Placental and Mammary Transfer of Vitamin A and Carotene by Beef Cows. (20421)

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The carotene and vit. A nutrition of cattle is greatly influenced by their ability to store large quantities of this vitamin while grazing green pasture. The major site of carotene and vit. A storage, according to Guilbert and Hart(1), is in the liver. It has been reported by Speilman *et al.*(2) that supplementation of dairy cow rations with high levels of vit. A during the last 30 days of gestation increased plasma levels and liver stores of vit. A in newborn calves. Fountain *et al.*(3) observed that calves from dairy cows maintained on green pasture during late gestation had significantly increased plasma vit. A levels at birth. Eaton and coworkers(4) reported that the vit. A content of the liver was significantly higher at birth and at 30 days of age in pigs and lambs whose dams received supplementary vit. A during late gestation. Henry and associates(5) have shown that the vit. A content of the milk of rats and the liver stores of the nursing offspring are influenced to a greater degree by the amount of vit. A in the diet of the mothers during lactation than by their liver reserves.

As a part of a long-time study of carotene metabolism in beef cattle, investigations have been made of the effect of level of carotene intake on liver stores of cows at parturition and of their newborn calves. Also included in the study were the effects of maternal liver stores and level of carotene intake on the vit. A content of the milk and on the blood and liver levels of the calves at 3 months of age.

Experimental. Ten 2-year-old Hereford cows in the second and third month of pregnancy were selected in March, 1952, for the experiment. During gestation, each of the cows was fed the following low-carotene ration daily: cottonseed meal, 2½ lb; ground milo, 1 lb; dried beet pulp, 2 lb; cottonseed hulls, 2 lb; and wheat straw or dry weathered range grass, *ad libitum*. During lactation, the low-carotene ration for each cow was composed of cottonseed meal, 3 lb; ground milo, 4 lb; and wheat straw, *ad libitum*. The cattle had access to a mineral mixture† and salt at all times. The basal ration was calculated to meet the recommended allowances of the National Research Council for all essential nutrients for beef cattle with the exception of carotene. During the experiment, the cows were maintained on concrete floors and had no access to carotene-containing feeds. At the beginning of the experiment, blood samples and liver biopsy samples were collected by the technic of Whitehair and associates(6). Assignment of the cows to the various treatments was based on liver stores of carotene and vit. A. To study the ability of cows to mobilize liver stores during lactation, high liver levels were desirable; consequently those animals with the highest liver stores were assigned Lots III and IV. The treatments and number of cows in each were: Lot I, no supplemental carotene, 2; Lot II, supplemental carotene during lactation, 3; Lot III, supplemental carotene during gestation, 3; Lot IV, supplemental carotene during gestation and lactation, 2. The levels of carotene administered were in accordance with the National Research Council's recommended daily allowances for beef cows of 60 μg of carotene/lb body weight during gestation and 330 μg/lb during lactation. A crude carotene concentrate‡ derived from alfalfa and containing 6 mg of carotene per g was administered in gelatin capsules to meet the specified allowances. The capsules were administered twice weekly during gestation and 3 times weekly during lactation. In addition to the liver and blood samples collected

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† The mineral mixture contained equal parts of ground limestone, steamed bone meal and salt.

‡ The carotene concentrate was supplied by the Chlorophyll Chemical Corp., McAllen, Texas.

TABLE I. Average Vit. A and Carotene Content of Livers of Beef Cows, and Vit. A Content of Livers of Their Calves (μg/g dry matter).

	Lot No.	6 mo prepartum	At parturition	3 mo post partum
		Cows		
Liver vit. A	I	68.9	5.7	1.7
	II	70.6	9.4	18.9
	III	122.6	20.6	7.4
	IV	92.2	14.3	23.4
" carotene	I	4.0	2.3	1.1
	II	4.7	2.7	3.7
	III	5.9	2.6	2.4
	IV	3.9	3.5	5.2
		Calves		
" vit. A	I		4.2	1.9
	II		3.4	14.2
	III		2.6	5.1
	IV		3.0	22.3

at the beginning of the experiment, liver samples were taken from the cows and their calves immediately after parturition and at the end of 3 months of lactation. Blood samples were collected from the cows approximately 3 months before parturition. Additional blood samples were taken from the cows and their calves immediately after parturition and at 2 weeks, one month, 2 months, and 3 months post partum. Colostrum samples were collected after parturition and milk samples were obtained each time a blood sample was taken from the cow during lactation. The calves were separated from the cows 4 hours before milk samples were collected. One quarter of each cow's udder was milked completely to obtain a representative milk sample. The blood, milk and liver samples were analyzed for carotene and vit. A. Liver samples were analyzed according to the method of Gallup and Hoefer(7), plasma by the method of Kimble(8), and milk by the method of Lesher *et al.*(9).

Results and discussion. The average liver levels of vit. A and carotene for the 4 lots of cows and calves are presented in Table I. Plasma and milk levels of vit. A and carotene for the cows and calves are shown in Table II. Negligible amounts of carotene were found in the milk, or in the blood and liver of the calves; consequently, the carotene contents of these samples are not given.

Examination of the liver composition of the cows at the beginning of the experimental period and at parturition shows that the amount of carotene administered to the cows of Lots III and IV was insufficient to maintain liver stores of vit. A at their initial level. Cows receiving carotene supplementation during gestation had about twice as much vit. A in their livers at parturition as those maintained on the depletion ration. The amount of carotene provided the cows of Lots II and IV during lactation resulted in an increase in liver stores of vit. A despite milk production.

TABLE II. Average Vit. A and Carotene Content of Plasma and Milk of Beef Cows and Vit. A Content of Plasma of Their Calves (μg/100 ml).

	Lot No.	6 mo prepartum	3 mo prepartum	At parturition	Lactation 2 wk	Lactation 1 mo	Lactation 2 mo	Lactation 3 mo
			Cows					
Plasma vit. A	I	24.9	8.3	11.3	11.1	10.1	14.2	13.3
	II	22.5	8.7	15.1	18.3	18.7	15.7	24.2
	III	22.3	10.1	16.3	20.5	14.6	15.0	15.1
	IV	21.1	14.2	16.3	17.7	21.4	21.8	23.5
" carotene	I	40.3	11.6	11.8	8.8	6.0	5.4	12.0
	II	45.8	12.9	10.3	52.6	34.5	31.2	43.0
	III	46.6	31.1	23.4	15.7	13.1	11.1	13.0
	IV	35.2	25.8	24.5	87.8	54.8	36.8	46.7
Milk and colostrum vit. A	I			45.6	4.9	4.1	3.3	2.8
	II			72.6	12.6	4.6	2.3	5.4
	III			163.9	4.4	2.5	3.6	3.2
	IV			90.6	7.7	4.0	7.7	4.5
			Calves					
Plasma vit. A	I			3.1	6.2	6.0	6.0	4.1
	II			1.3	9.3	5.7	8.1	9.7
	III			3.6	6.8	5.0	6.3	6.7
	IV			4.1	14.4	6.2	8.0	11.9

If maintenance of liver stores of vit. A is used as a criterion for determining the requirement of this nutrient, 60 μg per lb of body weight appears inadequate for the cow during gestation. However, no symptoms indicative of avitaminosis A were observed in any of the cows at any time during the experimental period. It is recognized that the stores of vit. A at the beginning of the experimental period were high, reflecting a high intake of carotene during the previous summer, and that failure to maintain them is not necessarily indicative of an inadequate carotene intake.

Liver stores of vit. A in newborn calves were not related to the carotene intake during gestation or liver stores of the cows at parturition. It should be noted that the amounts of carotene administered in these studies are distinctly less than those fed by Fountain *et al.* (3) or the vit. A fed by Speilman *et al.*(2) or by Eaton *et al.*(4). This may account for the failure to observe substantial placental transfer of vit. A. These findings are in agreement with the report of Thomas *et al.* (10) who found only small amounts of vit. A in the livers of newborn kids whose dams had been fed natural rations containing the recommended allowance of carotene for the goat. The vit. A content of the livers of the calves at 3 months of age appeared to be affected more by the carotene intake of the dam during lactation than by her liver stores at parturition. The higher liver stores of the cows are reflected in an increase in the liver vit. A of the calves at 3 months of age. Examination of the plasma vit. A levels of the calves reveals a similar trend. It will be noted that there was an actual decrease in the vit. A stores in the livers of calves of Lot I from birth to 3 months of age.

The plasma carotene levels of the cows reflected carotene supplementation, but in no case do the average values approach those commonly encountered when cattle are grazing winter range, or consuming good quality roughage(11). At the beginning of the experimental period, plasma vit. A content was normal. By mid-gestation, all animals showed a decline to levels usually considered indicative of a low plane of carotene or vit. A nutrition. Following parturition, the animals receiving supplemental carotene showed normal values for plasma vit. A, a finding consistent with the increase in liver vit. A stores in these animals.

The vit. A content of colostrum was somewhat higher for cows receiving supplemental carotene during gestation than from those that did not. Substantial amounts of this vitamin were found in the colostrum of cows receiving the low-carotene ration. Evidently the cow is able to mobilize her liver reserves to produce a colostrum relatively high in vit. A. As has been previously reported(12), the vit. A content of the milk rapidly declined and remained fairly constant during the second and third months of lactation. Milk vit. A was generally related to carotene supplementation during the lactation period; the effect of supplementation during gestation and liver vit. A stores at parturition was less apparent.

One calf in Lot I developed symptoms of a vit. A deficiency at about 3 months of age. After the termination of the experimental period, this calf responded to the administration of 480,000 I.U. of vit. A. None of the cows in this experiment showed symptoms of vit. A deficiency at any time.

Summary. Beef cows receiving a carotene allowance equivalent to 60 μg per pound of body weight daily were unable to maintain liver stores or plasma vit. A levels during the last 6 months of gestation. When the carotene allowance was increased to 333 μg during lactation, liver stores and plasma vit. A were increased. Vit. A in the plasma and liver of the calf was closely associated with the carotene intake of the dam during lactation, but was also influenced by the liver stores of the cow at parturition.

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Chromatographic Separation of the Plasma Lipids.* (20422)

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Although technics for the analysis of the various components of the plasma lipids are available, methods for their separation are neither simple nor quantitative. Bloor's method(1) of phospholipid separation has been the subject of considerable discussion (2-8) with general agreement that not all the phospholipid is precipitated by acetone-magnesium chloride(3,4,6,9,10) while significant amounts of other water soluble materials such as urea, creatinine, amino acids and Na, K, and Cl salts generally accompany it as impurities(11-13). Resolution in petroleum ether of the alcohol-ether extract may leave as much as 11.8% residue undissolved(13). The separation of the crude mixture into saponifiable and non-saponifiable fractions involves the destruction of both triglycerides and sterol ester fractions and thus precludes their separate analysis. Indeed, the separation and analysis of the sterol and sterol ester fractions has been the subject of considerable investigation, with the resultant disclosure of several procedures(14-21). The more successful of these methods have made use of the technic of chromatography. Trappe(20) investigated the use of alumina and later of silicic acid to separate glycerides from cholesterol esters, whereas Hess(14) used alumina for the separation of sterols and sterol esters. We were unable to repeat the work of Hess, who reported the elution of sterol ester with 10% ether in petroleum ether and of sterols with 10% alcohol in petroleum ether. In our hands both fractions appeared together in the eluate with the alcoholic mixture. Part of the difficulty may lie in the well-known activity of alumina in altering the adsorbed lipid, for example, by hydrolysis of fatty esters(2,20). Silicic acid seemed most suitable for the separation since it is not known to alter the adsorbed materials and shows no reaction with the solvents. Borgstrom(2) used this material to separate phospholipids or cholesterol palmitate from a mixture of stearic acid and glycerides. The last 2 substances could then be separated by extraction of their alkaline alcohol solution with petroleum ether. However, when mono- or diglycerides were present they were only partly extracted and it was necessary to use an ion exchange resin (IRA-400), to effect the separation.

With these considerations in mind, the complete separation of the plasma lipids was attempted with only one column and alterations in the eluting mixture. Regular changes in the eluant were not attempted since it was thought that some advantage could be gained by continuing the elution with a particular solvent mixture until little or no material appeared in the eluate. In this manner, sub-

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