SECRETION OF SULFONAMIDES IN MILK FOLLOWING INTRAMAMMARY ORAL AND PARENTERAL ADMINISTRATIONS

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SUMMARY

Commercial preparations of sulfonamides were administered to lactating dairy cows by one or more routes to determine the duration of their secretion in milk. Neoprontosil injected intramuscularly at the rate of 240 gr/cow was not detected in milk during the first 24-hr post-injection. Intravenous injection of sulfabrom (0.25 gr/lb of body wt), suljex (772 gr/cow), and sulfamerazine (0.5 and 1.0 gr/lb of body wt) resulted in detectable levels of drug residues for maximum periods of 21, 38, 54, and 54 hr, respectively. After intrauterine infusion of sulfaura (77 gr of sulfonamides), the drug was detected in milk a maximum of 45 hr. Orally administered sulfonamides, dose in grains/pound body weight, and the maximum period the drugs were detected in milk were: (a) sulfanilamide, 1.0 gr, 86 hr; (b) sulfadiazine, 0.5 gr, 62 hr; and (c) sulfamerazine, 0.5 gr, 60 hr.

Following intramammary infusion of suljex at the rate of 77 gr/quarter, the drug was detected in milk a maximum of 7 hr. Chromatographic determinations indicated that the concentration of sulfamerazine (from suljex) in milk was 4.86 times greater than that of sulfapyridine. After infusion of sulfamerazine as a single drug at the rate of 80 gr/quarter, it was detected in milk for a maximum of 24 hr.

Sulfonamide preparations are used extensively in the treatment of bacterial diseases of dairy cattle. Results of a few studies of individual sulfonamides show that they are secreted in milk following oral (1, 3, 5, 6) and intravenous (5) administration to lactating cows; yet, the literature on sulfonamide secretion in milk reveals that many facets of the problem have not been investigated adequately, including intramuscular injection. For this reason several commercial preparations of sulfonamides were tested to determine the duration of their secretion in milk following administration.

EXPERIMENTAL PROCEDURE

Seven commercial preparations of sulfonamides were administered to lactating dairy cows (Table 1). Milk production levels were in the range of 10 to 50 lb per cow daily. During oral and parenteral investigations, an aliquot of the total milk yield at each milking was sampled for analysis. For the intramammary infusion studies, only one quarter of the udder was infused and an aliquot of the total milk from each quarter sampled.

The commonly employed Bratton and Mar- shall reagent, as used by Silverman and Kosi-kowski (7) for determination of sulfonamides, did not consistently yield reproducible results in our laboratory. Hence, the following method that has a higher degree of reproducibility was employed.

A standard milk solution of the sulfonamide was prepared by accurately weighing 0.1 g of the desired drug and dissolving in a minimum amount of distilled water. A drop of concentrated NH₄OH was added for sulfonamides of low solubility. The solution was then diluted to 500 ml with homogenized milk. The concentration of the drug in the standard solution was 10 ppm.

To 20-ml aliquots of milk samples and standards was added 6 ml of 10% aqueous trichloroacetic acid. The mixtures were allowed to stand for 15 min, then centrifuged for 10 min at approximately 2,000 rpm. Following centrifugation, the samples were filtered through Whatman No. 1 paper. To a 5-ml aliquot of filtrate, 5 ml of the color reagent was added. The color reagent was prepared by adding 0.5 g p-di-methylaminobenzaldehyde, 0.3 g citric acid, and 0.5 ml concentrated HCl in 100 ml of 50% ethanol. Following addition of the color reagent, the sample was allowed to stand for 5 min, filtered through Whatman No. 42 paper, and the optical density of the filtrate read at 470 mµ with a Beckman DU spectrophotometer.
### TABLE 1
Mean, maximum, and minimum duration of secretion of sulfonamides in milk following different routes of administration

<table>
<thead>
<tr>
<th>Sulfonamide preparation</th>
<th>Method of administration</th>
<th>Dose</th>
<th>Replications</th>
<th>Duration (hr)</th>
<th>Mean</th>
<th>Maximum</th>
<th>Minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoprontosil</td>
<td>Intramuscular</td>
<td>240 gr/cow</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sulfabrom</td>
<td>Intravenous</td>
<td>0.25 gr/lb wt</td>
<td>12</td>
<td>10</td>
<td>21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Suljex</td>
<td>Intravenous</td>
<td>772 gr/cow</td>
<td>4</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
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<tr>
<td>Sulfamerazine</td>
<td>Intravenous</td>
<td>1.0 gr/lb wt</td>
<td>6</td>
<td>42</td>
<td>54</td>
<td>30</td>
<td>30</td>
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<tr>
<td>Sulfamerazine</td>
<td>Intravenous</td>
<td>0.5 gr/lb wt</td>
<td>6</td>
<td>44</td>
<td>54</td>
<td>30</td>
<td>30</td>
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<tr>
<td>Sulfanurea</td>
<td>Intraterine</td>
<td>76 gr/cow</td>
<td>13</td>
<td>23</td>
<td>45</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Sulfanilamide</td>
<td>Oral</td>
<td>1.0 gr/lb wt</td>
<td>12</td>
<td>72</td>
<td>86</td>
<td>62</td>
<td>62</td>
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<tr>
<td>Sulfadiazine</td>
<td>Oral</td>
<td>0.5 gr/lb wt</td>
<td>12</td>
<td>43</td>
<td>62</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Sulfamerazine</td>
<td>Oral</td>
<td>0.5 gr/lb wt</td>
<td>6</td>
<td>52</td>
<td>60</td>
<td>48</td>
<td>48</td>
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<tr>
<td>Suljex</td>
<td>Intramammary</td>
<td>77 gr/quarter</td>
<td>24</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Sulfamerazine</td>
<td>Intramammary</td>
<td>80 gr/quarter</td>
<td>16</td>
<td>18</td>
<td>24</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

* None detected during first 24 hr.

+ Determined by chromatography-densitometry (4).

Sulfonamide concentration (ppm) in the milk sample was calculated by the following formula:

\[
\text{Sulfonamide concentration (ppm)} = \left( \frac{\text{Optical density of sample}}{\text{Optical density of standard in ppm}} \right) \times \text{concentration of standard in ppm}
\]

### RESULTS

**Intramuscular administration.** Twelve cows received intramuscular injections of neoprontosil (disodium-4-sulfamido-phenyl-2-azo-7-acetylamino-1-hydroxynaphthalene 3,6-disulfonate) at the rate of 240 gr, divided into three equal doses of 80 gr, over an 8-hr period. As may be noted in Table 1, this sulfonamide was not detected in milk during the first 24 hr post-administration. The red coloration of urine during this period indicated that the drug was rapidly eliminated.

**Intravenous administration.** Sulfabrom (sodium sulfbromomethazine) given at the recommended dosage of 0.25 gr per pound of body weight was detected in the milk produced by 11 of 12 cows at 21 hr post-injection (Table 1). Also, there was a suggestion of traces of the drug in milk of each of these cows at 31-hr post-treatment, although the limit of accuracy of the method did not permit a definite identification of the drug. Milk from one of the 12 cows did not contain a detectable amount of sulfabrom at the 7, 21, or 31-hr post-injection periods.

Suljex, a solution containing 5% sodium sulfamerazine and 5% sodium sulfapyridine, was detected in milk of four cows, Table 1, for a maximum of 38 hr after injection of 772 gr. The duration of secretion in milk was the same for all cows and concentrations of the drug in milk from the four cows at each post-injection milking were quite similar. The ranges in concentrations in ppm by milkings were: (a) 14 hr, 41.6 to 42.9; (b) 24 hr, 11.6 to 16.0; (c) 38 hr, 3.0 to 5.9; and (d) 48 hr, 0 to 0.

Sulfamerazine was secreted in milk up to 54 hr following intravenous doses of 0.5 and 1.0 gr per pound of body weight. Although the maximum duration was the same for both dose levels, the average concentration in milk was lowest for those that received 0.5 gr. At the 54-hr post-treatment milking, the highest concentrations in milk from cows receiving the 1.0 and 0.5 gr doses were 0.8 and 0.5 ppm, respectively.

**Intrauterine infusion.** Two sulfaurea tablets (66 gr of sulfanilamide, and 10 gr of sulfathiazole) administered by intrauterine infusion to 13 cows resulted in milk containing detectable levels of sulfonamides for a maximum of 45 hr post-infusion. The response of individual cows, however, was highly variable. Milk from all cows contained from 4.9 to 25.7 ppm of sulfonamides at the first milking, which was only 5 to 6.5 hr post-infusion for six of the cows and 14 hr for the other seven. The shortest post-infusion milking at which an individual cow produced milk free of detectable level of the sulfonamides was 19 hr.

**Oral administration.** Sulfanilamide administered per os, at the rate of 1 gr per pound of body weight, was detected in milk from each of the 12 cows during the first 62 hr post-treatment, and persisted in the milk of four cows as long as 86 hr (Table 1). Sulfanilamide was not detected in the milk of any of the cows beyond 86 hr.
The sulfadiazine dose was 0.5 gr per pound of body weight. The maximum post-treatment period at which sulfadiazine was detected in milk was 62 hr. Milk from one cow did not contain a detectable level of the drug after the first 24 hr. This cow had an edematous udder, which may have reduced the permeability of the mammary gland to the sulfadiazine.

Sulfamerazine was detected in milk a maximum of 60 hr after oral administration at the level of 0.5 gr per pound of body weight. However, at the 60-hr milking interval, milk from only two of six cows contained the drug in measurable amounts. The post-treatment milking intervals were different for the oral and intravenous administrations of this drug. Charting of the concentration data, however, indicated that oral administration resulted in the highest levels of residue in milk after the first 12 hr.

Intramammary infusions. Suljex containing a total of 77 gr of sulfonamides (38.5 gr of sodium sulfamerazine and 38.5 gr of sodium sulfapyridine) was infused into single quarters of four cows. Milk produced at the 10-hr post-infusion milking did not contain a detectable level of either sulfonamide by the descending paper chromatographic method of Longenecker (2) as modified by Paar (4). Consequently, single quarters of the cows were infused at subsequent dates at 3- to 8-hr pre-milking intervals. The longest post-infusion interval at which the sulfonamides were detected in milk using this procedure was 7 hr (Table 1). The maximum concentration of sodium sulfamerazine in the milk was 4.86 times greater than that of sodium sulfapyridine.

Sulfamerazine given by intramammary infusion as a single drug at the rate of 80 gr was detectable in milk secreted a maximum of 24 hr post-infusion. The photometric method described in the procedure was used for this assay. Only four cows were used in this test, with one quarter infused at each treatment. Over the period of the investigation, however, each quarter was infused once, making a total of 16 quarters tested.

DISCUSSION

The duration of sulfonamide secretion in milk following intravenous injection of 772 gr of combined sulfas (sodium sulfamerazine and sodium sulfapyridine) was similar to that reported by Schipper and Eveleth (5) for the individual drugs. Cows used in the current study averaged 1,250 lb. Hence, the intravenous dosage of the combined drugs averaged 0.62 gr per pound of body weight, as compared to the 0.5 and 1.0 gr given by Schipper and Eveleth.

The 86-hr maximum duration of sulfanilamide in milk following an oral dose of 1 gr per pound of body weight was similar to the duration reported by Miller et al. (3), for cows given repeated doses of the drug. In other studies following administration of sulfanilamide at approximately 1 gr (5, 6) to 2.3 gr (1) per pound of body weight, the maximum period that the drug residue was detected in milk was 48 hr.

Sulfadiazine was secreted in milk up to 62 hr following an oral dosage of 0.5 gr per pound of body weight. In contrast, Schipper and Eveleth (5) reported a maximum duration of only 36 hr for the same dosage of this drug, and Schubarth et al. (6) detected the drug only at 22 hr post-administration of larger doses. It appears that the greater duration of secretion of sulfonamides in milk found for oral administrations in this investigation, as compared with findings of others, may have been the result of a higher degree of sensitivity by the method employed in the current investigations.

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REFERENCES