LEVELS OF CAROTENE AND VITAMIN A IN BLOOD AND COLOSTRUM OF HEREFORD AND ABERDEEN-ANGUS CATTLE

by

ROBERT RODERICK WHEELER

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APPROVED:

[redacted]

Assistant Professor of Animal Husbandry

In Charge of Major

[redacted]

Head of Department of Animal Husbandry

[redacted]

Chairman of School Graduate Committee

[redacted]

Dean of Graduate School

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INTRODUCTION

One of the most important vitamins discovered and investigated to date in terms of its far-reaching effects on animal metabolism is vitamin A. All its functions in the animal body are not completely known, but the broad significance in animal metabolism of vitamin A and its precursors is realized. A most important role of vitamin A is in the visual cycle, yet this is perhaps not its principal activity.

Information is not available to allow accurate estimation of the monetary losses suffered annually by stockmen, cattle feeders and farmers due to avitaminosis A. Madsen and Earle (30, p.617) observed that 651 beef carcasses were condemned for generalized edema or anasarca, one symptom of vitamin A deficiency, by federal meat inspectors from July, 1941 to December, 1946. Moreover, Madsen (29) unofficially places the loss from anasarca during the 1947-1953 period at 2084 head. These figures may be misleading by inferring that all anasarca losses are due to vitamin A deficiency. This has not been proven, but anasarca has been developed on low vitamin A diets and, in most cases has been cured by

feeding carotene and vitamin A. Numerous other losses from vitamin A occur, but detailed statistics are not available. A percentage of calf losses at calving time can surely be attributed to the deficiency. Deaths of calves from diarrhea and pneumonia may be partly caused by avitaminosis A. In addition, widespread monetary losses from vitamin A deficiency may be attributed to reproductive failure in males and females, general unthriftiness and "poor doing". Such losses, because of their very nature are difficult to classify or estimate accurately, yet they may well be important items to the livestock industry. Vitamin A deficiency has been demonstrated experimentally in almost all species of farm animals, from the newborn chick, calf or pig to mature animals and has been linked with vision, growth, reproduction, lactation and general health.

Visual troubles have been observed in two or three Aberdeen-Angus calves for the past several years in the beef cattle herd at Oregon State College. A detailed diagnosis of one Angus calf exhibiting this condition is presented in Appendix A. Since no similar disturbances have been evident in the Hereford calves of the herd this study has been initiated to investigate the carotene and vitamin A values of blood plasma and colostrum of the cows and of the blood plasma of calves of the two breeds.

Breed comparisons of these items are not numerous in the literature, and it was felt that knowledge of the carotene and vitamin A levels in these animals might be worthwhile apart from the possible implication of visual impairment.

REVIEW OF LITERATURE

General Aspects of Vitamin A and Carotene Nutrition

A wealth of information may be found in the literature on the role of vitamin A in higher vertebrates. An exhaustive search on the subject of vitamin A is far beyond the scope of this thesis, which will be confined to some implications of vitamin A in the nutrition of the domestic ruminant animals with particular emphasis on beef cattle. Much of the work done on vitamin A in ruminants has been concerned with dairy cattle, therefore some reference will be made to them.

A description of the chemistry of vitamin A may be found in most biochemistry and nutrition texts, e.g., Peterson and Strong (41, pp.203-210), Fruton and Simmonds (15, pp.901-903) and Maynard (33, pp.182-187).

The classical postulation of Thomas Moore which suggested that beta-carotene was converted to vitamin A principally in the liver has undergone revision in the past decade. Laboratories in America and Great Britain have not completely ruled out the liver as a carotenoid conversion site, but the intestinal wall is the only tissue that has been conclusively proven to be involved in this process.

Studies in vivo and in vitro on the liver as a site in carotenoid conversion by number of investigators (58, pp.85-88), (9, pp.1342-1347), (10, pp.236-242) (44, pp.299-319) have shown conflicting results. Bessey and Wolbach (3, pp.2072-2080) stated in a review that while no direct proof was available, circumstantial evidence suggested the liver as the site for carotene conversion.

Mattson, Mehl and Deuel (32, pp.65-73) fed carotene in oil to vitamin A deficient rats and concluded that the intestine was the site of conversion. Analyses showed that vitamin A appeared in the intestinal wall within 1.5 hours but none was observed in the liver until 2.75 hours. The amount in the intestine was greater than in the liver during the first four hours. Wiese, Mehl and Deuel (57, pp.75-79) demonstrated intestinal conversion in vitro. Glover, Goodwin and Morton (17, pp.512-518) also observed vitamin A in the intestinal walls of rats following high carotene intakes. Elliot (14, pp.711-712) noted vitamin A increases in the blood draining the small intestine of anesthetized dairy calves. Klosterman, Bolin and Light (24, p.624) found no traces of vitamin A in the intestinal walls of sheep unless dietary carotene was present. Control animals on low carotene diets, with

adequate vitamin A stores, had no vitamin A in the intestine. They concluded that the intestinal wall was the site of conversion.

Ronning and Knodt (43, p.290) noted in dairy calves that the most active absorption of vitamin A takes place in the upper two-thirds of the small intestine. The concentration of vitamin A in the digestive tract was lowest in this area after the administration of vitamin A. Mucous tissue in this area showed a greater concentration of vitamin A than elsewhere. There was a highly significant correlation between the concentration of vitamin A in the blood plasma and in the mucous tissue of this area after a single administered dose. Limited absorption was observed in the lower one-third of the small intestine and in the large intestine.

A most common symptom observed in vitamin A deficient animals is "night blindness" or the inability to see in dim light. Guilbert and Hart (18, pp.409-427) state that night blindness is the first detectable clinical symptom of vitamin A deficiency and constitutes a delicate index upon which minimum requirements may be based. The importance of vitamin A and its precursors in the visual cycle is fully realized and a great deal of literature may be found on this subject. Wald (53, p.73)

notes that the visual function of vitamin A is the only physiological activity of vitamin A that is at all understood. The visual pigment relationships involving rhodopsin, porphyropsin and iodopsin have been clearly outlined by Wald (53, p.74). Guilbert and Hart (18, pp.409-427) observed that about 225 days are required to deplete vitamin A reserves before night blindness develops. Riggs (42, p.491) states that wide variation exists in the time for depletion to occur and for night blindness to become apparent. He found the time for depletion of yearling steers was from 128 to 266 days while in calves, the range was from 101 to 206 days.

The importance of vitamin A and its precursors in the reproduction of ruminants has been noted by a number of authors, e.g., Brody, Cunha, Warwick and Evans, Hart and Guilbert, Hodgson, et al. and Lindley, et al. Brody (7, p.118) states that there is an absence of spermatazoa and a degeneration of the germinal epithelium of the testes following severe avitaminosis A. Cunha, Warwick and Evans (8, p.415) observed very little difference in reproductive ability of ewes on high vitamin A rations and those on a deficient ration. Lindley, et al. (27, pp.601-602) studying the effect of vitamin A deficiency on the quality of ram semen found that there was production of

normal semen and also normal growth and normal blood plasma vitamin A levels in rams that were on a ration supplemented with 20,000 I. U. of vitamin A daily. Rams on unsupplemented rations developed vitamin A deficiency symptoms after periods of seven to twenty-one weeks. Vitamin A plasma levels and semen quality were low in the unsupplemented group. Hodgson, et al. (22, pp.685-686) observed, in studies on fertility in young dairy bulls, that bulls on a ration deficient in vitamin A showed a high percentage of abnormal sperm, a high pH of the semen and low storage qualities of the semen. A degeneration in the epithelium of the seminiferous tubules was also noted. These investigators state that gross vitamin A deficiency symptoms are likely to appear before there is severe impairment in reproductive performance. Madsen, et al. (31, pp.391-392) observed that sexual activity and ability in beef bulls decreased rapidly as vitamin A deficiency progressed. Payne and Kingman (40, pp.50-55) noted that first-calf range heifers must have higher blood plasma carotene levels than aged cows to avoid clinical symptoms of carotene deficiency-nutritional abortions and retained placentas. Retention of placentas was also observed by Wise, et al. (59, pp.502-503) in dairy cows. Hart (20, p.263) states that cows on a

vitamin A deficient ration during the early months of pregnancy only may give rise to permanently blind, but otherwise normal calves.

Vitamin A, in the past, has been termed the anti-infective vitamin, but the terminology seems to be passe. Eichhorn, Sarles and Ellis (13, p.151) hypothesize that the increased susceptibility to diseases in vitamin A deficient animals may be due to the changes in the epithelial tissues, both internal and external, by the substitution of normal tissues with horny or keratinized tissue. These changes occur in the genitourinary, respiratory and alimentary tracts, the glands of the throat, ear and eye. They state that this keratinization increases susceptibility, but does not necessarily decrease immunity.

Wald (53, p.73) states:

> It is not enough to recall that animals deprived of vitamin A stop growing and eventually die, and neither as the result of night blindness. Vitamin A must play some very general role in cellular metabolism or structure, a role perhaps particularly associated with epithelial cells, since these undergo such marked changes early in vitamin A deficiency.

Wolbach and Bessey (60, p.234) regard epithelial tissue changes in vitamin A deficiency as the most characteristic consequence. These changes appear regardless of age

and, presumably, in all vertebrates.

Though the concentration of vitamin A investigations has been on the subjects of vision, reproduction and epithelial tissue transformations, studies have been conducted to some extent on the effect of vitamin A on growth, urolithiasis and bone and teeth formation.

Levels of Vitamin A and Carotene in Ruminants

Three methods are used extensively in determing the state of vitamin A metabolism in the animal body. These are the determination of: (1) Blood plasma levels of vitamin A and carotene, (2) levels in samples obtained by liver biopsy, and (3) cerebrospinal fluid pressures. Of the three, blood levels are probably the most widely used at the present time, but liver levels and cerebrospinal fluid pressures have been investigated to a considerable degree.

Moore, Berry and Sykes (34, pp.649-658) state that spinal fluid pressure measurements in calves are a critical method for showing adequate or inadequate carotene intake. They found that blood plasma vitamin A and carotene values will not distinguish between carotene intake variations of 62-72 micrograms per kilogram body weight, but plasma carotene and vitamin A may distinguish carotene intakes from 44-75 micrograms per kilogram. Moore and Sykes (36, pp.684-689) observed that avitaminosis A in the young bovine resulted in increased cerebrospinal fluid pressure. Vitamin A deficiency symptoms and low blood plasma carotene were correlated with spinal fluid pressure increase. Calves on a low carotene ration showed a decrease in blood plasma carotene and a

concurrent increase in spinal fluid pressure from 100 millimeters saline, which was noted on an adequate carotene intake, to 250-300 millimeters of saline. Upon carotene supplementation, blood plasma carotene increased and spinal fluid pressure decreased to about 150 millimeters of saline. Moore and Sykes (37, pp.436-439) showed that calves on a low carotene ration had spinal fluid pressure values of 400-600 mm. saline - four to six times normal values. These values were obtained at the terminal stage of vitamin A deficiency: diarrhea, lack of appetite and moribund state. Moore, et al. (35, pp.533-538) state that cerebrospinal fluid pressures greater than 120 millimeters saline are beleived to denote vitamin A deficiency.

Vitamin A and carotene investigations of the liver, the major storage site of vitamin A in the animal body, may give an accurate measure of vitamin A metabolism. A copious amount of literature may be found on liver studies in laboratory and farm animals. Lewis, et al. (26, pp. 351-363) observed that when rats were placed on vitamin A free diets, blood plasma vitamin A concentrations remained high in rats with high hepatic stores, but fell rapidly in rats with low liver vitamin A storage. Krause (25, pp.535-542), working with rats, found inverse

relationships existing between blood plasma vitamin A and liver vitamin A concentrations. When total liver vitamin A content fell below 600 international units per total liver, a parallelism was found to exist between blood plasma vitamin A and liver vitamin A concentrations. Sobel, et al. (46, p.236) found no definite relationship between serum vitamin A levels and liver storage in rat studies.

Liver biopsy methods for cattle have been developed whereby a small sample (usually 0.3 to 0.8 gram) of liver may be obtained for vitamin A and carotene analysis. Whitehair, et al. (56, pp.285-287) and Bone (4, pp.747-752) have presented techniques for liver biopsys.

Thomas, Gallup and Whitehair (50, pp.372-378) in a carotene and vitamin A investigation with Hereford cows found no definite constant relationship between hepatic vitamin A and that found in blood plasma. Similar results were noted by Gallup, et al. (16, pp.715-721) and by Baker, Pope and MacVicar (2, pp.802-807). Thomas and Moore (51, pp.687-692) state that blood plasma vitamin A levels do not give reliable estimates of liver storage or carotene intake in rachitic dairy calves or in calves on a semi-synthetic milk regime.

Boyer, et al. (5, pp.433-440) state that blood plasma vitamin A is a more delicate measure of the

vitamin A nutrition of an animal than either blood plasma carotene or growth. These writers found that blood plasma levels of vitamin A of 10 micrograms or more per 100 milliliters of blood plasma were necessary for adequate vitamin A nutrition of the growing dairy calf. Vitamin A plasma levels of 7 to 8 micrograms per cent were termed borderline levels. An inadequate vitamin A nutrition was found to exist below these latter levels. Daily intakes of vitamin A which would maintain deficient, borderline and adequate blood plasma levels were 6, 12 and 18 micrograms per kilogram body weight, respectively. Carotene blood plasma levels which would maintain adequate vitamin A plasma levels were 50-70 micrograms of carotene per 100 milliliters of blood plasma for Holstein calves and 100-140 micrograms per cent for Guernsey calves.

Davis and Madsen (12, p.145) observed that carotene and vitamin A blood plasma levels were dependent, but not directly correlated with intake and liver storage. Carotene levles were dependent upon carotene intake in a carotene and vitamin A depleted animal. Blood plasma vitamin A was found to be closely related to plasma carotene content. Beef heifers of the Hereford and Shorthorn breeds produced normal calves on a ration providing 60

micrograms per kilogram body weight of carotene daily. Heifers on 30 and 45 micrograms per kilogram body weight produced vitamin A deficient calves. The heifers on this carotene level, however, remained normal.

Phosphorus, according to Gallup, et al. (16, pp.715-721), has an effect on carotene and vitamin A metabolism. Steers whose phosphorus and vitamin A reserves were depleted, were placed on a low phosphorus but high (10 times the minimum requirement) carotene ration. Blood plasma carotene levels were consistently higher in the steers on a low phosphorus plane compared to a phosphorus-supplemented control group. Average plasma carotene levels were 119 micrograms per cent for the low phosphorus group and 76 micrograms per cent for the control group. Blood plasma vitamin A levles were 17 and 22 micrograms per cent, respectively. A decrease in the storage of vitamin A in the liver was also indicated in the low phosphorus ration. A brief examination of unpublished phosphorus-carotene data obtained at the Squaw Butte Harney Experiment Station at Burns, Oregon, seems to show no consistent relationship between blood plasma levels of phosphorus and of carotene in Hereford cows. Thomas,Gallup and Whitehair (50, pp.372-378) found plasma carotene levels to be generally higher in phosphorus-deficient cows than in cows fed adequate

phosphorus. Plasma vitamin A was found to be unaffected by phosphorus deficiency.

Baker, et al. (1, pp.571-574) present blood plasma carotene and vitamin A levels obtained before and after parturition in Hereford cows on different levels of carotene supplementation. Four lots of two year old Hereford cows were on a low carotene ration of cottonseed meal, ground milo, dried beet pulp, cottonseed hulls and wheat straw or dry weather range grass. Lot I received no carotene supplementation; Lot II received carotene during lactation; Lot III supplemental carotene during gestation and Lot IV supplemental carotene during gestation and lactation. Table 1 shows the values obtained by these authors.

Table 1 Blood plasma carotene and vitamin A values. Micrograms per 100 milliliters.

	Plasma vitamin A		Plasma carotene	
Lot No.	3 months prepartum	At parturition	3 months prepartum	At parturition
I	8.3	11.3	11.6	11.8
II	8.7	15.1	12.9	10.3
III	10.1	16.3	31.1	23.4
IV	14.2	16.3	25.8	24.5

Plasma carotene and vitamin A levels reflected carotene supplementation, but these average values do not approach the levels observed by Long, et al. (28, p.10)

investigating blood composition of normal beef cattle. Thomas, Gallup and Whitehair (50, p.375) show blood plasma carotene at parturition in mature Hereford cows to be 165 micrograms per 100 milliliters while plasma vitamin A levels were 9.3 micrograms per cent. Baker, Pope and MacVicar (2, p.805), in an experiment similar to that by Baker, et al. (1, pp.571-574), but differing in time of experimental initiation, present blood plasma carotene and vitamin A values at one month prepartum and at parturition. Two year old Hereford cows who had calved the previous summer were started on a carotene study in November. Four lots received a low carotene ration and carotene supplementation, as cited above (1,pp.571-574). Table 2 depicts levels observed in this investigation.

Table 2 Blood plasma carotene and vitamin A levels. Micrograms per 100 milliliters.

Item	Lot No.	5 months prepartum	1 month prepartum	At parturition	Lactation 1 wk.
Plasma vitamin A	I	32.5	25.0	17.3	14.4
	II	30.0	21.1	12.2	14.3
	III	27.4	20.9	13.4	12.2
	IV	34.0	25.5	16.8	12.3
Plasma carotene	I	111.6	22.9	21.3	19.8
	II	102.9	21.6	17.5	89.4
	III	73.8	41.4	48.1	28.1
	IV	97.5	49.1	63.0	89.6

Blood plasma carotene levels at the beginning of the

experiment were indicative of previous high carotene intake.

Sutton, Kaeser and Soldner (48, pp.933-939) in studying the effect of parturition in dairy cows, of the Ayrshire, Jersey, Guernsey and Holstein breeds, on normal barn rations, found that the average vitamin A concentration in blood plasma was 22 micrograms per 100 milliliters at three weeks prepartum and decreased to an average concentration of 12.5 micrograms per cent at parturition. A continued decrease was noted to 10 micrograms at 2 days postpartum, but at two weeks postpartum average vitamin A plasma levels had increased again to 15 micrograms. Blood plasma carotene was approximately 600 micrograms per cent three weeks prepartum, 440 micrograms at parturition, 325 micrograms at one week postpartum and 380 micrograms per 100 milliliters at two weeks after calving. The maximum decrease in blood plasma carotene was reached one week postpartum and the maximum decrease in plasma vitamin A was reached three days after parturition.

Watkins, Knox and Benner (54, p.8) presented blood plasma carotene and vitamin A values in mature Hereford cows for a five and one half year experimental period. Average blood plasma carotene levels at 28 day intervals

during this period showed variations from 149 micrograms per 100 milliliters in the January observations to as high an average as 734 micrograms noted in the May observations. Blood plasma vitamin A values ranged from 44 micrograms per cent in January to 60 micrograms per cent observed in May, June and September.

Long, et al. (28, pp.8-12) investigated the seasonal trend of vitamin A and carotene in blood plasma of heifers from the Hereford, Aberdeen-Angus and Shorthorn breeds. The highest values observed for both carotene and vitamin A occurred in the mid-summer months and the lowest levels were reached in February and March. Plasma carotene varied from a low of approximately 100 micrograms observed in February to a mid-summer high of 850 to 900 micrograms. Vitamin A plasma values ranged from 12 micrograms observed in March to 50 micrograms noted in July. Seasonal trends and carotene plasma concentrations of the three breeds were very similar, but somewhat higher blood plasma vitamin A values were noted in the heifers from the Shorthorn breed.

Nelson, et al. (39, pp.50-52) compared vitamin A and carotene levels in the blood plasma of dairy and beef calves. Table 3 shows the values obtained by these authors. The dairy calves were taken from their

dams at two days of age while the beef calves were kept with the dams constantly for four weeks and then were able to nurse twice daily. The beef calves also had access to a grain mixture and alfalfa-bromegrass hay. Carotene content increased considerably after forty days of age for dairy calves and after sixty days of age for beef calves.

Table 3 Vitamin A and carotene content of blood plasma of beef and dairy calves. Micrograms per 100 milliliters.

	Dairy calves		Beef calves	
Age in days	vitamin A	carotene	vitamin A	carotene
1-20	12	22	16	26
21-40	10	23	13	18
41-60	10	50	13	23
61-80	12	44	16	43
81-100	12	48	16	52
101-120	12	50	16	53

Baker, et al. (1, pp.571-574) show Hereford calf blood plasma levels from cows on four carotene supplemented regimes. Table 4 depicts calf levels from cows on no carotene supplementation (Lot I), carotene supplementation during lactation (Lot II), supplemental carotene during gestation only (Lot III) and supplemental carotene during gestation and lactation (Lot IV).

Table 4 Calf blood plasma vitamin A levels from cows on various carotene supplementation regimens. Micrograms per 100 milliliters.

Lot No.	At partu-rition	2 weeks post-partum	1 month	2 month	3 month
I	3.1	6.2	6.0	6.0	4.1
II	1.3	9.3	5.7	8.1	9.7
III	3.6	6.8	5.0	6.3	6.7
IV	4.1	14.4	6.2	8.0	11.9

The higher plasma vitamin A noted in lots II and IV at two weeks postpartum may be attributed to the supplementation of the cows of these two lots during lactation. Table 5 evinces vitamin A levels of calves from cows on carotene supplemented rations from data by Baker, Pope and MacVicar (2, p.805).

Table 5 Calf blood plasma vitamin A levels from cows on various carotene supplemented rations. Micrograms per 100 milliliters.

Lot No.	At Partu-rition	1 week	2 weeks	1 month	2 months	3 months
I	4.9	13.2	11.6	7.9	6.0	4.5
II	3.8	12.0	11.5	9.5	9.0	12.6
III	6.2	8.6	7.3	5.9	4.6	5.6
IV	4.9	13.6	13.8	10.3	10.0	11.5

An abundance of literature is available on blood plasma carotene and vitamin A levels in various dairy breeds, but most of the investigations to date with

beef cattle involve animals from the Hereford breed only. In some cases, plasma levels may not be comparable because of variable dietary regimens, but the presentation of these levels is valuable in providing basic data on this important subject.

Colostrum as a Source of Vitamin A and Carotene

The role of colostrum in the nutrition of the newborn is recognized by many and has been investigated to a considerable degree in farm animals. Dann (11, pp.1999-2005) states that colostrum vitamin A concentration in cattle may be from 10 to 100 times greater than later milk, independently of season. Colostrum carotene may be up to 70 times greater than that in later milk unless calving occurs during late winter feeding. Heifer colostrum, according to Dann, is generally richer in vitamin A than colostrum from cows. He states that a calf receives on its first day post-partum vitamin A supplies greater than later milk could provide in 20-50 days. Hansen, Phillips and Smith (19, pp.809-814), and Henry, Houston and Kon (21, pp.1-14), in agreement with Dann, found heifer colostrum twice as rich as older cow colostrum.

Hansen, Phillips and Smith (19, pp.809-814) observed that the chief drop of vitamin A concentration in colostrum occurred during the first three milkings. From that time, a more gradual decline was noted. Sutton, Warner and Kaeser (49, pp.927-932) also noted a rapid decline of vitamin A in colostrum and stated that vitamin A concentrations similar to those found in

normal milk were reached on the third day or at the sixth milking. Table 6 depicts the carotene and vitamin A concentrations observed by these authors at the first and sixth milkings.

Table 6 Carotene and vitamin A levels observed in colostrum and normal milk. Micrograms per 100 milliliters of milk.

Breed	First Milking	Sixth Milking	First Milking	Sixth Milking
Ayrshire	373	58	182	42
Guernsey	864	102	279	33
Holstein	289	57	169	49
Jersey	335	55	144	84
Brown Swiss	497	87	348	54
All breeds	473	90	240	51

In contrast to the work of Hansen, Phillips and Smith, Sutton, Warner and Kaeser report carotene values higher than vitamin A levels. Hansen, Phillips and Smith (19, pp.809-814) report vitamin A concentration in colostrum from the first lactation varying from 70-480 micrograms per 100 milliliters and carotene levels ranging from 77-228 micrograms per cent.

Van Arsdell, Ross and MacVicar (52, p.548) investigating blood and milk carotene and vitamin A concentration in Hereford cows found a rapid decrease in carotene and vitamin A in colostrum. A fairly constant

colostrum carotene level was reached after the third day following parturition.

The levels observed on the third day, both of carotene and vitamin A approached the levels found in normal milk. Carotene colostrum levels noted the third day postpartum were about 7.5 micrograms per cent and the vitamin A concentrations averaged 17.9 micrograms per 100 milliliters. Both levels were considerably lower than those observed in the dairy breeds (49, pp. 927-932).

Baker, Pope and MacVicar (2, p.805) found vitamin A colostrum levels varying from 128.3 to 224.5 micrograms per 100 milliliters in a study on various regimens of carotene supplementation. Baker, et al. (1, pp.571-574) noted vitamin A colostrum levels with a range from 45.6 to 163.9 micrograms per cent in a similar investigation as above. Baker, Pope and MacVicar state that the higher levels observed in their study compared to those by Baker and coworkers may have been due to higher liver reserves achieved by high carotene intake from range forage.

All these findings suggest the value of colostrum as a source of carotene and vitamin A for new born calves. Moreover, they indicate that sampling carried out to

assess the value of colostrum should be carried out within 48 hours of parturition.

EXPERIMENTAL INVESTIGATIONS

Biological Methods

1. General Management

Experimental animals used in this study were forty-four beef cows and their calves in the breeding herd of the Oregon Agriculture Experiment Station. The cows and calves were registered animals from the Hereford and Aberdeen-Angus breeds. The investigation covered the 1954 calving period and the early post-natal life of the calves, that is, from March 8, 1954 to June 30, 1954.

The management procedure may be generally described as that of a cow-calf operation on irrigated and non-irrigated pastures. From the period of March 1, to May 5, 1954, the cows and calves were on irrigated pastures consisting of perennial ryegrass, alta fescue, meadow foxtail and ladino clover. On May 6 they were rotated to non-irrigated pastures where the predominant species were perennial ryegrass and alta fescue. Because of the extreme variability in pasture species, both in nutrient content and in abundance at different times of the season, no attempt was made to analyze them for carotene content. Abundant lush green pasture of grasses and legumes is usually considered adequate in carotene

(38, p.145). A medium quality hay and grass clippings were fed when the irrigated pastures became low in forage and the non-irrigated pastures were not ready for stock.

The cows were wintered on a medium quality grass hay and mixed grass-legume silage, the composition of which was grasses of the Festuca spp., Lotus spp. and Ladino clover. No preservative was added when this mixture was ensiled. An abbreviated analysis of the silage, upon opening of the silo, revealed, a dry matter content of 30.0 per cent and crude protein content of 7.3 per cent. As the silage was used, and lower levels were opened, the dry matter content decreased to 19.9 per cent while the crude protein increased to 11.55 per cent, both changes probably being due to downward percolation of juices.

2. Blood Collection

Forty milliliter samples of blood were drawn by venous puncture from cows within one week of the expected calving date taken from breeding records. In fact, however, variation of calving dates were 10 days before the expected date and twenty-four days after this date. Blood samples from the cows were collected within 36 hours post partum and from the calves at approximately one month of age. Stainless steel bleeding needles of

14 and 15 gauge were used and the blood collected in pyrex tubes using 0.5 ml. of saturated sodium citrate per 40 ml. of sample as the anticoagulant. The samples were then refrigerated and the plasma drawn off by centrifugation at 1700 r.p.m. for 45 minutes after which the plasma was frozen until analyses could be made. Weswig (55) states that the vitamin A and carotene values in blood plasma are unaffected by freezing.

3. Colostrum Collection

Some variation occured in the time of colostrum collection due to the practical difficulties involved in bringing cows from distant pastures, with their calves, to the barn where they might be restrained for collection of the sample. Attempts were made to collect all colostrum samples within 24 hours post-partum. In one or two instances, this was not possible. The colostrum samples were collected in wide mouth 150-200 ml. jars, with approximately equal amounts being obtained from each quarter of the udder. The samples were immediately frozen until analyses could be made, when they were thawed and homogenized before final aliquots were taken.

Chemical Methods

The chemical methods used to determine the concentrations of vitamin A and carotene in the blood plasma of

the cows and calves and the colostrum of the cows were those that have found extensive application in work with humans and other species.

1. Blood Plasma Analysis

Blood plasma was analyzed by the method of Kimble (23, pp.1055-1065) modified by the addition of 2 per cent acetic anhydride to the Carr-Price reagent. (25 per cent antimony trichloride in chloroform).

2. Colostrum analysis

The scheme of analysis of the colostrum obtained was that proposed by Boyer, et al. (6, pp.101-102) involving the use of a homogenate. For photometric determinations, optical density was determined with a Coleman Photoelectric Spectrophotometer, model 14. Standard curves developed are presented in Appendix B.

Results and Discussion

The individual blood plasma carotene and vitamin A values from cows of the Hereford and Aberdeen-Angus breeds in the Oregon State College herd are presented in tables 7a and 7b below. Means of blood plasma carotene and vitamin A from the cows of both breeds are presented in table 8.

Table 7a Individual blood plasma carotene and vitamin A values in Hereford cows. Micrograms per 100 ml. plasma.

	Herefords			
Cow No.	Prepartum carotene	Postpartum carotene	Prepartum vitamin A	Postpartum vitamin A
14	219.0	222.8	73.1	62.2
2	247.5	243.0	59.3	79.4
10	211.6	223.5	50.3	57.2
5	167.4	136.8	48.9	35.7
7	158.0	206.9	40.1	64.4
B-11	148.8	190.9	48.4	61.1
3	193.7	199.7	62.4	66.9
A-5	235.7	205.8	96.2	95.9
2-0	228.7	246.8	59.3	49.3
A8	259.7	237.9	70.5	64.3
80	206.8	168.5	52.8	41.9
82	198.3	169.4	6.8	58.1
83	-----	-----	46.9	57.2
6-0	265.9	234.8	----	----
A-18	185.4	225.4	54.0	69.0
16-0	253.3	244.3	54.3	85.4
A-15	226.9	271.6	10.7	102.2
69	260.2	225.1	105.5	49.4
16P	191.6	225.5	40.3	90.6
10-0	181.4	167.6	52.0	35.0
A6	192.1	184.4	67.5	48.1
4-0	233.4	168.7	54.5	46.5
5-0	275.6	210.9	61.2	76.1
35	202.6	204.4	38.1	44.2
A10	156.1	142.6	66.4	51.8
B25	149.2	154.5	80.6	49.3
20P	185.7	174.9	54.3	49.2

Table 7b Individual blood plasma carotene and vitamin A values in Aberdeen-Angus cows. Micrograms per 100 ml. plasma.

Aberdeen-Angus				
Cow No.	Prepartum carotene	Postpartum carotene	Prepartum vitamin A	Postpartum vitamin A
36	150.6	169.4	37.9	33.2
110	236.3	215.1	50.3	43.6
111	212.3	180.6	81.2	37.9
10	224.9	236.9	41.8	50.9
67	235.8	162.7	40.0	52.9
3	250.4	222.0	50.8	37.5
5	175.2	195.9	46.7	46.6
29-0	211.7	184.2	66.9	49.2
2	173.2	154.4	45.0	77.2
B-64	165.2	165.9	46.2	38.1
62	214.3	174.9	72.6	43.1
109	219.4	206.8	51.4	64.3
A-57	221.5	210.9	66.6	59.7

Table 8 Means of blood plasma carotene and vitamin A before and after parturition. Micrograms per 100 ml. of blood plasma.

Breed	Prepartum carotene	Postpartum carotene	Prepartum vitamin A	Postpartum vitamin A
Hereford	209.0 ± 7.32[1]	203.3 ± 6.57	55.9±8.49	61.1±7.25
Angus	207.0±18.59	190.7±15.60	53.6±8.26	48.8±7.52

[1] Standard error of the mean, $p < .05$.

Considerable variations may be noted among the individual observations. These variations are depicted in table 8 by the magnitude of the standard error of the

mean. The probable reason for the higher standard error of the mean noted in the Angus cows may be that fewer numbers of that breed were involved in this study.

The decrease of blood plasma carotene in the Hereford cows at parturition was not significant at the five per cent level by an analysis of variance. However, the decrease noted in blood plasma carotene in the Aberdeen-Angus cows was significant. This decrease may be reflected in higher colostrum carotene values noted in table 12 indicating that there might be a greater loss of carotene due to lactation in Angus cows than in Hereford cows.

The increase in blood plasma vitamin A in the Hereford cows and the decrease of vitamin A in the blood plasma of the Angus cows at parturition was not significant at the five per cent level.

Baker, et al. (1, pp.571-574) observed increases in the vitamin A levels of blood plasma at parturition compared to three month prepartum levels. Increases from 3 to 6.4 micrograms per cent were noted by these authors. In contrast, however, Baker, Pope and MacVicar (2, pp.802-807) noted plasma vitamin A decreases at parturition varying from 2.7 to 8.9 micrograms per 100 milliliters compared to one month prepartum observations.

A number of variables existed in connection with the animals available for this experiment. The experimental groups contained cows of different ages, and also prepartum sampling time varied due to differences between actual and calculated calving dates. It was thought advisable to try to determine if these variables might be exerting any effect on the values obtained. Graphing these factors against the levels obtained, illustrated that there was no significant correlation between the variables mentioned and vitamin A and carotene contents of blood plasma or colostrum.

Correlations calculated by the method of Snedecor (45, pp.138-168) and by graphing showed that there was no significant correlation between prepartum carotene and vitamin A in the Hereford cows, but there was a highly significant correlation between postpartum carotene and vitamin A in that breed. No significant correlations, either in prepartum or postpartum carotene and vitamin A plasma levels, were demonstrated in the Angus cows. One possible explanation for the lack of correlation in postpartal carotene and vitamin A in the Angus cows in this study may be that there is a breed difference in time of removal of hepatic stores of vitamin A due to lactation.

On the other hand, a brief examination of data from Baker, Pope and MacVicar (2, p.805) and Baker et al. (1, pp.571-574) shows that there is very little correlation between prepartal carotene and vitamin A or between postpartal carotene and vitamin A in Hereford cows under their experimental conditions.

Carotene and Vitamin A Blood Plasma Levels in Calves One Month of Age.

Individual blood plasma carotene and vitamin A values from one month old Hereford and Angus calves are presented in table 9. Means of plasma carotene and vitamin A from the calves of the two breeds are indicated in table 10.

The differences noted between the two breeds, both of carotene and vitamin A were not demonstrated to be significant by an analysis of variance. No significant correlations between plasma carotene and vitamin A in the two breeds could be demonstrated.

Table 9 Individual blood plasma carotene and vitamin A values in Hereford and Aberdeen-Angus calves.

Calves one month

Herefords			Angus		
Calf No.	Plasma carotene	Plasma vitamin A	Calf No.	Plasma carotene	Plasma vitamin A
3	43.7	25.0	48	20.1	9.7
15	8.9	79.1	49	13.9	7.6
16	75.7	36.5	52	25.3	21.4
17	18.7	23.2	53	15.7	8.3
18	16.0	16.9	55	22.0	16.7
19	28.8	16.2	56	4.7	8.7
22	15.1	14.4	57	21.7	8.2
30	14.3	----	59	16.3	5.8
31	47.0	15.0	60	13.9	36.7
33	5.6	19.7	61	8.7	9.3
35	29.7	17.8	62	15.0	25.1
1	59.4	----	45	1.7	14.1
2	46.5	16.1	46	34.7	22.5
4	20.8	23.7	47	28.4	17.5
5	----	45.6	50	19.4	31.6
6	48.3	56.2	51	25.9	31.6
7	23.3	31.4	54	27.2	24.2
8	61.1	21.1	58	15.3	26.3
9	10.5	18.9			
10	14.9	8.9			
21	20.1	19.4			
23	20.8	29.1			
24	9.2	6.2			
25	4.7	9.5			
32	23.5	8.7			
34	2.5	22.2			
36	13.6	14.3			
37	29.9	6.8			
38	16.6	----			

Table 10 Means of blood plasma carotene and vitamin A in one-month old beef calves. Micrograms per 100 ml. of blood plasma.

Breed	Plasma Carotene	Plasma Vitamin A
Hereford	26.0 ± 7.32 [1]	23.1 ± 6.57
Angus	18.3 ± 4.20	18.1 ± 4.83

[1] Standard error of the mean, $P < .05$.

Colostrum Carotene and Vitamin A

Colostrum samples were obtained from twenty-five Hereford cows and from eighteen Angus cows. Tremendous variation may be noted in the individual values shown in table 11 and are exemplified by the large mean standard errors presented in table 12.

Table 11 Individual colostrum carotene and vitamin A in Hereford and Angus cows.

Herefords			Angus		
Cow No.	Colostrum carotene	Colostrum vitamin A	Cow No.	Colostrum carotene	Colostrum vitamin A
10	123.7	284.0	36	128.8	217.9
2-0	44.6	62.6	110	267.1	242.0
A-8	77.6	121.8	111	56.3	86.5
80	84.1	93.1	10	129.7	126.2
82	291.1	245.0	67	193.7	206.5
83	107.0	134.8	3	214.4	244.2
16-0	93.8	166.8	5	282.2	335.7
16P	192.9	228.7	29-0	96.5	259.0
10-0	214.5	379.2	2	100.7	87.0
4-0	221.9	-----	B-64	59.6	50.6
35	243.3	364.6	62	296.2	283.2
2	133.1	151.1	109	280.0	386.8
5	173.8	146.2	A-57	138.9	175.8
A-6	110.3	202.5	112	150.8	337.8
5-0	-----	-----	37	120.1	217.0
6-0	179.9	272.5	12	143.5	252.2
7	278.5	355.6	27-0	95.2	88.1
A-15	192.9	154.4	107	161.4	212.5
B11	168.5	321.9			
A-10	65.5	59.6			
B25	205.3	312.8			
20P	211.0	275.4			
A5	230.3	350.6			
69	89.3	202.6			
3	203.5	171.8			
4	116.8	69.8			

Table 12 Means of colostrum carotene and vitamin A. Micrograms per 100 ml.

Breeds	Carotene	Vitamin A
Herefords	162.1 ± 28.26[1]	213.6 ± 42.66
Angus	169.3 ± 42.53	219.3 ± 51.08

[1] Standard error of the mean, $P < .05$.

There was no significant difference in the carotene or vitamin A between the two breeds.

No significant correlations were obtained when colostrum carotene was calculated with offspring plasma carotene or when colostrum vitamin A was compared with calf plasma vitamin A.

Spielman, et al. (47, pp.343-350) and Hansen, Phillips and Smith (19, pp.809-814) similarly report large variations in colostrum vitamin A values in dairy cattle. Spielman, et al. observed mean standard errors approximating 40-50 micrograms per cent and Hansen, Phillips and Smith state that a seven fold variation existed in colostrum vitamin A values in cows on identical rations and under uniform conditions.

The possibility of calves of either breed involved in this study of developing typical symptoms of vitamin A deficiency, i. e. night blindness, diarrhea or unthriftiness, would seem rather remote because of the comparatively high blood plasma carotene values obtained. One would not expect severe vitamin A shortages in cows or calves that are on a high plane of carotene intake the majority of the year. The blood plasma carotene and vitamin A values of the Angus calves are well above levels considered to be marginal or deficient.

SUMMARY AND CONCLUSIONS

1. Blood samples from forty-four cows of Aberdeen-Angus and Hereford breeds were obtained prepartum and postpartum. Plasma from these samples was analyzed for carotene and vitamin A. Mean blood plasma carotene v alues observed prepartum were 207.0 micrograms per cent in the Angus cows and 209.0 micrograms in the Hereford cows. Mean postpartum plasma carotene values were 203.3 and 190.7, respectively. Mean prepartum plasma vitamin A levels were 53.6 and 55.9 in the Angus and Hereford cows, respectively. Postpartum plasma vitamin A values were 48.8 and 61.1 micrograms per cent in the Angus and Hereford cows, respectively.
2. Colostrum samples from Angus and Hereford cows were analyzed for carotene and vitamin A. Mean colostrum carotene values obtained in this study were 169.3 micrograms per cent in Angus cows and 162.1 micrograms per 100 ml. in Hereford cows. Vitamin A values obtained were 219.3 and 213.6 micrograms per cent in Angus and Hereford cows, respectively.
3. Vitamin A and carotene concentrations of blood plasma were obtained in calves of the cows studied at one month of age. Carotene values were 18.3 micrograms

per cent in Angus calves and 26.0 micrograms per cent in Hereford calves. Vitamin A levels were 18.1 and 23.1 micrograms per cent in Angus and Hereford calves, respectively.

4. A significant drop in blood plasma carotene postpartum in the Angus cows was observed in this study. This is in agreement with investigations on the effect of parturition in dairy cattle and may demonstrate a faster removal of carotene and vitamin A from liver stores of Angus cows than in Hereford cows.
5. A highly significant correlation between postpartum blood plasma carotene and vitamin A was noted in the Hereford cows.
6. Blood plasma carotene and vitamin A in one-month-old calves were not significantly correlated with carotene and vitamin A in the colostrum of their dams. Calves at one month of age are beginning to consume some green forage which may supplement the vitamin A and carotene in their blood.
7. The levels of carotene and vitamin A noted in this study in the Hereford and Aberdeen-Angus cows and in their calves are well above values reported as marginal or deficient. While Angus calves had slightly lower carotene and vitamin A blood plasma levels than

Hereford calves, statistical significance of the difference could not be demonstrated. It would appear that visual impairment in the Angus calves studied cannot be attributed to low blood vitamin A.

8. Plotting the distribution of the levels of blood plasma carotene of the cows and calves of the two breeds indicated that there might be a bimodal distribution in these values. No such distribution could be demonstrated in the vitamin A levels of cows of either breed. This would suggest that the population studied was not uniform.

9. Since the liver is the principle storage site of vitamin A, investigations of vitamin A and carotene values of this organ may be used to supplement blood plasma information.

10. The wide variations in concentrations of the items studied among animals of the same breed observed in this investigation have been noted in the literature by other workers in this field. Fluctuations of this type coupled with small numbers of experimental animals resulted in large mean standard errors and consequent difficulty in demonstrating significance of differences observed.

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APPENDIX A

Angus Calf B67 Blind. Male.

Calf killed: 12/4/52 3:00P.M.

Notes made: 12/5/52 11:00 - 4:00 P.M.

5:00 - 6:00 P.M.

12/6/52 8:00 - 9:00 A.M.

12/7/52 10:00 A.M.

12/5/52: Right eye

Marked white opacity in center of cornea.

Palpation indicates a one mm. concretion in the opacity.

Black thread in center of white.

Thread angles at 90°

Lens clear. No opacity.

Vitreous humour: clear, no opacity

Vitreous more adherent then left.

On cutting through eye:

Cornea: thick. Opaque as above.

Left eye

Cornea grey-white throughout.

Whiter in center.

Softer than right eye

No hard spot in center of cornea

Black line through white area

One mm. wide at medial and thinning to a line at the lateral end.

Lens clear. No opacity.

Vitreous: clear, no opacity

Vitreous more friable and stringy than right.

	Cornea thick and opaque. Difficult to decide whether right or left is thicker. Check this item histologically.
3:30-4:00 P.M. Retina: Most retinal area black. About 1/4 of area of retina blue white at the posterior pole. More blue than similar area of left eye. Brown pigment between blue area and black area.	About 1/4 of retinal area at posterior pole blue white. Whiter than right eye.
	Very little brown pigment at light area-dark area border
5:00 P.M. The light blue-white area had turned dark blue after direct exposure of inverted retina to light for one hour.	white area turned dark blue on exposure to light.
Sample of retina placed in Bouins showed disappearance of light area i.e. conversion into dark blue.	Light area of retina disappeared in bouins.

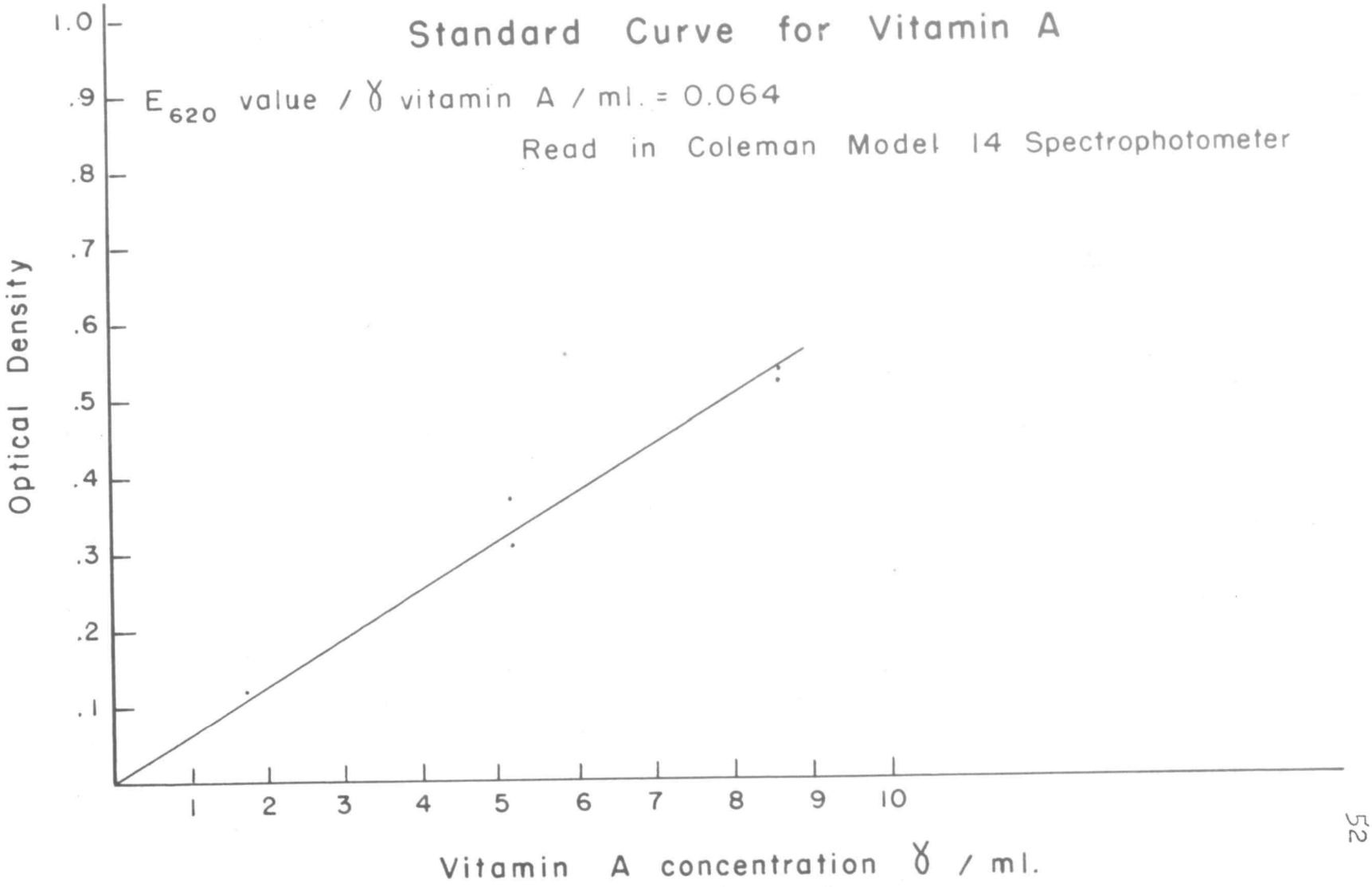
Both eyes: In Bouins the light area (possible some other area) became oedematous after one hour.

Both eyes: After exposure in air for one hour striations were visible in the blue areas of both eyes.

12/6/52 Retinas of both eyes had turned very dark blue black. All traces of light areas had disappeared.

Samples of half of left and half of right eye(without lens, vitreous or aqueous humor) in Bouins. Lens of right and left eyes placed in Bouins fluid.

APPENDIX B


Standard Curve for Vitamin A
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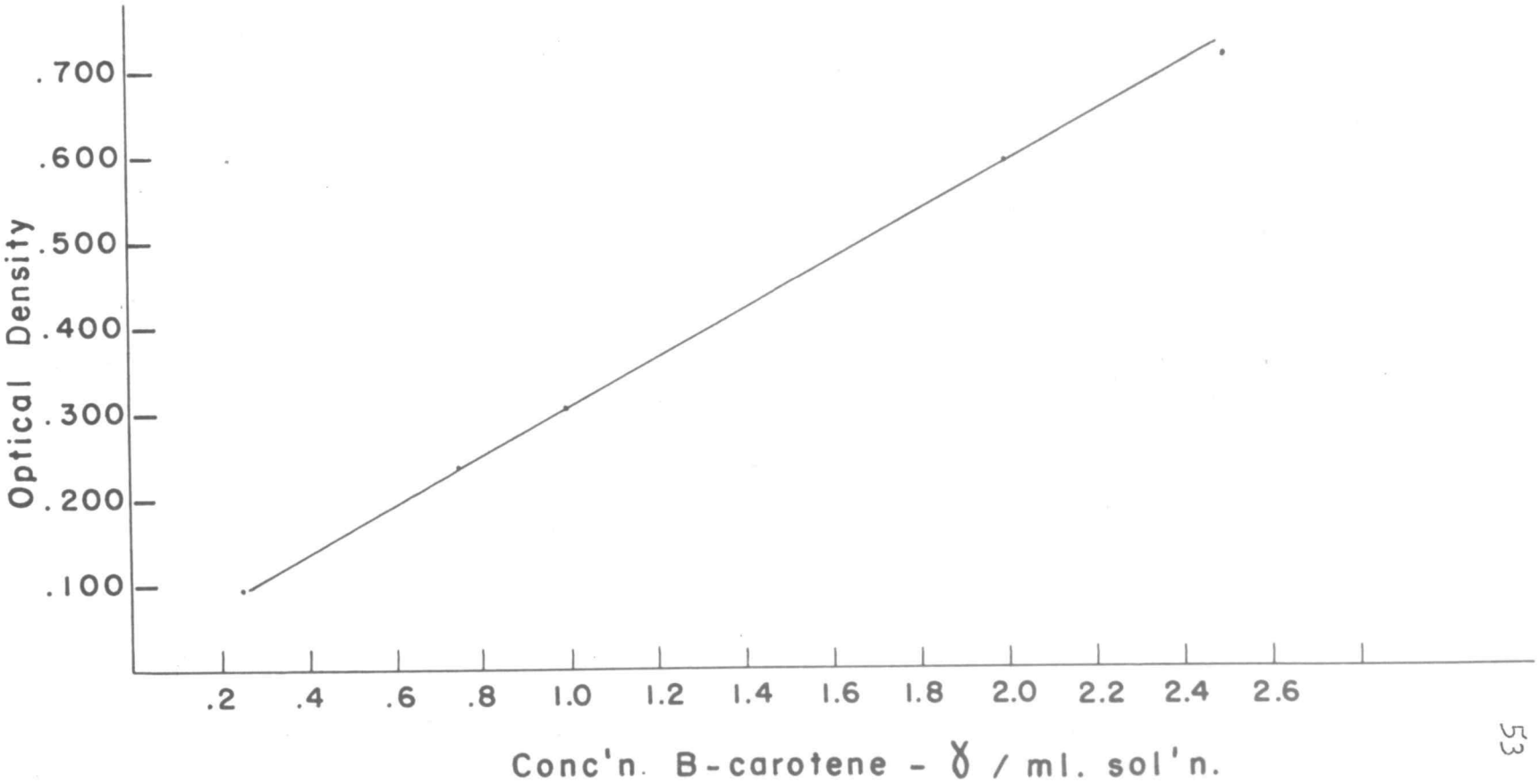
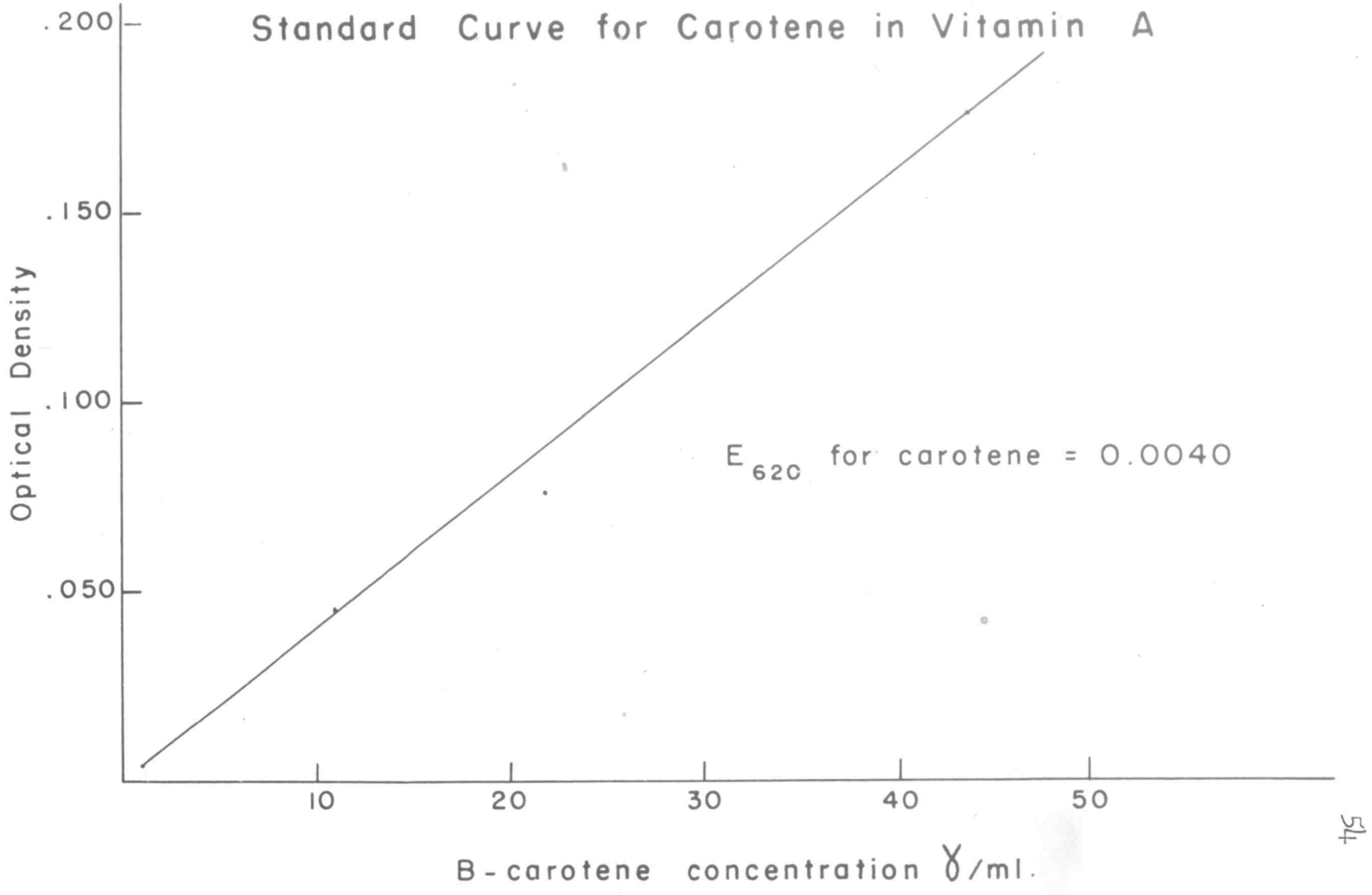
Read in Coleman Model 14 Spectrophotometer
Optical Density
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
1
2
3
4
5
6
7
8
9
10
Vitamin A concentration γ / ml.

Standard Curve for B-Carotene

Run with Hoffman-La Roche pure B-carotene supplied by G. A. Richardson

E_{440} det'nd = 0.307




Standard Curve for Carotene in Vitamin A
.200
.150
.100
.050
Optical Density
E_{620} for carotene = 0.0040
10
20
30
40
50
B-carotene concentration γ/ml.