UTILIZATION OF INTRAVENOUSLY ADMINISTERED CAROTENE

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Preface

An interesting aspect of comparative biochemistry is the fact that beta carotene is normally present in the blood of cattle but not in the blood of sheep, goats and swine. This observation may have practical importance when it is recognized that carotene is the precursor of vitamin A, and that this nutrient often assumes a critical role in the nutrition of livestock. It appeared appropriate, therefore, to undertake studies on the metabolism of carotene using intravenous administration of beta carotene and following the blood level of vitamin A. This study was undertaken in an attempt to determine whether or not the absence of carotene in the circulation reflects the existence of extra intestinal sites for the conversion of carotene to vitamin A. Wherever evidence pointed to the existence of such sites, an attempt was made to determine their actual location. Since the dairy breeds, Holstein and Guernsey, appear to differ in their metabolism of carotene, calves of both breeds were tested with respect to their ability to utilize parenterally administered carotene.

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CHAPTER I

INTRODUCTION

Steenbock (29) first recognized a relationship between the pigment, carotene, in yellow corn and vitamin A. Investigators subsequently have found these carotenoid pigments to be precursors of vitamin A in the animal body. Attempts to establish the locus of this transformation has led to much conjecture and investigative work, which continues to this day.

In 1930, Moore (25) showed that vitamin A was formed from carotene in the animal body. He assumed (26), therefore, that the liver, long recognized as a main site of metabolic activity, was the site where conversion took place. Though this view was generally accepted for approximately 15 years, the evidence was actually inconclusive and contradictory.

The theory of hepatic conversion did not satisfactorily explain why the blood of mammals, such as the rat, pig, sheep and goat contained only traces of carotenoids. The possible explanation that the carotene passes directly from the intestinal tract via the portal vein to the liver, without ever appearing in the general circulation, was discredited by Goodwin, Dewar, and Gregory (14), and Goodwin and Gregory (15), who were unable to find any carotene in the portal or systemic blood of sheep, goats or rabbits at intervals after introducing carotene into the abomasum, though vitamin A similarly given appeared in large quantities.

Verzar and McDougall (37) ventured that the intestinal mucosa, rather

than the liver, might be the site of conversion. The first evidence in support of this hypothesis came almost simultaneously from three different laboratories. From the University of Liverpool, Glover, Goodwin, and Morton (16) announced that the intestines of sacrificed rats, which had first been depleted of their vitamin A stores and then fed carotene, were found to contain very appreciable amounts of vitamin A. Mattson, Mehl, and Deuel (23), at the University of California, reported that orally fed carotene caused an increase in the intestinal level of vitamin A in rats to a point where it was higher than the liver level, and where it remained for four hours. Weise, Mehl, and Deuel (40) found also that carotene was converted to vitamin A by incubation of the provitamin with rat intestine under anaerobic conditions. From the University of Reading, then, Thompson, Ganguly, and Kon (31) reported that pigs which had been fed carotene before killing had greater amounts of vitamin A in the lymphatics than did the control animals. Further evidence of intestinal conversion came thereafter from a host of workers. Experimentation included in vitro incubation of carotene with intestine (Stallcup and Herman (28), and McGillivray (24)), analysis of various tissues after feeding carotene (Swick, Grummer, and Baumann (30), Goodwin and Gregory (15), and Mattson (22)), as well as cannulation of the lymphatics (Amber, Cheng, and Deuel (2), Coates, Thompson, and Kon (8), and Thompson and others (32)(33)(34)(35)) on a variety of higher vertebrates. The weight of all this evidence quite conclusively proved that carotene is converted to vitamin A in the intestinal mucosa of higher animals.

The suggestion of Zechmeister (44), that there might exist a

species difference as to the locus of carotene conversion, acquires support when we note that rats (Bieri and Pollard (4)) and pigs (Hentges, Grummer, and Sorenson (18)) can utilize intravenously administered cartoene, while Guernsey and Holstein calves (Elliott (12)) and Hereford calves (Church, MacVicar, Bieri, Baker, and Pope (7)) apparently make little use of the parenterally administered provitamin. Discrepancies still exist, however, between the observations of Eaton, Matterson, Decker, Helmboldt, and Jungherr (11), and Warner and Maynard (39), who found intravenously administered aqueous carotene to be utilized by Guernsey and Holstein calves, and Elliott (12) who was unable to demonstrate such utilization in the same type of animals.

Some of the contradictory evidence concerning the utilization of intravenously administered carotene by higher animals might well be due to the difference in the type of carotene dispersion used. Since the work of Drummond, Gilding, and MacWalter (9), it has been known that when colloidal carotene is introduced directly into the blood stream of animals, the liver rapidly removes the pigment as it would other colloidal material. Lease, Lease, Steenbock, and Baumann (21), and Sexton, Mehl, and Deuel (27), found that such colloidal carotene is essentially unavailable to the animal as a source of vitamin A and may be found in the liver after the animal has died from an apparent deficiency of the vitamin. The discovery by Tomarelli, Charney, and Bernhart (36) that parenteral carotene can serve rats as an effective source of vitamin A when it is solubilized in water with a surface active agent, supports the view that the parenteral utilization of the provitamin is, to a large

extent, determined by the physical state in which it is administered. This was further demonstrated by Hentges, Grummer, and Sorenson (18) who made intravenous and intramuscular injections of carotene, dispersed in cottonseed oil, to pigs and found no relief from avitaminosis A symptoms. Carotene in aqueous solution with Tween 80 (polyoxyethylene sorbitan monooleate), however, when similarly given, did give rise to vitamin A and relieved deficiency symptoms. Klosterman, Bolin, and Light (20) found no conversion of carotene to vitamin A after injecting colloidal carotene in cottonseed oil into the veins of sheep. Using an aqueous preparation of carotene with Tween 40 (polyoxyethylene sorbitan monopalmitate) as the dispersing agent Church, et al (7) did find significant conversion in the sheep.

This evidence of effective utilization of parenterally administered carotene suggests that some secondary site or sites of conversion, in addition to the intestine, exists at least in some animals. Such reports as listed above, plus those of Bieri and Sandman (5), and With (42) showing increases in vitamin A after parenteral administrations of carotene to rats, and Ahmad, Grewal, and Malik (1), Wolff, Overhoff, and Von Eckelen (43), and Vinet and Plessier (38), showing the same with rabbits, indicate the actual existence of secondary conversion sites. Bieri and Pollard (4) went on to quite definitely prove the presence of such extraintestinal conversion sites in rats. The formation of vitamin A from injected aqueous carotene occurred essentially unimpaired after ligation of the bile duct, removal of the small intestine or kidneys or removal of 60 - 75% of the liver.

The existing evidence seems definitely to denote the intestinal wall

as the main locus of carotene transformation to vitamin A in higher animals. There is much evidence to suggest, however, that some species have one or more secondary sites capable of performing this conversion.

CHAPTER II

MATERIALS AND METHODS

Injection Materials:

After surveying the previous work accomplished in this field of study, it was decided that the best type of carotene preparation to use in these experiments would be one in which the carotene exists as a molecular dispersion. This is approached in the preparation described by Bieri (3), wherein Tween 40 acts as the dispersing agent[1]. The carotene solution was made by dissolving the crystalline carotene (90% beta and 10% alpha)[2] in a minimum amount of chloroform, adding an adequate portion of Tween 40[3] at 100°C, and with constant stirring taking the solution to desired volume with distilled water at the same temperature. This solution was freshly prepared within 24 hours prior to use, and was stored in a brown glass bottle under refrigeration until then.

The vitamin A solution was prepared by dissolving vitamin A palmitate[4] in a small portion of Tween 40 at 40°C and then taking the solution to the desired volume with a citrate-phosphate buffer solution of pH 4.2 at the same temperature.

The Tween 40 solution was prepared in the same manner as was the carotene solution, with the exception that no carotene was added.

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[1]Bieri, J.G., of the University of Texas, Medical Branch, Galveston, Texas, kindly supplied a sample of the carotene preparation along with detailed instructions for its preparation.
[2]Supplied by Barnett Laboratories, Long Beach, California.
[3]Supplied by Atlas Powder Co., Wilmington, Deleware.
[4]Supplied by Chas. Pfizer and Co., Inc., New York City, New York.

Surgical Techniques:

All intravenous injections were made directly into the jugular vein, and the blood samples were withdrawn from the opposite jugular vein.

Liver biopsy samples were obtained by the method of Whitehair et al. (41), with exception of those obtained post mortem, in which case a sample was obtained by cutting off a portion of one of the liver lobes.

The surgical technique used in ileectomizing the sheep was developed for the particular experiment by Doctors E. L. Blevins, E. W. Jones, and C. N. Murphy of the Department of Veterinary Surgery of Oklahoma A & M College. The animals were anesthetized by giving 4% procaine hydrochloride by a para vertebral block of the last thoracic, and the first and second lumbar nerves. A ten to twelve inch incision was made into the right flank. Once the abdomen was open, the ileocecal valve was located and the gut ligated in this area. The small intestine from this point forward to the pylorus was resected, the mesenteric vessels being ligated in a series of mass ligations. The pyloric stump was ligated, and the incision closed.

Rations Fed To Experimental Animals:

The dairy calves were depleted of their vitamin A stores by placing them on a ration consisting of wood pulp or beet pulp and cottonseed hulls as the roughage, molasses, cottonseed meal, and white hominy feed.

The normal ration for the calves included prairie hay (free choice), and four pounds of standard calf starter (corn, oats, cottonseed meal, wheat, dried molasses, bran, dehydrated alfalfa meal, and dried butter milk).

The goats were maintained on prairie hay and ground oats.

Blood Analysis:

The blood samples were drawn into tubes containing lithium citrate as the anticoagulant. The samples were centrifuged and the plasma removed from the cells, frozen and stored at -14°C until analysis was made.

The determinations for carotene and vitamin A were carried out according to the method of Kimble (19). In order to precipitate the proteins, five ml. of aldehyde-free ethanol were added to a test tube containing five ml. of plasma. The fat soluble pigments were then extracted by five minutes of vigorous shaking with fifteen ml. of Darco-treated petroleum ether (Skelly Solve F). A ten ml. aliquot of the petroleum ether portion was transferred to a colorimeter tube and its optical density measured, against a blank of petroleum ether, in an Evelyn colorimeter at a wavelength of 440 mu. By comparing this reading with a standard curve previously prepared, a quantitative measurement of the carotene in solution was obtained. The concentrations were expressed in micrograms per 100 ml. (mcg. %) of plasma.

The petroleum ether was now removed from the aliquot by evaporation under reduced pressure, with the aid of a hot water bath to speed the operation. The last few drops of petroleum ether were allowed to evaporate at room temperature. The remaining residue was then taken up with 1 ml. of chloroform, and several drops of acetic anhydride were added to take up any traces of water. To this were then added 9 ml. of a 25% solution of antimony trichloride in chloroform (Carr Price Reagent) and the optical density of the blue color which developed was read within 6 seconds at a wavelength of 620 mu. in the Evelyn Colorimeter.

Since carotene also forms a blue color with Carr Price Reagent, though only about one tenth as intense as that given by an equivalent amount of vitamin A, a correction was made for any of this pigment present in the sample. This was done by subtracting correction values, obtained from a curve prepared by developing standard beta carotene solutions with Carr Price Reagent, from total blue color values. These concentrations, like those for carotene, were also expressed in mcg. %.

Liver Analysis:

Liver samples were stored in the deep freeze at -14°C until convenient to analyze them. The method of analysis, developed by Gallup and Hoeffer (13), consisted of digesting the liver, weighing from 0.5 to 1.0 grams, with 5ml. of 5% alcoholic KOH in a hot water bath until the samples were completely saponified. After cooling, the volume was brought up to twice the original volume with water, and 15 ml. of Darco-treated petroleum ether were added. The samples were then shaken vigorously for 5 minutes to extract the pigments, which were then analyzed for in the same manner as described in the analysis of plasma. The per cent dry matter in the liver was obtained by drying a tared sample at 100°C for 24 hours and reweighing the dried residue. Both the carotene and vitamin A values were expressed in mcg. per gram of dry matter.

CHAPTER III

UTILIZATION OF INTRAVENOUSLY ADMINISTERED CAROTENE BY HOLSTEIN AND GUERNSEY CALVES

Metabolism of Intravenously Administered Carotene by Depleted Dairy Calves.

It was the purpose of this experiment to attempt to determine whether or not the Holstein and Guernsey breeds of dairy animals are able to utilize, equally well, intravenously-administered beta carotene as a source of vitamin A.

Eight dairy calves, 3 Holstein and 5 Guernsey, were placed on a low carotene ration until their blood vitamin A levels ranged from 1 to 12 mcg.%. The animals were then injected intravenously with carotene preparation, the doses of which were varied from animal to animal as listed in Tables 1 and 2. Blood samples were then taken from the animals, at intervals covering a 9 day period following carotene administration, and analyzed for carotene and vitamin A.

Figures 1 and 2 are typical of the results, which are given in detail in Tables 1 and 2, obtained in this experiment. It was noted here that as the carotene level, which reached a peak value almost immediately after injection, fell off, no significant rise took place in the vitamin A level. There is, therefore, no positive indication of carotene conversion to vitamin A in these results. There was no apparent difference between the two breeds in their ability to use intravenous carotene. The rise in the apparent blood vitamin A within the first hour, was similar to several others found in this experiment. It was not, however, unique for either of the breeds. The fact

Figure 1

Changes in plasma carotene and vitamin A levels of Holstein Calf No.95 injected with 0.34 mg. carotene/kg.

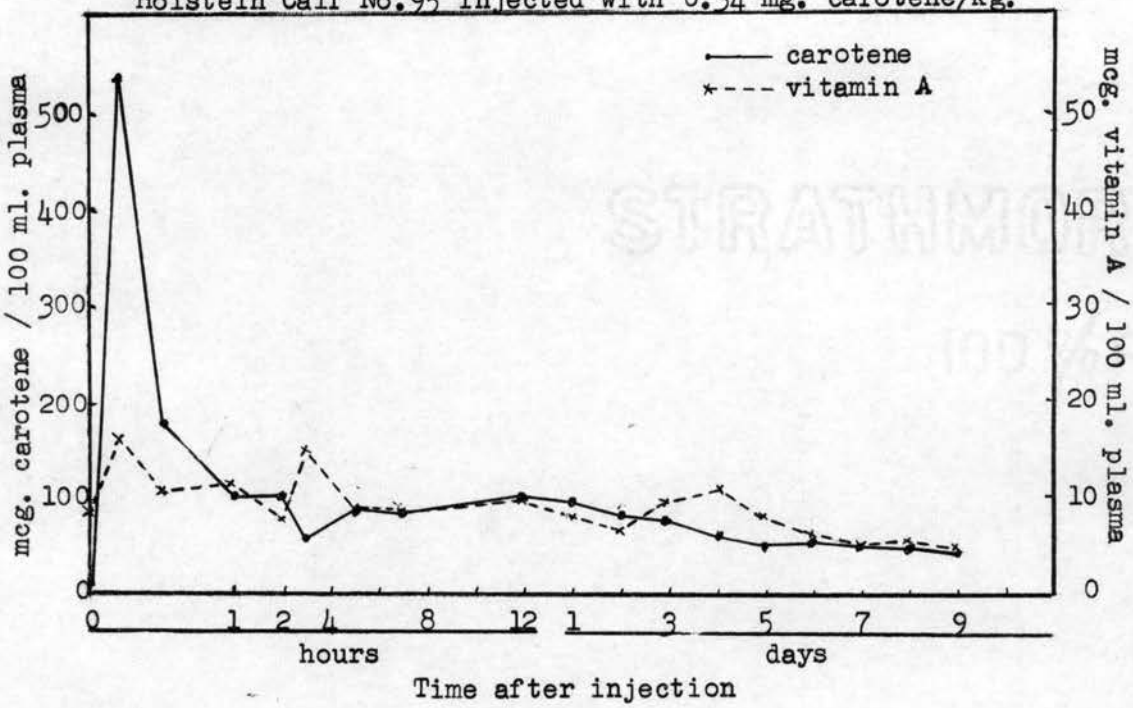


Figure 2

Changes in plasma carotene and vitamin A levels of Guernsey Calf No.16 injected with 0.25 mg. carotene/kg.

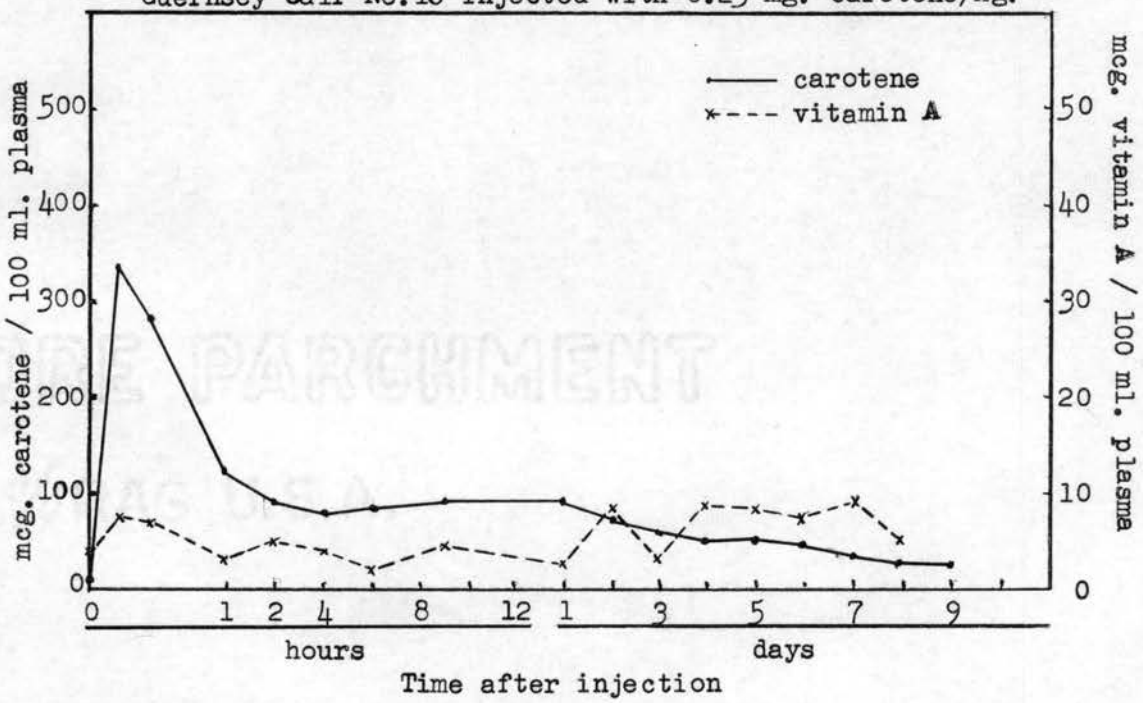


Table 1

Time Interval	Changes in plasma carotene conc. (mcg.%) following injection of carotene into depleted calves. (A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)
0 hrs.	37	11	12	14	20	21	16	11
1/6 "	360	540	198	339	321	372		58
1/3 "							1095	
1/2 "	180	180	111	288	140	195	1073	55
2/3 "							1050	
1 "	114	103	63	116	86	119	1020	28
2 "	100	103	56	94	79	114	825	
3 "		56					735	
4 "	81		64	86	86	123		20
5 "		83						
6 "	123		59	90	86	144	603	
7 "		80						
8 "								23
9 "			68	93	96	135	606	
12 "	109	105					615	21
1 days	123	109	84	90	85	135	540	17
2 "	121	96	68	71	79	107	533	14
3 "	117	88	65	57	56	90	458	14
4 "		70	56	54	51	85	443	12
5 "		55	47	56	48	72	365	10
6 "		55	43	50	39	71	338	12
7 "		54	34	36	38	50	310	14
8 "		54	32	32	41	50	267	14
9 "		50	32	30	34	44	240	
10 "							210	

(A) Holstein Calf No. 2 (578 lb.) inj. 0.45 mg. / kg.
(B) Holstein Calf No.95 (260 lb.) inj. 0.34 mg. / kg.
(C) Holstein Calf No.95 (277 lb.) inj. 0.25 mg. / kg.
(D) Holstein Calf No.16 (409 lb.) inj. 0.25 mg. / kg.
(E) Guernsey Calf No. 7 (308 lb.) inj. 0.23 mg./kg.
(F) Guernsey Calf No.14 (390 lb.) inj. 0.27 mg./kg.
(G) Guernsey Calf No.24 (307 lb.) inj. 0.86 mg./kg.
(H) Guernsey Calf No.24 (380 lb.) inj. 0.35 mg./kg.

Table 2

Time Interval	Changes in plasma vitamin A conc. (mcg.%) following injection of carotene into depleted calves. (A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)
0 hrs.	8.1	7.2	12.3	4.3	7.5	1.6	6.0	9.6
1/6 "		15.7	21.7	7.6	8.5	28.5		28.1
1/3 "							36.0	
1/2 "	13.2	11.7	22.4	7.4	8.3	14.9	36.6	18.2
2/3 "							32.2	
1 "	11.7	12.0	16.0	3.8	6.2	9.1	29.2	----
2 "	11.3	7.2	11.8	5.9	6.2	8.7	26.9	
3 "		15.2						
4 "	7.0		10.5	4.8	6.6	9.3		10.6
5 "		8.3						
6 "	11.9		15.1	2.1	7.6	11.0	23.1	
7 "		8.1						
8 "								6.8
9 "			11.4	4.7	5.4	10.4	21.1	
12 "	10.1	9.9					18.2	7.4
1 days	10.9	8.2	12.3	3.5	6.2	10.4	18.0	5.8
2 "	15.4	7.3	13.6	7.7	8.1	8.1	15.7	7.9
3 "	7.6	9.3	10.1	2.9	3.5	6.8	----	7.9
4 "		10.2	13.2	8.7	6.9	6.4	17.6	4.9
5 "		8.0	9.9	8.0	6.3	5.4	12.2	4.6
6 "		5.2	9.2	7.0	6.0	5.4	12.8	8.1
7 "		4.5	8.3	9.9	5.2	5.1	13.4	4.8
8 "		5.0	8.4	4.7	6.8	8.0	16.5	5.7
9 "		3.7	9.3	---	8.3	7.5	11.5	
10 "							12.5	

See key for Table 1

that these rises were so rapid, and that they fell along with the carotene levels, plus the fact that these rises were not found throughout this and later experiments, lends us to believe that this is an artifact introduced by the injection of Tween 40. Efforts to separate the carotene and the vitamin A by chromatographic methods, in order to account for the true origin of the blue color with Carr Price Reagent, were unsuccessful. Additional experiments which follow, were undertaken, therefore, in attempting to establish that the failure to observe an increase in blood vitamin A following carotene injection was due, essentially, to a failure to effect conversion of carotene to vitamin A.

Metabolism of Intravenously Administered Carotene by Normal Dairy Calves.

Perhaps the reason that no significant signs of carotene conversion were observed in the previous experiment was that the animals were so in need of the vitamin that the tissues absorbed it from the blood as rapidly as it was produced. In an attempt to rule out such a demand for the vitamin by the tissues, an experiment was conducted in which carotene was administered to normal animals that had not been depleted of their vitamin A stores.

Two Holstein calves and a Guernsey calf, whose plasma vitamin A levels ranged from 8.0 to 11.0 mcg. % were injected with 0.15 and 0.25 mg./kg. respectively. At intervals following injection, blood samples were taken and analyzed for carotene and vitamin A.

Here again, as the results listed in Table 3 and described in Figures 3 and 4 demonstrate, no rise in the vitamin A levels occur after injection. This would indicate that no conversion of the provitamin takes place, since any significant amount of transformation should result in

Figure 3

Changes in plasma carotene and vitamin A levels of Holstein Calf No.81 injected with 0.15 mg. carotene/kg.

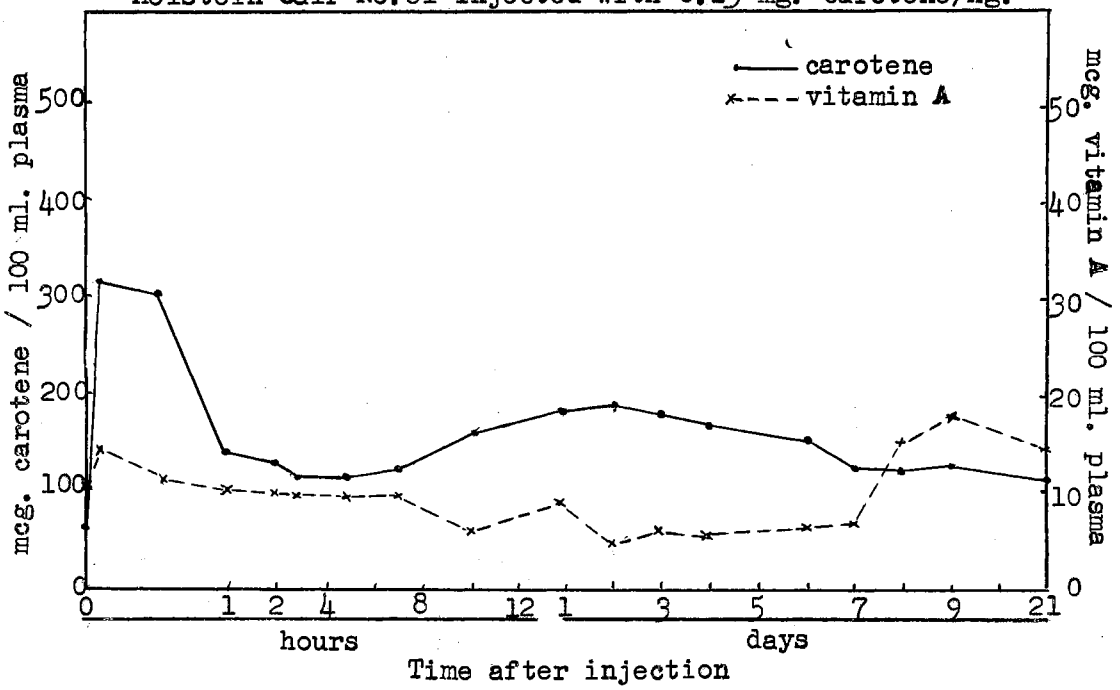


Figure 4

Changes in plasma carotene and vitamin A levels of Guernsey Calf No.29 injected with 0.25 mg. carotene/kg.

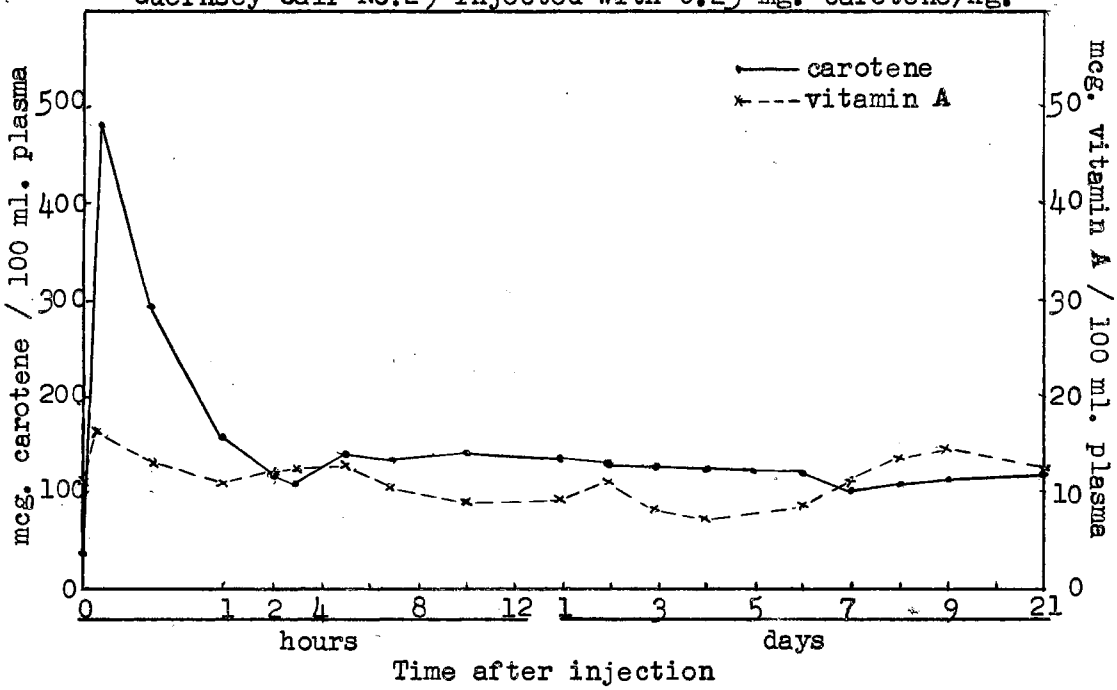


Table 3

Changes in plasma carotene and vitamin A concentrations following injection of carotene into normal dairy calves.

Time Interval	mcg. % of carotene (A)	(B)	(C)
0 hrs.	42	69	61
1/6 "	486	282	309
1/2 "	297	225	252
1 "	160	145	146
2 "	117	128	128
3 "	112	121	123
5 "	145	138	123
7 "	140	135	134
10 "	146	146	165
1 days	141	185	182
2 "	137	209	191
3 "	133	204	180
4 "	125	212	168
6 "	125	187	152
7 "	113	174	125
8 "	116	183	121
9 "	119	170	126
21 "	120	177	108

Time Interval	mcg. % of vitamin A (A)	(B)	(C)
0 hrs.	10.9	8.9	10.1
1/6 "	16.8	16.3	14.3
1/2 "	13.1	11.2	11.2
1 "	10.1	10.7	10.7
2 "	11.4	11.0	10.5
3 "	11.8	9.2	9.9
5 "	11.8	12.9	9.1
7 "	10.2	11.0	9.2
10 "	9.1	12.2	5.8
1 days	9.6	11.8	8.5
2 "	11.4	7.4	4.8
3 "	8.3	8.7	5.7
4 "	7.4	6.6	5.6
6 "	8.9	9.2	6.8
7 "	10.8	9.1	6.9
8 "	13.2	14.4	16.1
9 "	14.0	15.0	19.2
21 "	10.3	13.8	14.1

(A) Guernsey Calf No.29 (108 lb.) inj. 0.25 mg. / kg. of beta carotene.
(B) Holstein Calf No.50 (300 lb.) inj. 0.15 mg. / kg. of beta carotene.
(C) Holstein Calf No.81 (257 lb.) inj. 0.15 mg. / kg. of beta carotene.

an increase in the vitamin A plasma levels of these non-depleted animals. There is no explanation for the appearance of a second peak in the carotene curves of the Holstein animals.

Effect of Intravenously Administered Tween 40 on the Plasma Carotene and Vitamin A Levels of a Guernsey Calf.

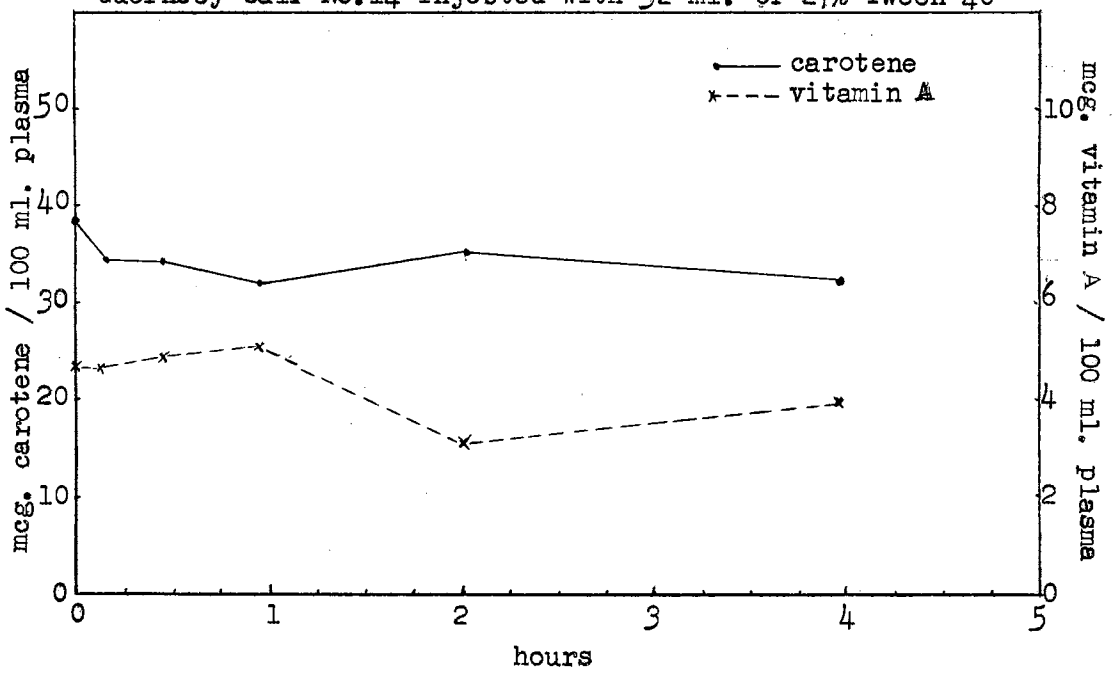
In the interpretation of these data it was necessary to learn whether or not Tween 40 had an effect upon the blood levels of carotene and vitamin A. It was the purpose of this experiment, therefore, to find out what effect, if any, the Tween 40 had on blood carotene and vitamin A concentrations.

An injection of 32 ml. of a 27% solution of Tween 40 in water was made into the veins of a normal Guernsey calf. The blood picture for carotene and vitamin A was followed for four hours after injection.

The results, given in Table 4 and described in Figure 5, show no appreciable changes in either the carotene or vitamin A levels after the injection. It can be said, therefore, that these two levels are not affected by the dispersing agent used in the intravenous preparations. Church and co-workers (7) found the same absence of any adverse effect on the part of the Tween 40 on sheep. The unexplained peaks in the vitamin A curves in Part 1 were suggested to be a result of some effect of the Tween 40. Though the results of this experiment do not support this suggestion, they do not rule out the possibility that the so called artifacts are results of some effect of the Tween 40 and the high carotene concentrations on the analysis.

Figure 5

Changes in plasma carotene and vitamin A levels of Guernsey Calf No.14 injected with 32 ml. of 27% Tween 40



Time after injection

Table 4

Changes in plasma carotene and vitamin A concentrations of Guernsey Calf No.14 following injection of 35 ml. of 27% Tween 40 solution.

Time Interval	carotene mcg. %	vitamin A mcg. %
0 hrs.	38	4.7
1/6 "	34	4.6
1/2 "	34	5.0
1 "	32	5.2
2 "	35	3.1
4 "	32	3.9

Metabolism of Carotene Injected Directly into the Duodenum of Calves.

This experiment was carried out to make certain that the carotene, as held in solution with Tween 40, becomes available to the animal and that the resulting vitamin A can be detected in the blood.

Liver biopsy samples were obtained from each of two vitamin A deficient calves, one Holstein and one Guernsey, by the method of Whitehair at al (41). Each animal then had 90 mg. of carotene injected directly into the duodenum, which had been exposed by an incision through the abdominal wall and peritoneum. The incision was sutured after injection and blood samples were taken at various intervals throughout a five day period. The animals were then sacrificed and liver samples again taken.

The results are listed in Table 5 and described in Figures 6 and 7. Here it is noted that as the carotene level rose in the Guernsey calf, the vitamin A level also increased from 1.3 mcg. % at the time of injection to 8.4 mcg. % 12 hours thereafter. Also, the liver level rose from 1.5 mcg. per gram to 2.1 mcg. per gram over this five day period. Very similar increases resulted with the Holstein calf. The increases are pronounced enough to indicate that such administration of carotene is an efficient source of vitamin A.

Effect of Periodic Administrations of Intravenous Carotene to a Guernsey Calf.

The failure to observe increased plasma vitamin A in the first two experiments suggested that perhaps a lower efficiency of utilization than sheep or goats made the dosages of carotene used so small that the amounts of vitamin A synthesized were not detected by the methods employed. The following experiment was carried out, therefore, in an effort

Figure 6

Changes in plasma carotene and vitamin A levels of Guernsey Calf No.7 injected with 90 mg. carotene into duodenum

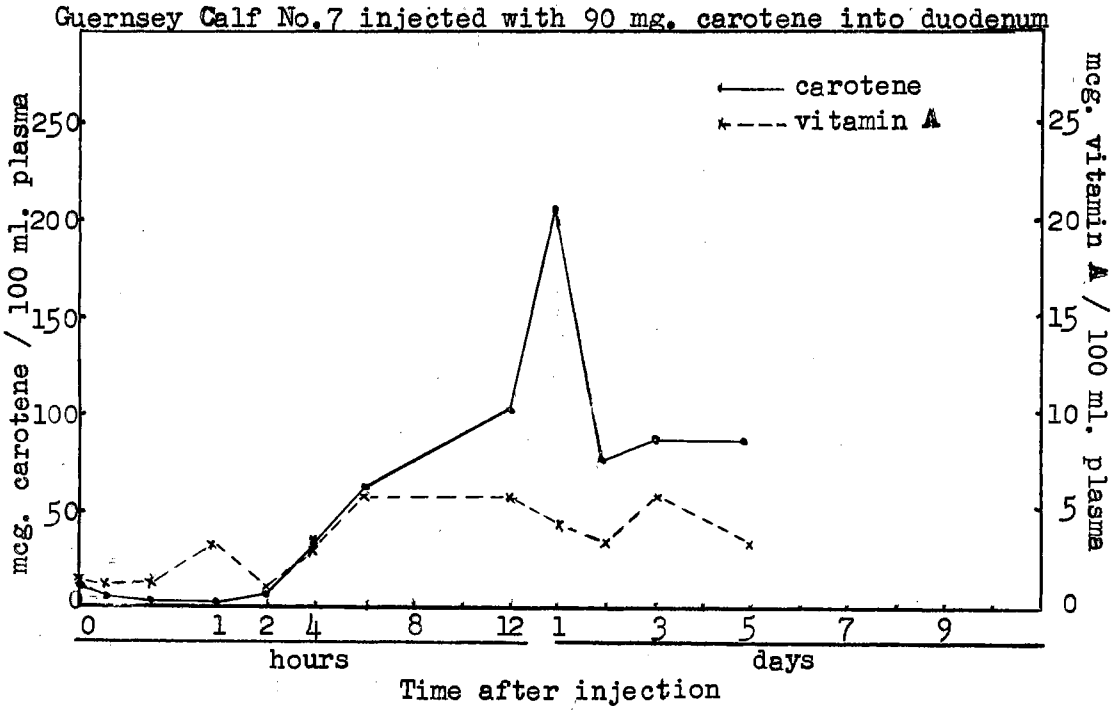


Figure 7

Changes in plasma carotene and vitamin A levels of Holstein Calf No.95 injected with 90 mg. carotene into duodenum

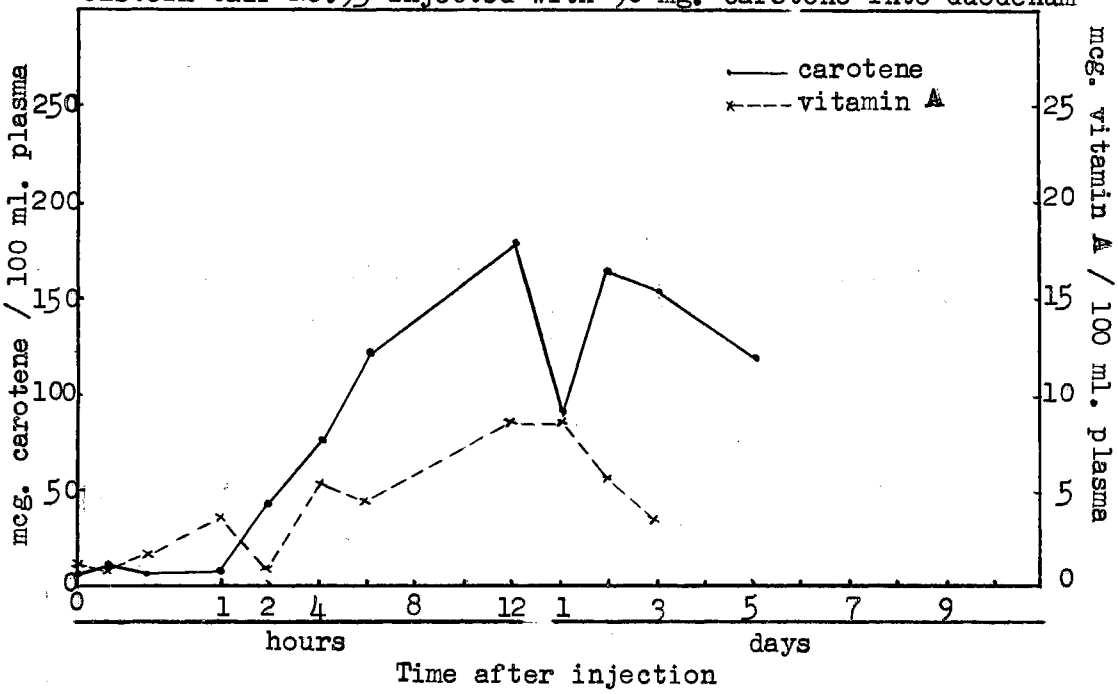


Table 5

Changes in plasma carotene and vitamin A concentrations of depleted dairy calves following injection of carotene into the duodenum.

Time Interval	Carotene mcg. / 100 ml. (A)	Carotene mcg. / 100 ml. (B)	Vitamin A mcg. / 100 ml. (A)	Vitamin A mcg. / 100 ml. (B)
0 hours	11	7	1.9	1.3
1/4 "	5	13	1.4	0.8
1/2 "	3	7	1.5	1.7
1 "	3	7	2.9	3.1
2 "	7	43	1.3	1.1
4 "	36	77	3.1	5.2
6 "	69	123	5.9	4.3
12 "	101	179	5.7	8.4
24 "	212	91	4.4	8.2
48 "	79	169	3.7	5.9
72 "	86	155	6.4	3.4
120 "	85	89	3.9	---

Carotene and vitamin A concentrations of liver samples.

	Carotene mcg./gm. (A)	Carotene mcg./gm. (B)	Vitamin A mcg./gm. (A)	Vitamin A mcg./gm. (B)
Before Injection	1.3	1.9	1.2	1.5
After 5 days	8.8	23.0	2.2	2.1

(A) Guernsey Calf No. 7 (326 lb.) injected into duodenum 0.7 mg. beta carotene / kg.
(B) Holstein Calf No.95 (296 lb.) injected into duodenum 0.7 mg. beta carotene / kg.

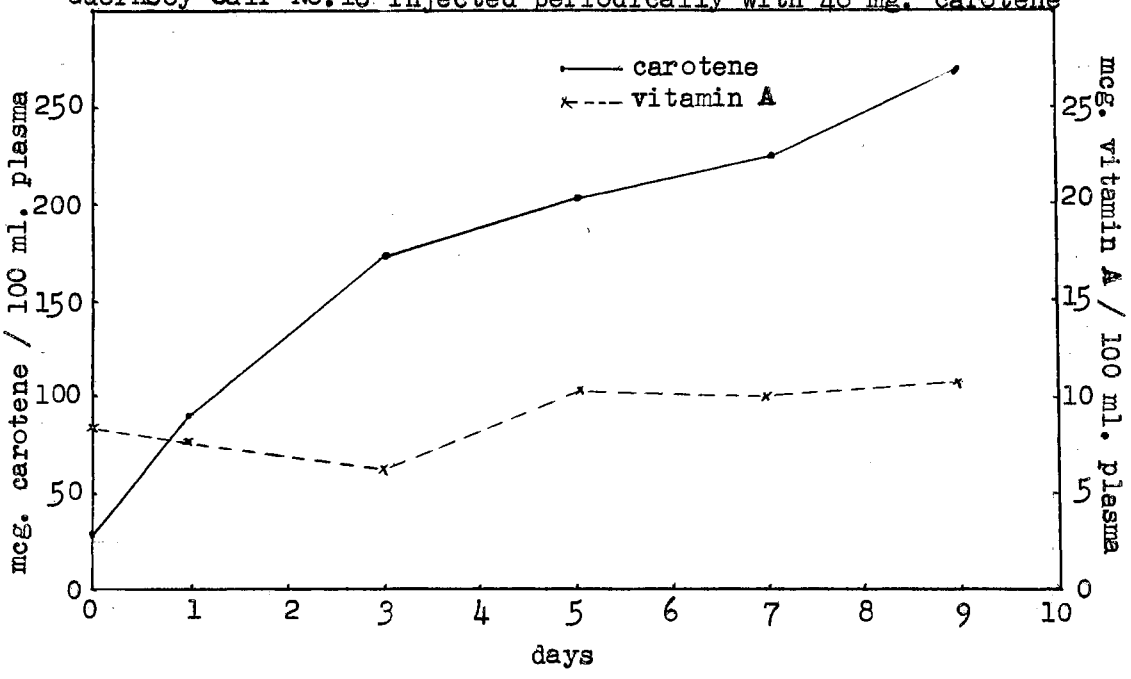
to observe the effect of repeated injections of carotene on the plasma vitamin A level of a depleted calf on a carotene deficient ration.

A Guernsey calf was injected every other day for nine days with 48 mg. of carotene. Blood samples were withdrawn on the alternate days and analyzed for carotene and vitamin A.

The results, given in Table 6 and described in Figure 8, showed a steady increase in the carotene blood level. The vitamin A level, however, varied from 6.3 to 11.6 mcg. %, an increase of about 3 mcg. % above the initial level. Such a change is not significant when it is noted that 90 mg. of carotene given into the duodenum brought about an increase of 8 mcg. % within 12 hours after injection. This all suggests that intravenous carotene is not effectively utilized as a vitamin A source.

Figure 8

Changes in plasma carotene and vitamin A levels of Guernsey Calf No. 16 injected periodically with 48 mg. carotene



Time after injection

Table 6

Changes in plasma carotene and vitamin A concentrations of Guernsey calf No.16 injected periodically with 48 mg. carotene.

Time Interval	Carotene mcg. %	Vitamin A mcg. %
0 days	31	8.4
1 "	88	7.9
3 "	171	6.3
5 "	201	11.1
7 "	229	11.0
9 "	273	11.6

Metabolism of Intravenously Administered Vitamin A by Dairy Calves.

To evaluate the rate at which the vitamin A, if it was being formed from carotene, was being absorbed from the blood by the tissues, the following procedure was followed.

Vitamin A palmitate was administered to each of four calves, 2 Holstein and 2 Guernsey. Three trials, involving the same four animals, with three different dosages of the vitamin being given, were carried out. In the first trial the animals were not depleted and were given 20 mcg. / kg. of vitamin A. In the second trial, the animals were depleted to the point where their plasma vitamin A levels were between 3.7 and 4.5 mcg. %. Each animal was given 11 mcg. / kg. of vitamin A. In the third trial, the animals were depleted to the point where gross symptoms of vitamin A deficiency (lachrymation and incoordination) had set in. The plasma levels of the vitamin at this point ranged from 2.0 to 4.6 mcg. %. These animals were then injected with 180 mcg. / kg. of vitamin A.

The resulting data is shown in Tables 7 and 8 and described in Figures 9, 10, 11, and 12. It can be seen that the vitamin A was removed from the blood stream quite rapidly after administration. There seemed

to be no appreciable difference in the removal rates of the non-depleted and the fully depleted animals, or between the high and low dosage injections. The rapid disappearance of intravenously administered vitamin A from the blood raises some question concerning the interpretation of previously repeated failure to observe vitamin A increases following carotene injection. It should be noted, however, that the largest dose of vitamin A used in this experiment (180 mcg. / kg.) compares with only the smallest of carotene doses (150 mcg. / kg.) used in the carotene injection experiments.

Figure 9

Changes in plasma vitamin A concentration of
Holstein Calf No.81 injected with 11 mcg. vitamin A/kg.

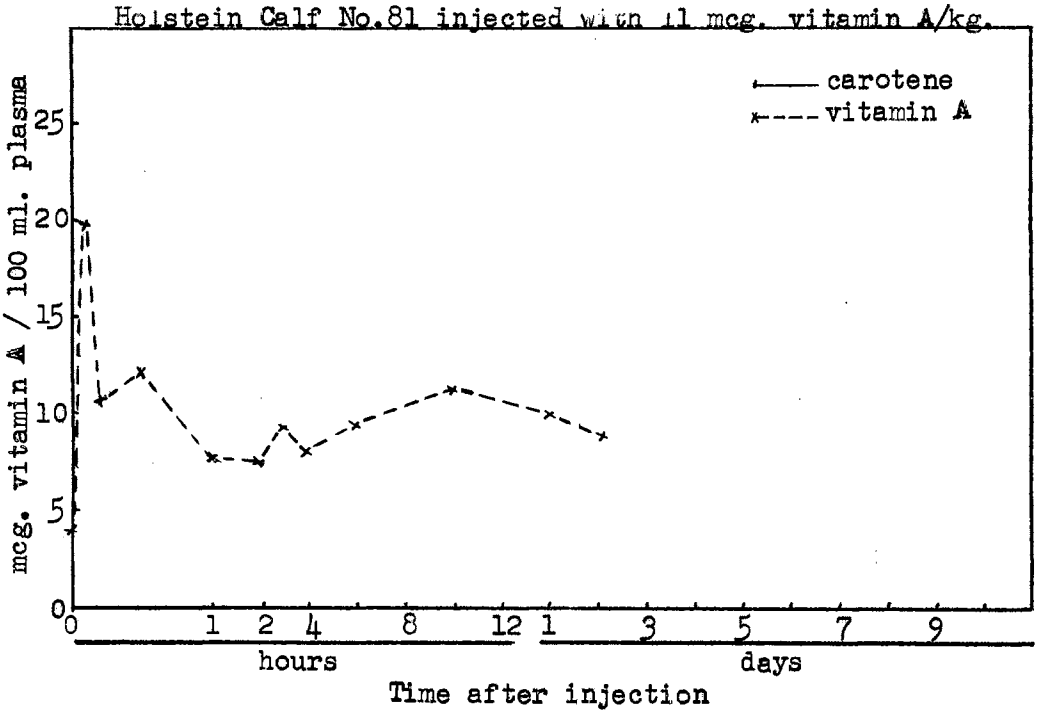


Figure 10

Changes in plasma vitamin A concentration of
Holstein Calf No.81 injected with 20 mcg. vitamin A/kg.

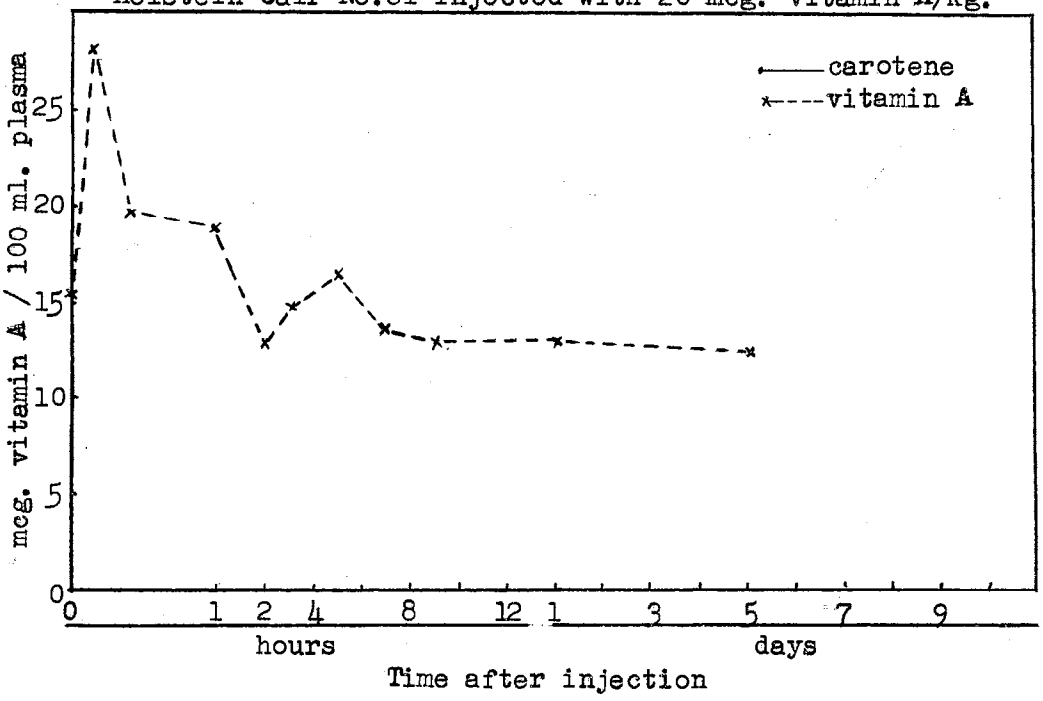


Figure 11

Changes in plasma vitamin A concentration of
Guernsey Calf No.29 injected with 11 mcg. vitamin A/kg.

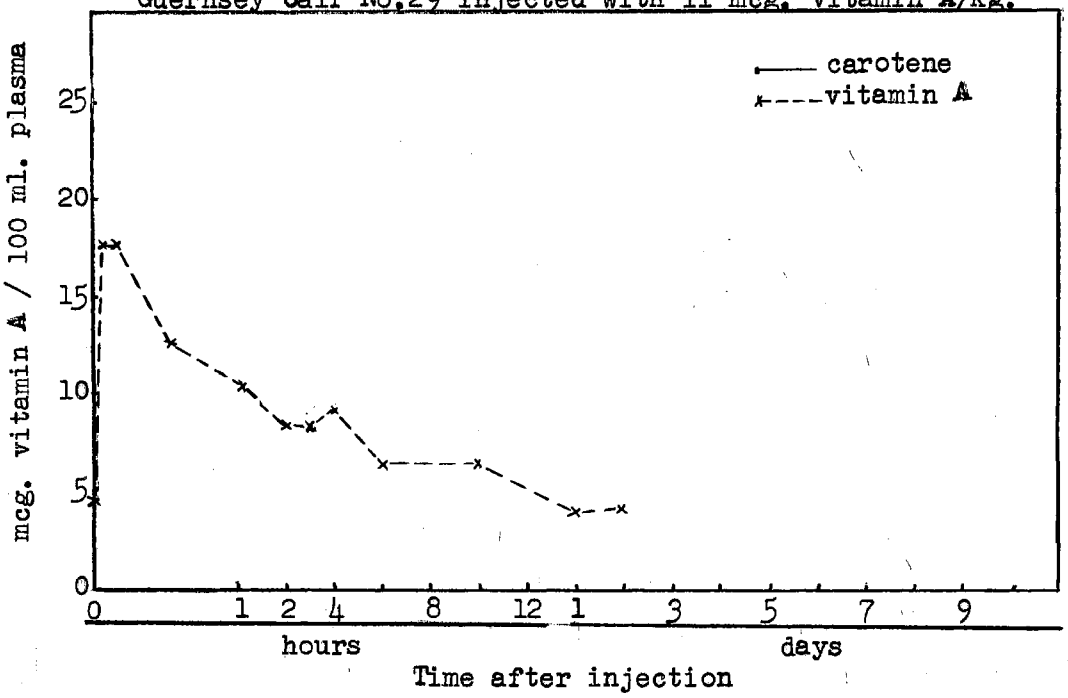


Figure 12

Changes in plasma vitamin A concentration of
Guernsey Calf No.29 injected with 180 mcg. vitamin A/kg.

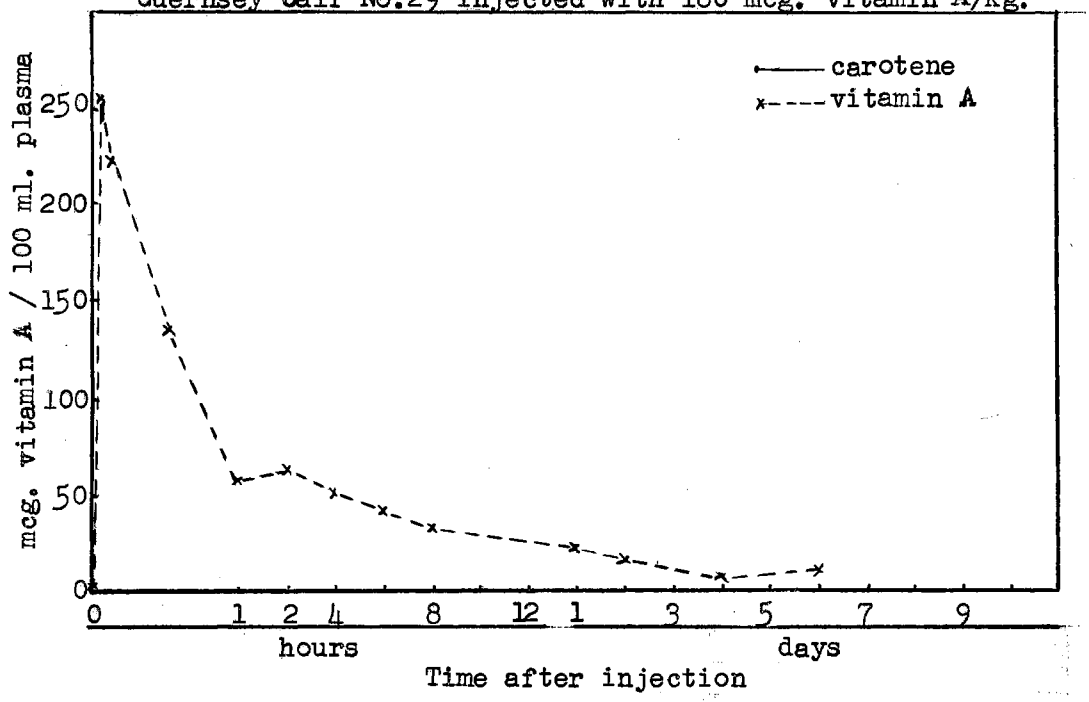


Table 7

Time Interval	Changes in plasma carotene conc. (mcg. %) following injection of vitamin A into dairy calves.											
	(A1)	(B1)	(C1)	(D1)	(A2)	(B2)	(C2)	(D2)	(A3)	(B3)	(C3)	(D3)
0 min.	128	69	124	99	11	25	21	19	12	10	19	9
5 "					11	25	19	20	14	7	19	12
10 "	121	65	153	95	11	25	19	19			19	11
30 "	121	56	133	88	11	25	20	19	12	7	19	9
1 hrs.	125	61	146	93	11	21	20	21	10	9	21	9
2 "	125	62	149	105	12	20	21	20	12	11	19	6
3 "	133	62	135	95	10	22	19	20				
4 "					12	24	21	19	8	11	19	9
5 "	135	62	133	85								
6 "					13	20	22	18	12	9	19	9
7 "	133	63	141	83								
8 "									10	9	28	7
9 "	128	59	141	87	21	17	23	14				
1 days	128	58	146	95	25	17	21	9	14	9	19	7
2 "					19	18	21	7	9	10	19	9
3 "												
4 "									7	17	14	7
5 "	125	62	165	103								
6 "									10	9	14	8

(A) Guernsey Calf No.29 (143 lb.)
(B) Guernsey Calf No.30 (94 lb.)
(C) Holstein Calf No.50 (340 lb.)
(D) Holstein Calf No.81 (300 lb.)

(1) Injected 20 mcg. vitamin A per kilogram.
(2) Injected 11 mcg. vitamin A per kilogram.
(3) Injected 180 mcg. vitamin A per kilogram.

Table 8

Time Interval	Changes in plasma vitamin A conc. (mcg. %) following injection of vitamin A into dairy calves.											
	(A_1)	(B_1)	(C_1)	(D_1)	(A_2)	(B_2)	(C_2)	(D_2)	(A_3)	(B_3)	(C_3)	(D_3)
0 min.	15.5	9.8	18.9	15.3	4.5	5.7	3.7	3.9	2.7	4.6	3.0	4.2
2 "									255.8	309.2	200.7	175.5
5 "					17.7	13.6	25.4	19.9	221.6	228.6	182.1	168.3
10 "	24.4	20.5	31.8	28.0	17.7	4.3	13.6	10.8				
30 "	21.2	18.1	24.5	19.8	12.9	6.2	8.9	12.2	139.6	107.3	140.1	110.3
1 hrs.	18.8	12.8	23.5	19.2	10.1	5.5	8.0	7.6	57.9	65.5	99.8	74.9
2 "	19.8	11.8	19.1	13.3	8.2	5.6	7.1	7.6	60.2	56.8	67.3	48.5
3 "	18.2	9.9	22.1	14.5	8.2	8.7	5.2	9.4				
4 "					8.7	6.8	8.0	8.5	50.9	35.0	52.4	77.5
5 "	20.1	10.8	19.2	16.9								
6 "					7.3	5.7	6.6	9.4	47.1	29.8		
7 "	17.2	12.6	18.5	13.1								
8 "									37.2	24.7	37.0	21.9
9 "	14.5	7.3	14.7	12.8	7.4	6.8	10.5	11.6				
1 days	12.3	7.4	14.2	13.1	4.3	5.8	11.1	10.1	25.3	12.0	21.9	14.6
2 "					4.8	5.3	11.1	8.5	18.8	9.2	18.7	13.9
3 "												
4 "									7.6	9.4	15.0	10.7
5 "	11.8	7.5	12.8	12.5								
6 "									9.1	3.7	9.8	10.7

See Key for Table 7

CHAPTER IV

UTILIZATION OF INTRAVENOUSLY ADMINISTERED CAROTENE BY ILEECTOMIZED SHEEP

Unlike Cattle whose blood contains small quantities of carotenoids under normal dietary conditions, sheep do not circulate carotenoids to any appreciable extent. The work of Church and co-workers (7) showed that sheep are able to utilize intravenously administered carotene as a source of vitamin A. This being the case, it seemed likely that some extra intestinal site existed where this transformation took place. In an attempt to rule out the possibility that the intestine was somehow involved, it was decided to perform an experiment similar to that carried out by Church and co-workers, but using as the experimental subject sheep whose ilea have been removed.

The entire small intestinal tract, from the stomach to the cecum, was removed from each of three sheep. A small liver sample was taken from one of the liver lobes during surgery and another post mortem. After completion of the surgery, carotene was injected intravenously. The dosages used varied from animal to animal as listed in Table 9. Following injection, blood samples were taken at intervals covering a 24 hour period.

The results of the three trials of this experiment are presented in Table 9 and shown graphically in Figure 13. In each case, the vitamin A level rose as the carotene level fell from its maximum value. The liver vitamin A levels were too high to allow detection of changes of the magnitude one could expect even if the injected carotene was wholly converted to the vitamin. These blood pictures are very similar to those reported

for the intact animal (7). It seems certain, therefore, that the ileum is not involved to any appreciable extent in the metabolism of intravenously administered carotene by sheep.

Figure 13

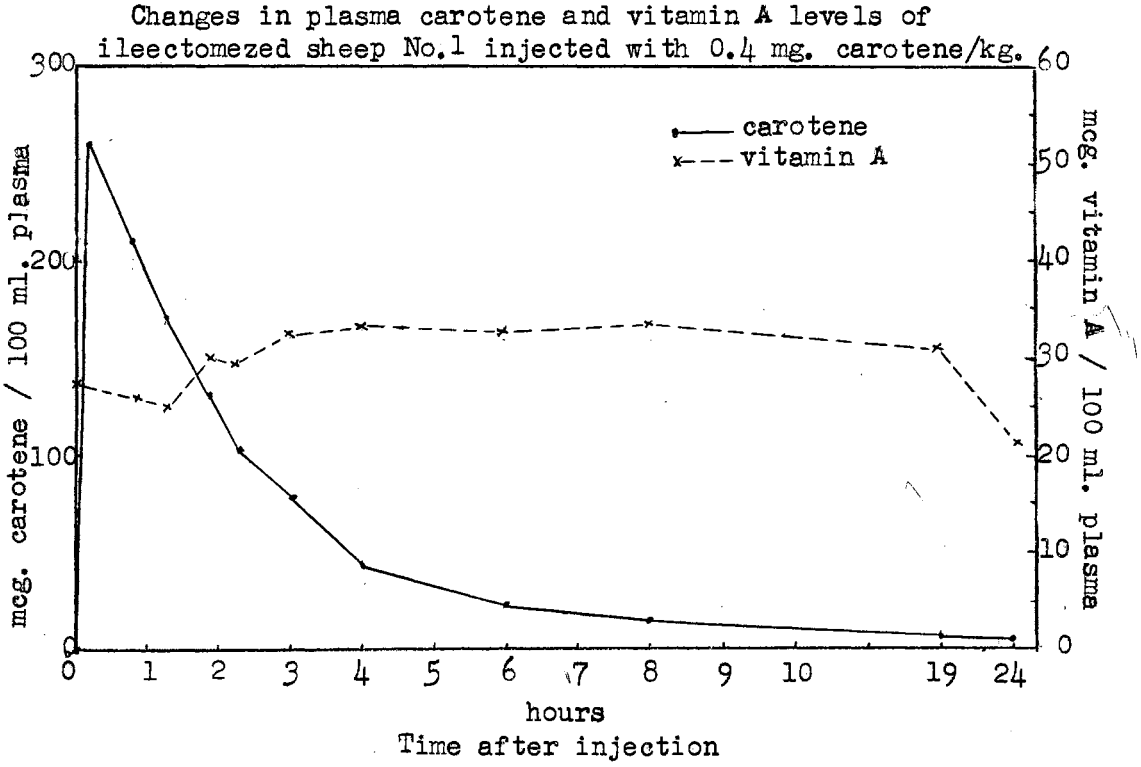


Table 9

Changes in plasma carotene and vitamin A concentrations following injection of carotene into ileectomized sheep.

Time Interval	mcg. % of carotene (A)	(B)	(C)
0 min.	0	8	0
10 "	261		765
15 "		470	
30 "			713
45 "	210	430	
60 "		380	623
75 "	172		
105 "	126	342	
2 hrs.			546
2.3 "	102		
3 "	72	170	325
4 "	43	140	
4.5 "			168
6 "	28		
6.5 "			180
7 "		95	
8 "	19		
10 "			120
19 "	11		
26 "	9		58

Time Interval	mcg. % of vitamin A (A)	(B)	(C)
0 min.	27.4	29.0	21.4
10 "			59.0
15 "		51.2	
30 "			46.9
45 "	26.8	56.7	
60 "		53.6	30.5
75 "	25.7		
105 "	31.4	61.7	
2 hrs.			39.3
2.3 "	31.2		
3 "	35.2	46.6	44.7
4 "	35.0	48.6	
4.5 "			45.4
6 "	35.9		
6.5 "			39.7
7 "		48.9	
8 "	33.7		
10 "			31.2
19 "	31.0		
26 "	21.5		25.0

(A) Sheep No.1 injected with 10 mg. of carotene.
(B) Sheep No.2 injected with 10 mg. of carotene.
(C) Sheep No.3 injected with 15 mg. of carotene.

CHAPTER V

UTILIZATION OF INTRAVENOUSLY ADMINISTERED CAROTENE BY GOATS

Like sheep, swine and rats, goats circulate no appreciable quantities of carotene in the blood. Work has been done on sheep, swine and rats in studying utilization of intravenously administered carotene; none, however, had been done with goats. It seemed appropriate, therefore, to determine whether or not the goat could satisfactorily convert parenterally administered carotene to vitamin A under the conditions employed in these studies.

Each of four goats were injected with 15 mg. of carotene. At intervals following injection, blood samples were taken and later analyzed for carotene and vitamin A.

The resulting data are given in Table 10 and Figure 14. This is similar to the results obtained with sheep (7). As the carotene level fell from the maximum, there appears a distinct and substantial rise in the vitamin A curve. If one assumes a metabolic pattern similar to sheep, it must be presumed that some extra-intestinal site exists for the conversion of carotene to vitamin A.

FIGURE 14

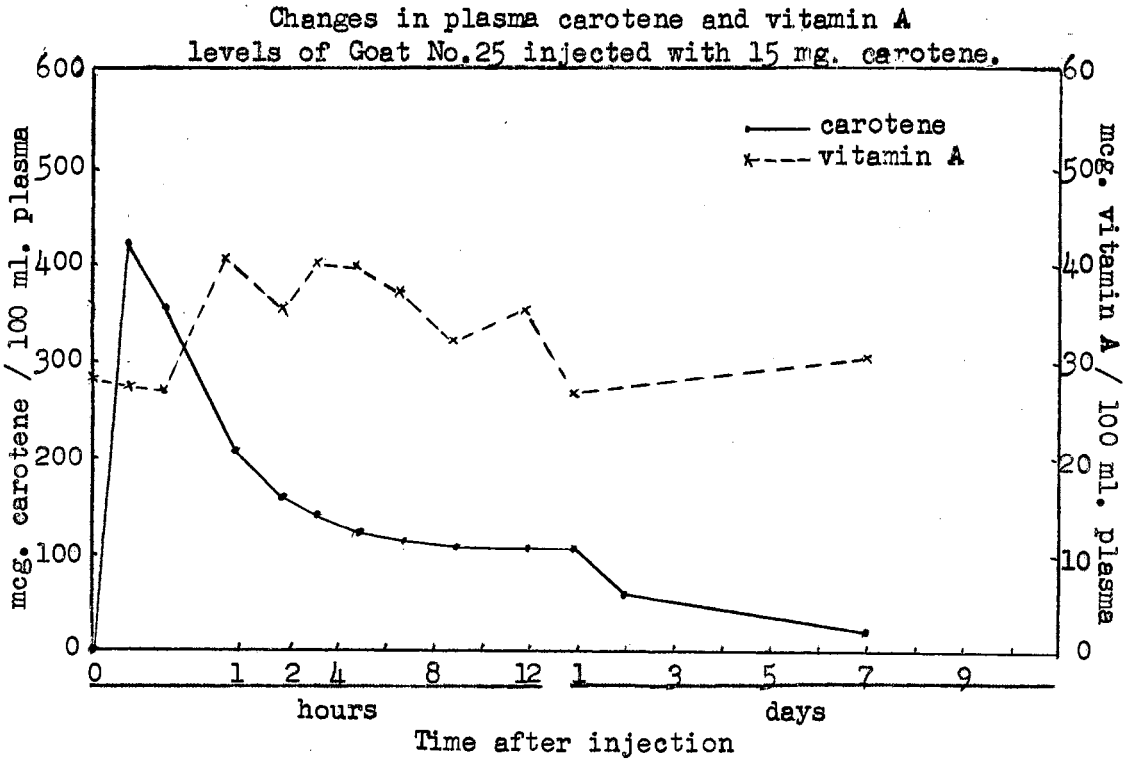

Changes in plasma carotene and vitamin A
levels of Goat No.25 injected with 15 mg. carotene.
carotene
vitamin A
mcg. carotene / 100 ml. plasma
mcg. vitamin A / 100 ml. plasma
600
500
400
300
200
100
0
60
50
40
30
20
10
0
0 1 2 4 8 12 1 3 5 7 9
hours
days
Time after injection

Table 10

Changes in plasma carotene and vitamin A concentrations following injection of carotene into goats.

Time Interval	mcg. % of carotene (A)	(B)	(C)	(D)
0hours	7	3	0	3
1/4 "	1155	627	425	900
1/2 "	1140	487	353	840
1 "	1020	248	210	665
2 "	772	248	168	386
3 "	501	160	138	273
5 "	301	149	114	198
7 "	168	135	107	175
9 "	243	119	105	158
12 "	168	119	105	121
1 days	110	110	105	86
2 "	66	62	63	46
7 "	11	17	17	--

Time Interval	mcg. % of vitamin A (A)	(B)	(C)	(D)
0 hours	38.5	30.2	28.4	36.6
1/4 "	31.4	20.8	27.5	25.6
1/2 "	28.7	23.9	28.0	24.9
1 "	41.5	25.6	41.4	32.9
2 "	91.6	33.7	36.3	48.5
3 "	48.2	36.3	40.2	36.5
5 "	48.9	46.2	40.0	39.9
7 "	42.2	36.5	37.5	38.7
9 "	45.1	33.3	33.2	41.7
12 "	43.3	30.0	35.4	36.5
1 days	39.0	29.5	26.1	38.0
2 "	37.8	30.0	----	35.5
7 "	31.8	28.1	30.2	

(A) Goat No. 23
(B) Goat No. 24
(C) Goat No. 25
(D) Goat No. 75

DISCUSSION

In an attempt to determine whether or not the absence of carotene in the circulation of animals reflects the existence of extra-intestinal sites of carotene to vitamin A conversion, eight dairy calves, three ileectomized sheep, and four goats were given single intravenous injections of carotene, solubilized in water with Tween 40. Blood samples were collected at intervals and analyzed for carotene and vitamin A.

The results of the injection studies with dairy calves displayed no distinguishable differences between Holstein and Guernsey breeds with respect to utilization of intravenously administered carotene. None of the experiments with calves gave positive evidence of carotene transformation. Such conversion may actually have occurred, however, but not have been reflected in an increases vitamin A blood level. Tween 40 displayed no effect upon the carotene or vitamin A blood levels, nor did it affect the availability of the carotene to the animal. Although intravenously administered vitamin A was found to disappear quite rapidly from the blood stream, the inability to produce vitamin A increases with repeated injections of carotene suggests that such carotene preparations are not effectively utilized as sources of vitamin A by Holstein or Guernsey calves.

Significant increases in vitamin A levels were obtained with ileectomized sheep. The results obtained from the experiments with these animals were very similar to those found with the intact sheep by Church and co-workers. This would indicate that intravenous carotene administration effectively

bypasses the small intestine to be converted to vitamin A by some other organ or organs.

It was also found that goats can effectively utilize parenteral carotene administration of this type as a source of plasma vitamin A. Assuming a metabolic pattern similar to sheep, it can be said that injected carotene also bypasses the small intestine of the goat and is converted to vitamin A by some extra-intestinal site.

The marked difference between the results of the carotene injection studies on calves and those on goats and sheep strongly suggests that utilization of intravenously administered carotene as the provitamin is reflected in increased vitamin A blood levels. These data also indicate that the absence of carotene in the circulation reflects the existence of extra-intestinal sites for the conversion of carotene to vitamin A.

SUMMARY

It is postulated that the absence of carotene in the circulation of animals reflects the existence of extra-intestinal sites of carotene conversion . In support of this hypothesis, it was found that:

1. Neither Holstein nor Guernsey calves were able to utilize intravenously administered carotene as a vitamin A source.
2. Sheep, deprived of their small intestine, were able to utilize carotene when given intravenously.
3. Goats were able to convert intravenously administered carotene to vitamin A.

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