glucosinolates in the leafy portion are 3-indolylmethyl-GS's. Because the leafy part averages 94% of the entire cabbage head, the 3-indolylmethyl-GS's make the major contribution of glucosinolates in cabbage.

Certain insects such as *Pieris rapae* and *Hylemya brassicae* (Bouche) display a parasite–host relationship with *Brassica* (Thorsteinson, 1960). The glucosinolates and/or their hydrolytic products are involved in attracting the adult female and in her oviposition. These compounds also act as feeding stimulants for the larvae (Nair and McEwen, 1976; Blau et al., 1978). A knowledge of the variation in the amount and the kind of glucosinolates with respect to the parts of the cabbage plant may require a reevaluation of the relationship of host to parasite.

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25-Hydroxycholecalciferol in Cow Milk as Determined by High-Performance Liquid Chromatography

A high-performance liquid chromatographic (LC) procedure was developed for the determination of 25-hydroxycholecalciferol (25-OH-D_3) in cow milk. The procedure involved extraction with an ethanol–ether mixture, a set of solvent partitions, adsorption chromatography on silica gel, a partition chromatography on diatomaceous earth support, and final determination by reversed-phase LC on a C_{18} bonded microparticulate silica column using a 254-nm fixed wavelength detector. The method was quantitative at the 10-ppb level and capable of detection at the 2–3-ppb level. The method was applicable to milk and colostrum. The endogenous level of 25-OH-D_3 in the milk was below the detection limit. It was very significant that, even in cows whose serum concentrations were elevated fivefold after treatment with 25-OH-D_3, the concentration in the milk was below the detection level.

The metabolite of cholecalciferol (vitamin D_3), 25-hydroxycholecalciferol (25-OH-D_3) was found useful in reducing the incidence of parturient paresis in cows (Bringe et al., 1971; Olson et al., 1973). We have reported procedures for the determination of 25-OH-D_3 in cow plasma (Koshy and VanDerSlik, 1976, 1978) and in cow liver, kidney, and muscle (Koshy and VanDerSlik, 1977). Since it was known to us that the serum level of 25-OH-D_3 was elevated after oral, intramuscular, or intravenous administration of 25-OH-D_3, it was of great importance to know if there was any corresponding increase in the concentration of 25-OH-D_3 in the milk. The present study was undertaken to determine the concentration of 25-OH-D_3 in the milk and colostrum of treated and untreated dairy cattle.

EXPERIMENTAL SECTION

Sample. A 100-g sample was used for each analysis. If the sample was to be stored for any length of time, it was weighed into individual plastic containers and frozen.

Solvents. All solvents except 3A alcohol and anhydrous ethyl ether were distilled in glass (Burdick and Jackson, Inc., Muskegon, MI).

Extraction. The thawed sample was transferred to a

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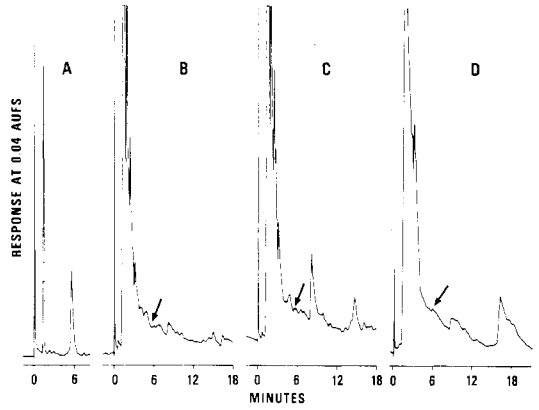

Figure 1. Liquid chromatograms of (A) 25-OH-D_3 standard (10 ng/μL, 50 ng on column) and unfortified milk extracts from (B) Holstein, (C) Jersey, and (D) Guernsey cows.

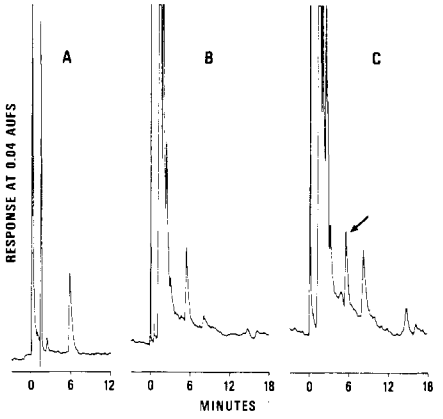

Figure 2. Liquid chromatograms showing recovery of 25-OH-D_3 from milk fortified at 10 ppb; (A) standard (10 ng/μL, 50 ng on column), (B) Holstein, (C) Jersey.

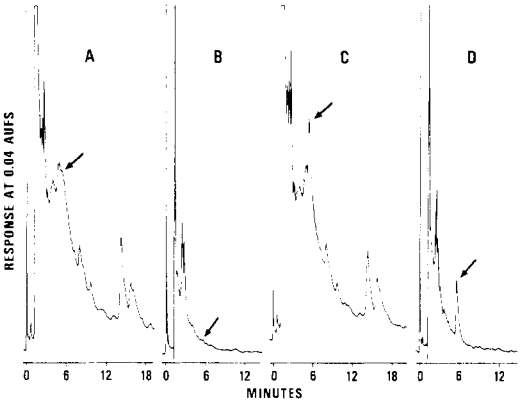

Figure 3. Liquid chromatograms showing the effectiveness of the Celite partition column in removing interferences from milk extracts; (A) unfortified Guernsey milk, (B) A after Celite column, (C) fortified Guernsey milk (10 ppb), (D) C after Celite column.

1-L mixing cylinder to which 0.5 g of $NaHCO_3$ and 0.25 g of sodium ascorbate and 200 mL of 3A alcohol (or 95% alcohol) were added. The contents were mixed thoroughly to precipitate the proteins and then further shaken vigorously with the 300 mL of anhydrous ethyl ether AR. The precipitates were allowed to settle. The supernatant extract was poured into a 1-L separatory funnel and gently shaken with 100 mL of water. The phases were allowed to separate, and the lower aqueous portion was discarded. The solid residue in the mixing cylinder was reextracted with 100 mL of ethanol and 200 mL of ethyl ether and allowed to settle. The supernatant was added to the separatory funnel and the combined extract was washed with 100 mL of saturated NaCl solution to remove most of the residual water. The extract was filtered through a wad of cotton into a 1-L round-bottom flask and rotary evaporated under vacuum to about 25–30 mL of a milky residue in a water bath at 40 °C.

Solvent Partition. One hundred milliliters of 5% $NaHCO_3$ solution was added to the concentrated extract and the mixture was transferred to a 250-mL separatory funnel. It was extracted with 4 × 50 mL of methylene chloride using each portion to first rinse the flask. The extracts were combined in a 500-mL round-bottom flask and evaporated just to dryness under vacuum. The residue was immediately dissolved in 100 mL of hexane, transferred to a 250-mL separatory funnel, and extracted with 4 × 50 mL of acetonitrile using each portion to rinse the round-bottom flask (the hexane and acetonitrile were mutually saturated with each other prior to use). The acetonitrile extracts were combined in a 500-mL round-bottom flask and evaporated just to dryness under vacuum in a water bath at about 40 °C.

Silica Gel Column Chromatography. The residue above was subjected to chromatography on 10 g of silica gel 60 (E. M. Reagents 70-230 ASTM) as described in our procedure for cow plasma (Koshy and VanDerSlik, 1976).

Celite Partition Chromatography (Optional). When milk and colostrum samples from different geographical locations in the United States were analyzed after the silica gel chromatographic step, there were some that showed interference on the liquid chromatograms. These samples were subjected to an additional partition column chromatography on Celite 545 as described (Koshy and VanDerSlik, 1978). In addition to removing the interference, the use of this column assured removal of any 25-hydroxyergocalciferol that could be present in the milk from the ergocalciferol (vitamin D_2) in the cow diet.

High-Performance Liquid Chromatography. The LC system was as described in our procedures for cow tissues (Koshy and VanDerSlik, 1977).

RESULTS

Figure 1 shows chromatograms of unfortified whole milk extracts from Holstein, Jersey, and Guernsey breed of cows, all showing no detectable levels of 25-OH-D_3. The peak height response of the standard (Figure 1A) was equivalent to 10 ppb in the milk. A number of milk samples from different geographical locations in the United States were analyzed and all showed nondetectable levels of 25-OH-D_3. Figure 2 shows chromatograms of milk from Holstein and Jersey cows that were fortified at the level of 10 ppb and carried through the procedure. Recovery of 25-OH-D_3 from milk from Guernsey and Holstein cows fortified at 10 ppb were 100.6 ± 4.5% (n = 7, range 97–109%) and 96.3 ± 4.1% (n = 7, range 91–104%), respectively. Milk samples from a Guernsey cow that were fortified at 10 ppb and frozen for 7 months were analyzed. The recovery was 90.8 ± 6.9% (n = 6, range 82–103%), indicating good stability in the frozen state. All the above samples were analyzed by LC after the silica gel column chromatographic step. Figure 3B,D shows the effectiveness of the Celite 545 partition column chromatography as an additional clean up step for samples that showed interference after the silica gel column step (Figure 3A,C). This column was also very useful for colostrum.

A number of milk samples from lactating cows that were treated with 25-OH-D_3 was analyzed. Milk from (a) two cows that had received single 8-mg oral doses, (b) four cows that had received single 8-mg intravenous doses, and (c) four cows that had received three 8-mg intramuscular doses

at weekly intervals were analyzed. The pretreatment serum levels of 25-OH-D_3 in these cows were in the 40–50-ppb range. The serum level in the oral group reached a peak of 90 ppb after 2 days. In the intravenous group, the serum level was elevated to 240 ppb immediately and had not returned to the endogenous level by the 51st day after treatment. In the four intramuscularly treated cows, the serum was elevated to 185 ppb on the 19th day and was 82 ppb on the 51st day after the first injection. The milk from all these cows showed nondetectable levels of 25-OH-D_3, i.e., less than 2 ppb.

The above LC findings were confirmed from a tissue distribution and excretion study conducted on a Jersey cow that had received a single intramuscular injection of 4.85 mg of [26,27-^{14}C]-25-OH-D_3 (sp act., 33.3 dpm/ng). Gross radioactivity in the serum after parturition (induced 110 h postinjection) was 58 ppb. However, the total radioactivity in the colostrum was only 0.62 ppb which was less than the detectable limit by LC.

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