CHANGES IN THE CONCENTRATION OF TOCOPHEROLS IN THE BLOOD SERUM OF THE PARTURIENT DAIRY COW AND HER NEONATAL CALF

by

CARL ERNEST LATSCHAR

B. S., Kansas State College of
Agriculture and Applied Science, 1941

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE

OF AGRICULTURE AND APPLIED SCIENCE

1947

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INTRODUCTION AND REVIEW OF LITERATURE

The elucidation of the role of tocopherol (vitamin E) in the nutrition and physiology of the parturient dairy cow and her neonatal calf suggests a study of the changes in the concentration of this vitamin in the blood serum of the cow during the terminal stages of gestation, and in the blood serum of both the cow and her calf for a period following parturition.

With the possible exception of the crustacean, Daphnia magna, Mason (1) points out there is no evidence that the tocopherols were required, or stored, by invertebrates. Various vertebrates, however, have been shown to require, or to benefit from, inclusion of the tocopherols in the diet.

In a review, Wolbach and Bessey (2) enumerated the following pathological lesions preventable by pure tocopherols: (a) failure of early embryonic development in the rat and mouse, (b) irreparable degeneration of germinal epithelium in the rat, (c) a degeneration (nutritional dystrophy) of the skeletal musculature in guinea pigs, rabbits, rats, dogs, and ducklings, and (d) a nutritional encephalomalacia in the chick. In addition, degeneration of the testes of male fowls, early embryonic death of chicks, idiopathic degeneration of the smooth musculature of the young turkey, and severe spinal cord lesions in adult rats have resulted from feeding vitamin E low rations, but these conditions have not yet been shown to be preventable

by tocopherols. Indirect and controversial evidence of the need for the vitamin by other species consists of reports of improvement in reproduction (human, cattle, pigs) in cases of repeated spontaneous abortion, exclusive of that due to pathological states of the uterus, and of contradictory reports of benefits from treatment with α-tocopherol in human neuromuscular and muscular dystrophies.

The method of biological assay with female rats was taken by Devlin and Mattill (3) as being in itself evidence that the tocopherol requirements for maintenance of the structural and functional integrity of skeletal muscle are, at least in the rat, smaller than that needed to prevent reproductive failure. Adequate knowledge of the vitamin E levels in various animal tissues has been curtailed by the necessity, until recently, of employing the time-consuming and expensive biological assay.

Reports of tocopherol levels in normal human blood serum include those of Couperus (4, 5), Mayer and Sobotka (6), Meunier and Vinet (7), Minot (8), Minot and Frank (9), Quaife and Harris (10), Varangot (11), Varangot et al. (12, 13), and Wechsler et al. (14, 15).

The effects on the tocopherol levels in blood serum of vitamin E supplementation of the human diet have been reported by Wechsler et al. (14, 15), Quaife and Harris (10), and Couperus (4, 5); the latter two also investigated the rate of absorption of this vitamin. Little information has been obtained concerning the normal tocopherol levels in blood serum, other than from humans. Mayer and Sobotka (6) reported a value for dog

serum and for preserved horse serum. The levels of tocopherols in the serum of dogs, according to Vinet and Meunier (16), fluctuated with the amount administered. Minot (8) reported the tocopherol levels in the blood serum of rabbits in varying stages of vitamin E deficiency, and the effect of administration of vitamin E upon those levels. The tocopherol content of a sample of reconstituted dried beef serum was reported by Quaife and Harris (10). The present knowledge of the tocopherol levels in the various animal tissues stresses the need for more information in order that the functions of this vitamin under various normal and pathological conditions may be better understood.

Conflicting data concerning the trend of the tocopherol levels in the blood serum of pregnant women with the approach of childbirth include the reports of Rauramo (17), Straumfjord and Quaife (18), and Varangot (11) that the serum tocopherol levels rose with the progress of pregnancy, and the report of Varangot et al. (13) which indicated the opposite trend as parturition approached.

Employing a biological assay, Mason and Bryan (19, 20) and Mason (21) have shown that the maternal intake of high levels of vitamin E permitted a demonstrable but exceedingly limited amount of this vitamin to pass the placental barrier, but resulted in an appreciable increase of the mammary transfer over that occurring at lower levels of vitamin intake. They concluded that the mammary gland was almost entirely responsible for the transfer of tocopherol to the offspring. The reports of Varangot et al. (22) and Straumfjord and Quaife (18) agreed that the vitamin E

levels in the cord blood of infants at birth was much lower than in the venous blood of the mothers. Injection of tocopherol into the mothers previous to delivery was shown by Varangot et al. (22) to increase the tocopherol levels in the cord blood, indicating that some placental transfer of this vitamin took place.

The present investigation was undertaken to obtain information on the nutritional and physiological significance of tocopherol to the dairy cow during the parturient period and to her calf following birth through a study of the tocopherol levels in the blood serum.

PROCEDURE

Feeding and Management of Experimental Animals

Dairy cows of four breeds, Holstein, Ayrshire, Jersey, and Guernsey, were divided into the experimental groups shown in Table 1. The cows were placed, at least six weeks prepartal, on a standard control diet consisting of concentrate mixture, 12.5 per cent crude protein, good quality Atlas silage and high grade alfalfa hay. The respective vitamin supplements were added one month before date of expected parturition.

Silage and hay in the ratio of 2:1 were fed morning and evening, respectively, at levels adjusted to the appetite of the individual cows; the concentrate was fed in equal portions at both feeding periods, at the rate of one pound per 100 pounds of initial weight of the cow. Seven days after parturition all cows were placed on the standard milking-herd rations.

The supplements of vitamin A[1] and tocopherols[2] were given in

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[1] Three forms of vitamin A concentrates were used: (a) vitamin A alcohol in a powdered soybean-like base, (b) vitamin A alcohol in an oil base, and (c) natural vitamin A ester in an oil base. The first product was supplied by Distillation Products, Inc., Rochester, N. Y., and the latter two by the Borden Co., New York, N. Y.

[2] Mixed and α-γ tocopherols, both in a powdered soybean-like base, were supplied by Distillation Products, Inc., Rochester, N. Y.

Table 1. The breed and the number of lactations of the cows within experimental groups.

	Breed and herd number	Number of lactation		Breed and herd number	Number of lactation
Group A	H-175	6	Group D	H-135	1
	H-105	4		H-136	1
	A-230	3		J-374	1
	G-433	5		J-380	1
	G-432	5		G-458	2
				G-459	2
Group B	H-137	1			
	H-115	2	Group E	H-138	1
	A-252	1		A-200	7
	A-243	2		J-356	2
	J-365	2			
	J-379	1	Cow	G-451	1
	G-465	1			
	G-452	2			
			Cow	A-240	2
Group C	H-128	2			
	A-241	2			
	J-378	1	Cow	G-467	1
	J-358	2			
	G-468	1			
	G-453	2	Cow	H-126	2

the evening, in quantities as shown in Table 2. All supplements, except tocopherols in excess of one gram, were administered orally in gelatine capsules. When both vitamin A and E were given, they were premixed and administered in the same capsule. The larger amounts of tocopherols were combined with the concentrate mixture immediately before feeding. Supplementation was discontinued at parturition. Originally group D was divided into two subgroups, which differed only in that one received α-γ tocopherols (50-50), and the other α-γ tocopherols (10-90). Since the data collected showed no significant differences between these subgroups, they were combined in one lot, group D.

The experiment was conducted during the months from October to February, and, whenever the weather permitted, the cows exercised in a dry lot with free access to water and common salt. The rations were consumed readily by all animals except an Ayrshire, A-200, which refused some concentrate feed when the tocopherol supplement was first introduced; however, she was eating normally again about two weeks before calving. One Holstein, H-126, was maintained on standard winter milking-herd rations and was milked through gestation without the usual dry-rest period.

Newborn calves were not allowed to nurse; this was accomplished by keeping the udder of the dam covered during the parturient period. The first colostrum was withdrawn from the udder as completely as possible by standard procedures, usually less than two hours after calving, but always within six hours, and thereafter at the regular morning and evening milking periods.

Table 2. Vitamin supplements given to pregnant cows.

	Number of animals	Days previous to date of expected parturition	
		28-14	14-0
Group			
A	5	No supplement	No supplement
B	8	500,000 I U vitamin A alcohol	1,000,000 I U vitamin A alcohol
C	6	500,000 I U vitamin A ester	1,000,000 I U vitamin A ester
D	6	500,000 I U vitamin A alcohol and 0.5 g. α-γ tocopherols	1,000,000 I U vitamin A alcohol and 1 g. α-γ tocopherols
E	3	10 g. mixed tocopherols	10 g. mixed tocopherols
Cow			
G-451	1	0.5 g. α-γ tocopherols	1 g. α-γ tocopherols
G-467	1	4 g. mixed tocopherols	4 g. mixed tocopherols
A-240	1	5 g. α-γ tocopherols	5 g. α-γ tocopherols
H-126*	1	No supplement	No supplement

*Cow milked through gestation without the usual dry-rest period.

The dietary regimen of the calves was as indicated in Table 3. Each calf was hand-fed the colostrum from its own dam immediately after milking. After the third day, whole milk, from a combined herd receiving standard dairy rations, was fed morning and evening at the daily rate indicated in the table. After the calves were two weeks of age, they were given a simple concentrate mixture immediately following each milk feeding and were supplied with fair quality prairie hay and green leafy alfalfa hay at all times. Whenever weather conditions were favorable, the calves were released in an open paddock where water and common salt were accessible.

Collection of Blood Samples

Blood samples were obtained by puncturing the jugular vein of the cows and calves. The periods at which blood samples were collected from the experimental cows are outlined in Table 4. The first samples of blood were collected from the respective calves before colostrum consumption. Many of the male calves, immediately after collection of this first blood sample, were sacrificed as a part of another study. Other blood samples were obtained, six to eight hours after the regular morning feeding, from the other calves according to the schedule shown in Table 5.

Samples were collected in 50 ml. centrifuge tubes, which were either taken to the laboratory immediately, or placed in cold storage until taken to the laboratory a few hours later. As soon as the blood samples were received in the laboratory and

Table 3. Feeds given daily to experimental calves.

Age	Lacteal products fed		Concentrate mixture fed	Hays fed (Prairie and alfalfa)
	Kind	Rate of feeding		
Days				
1-3	Fresh colostrum	1 lb./10 lb. body weight**	None	None
4-14	Whole milk*	1 lb./10 lb. body weight	None	None
15-28	Whole milk	1 lb./10 lb. body weight	To extent of appetite	Free access

*Combined herd, Ayrshires, Holsteins, Guernseys and Jerseys, but predominately Holstein.

**Maximum quantity limited to 12 pounds daily.

Table 4. Periods at which blood samples were collected from experimental cows.

Period	Days before estimated parturition	Notes
1	-35*	Start of experiment
2	-28*	Immediately previous to start of vitamin supplementation
3	-14*	Immediately previous to increase of vitamin supplementation
4	-7*	
5	-3	
6	-1	
	Days postpartum	
7	0	Immediately after birth of calf
8	1	
9	2	
10	3	
11	7	
12	14	
13	21	
14	28	

*The comparison of the number of days in the estimated and in the actual prepartal periods is shown in Table 7.

Table 5. Periods at which blood samples were collected from newborn calves.

Period	Days postpartum	Notes
7*	0	Immediately after birth, before ingestion of colostrum
8	1	
9	2	
10	3	
11	7	
12	14	
13	21	
14	28	

*Identical period designations were maintained for the calves and the cows, to permit easier comparison of data.

had clotted, centrifugation was employed to obtain the blood serum, which was stored in screw-top bottles in the dark at 4° C. until analyzed. All samples were analyzed in duplicate as soon after collection as possible.

Analytical Procedures Proposed for Tocopherol Determination

Mason (1) has outlined the principle methods which have been devised for the physical or chemical measurement of tocopherols; these differ primarily in the procedure used for estimation of the oxidation products, the quinones, after treatment with various oxidizing reagents. The methods are: (a) spectroscopic analysis after oxidation with alcoholic silver nitrate, (b) potentiometric estimation after gold chloride treatment, (c) colorimetric measurement after oxidation with alcoholic nitric acid, (d) colorimetric measurement after oxidation with ferric chloride and conversion of the ferrous ions into a red complex upon addition of α,α'-dipyridyl, (e) colorimetric oxidation-reduction method permitting differentiation between tocopherols and their oxidation quinones, based on methods used for vitamin K, and (f) amperometric titration at the dropping mercury electrode after gold chloride oxidation.

Certain disadvantages become apparent when these methods are applied to natural sources of tocopherol. The methods usually require a rather concentrated source of the vitamin, often necessitating a preliminary saponification treatment which may cause losses unless proper precautions are taken (Devlin and

Mattill, 3; Ritsert, 23). Some methods are affected by the presence of other products, such as vitamins, carotenoids, cholesterol, etc.

The method selected for the present investigation is that of Quaife and Harris (10), as modified by Quaife and Biehler (24), wherein mild hydrogenation is employed to remove the substances such as vitamin A and carotenoids which interfere with the determination of tocopherols upon use of the Emmerie and Engel (25, 26) reagents. The method is advantageous in that it is relatively simple, rapid, and rather specific for tocopherols. However, it does not allow for differentiation of the relative concentrations of α-, β-, and γ-tocopherols, and therefore, as is the case for most other methods, probably overestimates the biological activities of tocopherol mixtures.

Method

Five ml. of blood serum were pipetted into a 50 ml. glass-stoppered centrifuge tube, and five ml. of absolute ethyl alcohol were added. After shaking the mixture gently to precipitate the proteins by denaturation, exactly 12 ml. of purified (Hines and Mattill, 27) Skellysolve B were added. The stopper was sealed securely in the vessel by an application of starch-glycerol jelly. The lipides were extracted by continuous vigorous shaking for 10 minutes in a special motor-driven apparatus[3].

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[3] Designed and built by Dr. Marion J. Caldwell.

After separation of the phases by centrifugation, the skellysolve layer was drawn by gentle suction into a small test tube, and exactly 10 ml. of this solution were transferred to a 7 x 7/8 inch Evelyn absorption tube. To obtain the lipide residue, the Skellysolve B was removed by evaporation using suction from a water aspirator and a water bath at 50 to 60° C. Upon evaporation just to dryness, the residue was redissolved in exactly 10 ml. of absolute ethanol, using the hot water bath to aid in the complete re-solution, which was essential for accurate results. This solution, upon cooling to room temperature, was transferred to a special 50 ml. conical centrifuge tube. Approximately 0.2 g. of palladium catalyst[4] was added and the mixture stirred to form a suspension; the tube then was clamped into position on the hydrogenation apparatus.

The essentials of the apparatus[5] for hydrogenation (Quaife and Biehler, 24) included a needle valve to regulate pressure, clamps to hold two 50 ml. conical centrifuge tubes in proper position, micro porous disperser tubes[6] to break the flow of hydrogen gas into numerous very small bubbles, and a system of

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[4] "Five per cent Pd-$CaCO_3$ catalyst", purchased from Baker and Company, Inc., Newark, New Jersey.

[5] Purchased from the Vacuum Equipment Division, Distillation Products, Inc., Rochester, New York.

[6] Reused after acetone wash and air drying; discarded after the pores became clogged upon prolonged use.

tubing and connections arranged to pass the entering hydrogen through one disperser tube into pure absolute ethanol contained in the first conical tube, before passing through the second disperser tube and into the solution to be hydrogenated. From the tube in which hydrogenation occurs, the gas passes through a needle valve and finally the exhaust. Passage of the hydrogen through the pure solvent in the first tube resulted in saturation of the gas with alcohol, thus minimizing loss of this solvent from the second tube containing the sample.

Hydrogenation was accomplished by opening the two-stage hydrogen reduction valve on the supply tank to 15 pounds and adjusting the needle valve to permit a rapid but smooth flow of gas[7], which aids in the maintenance of a good suspension of the catalyst. Hydrogenation was continued for one minute. This treatment did not affect the tocopherol, but the ability of vitamin A and carotenoids to react with the Emmerie-Engel reagents was removed through reduction. After reduction, the tank valve was closed, and atmospheric pressure was restored by gradual opening of the needle valve. The tube containing the hydrogenated solution was removed, corked, and centrifuged.

Quaife and Biehler (24) found that the hydrogenated extract tended to be unstable, especially to air, intense light, heat, and continued contact with the activated catalyst. Accordingly, not more than eight samples, including blanks, were hydrogenated

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[7] Contact between the solution and the rubber joint on the disperser tube, caused by too rapid flow of gas, was avoided since the ethanol extracted impurities from rubber which react with the Emmerie-Engel reagents.

successively, followed by centrifugation and completion of the analysis.

After centrifugation, the clear supernatant liquid was siphoned into a small test tube, of which exactly eight ml. were pipetted into a 7 x 7/8 inch Evelyn absorption tube. It had been found that warming of the hydrogenated solution to 35° C. prevented occasional formation of slightly cloudy solutions, which would give incorrect readings in the spectrophotometer. Consequently, the tubes were placed in a water bath at 35° C. for a few minutes before the addition of the analytical reagents. A Coleman spectrophotometer with a special cell holder that accommodated the 7 x 7/8 inch tubes was used in the assay; readings were made at a wave length setting of 520 mμ.

After the addition of one ml. of the α,α'-dipyridyl reagent[8] to the eight ml. of hydrogenated solution, the tube was placed in the cell holder, and the galvanometer reading was adjusted to read 100 per cent transmission. The tube was then removed from the instrument, one ml. of the ferric chloride reagent[9] was added from a rapid delivery pipette (less than five seconds), the contents of the tube were mixed by vigorous shaking, and the tube was replaced in the cell holder.

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[8] Dissolve 0.25 g. of α,α'-dipyridyl in 50 ml. of absolute ethyl alcohol, and store in a dark bottle.

[9] Dissolve 0.10 g. of ferric chloride hexahydrate in 50 ml. of absolute ethyl alcohol; check the reading (about 90) of this solution in the spectrophotometer against distilled water set at 100 per cent; store in a dark bottle.

In the presence of α,α'-dipyridyl, tocopherol is said by Emmerie and Engel (26) to be quantitatively oxidized by ferric chloride to tocopheryl quinone, the simultaneously formed ferrous chloride giving with α,α'-dipyridyl a red color, the intensity of which, measured in the photometer, is used to estimate the original tocopherol concentration. The intensity of the color developed, as shown by the galvanometer reading, was recorded at exactly 15 seconds after the addition of the final drop of the ferric chloride reagent. On duplicate samples, readings differing by one unit or less on the galvanometer scale were considered to be within the range of the experimental error.

To correct for the color present in the ferric chloride reagent and for possible interfering impurities in the absolute ethyl alcohol, a blank was determined each day. The procedure for the blank started with the treatment of exactly 10 ml. of absolute ethanol in the hydrogenation apparatus in the same manner as the sample, followed by the usual steps for the completion of the analysis. In case the reading of the blank changed several per cent from the original reading, which was about 80, the reagents were replaced by new ones.

Calibration Curve and Calculations

The tocopherol content of the sample was found from a calibration curve prepared using a sample of pure natural

α-tocopherol[10] dissolved in absolute ethyl alcohol. The calibration samples were started with the hydrogenation process and carried through the subsequent treatment. The formula used for calculating tocopherol concentration was:

$$\text{Tocopherol conc., in } \gamma/100 \text{ ml.} = \frac{100}{\text{ml. plasma}} \times \frac{\text{total ml. SSB}}{\text{aliquot SSB}} \times \frac{\text{total ml. EtOH}}{\text{aliquot EtOH}} \times \gamma \text{ tocopherol in aliquot EtOH.}$$

Sample calculation: If five ml. of blood serum were taken, eight ml. of the alcohol solution used, and 40 γ of tocopherols found in the solution analyzed, then:

$$\text{Tocopherol conc., in } \gamma/100 \text{ ml. of blood serum} = \frac{100}{5} \times \frac{12}{10} \times \frac{10}{8} \times 40 = 1200 \ \gamma/100 \text{ ml.}$$

In routine analysis, a formula was employed for the calculation of the tocopherol concentration in the blood serum, since constant amounts of serum and aliquots were used. Thus:

$C = K \times L$, in which C is the concentration of tocopherols expressed in γ/100 ml., K is a constant, L is the customary (2 - log G), and G is the recorded galvanometer reading. Before substitution into the formula, the value of L for the blank must be subtracted from the value of L for the sample.

With a composite calibration curve constructed from repeated standardizations of a sample of pure natural α-toco-

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[10] Supplied by M. L. Quaife, of Distillation Products, Inc. Rochester, New York.

pherol, a value of 6190 was found for the constant, K, in the above formula. This value differs from the value of K = 4270 as reported by Quaife and Harris (10), using the Evelyn photometer.

Method of Expressing Results

An attempt was made to obtain from the cow, before calving, blood samples which would be characteristic of definite periods of vitamin supplementation and also of definite prepartal periods, as shown in Table 4. As might be anticipated, parturition seldom occurred on the expected date. Thus the earlier prepartal sampling periods were not representative of an exact number of days before calving, but represented rather periods or stages in the vitamin supplementation. The samples obtained from the cows and the calves after parturition obviously presented no such difficulties as that indicated above, since they could be collected using parturition as the reference point.

It was desired to have the final data correspond as nearly as possible to the periods set up in Table 4. To accomplish this, a separate graph was constructed utilizing the data from each individual cow. For the earlier prepartal samples the concentrations of tocopherols were plotted against a time scale adjusted to fit into the various periods of supplementation in the best possible manner; then starting four days before parturition and continuing after calving, the data were plotted on a daily basis.

To illustrate the procedure employed in the construction of

these individual graphs, three examples have been considered in detail. The data representing the tocopherol content of samples collected from three different cows are given in Table 6. Application of the indicated method of adjusting the data to the periods (Table 4) gave the three curves of Fig. 1. Similar curves were constructed for all animals, the data for the calves being handled in a manner identical to that used for the postpartum data for the cows. The individual graphs were obtained by constructing straight lines between successive points. From the graph for each animal, data were taken to correspond to the periods set up in the experimental design. In most cases the data for each individual period represented values obtained from samples collected at that specific time, but in other cases it was necessary to obtain the data from the graph. In the case of cow A-240, the values obtained from Fig. 1 are to be found in Table 11. The remainder of the original data is too voluminous to present here.

The comparison, within groups, between the estimated number of days and the actual number of days in the first four prepartal periods is shown in Table 7. Although each of these prepartal periods was somewhat longer than had been estimated, it was considered that the range within periods was not excessive in view of the difficulties of estimation of the date of parturition.

In the case of cows of group A and cow H-126, which received no vitamin supplementation, the prepartal portion of the graph was constructed on a daily basis with respect to parturition.

Table 6. Typical sample data used in plotting Fig. 1.

Cow*	Number of days prepartal											
	-42	-35	-32	-25	-24	-18	-16	-11	-9	-7	-5	-3	-2
	Tocopherol concn., γ/100 ml.												
A-230			644		470		433		334		316	316	
A-240	619	378**		984		941		799		879		805	
J-380		520			495**				427***				340

Cow	Number of days postpartum									
	0****	1	2	3	7	15	16	22	28	29
	Tocopherol concn., γ/100 ml.									
A-230	254	235	223	198	217	310		489		594
A-240	817	650	582	557	396		594		799	
J-380	285	266	235	229	266					

*See Table 1 for experimental grouping.
**Immediately previous to start of vitamin supplementation.
***Immediately previous to increase of vitamin supplementation.
****Immediately after birth of calf.

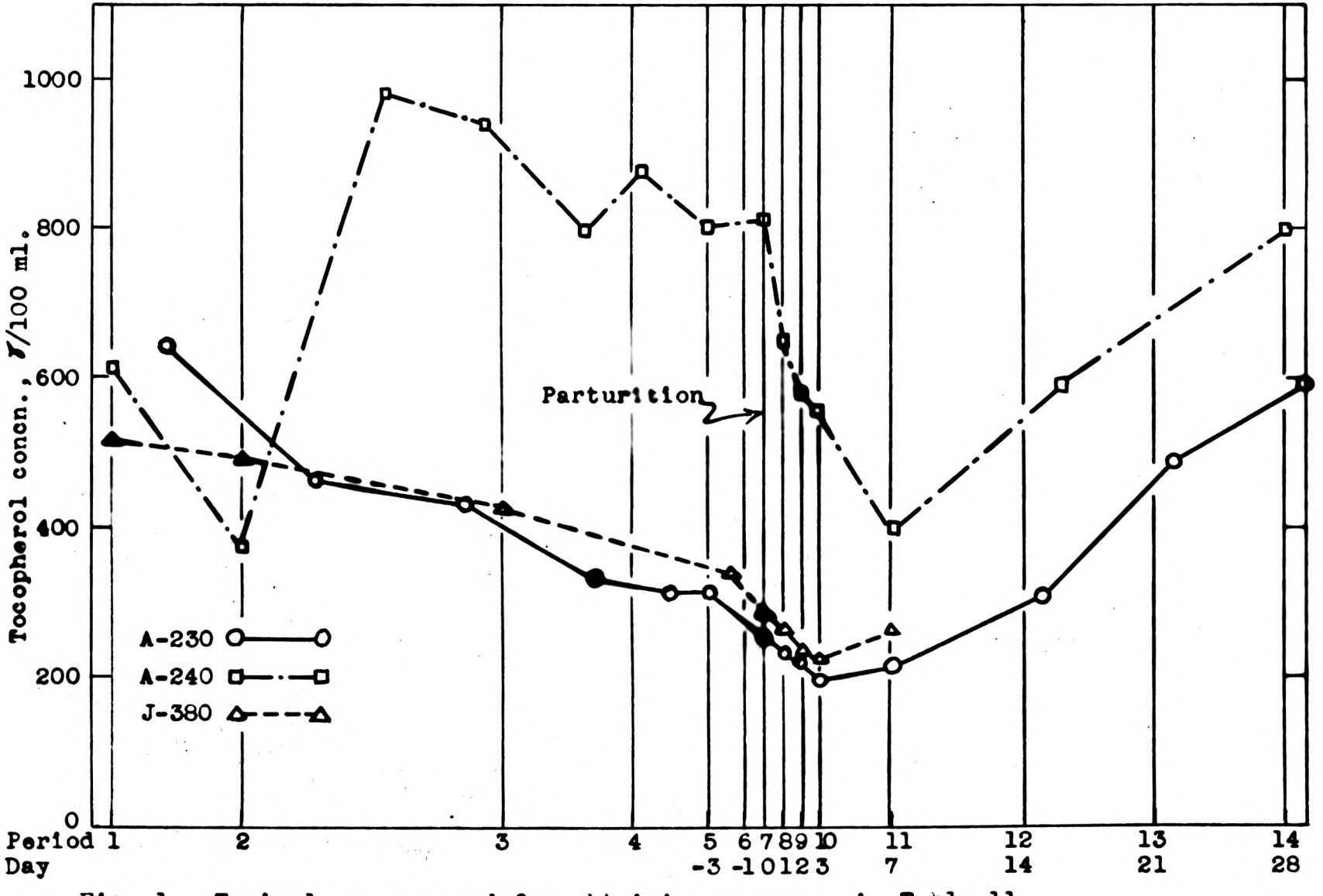

Fig. 1. Typical curves used for obtaining averages in Table 11.

Table 7. Comparison of estimated and actual number of days in prepartal periods.

	Prepartal periods			
	1	2	3	4
	Days (estimated)			
	35	28	14	7
	Days (actual)			
Group				
A	--	28	14	7
B	40	30	18	8.2
C	36	35	18	8.0
D	44	32	19	8.4
E	39	33	16.1	7.6
Cow				
G-451	41	34	26	10.6
G-467	49	37	17.7	8.6
A-240	42	35	16.9	7.9
H-126	--	28	14	7

For the cows receiving vitamin supplementation with no increase, the third period represents a point midway between the start of supplementation and parturition, and the fourth period represents the three-quarter point between the start of supplementation and parturition. For cows receiving supplemental vitamins, first at the lower and then at the higher level, the fourth period represents a point midway between the increase of supplementation and parturition.

The values obtained by the procedure outlined above were used in the construction of Tables 11 and 12, and of Figs. 2 to 10, inclusive.

Recovery Studies

To determine the recovery of amounts of natural α-tocopherol added to extracts of blood serum, as a check on our modifications of the method, the following experiment was devised. A sample of blood serum was given three treatments in duplicate as follows: One sample was carried through by the regular procedure; to the other two sets of duplicates different levels of natural α-tocopherol in absolute ethanol were added by replacing part of the five ml. of alcohol used as a protein denaturant with an equivalent volume of an ethanol solution of tocopherol. The remainder of the procedure was unchanged.

The average recovery of 97.1 per cent based on eight samples, as shown in Table 8, was in satisfactory agreement with

Table 8. Recovery of natural α-tocopherol added to blood serum.

Experimental animal	tocopherol content of sample	Added tocopherol	Total tocopherol found	Recovery
	γ/100 ml.	γ/100 ml.	γ/100 ml.	per cent
J-374*	25	191	210	97.2
	25	538	545	96.8
G-433*	167	207	359	96.0
	167	414	588	101.2
G-452	359	207	545	96.3
	359	414	792	102.5
A-240	619	191	755	93.2
	619	538	1083	93.6
Average recovery----------				97.1

*Calf.

similar studies of blood plasma by Quaife and Harris (10) and by Quaife and Biehler (24) who, respectively, reported recoveries of 100.0 per cent based on four samples, and 97.8 per cent based on six samples. The recovery experiment covered samples from different breeds and dietary groups, and included a wide range of original tocopherol content and of added amounts of tocopherol.

Stability Studies

To investigate the effects of various storage conditions upon the stability of tocopherols in blood serum, in order to determine the possible necessity for analysis of the serum immediately after collection, a study was made of the changes in the tocopherol concentrations of blood serum held at 4° C. and at 38° C. Blood serum samples were placed, as soon as received, in the dark at 4° C. An aliquot was withdrawn from each for immediate analysis; further aliquots were analyzed at regular intervals. The stability of the tocopherols in blood serum held at 4° C., as evidenced by the tocopherol values for storage periods ranging up to 100 days, is indicated in Table 9. Aliquots of two blood serum samples were taken from the 4° C. refrigerator for immediate analysis; other aliquots of these samples were placed in an incubation oven at 38° C. for periods of 12, 24 and 48 hours before being analyzed. As shown in Table 10, the tocopherol concentration in blood serum held at 38° C. remained essentially constant.

Table 9. Stability of samples held at 4° C.

Sample number	Age of sample in days							
	0*	5-15	16-30	31-45	46-60	61-75	76-90	90-105
	Tocopherol concn., γ/100 ml.							
1344	217	217	223	---	---	260**	---	---
1425	204	---	266	---	291	---	291**	285**
1426	446	---	---	---	470	---	470	477**
1444***	25	--	--	37	--	68	--	68
1489	254	310	285	---	279	322	---	254**
1547	217	---	217	---	254	---	229	---
1566	217	---	210	204	210	---	192	---

*Analyzed as soon after collection as possible.
**Definite bacterial growth.
***Calf.

Table 10. Stability of samples held at 38° C.

Sample number	Hours held at 38° C.			
	0	12	24	48
	Tocopherol concn., γ/100 ml.			
1842	693	681	693	669
1846	904	904	904	929*

*Definite bacterial growth.

The above experiments indicate that the concentration of the tocopherol in stored blood serum either remains constant (within the range of experimental error) or tends to show a slight increase. The possibility of evaporation from the screw-top bottles holding the samples was considered to be slight. Enzymatic changes or bacterial action within the sample might result in the reduction of tocopheryl quinones in the serum, in the freeing of the tocopherols from their esters, etc., tending to vary the amount of apparent tocopherols detected by this method. A few samples upon prolonged storage did show visible evidences of bacterial growth or mold formation; no correlation with stability was noted.

It was concluded that the tocopherol in the blood serum was stable for reasonable lengths of time under the conditions of ordinary laboratory treatment of the serum samples. This is in agreement with the report of Mason (1) that tocopherols were sensitive to ultraviolet light but were fairly stable to visible light, and that the tocopherols were destroyed slowly by alkalis and were highly sensitive to oxidation but were stable in oil concentrates to 200° C. in the absence of oxygen and to 100° C. in sulfuric and hydrochloric acids.

EXPERIMENTAL RESULTS

The averages and the ranges of the tocopherol levels of the blood serum for dairy cattle during the parturient period are tabulated in Table 11, and for the calf during the period following birth in Table 12. These data are plotted on semi-logarithmic graph paper, for by so doing, the rate of change in levels can be followed, and the different portions of the curves can be compared directly. The original data, which was too voluminous to be presented here, revealed no significant differences due to breed or to the number of previous lactations.

Normal Tocopherol Levels of Blood Serum

Tocopherol Levels of Cows During Parturient Period. The trends and the levels of the tocopherols in the blood serum of the cows of group A which received no vitamin supplements, Fig. 2, of the cows of group B which received vitamin A alcohol supplements, Fig. 3, and of the cows of group C which received vitamin A ester supplements, Fig. 4, were very similar; groups A, B and C, therefore, will be considered as control groups, with respect to other groups receiving tocopherol supplementation.

As parturition approached, a downward trend was exhibited by the tocopherol levels in the blood serum of cows of the control

Table 11. Tocopherol levels in serum from the blood of the parturient dairy cow.

Group		Periods* 1	2	3	4	5	6	7***	8	9	10	11	12	13	14
		γ/100ml	γ/100ml	γ/100ml	γ/100ml	γ/100ml	γ/100ml	γ/100ml	γ/100ml	γ/100ml	γ/100ml	γ/100ml	γ/100ml	γ/100ml	γ/100ml
A	Av.	---	433 (3)**	340 (5)	347 (5)	328 (5)	290 (5)	276 (5)	260 (5)	240 (5)	261 (5)	305 (5)	410 (5)	420 (5)	575 (5)
	Range	---	351-557	302-405	325-373	306-352	266-353	254-316	210-297	192-285	198-310	186-458	298-613	322-463	490-631
B	Av.	588 (5)	508 (6)	402 (6)	365 (7)	298 (8)	286 (8)	271 (8)	248 (8)	227 (8)	236 (8)	329 (8)	454 (2)	528 (2)	534 (1)
	Range	371-898	396-755	266-588	286-427	204-384	180-371	155-371	136-340	142-291	99-365	204-508	314-593	424-632	
C	Av.	384 (3)	530 (5)	395 (6)	320 (6)	268 (6)	248 (6)	234 (6)	223 (6)	213 (6)	228 (6)	275 (6)	661 (1)		
	Range	266-470	279-873	266-526	237-483	167-483	176-450	155-433	149-384	155-340	155-340	192-470			
D	Av.	690 (5)	535 (6)	446 (6)	414 (6)	391 (6)	388 (6)	350 (6)	292 (6)	255 (6)	279 (6)	323 (6)			
	Range	310-1473	390-792	359-532	301-518	328-510	307-545	285-508	229-390	192-297	210-340	266-458			
E	Av.	351 (3)	479 (3)	1268 (3)	1333 (3)	1201 (3)	1164 (3)	1011 (3)	1044 (3)	1007 (3)	852 (3)	593 (3)	582 (3)	643 (3)	659 (3)
	Range	198-427	297-724	890-1764	930-1836	965-1594	919-1622	848-1281	848-1269	904-1151	563-1003	303-899	378-885	452-900	435-896
Cow															
G-451		384	396	569	559	557	378	396	279	347	285	369	501	513	616
G-467		724	588	1187	1278	1271	1136	1003	891	848	823	836	799	799	842
A-240		619	378	919	861	805	813	817	650	582	557	396	550	680	799
H-126		---	283	341	353	353	365	347	396	359	316	241	379		

*See Table 4.
**Number of samples represented in the average.
***Sample immediately after parturition.

Table 12. Tocopherol levels in serum from the blood of the neonatal calf.

		Age of calf in days							
		0*	1	2	3	7	14	21	28
		γ/100ml	γ/100ml	γ/100ml	γ/100ml	γ/100ml	γ/100ml	γ/100ml	γ/100ml
Group A	Av.	44 (7)**	159 (6)	175 (6)	202 (6)	238 (4)	307 (4)	227 (4)	183 (4)
	Range	12-105	105-204	136-248	149-272	198-266	272-378	203-260	99-260
B	Av.	56 (8)	144 (4)	189 (4)	203 (4)	248 (2)	245 (2)	221 (2)	184 (2)
	Range	0-111	111-186	87-297	74-291	186-310	204-285	161-281	118-250
C	Av.	48 (6)	103 (4)	152 (4)	188 (3)	162 (2)	161 (1)	75 (1)	76 (1)
	Range	25-74	62-149	68-260	118-272	157-167			
D	Av.	84 (5)	223 (2)	334 (2)	306 (2)				
	Range	56-136	217-229	322-347	285-328				
E	Av.	79 (3)	371 (2)	597 (2)	452 (2)	706 (1)	415 (1)	210 (1)	162 (1)
	Range	56-93	241-501	464-730	285-619				
Calf of Cow									
G-451		43	334	260	229	199	282	292	206
G-467		68	520	588	520	198	260	186	115
A-240		43							
H-126		25	68	62	80				

*Sample collected immediately after birth, before ingestion of colostrum.

**Number of samples used in obtaining the average. In group A, two control calves were from cows for which tocopherol levels had not been determined.

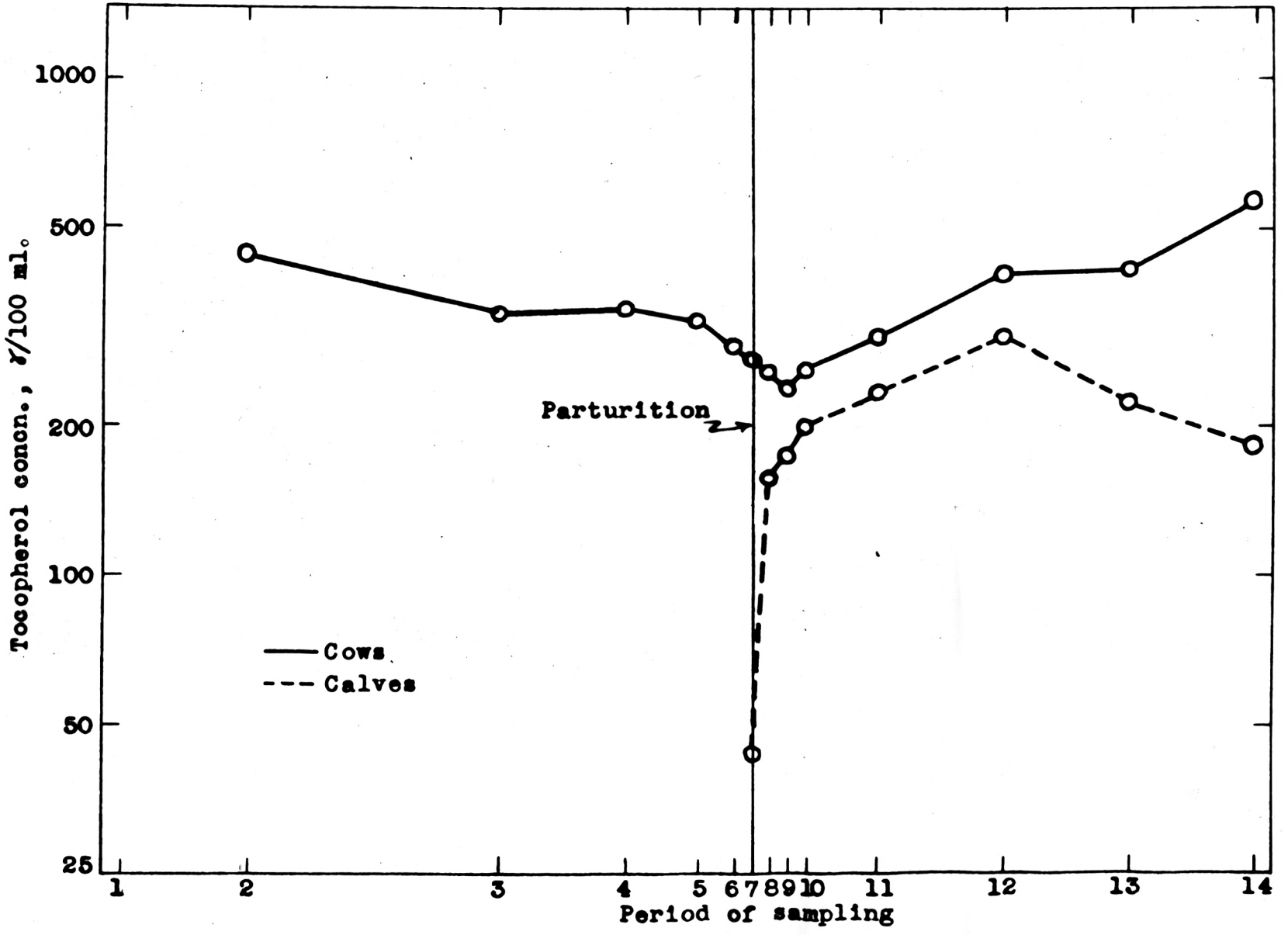

Fig. 2. Changes of tocopherol levels in blood serum; group A.

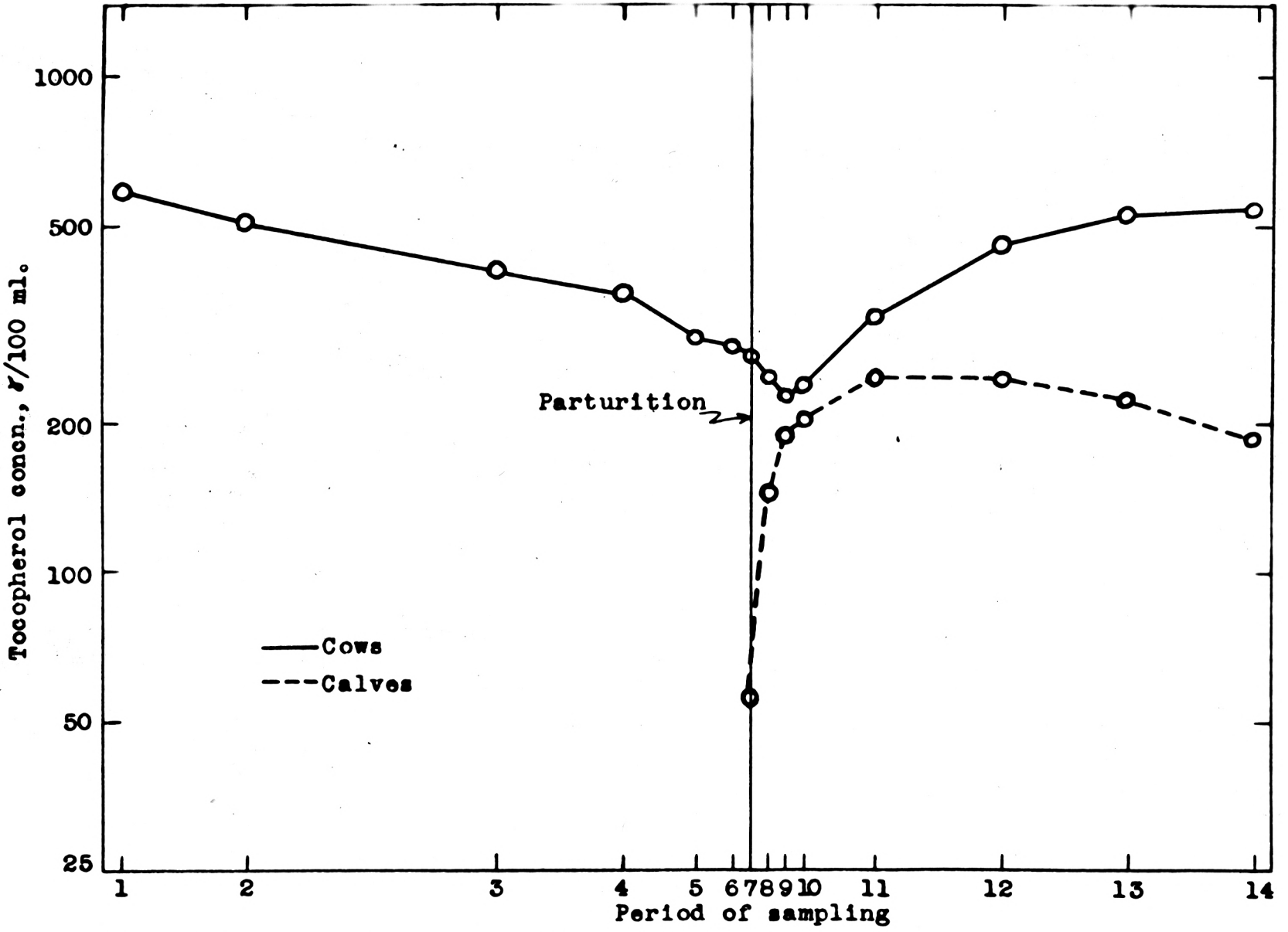

Fig. 3. Changes of tocopherol levels in blood serum; group B.

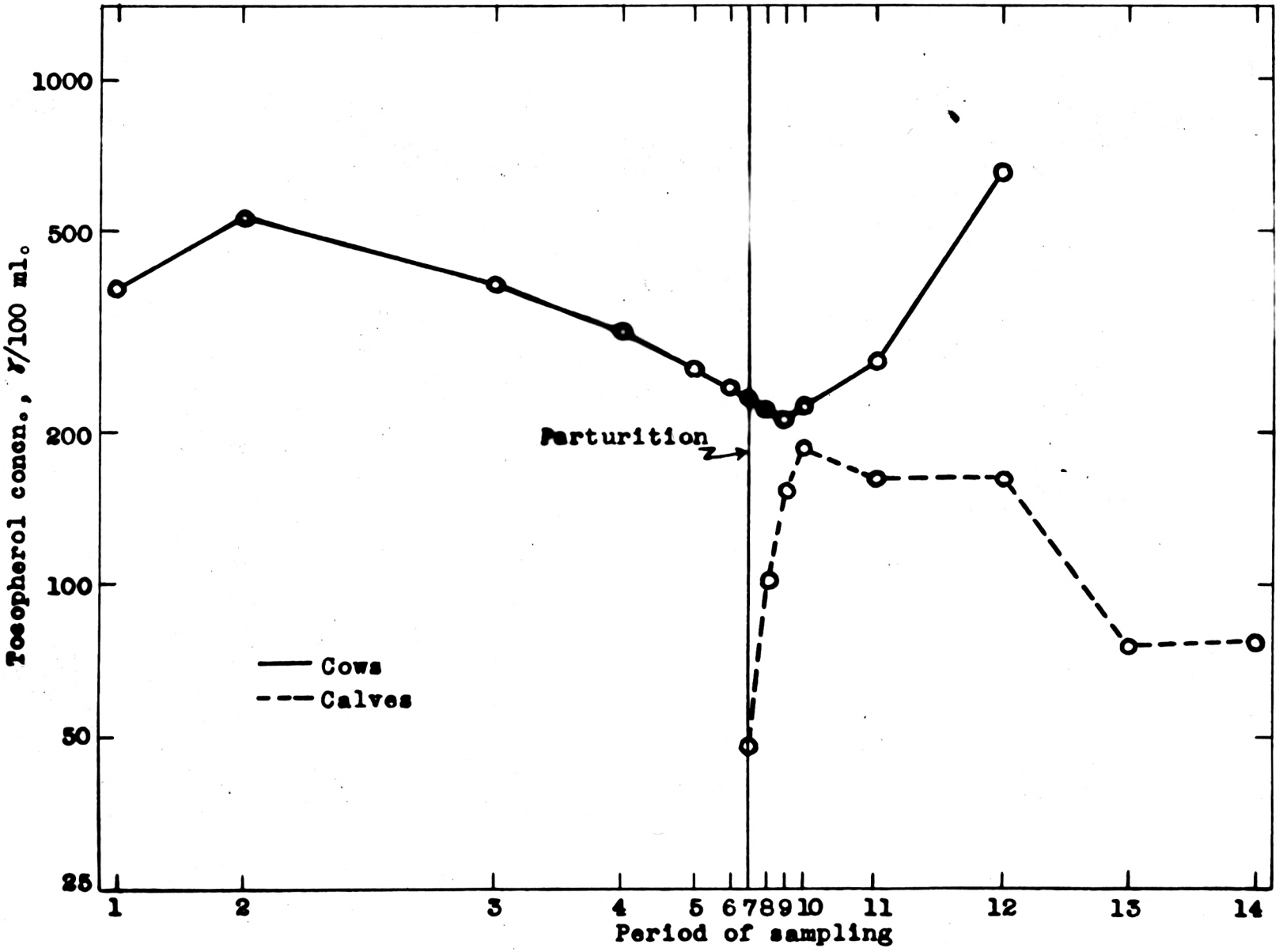

Fig. 4. Changes of tocopherol levels in blood serum; group C.

groups, the rate of decrease usually becoming most pronounced after the third day prepartal. The minimal value usually was attained on the second day postpartum, after which the trend reversed and a gradual, though continuous, increase occurred. At the end of the first month after parturition, the tocopherol levels were appreciably higher than at the start of the experiment, one month prepartal.

The levels of tocopherols in the blood serum of cow H-126, in the prepartal period, Fig. 5, were comparable to those of the control groups, but showed the opposite trend, increasing levels, with approaching parturition. The maximum level was attained on the first day postpartum, followed by a decline to a minimum level on the seventh day postpartum.

Tocopherol Levels of Calves. At birth the average tocopherol levels in the blood serum of the calves from the control groups were very low, about 20 per cent of that of the dams at parturition. However, due to the ingestation of colostrum, a rich source of tocopherols (Parrish et al., 28), a marked increase (averaging 175 per cent) in tocopherol levels occurred during the first 24 hours after birth. The maximum values were usually attained at 7 or 14 days after birth; the slow decrease thereafter resulted in tocopherol levels for the calves at 28 days of age which were one-third of those of the dams at the same period.

The trend of the tocopherol levels of the blood serum of the

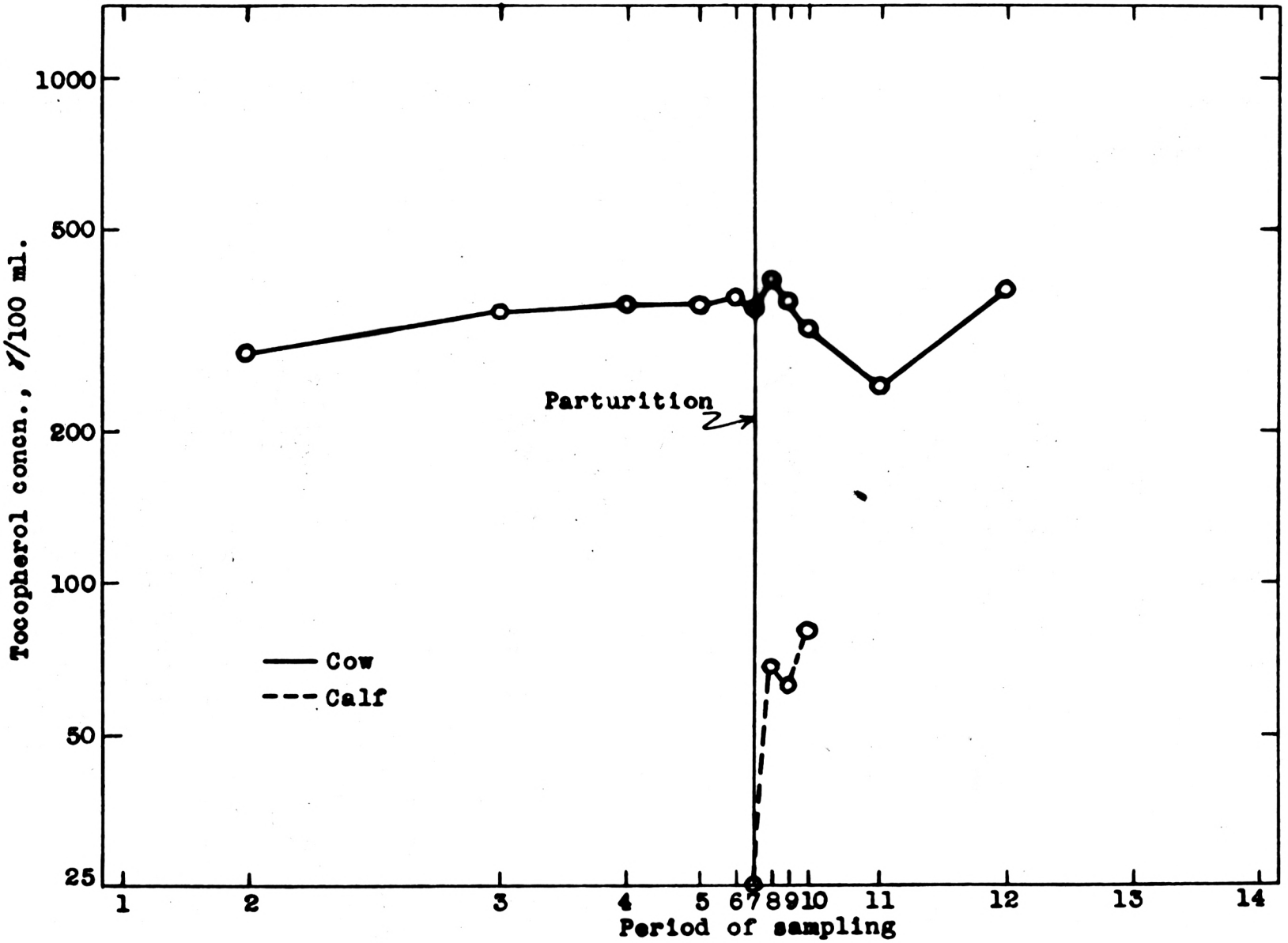

Fig. 5. Changes of tocopherol levels in blood serum; cow H-126.

calf of H-126 followed the same general pattern as the calves from the control groups, but the values for this calf were 35 to 50 per cent lower from birth to three days thereafter.

Tocopherol Levels of Normal Dairy Cattle. Although this investigation was designed primarily to study the changes in the tocopherol levels during the parturient period, additional, though limited, data were collected from dry cows and heifers one to seven days off late pasture and at least one month prepartal, and from normal milking cows on standard winter rations at least one month postpartum. Although some effects of the parturient period possibly were reflected in the tocopherol levels of the blood serum obtained at least one month prepartal, these values, Table 13, may be considered representative of the tocopherol levels of the blood serum of the normal dairy cow. The average level for groups A, B and C at parturition, 260 γ/100 ml., when compared with the values of 702 γ/100 ml. and 745 γ/100 ml. for heifers and dry cows and for milking cows, respectively, emphasizes the decreased amounts of tocopherol in the blood serum during the terminal stages of gestation.

Effect of Tocopherol Supplements on Tocopherol Levels of Blood Serum

Tocopherol Levels of Cows During Parturient Period. The tocopherol levels of the blood serum of the cows of group D,

Table 13. Normal tocopherol levels in the serum from the blood of dairy cattle.

At least one month prepartal		One month or more postpartum	
Experimental animals	Tocopherol concentration	Experimental animals	Tocopherol concentration
	γ/100 ml.		γ/100 ml.
H-135	1473	H-136	959
G-452	898	J-378	811
J-374	737	H-132	774
G-467	724	G-465	693
A-252	699	J-358	693
A-230	644	A-243	675
J-365	532	J-365	607
J-380	520		
G-458	409		
G-433	384		
Average----------	702	Average----------	745

which received vitamin A alcohol and a low level (0.5 - 1 g.) of tocopherol supplementation (Fig. 6), were 25 to 30 per cent higher than that shown by the groups receiving no tocopherols during the prepartal period. The trend of the tocopherol levels of group D and the control groups was similar, with the rapid decrease for group D beginning one day prepartal. The tocopherol levels of group D after parturition were 15 to 20 per cent higher than those of the control groups, with the levels becoming nearly alike in the latter periods.

The prepartal data (Fig. 7) of cow G-451, receiving the 0.5 - 1 g. level of tocopherols without vitamin A supplementation, exhibited the same general prepartal levels of blood serum tocopherols as those of the cows of group D, with, however, a rising trend upon approaching parturition, which was opposite to the trend shown by group D. Data for the postpartum periods were similar for group D and cow G-451.

The data for cow G-467, cow A-240, and group E, receiving 4 g., 5 g., and 10 g. of tocopherol supplementation, are shown in Figs. 8, 9, and 10, respectively. In each case the tocopherol levels of the blood serum increased rapidly after the start of supplementation, the rate of increase during the period just after the start of supplementation being in the same order as the relative levels of supplementation. In spite of this more rapid rate of increase for A-240 as compared to G-467, the latter maintained a consistently higher level of blood serum tocopherol, due to the greater amounts of tocopherols initially present.

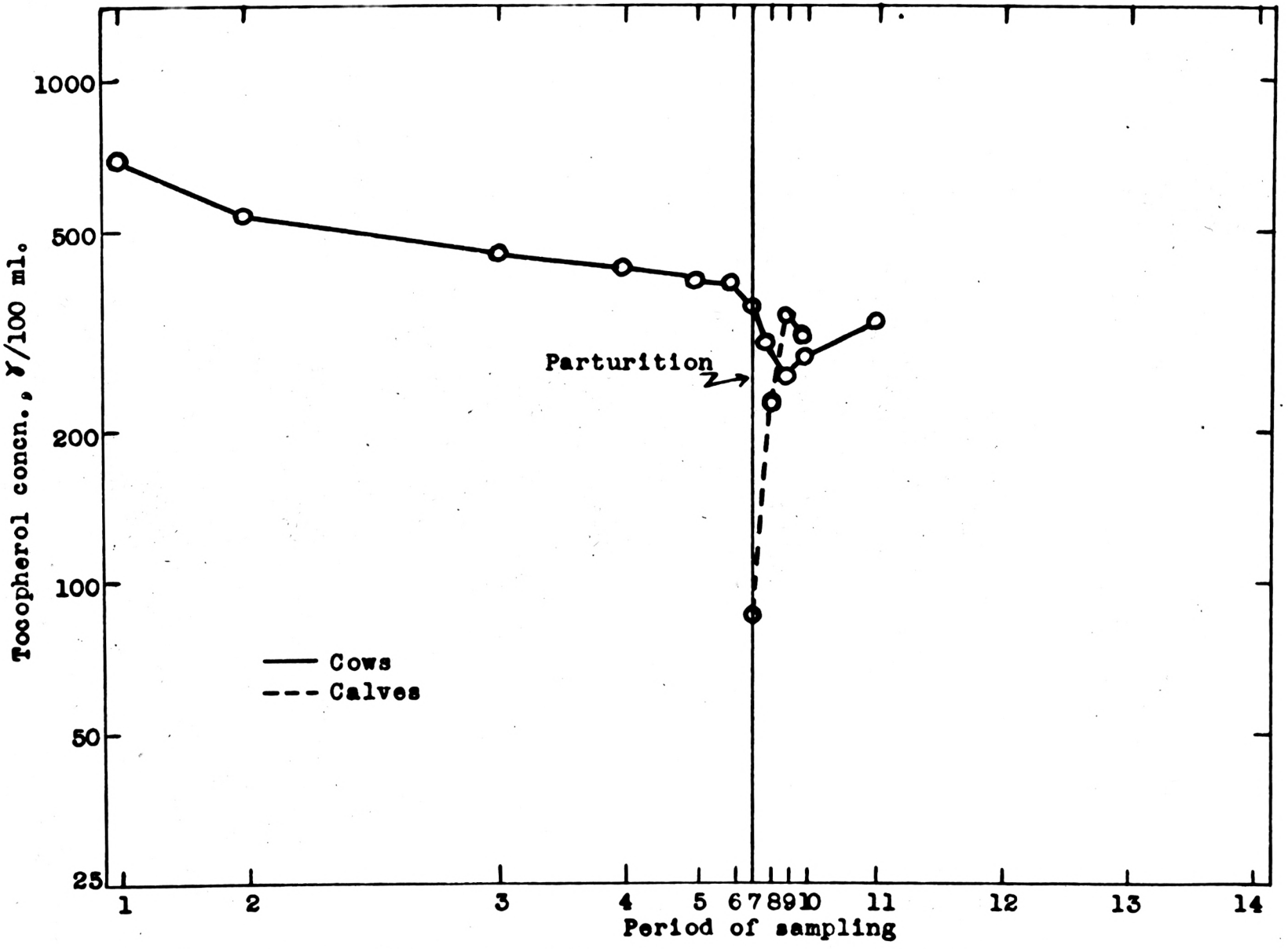

Fig. 6. Changes of tocopherol levels in blood serum; group D.

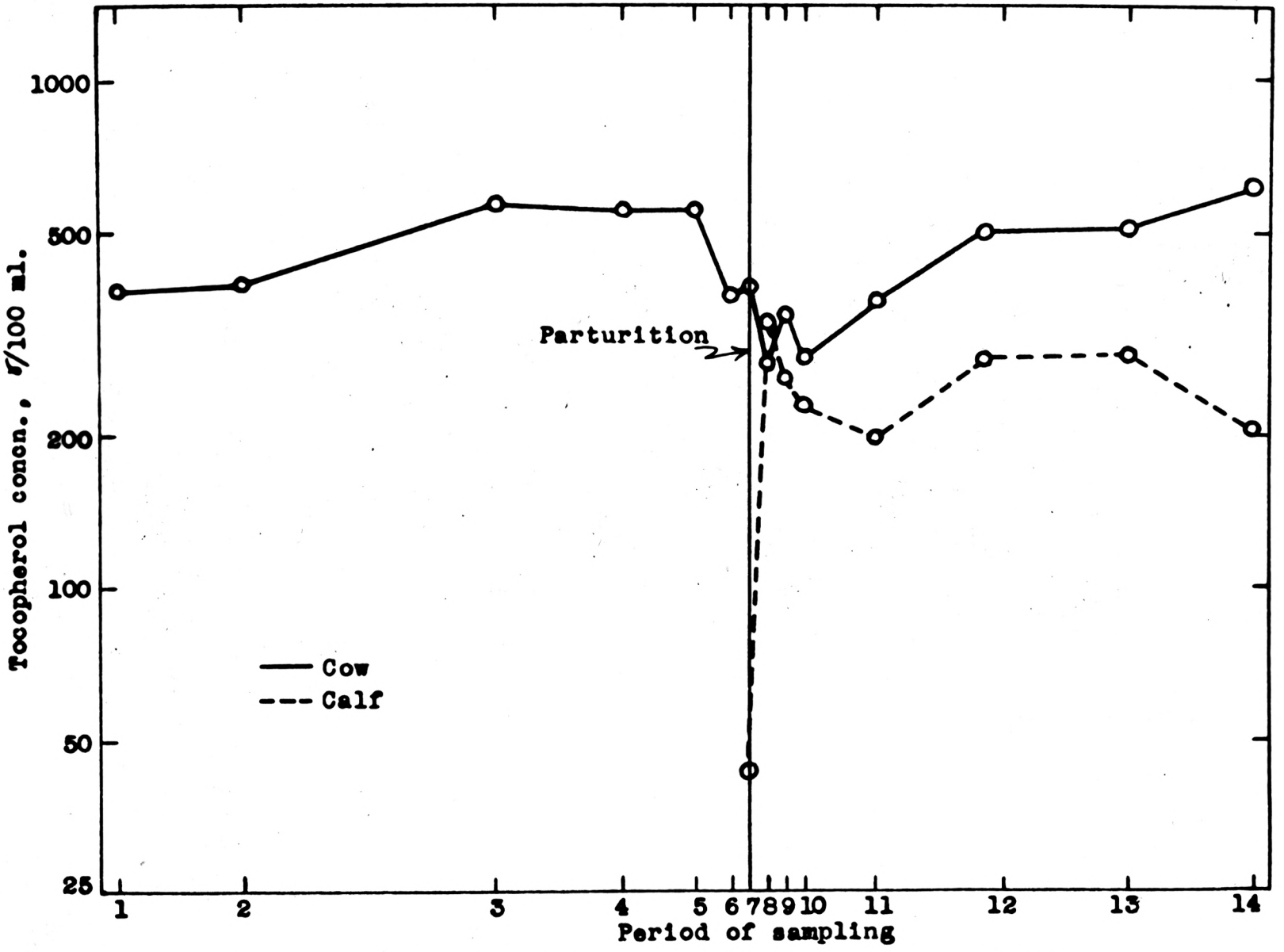

Fig. 7. Changes of tocopherol levels in blood serum; cow G-451.

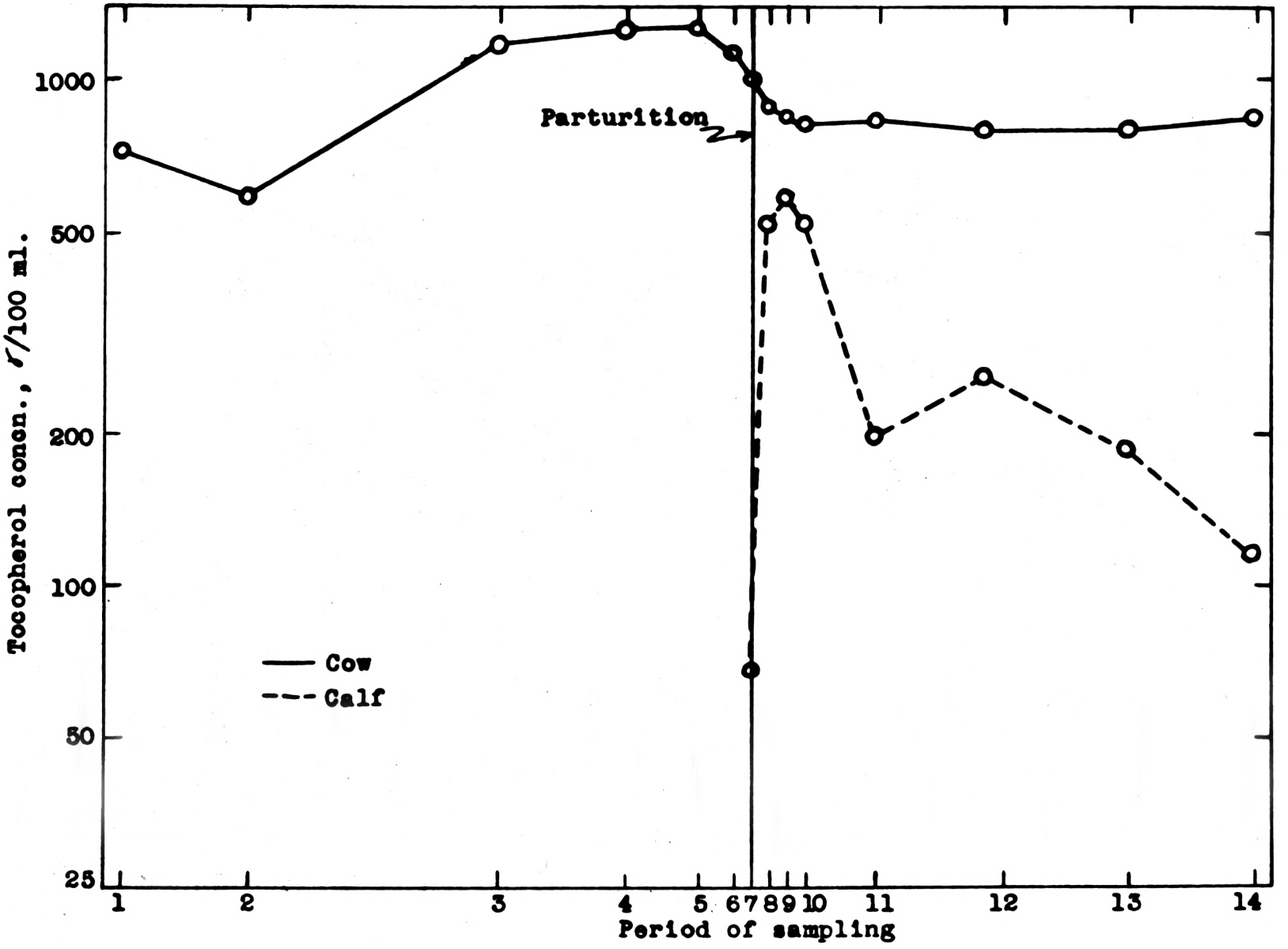

Fig. 8. Changes of tocopherol levels in blood serum; cow G-467.

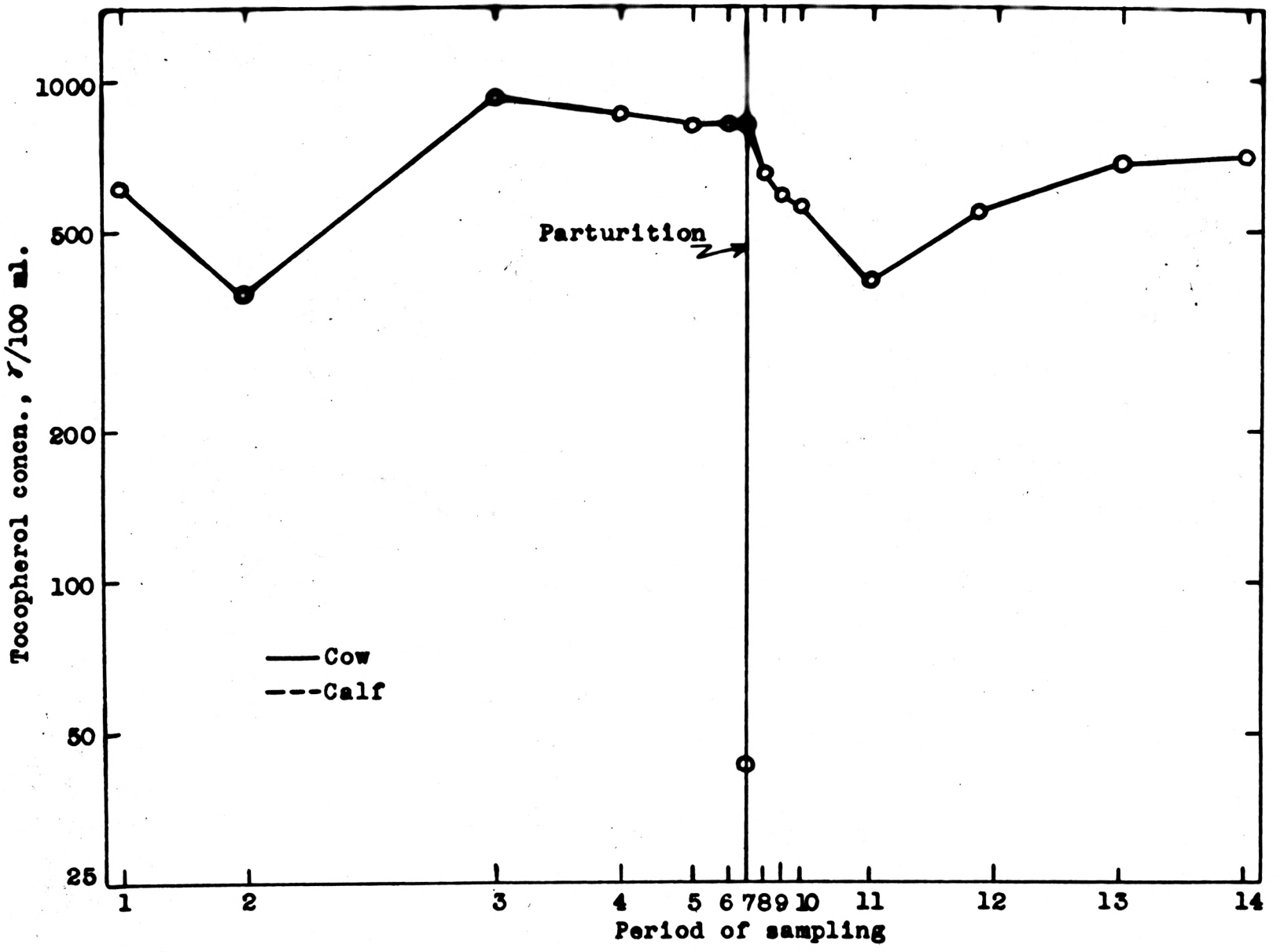

Fig. 9. Changes of Tocopherol levels in blood serum; cow A-240.

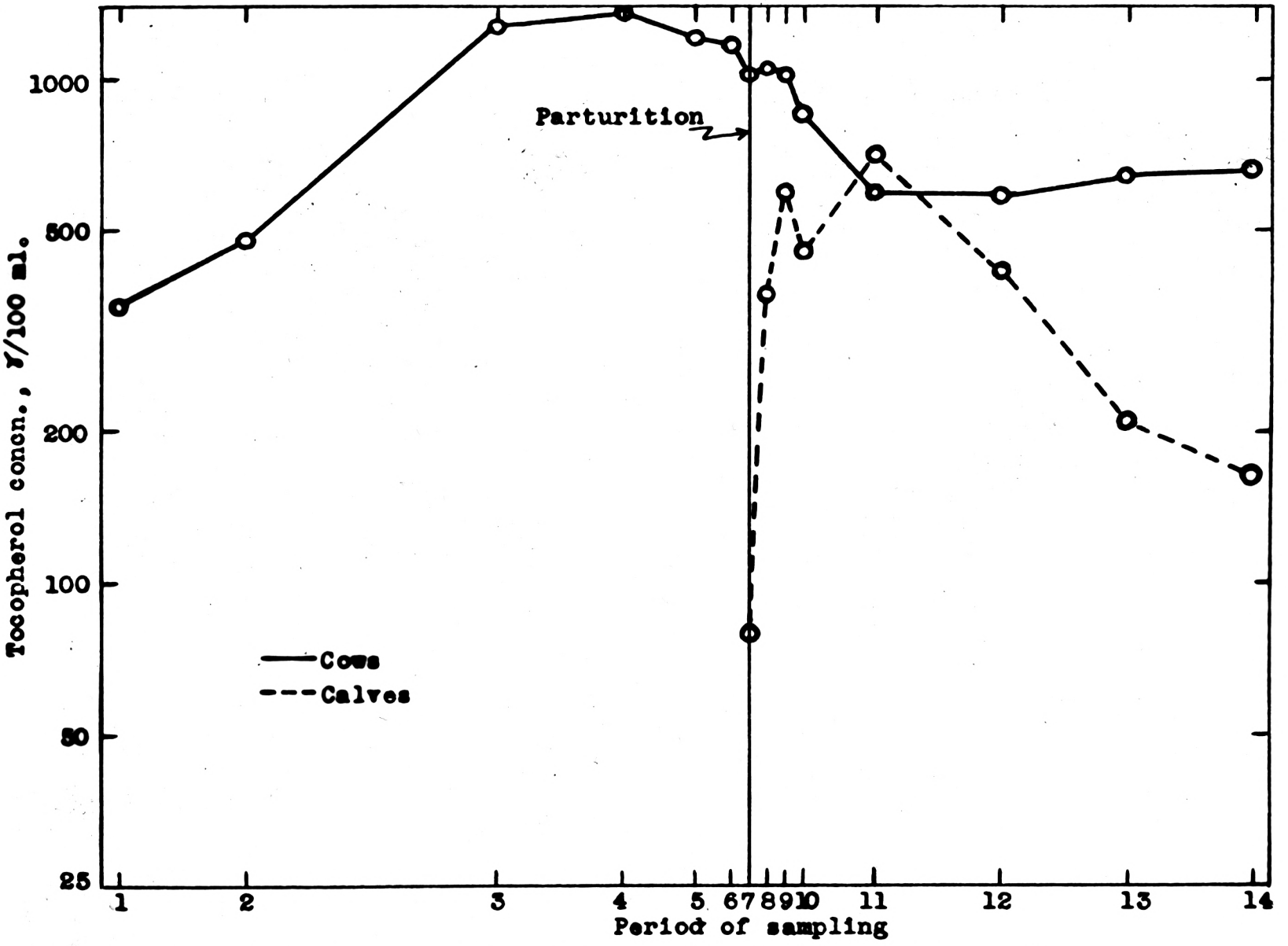

Fig. 10. Changes of tocopherol levels in blood serum; group E.

During the prepartal periods in which high tocopherol supplementation was practiced, the tocopherol levels in the blood serum of the cows of group E were from 280 to 300 per cent higher than those of the control groups for the same periods. After parturition, with cessation of tocopherol supplements, the difference in the tocopherol levels between these groups steadily diminished, until at four weeks postpartum the blood serum tocopherol levels of group E were only 16 per cent higher than those of the control groups.

The normal decreasing trend of tocopherol levels in the blood serum with approach of calving was not entirely eliminated by tocopherol supplementation at the three levels employed. As might be anticipated, the relative levels of supplementation of cow G-467, cow A-240 and group E affected the times of attainment of the maximum blood serum tocopherol levels, which occurred between 7 and 14 days prepartal. In addition the levels of supplementation influenced the times of occurrence of the more rapid decline, which became apparent at three days prepartal, at parturition, and at two days postpartum, respectively; and the levels influenced the times of attainment of the minimum blood serum tocopherol levels, which occurred at 3, 7, and 14 days postpartum, respectively.

Tocopherol Levels of Calves. The blood serum tocopherol levels of the calves of cows from group D showed the same general trend and rate of tocopherol increase as that of the calves from

the control groups, except the levels at birth and up to the third day postpartum were 70 to 75 per cent higher. The rate of increase was such that the tocopherol levels in the blood serum of the calves of group D became higher than those of the cows on the second day postpartum. The data for the calf of cow J-380 were considered abnormal and were eliminated entirely since the calf died before the third day postpartum sample was collected. The tocopherol content of the blood serum of this calf was very low, being 24, 56, and 12 γ/100 ml. at birth, first, and second day postpartum, respectively. These low levels might have been the result of poor absorption of tocopherol from the colostrum consumed, or of increased requirements for the vitamin.

The data for the calf of G-451 showed that the tocopherol level in the blood serum at birth was slightly lower than, and that the rate of increase during the first 24 hours was about four times as great as that of the calves of the control groups. This calf, as did those of group D, attained a tocopherol level higher than that of the dam within two days after birth.

At birth and at one day postpartum the tocopherol levels of the calf of G-467, which received five times as much tocopherol supplement as G-451, were about 55 to 60 per cent higher than those of the calf of G-451, but the rate of increase during that time was very nearly the same. At the second and the third day postpartum the tocopherol levels of the former were about 125 to 130 per cent higher than those of the latter calf. The calf of A-240 was sacrificed immediately after birth, at which time the

tocopherol level of the blood serum was slightly lower than that of the calves from control groups.

The tocopherol levels in the blood serum of calves of group E were 60 to 65 per cent higher than those of the calves of control groups, with the rate of increase of tocopherols during the first 24 hours about the same for these groups. From two to seven days postpartum the tocopherol levels of calves of group E were 210 to 245 per cent higher than those of the calves from control groups; however, these differences in levels diminished, and at 28 days postpartum the tocopherol levels of the blood serum of these two groups were essentially the same.

DISCUSSION

Normal Tocopherol Levels of Blood Serum

The tocopherol content of the blood serum of the dairy cow under normal conditions (e.g., not affected by the immediate parturition process) showed a range of 700 to 750 γ/100 ml. (Table 13). This was somewhat higher than the value of 360 γ/100 ml. for reconstituted, dehydrated beef serum reported by Quaife and Harris (10) using essentially the same method as the author. The latter value corresponded reasonably well with the value of 400 γ/100 ml. obtained by bioassay of the beef serum by the same workers. It is difficult to analyze these differences since they did not report the type of ration consumed by their animals. Further, the effect of treatment of their samples compared with the author's has not been investigated.

For other animals, Mayer and Sobotka (6) reported a value of 233 γ/100 ml. for preserved horse serum (with the note that this low value might be due to tocopherol destruction during storage), and a value of 1233 γ/100 ml. for the serum of dogs. A value of 3000 γ/100 ml. for dog serum was reported by Vinet and Meunier (16), the level fluctuating according to the quantity of vitamin E administered.

Many investigations have been made of the normal tocopherol

levels in the human blood serum: Varangot et al. (12, 13), reported values of 190 to 220 γ/100 ml.; Meunier and Vinet (7), 300 to 600 γ/100 ml.; Couperus (4, 5), 500 to 900 γ/100 ml.; Mayer and Sobotka (6), 600 to 1400 γ/100 ml.; Wechsler et al. (14, 15), 600 to 1600 γ/100 ml. with an average of 960 γ/100 ml.; Quaife and Harris (10), 900 to 1600 γ/100 ml.; Minot (8), 100 to 1300 γ/100 ml.; and Varangot (11), 1380 γ/100 ml. For children values of 640 to 1120 γ/100 ml. were reported by Minot and Frank (9), and 1000 γ/100 ml. by Minot (8).

Changes of Tocopherol Levels of Blood Serum During the Parturient Period

A decrease of tocopherols in the blood serum of the dairy cow with the approach of calving was found to be the normal situation. A similar phenomenum has been reported by Wise et al. (29) and Sutton et al. (30), who found that in dairy cows the blood serum carotene and vitamin A decreased with approaching parturition and the beginning of lactation, with the maximum decrease in blood plasma vitamin A occurring three days after parturition.

Results of a similar downward trend of the tocopherol levels in human serum have been published by Varangot et al. (13) who found that the vitamin E of human blood serum during pregnancy was 340 γ/100 ml. during the first third, 320 γ/100 ml. during the second third, and 310 γ/100 ml. during the final third of the period of pregnancy; an average of 220 γ/100 ml. was found at 10

days postpartum. These workers noted that, although the same method was used, the values were lower than those previously reported by Varangot (11), who had reported, in that earlier paper, that the tocopherol level increased during the period of pregnancy and decreased rapidly after delivery.

This upward trend also was reported by two other laboratories. Rauramo (17) found that the vitamin E content of the human blood serum was 600 to 1000 γ/100 ml. in the sixth to the ninth month of pregnancy, and was 800 to 1300 γ/100 ml. at parturition. In an excellent paper, Straumfjord and Quaife (18) reported that 11 women in the first 24 weeks of pregnancy had a mean tocopherol level of 1170 γ/100 ml., and that 12 other women in the twenty-fifth to thirty-sixth week of pregnancy had a significantly higher level of 1620 γ/100 ml.

In order to obtain a better understanding of these conflicting results, the differences in the basic experiments must be kept in mind. The trends as reported for the human blood serum in pregnancy covered a much longer period of time than that for dairy cows. The present experiment concentrated the investigation upon the changes occurring immediately preceding and following parturition. Furthermore, the degree to which the tocopherol content of the diet of the pregnant women influenced the results apparently was not investigated.

Effect of Tocopherol Supplements on Tocopherol Levels of Blood Serum

Comparison of the data of groups A, B, and C showed that supplementation of the diet with the vitamin A had no apparent effect upon the tocopherol levels in the blood serum of the cow or the calf; the data of group D and cow G-451 also tended to support this conclusion.

Cows receiving the various levels of vitamin E supplementation showed a definite response in the tocopherol levels of the blood serum during the periods in which the vitamin supplement was administered. The highest level of vitamin E supplementation, 10 g. daily, produced in the tocopherol levels of the blood serum of the cow an increase of 265 to 300 per cent above that of the cows receiving no tocopherol during the same periods. The highest value attained was that of a cow receiving this high tocopherol supplementation; 1888 γ/100 ml. were found seven days after the start of daily 10 g. supplementation.

Group D, receiving the lower level of tocopherol supplementation, exhibited the downward trend of the tocopherol levels with approaching parturition, similar to that of the groups A, B, and C; however, the rate of decrease was smaller for group D than for the latter groups. As might be anticipated, the groups receiving the higher tocopherol supplementation exhibited an in-

crease of tocopherol in the blood serum with the start of the supplement, instead of the downward trend that usually accompanies parturition. The increase of tocopherol levels was of the greatest magnitude during the period of the start of the vitamin supplementation.

Other workers also have found increased tocopherol levels of blood serum following administration of vitamin E. Minot (8), after producing vitamin E deficiency in rabbits, reported the following values of tocopherol in the blood serum: less than 50 γ/100 ml. in a rabbit dying from vitamin E deficiency; 430 γ/100 ml. in an E-deficient rabbit beginning to show weakness, increasing to 1740 γ/100 ml. upon administration of quantities of the vitamin; and 260 γ/100 ml. in a rabbit exhibiting marked muscular weakness as a result of prolonged E-deficiency, increasing to 2760 γ/100 ml. after treatment with liberal amounts of vitamin E.

Daily administration of oral doses of tocopherol to clinical patients was found by Wechsler et al. (14, 15) to result in a rise of the tocopherol levels of the blood serum; the response to daily administration of 75 to 740 mg. of tocopherols was parallel to the dosage and attained levels of more than 2000 γ/100 ml. of serum, as compared to levels of 610 to 670 γ/100 ml. before vitamin therapy. Couperus (4, 5) reported that the tocopherol content of the serum attained a maximum six hours after oral administration of 300 mg. of dl-α-tocopherol acetate and returned to normal in 24 hours; administration of 30 mg. of toco-

pherol acetate three times daily by mouth was found to increase the tocopherol levels of human blood serum from the normal of 500 to 900 γ/100 ml. to 1400 to 2000 γ/100 ml. A single oral dose of 1.5 g. of natural mixed tocopherols was found by Quaife and Harris (10) to result in the change of the tocopherol levels in human blood plasma from 1340 γ/100 ml. to a maximum of 2600 γ/100 ml. at six hours, to 2040 γ/100 ml. at 24 hours, and to 1920 γ/100 ml. at 48 hours. Thus an increase in the tocopherol level of the blood can be produced in various mammals by incorporation of tocopherol in the diet.

An interesting experiment was conducted on the calf of H-138 of group E. The tocopherol level of the blood serum collected immediately after birth was found to be 56 γ/100 ml. Four hours after birth the calf was fed five pounds of colostrum from the dam. At seven hours of age the tocopherol level of the serum was found to be 111 γ/100 ml. A final sample obtained at 10 hours of age had a tocopherol content of 105 γ/100 ml. The calf then was sacrificed. These data indicated that rapid absorption of tocopherols took place in the dairy calf, as evidenced by the 100 per cent increase of the tocopherol level of the serum three hours after ingestion of the colostrum, which is a rich source of tocopherols (Parrish et al., 28).

Cabell and Ellis (31) have published results which make questionable the value of small tocopherol supplements for dairy cattle. By bioassay these workers found that the vitamin E content of wheat ranged from 2.3 to 5.4 mg. per 100 g. of grain,

that the range for corn was 1.5 to 3.6 mg., and that dried and green grasses and legumes had a range from 7.1 to 28.1 mg. They calculated that a 1000 pound cow consuming 100 pounds of grass during a 24 hour period of grazing would have an α-tocopherol intake of approximately 7.1 g. based on their assays. Therefore, a one gram level of tocopherol supplementation appears of doubtful significance, in view of the amounts of tocopherols which may normally be in the diet.

Placental Transmission of Tocopherols

The tocopherol levels in the blood serum of the calves, immediately after birth and before ingestion of colostrum, were uniformly low. The range of values obtained on the first sample of blood from calves of all groups was from the extreme low of 0 γ/100 ml. (group B) to the high of 136 γ/100 ml. (group D); the average was 57 γ/100 ml. Although there was considerable variation within each group, newborn calves of those groups receiving no tocopherol supplementation averaged lower than 57 γ/100 ml. Calves whose dams received tocopherol supplements tended to run above 57 γ/100 ml., with the highest less than 50 per cent greater. Therefore, prepartal vitamin supplementation of the dam had only small effect on the tocopherol level of the blood serum of the calf at birth.

Similar results in the case of infants have been found by Varangot et al. (22), who reported that the tocopherol content in

18 samples of blood collected from the umbilical vein at birth was approximately 100 γ/100 ml., with the amount in six samples being too small to determine. Considerably higher values were reported by Straumfjord and Quaife (18) who found a mean of 340 γ/100 ml. in the cord blood. However, an essential difference existed between the studies on infants and on calves. It has not been established whether the blood of the umbilical cord is of the same composition as the blood circulating within the infant at birth, while the sample of calf blood, obtained from the jugular vein, was representative of the conditions within the calf.

After injection of 250 mg. of tocopherol into the mothers four to eight hours before delivery, Varangot et al. (22) found that the cord blood contained 250 γ/100 ml., an increase of 150 per cent over the normal value, indicating that increased placental transfer of the tocopherol occurred. Wise et al. (32) and Wise et al. (33) found limited placental transfer of carotenoid and vitamin A reserves when the dams were receiving normal feeds. However, the amount of vitamin A reserves transferred was increased appreciably by supplementation of the dam during pregnancy with large amounts of vitamin A. The limited transfer observed by Dann (34, 35) in rats and rabbits could be augmented but little by feeding large amounts of vitamin A preceding parturition. The same appears to be true of the placental transfer of vitamin D in the rat, as shown by the report of McCollum et al. (36).

A comparison of the tocopherol levels of the blood serum of the calf at birth and of the cow at the same period showed that,

in spite of considerable variations within groups, the mean concentration in the blood of the cows of groups A, B, C, D, and E was 5.2, 4.6, 4.8, 5.0, and 12.5 times that of the calves of the respective groups. The mean ratio of plasma vitamin E in the mother to that in the infant was reported by Straumfjord and Quaife (18) as 5.7; Mason and Bryan (20) found for the rat that the concentration of tocopherol on the maternal side of the placental barrier was more than five times that present in the fetuses or newborn. The general agreement of the above workers with the results of the present investigation, except for group E, serves to emphasize the fact that in spite of the greatly increased amounts of blood serum tocopherols of the dams of group E as a result of tocopherol supplementation, placental transfer of the vitamin did not keep pace.

As previously indicated on pages 54-55, the reports of Couperus (4) and Quaife and Harris (10) showed the rapid absorption of tocopherols administered to the human, leading to high temporary concentrations of tocopherols in the blood. Since, in the case of the dairy cow, solid food first goes to the rumen, the tocopherols administered would probably be released more slowly for absorption, and thus one would not expect a large temporary rise of blood tocopherols following a single supplemental dose. However, for the newborn calf which has no functioning rumen, the response (as indicated on page 55) was similar to that of the human.

Mason and Bryan (20), who found that the placental trans-

mission of tocopherol in the rat was exceedingly limited, suggested that the limiting effect must be attributed to the physiological selectivity of the placental barrier, and that, for the fat-soluble vitamins, this might be a function of their molecular size. Kofler (37) reported a relatively low tocopherol value of 500 γ/100 g. for the human placenta, itself.

Hickman and Harris (38) suggested that the low value of tocopherol in the blood serum of the newborn might be due to (a) normal tissue contents associated with an abnormal blood composition, (b) faulty transfer across the placenta, or (c) efficient placental transfer followed by rapid fixation by the fetus. They further point out that, with reference to the adult, a "maintaining dose" of the vitamin is employed, while for the fetus sufficient vitamin must be provided for manufacture, maintenance and endowment, in order that the young may be started in life with sufficient vitamin reserve to tide over the limited dietary resources of the first few months.

If the tocopherol levels found in the blood serum of the calf immediately after birth should be interpreted as the amount of "tocopherol reserves" present, the calf is <u>not started</u> in life with vitamin E reserves sufficient to carry it for any appreciable time, as is evidenced by the very low level of tocopherols at birth. The very marked increase in the tocopherol levels of the calf during the first 24 hours after birth indicates that the calf acquires by far the most of its tocopherol reserves after birth, through ingestion of colostrum. Furthermore, the produc-

tion of the fetus does not appear to cause a great strain upon the dairy cow since the bulk of the nutrients going into the daily production of milk is probably greater than that going into the production of the fetus over the nine month gestation period. The situation with respect to vitamin E might be expected to be similar to that of other metabolites.

Mammary Transmission of Tocopherols

The tocopherol levels in the blood serum of the calf were found in all groups to undergo a very marked increase within the first 24 hours after birth. As shown in Table 3, colostrum from its own dam was fed to each calf until the age of three days; in most cases the maximum level of tocopherols in the blood serum of the calf had been attained by that time. Parrish et al. (28) found that the initial colostrum had a high tocopherol content, with progressively lesser amounts in succeeding samples. Further, they found that the relative average differences in the tocopherol levels in the first samples of colostrum from the groups receiving levels of 0, 0.5 to 1 g., and 10 g. tocopherol supplementation were essentially the same as those in the blood serum of the respective groups of cows at the time of parturition. The same relationship applied when the comparison was made between the serum levels at parturition and the total tocopherols secreted during the four day colostric period. These results indicate that it is through mammary and not placental transfer

that the newborn calf is endowed with the large reserves of tocopherol.

These results do not bear out the contention of Hickman and Harris (38) that the fetus must be endowed with large amounts of tocopherol. Furthermore, that the tissues other than blood do not have large reserves of tocopherol is indicated by the report of Mason (21) that the newborn rat had only a negligible supply of tocopherol at birth, but after 24 to 48 hours the concentration of tocopherol per gram of tissue of the suckling rat had increased about four-fold and was approximately that of the body tissues of the mother.

Mason and Bryan (20) reported that tocopherol was much more readily transferred through the mammary gland to the colostrum than through the limited placental transmission to the fetus. The active mammary gland was found by Mason (21) to possess a greater storage capacity for tocopherol than did the liver, which seemed to have decidedly less ability to store this vitamin than vitamins A and D. However, the liver was found to be the chief repository for tocopherol when the intake was optimal or greater.

The principal mode of transfer of tocopherol reserves is through the mammary secretions, which furnish a large endowment within the first few days while the calf consumes colostrum. These observations concerning the mammary transmission of vitamin E is in close accord with the reports of Wise et al. (32), of Moore and Berry (39), and of Dann (40) regarding the mammary transmission of carotenoids and vitamin A in the dairy cow.

Similar observations also are reported concerning the mammary transmission of vitamin A and carotenoids in the rat by Dann (34), and of vitamin D in the rat by McCollum et al. (36).

CONCLUSIONS

1. The range of tocopherol levels in the blood serum of the dairy cow under normal conditions was found to be 700 to 750 γ/100 ml.

2. The trend of the tocopherol levels of the blood serum of the cows not receiving tocopherol supplements, control animals, exhibited a slow decrease as the calving date approached, reached a minimal value usually on the second day postpartum, and then a gradual but continuous increase ensued.

3. Addition of vitamin A supplements to the diet had no appreciable effect on the tocopherol levels in the blood serums of the dairy cow or her calf.

4. The addition of tocopherol supplements to the standard dairy ration resulted in an increase in the tocopherol levels in the blood serums of the dairy cow, with the highest level of tocopherol supplementation, 10 g. daily, eliciting the greatest response. The higher levels of tocopherol supplementation reversed the usual trend of tocopherol levels for the prepartal period as shown by the control animals, resulting in increasing levels of blood serum tocopherols with approaching parturition.

5. The level of tocopherols in the blood serum of the neonatal calf was very low, averaging 57 γ/100 ml. at birth.

6. The level of the highest tocopherol supplementation of

the dam had a minor effect upon the level of blood serum tocopherols of the newborn calf, indicating that there was only slight placental transfer of tocopherol reserves from the dam to the calf.

7. The concentration of the tocopherol on the maternal side of the placental barrier, as evidenced by the levels of tocopherols of the blood serum, compared to the concentration of the tocopherols of the blood serum of the calf at birth, was in a ratio of 5:1 for all cows except those receiving the highest level of tocopherol supplementation; for the latter the ratio was 12.5:1.

8. Following ingestion of colostrum, the tocopherol levels of the blood serum of the calf increased markedly, especially during the first 24 hours, with the response in the calf corresponding, in general, to the levels of tocopherol supplementation of the dam; thus mammary transfer was the principal mode of transfer of the tocopherol reserves from the dam to the calf.

9. The tocopherol in samples of blood serum was found to be stable for reasonable lengths of time under ordinary conditions of laboratory treatment.

10. The recovery of α-tocopherol added to blood serum samples was found satisfactory, averaging 97.1 per cent.

ACKNOWLEDGMENTS

The author wishes to take this opportunity to express his appreciation to Dr. J. S. Hughes of the Department of Chemistry and to Dr. G. H. Wise of the Department of Dairy Husbandry for their assistance in the selection of this interesting problem, the investigation of which was a constant challenge, and to Dr. G. H. Wise who supervised all phases concerned with the experimental animals. Use of the facilities of the Kansas Agricultural Experiment Station, under the supervision of Dr. J. S. Hughes, aided materially in the successful investigation of the problem. A special note of appreciation is due Mr. D. B. Parrish of the Department of Chemistry whose constant assistance and encouragement contributed materially to the completion of this work. To fellow students and members of the staff, the author is indebted for assistance at various times while carrying on this investigation.

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