Phenobarbital Metabolism in the Lactating Dairy Cow

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Abstract

Two Holstein cows were used to study the metabolism and excretion of phenobarbital in the lactating cow. Each cow received 6 g of sodium phenobarbital, intrarumenally, daily for 10 days. On the 10th day carbon 14 phenobarbital was mixed with the unlabeled material prior to dosing. Total milk, urine, and feces were collected for 6 days, and about 93% of the recovered radioactivity was in urine, 5% in milk, and 2% in feces. The biological half-lives of phenobarbital for milk, urine, and feces were 40.5, 29.5, and 46.5 h. From these values we calculated that 7 days after the last 6-g dose of phenobarbital the milk produced would contain less than .5 ppm of this compound.

A second study determined how long after withdrawal from phenobarbital feeding the drug could be detected in milk. Three Holstein cows received 6 g of sodium phenobarbital, intrarumenally, daily for 10 days. Starting on the 11th day milk samples were collected at each milking and analyzed for phenobarbital by gas liquid chromatography and spectrophotometrically. After 7 days withdrawal, phenobarbital could not be detected in milk by either method.

Introduction

Contamination of cattle with chlorinated hydrocarbons such as dieldrin, heptachlor, and DDT presents a problem to livestock producers. Since these chemicals are fat soluble, they are concentrated in the fat depots resulting in slow elimination from the body. Milk, because of its fat content, is a major route of elimination in lactating animals.

Numerous studies have sought ways to accelerate removal of pesticides from tissues of contaminated animals (1, 5, 6, 8, 11). The recommended practice is to identify and eliminate the source of contamination and to feed activated charcoal and phenobarbital to decontaminate the animal (3). Activated charcoal binds with the pesticide in the gut, thereby reducing the resorption process. Phenobarbital acts by stimulating the liver to produce enzymes which degrade the pesticides (2, 9). The charcoal is eliminated through the feces while the phenobarbital is absorbed from the gut and eliminated via the urine, feces, and milk. Little is known about the metabolism and excretion of barbituates in the lactating animal. Dairymen are interested in knowing how soon

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after withdrawal from the feeding of phenobarbital the milk will be free of the drug. Therefore, our study was of metabolism and excretion of phenobarbital by the dairy cow.

**Experimental Procedure**

**Trial 1.** Two Holstein cows were given 6 g of sodium phenobarbital in 200 ml of water daily, as a single dose into the tureen, for 10 days. This daily dosage (10 mg/kg body weight) was based on the recommendation by Cook and Wilson (3). On the 10th day, $^{14}$C-phenobarbital (5-ethyl-5-phenobarbituric-2-$^{14}$C acid, 3.15 Mc/mM) in 10 ml of absolute ethanol was mixed with the unlabeled sodium phenobarbital prior to dosing. Cow A and cow B received 311.5 uCi and 229.8 uCi of $^{14}$C-phenobarbital, respectively. Total milk, urine, and feces were collected and sampled for 6 days following administration of the radioactive dose. Urine was collected via indwelling bladder catheters as described by Crutchfield (4).

Phenobarbital was extracted from milk by shaking 10 ml of milk, which had been acidified with 1 ml of concentrated HCl, with 50 ml of chloroform. The chloroform extract was dried under reduced pressure at 50 C. The residue was dissolved in 2 ml of petroleum ether (bp 40 to 60) and transferred to a scintillation vial. The flask was rinsed twice more with 2 ml of petroleum ether, and the washes were combined and dried under a stream of N$_2$. The sample for TLC was prepared by extracting the radioactive component(s) from the urine. Following the procedure of Sunshine (10) only 33% of the total radioactivity was recovered within 144 h after administration.

**Trial 2.** Three Holstein cows were given 6 g of sodium phenobarbital in 200 ml of water daily, as a single dose into the rumen, for 10 days. On day 11 milk samples were collected at each milking for 1 wk and analyzed for phenobarbital by gas liquid chromatography (GLC) and ultraviolet spectrophotometry. Phenobarbital was extracted from the milk as described in Trial 1, and after concentration of the chloroform extract an aliquot was analyzed by GLC. The instrument was an Aerograph Hi-fi (model 600) equipped with a hydrogen flame ionization detector. A stainless steel column (2 mm ID by 90 cm) packed with 3% Poly I-110 on Chromosorb Q was used to analyze for phenobarbital. A quantification curve was prepared with pure phenobarbital from Applied Sciences Laboratory, College Park, Pennsylvania. Ultraviolet spectrophotometry was as described by Goldbaum (7).

**Results and Discussion**

Results of the first trial show that the main route of elimination of phenobarbital from the body of the dairy cow is via urine (Table 1). About 93% of the recovered radioactivity was in the urine while milk and feces contained approximately 5 and 2%. A majority (68 to 92%) of radioactivity was recovered within 144 h after administration.

We determined the chemical form of the radioactive compounds in the milk, urine, feces, and rumen fluid by thin-layer chromatography. In preparing the samples for TLC, a great deal of difficulty was experienced in extracting the radioactive component(s) from the urine. Following the procedure of Sunshine (10) only 33% of the total radioactivity in

<table>
<thead>
<tr>
<th>Cow no.</th>
<th>Recovery of dose*</th>
<th>Distribution of radioactivity</th>
<th>Biological half-life**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Milk (%)</td>
<td>Urine (%)</td>
<td>Feces</td>
</tr>
<tr>
<td>A</td>
<td>68.4</td>
<td>5.6</td>
<td>93.1</td>
</tr>
<tr>
<td>B</td>
<td>92.2</td>
<td>3.4</td>
<td>94.1</td>
</tr>
</tbody>
</table>

*After 144 h postdosing.
**See text for calculations.
Table 2. Excretion of phenobarbital in milk.

<table>
<thead>
<tr>
<th>Sampling time (h after last dose)</th>
<th>Phenobarbital concentration (PPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>3.7 ± .8**</td>
</tr>
<tr>
<td>60</td>
<td>1.7 ± .8</td>
</tr>
<tr>
<td>168 (1 wk)</td>
<td>N.D.*</td>
</tr>
</tbody>
</table>

*Not detectable by gas liquid chromatography or spectrophotometric determinations.

**Standard error of mean.

urine could be extracted. Attempts to render the radioactive components soluble in chloroform via treatment with strong acid (5 volumes 10 N H₂SO₄ at 100 °C for 10 h) and base (.4 volume 25% KOH at 100 °C for 2 h) failed. Of the radioactivity extracted from urine, primarily all migrated on a TLC plate with phenobarbital. Recoveries of radioactivity from milk, feces, and rumen fluid were high (> 90%) and TLC of the extracts showed that the major component was phenobarbital or a compound which had an Rₜ similar to phenobarbital by the solvent systems of Sunshine (10).

Excretion rates of phenobarbital through milk, urine, and feces were estimated from the differential equation dx/dt = -ax, where x is radioactivity, t is time, and a is a constant which is the reciprocal of turnover time. From such plots, the biological half-life of phenobarbital was about 40, 29.5, and 46.5 h in milk, urine, and feces (Table 1). The relatively high correlation coefficients of the plots indicated a high degree of linear association between the samples.

A second trial was to determine how long phenobarbital could be detected in milk by analytical techniques once it had been withdrawn from the diet of the cow. Forty-eight hours after the last dose, phenobarbital concentration in milk was 3.7 ppm and declined to 1.7 ppm at 60 h (Table 2). After 1 wk post-withdrawal, phenobarbital could not be detected in milk by either GLC or spectrophotometry. The lowest concentrations of phenobarbital detectable by spectrophotometry and GLC were .5 ppm and .1 ppm, respectively.

In summary, phenobarbital is eliminated from the body of the cow mainly through urine. Following a radioactive dose of phenobarbital, less than 5% of the recovered radioactivity was associated with the milk produced. After a 1-wk withdrawal, phenobarbital could not be detected in the milk by GLC or spectrophotometry.

References


