had some obstruction or disorder of the respiratory system which became more severe as the bull matured. Towards the end of his life the condition was so severe that it interfered with his ability to mount. Bull $252B_1$ frequently, on exercising, as just prior to serving, would go into a coma, drop to the floor and be unaware of his surroundings for about two minutes. On recovery he would be quite capable of serving.

Inasmuch as the carotene intake of the bulls on the low carotene ration was changed at the same calendar periods as the females, the semen data, as presented in Table IX, is grouped as closely as possible into four periods according to the carotene intake and age. Thus period 1 approximates one and one-half years of age and 50 micrograms of carotene per kilogram of body weight. Period 2 represents two and one-half years and 90 micrograms of carotene; period 3 represents three and one half years and 130 micrograms of carotene per kilogram of body weight. Period 4 represents the same carotene intake as period 3, but the bulls are four and one-half years old.

An observed characteristic of the semen of all the bulls on the low carotene ration was that the color appeared a grayish white as contrasted to the creamy color of the control bulls. Secondly, the amount of seminal plasma released prior to the true ejaculate appeared greater with the low carotene bulls. Under the microscope the raw semen of the low carotene bulls showed high percentages of tissue debris.

The differences between the two groups of bulls with respect to the various semen quality tests were not at any time great with the

TABLE IX

SEMEN DATA

Bull (no.)	Volume (mls.)	Total Sperm (mills/ml.)	Abnormal Sperm (%)	Live Sperm (%)	0 Hour Motility (%)	168 Hour Motility (%)
			Period 1--Controls			
29B	1.8	910	26.0		60.7	12.7
$200B_2$	4.7	847	21.1	69.8	54.7	15.6
$212B_3$	4.3	541	36.4	60.2	61.4	19.6
$264B_2$	2.2	763	15.1	69.2	70.0	22.7
$493B_2$	4.4	632	26.0	74.2	76.2	27.8
$493B_3$	5.2	696	21.0	75.7	70.9	28.1
$506B_2$	3.2	536	19.1	68.3	63.8	23.3
Average	3.7	703	23.5	69.5	65.3	21.4
			Period 1--Low Carotene			
496B	2.6	721	45.0		57.1	23.1
503B	4.3	666	23.4	68.4	43.1	21.5
221B	2.5	766	10.1	44.6	71.9	29.7
$200B_1$	2.4	936	16.6	69.5	61.0	29.1
$252B_1$	2.2	1,148	14.0	82.6	62.8	29.2
500B	4.5	841	43.7		53.1	30.8
218B	1.5	571	40.0		83.5	17.1
Average	2.8	807	27.5	66.3	61.8	25.8
			Period 2--Controls			
29B	5.0	1,004	22.0	75.3	73.8	24.2
$200B_2$	3.4	1,007	23.2	82.7	66.3	25.7
$212B_3$	4.6	1,022	18.5	73.9	59.3	18.7
$264B_2$	4.3	734	22.6	71.5	70.0	25.4
$493B_2$	5.2	1,220	17.9	77.1	74.8	33.0
$493B_3$	5.4	1,060	16.0	87.9	70.6	32.7
$506B_2$	3.8	721	18.6	69.2	72.6	28.4
Average	4.5	967	19.8	76.8	69.6	26.9

TABLE IX (Continued)

Bull (no.)	Volume (Mls.)	Total Sperm (mils/ml.)	Abnormal Sperm (%)	Live Sperm (%)	OHour Motility (%)	168 Hour Motility (%)
			Period 2--Low Carotene			
503B	5.7	1,220	14.9	80.4	79.4	32.2
221B	4.1	574	25.2	71.0	61.5	24.6
$200B_1$	4.7	953	18.9	77.4	73.2	32.5
$252B_1$	3.5	897	37.3	78.7	55.9	17.1
500B	5.2	442	34.1	59.4	45.3	12.7
Average	4.6	870	26.1	73.4	63.0	23.9
			Period 3--Controls			
29B	4.0	1,176	24.8	85.3	73.8	26.5
$200B_2$	3.9	884	41.6	75.6	54.6	18.6
$264B_2$	3.8	1,109	26.6	78.1	61.1	23.3
$493B_2$	5.9	1,448	20.8	89.4	73.8	25.9
$493B_3$	5.8	1,097	22.1	90.4	65.3	26.4
Average	4.7	1,203	27.1	83.7	65.7	24.1
			Period 3--Low Carotene			
503B	6.2	1,496	19.9	82.6	76.8	31.1
221B	4.6	1,087	25.9	82.9	68.1	33.9
$200B_1$	3.9	1,519	21.0	85.7	71.2	26.0
500B	8.2	757	36.0	77.0	35.4	10.0
Average	5.7	1,215	25.7	82.0	62.8	25.2
			Period 4--Controls			
29B	3.9	1,478	39.7	87.5	72.3	25.9
			Period 4--Low Carotene			
500B	6.5	755	36.1	78.3	34.8	13.6

possible exception of period four. In this period, however, only two bulls are compared. There appears in general a trend favoring the control group. In period 1 there is a greater number of abnormal spermatozoa and a lower percentage of live sperm in the low carotene group. In period 2 the control group has superior semen in all respects. In period 3, on the other hand, the control group surpasses the low carotene group in only the per cent live spermatozoa and 0 hour motility.

Bull 500B produced a large volume of semen at all times, but never of very high concentration. The semen usually contained a large number of abnormal spermatozoa. The most common abnormality was tails curled back over the head; see Plate VIII, page 51. Motility of the semen of bull 500B was never very high in spite of maintaining a reasonable live spermatozoa percentage.

Bulls $200B_1$ and 503B produced high quality semen for the majority of the time they were under observation. The volume was low in the case of $200B_1$, but the concentration, abnormality, live sperm and motility was equal to the average for normal bulls and frequently above the normal. Bull 503B generally produced excellent semen in all respects.

The semen of bull 221B resembled that of bull 500B, but not quite as abnormal. Bull $252B_1$ was quite erratic in the quality of semen production. On occasion his semen would rate excellent; while frequently it would rate quite poorly. Bulls 496B and 218B were slaughtered before trends in quality of semen could be established.

Examination of Table IX shows only slight differences in the quality of semen between the groups for the respective periods.

Although the bulls have been grouped as closely as possible by ages, there remains considerable range in age of the animals within a period that could account for the wide variability in the semen quality within a group and the lack of marked differences between the groups within a period. The data do show the semen quality of the low carotene animals dropping as the animals advance from period 2 to period 3. The individual animals within the group, however, did not all respond in the same manner. Bull 221B, for example, improved in motility both at zero hours and after 168 hours storage. Concentration improved in period 3 over period 2 in all cases with the low carotene bulls.

An additional test of semen quality used was the oxygen consumption of the semen as measured with a Warburg respirometer. Because of the time involved in determining oxygen consumption only a limited number of samples of semen were checked. The results for these samples are presented in Table X in which the data is presented on the basis of oxygen consumption per hour per 100 million live sperm and per milliliter of raw semen.

The differences between the control group and the low carotene group in oxygen consumption per milliliter of semen and per 100 million live sperm are not statistically significant. The fact that the low carotene bulls on the average respired at a higher rate than the control bulls may be due to the stress under which the spermatozoa are metabolizing due to the deficiency of the carotene which is known to influence spermatogenesis. Bull 503B respired at a rate far in excess of the other animals which agrees with other semen quality tests in which he was superior to the other low carotene bulls. The

TABLE X

SEMEN RESPIRATION

Bull (no.)	Samples (no.)	Microliters of O_2 per Semen (milliliter)	Microliters of O_2 per Live Sperm (100 million)
		Control Bulls	
29B	32	75.0	5.9
$200B_2$	23	43.4	6.5
$212B_3$	21	26.1	3.4
$264B_2$	18	46.8	5.4
Average	94	47.8	5.1
		Low Carotene Bulls	
200B	14	46.3	3.5
$221B_1$	12	35.3	3.9
$252B_1$	17	36.6	5.2
500B	14	44.9	7.7
503B	13	130.6	10.6
Average	70	58.7	5.9

stress under which he operated probably was quite severe since his pituitary gland was so cystic on autopsy. Wide variations in the respiration rate were noted frequently depending on whether the bull was collected off the dummy cow or a live animal.

BREEDING EFFICIENCY

A limited number of cows were available to check the fertility of the low carotene and control bulls. All cows were bred artificially with the exception of 44 services in the case of bull 29B. Semen in egg-yolk citrate diluter fortified with streptomycin and penicillin was never more than 72 hours old. In calculating the efficiency of the bull, the cow's condition was disregarded. All services were used in the calculations. The efficiency of the control and low carotene groups is presented in Table XI. In the low carotene group, bull 503B had a very creditable breeding record; while in the control group of bulls, $212B_3$ and $506B_2$ were rather poor in breeding efficiency. The difference in efficiency between the two groups is statistically significant.

Bulls 218B and 496B were slaughtered before they were old enough to have had many services. When he was four years old, bull 500B was not bred to many cows since his semen was of such low quality and his efficiency so low. This was also true of bull 221B.

In the control group, bull 506B was only about a year old when the majority of his breedings were made which may account for his low efficiency. $212B_3$ on the other hand was used mostly when he was about 18 months of age or older. Bull 29B was bred to 44 cows in natural

TABLE XI

BREEDING EFFICIENCY OF BULLS

Animal	Cows Bred	Services	Pregnancies	Efficiency
(no.)	(no.)	(no.)	(no.)	(%)
		Low Carotene Animals		
$200B_1$	31	45	14	31.1
218B	7	11	5	45.4
$221B_1$	29	42	11	26.2
$252B_1$	19	21	9	42.8
496B	3	5	0	0
500B	19	30	6	20.0
503B	48	52	25	48.1
Total	156	206	70	33.98
		Control Animals		
29B	81	128	69	53.9
$200B_2$	17	26	11	42.3
$212B_3$	23	27	10	37.0
$264B_2$	17	21	12	57.1
$493B_2$	51	81	45	55.5
$506B_2$	15	15	5	33.3
$493B_3$	75	124	65	52.4
Total	279	422	217	51.4

service and had an efficiency of 1.69 services per conception after he had been biopsied in the right testicle. Although the data presented in Table XI are not conclusive, they do at least indicate the bulls were not sterile. Bull 496B was not bred to sufficient cows to pronounce him sterile.

FEED UTILIZATION AND TISSUE RESPIRATION

Observing during the course of the study that animals on a low carotene ration when returned to a normal ration did not respond in milk production to a diet of irrigated pasture, grass silage and alfalfa hay, and when added quantities of either carotene or vitamin A were administered that the blood, liver and milk-fat carotene and vitamin A values did not increase proportionately, two digestion trials and a series of tissue respirometer studies were conducted with low carotene and control animals.

The first digestion trial was run with a pair of identical twin Jersey cows that were milking and had been on a normal ration all their lives, Holstein cow 553 who was late in her first lactation on a normal ration and Jersey cow 252 who was dry after her second lactation which was on the normal ration. The feed used in the first trial was alfalfa-grass silage (50 per cent alfalfa) preserved with 100 pounds of dried molasses beet pulp per ton of green material ensiled. Table XII gives the dry matter consumed and the digestion coefficients of the various ingredients together with the digestible protein and total digestible nutrients on the "as fed" basis.

Averaging the two controls and the two low carotene animals it

TABLE XII

DIGESTION COEFFICIENTS

Animal (no.)	Dry Matter Consumed (lbs.)	Dry Matter (%)	Crude Protein (%)	Fat (%)	Fiber (%)	NFE (%)	As Fed D.P. (%)	As Fed TDN (%)
			Alfalfa-Grass Silage (100# beet pulp)					
TG1	76.3	65.7	57.3	56.5	64.3	63.9	1.34	17.12
TG2	76.3	65.3	54.4	64.1	66.5	60.3	1.27	16.84
252	64.9	66.4	50.4	65.0	68.1	71.0	1.18	18.51
553	133.4	66.4	52.8	67.8	69.9	69.5	1.59	18.85
Average Control		65.5	55.8	60.2	65.4	62.1	1.35	17.83
Low Carotene		66.4	51.6	66.4	69.2	70.2	1.30	16.98
			Low Carotene Alfalfa, Tualatin Grass Hay					
TL1	50.7	56.9	60.8	22.0	54.2	65.5	7.1	52.7
TL2	52.1	57.3	60.1	26.2	54.8	64.4	7.1	52.4
250	60.2	56.2	61.7	25.3	56.2	62.5	7.3	52.3
266	49.5	53.3	57.4	23.8	52.5	60.8	6.3	47.4
Average Control		57.1	60.5	24.1	54.5	64.9	7.1	52.5
Low Carotene		53.3	57.4	23.8	52.5	60.8	6.8	49.8

is found that the coefficient of digestibility of the dry matter is nearly the same and the digestible protein and total digestible nutrients on the "as fed" basis differ only slightly. There is considerable difference, however, in the coefficients of digestibility of the fat, fiber and nitrogen free-extract in favor of the low carotene cows; while protein is digested by the control group more efficiently.

In the second digestion trial the low carotene hay of 1953, alfalfa and Tualatin oat grass, was used. Two dry identical twin Jersey heifers, TL1 and TL2, were used as controls; Jersey cow 250 that had never been on a normal ration and Jersey cow 266 that had been on the low carotene ration until her second lactation and then placed on a normal ration were the low carotene animals. After six months on the normal ration cow 266 was returned to the low carotene ration for three weeks prior to being used in the digestion trial. The data for the second digestion trial are also presented in Table XII. Cow 250 digested the dry matter as efficiently as the control heifers; while cow 266 did not. Digestible protein and total digestible nutrients on the "as fed" basis were the same for TL1, TL2 and 250, but were lower for cow 266. The lower digestion coefficients of all factors for the low carotene group are a result of the low values for cow 266.

Comparing the data from these two trials and for these two feeds, it may be said that there is no significant difference in the efficiency of digestion between the low carotene animals of this study and normal animals.

Since metabolism of tissues may be measured by oxygen consumption of the tissues and metabolism is a function of feed utilization,

selected tissues from five low carotene animals and two control animals were used in a Warburg respirometer to measure oxygen uptake. The tissues and the oxygen consumed per milligram of dry tissue are presented in Table XIII.

The tissues at the time of slaughter were placed in Ringers-phosphate solution and brought to the laboratory and within one hour of slaughtering oxygen uptake was being measured. The differences in tissue respiration do not appear to be great with the exception of the small intestine which showed a real difference in favor of the control animals. Also shown in Table XIII is the indication that the respiration of tissue of the testicle at the biopsy site was not materially less than from other areas of the testicle.

HISTOLOGICAL FINDINGS

All the bulls on the low carotene ration were slaughtered and the tissues of the pituitary, thyroid, adrenal and testicles were fixed in Bouin's solution within 30 minutes of killing. After imbedding, slicing and staining with Harris Hematoxylin, the tissues were examined for normality. Low carotene bull $557B_1$ was slaughtered at four months of age, $267B_1$ at ten months of age and bulls 218B and 496B were slaughtered at 16 and 18 months of age, respectively. The other bulls on the low carotene ration were slaughtered when they were two and one-half years old or older.

The tissues of bulls 218B and 496B were essentially normal with the exception that the testicles showed slightly reduced spermatogensis for young bulls and the pituitary gland of 496B showed evidence of

TABLE XIII

TISSUE QO_2 VALUES
(Per mg. of Dry Tissue)

Animal no.	Intestine at Jejunum	Liver	Pituitary	Left Adrenal	Right Adrenal	Left Testicle	Right Testicle	Right Testicle Biopsy Site	Left Testicle Biopsy Site	Ration
503B	1.68	0.73	0.97	1.34	1.04	2.12	1.02			Low Carotene
$200B_1$	0.49	0.44	0.95	1.23	1.06	1.24	1.23	0.50		Low Carotene
500B	1.40	0.75				0.94	0.83	0.83	0.74	Low Carotene
221B	0.93	0.70				1.25	1.48	1.53		Low Carotene
252	1.53	1.19								Low Carotene
Go.	4.31	0.72				0.93	1.40		0.97	Control
$493B_2$	5.79	0.70	0.34	0.48	0.91	1.09	1.66			Control

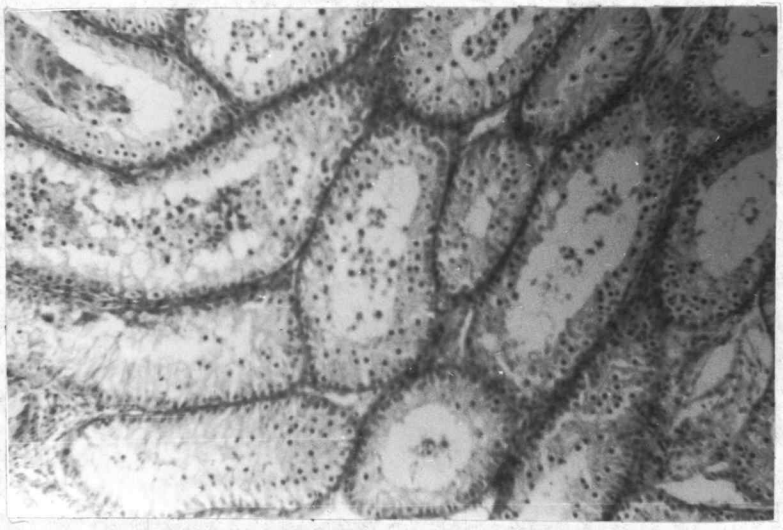
multiple small cysts of 10-20 microns in diameter in the pars nervosa.

Bull 500B, which went blind at 16 months of age, on slaughter at four and one-half years of age showed in both testicles the interstitial tissue reduced to strips of fibrin with the cells of Leydig reduced in number and those that remained showing evidence of nuclei degeneration. The basement membrane of the seminiferous tubules appeared thickened with the Sertoli cells showing slight degeneration. There was only mild spermatogenesis. Plates IX, X and XI show a degeneration of the testicle of 500B. Plate IX shows the testicle biopsy at 16 months of age. Plate X shows the slaughter specimen of the testicle at four and one-half years. Plate XI shows the testicle at the biopsy site.

It is apparent that not too much damage could have resulted from the biopsies since Plate XI of the biopsy site is very little different from the slaughter specimen which was taken far removed from the small fibrous area of the biopsy. The pituitary gland of bull 500B was not available for examination since it was destroyed by the bullet used to kill the animal. The adrenal glands showed a condition similar to that shown in Plate XII, although not as severe. Interpenetration of medulla and the cortex by each other was evident. There was slight evidence of mucoid degeneration of the glomerulosa with a slight pyknosis of the cells.

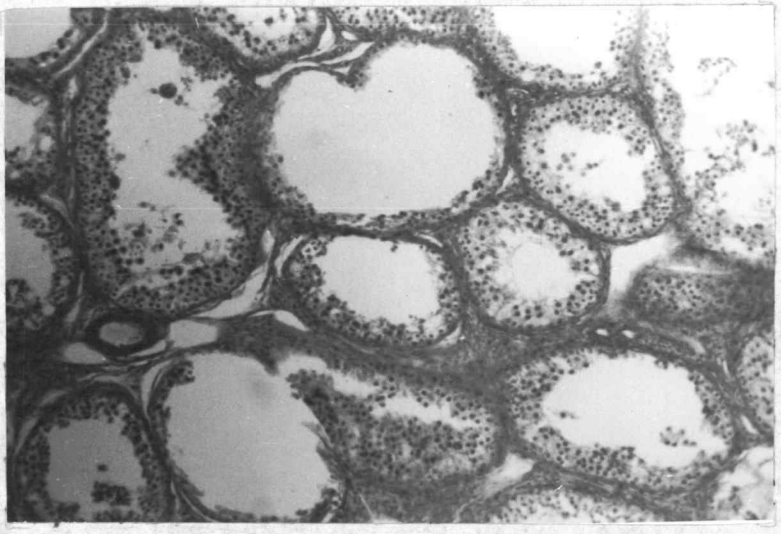
Bull 221B was slaughtered at about four years of age and on macroscopic examination appeared normal. Microscopic examination of the testicles revealed a similar condition to that found in bull 500B, though not quite as severe. The interstitial area appeared normal.

PLATE IX



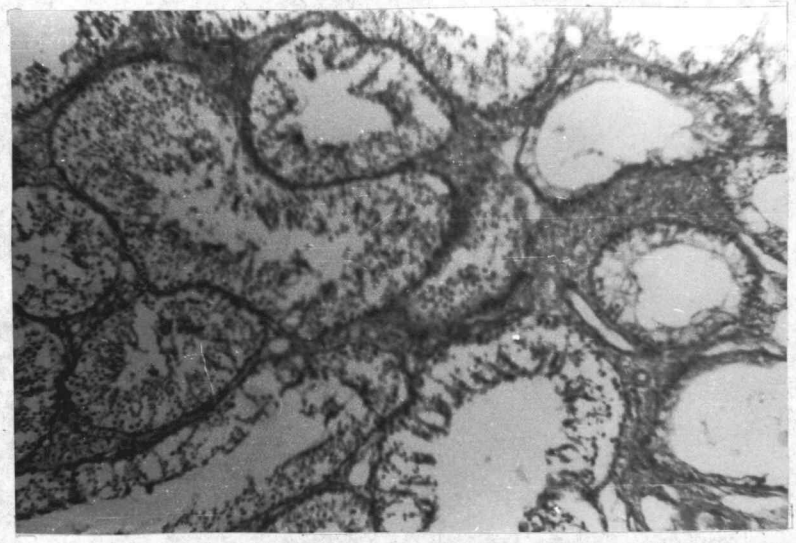
Biopsy specimen of left testicle of low carotene Holstein bull 500B at 16 months of age

PLATE X



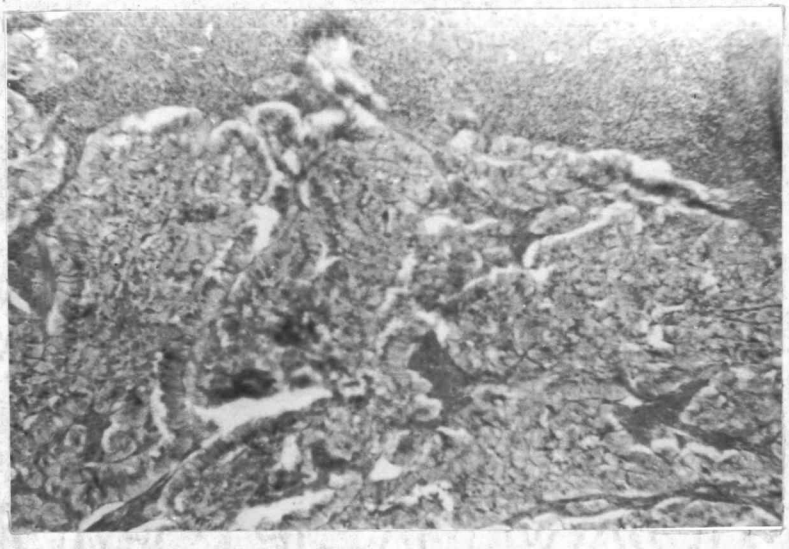
Slaughter specimen of left testicle of low carotene Holstein bull 500B at $4\frac{1}{2}$ years of age

PLATE XI



Biopsy site of left testicle of low carotene
Holstein bull 500B at time of slaughter

PLATE XII



Left adrenal gland of low carotene
Jersey bull 221B

The adrenal glands of 221B were abnormal, as shown in Plate XII. The capsule appeared thickened. The glomerulosa showed mucoid degeneration with pyknotic cells. The reticularis appeared normal with slight mononuclear infiltration from the medulla towards the periphery of the gland. The zona fasiculatis was reduced in size to being absent in areas. The cells of the medulla showed evidence of pyknosis and mucoid degeneration. The pituitary gland of 221B was essentially normal with evidence of minute cysts in the pars nervosa and pars distalis.

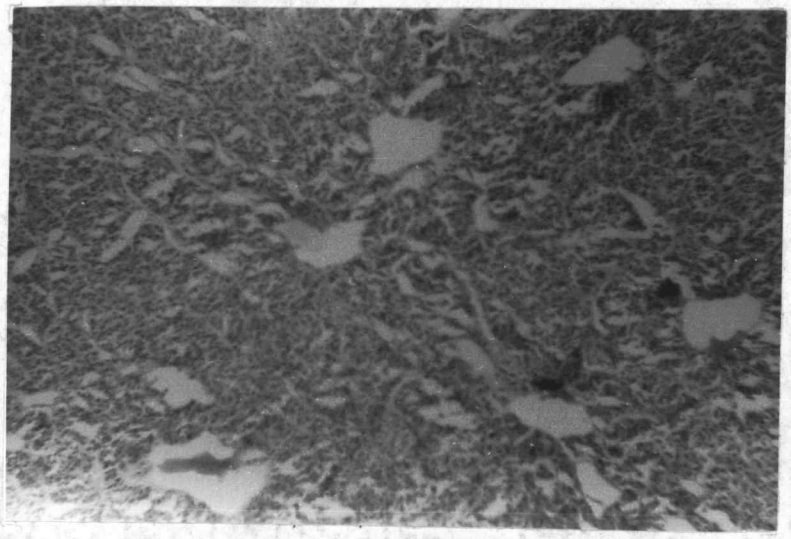
Bull $200B_1$, when slaughtered, appeared normal in all respects. The testicle showed slight degeneration of the seminiferous tubules and reduction in spermatogenesis. The pituitary gland appeared normal as were the adrenals except for a slight invasion of the medulla by the cortex.

Bull 503B, on slaughter, showed adhesions of the thoracic cavity with the lungs functioning at about 35 per cent efficiency. Bull 503B had testicles similar to 500B, but not as severely damaged. There was more interstitial tissue, but a reduction of Leydig cells. The lumen of the seminiferous tubules contained considerable tissue debris.

The pituitary of 503B, Plate XIII, consisted almost entirely of a cyst. Approximately 15 per cent of the pituitary gland appeared to be functioning with the majority of the anterior pituitary being cystic. The adrenal glands of 503B were essentially normal.

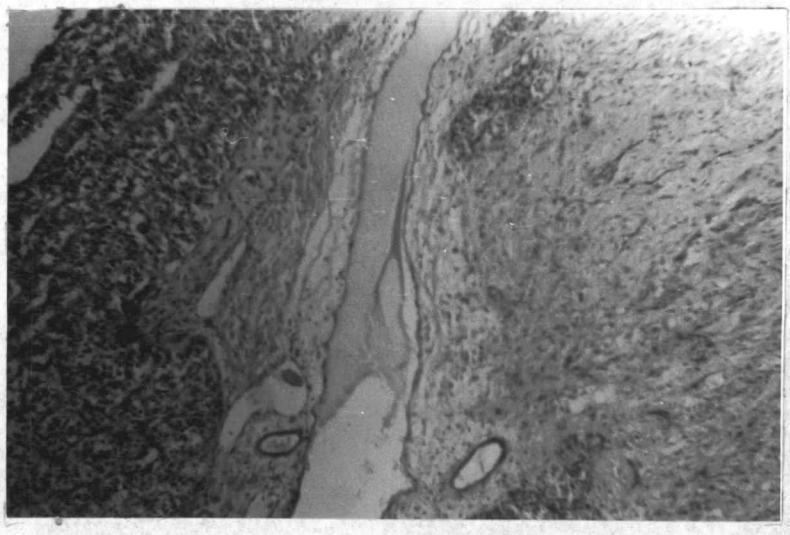
Macroscopically, bull $252B_1$, a third generation low carotene animal, appeared normal in all respects. Microscopically, the pituitary gland of $252B_1$ showed a cyst between the pars nervosa and the pars distallis approximately 40 microns wide and 350 microns long; see Plate

PLATE XIII



Cystic pituitary of low carotene
Holstein bull 503B

PLATE XIV



Cystic pituitary of third generation low carotene
bull $252B_1$ when slaughtered at $2\frac{1}{2}$ years of age

XIV. The adrenal glands showed evidence of small cysts eight to 100 microns in diameter in the zona fasiculatis and mucoid degeneration of the medulla. The testicles of $252B_1$ appeared essentially normal, but with slight degeneration of the basement membrane of the seminiferous tubules and a reduction in spermatogenesis.

Third generation calves $557B_1$ and $267B_1$ on slaughter appeared normal in all respects. Microscopically, the only abnormal condition found in the tissues examined was multiple minute cysts in the pars nervosa of both animals; see Plate XV.

Cow 250, when slaughtered, showed no observed macroscopic abnormalities. Microscopically, no unusual histological picture could be established with the exception of possible minute cysts in the pituitary and ovaries that appeared slightly inactive as indicated by the lack of developing follicles and a small number of corpus albicans.

Cow 252, when slaughtered, showed a large tumor on the right side in the flank area, otherwise she appeared normal. All organs and tissues examined microscopically appeared normal.

Cow 253 was normal in all respects except for an acute mastitic mammary gland. On microscopic examination the tissues observed were normal with the exception of the right adrenal which showed a small cyst in the medulla.

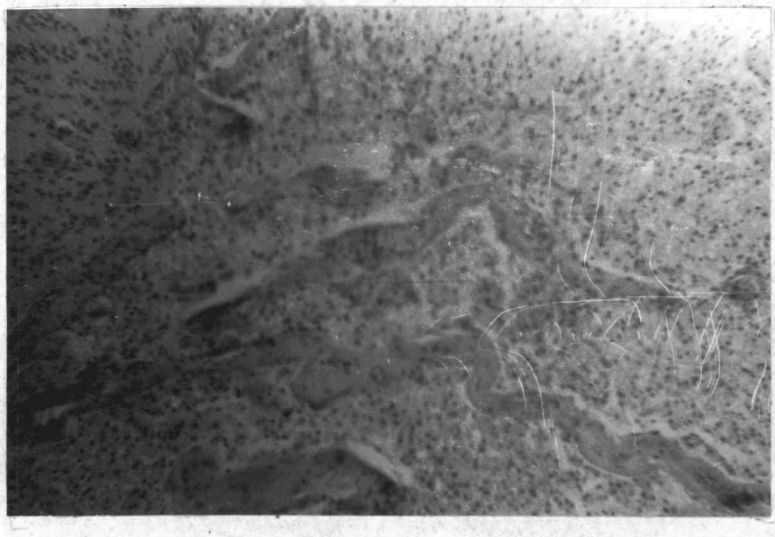
Blind cow 255 appeared normal on slaughter. Microscopically, the tissues examined appeared normal except for small cysts in the pars nervosa of the pituitary gland and apparent inactivity in the ovaries.

The calves born dead or so weak that they were autopsied shortly after birth on examination showed normal tissues except for the optic

PLATE XV

Cystic pituitary of third generation low carotene Holstein bull $557B_1$ when slaughtered at 4 months of age

PLATE XVI



Left optic nerve of low carotene calf G-8 showing necrosis

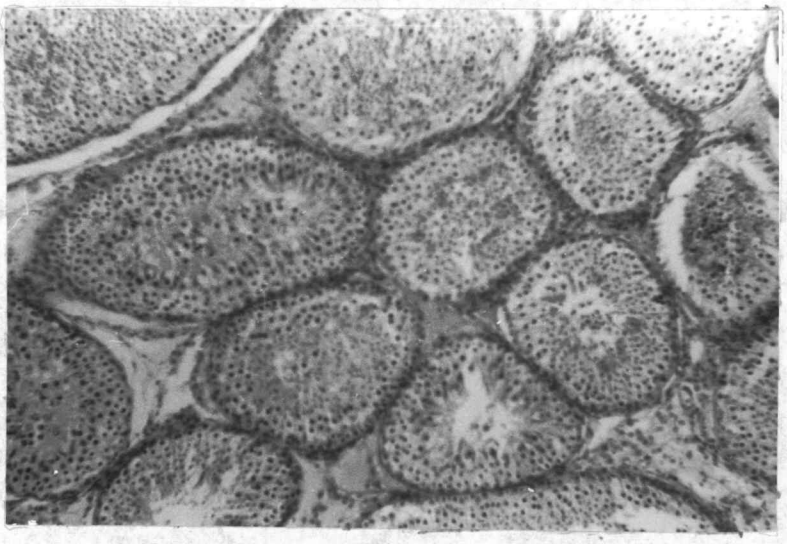
nerves at the point passing through the optic foramen where necrosis of greater or less degree was evident, as shown in Plate XVI.

Control bulls slaughtered appeared normal in all tissues examined. Plates XVII and XVIII show the biopsy and slaughter specimen of the right testicle of control bull 29B and Plates XIX and XX show the macroscopic effect of the biopsy technique. These plates are evidence that the biopsy of the testicles was not responsible for the degeneration of the testicles seen in the low carotene bulls when slaughtered.

The thyroid glands of all animals slaughtered appeared normal on macroscopic examination and showed no abnormalities when checked microscopically.

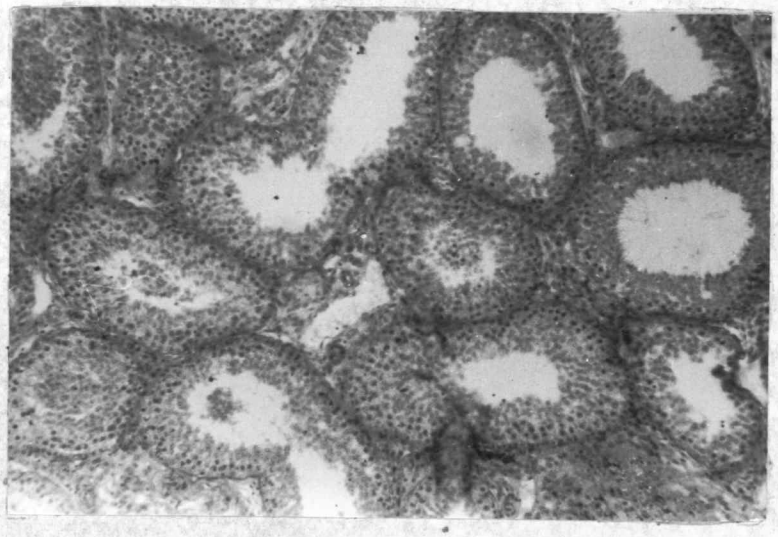
The histological findings on those tissues examined of the low carotene group were in agreement in respect to the testicles and pituitary gland with the findings of other investigators (21, pp.764-773; 39, pp.669-675; 45, pp.689-690). Apparently the low carotene ration at either 50 micrograms of carotene per kilogram of body weight, 90 micrograms per kilogram of body weight, or 130 micrograms of carotene per kilogram of body weight will affect the testicle or pituitary gland of the offspring of animals receiving this carotene intake, depending, to some extent, on the length of time the animal has been on the low carotene ration; i.e., whether the animal is a second or third generation low carotene animal. The effect of these carotene intakes is similar to what is recognized as a distinct carotene or vitamin A deficiency. In the second generation low carotene animals, degeneration of the seminiferous tubules and reduced spermatogenesis together with cystic pituitaries was noted when the carotene intake had been

PLATE XVII



Biopsy specimen of right testicle of control Jersey bull 29B at 16 months of age

PLATE XVIII



Slaughter specimen of right testicle of control Jersey bull 29B at 5 years of age

PLATE XIX

Right testicle of control Jersey bull 29B
showing adhesion at site of biopsy

PLATE XX

Right testicle of control Jersey bull 29B
Showing lack of testicular damage at biopsy site

increased to 130 micrograms of carotene per kilogram of body weight. The third generation bulls, $267B_1$ at 10 months of age and $557B_1$ at four months of age showed evidence of vitamin A or carotene deficiency even though they received 390 micrograms of carotene per kilogram of body weight and 130 micrograms per kilogram of body per kilogram of body weight, respectively. The pituitary gland of both animals showed evidence of small cysts. The testicles had not developed in $557B_1$ to show any effect, but $267B_1$ showed evidence of lack of development. Thus it would appear that recommended vitamin A or carotene allowances for dairy cattle will require modification depending on the length of time the animal has been exposed to a low carotene diet, or whether the animal might be a second or third generation individual exposed to the low carotene diet.

Pathological syndrome of the adrenal gland is unusual in animals suffering from what is supposed to be only a vitamin A and/or carotene insufficiency. It is possible, however, that the vitamin A and/or carotene deficiency affected the pituitary gland to such an extent t that the balance of adrenotropic hormones of the anterior pituitary has altered. The changing of the adrenotropic hormone balance might so affect the adrenal glands as to bring about such cellular disturbances observed in the adrenal glands of animals 221B, $252B_1$ and 253.

SUMMARY

This study has shown that the carotene requirements of dairy cattle for growth and reproduction are considerably increased, depending on the length of time the individual and her dam have been exposed to a sub-optimum carotene intake. It has been demonstrated that females raised on a normal ration, but exposed to a reduced carotene intake for the latter months of their pregnancy, produced normal, healthy calves, but calves that were considerably below normal in weight at birth. When these calves, after a normal milk feeding period, were placed on the same low carotene level as their dams had received during the latter months of pregnancy, they did not grow as rapidly as a comparable group of calves on a normal ration.

The study has shown that females maintained on a ration supplying 50 micrograms of carotene per kilogram of body weight and from dams which had received 50 micrograms of carotene per kilogram of body weight during the latter months of pregnancy were not able, on the average, to produce normal calves. When the carotene intake was raised to 90 micrograms of carotene per kilogram of body weight, the reproductive performance of the females was but little improved. When the carotene intake was further increased to 130 micrograms of carotene per kilogram of body weight, there was no improvement in reproduction as measured by the uniformity of estrous cycles. Completed reproduction data is not yet available.

Milk and butterfat production of the low carotene cows, when receiving 90 or 130 micrograms of carotene per kilogram of body weight,

was hardly 50 per cent of the expected. When some of the cows were returned to a normal ration of irrigated pasture or alfalfa hay and grass silage, milk and butterfat production did not markedly improve, indicating that some factor(s) in the physiology of milk secretion had been severely affected by the prenatal and postnatal exposure of the animal to the low carotene ration for an extended period.

With males the influence of the carotene intake does not appear to be as critical as with females. Semen quality deteriorated with advancing age regardless of an increasing carotene intake, indicating that the length of exposure to a low carotene ration of the animal, itself, or its ancestors influenced its reproductive performance as measured in the case of males by semen quality and breeding efficiency.

This study emphasizes that the only true measure of semen quality is fertility. Notwithstanding the small differences in semen quality between the low carotene group and the control group of bulls, there was a significant difference in the breeding efficiency of the two groups with the control group far exceeding the low carotene group.

Blood, liver and milk fat analyses suggest that animals in the second and third generation on a low carotene ration require a considerably higher intake of carotene than recommended by the National Research Council (68, p.6) in order to maintain the blood, liver and milk fat at values approximating the higher values found with normal animals. The data suggest that 130 micrograms of carotene per kilogram of body weight which is the equivalent of the 60 milligrams per 1000 pounds of body weight recommended by the National Research Council for growth (68, p.6) is not sufficient to maintain the liver values of

vitamin A at a normal level.

Although the animals on low carotene made no marked response to either a normal ration or large intakes of carotene, the efficiency of digestion of major feed constituents as measured during two digestion trials does not seem to have been altered. Low carotene animals digested grass silage and low quality hay as well as normal animals.

Blood values of carotene and vitamin A in this study should be considered with a great deal of caution as an indication of an animal's nutritional status with respect to vitamin A. Simply because the blood carotene and vitamin A values of an animal are high does not mean that the liver carotene and vitamin A values will be high.

The study also suggests that good quality green alfalfa hay of 30 to 40 p.p.m. carotene is unsatisfactory in maintaining blood carotene and vitamin A values as high as with animals receiving grass silage or irrigated pasture and by no means maintains the liver carotene and vitamin A values near the higher values of animals when on irrigated pasture or grass silage.

Neither 260 or 390 micrograms of carotene consumed per kilogram of body weight is sufficient carotene when supplied to either a second or third generation low carotene animal to maintain liver vitamin A near what might be considered a normal liver value.

The semen and the small intestine of the low carotene animals respired at a lower rate than the control animals. However, these differences were not statistically significant.

Histological analyses of the tissues of the low carotene animals slaughtered indicated that the carotene intakes resulted in cystic

pituitaries in mature animals and young calves. Degeneration of the testicles and reduction in spermatogenesis was evident in the low carotene bulls.

A pathological syndrome of the adrenal glands was noted in the adrenal glands that might be associated with the long-time exposure of the animal to low levels of carotene.

Pregnant cows fed the low carotene ration produced dead or weak calves that showed degeneration of the optic nerves and corneal opacity suggesting that levels of 50, 90 and 130 micrograms of carotene per kilogram of body weight is insufficient for the production of normal, healthy calves. Associated with the eye condition of a number of calves was a hydrocephalic condition that might be attributed to the low carotene status of the dam.

It appears from this study that the longer the animal is exposed to a low carotene ration the higher the carotene intake must be in order to maintain normal physiological functions. This study suggests that adequate carotene and/or vitamin A should be supplied dairy cattle for normal growth, well-being, reproduction and production, and that the critical period of the individual is during prenatal and early postnatal life.

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