ON THE VITAMIN A AND CAROTENE CONTENTS OF THE BLOOD PLASMA AND MILK OF THE COW WITH SPECIAL REFERENCE TO THE INFERTILITY AND THE EFFECT OF ADMINISTRATION OF COD LIVER OIL

ON THE VITAMIN A AND CAROTENE CONTENTS OF THE BLOOD PLASMA AND MILK OF THE COW WITH SPECIAL REFERENCE TO THE INFERTILITY AND THE EFFECT OF ADMINISTRATION OF COD LIVER OIL*

By

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The vitamin A and carotene contents of the blood plasma and milk of the cow have been the subject of many investigations. The seasonal changes are attributed mainly to changes in the feed, whether fresh (in summer) or dry (in winter) fodder (1–15). In our country the vitamin A content of milk becomes extremely low in late winter and early spring, particularly in the northern districts (16, 17).

While nutritional disorders due to feeds unbalance are now considered one of the main causes of infertility of the cow, the conclusion is open to some doubt on account of the lack of accurate knowledge in this field, despite the application of reproductive techniques. For many years this close relationship between reproduction and nutritional status had been suspected. However, little evidence has been given so far to clarify the relationship (18–21). On the other hand, Umezu and his co-worker attempted to standardize the methods for the detection of nutritional disorders in the dairy cow, and investigated the nutritional states of cows in their natural environment (22). On that occasion the present authors determined vitamin A and carotene of the blood plasma and milk, undertaking to correlate vitamin A deficiency with infertility. Secondly, they studied the effects of the administration of large amounts of vitamin A on the vitamin A and carotene contents of the blood plasma and milk of cows in their natural environment.

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Materials and Methods

I. Animals

i) *Cows studied in the infertility survey:* The cows of Holstein were selected from those subjected to the mass health examination held on January 18th, 1956, for dairy cattle in the Iwadeyama area of Miyagi Prefecture. One reason for the high frequency of infertility in this area (25%) might possibly be due to the feeding of excess "okara"-bean curd refuse during winter. "Okara" itself seemed not to be poor as a food since it is quite rich in crude protein (4.7%) and water (84%), but, given in large amounts, the diet tends to become unbalanced. Vitamin A and carotene in the blood plasma and milk were determined by the method described below. The condition of the genital organs was checked by rectal palpation. It was later found that some of the cows chosen, after a long period of infertility, had been pregnant at the time of examination.

ii) *Concentrated cod liver oil administration experiment and subsequent measurements:* The experiment was performed at the University Farm. Six cows were divided into two groups, experimental and control (Table 1).

Table 1. Conditions of the animals in the administration experiment.

No.	Breed	Age	Last parturition (Mos. of lactation)
	I. Control group		
1	Holstein	11	10/2/55(10)
2	Holstein	5	16/7/55(10)
3	Japanese Native Breed × Brown Swiss	4	30/11/55(2)
	II. Experimental group		
4	Ayrshire × Holstein	7	29/10/54(13)
5	Holstein	2	30/8/55(4)
6	Japanese Native Breed × Brown Swiss	4	10/12/55(1)

Rations were given making allowance of body weight and milk production and ranged between 3,400–4,160 and 450–600 g expressed as starch value and digestible protein, respectively.

The cows were fed as usual throughout the period from December 21st, 1955 to April 18th, 1956, and then were put on the pasture until the end of October. The carotene intake, calculated from the content in silage and hay (23) given was 150,000–200,000 i u or 90–120 mg (ca. 180–250 μg/kg body weight). Two and five g of the concentrated cod liver oil (Rikken Pharmacy Co., Ltd., Tokyo), 220,000 and 1,100,000 i u, respectively, in the form of

dumplings, mixed with wheat flour, were administered orally. The former dosage was given daily for 44 days from January 12th after the second sampling, and the latter was given for 54 days from February 23rd. The first three weeks were taken as the control (untreated) period, and samplings were performed (except the sixth) about every 20 days. During the administration experiment some cows dried up about two months before parturition. Three further samplings were made on June 21st, August 11th, and November 28th to determine the changes throughout the year. The conditions of the cows used, the manner of vitamin A administration and the sampling dates are presented in Tables 1, 3 and 4 and Figures 1 and 3, respectively.

II. Analytical Method

Vitamin A and carotene were determined in duplicate by a slight modification of the Bessey-Sahashi method (24, 25; Ref. 12, 26-30). The method is based on the measurement of the absorption at 328 mμ of the non-saponifiable fraction before and after exposure to ultraviolet light. The essential procedure employed was as follows: Five ml. of plasma or 2.5 ml. of milk was placed in a short test-tube (1.5×10 cm.) with a glass stopper and an equal volume of freshly-prepared 1 N KOH in methanol added. In the preparation of the plasma special attention was paid to the prevention of haemolysis in order to eliminate any possible analytical error due to the presence of haemoglobin (26, 31). After mixing well, the tubes were incubated at 60°C in a water bath for 20 minutes for saponification. After cooling the nonsaponifiable fraction was extracted by shaking with a half (for plasma) or equal (for milk) volume of redistilled xylene to that of the original samples. With higher concentrations of vitamin A and carotene a larger volume of xylene was used. Kerosene was not used as part of the extraction fluid. The emulsified phase was then eparated by centrifugation and the supernatant layer (xylene) was removed with a Pasteur pipette. In some cases, the xylene became turbid during or after ultraviolet irradiation so that it seemed safer to add granules of anhydrous calcium chloride after the separation to keep the solution clear for subsequent spectrophotometric measurements. The above procedures were carried out in a semi-darkened room to avoid destruction of vitamin A by sunlight.

Optical density measurements on the xylene solutions were made at 328 mμ with a Beckman Model DU spectrophotometer using silica cells and a tungsten lamp as the light source. The solutions were then irradiated for 60-90 minutes in glass-stoppered soft-glass tubes (5×100 mm). For the ultraviolet irradiation a SHL 200 UV lamp (Nippon Battery Co.) was used with a UVD-II filter (Toshiba Electric Co.) transmitting light in the range 300-400 mμ. The

distance between the lamp and racks of tubes was 12 cm. An electric fan was used to keep the tubes cool during the irradiation. After irradiation the optical densities were again determined at 328 mμ. The vitamin A content is given by the following formula:

$$\text{i u vitamin A per 100 ml.} = (E_0 - E_1)_{328} \times \frac{x}{y} \times 1{,}900$$

where E_0 = initial optical density at 328 mμ; E_1 = post irradiation optical density; x = ml. xylene used for extraction; y = ml. sample.

The spectrum of vitamin A of milk between 310-360 mμ corresponded moderately to that of the pure vitamin A standard (Figure 4). Recovery tests on known samples were carried out (Table 5).

Carotene was determined in the initial extract by reading at 460 mμ and proper dilutions made where the content was very high. The carotene content was calculated from the relation:

$$\mu\text{g carotene per 100 ml.} = E_{460} \times 480$$

Fat percent was determined with the Gerber method.

Total Vitamin A activity (TVA), calculated from the assumption that 0.6 μg carotene is equivalent to 1.0 i u vitamin A, is indicated in the tables for comparative purposes. Also, the ratio of i u Vitamin A to μg carotene (A/C), i u vitamin A per g fat, and the ratio of vitamin A in milk augmented by the treatment to the total vitamin A given in cod liver oil are given.

The differences between groups in the administration experiment were tested by the analysis of variance.

Results

I. *Results of the Analysis of Vitamin A and Carotene in Blood Plasma and of Cows in the Iwaderawa Area:*

The vitamin A and carotene contents of blood plasma and milk together with the animal conditions are shown in Table 2.

TVA (mainly attributed to the variable concentrations of carotene in this case) and carotene in the blood plasma fluctuated considerably. Consequently the A/C ratio in some cases was very low and quite variable. On the other hand, the vitamin A remained fairly constant. These values were more variable in the plasma in comparison with those in milk.

The carotene content of milk was relatively small and still moderately variable. TVA of milk was one-seventh, and A/C rose to four times that of plasma. From these findings it is seen that carotene in plasma accounts for the major part of TVA, but in milk vitamin A is of prime importance when the investigation is performed. In addition, vitamin A in milk fluctuated less than in blood plasma.

Table 2. The conditions of animal, and the results of vitamin A and carotene contents in the blood plasma and milk of cows in the Iwadeyama area.

(18th January, 1956)

Anim. No	Age	Fertility	Date of last parturition	Mos. of 'infertility'	Diagnosis	Milk yield (kg)	Feeding level (g) d.p.	Feeding level (g) s.v.	Blood plasma TVA	Blood plasma VA	Blood plasma Car.	Blood plasma A/C	Milk TVA	Milk VA	Milk VA/g fat	Milk Car.	Milk A/C
1	7	Preg. 2	—			15	−248	+ 768	774	93	409	0.23	223	196	29	16	12.3
2	7	Preg. 2	—			22	−585	− 270	621	111	306	0.36	102	90	47	7	13.0
3	5	Preg. 2	Mar., '55	12		7	+521	+8198	1735	155	949	0.17	108	91	25	10	9.1
4	9	Preg. 2	Mar., '55	12		11	+210	+4980	1171	121	631	0.20	195	170	54	15	11.3
5	7	Preg. 1	Feb., '54	22		9	+394	+ 600	435	102	198	0.52	136	126	22	6	21.7
6	6	Physiol. Infertile	Dec., '55		Postparturition Ketosis	26	+ 668	+2415	2383	143	1342	0.11	110	96	64	15	6.4
7	7	Physiol. Infertile	Dec., '55		Postparturition Ketosis	37	−1054	−4406	731	108	375	0.29	127	110	28	10	11.0
8	6	Physiol. Infertile	Dec., '55		Ovarian Cyst	20	0	−1819	317	85	133	0.64	140	132	28	6	21.2
9	6	Infertile	Sept., '55	4	Irregular & Weak oestrus	30	−1129	−2831	2330	140	1313	0.11	195	160	44	21	7.7
10	7	Infertile	Jul., '53	39	—	7	− 203	+ 330	758	135	375	0.36	146	120	21	16	7.4
11	3	Infertile	Jul., '55	6	Irregular & Weak oestrus	13	+ 570	+3034	498	928	244	0.38	116	102	33	8	12.7
12	7	Infertile	Aug., '55	5	Ovarian Dysfunction	13	− 19	− 227	670	132	350	0.37	131	111	35	12	9.2
13	4	Infertile	Jan., '55	12	—	9	+ 15	+ 146	618	100	311	0.32	180	167	26	8	21.7
14	7	Infertile	Apr., '55	9	—	13	− 30	−1597	2380	215	1303	0.17	242	204	42	23	9.1
15	7	Infertile	Aug., '54	17	Ovarian Sclerosis	13	− 41	− 360	523	106	250	0.42	132	114	24	11	10.4
Average									1063	123	566	0.31	153	132	35	13	11.0
±s.d.									728	32	411	0.15	42	12	12	5	5.5

Note: D. P. and s.v. indicate the digestible protein and starch value in g.
TVA and VA are expressed in iu/100 *ml*; carotene in μg/100*ml*.
'Physiological infertility' means cows of which oestrus does not come until three months after the parturition, or the cows are not mated even if oestrus begins.
'Infertility' means cows do not become pregnant even after mating three times.

Due to the small number of animals examined final conclusions cannot be drawn, but it seems unlikely that there is any close or direct relationship between the vitamin A and carotene contents of plasma and milk and reproductive disturbances. However, a tendency is noticed that the higher the carotene content, the higher the vitamin A content in the blood plasma (see Animal Nos. 3, 6,. 9, 14; reverse relation is found in 8, 11, 15), although the relationship is not necessary proportional.

II. *Effects Administration of Concentrated Vitamin A*

i) *Changes of vitamin A and carotene in blood plasma:* The results of this experiment are shown in Table 3, and Figures 1 and 2.

Table 3. Results of vitamin A and carotene analyses of the blood plasma in the administration experiment.

No. and date of sampling	Group* (animal no.)	Total vitamin A iu/100 *ml*	Vitamin A iu/100 *ml*	Carotene μg/100 *ml*	Vitamin A iu/Carotene μg
I	C(3)	894±326	95±26	482±196	0.22±0.09
	T(3)	1246±265	115±15	679±152	0.17±0.002
1/XII/55	av.	1072±344	105±23	580±210	0.18±0.10
II	C(3)	451± 86	76±20	231± 85	0.34±0.17
	T(3)	779±198	94±15	411±138	0.25±0.08
11/I/56	av.	617±237	83±23	321±137	0.29±0.14
III	C(3)	451± 86	76±20	225± 35	0.34±0.09
1/II/56	T(3)	545± 89	92±11	272± 47	0.35±0.10
IV	C(3)	396±100	76±11	192± 56	0.42±0.10
22/II/56	T(3)	402± 51	98±18	182± 31	0.56±0.17
V	C(3)	414± 90	106±34	184± 35	0.57±0.01
14/III/56	T(3)	344± 63	138±12	125± 32	1.16±0.23
VI	C(3)	405± 60	77±15	198± 32	0.40±0.08
18/IV/56	T(3)	284± 59	132±21	75± 20	1.82±0.19
VII 21/VI/56	(5)	941± 87	176±19	459± 59	0.39±0.07
VIII 11/VIII/56	(6)	1884±514	156±37	1039±261	0.15±0.02
IX 28/IX/56	(6)	958±282	83±23	526±158	0.17±0.03

± Indicates standard deviation * C : Control, T : Treated

First, in control animals, the major portion of TVA in plasma was accounted for by carotene at the beginning of the experiment (January 21st). Subsequently there was an abrupt decline in carotene levels at the second sampling (February 11th), followed by a levelling until the end of April since then fresh fodder gradually began to be available. Then the level

increased during the summer, reaching the highest value in August when the cows were in pasture. Stall feeding began at the end of October and toward the latter part of November carotene level showed a decrease. The vitamin A content remained fairly constant throughout winter and spring but in summer a moderate increase was detected. These variations in vitamin A and carotene seem to indicate annual changes.

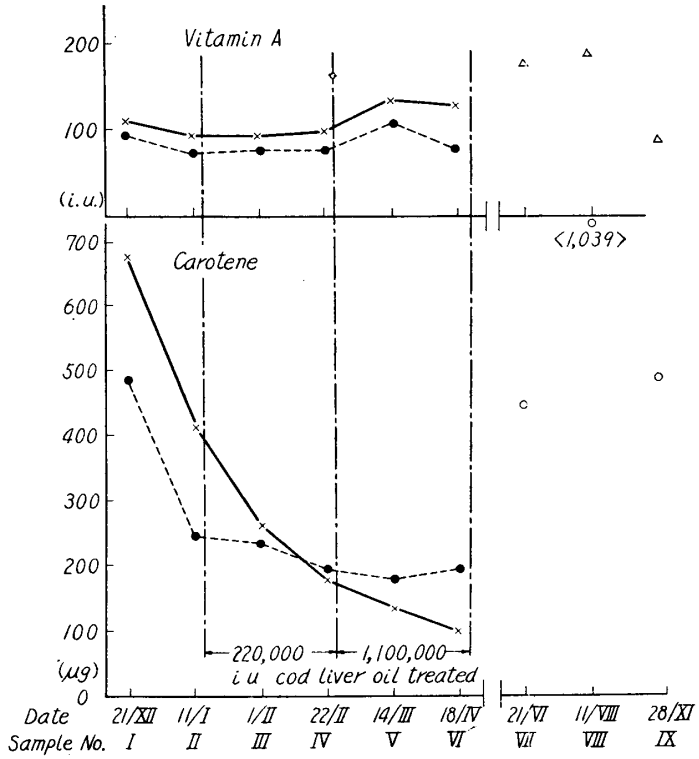

Fig. 1. Changes of the plasma vitamin A and carotene.
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Crosses and dots indicate the values of the treated and nontreated (control), respectively : triangle and hollow circle, those of summer and autumn.

With respect to the effects of vitamin A administration, it was found that the carotene levels in the experimental group declined contenuously and reached the lowest value in April, contrary to the relatively higher and plane levels in the control during late winter and early spring. It appears that vitamin A administration effects a decrease of carotene in the blood plasma. There was no increase of vitamin A in plasma levels even by administration of 220,000 i u daily, but when the dose was raised to 1,100.000 i u daily a slight increase was observed.

The relation between the A/C ratio (ordinate) and carotene level (abscissa) including both the Iwadeyama and University Farm animals is seen to follow a hyperbolic curve in Figure 2 representing the decrease of the ratio with increasing carotene levels, though somewhat deviational cases were seen with

the June samples. In the other words, the A/C ratio remained between 0.1 and 0.2 when the carotene levels were over 400 μg/100 *ml.* This shows that above the 400 μg/100 *ml.* level the increase in carotene is associated with the increase of vitamin A content, while in the range of 100-300 μg/100 *ml,* the relation is inverse; that is, the less the carotene content the higher the vitamin A content of blood plasma.

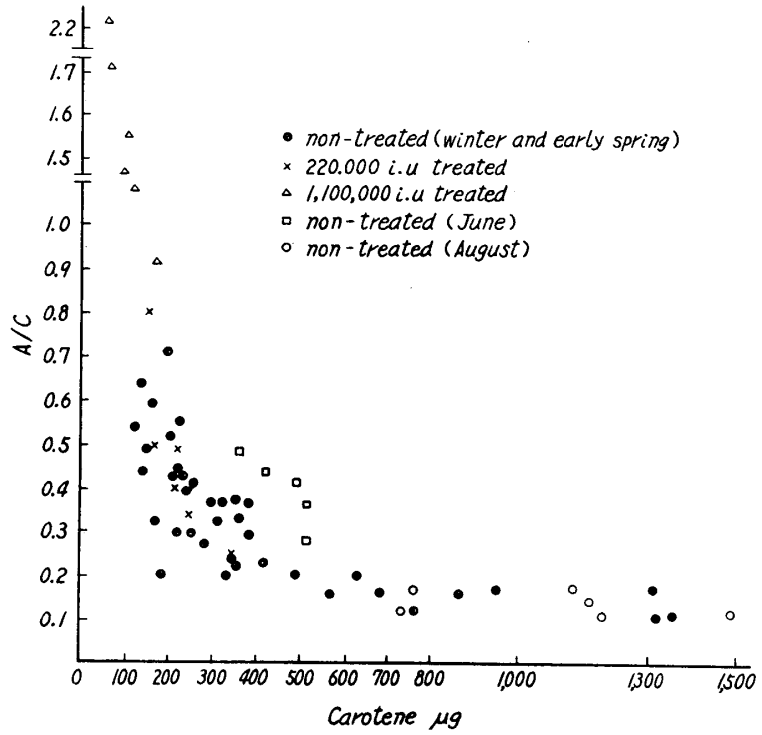

Fig. 2. Correlation of carotene and Vitamin A/carotene (A/C) in the blood plasma.

ii) *Changes of vitamin A and carotene in milk:* The results are given in Table 4 and Fig. 3.

The carotene level in milk was extremely smaller than that in blood plasma and consequently the vitamin A content was relatively larger. The vitamin A content was increased by about two times when 220,000 i u of the vitamin was given. When 1,100,000 i u was administered the increase was quite dramatic, being six times in the untreated period. Toward the end of the terms of both administrations a considerable drop in vitamin A level appeared to take place. In the control group there was a slight temporary increase in vitamin A at the beginning of February despite no change in the blood plas ma level. But this happened to coincide with the levelling off time of the plasma carotene. This was unknown origin and proved to be not statistically significant.

From 1.1 to 2.2 per cent of the ingested vitamin A was excreted in the milk.

The vitamin A levels in milk, in general, showed seasonal changes, the

Table 4. The results of milk analyses in vitamin A, carotene, milk yield and fat percentage in the administration experiment.

No. and date of sampling	Group* (anim. no.)	Total vitamin A iu/100 *ml*	Vitamin A iu/100 *ml* (iu/g fat)	Carotene μg/100 *ml*	Vitamin A iu/ Carotene μg	Fat %	Milk yield kg
I	C(3)	144±40	107±18(32)	23±10	5.4±2.2	3.3±0.6	8.1±4.4
	T(3)	162±23	122±16(35)	24± 8	5.6±1.6	3.5±0.03	5.3±1.4
1/XII/55	av.	153±34	114±19(34)	24± 8	5.5±1.8		6.7±4.9
II	C(3)	149±45	125±36(31)	15± 6	9.4±2.7	4.0±0.2	5.8±2.6
	T(3)	182±37	149±25(45)	20± 9	8.2±2.7	3.3±0.7	4.3±0.3
11/I/56	av.	165±44	137±33(37)	17± 8	8.8±2.5	3.7±0.7	5.1±2.9
III	C(3)	225±45	199±38(44)	16± 5	13.2± 1.8	4.5±0.8	6.2±2.9
1/II/56	T(3)	336±39	311±31(68)	15± 6	24.9±10.0	4.6±0.2	3.9±0.4
IV	C(3)	167±59	151±30(42)	10± 6	18.6± 6.0	3.6±1.3	5.7±3.7
22/II/56	T(3)	292±35	268±15(15)	14±13	42.0±31.3	4.4±0.2	3.1±1.3
V	C(2)	124	116(35)	5	28.5	3.3	8.4
14/III/56	T(2)	690	685(167)	3	265	4.1	3.2
VI	C(2)	103	100(37)	2	62	2.7	7.7
18/IV/56	T(1)	515	505(188)	6	84	2.7	4.4
VII 21/VI/56	(4)	305±45	259±39(70)	28± 4	9.3±0.8	3.7±0.9	—
VIII 11/VIII/56	(6)	822±149	223±15(80)	36± 8	0.7±0.3	2.7±0.7	—
IX 28/IX/56	(6)	239±90	188±65(53)	30±18	7.3±2.3	3.5±2.2	—

± Indicates standard deviation * C : Control, T : Treated

level in summer being roughly twice as high as that in winter and early spring. The carotene content of milk diminished gradually during the winter until April, then it increased in summer, reaching a maximum value in August. A similar trend in the seasonal variation of the carotene content between milk and blood plasma was noted. No marked difference in milk carotene was seen in both groups by the cod liver oil treatment.

III. *Examination of the Analytical Methods.*

Since some modifications in analytical methods were made the validation of these changes was required, particularly in their application to the measurement of vitamin A in milk. The following measurements were made by the modified methods: estimation of the pure standard vitamin A sample ; recovery of vitamin A added to blood plasma or milk ; measurement of the absorption spectra from 310-360 mμ of the non-saponifiable fraction extracted with xylene from milk.

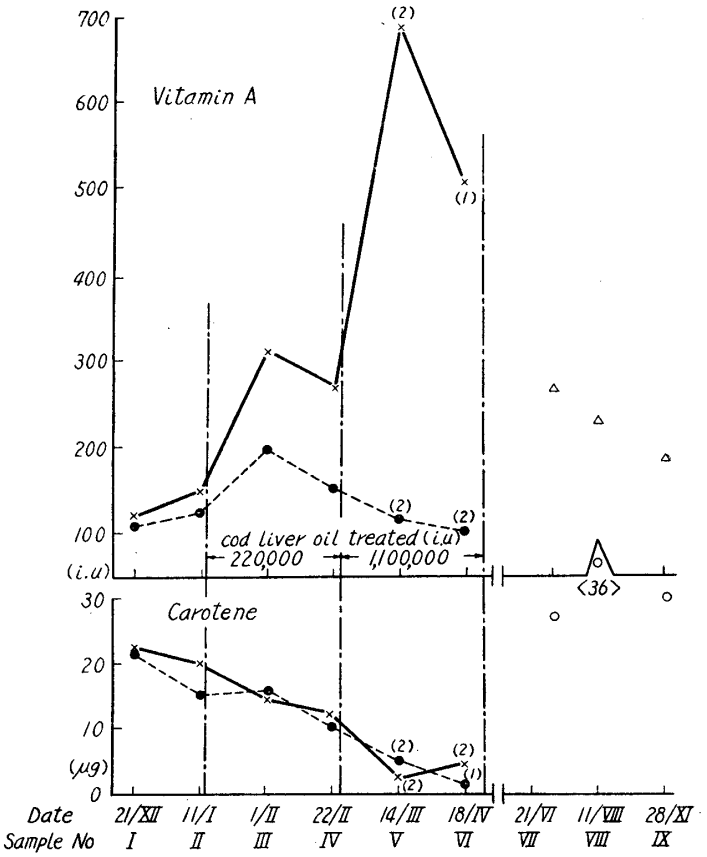

Fig. 3. Changes of the milk vitamin A and carotene.

Figures in the brachet and < > mean the cows number used for analyses and that of beyond the scale, respectively.
Crosses and dots indicate the values of the treated and nontreated (control), respectively : triangle and hollow circle, those of summer and autumn.

Table 5. Examination of the analytical method.

i) Determination of pure vitamin A by Bessey-Sahashi's method.

VA existed	VA found	% found
iu/dl	iu/dl	
500	494	98.7
1,000	991	99.1
1,500	1,438	96.6
		Av. 98.1

ii) Recovery tests of vitamin A in blood plasma.

VA added	Recovery VA found	%
—	(76)	—
200	272−76=196	98.0
400	447−76=371	92.8
600	645−76=569	97.2
		Av. 95.3

iii) Recovery of vitamin A in milk.

VA added	Recovery VA found	%
—	(225)	—
200	421−225=196	98.0
400	606−225=381	95.3
600	790−225=565	94.2
		Av. 95.8

i) Estimation of the pure standard vitamin A by the modified method was made with 98 per cent accuracy as shown in Table 5 (i).
ii) The spectrum of the non-saponifiable fraction from milk is illustrated in Figure 4, and showed a good agreement with that of the pure vitamin A.
iii) A 96 per cent recovery of vitamin A added to either blood plasma (ii) or milk was demonstrated (Table 5, (iii) for milk).

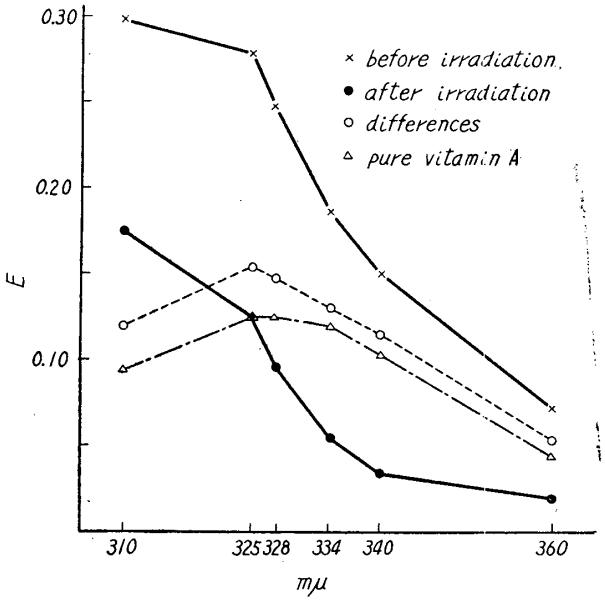

Fig 4 Absorption spectra of the milk between 310 and 360 mμ.

Discussion

I) It is well established that the vitamin A content of milk is low, especially in late winter and early spring in the northern part of Japan. This fact itself is not unusual since the vitamin A level is known to vary with the seasons, feeds, breed of animals, and other factors, (1-5, 8-17). The same relation can be said as to the vitamin A content in the blood plasma (6, 7, 10, 11, 32). However, if it is abnormally low, possible disturbances in the cows' physiological function must be taken into consideration. Vitamin A deficiency in the cow has been demonstrated previously to result in degeneration of epithelial tissue, the placenta and foetus; disturbances in vision; retardation in growth, and so on (1, 33-36). The report on foetal death, prolonged gestation and difficult parturition due to vitamin A deficiency in the rat has been clarified in details (36). The epithelial cells of the genital tracts, especially in the female, are quite vulnerable to degeneration and deficiency leads to characteristic morphological and functional changes in the sex organs. To maintain the regularity of vaginal cornification (the sign of oestrus in the sexual cycle) larger doses of vitamin v are required than for

survival and growth(1). Popper and his collaborators demonstrated (37) high levels of vitamin A content in the liver or adrenals. Atrophy of the rat ovary occurs in vitamin A deficiency independent of body weight changes (38). It is also well known that fairly high levels of vitamin A are required for reproduction and lactation (39-41). Furthermore, Russian workers demonstrated the disturbances in genital organs, particularly ovarian dysfunction in the heifers suffering from vitamin A insufficiency (42).

Frequent occurrence of infertility in the dairy cow affords a serious problem for dairy farming in our country, in spite of the rapid increase of the dairy cow population since the end of the War (163,000 heads in 1946, 587,000 heads in 1957 (43)). It results in a striking retardation of productivity in both milk and calves and can, if it becomes worse, damage the dairy industry seriously. According to the survey of the Ministry of Agriculture and Forestry made in 1949 (44), infertility in the dairy cows (more than 15.5%) is considerably higher than that occurring in European countries or in the United States. Furthermore, the number of farmers who have given up raising cows on account of this reproductive disorder make up 15.6 per cent of the total of those who have discontinued dairy farming. Generally speaking, to be economically profitable for the dairy farmer, cows must produce four calves per five years, but at present they produce only three on the average. Accordingly, it is one of the most desirable problems to improve the present situation in dairy farming eliminating infertility of the cows.

Considering the very fluctable values of vitamin A in milk described above it would seem possible that some cows could have a vitamin A deficiency. Furthermore, it is possible that these low values can be reckoned as one of the causes of the observed frequent infertility when the cows exposed in such condition for a long time. In the same sence, infertile cows should also be considered as suffering from malnutrition (22), since vitamin A deficiency frequently accompanies with low in protein and phosphorus, as Asdell pointed out (19). From the results of the first trial it is not possible to relate the reproductive disorder directly with malnutrition or vitamin A deficiency. However, it appears that many cows are being fed improperly under practical conditions since the vitamin A and carotene levels in the blood plasma and milk are very fluctable and in some cases low (Table 2). This may be partly due to lack of knowledge and experience in keeping dairy cows, so that the diet of the cows varies widely from one cow to another in a newly established dairy area. The relations between reproductive function and nutrition and in particular the role of vitamin A needs to be explained by further experimental work.

II) One of the present experiments was designed to test how far the vitamin A in blood plasma and milk can be elevated during the late winter

and early spring when it is lower, and to follow the changes in vitamin A levels in the blood plasma and milk throughout the year. There are numerous reports on both subjects in foreign countries, especially on the vitamin A content in the milk, because it is an important source of the vitamin supply for humans. But few employed the same conditions of management and circumstances as in Japan. Due to the difficulties in arranging for larger numbers and in controlling the conditions of the experimental animals final conclusions cannot be drawn. However, we have been able to ascertain the changes of vitamin A and carotene in the plasma and milk season by season in Japan and have found some interesting results on the effects of administration of large doses of vitamin A to the cow.

Administering vitamin A in the form of concentrated cod liver oil did not markedly influence the level of vitamin A in the blood plasma under the present experimental conditions, as the preceeding papers indicated (45-47; 40, for carotene supplement). Normally, the vitamin A content of blood plasma does not appear to change by depletion or supplementation of vitamin A unless exceptional conditions are encountered. Only with the huge dose of 1,100,000 i u did the vitamin A level in plasma increase, and then only slightly. On the other hand, in milk the vitamin A level was two and six times higher than that of the untreated controls with administration of 220,000 and 1,100,000 i u, respectively. Most reports on the effects of vitamin A administration have shown that a few times increase appear in the milk (17, 45, 47-50). Some of them (17, 48) postulated a threshold level of vitamin A. Deuel and his collaborators, however, observed (51, 52) that the vitamin A level in milk rose to 300 i u per 100 *ml.* when 700,000 i u was administered, and to 725 i u when 1,400,000 i u was given in the form of shark liver oil. These results come to a nice agreement with ours. In general, these differences in results can probably be ascribed to the differences in the conditions of the animals, degrees of vitamin A storage in the liver, dosage and period of vitamin A treated, and others.

In contrast to the marked elevation of the vitamin A in milk and a slight increase of vitamin A in the blood plasma on the administration of cod liver oil, the carotene level in the plasma continued to decrease considerably toward the end of the period of treatment in the later part of April. While in the untreated control the decline ceased in early January. Vitamin A treatment tended to stimulate the decreasing of plasma carotene. This phenomenon has also been reported by other workers (45-47, 52). The carotene in the milk seemed to show typical seasonal changes in both the treated and control groups beyond the effect of treatment, if any (Fig. 3).

The present results give no evidence of increased milk yield or fat percentage on administration of vitamin A despite some published reports to

the contrary (45, 48, 51). The increased amount of vitamin A which appeared in the milk on its oral administration was found to be exteremely small (1.1-2.2 %) in agreement with previous reports (5, 47), but Deuel *et al.* (51, 52) showed 3 per cent.

The relations between vitamin A and carotene in the blood plasma was seen to possess two phases. In the first, where the carotene content was more than 400μg, vitamin A increased in proportion to the carotene content so that the A/C ratio was constant, while below this range (100-300μg), roughly speaking, the vitamin A could be higher regardless to the carotene content, e.g. when vitamin A is supplemented. These results are in good agreement with those obtained by Braun (10) and Davis and Madsen (40).

In considering the analytical methods employed it is to be noted that the use of ultraviolet light to destroy blood plasma vitamin A has already been well established (24-26). Some investigators claim that the spectrophotometric determination of vitamin A without the ultraviolet irradiation treatment tended to give higher values in comparison to those given by the Carr-Price or activated glycerol dichlorohydrin methods (53). However, the advantages of the former method employed exceed the latter two by far in requiring much less time to carry out the analyses and in permitting the determination of a large number of samples simultaneously. Furthermore, the spectrophotometric method does not require control of the humidity during the analysis, a factor which greatly affects the Carr-Price method. The recovery tests of vitamin A from blood plasma and the determination of the standard vitamin A were very satisfactory (Table 5, i and ii).

Few workers have hitherto adopted this method for the estimation of vitamin A in milk. Taking into consideration the use of the spectrophotometric method in the determination of the milk vitamin A (4, 5, 12, 27-29), and the present results, in recovery tests, and measurement of the absorption spectrum of the non-saponifiable fraction of milk, the authors believe that the analytical method employed here is considerably reliable and, at least, can give relative values for comparative purposes.

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Conclusion

Measurements have been made of : (i) the vitamin A and carotene levels of the blood plasma and milk of normal and infertile cows to determine whether there is a correlation between these levels and the reproductive disorder; (ii) the effects of cod liver oil administration on the vitamin A and carotene levels to determine how much the vitamin A level in milk can be elevated; and (iii) the changes in vitamin A and carotene levels in plasma and milk throughout the year under practical conditions.

The vitamin and carotene contents of the blood plasma and milk varied greatly from cow to cow in the dairy area, and at this stage no clear relation between these levels and infertility could be deduced. Judging from the results obtained and a survey of the feeding standards, it is clear that both the quality and quantity of the rations must be given much attention.

On administration of large amounts of vitamin A, the vitamin A level in milk could be risen up to 700 i u per 100 *ml.*, six times the normal level in winter and early spring and nearly three times the normal level in summer. The carotene level in milk showed the same annual fluctuations regardless of the treatment. In the blood plasma, the vitamin A increased only on administration of 1,100,000 i u while the carotene level markedly decreased.

The vitamin A and carotene levels in both blood plasma and milk varied with seasonal changes in the typical manner reported by many other authors.

The reliability of the analytical method for vitamin A determination, especially in milk, was tested in several ways.

The results obtained were discussed in relation to previous reports on these subjects.

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Addendum:

Since writing this paper, Ushimi *et al.* have published a series of paper [Jour. Vet. Med (Japan), no. 267, 1959 and no. 272, 1960 (in Japanese)] dealing with the effect of vitamin A, three forms, administration on the vitamin content of blood plasma in the dairy cows, and survey of the nutritional status of dairy cows VII. On vitamin A.

They have shown in the former paper, i) the increase of vitamin A in the blood and milk is more effective by the adminisration of watwer-solublized vitamin than the ordinary oil. The latter paper demonstrated that ii) the plasma vitamin A and carotenoids show a seasonal change, iii) a close correlation exists between these two factors and iv) the plasma vitamin A of liver dysfunction cows (Gross test or distomatosis test positive) tends to be lower.