THE UTILIZATION OF INTRAVENOUSLY ADMINISTERED CAROTENE BY DAIRY CALVES

By

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BY DAIRY CALVES

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INTRODUCTION

Within a relatively recent period of time it has been shown in several species of higher animals that the wall of the small intestine acts as the primary site for the conversion of carotene to vitamin A. However, the possible existence of other or secondary sites of carotene metabolism has been indicated with several species.

In support of such extra-intestinal sites several workers have shown an apparent species difference between sheep and cattle with respect to their ability to convert intravenously administered carotene to vitamin A. These studies, including two at this experiment station, were primarily confined to short time observations of blood changes of vitamin A for estimating the rate of conversion. Due to the inconsistent increases of this vitamin in the blood of cattle after injection considerable controversy has developed concerning the ability of these animals to utilize carotene normally circulating in their blood.

This study was undertaken to determine whether or not a large amount of intravenously administered carotene given over a period of time would (1) affect the blood vitamin A values of depleted dairy calves; and (2) result in any improvement in the condition of calves showing definite clinical avitaminosis A symptoms.

REVIEW OF LITERATURE

In 1913 McCollum and Davis (31) reported the discovery of a necessary dietary growth factor in eggs and milk, which they referred to as the fat soluble A factor. The letter A was later prefixed with the term vitamin and the factor became known as vitamin A. After the extensive investigations of Steenbock (44) beginning in 1919, considerable evidence pointed to a relationship between the yellow carotenoid pigmentation of vegetable matter and vitamin A activity.

A chemical basis showing the relationship between vitamin A and its precursor was finally established in 1930 by Karrer and his associates (23). During this same period of time, Moore (36) obtained biological proof of this association by showing that carotene administered orally to rats was convertible to vitamin A in the body of the rat. Finding the greatest concentration of the vitamin to exist in the liver (37) he assumed that because of its many other important metabolic functions this organ was the site of the transformation of carotene to vitamin A within the body.

Though this theory was accepted for almost 15 years, the evidence presented during this time by various workers (1, 12, 38, 39, 52) was conflicting and unsatisfactory in confirming the liver as the site of conversion. In 1936, Verzar and McDougall (51) proposed the intestinal mucosa as the possible site of conversion but no definite evidence was obtained in support of this hypothesis until 1947 when

the answer came almost simultaneously from three separate groups of workers. Glover, Goodwin, and Morton (18) found appreciable amounts of vitamin A in the intestinal wall of rats which had previously been depleted of their vitamin A stores and then fed considerable amounts of carotene. Matterson, Mehl, and Deuel (30) reported that the oral administration of a carotene solution to vitamin A depleted rats resulted in a increase in the vitamin A content of the intestinal wall approximately one hour before it appeared in the liver, where the level remained lower than the intestinal level for almost four hours. Thompson, Ganguly, and Kon (48) stated that the administration of carotene to fasting vitamin A deficient rats was followed by the appearance of vitamin A in the intestinal wall in quantities larger than in the liver or circulating blood. They also found that pigs dosed with a carotene solution just before slaughter showed a higher vitamin A concentration in their blood plasma, mesenteric lymphatics and intestinal wall than did control pigs.

Numerous other workers immediately confirmed the intestine as a site of conversion by applying both *in vitro* and *in vivo* experiments on several species of animals. *In vitro* transformation was demonstrated in the small intestine of the rat by Weise, Mehl, and Deuel (54), in sheep by McGillivary (32), and in dairy calves by Stallcup and Herman (43). *In vivo* intestinal conversion has been shown in the rat by Alexander and Goodwin (3), and Mattson (29); in rats and pigs by Coates, Thompson and Kon (10), and by Thompson and co-workers (45, 46, 48, 49); in sheep by McGillivary (32) and Klosterman and co-workers (26); in sheep, goats and rabbits by Goodwin and co-workers (19, 20); in chickens by Thompson, Coates, and Kon (47) and Cheng and Deuel (8); and in dairy calves by Elliott (15).

That a difference might exist as to the sites of carotene conversion between different species became apparent when it was noted that rats (3), (49), rabbits (20), pigs (10), (49), sheep and goats (19), normally did not have any carotene in their blood while dairy cattle (15) exhibited large quantities of carotene in their circulatory system. It was recently shown by Ganguly, Mehl, and Deuel (16) that animals fall into at least four groups insofar as their ability to absorb and deposit the different carotenoids within their body.

The actual existence of sites of conversion other than the intestine has been indicated by the apparent effective utilization of parenterally administered carotene by some animals. Early work concerning the intravenous, intramuscular or subcutaneous injections of carotene solutions was contradictory and inconclusive. Ahmad, Grewal and Malik (2) and Vinet, Plessiner and Raoul (52) reported positive conversion with the intravenous injection of a colloidal suspension of carotene to vitamin A deficient rabbits. The latter workers, however, were unable to demonstrate such conversion in rats and dogs. Phillips and Bohsted (40) observed that large doses of subcutaneously injected carotene suspended in water were effective in improving deficiency conditions of vitamin A depleted rabbits but small doses were ineffective. Drummond and co-workers (11, 12, 13) were unable to obtain any conversion in rats, rabbits or cats by way of the intravenous route and stated that carotene introduced directly into the circulation in the form of aqueous, collodial suspensions was rapidly removed from the blood stream like any other foreign material by the Kupffer cells of the liver. Leese, Leese, Steenbock and Baumann (28), and Sexton, Mehl and Deuel (42) reported inefficient utilization of

parenteral administered aqueous or oil colloidal carotene solutions to rats since deficiency symptoms and death occurred even though considerable amounts of carotene were still present within the body.

Some of these discrepancies between the results of different investigators has been indicated to be due to the difference in the type of solvent and physical state of the carotene dispersion used. Tomarelli, Charney and Bernhart (50) reported that carotene solubilized in water with an active surface agent in the form of Tween 80 (polyoxyethylene-sorbitan monoleate) and injected intramuscularly could serve rats as an effective source of vitamin A while carotene dispersed in sesame oil was very poorly utilized. In the former solution the carotene is dissolved in an appropriate solvent such as chloroform and then dispersed in the Tween. This reduces surface tension producing a clear solution that remains stable when diluted with water. This was further confirmed by Hentges and Sorenson (22) who found that the intravenous and intramuscular injection of the above aqueous carotene solution into vitamin A deficient pigs resulted in the relief of avitaminosis A symptoms, while similar injections of carotene suspended as a colloidal solution in cottonseed oil failed to relieve deficiency symptoms. In order to rule out the possibility of the injected aqueous carotene returning to the intestinal site of conversion the anterior, posterior and colliac arteries were ligated in addition to cannulation of the common bile duct. The fact that these measures did not prevent the parenterally administered carotene from being converted to vitamin A strongly indicated the existence of secondary sites of conversion in the pig.

Bieri (4) injected an aqueous solution of carotene dispersed in Tween 40 (polyoxyethylene-sorbitan monopalmitate) intramuscularly into vitamin A deficient rats after surgical removal of the small intestine and found appreciable amounts of a vitamin A like substance in the blood serum. Later Bieri and Pollard (6) indicated further the existence of extra-intestinal sites of conversion in the rat by showing that the formation of vitamin A from injected aqueous Tween carotene occurred essentially unimpared after ligation of the bile duct, removal of the small intestine or kidneys or removal of 60-75 per cent of the liver. In more recent investigations McGillivary, Thompson and Worker (34) and Worker (55) (56) have added further conclusive evidence of extra-intestinal conversion by applying more extensively the methods used by Bieri. They have found with the rat that the formation of vitamin A from carotene administered intravenously in an aqueous Tween dispersion was unaffected by decapitation of the head, complete removal of the lungs, liver, stomach, small intestine, large intestine, pancreas, kidneys, adrenals, and gonads. The former workers (34) have demonstrated further that such conversion was little affected by the activity of the thyroid gland. Worker (55) has also shown that conversion is not adversely affected by complete removal of the liver in rabbits. From such results it has been concluded by various experimenters that, at least in the rat, the ability to convert injected carotene into vitamin A is not a function of any one but of several organs or tissues. McGillivary and associates (33) suggested that the intravenous conversion results primarily from a random oxidation of the carotene, the initial stages of which may occur in the blood.

Recently Bieri (5) has reported that vitamin A deficient chicks and rabbits successfully converted intravenously administered carotene to vitamin A which appeared in the liver and serum. Kon, McGillivary and Thompson (27) have also reported the conversion of intravenously administered aqueous Tween carotene solution by rabbits in addition to rats. However, they were unable to show any evidence of conversion by the injection of carotene dispersed in oil or in water without Tween. Of late, however, McGillivary and associates (35) have confirmed the work of Greenberg and co-workers (21) by demonstrating the appearance of vitamin A in the rat after intravenous administration of a specially prepared aqueous emulsion of carotene without the use of surface-active agents such as Tween. They reported that the conversion was effected in a similar manner; that is, the vitamin A appearing first in the blood as the alcohol which was subsequently esterfied by the liver, but not as efficiently as when injected as aqueous Tween dispersions. In contrast they found that goats injected with the same type emulsion did not show any significant increase in plasma vitamin A.

Bieri and Sandman (7) found that carotene in oil is not utilized intravenously and that for maximum growth in rats approximately 4 to 6 times as much aqueous Tween carotene is required parenterally as orally. Somewhat perplexing then is the recent report by McGillivary and Worker (33) that a carotene Tween dispersion was equally well utilized whether injected intravenously, intraperitoneally or given orally as indicated by increased hepatic vitamin A levels in rats. Intramuscular injections were less efficiently utilized while subcutaneous injections failed to increase liver vitamin A stores. They were unable to demonstrate any increase in plasma vitamin A following the intravenous injection of the solution into goats.

Klosterman, Bolin and Light (26) were unable to find any conversion after injecting aqueous Tween carotene or carotene suspended in cotton-seed oil into the veins of sheep. Contrary to this, Church, MacVicar and Bieri (9) found that sheep were able to convert the intravenously injected aqueous Tween 40 (polyoxyethylene-sorbitan monopalimtate) carotene solution into vitamin A rather rapidly. This work has been confirmed by Kirschman (25) who obtained significant increases in plasma vitamin A following the intravenous injection of aqueous Tween carotene in goats and ileoctomized sheep.

Carotene solubilized in an aqueous Tween solution and injected parenterally has apparently been utilized successfully as a source of vitamin A by several species of animals. However, the ability of cattle to utilize this solution when administered intravenously has been questionable. Although both Church and his co-workers (9) and Kirschman (25) were able to show conversion in sheep, they were unable to show any conversion when the same type solution was injected into Hereford, Guernsey, or Holstein calves.

Church (9) injected seven vitamin A deficient Hereford calves weighing between 150-200 lb. with single injections containing 13.7 mg. to 17.0 mg. of carotene each. The analysis of blood samples and liver biopsies taken before and after injection when correlated with clinical deficiency symptoms did not reflect any significant conversion of carotene to vitamin A in any of the calves.

Kirschman (25) depleted and then injected three Holstein and five Guernsey calves, having an average weight of 355 lb, with single injections containing between 0.23 mg. to 0.86 mg. of carotene per kg. of body weight. Blood samples taken at various intervals over a nine day per-

iod did not show any positive indication of carotene conversion to vitamin A. Similar intravenous injections to three undepleted calves and five alternate day injections containing 48 mg. of carotene each to one Guernsey calf did not result in any appreciable increase in plasma vitamin A. However, two calves receiving direct injections of the Tween dispersion containing 90 mg. of carotene into the duodenum showed increases in plasma vitamin A.

Kon, McGillivary and Thompson (27) were unable to show any appreciable conversion with the injection of colloidal carotene or carotene solubilized in Tween to depleted Ayrshire and Shorthorn calves. Each of three calves was injected on three occasions at intervals of 6-10 days with 10 ml. of aqueous Tween containing 10 mg. of carotene. Three additional calves received a single injection of 10 ml. of the same solution. A small but reproducible increase in vitamin A alcohol occurred after each injection but it was considered too limited to be of value to the calf for preventing vitamin A deficiency. In contrast the oral administration of aqueous Tween carotene produced marked increases in plasma vitamin A.

Elliott (15) injected dairy calves intravenously with large amounts of carotene rich plasma but did not obtain any positive evidence of conversion by this method. On the other hand Eaton and associates (14) and Warner and Maynard (53) found that intravenously injected aqueous carotene was utilized by Guernsey and Holstein calves.

Eaton and co-workers (14) depleted six Holstein and six Guernsey calves and then divided them with restriction as to breed and sex into the following treatment groups: 1. no treatment, 2. intravenous and 3. oral administration of carotene dispersed in an aqueous Tween sol-

ution. All calves had diarrhea and exhibited muscular incoordination and convulsive attacks during the experiment. The carotene was administered twice daily for six days either by injection or orally in gelatin capsules at the rate of 120 mcg. per lb. of body weight. Injected carotene resulted in higher blood plasma levels of carotene and vitamin A than orally administered carotene. Spinal fluid pressures as well as degree of diarrhea, muscular incoordination and convulsive seizures were decreased in both groups receiving carotene.

Warner and Maynard (53) depleted four 300 lb. Holstein bull calves until distinct deficiency symptoms were apparent. Two calves received 40 daily injections of 5.4 mg. of carotene stabilized in an aqueous 10% coconut oil solution with 2% intravenous gelatin. Two other calves received the same number of injections of 0.95 mg. of vitamin A alcohol in the same solution. No beneficial effects were noted from the carotene injections but the calves receiving vitamin A improved noticeably. In contrast to these results, however, aqueous colloidal carotene proved beneficial in a second trial. Six 300 lb. Holstein calves were depleted and then injected for six straight days with an aqueous colloidal suspension of carotene at the rate of 30 mg. per 100 lb. of body weight. They stated that a statistically significant increase in plasma carotene and vitamin A was observed as estimated by three independent analytical procedures.

The existing evidence seems to show definitely that the intestinal wall is the main site of conversion of carotene to vitamin A. However, the apparent effective utilization of parenterally administered carotene by some species suggests the possibility of some secondary site or sites of conversion in addition to the intestine in some animals.

EXPERIMENTAL PROCEDURE

This experiment was conducted to study the ability of dairy calves to convert intravenously administered carotene to vitamin A. Calves were depleted until their blood vitamin A values were 5 mcg. or less per 100 ml. of plasma for a period of 12 or more consecutive days. They were then injected intravenously at varying intervals with carotene suspended in an aqueous solution of Tween 40 (polyoxyethylene sorbitan monopalimtate). Blood samples were obtained periodically after injection and analyzed for carotene and vitamin A.

Early observations following the injection of several calves indicated that other criteria in addition to blood vitamin A level would be helpful in establishing any possible conversion of the injected carotene to vitamin A. To study the physiological value of the injected carotene, calves were depleted until clinical symptoms of avitaminosis A were established. Carotene was then injected intravenously and the response of the animals in terms of the remission of avitaminosis A symptoms was observed.

Clinical deficiency symptoms included severe scours, muscular incoordination, poor physical appearance and ocular changes such as papilledema, exopthalmia and nyctalopia. Tests were made so rigorous that there could be no reasonable doubt about the remission of any clinical symptom. Scouring was considered as a symptom only after the

condition had persisted continuously in a mild-to-severe form for several days. Muscular incoordination was accepted as a symptom when a calf showed poor control of his hind legs and would stagger and lose his balance. The general physical condition of each animal was classified as good, fair or poor according to daily observations and weekly live weight changes. Papilledema was observed with the aid of an opthalmoscope. At various intervals the animals were tested for nyctalopia by observing their reactions in dim light (evening) to objects placed in their path.

Purebred male Holstein, Ayrshire, Guernsey and Jersey calves not needed for future service were obtained from the college dairy herd for this study. The calves were tied in individual solid-partitioned stalls located in a heated and ventilated barn. Wood shavings were used for bedding. The normal diet for the calves consisted of whole milk, a calf starter and prairie hay. The whole milk was fed twice daily at the rate of 10% of live weight up to a maximum of 10 lb. per day for each calf. The standard calf starter was fed according to appetite up to a limit of 4 lb. daily. Prairie hay was fed ad libitum. The calves were depleted of their carotene and vitamin A stores by a diet deficient in those two materials. The roughage portion of the diet consisted of a mixture of 40 lb. of wood cellulose[1], 20 lb. of cottonseed hulls and 60 lb. of molasses. These ingredients were mixed thoroughly and then passed through a roller blender. The concentrate portion was a combination of equal parts of 41% cottonseed meal and white hominy feed plus 1% each of steamed bonemeal, finely ground limestone and

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[1]Solka-Floc, an acid-treated wood pulp cellulose.

trace mineral salt. Skimmilk was fed twice daily at the rate of 10% of live weight to a maximum of 10 lb. daily. As the consumption of roughage and concentrate increased the milk was appropriately removed from the diet. Approximately 25,000 to 50,000 I. U. of vitamin D was administered orally at two-week intervals.

Some calves were fed a normal diet for short periods before being placed on the depletion diet where as other calves were placed directly on the depletion diet. The dietary regimen for each calf is described in its case history.

The carotene solution wherein Tween 40 acted as the dispersing agent was prepared as described by Bieri (4). Crystalline carotene (90% beta and 10% alpha) was dissolved in about 2 ml. of warm chloroform and then an adequate portion of Tween 40 at 100° C. was added rapidly with constant stirring. Heating was continued for about one minute or less to drive off the excess chloroform. The solution was brought to near the desired volume with distilled water at 80° C. The mixture was then transfered to a brown volumetric flask and brought to the correct volume at room temperature with a small amount of distilled water. The solutions were kept in the dark and were made up frequently to avoid prolonged storage.

The amounts of carotene administered intravenously were based on the National Research Council (41) recommended daily dietary intake of 6 mg. of carotene per 100 lb. live weight and on results of previous work. All of the carotene injections were made directly into the jugular vein; the amounts injected, number of injections, and length of time between injections were adjusted according to the increase in blood carotene and the response of the animal following each injection.

Blood samples were drawn from the jugular vein into tubes containing lithium citrate as the anticoagulant. The samples were centrifuged immediately and placed in a refrigerator at 5-10° C. for not more than one week until analysis could be made. Blood analyses for vitamin A and carotene were made according to the method of Kimble (24).

Autopsies were performed on calves that died during the experiment and samples of bile and liver were obtained. Bile was analyzed both chemically and biologically for the presence of carotene. Liver samples were analyzed for carotene and vitamin A to determine the effectiveness of depletion of this organ in negative control animals, and for comparison with animals that had been injected with carotene. The samples were stored in a deep freeze until analyzed by the method developed by Gallup and Hoefer (17).

RESULTS

The results for each calf are presented in individual case histories. The age of the calf and the diet received are given at the beginning of each case history. This is followed by a description of clinical deficiency symptoms, the condition of the animal and blood levels of vitamin A and carotene before and during the time of each series of carotene administration. The rate of carotene administration, length of each injection series and the time blood samples were taken are given for each series of injections. The plasma vitamin A and carotene values for each series of injections are represented graphically with each case history. Complete plasma vitamin A and carotene values for each calf for the entire experiment are listed in the appendix.

Jersey calf number 71 was 78 days of age and was receiving a normal dry ration when placed on the vitamin A deficient diet. After 72 days on the deficient diet the plasma vitamin A and carotene levels had decreased to approximately 4 mcg. and 25 mcg. per 100 ml., respectively. At this time the calf weighed 200 lb. and did not show any clinical symptoms of vitamin A deficiency. Carotene was then injected at the rate of 24 mg. per 100 lb. live weight every day for 20 days. Blood samples were taken on alternate days. A total of 495 mg. of carotene was injected during the 20 day injection period.

As shown in Figure 1, plasma vitamin A increased about 20 mcg. and plasma carotene increased 735 mcg. per 100 ml. during the period of injection. Vitamin A decreased sharply following the sixth injection and after the last injection the vitamin A values became very erratic. On two successive days the values were masked by the correction factor applied for the blue color produced in the presence of carotene. Plasma carotene began to level off after the seventh injection and decreased with the last injection.

Eighty-seven days after the last injection the calf showed deficiency symptoms including nyctalopia, exophthalmia, muscular incoordination and a poor physical condition. Vitamin A and carotene levels had decreased to approximately 3 mcg. and 31 mcg. per 100 ml. of plasma, respectively. The calf now weighed 230 lb. During the next 37 days he received a total of 637 mg. of carotene in 12 injections. The schedule of administration was as follows: two injections, 28 mg. each, four days apart; six injections, ranging from 57 to 59 mg., each at four day intervals; four injections, 60 mg. each at two day intervals. This represents from 12 to 24 mg. per 100 lb. live weight. A blood

sample was taken prior to each injection. Carotene was injected at the lower level to determine if high plasma carotene was interfering with the determination of plasma vitamin A. The amount was gradually increased in an attempt to reverse the deficiency symptoms.

By the third injection the deficiency symptoms had increased in intensity and the calf was almost completely blind. A stigma had developed in the center of the right eye and a white cloudy condition had appeared in the left eye. Following the fifth injection the stigma and cloudy condition of the eyes disappeared and day vision appeared to be normal. After the ninth injection there was no evidence of muscular incoordination and the physical condition was improved. At the end of the injection period the physical condition was good but papillidema and complete night blindness still existed. Vitamin A increased about 15.5 mcg. and carotene 357 mcg. per 100 ml. of plasma.

The possibility that the symptoms had proceeded beyond the point of reversion prompted the oral administration of several large (200,000 I.U.) doses of vitamin A alcohol in capsule form. Following this administration plasma vitamin A increased sharply to 44 mcg. per 100 ml. Within a few days the calf had normal night vision but the conditions of exophthalmia and papillidema improved only slightly during the next several weeks. The calf had been maintained on the deficient diet 220 days, supplemented at intervals with intravenous injections of carotene before any vitamin A was administered orally.

BLOOD PLASMA CAROTENE AND VITAMIN A JERSEY NO. 71

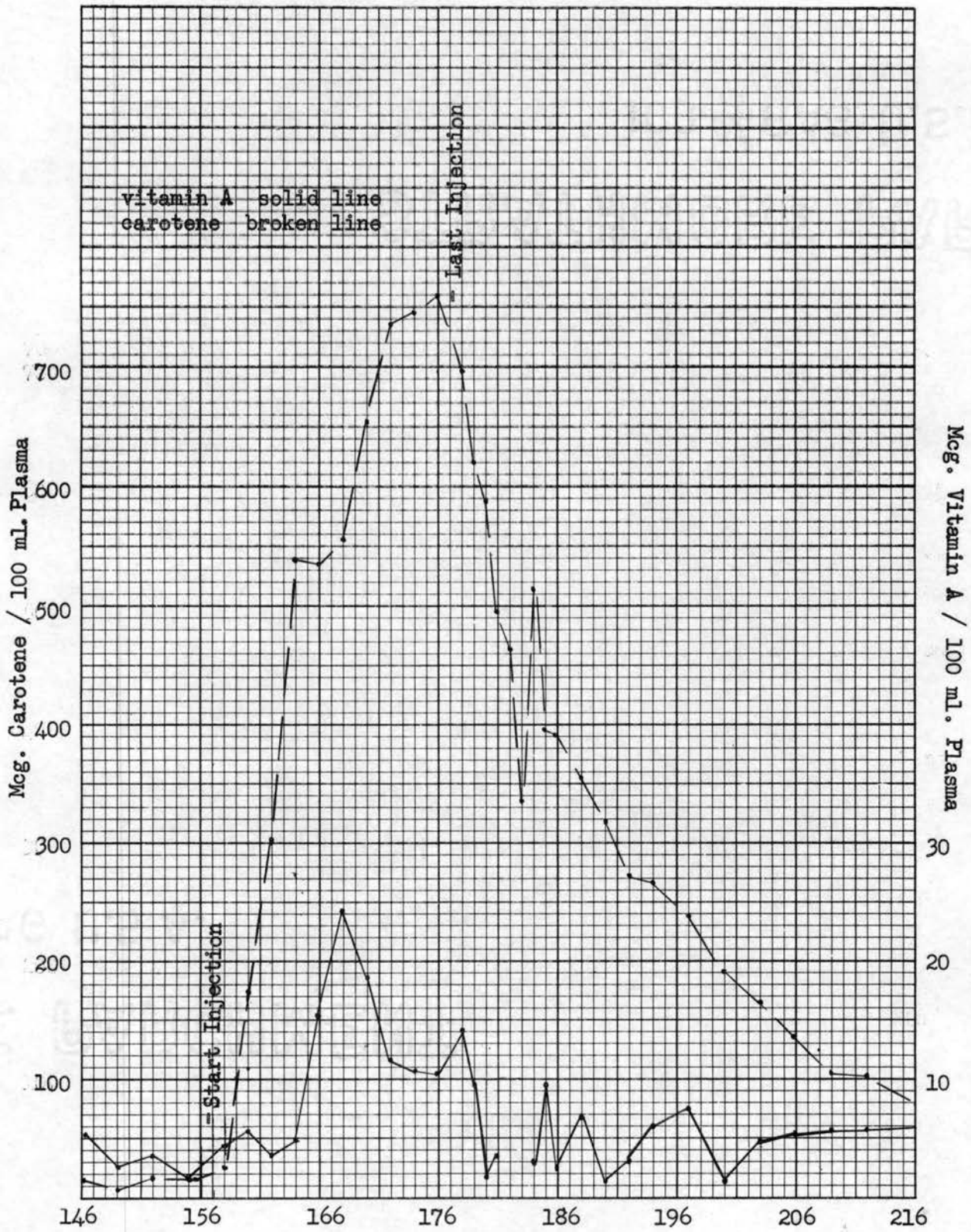


Fig. 1

AGE, da.

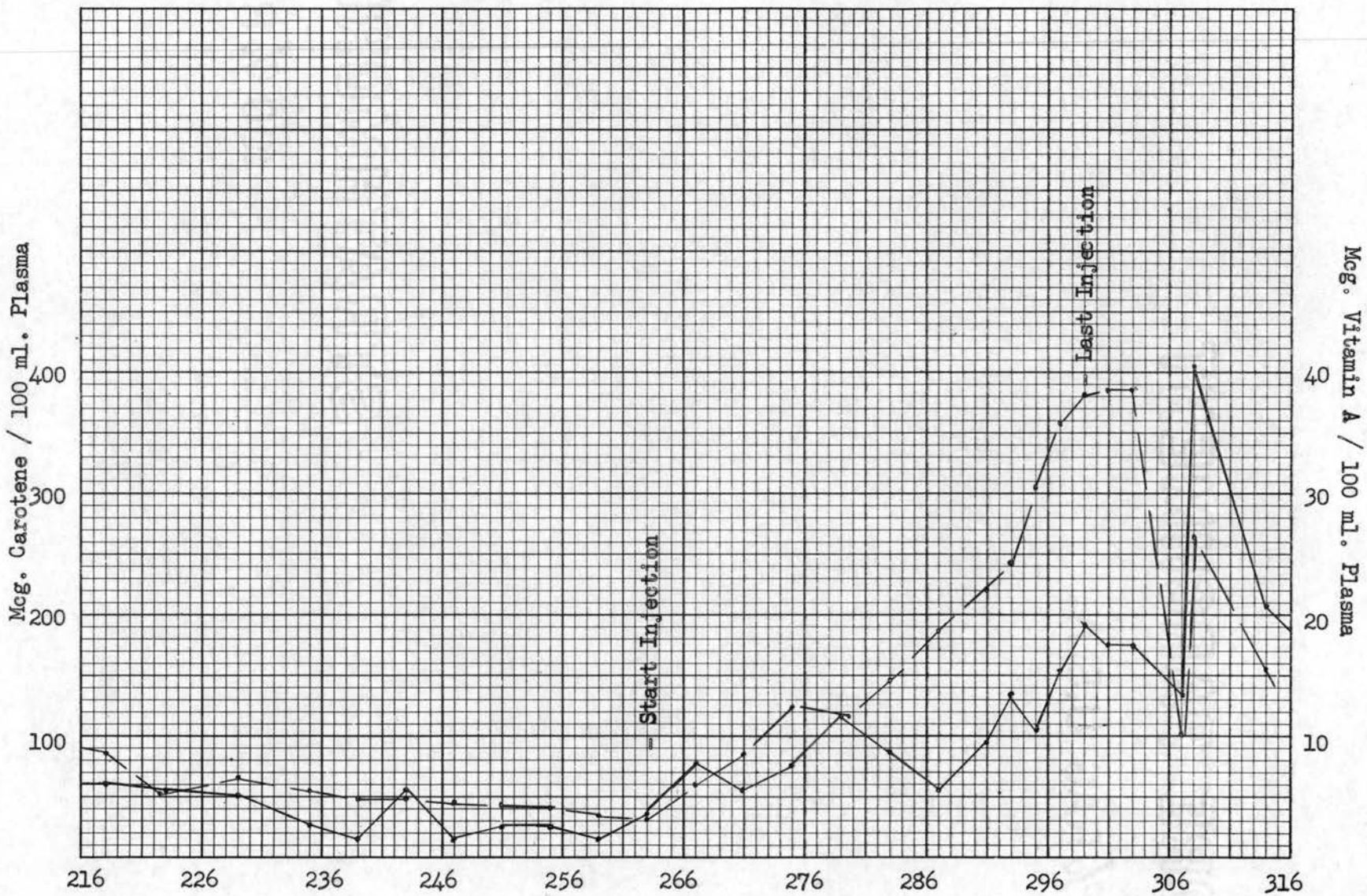

Mcg. Carotene / 100 ml. Plasma
400
300
200
100
Mcg. Vitamin A / 100 ml. Plasma
40
30
20
10
Start Injection
Last Injection
216
226
236
246
256
266
276
286
296
306
316
AGE, da.

Fig. 1 (contd.)

Ayrshire calf number 118 was placed on trial 7 days after birth. He received a normal diet including whole milk for 36 days after which he was gradually changed over to the depletion diet. Carotene injection was started after 36 days on the deficiency diet when his blood values for vitamin A and carotene decreased to approximately 4 mcg. and 19 mcg. per 100 ml. of plasma, respectively. He weighed 135 lb. and did not show any definite vitamin A deficiency symptoms.

A total of 364 mg. of carotene was then injected at the rate of 24 mg. per 100 lb. live weight every other day for 20 days, blood samples being taken on alternate days. Plasma vitamin A and carotene changes are shown in Figure 2. Plasma vitamin A increased to a maximum of approximately 10 mcg. and plasma carotene increased 725 mcg. per 100 ml. After the last injection the vitamin A values became very irregular and two of the values were masked by the carotene correction factor.

Sixty-three days after the last injection blood vitamin A and carotene had decreased to about 2 mcg. and 33 mcg. per 100 ml. of plasma, respectively. During this time a severe condition of exophthalmia developed but no other signs of vitamin A deficiency were apparent.

The calf weighed 197 lb. and the physical condition was good. He was injected 14 times with a total of 340.5 mg. of carotene at the rate of 12 mg. per 100 lb. live weight every four days, blood samples being taken immediately before each injection.

At the end of the injection period the exophthalmic condition had improved but was still present. Plasma vitamin A increased about 12 mcg. and plasma carotene 120 mcg. per 100 ml. of plasma. After the last injection plasma carotene gradually decreased while plasma vitamin A dropped slightly and then increased to a high of 16 mcg. per 100 ml. of plasma.

Sixty-four days after the last injection of the second period the calf showed symptoms of mild night blindness and 16 days later developed complete night blindness, complicated by an exophthalmic condition. After 93 days without carotene the vitamin A was variable at approximately 3 mcg. and the carotene 18 mcg. per 100 ml. of plasma. The calf weighed 301 lb. and its physical condition was good.

He was then injected three days in succession at the rate of 6 mg. of carotene per 100 lb. live weight. On the third day of injection the calf was classified as only slightly night blind and four days later his night vision was normal, but the exophthalmic condition was still present.

Vitamin A increased about 12 mcg. and carotene 58 mcg. per 100 ml. of plasma. During 72 days after injection the calf did not show any definite vitamin A deficiency symptoms other than the exophthalmia and this condition improved. The physical condition appeared good and the calf was removed from the experiment after being maintained on the completely deficient diet for 336 days with periodic intravenous injections of carotene.

BLOOD PLASMA CAROTENE AND VITAMIN A AYRSHIRE 118

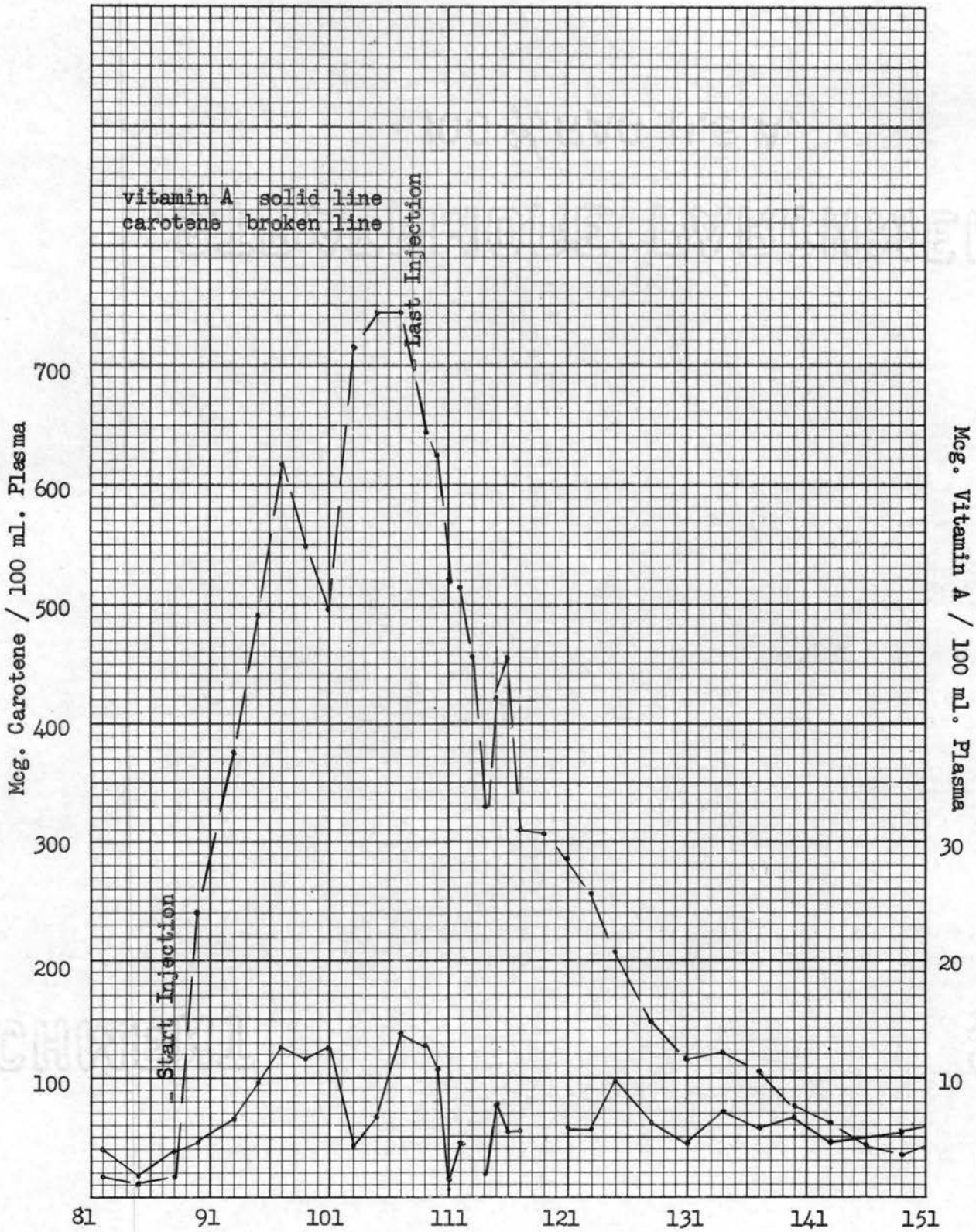
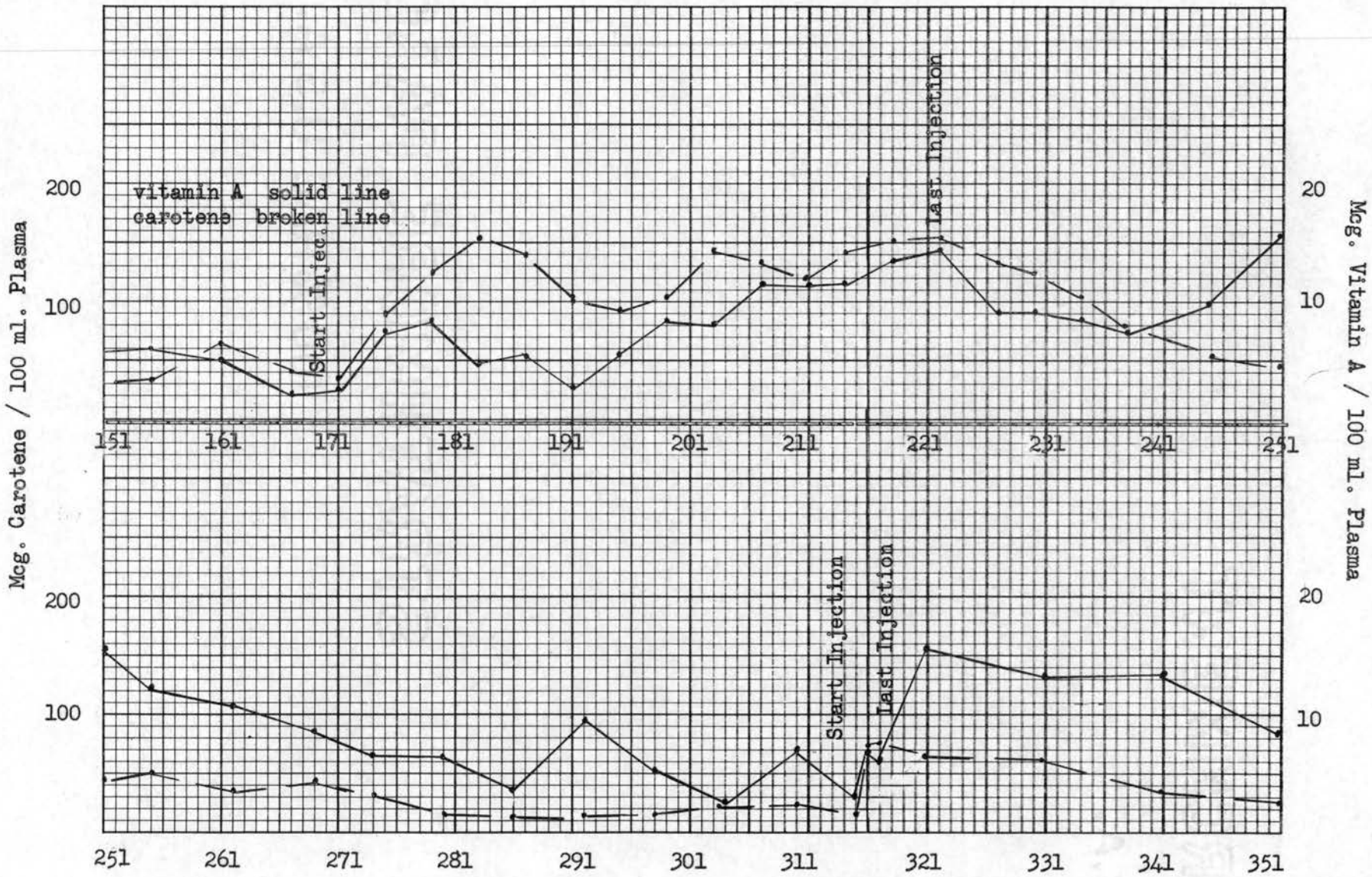


Fig. 2


vitamin A solid line
carotene broken line
Start Inject.
Last Injection
Start Injection
Last Injection
Mcg. Carotene / 100 ml. Plasma
Mcg. Vitamin A / 100 ml. Plasma
200
100
20
10
151
161
171
181
191
201
211
221
231
241
251
261
271
281
291
301
311
321
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341
351
AGE, da.

Fig. 2 (contd.)

Holstein calf number 8 received a normal diet for 30 days before being placed on the depletion diet. After 66 days on the deficient diet his blood levels of vitamin A and carotene were less than 2 mcg. and 15 mcg. per 100 ml. of plasma, respectively. The calf weighed 185 lb. and did not show any signs of vitamin A deficiency. A total of 407 mg. of carotene was then injected at the rate of 12 mg. per 100 lb. live weight every other day for 32 days. Blood samples were taken on alternate days and these values are shown in Figure 3.

Plasma vitamin A increased approximately 12 mcg. and plasma carotene 438 mcg. per 100 ml. Immediately following the sixteenth inject- the calf went into a state of shock and gasping for breath died within a few minutes. An autopsy failed to show the cause of death. Liver vitamin A was 52.2 mcg. and carotene 56 mcg. per 100 g. fresh weight. The bile had an orange color instead of the characteristic green color. This suggested a high concentration of carotene however analysis failed to show the presence of carotene. The calf had been on the deficient diet 96 days at the time of death.

BLOOD PLASMA CAROTENE AND VITAMIN A

JERSEY NO. 6 HOLSTEIN NO. 8

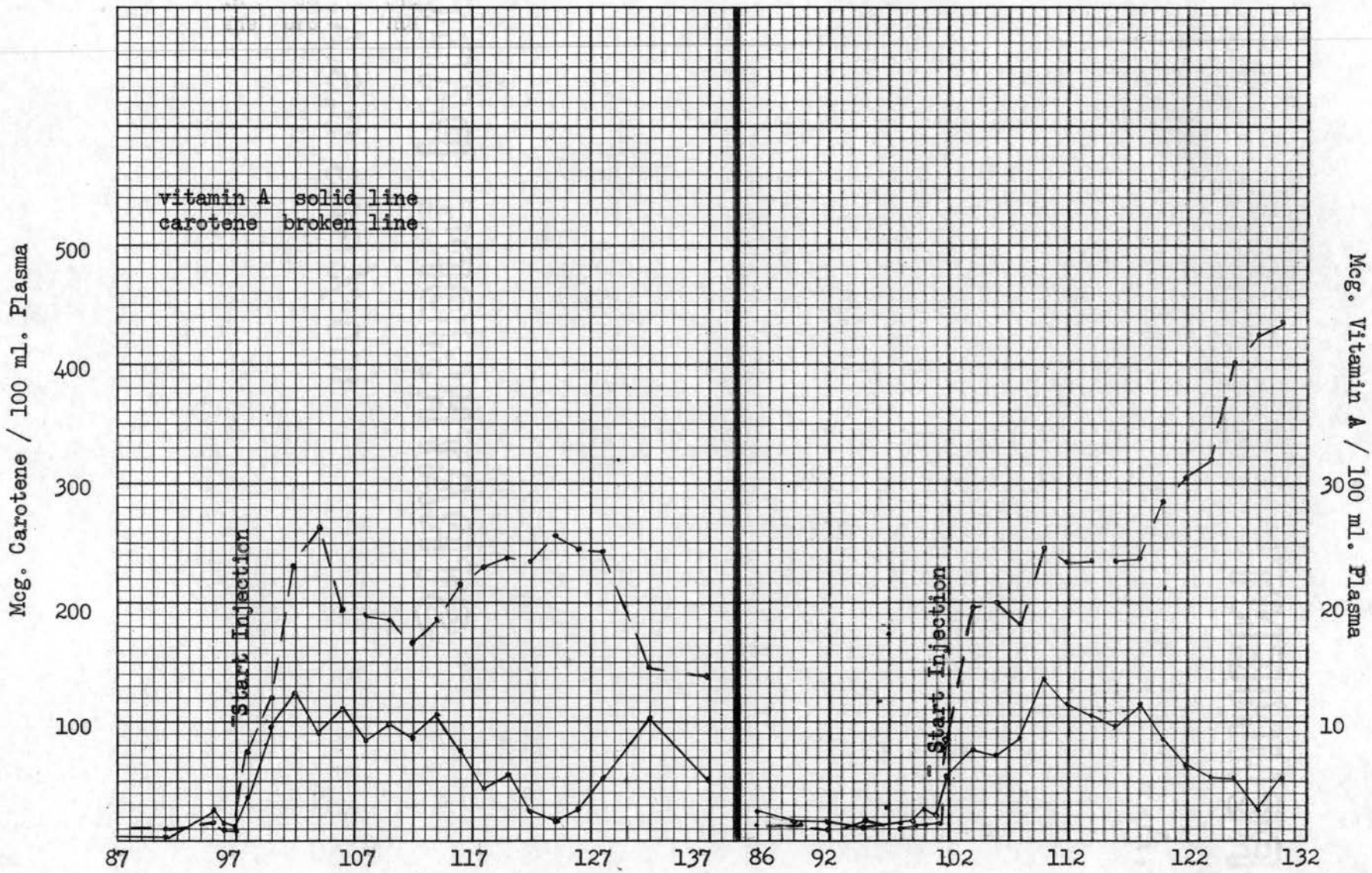

Fig. 4 AGE, da. Fig. 3

Jersey calf number 6 received a normal diet for 36 days before being placed on the depletion diet. After 55 days on the depletion diet the calf's vitamin A and carotene blood levels were less than 1.5 mcg. and 10 mcg. per 100 ml. of plasma, respectively. Lacrimation and a mild scouring condition had developed but otherwise the physical condition of the calf appeared good. It weighed 105 lb.

A total of 248 mg. of carotene was administered in 18 injections at the rate of 12 mg. per 100 lb. live weight. The first 16 injections were made on alternate days, blood samples being taken the day after each injection. The last two injections were made 5 days apart, blood samples being taken immediately prior to each injection. Plasma changes are shown in Figure 4.

After 9 injections the calf had stopped scouring and there was no sign of lacrimation. About 5 minutes after the sixteenth injection the calf went into a state of shock. He was immediately given an injection of antihistamine and within a few minutes appeared normal and was able to stand.

There seemed to be no adverse effect from the seventeenth injection but a few minutes after the eighteenth injection the calf died. An autopsy failed to show the cause of death. Liver values for vitamin A and carotene were 76.5 mcg. and 46.8 mcg. per 100 g. fresh weight, respectively. It was noted that the bile was orange in color but the presence of carotene was not detected. During the injection period vitamin A increased approximately 11 mcg. and carotene 246 mcg. per 100 ml. of plasma. The calf had been on the deficient diet 96 days at the time death occurred.

Guernsey calf number 76 received a normal diet from birth to 31 days of age before being changed over to the depletion diet. After 55 days on the depletion diet the calf's plasma values for vitamin A and carotene had decreased to approximately 3 mcg. and 15 mcg. per 100 ml. of plasma, respectively. The calf weighed 131 lb. and was in fair physical condition but had a very severe case of scours.

A total of 240 mg. of carotene was injected at the rate of 12 mg. per 100 lb. live weight every other day for 32 days. Blood samples were taken the day after each injection. These values are shown in Figure 5. Plasma vitamin A reached a maximum increase of about 12 mcg. on the eighth injection and plasma carotene, 363 mcg. per 100 ml. of plasma on the thirteenth injection. The scouring condition began to improve after the fourth injection and by the nineteenth day it had ceased completely and the calf's physical condition was good.

Sixty-five days after the last injection the calf showed clinical vitamin A deficiency symptoms of complete night blindness and papilledema. Plasma vitamin A was about 5 mcg. and plasma carotene 18 mcg. per 100 ml. of plasma. The calf weighed 178 lb. and was in good physical condition.

A total of 172 mg. of carotene was then administered in six injections during an eight-day period. The first two injections were made on alternate days at the rate of 24 mg. of carotene per 100 lb. live weight and the other four on successive days at the rate of 12 mg. per 100 lb. live weight. Blood samples were taken the day following the first and second injection and just before each of the daily injections. Plasma vitamin A increased approximately 12 mcg. and

plasma carotene 535 mcg. per 100 ml. of plasma. Night blindness had completely reverted and the condition of papilledema had improved by the end of the period.

The calf became completely blind 164 days after the second series of injections. The loss of sight developed suddenly within a week after a regular ocular examination at which time a marked increase in the severity of papilledema was noted. Prior to this time the calf had shown no signs of sight impairment since the last period of injection. The blood plasma levels had declined to 2.6 mcg. of vitamin A and 31 mcg. of carotene per 100 ml. of plasma and the physical condition was good.

The calf was injected five times over a period of 15 days with a total of 179.0 mg. of carotene at the varying rate of 9 to 24 mg. per 100 lb. live weight. Plasma vitamin A and carotene increased approximately 7 mcg. and 184 mcg., respectively. In an attempt to determine if the blindness was permanent the calf was given three oral doses (150,000 I.U. each) of vitamin A alcohol on alternate days. He was then placed on a normal ration including alfalfa hay but after 30 days was still blind. The calf was maintained 342 days on the deficient diet supplemented with intravenous carotene injections before becoming completely blind.

BLOOD PLASMA CAROTENE AND VITAMIN A GUERNSEY NO. 76

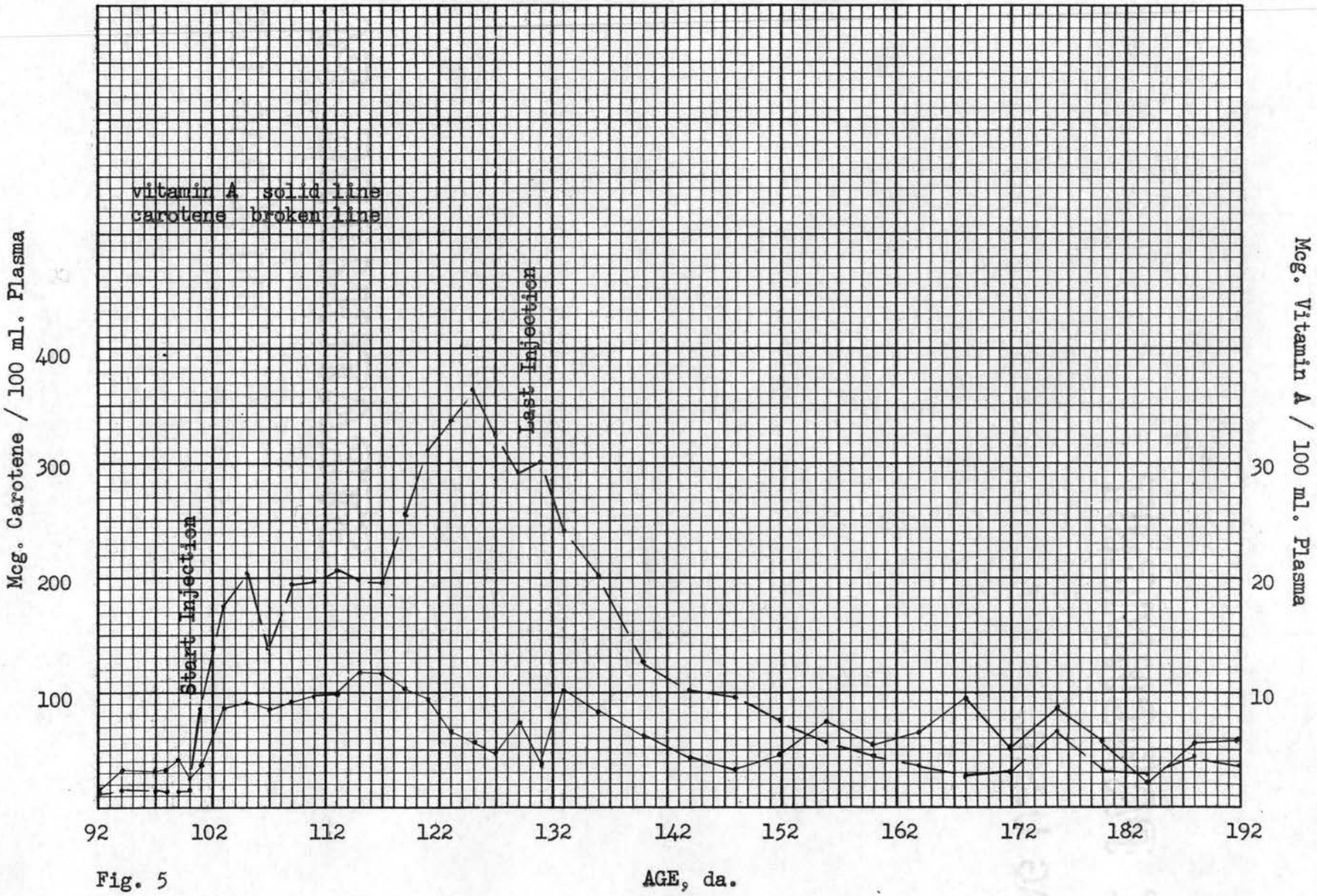

Fig. 5

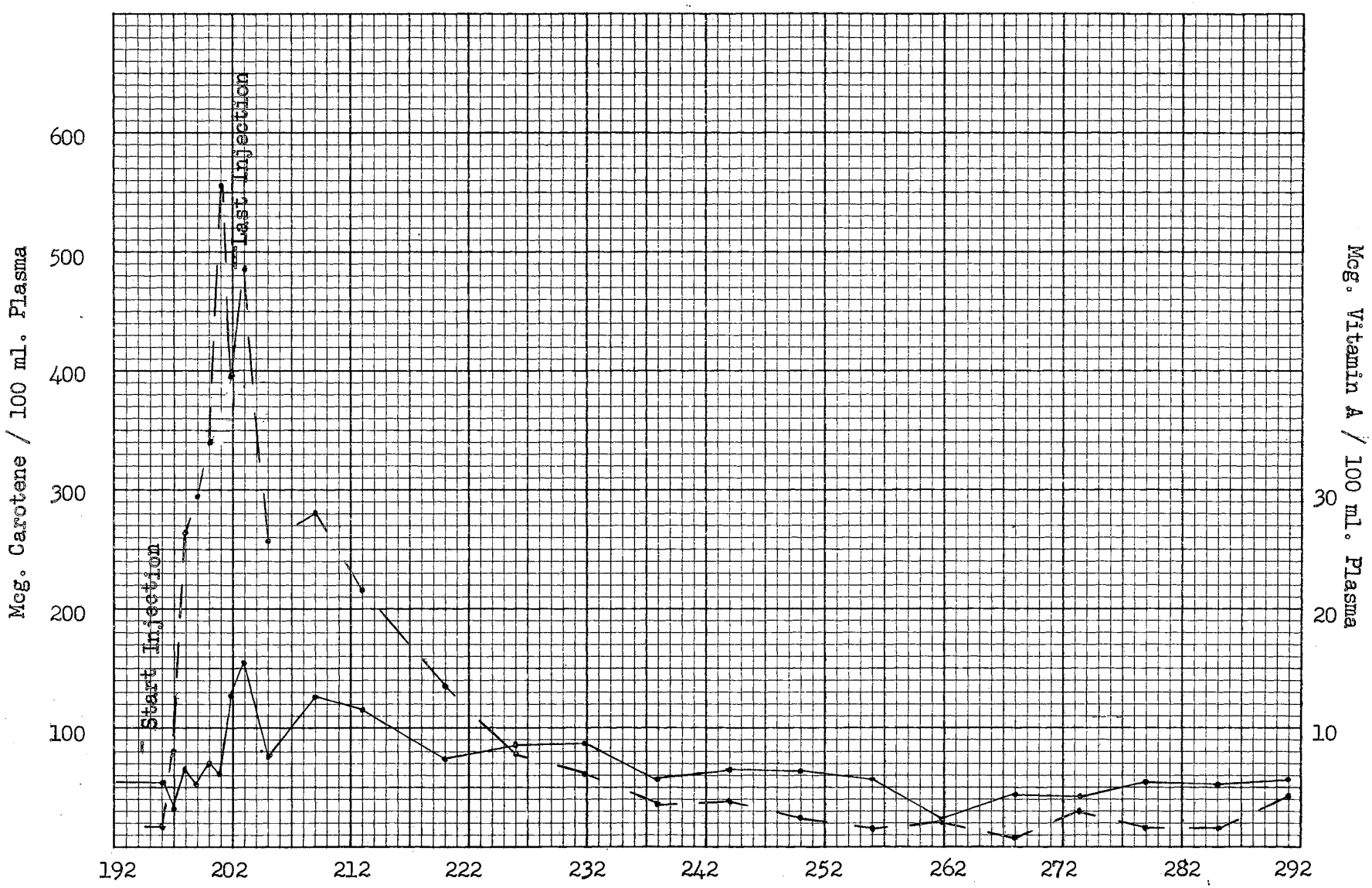
Mcg. Carotene / 100 ml. Plasma
600
500
400
300
200
100
Mcg. Vitamin A / 100 ml. Plasma
30
20
10
Start Injection
Last Injection
192
202
212
222
232
242
252
262
272
282
292
AGE, da.

Fig. 5 (contd.)

Ayrshire calf number 85 after receiving a normal diet for 29 days was gradually changed over to the depletion diet. After 53 days on the depletion diet the calf's blood values for vitamin A and carotene had decreased to about 2 mcg. and 3 mcg. per 100 ml. of plasma, respectively. This calf, which had a severe case of scours, was in only fair physical condition and weighed only 101 lb.

During the next 65-day period the calf received a total of 334 mg. of carotene. Sixteen injections were given on alternate days at the rate of 12 mg. of carotene per 100 lb. live weight, followed by eight injections at the same rate every fourth day. (Six days were allowed to elapse between the sixteenth and seventeenth injection so that the day of injection would coincide with that of another calf.) Blood samples were taken the day after each of the alternate-day injections and just before each of the fourth-day injections. The plasma changes for the period are shown in Figure 6.

Plasma vitamin A increased approximately 12 mcg. and carotene increased 253 mcg. per 100 ml. of plasma. The scouring condition began to improve after the seventh injection and by the tenth injection the calf had stopped scouring and his physical condition had improved.

Thirty-two days after the last injection the calf had developed almost complete night blindness and a mild case of papilledema. He was not carried to complete night blindness because it was desired to study the effect of carotene injection on a mild case. Plasma vitamin A and carotene levels were approximately 7 mcg. and 40 mcg. per 100 ml., respectively. The calf weighed 183 lb. and his physical condition was good. At this time he was injected with a total of 88 mg. of carotene at the rate of 12 mg. per 100 lb. live weight on 4 successive days.

Two days after the last injection the calf had recovered normal night vision and the condition of papilledema was slightly improved. Vitamin A increased about 12 mcg. and carotene 500 mcg. per 100 ml. of plasma.

Seventy-three days after this second series of carotene injection the calf had developed complete night blindness. His physical condition was good, however, and he weighed 223 lb. The plasma levels for vitamin A and carotene were about 4 mcg. and 27 mcg. per 100 ml., respectively. On two successive days 13.4 mg. of carotene was administered intravenously at the rate of 6 mg. per 100 lb. live weight. Night vision was improved and within 3 days there was no sign of nyctalopia. Plasma vitamin A showed an increase of approximately 6 mcg. and plasma carotene, 40 mcg. per 100 ml.

During the following 101 days varying degrees of night blindness were indicated on different occasions but the condition was never diagnosed as definite nyctalopia. The physical condition was good and the calf was removed from the trial after being maintained 329 days on the deficient diet supplemented with intravenous injections of carotene.

BLOOD PLASMA CAROTENE AND VITAMIN A AYRSHIRE NO. 85

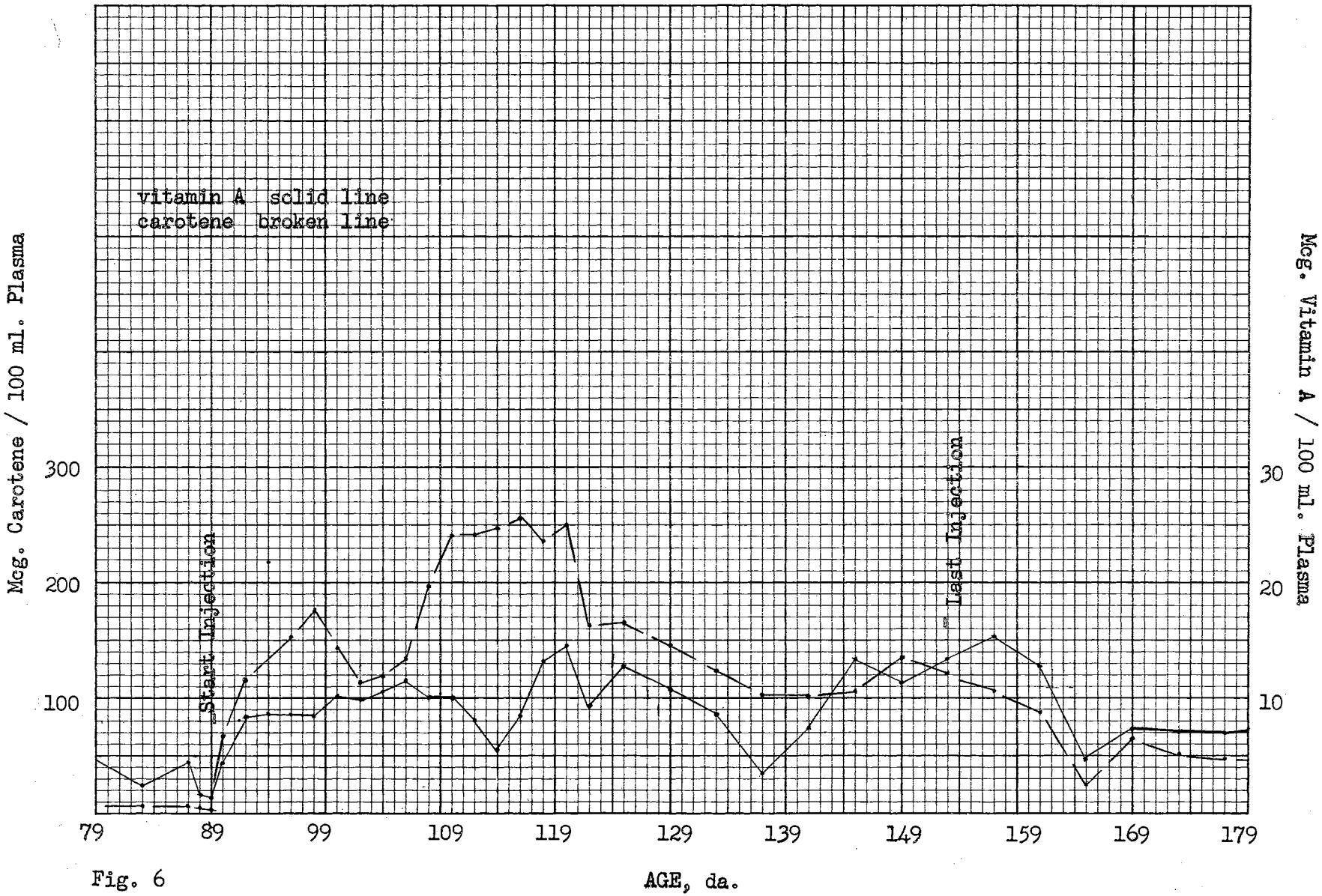

Fig. 6

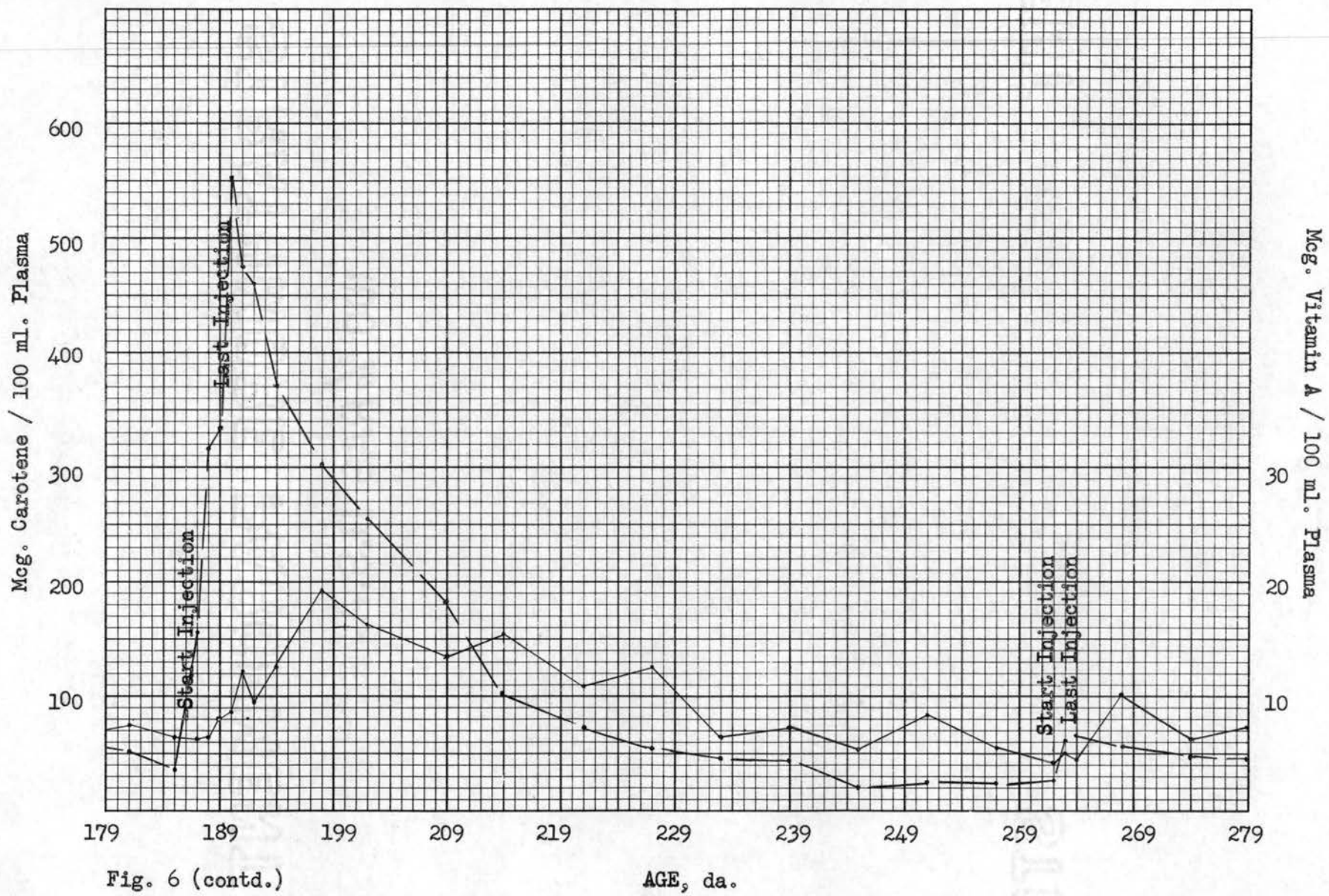
Mcg. Carotene / 100 ml. Plasma
600
500
400
300
200
100
Mcg. Vitamin A / 100 ml. Plasma
30
20
10
Start Injection
Last Injection
Start Injection
Last Injection
179
189
199
209
219
229
239
249
259
269
279
AGE, da.

Fig. 6 (contd.)

Ayrshire calf number 3 received a normal diet from birth to 20 days of age and then was changed over to the depletion diet. After 71 days on the depletion diet he developed a very severe case of scours. Blood vitamin A was less than 3 mcg. and carotene 7 mcg. per 100 ml. of plasma. The calf weighed 134 lb. and appeared to be in good physical condition except for the scouring.

Carotene was injected in nine doses totalling 153.3 mg. at the rate of 12 mg. per 100 lb. live weight every fourth day. Blood samples were obtained prior to each injection. By the time of the second injection the scouring condition had become extremely severe and the calf's physical condition was poor. After the fourth injection both the scouring and physical condition began to improve and by the time of the fifth injection scouring had stopped completely. Plasma vitamin A increased about 5 mcg. and carotene 43 mcg. per 100 ml. during the injection period (Figure 7). Despite this apparent favorable response the calf died two days after the fifth injection. He had refused feed the previous day. An autopsy showed an impaction of the omasum apparently resulting from the consumption of a large amount of wood shavings. The concentrations of vitamin A and carotene in the liver were 69.98 mcg. and 28.73 mcg. per 100 g. fresh weight, respectively. Examination of the gall bladder showed that the bile was light orange in color but neither chemical or biological analysis indicated the presence of carotene. The calf had been maintained on the deficient diet 106 days before death.

BLOOD PLASMA CAROTENE AND VITAMIN A

AYRSHIRE NO. 3 JERSEY NO. 62

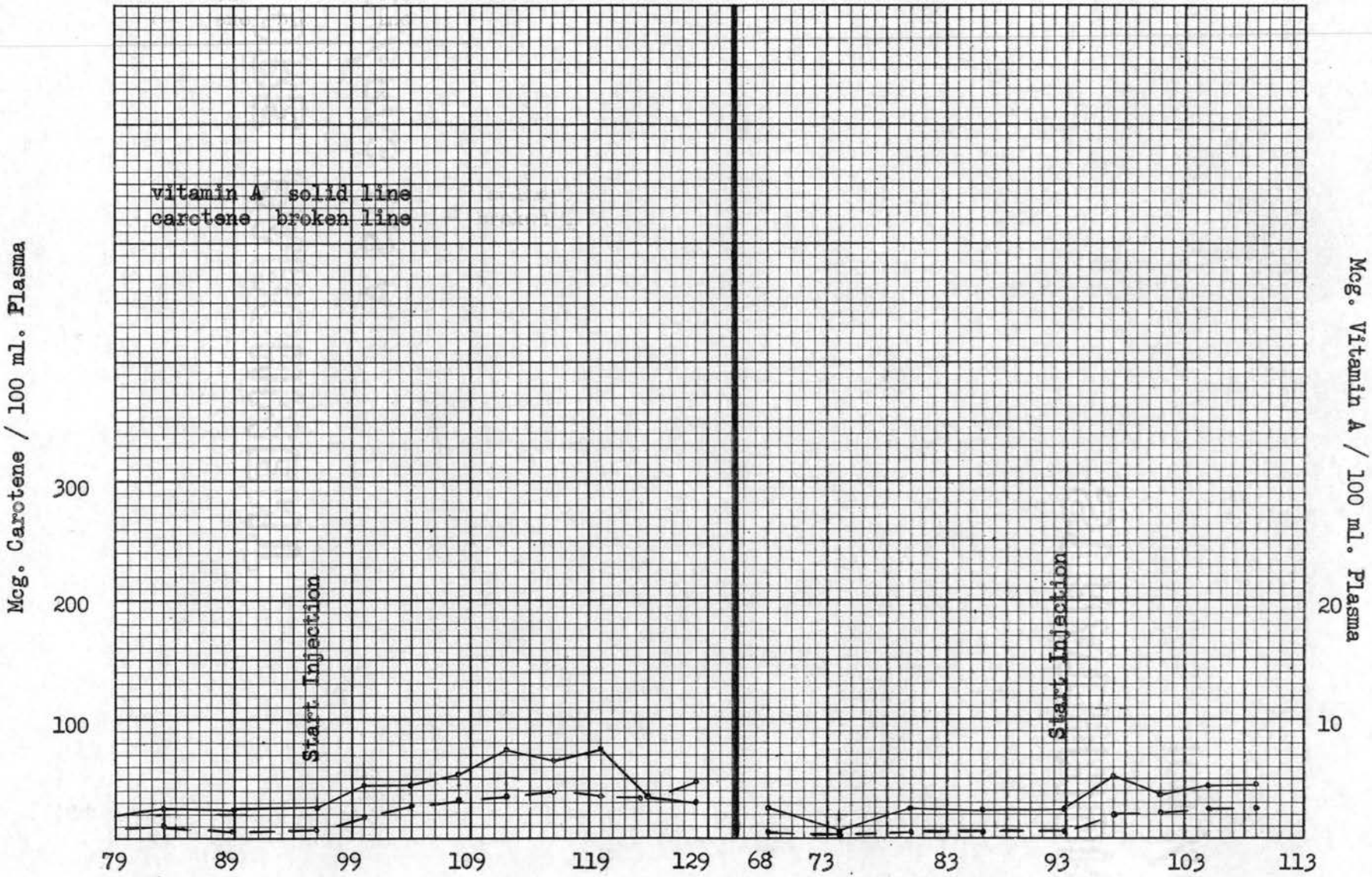

Fig. 7 Fig. 8

Jersey calf number 62 was placed on the depletion diet 18 days after birth and carried until the development of avitaminosis A symptoms which included serious vision impairment, severe scours and muscular incoordination. The calf weighed 92 lb. and vitamin A and carotene blood levels were less than 2 mcg. and 5 mcg. per 100 ml. of plasma, respectively. A total of 56.5 mg. of carotene was injected during 16 days at a rate of 12 mg. per 100 lb. live weight every four days.

The severity of the deficiency conditions continued to increase after the second injection and the physical condition became poor. A slight rise in body temperature above normal and heavy breathing indicated pneumonia. The calf died two days after the fifth injection and an autopsy showed the lungs to be severely damaged by pneumonia. Plasma vitamin A increased about 2.5 mcg. and plasma carotene increased 20 mcg. per 100 ml. of plasma (Figure 8). The concentrations of vitamin A and carotene in the liver were 54.68 mcg. and 45.08 mcg. per 100 g. fresh weight, respectively. The gall bladder was distended and the color of the bile was orange but the presence of carotene was not detected by a biological test. The calf had been on the deficient diet 93 days at the time death occurred.

Jersey calf number 61 received a normal diet until he was placed on the depletion diet 18 days after birth. After 62 days avitaminosis A symptoms including complete night blindness, severe scouring and muscular incoordination had developed. Blood plasma levels of vitamin A and carotene were about 4 mcg. and 8 mcg. per 100 ml., respectively. The calf weighed 93 lb. and was in fair physical condition. Carotene was then injected at the rate of 12 mg. per 100 lb. live weight, four such injections being made during a six-day period. The first two injections were made on successive days and the last two on alternate days. A total of 44.6 mg. of carotene was supplied.

Deficiency symptoms improved after the last injection and within 7 days the calf showed no symptoms of night blindness, scouring, nor muscular incoordination, his physical condition was good. As shown in Figure 9 plasma vitamin A increased approximately 4 mcg. and plasma carotene 166 mcg. per 100 ml.

Within eighty-eight days after the last injection the calf had again developed complete night blindness and severe scours. The plasma vitamin A and carotene had decreased to approximately 3 mcg. and 15 mcg. per 100 ml., respectively. The calf now weighed 165 lb. and was in fair physical condition. Carotene was then injected six times over a period of 15 days with a total of 65.7 mg. being given. In the first five injections carotene was administered at the rate of 6 mg. per 100 lb. live weight and in the last injection the dosage was doubled. The time between injections varied from 1 to 5 days. Blood samples were taken just prior to each injection.

At the end of the injection period the conditions of night blindness and scouring had cleared up and the physical condition of the calf had improved. Plasma vitamin A increased approximately 3 mcg. and plasma carotene 266 mcg. per 100 ml. of plasma. Plasma vitamin A did not change appreciably until after the last injection when it began to rise gradually. Two blood samples were taken within six days after the last injection and the animal was observed for another week and then removed from the experiment. The calf had been maintained for 180 days on the deficient diet during which time he recovered twice from vitamin A deficiency symptoms following the intravenous administration of carotene.

BLOOD PLASMA CAROTENE AND VITAMIN A JERSEY NO. 61

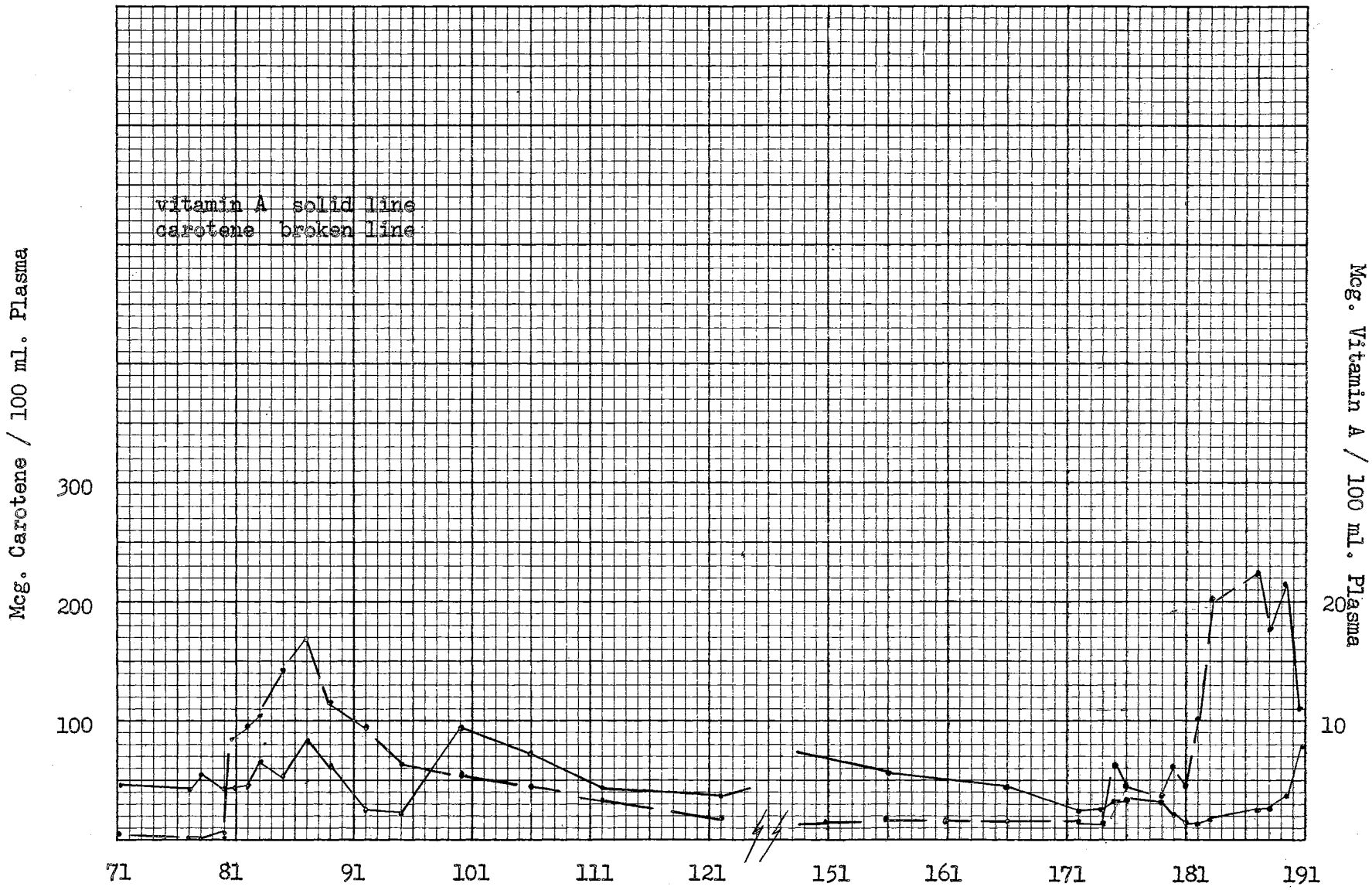

Fig. 9

Ayrshire calf number 28 was started on the deficient diet 17 days after birth. After 53 days on the deficient diet a severe case of scours developed and a few days later body temperature increased to 104° F. The calf died at the age of 77 days and an autopsy showed that death was caused by pneumonia. At the time of death blood plasma vitamin A was approximately 2 mcg. and carotene 4.0 mcg. per 100 ml. An examination of the gall bladder showed the bile to be dark green in color. The liver contained 59.85 mcg. vitamin A and 4.0 mcg. carotene per 100 g. fresh weight.

Ayrshire calf number 81 received a normal diet for 15 days after birth and then was placed on the deficient diet. As the result of a weak condition at birth the calf had been administered about three ounces of cod liver oil twice daily for a period of one week before he was obtained for this experiment.

After 94 days on the deficient diet the calf developed complete night blindness, muscular incoordination and severe scours. As these deficiency symptoms increased in severity the physical condition became poor and death occurred after 107 days on the depletion diet. At the time of death the blood plasma levels of vitamin A and carotene were less than 2 mcg. and 3 mcg. per 100 ml., respectively. An autopsy indicated pneumonia. Liver values for vitamin A and carotene were 44.31 mcg. and 9.40 mcg. per 100 g. fresh weight, respectively. The color of the bile was dark green.

Holstein calf number 84 was placed on the depletion diet 3 days after birth. Within 47 days mild night blindness, severe scours and muscular incoordination had developed. The severity of the deficiency symptoms increased, the physical condition became poor and the calf died after 51 days. Plasma vitamin A and carotene levels at the time of death were less than 3 mcg. and 1 mcg. per 100 ml., respectively.

Although the calf had difficulty in breathing the last two days before death, an autopsy did not show any signs of pneumonia. The color of the bile was dark green. Liver values for vitamin A and carotene were 24.20 mcg. and 3 mcg. per 100 g. fresh weight, respectively.

DISCUSSION

At the start of the experiment only blood analyses were used as criteria to test for conversion of the injected carotene to vitamin A. This procedure is exemplified by calves 8, 71, and 118 when injections were begun in the absence of deficiency symptoms other than low vitamin A blood levels. The peculiar fluctuations of plasma carotene and vitamin A of calves 71 and 118 during the first series of injections were confusing. The high plasma carotene concentrations might have interfered some with vitamin A determinations and thus account for some of the erratic plasma vitamin A values. Carotene interference in the determination may also suggest that some of the apparent vitamin A increase may have been due to artifacts. Both calves were injected at the same time with the same carotene solutions.

In the case of calves 6, 8, 76 and 85 which received lower carotene doses the plasma vitamin A values were more uniform during the early part of the first injection series. However, as the injection time proceeded plasma vitamin A decreased and plasma carotene increased. This inverse relationship between plasma vitamin A and carotene was apparent at about the same time in all four calves and seemed to be directly associated with a newly prepared carotene solution, portions of which were being injected into each calf. It appeared that the calves were unable to utilize the carotene in this solution to the same extent as that of other batches. A new solution resulted in an increase in plasma

vitamin A, but this observation was limited because of the deaths of calves 6 and 8. Since the same carotene supply was involved in all solutions used during these observations it would seem that the difference was in the makeup of the solution.

McGillivary and associates (33) (34) have reported similar differences between dispersions prepared from the same sample of carotene when injected into rats. They proposed that the variation in vitamin A formation from different carotene solutions might represent differences in the physical state of the carotene in the solutions.

In spite of some of these discrepancies and confusing responses the plasma vitamin A values increased in all the injected calves with each series of injections. Avitaminosis A symptoms were reverted by the injection of carotene although the remission was not complete in some cases. This proved that at least a portion of the apparent increase of plasma vitamin A was used by the animals. Jersey calf number 71 did not regain normal night vision after the injection of carotene but the eye stigma and muscular incoordination cleared up and his physical condition improved. Had this not been the first calf examined more time would have been allowed before the oral administration of vitamin A. It was necessary, however, to determine if the condition was permanent so that other calves would not be allowed to proceed too far as later occurred in the case of calf number 76.

Calves number 3 and 62 died while receiving low levels of injected carotene. The deficiency condition of the latter calf was extremely severe at the start of injection and he succumbed to pneumonia the occurrence of which made it very difficult to deplete and maintain the calves in the state of deficiency desired. Further proof of the conversion and

utilization of the injected carotene was demonstrated by several calves which were injected and survived on the deficient ration between 180 and 336 days while uninjected calves died within about 100 days.

Calves number 6 and 8 died as a direct result of the injected solution. Calf number 6 recovered from the first attack but died two injections later from a different solution than the one which had previously affected him. The solutions in both cases did not result in any ill effects when used on other calves.

Kon and his associates (27) have reported the same general type of symptoms following the injection of large amounts of Tween to dairy calves. They attributed the cause of death to a toxic condition produced by the Tween.

The formation of vitamin A from the injected carotene was apparent but it was difficult to determine the rate or efficiency of conversion. This was undoubtedly complicated by such things as the state of depletion, the individual ability of conversion, oxidation of the carotene in the blood and the previously mentioned possible variations between and within the solutions. In some cases large amounts of injected carotene were needed to clear up deficiency symptoms while at other times such as the last injections of calves 85 and 118 smaller amounts were effective. These differences were also noted in the re-occurrence of deficiency symptoms which sometimes appeared earlier after larger doses of carotene than after smaller ones. It appeared that the best results were obtained when injections were given daily or every other day indicating that perhaps only a small amount of the carotene in each injection could be utilized by the calves.

An examination of bile in the gall bladder showed that injected calves had an orange colored bile while the bile of uninjected calves had a more characteristic dark green color. The possibility of the bile acting as a mode of transportation of injected carotene from the blood to the intestine for conversion was investigated. The color could not be identified chemically as carotene or bilirubin. The bile from calves number 3 and 62 which was orange in color was fed to vitamin A deficient rats in a biological test to determine if it contained vitamin A activity. Orange colored bile obtained from two other calves which had been injected for this purpose only, was also fed. The rats died from apparent avitaminosis A confirming the chemical analysis that no carotene or at least biologically available carotene was present in the bile. It has been reported (41) that during vitamin D deficiency the gall bladder is frequently distended by the accumulation of a viscous ropy orange-yellow bile. However in the present experiment all calves received vitamin D and the condition was found only in the injected calves that died and not the uninjected calves.

SUMMARY

Studies were conducted to determine whether intravenously administered carotene could be utilized as a source of vitamin A by Holstein, Jersey, Guernsey and Ayrshire male calves. The calves were depleted of their vitamin A reserves until their blood plasma levels were less than 5 mcg. per cent for 12 consecutive days or until they had developed distinct avitaminosis A symptoms or both. The deficiency symptoms included complete night blindness, papilledema, marked diarrhea and muscular incoordination.

Carotene suspended in an aqueous solution of Tween 40 was injected at various arbitary levels from 6 to 24 mg. per 100 lb. live weight. The animals were injected daily, on alternate days or every four days over periods ranging from 2 to 65 days in length. Blood samples were taken prior to injection and at regular intervals thereafter.

Utilization of the intravenously injected carotene was apparent as shown by the reversal of the various deficiency symptoms. Several calves receiving injections were maintained on the deficient diet. between 180 and 336 days while uninjected calves died within 100 days. Following injections there was a marked increase in plasma carotene with moderate and irregular increases in plasma vitamin A.

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A P P E N D I X

A P P E N D I X

Plasma Carotene and Vitamin A Values, Treatments, and Observations of Individual Calves

TABLE I

JERSEY NO. 71

Age da.	Vitamin A mcg./100 ml. plasma	Carotene mcg./100 ml. plasma	Remarks
78	13.64	154.65	
84	11.73	128.10	
87			depletion diet
92	13.38	84.90	
99	2.28	38.10	
106	3.66	17.80	
109	6.94	15.30	
113	6.94	12.38	
116	6.34	13.65	
120	5.13	19.05	
123	6.85	13.05	
127	8.80	9.30	
130	5.76	9.75	
134	5.61	12.08	
137	5.61	14.55	
140	4.90	11.10	
143	5.32	14.10	
146	5.20	12.15	
149	2.74	7.05	
152	3.57	17.02	
155	1.49	17.25	
158	4.32	25.57	
159			start injection
160	5.61	174.82	
162	3.68	301.80	
164	4.96	539.60	
166	15.42	534.20	
168	24.14	555.30	
170	18.65	654.00	
172	11.68	738.75	
174	10.80	744.75	
176	10.50	760.00	
177			last injection
178	14.06	698.13	
179	9.60	620.50	
180	1.80	589.50	
181	3.35	496.50	
182	0.00	463.50	
183	0.00	334.00	
184	2.90	513.00	
185	9.58	397.50	
186	2.30	391.50	
188	6.95	355.25	
190	1.12	319.00	
192	3.06	273.75	
194	6.00	269.10	
197	7.55	239.25	

(continued)

Table 1 (continued)

Age da.	Vitamin A mcg./100 ml. plasma	Carotene mcg./100 ml. plasma	Remarks
200	1.17	191.70	
203	4.95	165.90	
206	5.25	137.10	
209	5.72	105.00	
212	5.72	101.40	
218	6.00	78.75	
224	5.55	53.25	
230	5.04	66.15	
236	2.72	55.95	
240	1.38	49.20	
244	5.67	49.20	
248	1.35	46.05	
252	2.24	43.50	muscular incoordination
256	2.48	40.05	
260	1.44	33.90	nyctalopia
264	3.50	31.65	start injection
268	7.91	60.30	
272	5.40	86.70	eye stigma
276	7.35	122.85	
280	11.82	114.20	stigma cleared up
284	8.82	147.90	
288	5.43	188.70	
292	9.47	220.50	
294	13.43	241.80	incoordination cleared up
296	10.32	303.60	
298	15.26	358.00	
300	19.08	381.80	last injection
302	17.60	383.00	
304	17.37	389.10	
308	13.28	100.20	orally administered vitamin A
309	43.95	264.10	
315	27.59	153.90	nyctalopia cleared up
317	25.11	135.80	
321	28.49	138.45	
325	47.01	136.50	
332	24.27	100.20	
338	22.14	69.20	
344	18.12	70.50	
351	19.50	42.0	
357	15.57	38.10	

TABLE II

AYRSHIRE NO. 118

Age da.	Vitamin A mcg./100 ml. plasma	Carotene mcg./100 ml. plasma	Remarks
29	3.72	24.30	
36	5.90	14.20	
39	5.35	19.05	
43	9.55	40.20	
46	8.04	37.95	
50	11.06	82.35	
53	8.04	68.25	
54			depletion diet
57	8.29	28.20	
60	4.90	21.45	
64	4.90	19.50	
67	3.46	17.55	
70	3.91	15.00	
73	3.91	16.95	
76	2.52	14.55	
79	3.00	13.65	
82	4.05	17.55	
85	1.83	11.70	
88	3.98	18.77	
89			start injection
90	4.42	240.15	
93	6.42	378.80	
95	9.84	490.80	
97	12.74	618.40	
99	11.80	594.30	
101	12.63	497.28	
103	4.15	716.00	
105	6.98	744.75	
107	13.91	744.40	
108			last injection
109	12.72	643.44	
110	10.07	626.00	
111	1.50	520.50	
112	4.30	515.50	
113	0.00	457.75	
114	1.95	330.00	
115	7.98	421.50	
116	5.50	457.75	
117	5.65	360.75	
119	0.00	307.00	
121	6.90	286.40	
123	5.81	257.85	
125	9.98	207.45	
128	6.27	149.25	
131	4.53	114.19	
134	7.14	121.65	
137	5.87	105.00	

(continued)

Table 2 (continued)

Age da.	Vitamin A mcg./100 ml. plasma	Carotene mcg./100 ml. plasma	Remarks
140	6.50	74.85	
143	4.47	61.35	
149	5.28	43.95	
155	6.05	34.05	
161	5.04	66.15	
167	2.09	40.75	
171	2.40	33.00	start injection
175	7.89	91.80	
179	8.72	126.75	
183	4.82	153.15	
187	5.21	140.40	
191	2.79	105.00	
195	6.38	93.00	
199	8.24	106.70	
203	8.09	147.90	
207	11.45	134.55	
211	11.33	112.20	
214	11.72	140.40	
218	13.45	152.00	
222	14.49	154.80	last injection
226	9.14	137.85	
230	9.18	124.20	
234	8.67	103.80	
238	7.23	74.70	
245	10.08	52.35	
251	15.84	47.25	
255	12.15	50.10	
262	10.88	37.50	
268	8.80	41.10	
274	6.62	30.20	
280	6.45	16.50	
286	3.57	12.00	
292	9.54	13.80	
298	5.11	15.60	
302			nyctalopia
304	2.43	26.10	
310	7.02	22.20	
315	3.03	17.70	start injection
316	6.98	72.15	
317	6.17	76.05	last injection
321	15.63	63.00	nyctalopia cleared up
331	13.68	61.80	
341	13.92	33.00	
351	8.25	24.30	
357	5.24	22.40	
367	7.26	24.75	

TABLE III

HOLSTEIN NO. 8

Age da.	Vitamin A mcg./100 ml. plasma	Carotene mcg./100 ml. plasma	Remarks
20	10.80	31.50	
23	7.54	27.15	
26	12.61	25.65	
29	9.04	29.85	
32	7.32	29.85	
35	4.90	22.05	depletion diet
41	8.79	34.42	
44	7.03	43.65	
47	7.02	43.65	
50	6.95	41.73	
53	4.43	30.90	
56	2.87	23.85	
59	4.04	19.28	
62	2.13	19.30	
65	3.35	16.00	
68	2.87	15.55	
71	0.56	18.75	
74	0.78	16.05	
77	1.31	13.80	
80	0.96	13.65	
83	1.15	11.80	
86	2.90	14.70	
89	1.70	14.20	
92	1.50	10.35	
95	1.06	12.20	
98	1.51	14.00	
100	2.77	13.15	start injection
101	2.23	15.05	
102	5.24	85.05	
104	7.82	194.85	
106	7.11	200.25	
108	8.62	181.95	
110	13.77	247.35	
112	11.39	232.35	
114	10.30	234.15	
116	9.80	237.60	
120	8.55	284.25	
122	6.72	305.05	
124	5.21	320.25	
126	5.25	401.55	
128	2.66	424.50	
130	5.16	437.85	
131			calf died

TABLE IV

JERSEY NO. 6

Age da.	Vitamin A mcg./100 ml. plasma	Carotene mcg./100 ml. plasma	Remarks
19	10.71	18.60	
25	9.55	24.15	
31	8.30	44.85	
37	7.18	36.30	
42			depletion diet
43	6.05	40.22	
49	2.00	57.60	
55	4.17	25.30	
61	3.35	16.00	
67	1.04	11.90	
73	1.33	7.13	
79	2.56	9.85	
85	0.78	6.20	
91	0.57	9.98	lacrimation, mild scours
95	2.84	12.10	
96	1.48	9.75	
97	1.15	9.45	start injection
98	3.68	74.10	
100	9.51	120.30	
102	12.50	230.40	
104	9.00	262.50	
106	11.18	195.00	
108	8.46	190.95	
110	9.97	185.70	
112	8.75	166.80	lacrimation cleared up
114	10.40	187.20	scours cleared up
116	7.63	218.35	
118	4.25	230.55	
120	5.43	239.25	
122	2.27	236.70	
124	1.71	256.95	
125			state of shock
126	2.80	243.00	
128	5.18	242.70	
132	11.13	147.15	
137	5.06	139.05	calf died

TABLE V

GUERNSEY NO. 76

Age da.	Vitamin A mcg./100 ml. plasma	Carotene mcg./100 ml. plasma	Remarks
22	9.55	21.45	
25	7.32	32.55	
28	10.05	26.10	
31	6.47	29.25	
34	7.06	35.85	
37	6.94	46.90	
40	9.42	55.97	
45			depletion diet
46	8.30	51.60	
49	6.11	31.95	
52	5.98	22.80	
55	5.10	17.78	
58	4.65	18.53	
61	5.53	16.05	
64	3.30	16.50	
67	4.87	17.43	
70	0.78	19.05	
73	1.22	16.05	
76	2.52	13.35	
79	1.81	14.20	
82	1.57	15.55	
85	3.01	16.05	
88	2.59	16.55	
91	1.73	13.15	severe scours
94	3.17	15.40	
97	3.06	14.50	
98	3.63	14.20	
99	4.03	13.80	
100	2.63	14.70	start injection
101	3.78	85.65	
103	8.87	174.60	
105	9.14	204.30	
107	8.54	139.05	slight scours
109	9.15	194.85	
111	9.69	197.25	
113	9.90	207.60	
115	11.88	199.65	
117	11.69	194.85	
119	10.32	257.85	scours cleared up
121	9.41	310.20	
123	6.56	337.50	
125	5.69	363.45	
127	4.88	328.65	
129	7.38	292.50	
130			last injection
131	3.53	300.00	
133	10.13	241.05	
136	8.34	201.15	

(continued)

Table V (continued)

Age da.	Vitamin A mcg./100 ml. plasma	Carotene mcg./100 ml. plasma	Remarks
140	6.20	127.90	
144	4.23	101.40	
148	3.24	97.80	
152	4.49	78.75	
156	7.41	59.10	
160	5.36	43.50	
164	6.50	36.90	
168	9.60	29.10	
172	4.91	30.60	
176	8.55	68.25	
180	5.73	31.70	
184	2.18	29.05	
188	5.52	44.55	
192	5.90	36.60	nyctalopia
195	5.33	18.30	start injection
196	3.00	81.30	
198	6.65	263.85	
199	6.08	296.25	
200	8.02	340.40	
201	7.71	555.75	
202	12.75	396.00	last injection, nyctalopia
203	15.30	477.25	cleared up
205	7.68	258.80	
209	17.60	280.65	
213	11.46	217.20	
220	7.37	134.40	
226	8.45	78.80	
232	8.76	61.80	
238	5.73	35.40	
244	6.49	38.10	
250	6.34	24.30	
256	5.31	16.05	
262	2.37	21.30	
268	4.23	9.00	
274	3.06	30.00	
279	4.59	17.70	
285	4.14	16.50	
291	5.79	44.10	
301	7.77	18.60	
309	5.58	20.40	
315	8.04	36.45	
325	10.97	36.00	
335	7.05	36.60	
345	5.45	45.60	
355	4.19	37.05	calf completely blind
365	3.80	26.65	start injection
366	2.60	31.00	
368	4.79	87.90	
371	8.19	109.80	
375	5.34	176.70	
380	4.91	147.20	last injection
387	7.34	214.05	

TABLE VI

AYRSHIRE NO. 85

Age da.	Vitamin A mcg./100 ml. plasma	Carotene mcg./100 ml. plasma	Remarks
11	20.20	11.10	
17	20.47	16.95	
23	11.84	37.95	
29	9.17	21.82	
35	8.92	19.50	
36			depletion diet
41	4.36	24.75	
47	5.85	15.80	
53	6.64	9.50	
59	4.71	7.88	
65	5.20	3.65	
71	4.24	5.40	
77	4.97	4.60	
83	2.39	3.54	severe scours
87	4.46	4.60	
88	1.87	3.15	
89	1.43	2.85	start injection
90	4.40	67.80	
92	8.37	115.35	
94	8.90	219.75	
96	8.72	152.55	
98	8.58	174.60	
100	10.26	142.95	
102	9.96	112.95	
104	10.64	198.75	
106	11.65	134.40	
108	10.07	198.75	scours cleared up
110	10.05	240.15	
112	8.15	241.05	
114	5.52	248.25	
116	8.52	256.35	
118	13.34	235.80	
120	14.66	250.01	
122	9.27	162.30	
125	12.98	164.40	
129	10.83	145.05	
133	8.75	122.25	
137	3.56	104.40	
141	7.32	100.50	
145	13.30	108.00	
149	11.36	135.15	
153	13.46	120.90	last injection
157	15.48	106.80	
161	12.91	89.10	
165	4.71	24.30	
169	7.32	64.50	
173	6.98	51.15	

(continued)

Table 6 (continued)

Age da.	Vitamin A mcg./100 ml. plasma	Carotene mcg./100 ml. plasma	Remarks
177	6.99	48.60	
181	7.74	52.80	
185	6.40	38.10	mild nyctalopia
186			start injection
187	6.32	155.40	
188	6.47	314.40	
189	8.04	334.40	last injection
190	8.75	551.50	
191	12.20	475.25	nyctalopia cleared up
192	9.55	460.00	
194	12.70	371.40	
196	19.26	309.00	
202	16.17	255.90	
209	13.10	182.70	
215	15.42	102.60	
221	10.98	71.70	
227	12.48	55.30	
233	6.66	48.15	
239	7.22	45.15	
245	5.20	19.90	
251	8.25	24.30	
257	5.46	23.40	nyctalopia
262	4.29	27.30	start injection
263	5.03	60.15	last injection
264	4.44	66.15	
268	10.10	57.60	nyctalopia cleared up
274	6.30	49.20	
280	7.23	46.20	
290	7.56	27.30	
298	5.52	22.20	
304	5.70	19.05	
314	12.84	18.00	
324	6.69	21.75	
334	5.18	20.85	
344	4.41	20.40	
354	5.30	22.20	
364	8.13	26.10	

TABLE VII

AYRSHIRE NO. 3

Age da.	Vitamin A mcg./100 ml. plasma	Carotene mcg./100 ml. plasma	Remarks
11	14.98	6.45	
17	13.36	10.20	
23	10.86	8.85	
24			depletion diet
29	6.05	3.15	
35	8.51	3.60	
41	7.08	3.35	
47	1.28	3.60	
53	1.89	4.53	
59	1.77	4.50	
65	1.81	4.73	
71	2.24	2.93	
77	1.15	8.33	
83	2.45	10.65	
89	2.34	3.90	
92			severe scours
96	2.61	6.30	start injection
100	4.83	19.50	bloody scours
104	4.65	28.00	
108	5.34	31.01	scours cleared up
112	7.52	33.45	
116	6.62	39.60	
120	7.50	35.10	
124	3.57	33.45	
128	3.05	42.20	last injection
130			calf died

TABLE VIII

JERSEY NO. 62

Age da.	Vitamin A mcg./100 ml. plasma	Carotene mcg./100 ml. plasma	Remarks
14	8.07	8.78	
18			depletion diet
20	5.90	7.80	
26	6.60	9.98	
32	5.69	18.75	
38	4.38	12.08	
44	2.80	7.80	
50	1.11	5.40	
56	1.23	2.70	
62	2.96	3.35	
68	2.30	3.35	
74	0.68	1.80	
80	2.53	4.75	
86	2.30	4.00	mild scours, mild nyctalopia
93	2.39	4.95	start injection
97	5.18	20.85	nyctalopia and almost blind,
101	3.94	21.30	/ severe scours
105	4.47	25.65	
109	4.64	25.20	last injection
111			calf died

TABLE IX

JERSEY NO. 61

Age da.	Vitamin A mcg./100 ml. plasma	Carotene mcg./100 ml. plasma	Remarks
18			depletion diet
34	8.85	4.50	
47	4.44	12.00	
53	4.71	9.00	
59	4.12	0.50	
65	4.80	0.50	
71	4.78	4.95	nyctalopia
77	4.17	0.50	muscular incoordination
78	5.49	0.50	start injection
80	4.32	7.65	severe scours
81	4.38	87.30	
82	4.95	99.00	
83	6.78	103.80	
85	5.29	140.95	last injection
87	8.47	173.10	
89	6.03	116.10	scours, incoordination cleared
92	2.67	96.60	nyctalopia cleared up
95	2.19	62.85	
100	9.45	53.40	
106	7.22	45.15	
112	4.37	32.10	
122	3.96	19.50	
130	5.11	15.60	
136	6.22	16.65	
146	10.53	15.15	
156	5.70	16.35	
166	4.22	18.15	nyctalopia
172	2.69	17.10	severe scours
173	2.65	15.60	start injection
174	3.21	64.05	
175	3.38	47.70	
178	3.05	32.10	
179	2.07	61.20	scours cleared up
181	1.32	48.15	
182	1.32	101.40	
183	1.92	205.20	
186	2.46	227.60	
187	2.76	179.70	last injection
189	3.80	216.25	nyctalopia cleared up
192	8.19	109.80	

TABLE X

AYRSHIRE NO. 81

Age da.	Vitamin A mcg./100 ml. plasma	Carotene mcg./100 ml. plasma	Remarks
16			depletion diet
36	12.03	6.30	
49	8.49	3.60	
55	6.00	7.20	
61	7.83	0.50	
67	9.84	0.50	
73	4.32	4.95	
79	5.50	0.40	
85	3.99	12.00	
91	3.63	5.40	
98	0.00	4.50	
108	2.28	4.50	
114	1.22	3.60	nyctalopia, severe scours, / muscular incoordination
122			calf died

TABLE XI

AYRSHIRE NO. 28

Age da.	Vitamin A mcg./100 ml. plasma	Carotene mcg./100 ml. plasma	Remarks
6	7.31	6.90	
12	9.43	18.78	
18	8.51	10.20	depletion diet
24	6.77	7.80	
28	7.19	9.30	
35	3.40	7.25	
41	2.07	4.60	
47	1.31	5.40	
53	2.77	6.95	
59	1.72	2.10	
65	1.61	3.15	
71	2.52	4.90	severe scours
77			calf died

TABLE XII

HOLSTEIN NO. 84

Age da.	Vitamin A mcg./100 ml. plasma	Carotene mcg./100 ml. plasma	Remarks
23	5.34	6.30	
36	11.73	4.50	
42	4.01	5.85	
48	4.59	0.00	
54	1.10	0.00	calf died

VITA

JAMES D. SCHUH

Candidate for the Degree of

Master of Science

Thesis: THE UTILIZATION OF INTRAVENOUSLY ADMINISTERED CAROTENE BY DAIRY CALVES

Major: Dairy Production

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Date of Final Examination: July , 1957