TRANSFER OF VITAMIN A FROM BOVINE LIVER TO MILK$^{1,2,3}$


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Summary

The contributions of liver stores and the diet to the vitamin A contents of colostrum and milk were studied in eight mature Angus cows with previously established stores of tritium-labeled vitamin A. Samples of liver, serum and milk were taken within 2 hr. after parturition and at intervals during the first 12 weeks of lactation. Total vitamin A and alcohol and ester forms of vitamin A were determined on these samples as well as the radioactivity associated with each fraction. Average results indicated that liver stores furnished 36% of the vitamin A in colostrum and 55% of the vitamin A in milk. Most of the vitamin A activity and radioactivity in liver, colostrum and milk was present in the ester fraction. In serum, about 70% of the vitamin A activity and all of the radioactivity appeared in the alcohol fraction. Specific activities for vitamin A in colostrum and milk were consistently higher than for serum. The results indicate that vitamin A is removed from the liver as the alcohol and that the mammary gland is capable of transferring vitamin A alcohol from blood to milk in preference to vitamin A ester.

Introduction

Newborn calves (Guilbert and Hart, 1935), like all newborn mammals which have been studied (Moore, 1971), typically have quite limited stores of vitamin A. Consequently, they are heavily dependent on colostrum for initial protection against vitamin A deficiency and milk for continued maintenance of adequate vitamin A status until this role is assumed by other feedstuffs containing adequate vitamin A or carotenoids to meet requirements. The influence of the diet on carotene and vitamin A levels in both colostrum and normal milk has been well documented (Kramer et al., 1938; Thomas, Spielman and Turk, 1946; Wise et al., 1947; Spielman et al., 1947). The contribution of liver stores has received relatively little attention. Branstetter et al. (1973) estimated that an average of 52% of the vitamin A appearing in the colostrum and milk collected from Angus cows during the first 15 weeks of lactation originated in the liver. Isotope data suggested that the bovine mammary gland was taking up labeled vitamin A from the liver more efficiently than unlabeled vitamin A from the diet. The experiment reported here was conducted to verify these observations on the contribution of liver stores to milk and to further evaluate the possibility that this contribution is mediated by a selective mammary transport mechanism.

Experimental Procedure

Eight mature Angus cows were used in the experiment. Stores of radioactive vitamin A were established by intravenous injections of tritium-labeled vitamin A acetate. At parturition, biopsy samples of liver contained $225\pm17$ (SE) mcg of vitamin A per gram with a specific activity of $119\pm11$ disintegrations per minute per microgram of vitamin A (DPM/mcg). Corresponding values during the first 12 weeks of lactation were $211\pm19$ and $99\pm8$, respectively. Each cow was fed daily 9.08 kg.
alfalfa-grass, hay, s-c, 40% grass, (1) #1-08-744 and 0.45kg corn, grain extn. unspecified grind, (4) #4-02-857 containing 50,000 IU of added vitamin A palmitate.

Samples of blood, liver and milk were taken within 2 hr. after parturition, 5 to 21 days after parturition, and at four subsequent 3-week intervals. All cows were sampled on the same days, introducing some variation in the time of the second sample after parturition due to variable calving dates. Sampling of three cows was terminated before the final period because of low milk production. Liver was obtained by aspiration biopsy (Erwin et al., 1956), blood by jugular puncture and milk by hand milking one quarter while the calf nursed the other three.

Vitamin A was extracted from serum by the method of Kimble (1939). Milk was homogenized by sonification, then handled similarly as was blood. Liver was extracted as described by Ames, Risley and Harris (1954). Vitamin A was determined by the trifluoroacetic acid method (Dugan, Frigui and Seibert, 1964), alcohol and ester separations by column chromatography on alumina (Thompson, Ganguly and Kon, 1949) and radioactivity by liquid scintillation counting (Mitchell et al., 1967). Statistical analysis of variance was conducted according to Snedecor and Cochran (1967).

Results and Discussion

If one assumes steady state conditions and proportionate withdrawal of labeled vitamin A from the liver, the critical values for estimating the contribution of liver stores to the vitamin A in milk and evaluating the inference by Branstetter et al. (1973) of selective transfer of vitamin A from the liver to the mammary gland are the relative specific activities of a vitamin A in the various compartments. Total vitamin A stores did not vary significantly, and the labeled stores were established far enough in advance to eliminate transient effects associated with their establishment, supporting steady state assumptions. Specific activities for liver vitamin A declined moderately as would be expected from previous work with cows (Branstetter et al., 1973), steers (Hayes, Mitchell and Little, 1967), rams (Boling et al., 1969), and wethers (Mitchell et al., 1967). Uniform isotope removal can only be assumed but was supported by a gradually declining specific activity pattern in blood similar to that in liver.

Comparison of the mean specific activity of vitamin A in liver at parturition (119 ± 11 DPM/mcg) with that for colostrum (56 ± 7 DPM/mcg) yields an estimate that 46% of the colostral vitamin A was derived from liver stores. Similar calculations indicate that 40% of the vitamin A in milk came from liver stores. Considering the small number of animals used for previous estimates and expected biological and analytical variation, these values are in reasonable agreement with the estimates by Branstetter et al. (1973) that 36% of colostral vitamin A came from liver while the liver furnished 55% of the vitamin A in subsequent milk came from liver. Contributions of this magnitude when dietary intake (in both experiments) was more than twice N.R.C. (1970) recommendations, furnish strong evidence of a major role for liver stores in determining the amount of vitamin A in milk.

Substantial evidence for preferential transfer of vitamin A by the mammary gland from liver stores in preference to dietary vitamin A is presented in table 1. Without such preferential transfer, specific activities of vitamin A in serum and milk should be similar because milk vitamin A is derived primarily from vitamin A in the blood. Any contribution of non-radioactive precursors would only tend to lower the specific activity in milk. However, specific activities of vitamin A in colostrum and within animal means for milk were higher than the corresponding values for serum in 15 of 16 comparisons (P < .05). Mean increases in specific activity were 70% for colostrum and 23% for subsequent milk. Although the magnitude of concentration of radioactivity was greater for colostrum and less for milk than observed previously, this confirms the observations by Branstetter et al. (1973) that the
mammary gland is capable of transferring vitamin A from liver stores in preference to dietary vitamin A.

Differential handling of vitamin A alcohol and vitamin A esters provides one possibility for explaining the preferential transfer of liver stores to milk. Regardless of form of administration, dietary vitamin A appears in the blood almost entirely as the palmitate ester (Clausen, 1943; Eden and Sellers, 1950; Krinsky, Cornwell and Oncley, 1958). Vitamin A is mobilized from the liver to blood primarily in the alcohol form (Hoch, 1946; Moore, 1957; Ganguly, 1960; McGillivray, 1961). Thus preferential transfer could be accomplished by a transport system which favors the alcohol over the ester. The discrimination could involve direct reaction with the vitamin A molecule or could be mediated by the carrier complex, which is apparently different for vitamin A alcohol than for vitamin A esters (Goodman, 1969; Krinski et al., 1958; Peterson, 1971; Roels, 1966). Chromatographic separation of vitamin A alcohol from its esters was used to obtain evidence for use in evaluating this hypothesis.

Results of total vitamin A determinations and of separations of vitamin A alcohol and vitamin A esters are summarized in table 2. Total vitamin A in the livers was somewhat variable but was at consistently high levels and did not change significantly during the experiment. Serum total vitamin A values were quite uniform, showing a depression of about 20% at parturition based on previous measurements followed by stable values in normal ranges during the lactation phase. This lower serum vitamin A at parturition is consistent with previous reports (Baker, Pope and MacVicar, 1953; Kendall and Harshbarger, 1953; Moore, 1957). As expected, colostrum contained more vitamin A than milk (Dann, 1933; Chanda, 1953; Moore, 1957) although the vitamin A content was highly variable (Moore, 1957; Voightlander, 1970). The milk contained less vitamin A than reported by some other workers (Lichvar, 1969; Voightlander, 1970) but the amount was in close agreement with previous observations under similar conditions (Branstetter et al., 1973).

Although not true in every cow, specific activity of serum vitamin A tended to decline during the experiment. This decline is consistent with a concept of continuous turnover of radioactive liver stores (Mitchell et al., 1967; Hayes et al., 1967) and loss of labeled vitamin A via the milk and other excretory pathways and agrees with the results of Branstetter et al., 1973). They reported increasing specific activities in milk during early lactation, while a decline similar to that in serum was observed in the present study.

Data for the separation of the alcohol and ester forms of vitamin A are expressed as percentages of the total vitamin A recovered from the column. Recoveries averaged 89.4% for liver extracts, 94.7% for serum and 93.2% for milk. The observed proportions of vitamin A alcohol and vitamin A ester were similar to literature values in most cases. The predominance of vitamin A ester in the liver has been generally accepted (Moore, 1957; Ganguly, 1960) with quantitative estimates mainly above 90% as observed consistently in this study. However, Sewell et al. (1967) found only 62% of the liver vitamin A of rats in the ester form, and High and Wilson (1956) reported percentages from 68 to 91 for rats. Division of

| TABLE 2. PARTITIONING OF VITAMIN A AND RADIOACTIVITY BETWEEN ALCOHOL AND ESTER |
|-----------------|-----------------|-----------------|
|                 | Vitamin A        | Radioactivity    |
|                 | Totala           | % alcohol        | % ester        | % alcohol | % ester |
| At parturitionb |                 |                  |                |
| Liver           | 225 ± 17         | 8.6 ± 1.8        | 91.4 ± 1.8     | 14.0 ± 2.5| 86.0 ± 2.5|
| Serum           | 28 ± 2           | 70.3 ± 4.0       | 29.7 ± 4.0     | 100.0 ± 0.0| 0.0 ± 0.0 |
| Colostrum       | 261 ± 39         | 2.8 ± 0.3        | 97.2 ± 0.3     | 1.7 ± 0.4 | 98.3 ± 0.4|
| During lactationc |                |                  |                |
| Liver           | 211 ± 19         | 7.7 ± 0.4        | 93.3 ± 0.4     | 9.7 ± 0.7 | 90.3 ± 0.7|
| Serum           | 34 ± 1           | 69.1 ± 0.9       | 30.9 ± 0.9     | 100.0 ± 0.0| 0.0 ± 0.0 |
| Milk            | 28 ± 2           | 26.5 ± 1.2       | 73.5 ± 1.2     | 6.1 ± 1.5 | 93.9 ± 1.5|

aMicrograms per gram of liver, mg per 100 ml of serum or milk.
bMeans for eight cows ± standard errors.
cMeans for eight cows ± standard errors (five observations each for five cows, four observations each for three cows).
radioactivity between alcohol and ester in the liver was in close agreement with the vitamin A activity in these fractions.

Vitamin A alcohol concentrations in blood apparently remain stable except in times of stress (Ganguly and Krinsky, 1953; High and Wilson, 1956), while the level of vitamin A ester in blood depends mostly on level of dietary vitamin A (Clausen, 1943; Eden and Sellers, 1950). Throughout the present experiment, about 70% of the total vitamin A activity was found in the alcohol form with the remaining 30% in the ester fraction. With vitamin A intake constant, this result is consistent with present theory. The separation of radioactivity between the two fractions in serum was quite dramatic. The radioactivity in the serum appeared only in the alcohol fraction. This shows clearly that two separate pools of vitamin A activity exist in the blood. Since the only source of radioactivity was in the liver, the radioactivity of the serum vitamin A identifies it with the liver stores. The higher specific activity of the vitamin A in colostrum and milk (table 1) compared with that in serum could be readily explained by preferential transfer from this pool to the colostrum and milk.

Another explanation of higher specific activity in milk than in serum might be a time lag between uptake by the mammary gland and secretion in the milk. With declining specific activity in serum, milk vitamin A secreted at a given time would be derived from serum vitamin A with a higher specific activity than that at the time the milk was secreted. However, a very large time lag would be required. It seems highly improbable that this effect could account for differences as large as those observed. Half-times for radioactive vitamin A in the liver during lactation was estimated to be 300 days for the study of Branstetter et al. (1973) and 248 days for the present study. Both are quite slow in relation to probable residual time in the mammary gland. Mean values at parturition (33DPM/mcg of vitamin A) and at the final lactation sample (27DPM/mcg) showed the expected decline but were not significantly different.

Only a small fraction of the vitamin A in colostrum and milk has been reported to be in the alcohol form (Ganguly, Kon and Thompson, 1947; Parrish, Wise and Hughes, 1947; Chanda, 1953). This agrees with the result obtained for colostrum (2.8% alcohol and 97.2% ester) but not that obtained for milk (26.5% alcohol and 73.5% ester). Although rather consistent (about 5% SE), the finding of this high level of vitamin A alcohol in milk is suspect because the vitamin A assay was operating near its maximum sensitivity and the recovery of radioactivity in this fraction from milk was less than one-fourth the apparent recovery of vitamin A. This is particularly true since all the serum radioactivity was associated with vitamin A alcohol but only 6.1% of the milk radioactivity. In addition, the specific activity of the alcohol (23 ± 7DPM/mcg) was considerably lower than the specific activity of the ester (37 ± 5DPM/mcg) or that of the vitamin A in the whole milk extract before chromatographic separation (39 ± 2DPM/mcg). Overestimation of the vitamin A alcohol would reduce its apparent specific activity.

The data obtained from this experiment are consistent with generally held concepts that dietary vitamin A is transported in the blood in ester form, storage in the liver is mainly in ester form, circulating vitamin A withdrawn from stores is mainly in the alcohol form. We conclude further that the mammary gland is capable of preferential uptake of the alcohol from blood. Much of it is apparently then esterified and secreted in milk. The mechanism involved, the physiological significance of preferential uptake and possible effects of dietary, physiological or other variables or preference are yet to be elucidated. Preferential uptake by other tissues utilizing vitamin A is also considered an interesting possibility.

Although the data are inadequate for conclusive results, the extent of preferential uptake can be estimated from the observed specific activities and the ratio of vitamin A alcohol to vitamin A ester in the blood. Since the specific activity of the vitamin A alcohol in serum during lactation averaged 48DPM/mcg while that of milk vitamin A averaged 39DPM/mcg, it can be calculated that 81% (39 ÷ 48) of the vitamin A in the milk was derived from vitamin A alcohol while only 69% of the vitamin A in the blood was in the alcohol form. A similar calculation for colostrum (56 ÷ 52) suggests that all of its vitamin A came from labeled vitamin A alcohol in the blood. Branstetter et al. (1973) did not separate the alcohol and ester forms of vitamin A. However, if it is assumed that the proportion of vitamin A in alcohol form in the blood was the same as in the present study and that all the serum radioactivity was in this form, similar calculations based on their data indicate that all the vitamin A in both colostrum and milk came from vitamin A alcohol in blood. While these calculations are obviously imprecise, they do suggest that the potential for the mammary
gland to preferentially transfer vitamin A alcohol is substantial.

Literature Cited
Clausen, S. W. 1943. The absorption of vitamin A and its storage in the tissues. Harvey Lectures Ser. 38:199.