

PENICILLIN LEVELS IN MILK FOLLOWING PARENTERAL ADMINISTRATION OF PROCAINE PENICILLIN G¹

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Penicillin levels in milk were determined following the intramuscular administration of two types of procaine penicillin G, the aqueous suspension and the oil base with aluminum monostearate. Three injections of the aqueous suspension type were administered, at intervals of 24 hours, to each of twelve cows. A single injection of the oil base type was given to each of twelve cows. In all instances the dose administered was at the approximate level of 5000 u/lb. of body weight.

Higher concentrations of penicillin were found in the milk from cows receiving the aqueous suspension type. The highest levels for the two types were 0.52 and 0.15 units per ml. respectively. However, penicillin persisted longer in the milk from cows to which the oil base type was administered. Following the use of each type of procaine penicillin G the levels of this drug in the milk from the different cows of the same series varied considerably.

The results of this study indicate a withholding period of at least 60 hours following the last intramuscular injection of procaine penicillin G, aqueous suspension and 108 hours after procaine penicillin G, oil base with aluminum stearate.

The intramuscular injection of dairy cows with antibiotics for the treatment of various infections, including mastitis, is a common veterinary practice. However, only a limited amount of information is available relative to the levels of these drugs in the milk following this method of treatment.

Soon after the introduction of penicillin for veterinary use, several investigators (2, 11, 12) reported that this antibiotic did not appear in the milk following intravenous and intramuscular administration. Dosages, in these studies, ranged from 80,000 to 2,681,000 units, the highest being a penicillin preparation in a carrier of beeswax and oil.

Welch *et al.* (13) were the first to show the presence of penicillin in milk following intramuscular injection. They used sodium penicillin and sodium penicillin G at a dosage level of 12,500 u/lb. of body weight. Edwards and Haskins (3) detected penicillin, aureomycin, and streptomycin in the milk of lactating cows after parenteral injection. They used

penicillin at the rate of 11.0 mg/lb. of body weight. Sadek, (10) using crystalline penicillin G and procaine penicillin G, at the rate of 5000 u/lb. of body weight, confirmed the observation of Welch *et al.* and Edwards and Haskins. He pointed out the advantages of treating mastitis of the dairy cow by the intramuscular administration of the antibiotic.

Randall *et al.* (9) administered procaine penicillin G, aqueous suspension and oil base types, at the rate of 5000 u/lb. of body weight. Milk from the cow receiving the aqueous suspension showed measurable amounts of penicillin up to 72 hours as compared with 120 hours for the milk from the cow that was given the oil base procaine penicillin G. Hollister *et al.* (6) administered procaine penicillin G, aqueous suspension to a cow at the rate of 3,000,000 units. Detectable penicillin was present in the milk after 24 but not after 48 hours. Hollister *et al.* (7) used benzathine penicillin V aqueous at the rate of 6,000,000 units per cow administered in a single dose. They found the drug to persist in the milk up to 144 hours.

The Food and Drug Administration (4) on September 22, 1959 certified benzathine penicillin V aqueous for injection of dairy cows. Preparations of this antibiotic are required to carry the following statement: "Warning—Milk taken from cows seven days after the latest treatment must not be used for human consumption." According to McFarland (8) this is the only antibiotic product for injection for which the Food and Drug Administration has issued a specific regulation with respect to withholding the milk following its use.

METHODS

Procaine penicillin G, in aqueous suspension and oil base with aluminum monostearate was used in this study. Three injections of the aqueous suspension were given at 0, 24, and 48 hours. Dosages ranged from 4316 to 5725 u/lb. of body weight and averaged 5176 units. The oil base type was administered in a single dose, ranging from 4667 to 5600 u/lb. of body weight and averaged 5146 units.

A series of twelve cows was employed in the study

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of each type of procaine penicillin G. None was a clinical case of mastitis. The daily production of milk ranged from 9 to 74 pounds. Only one cow produced less than 22 pounds per day.

A sample of milk was collected from each cow before the injection was given to insure that no antibiotic was being excreted in the milk prior to the intramuscular injection. After the injection, samples of milk were collected at every milking period up to and including 108 hours. The samples were held in a frozen state until they were analyzed for penicillin.

The plate-cylinder bio-assay method (5) was employed, using *Sarcina lutea* ATCC strain 9341, as the test organism.

RESULTS AND DISCUSSION

Data showing penicillin levels in the milk from the series of 12 cows that received intramuscular injections of the aqueous suspension type of procaine penicillin G are in Table 1 and Figure 1. The horizontal broken line in figure 1 at the 0.05 level represents the minimum working level of the 2-1/2 hour Food and Drug Administration method as described by Arret and Kirshbaum (1). The broken line at the 0.10 level represents the approximate penicillin concentration at which the activity of lactic dairy starters is retarded.

An average concentration of 0.145 units of penicillin per ml. of milk was observed after 12 hours. This

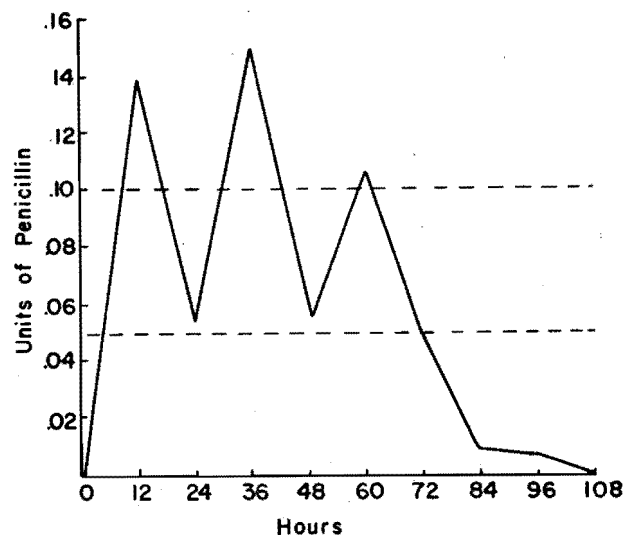


Figure 1. Penicillin levels in milk following the intramuscular injections of procaine penicillin G, aqueous suspension. Three injections of approximately 5000 u/lb. of body weight. Average of 12 cows.

fell to 0.054 units per ml. at 24 hours, at which time the second injection was given. At the 36-hour period a second peak was observed at a concentration of 0.154 units per milliliter. This again fell to 0.056 units per ml. at 48 hours, at which time the third and last injection was given. At the 60-hour period a lower peak was observed at a concentration of 0.113 units per milliliter. This gradually declined to 96 hours and the milk was negative for penicillin at 108 hours.

TABLE 1 — PROCAINE PENICILLIN G, AQUEOUS SUSPENSION, UNITS PER MILLILITER OF MILK

| Cow No. | Units/lb body wt. | Milk lbs/day | Hours | | | | | | | | | |
|---------|----------------------|-----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----|
| | | | 0* | 12 | 24* | 36 | 48* | 60 | 72 | 84 | 96 | 108 |
| 506 | 4316 | 46 | 0 | 0.10 | 0.076 | 0.215 | 0.088 | 0.185 | 0.064 | 0 | 0 | 0 |
| 401 | 5421 | 23 | 0 | 0.14 | 0.084 | 0.255 | 0.032 | 0.135 | 0.045 | 0 | 0 | 0 |
| 2162 | 4873 | 24 | 0 | 0.085 | 0.060 | 0.115 | 0.062 | 0.096 | 0.044 | 0 | 0 | 0 |
| 455 | 4845 | 74 | 0 | 0.52 | 0.04 | 0.086 | 0.047 | 0.08 | 0.044 | 0 | 0 | 0 |
| 451 | 5522 | 64 | 0 | 0.185 | 0.055 | 0.041 | 0.082 | 0.11 | 0.052 | 0.032 | 0.021 | 0 |
| 480 | 5444 | 59 | 0 | 0.245 | 0.062 | 0.142 | 0.041 | 0.087 | 0.032 | 0.021 | 0.016 | 0 |
| 461 | 5106 | 59 | 0 | 0.09 | 0.050 | 0.08 | 0.042 | 0.13 | 0.046 | 0 | 0 | 0 |
| 437 | 4922 | 56 | 0 | 0.042 | 0.042 | 0.067 | 0.031 | 0.095 | 0.029 | 0 | 0 | 0 |
| 490 | 5297 | 53 | 0 | 0.13 | 0.064 | 0.165 | 0.045 | 0.149 | 0.027 | 0 | 0 | 0 |
| 2208 | 5725 | 34 | 0 | 0.073 | 0.048 | 0.330 | 0.073 | 0.048 | 0.039 | 0.021 | 0.024 | 0 |
| 2203 | 5504 | 22 | 0 | 0.048 | 0.031 | 0.145 | 0.033 | 0.066 | 0.036 | 0.022 | 0.018 | 0 |
| 2234 | 5335 | 38 | 0 | 0.079 | 0.043 | 0.207 | 0.10 | 0.18 | 0.155 | 0.043 | 0.018 | 0 |
| Ave. | 5176 | | 0 | 0.145 | 0.054 | 0.154 | 0.056 | 0.113 | 0.051 | 0.012 | 0.008 | 0 |

*Three injections — time of injection.

TABLE 2 — PROCAINE PENICILLIN G IN OIL, UNITS PER MILLILITER OF MILK

| Cow No. | Units/lb. body wt. | Milk lbs/day | Hours | | | | | | | | | | |
|---------|--------------------|--------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----|---|
| | | | 0* | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | 108 | |
| 460 | 5501 | 65 | 0 | 0.017 | 0.012 | 0.006 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2167 | 5497 | 45 | 0 | 0.018 | 0.019 | 0.019 | 0.017 | 0.008 | 0.007 | 0 | 0 | 0 | 0 |
| 380 | 4791 | 58 | 0 | 0.042 | 0.057 | 0.048 | 0.018 | 0.016 | 0.014 | 0 | 0 | 0 | 0 |
| 309 | 4740 | 9 | 0 | 0.15 | 0.05 | 0.013 | 0.014 | 0 | 0 | 0 | 0 | 0 | 0 |
| 459 | 4734 | | 0 | 0.085 | 0.033 | 0.032 | 0.030 | 0.028 | 0.026 | 0.021 | 0.021 | 0 | 0 |
| 403 | 4667 | 59 | 0 | 0.027 | 0.045 | 0.020 | 0.026 | 0.021 | 0.018 | 0 | 0 | 0 | 0 |
| 429 | 5471 | 63 | 0 | 0.036 | 0.039 | 0.029 | 0.023 | 0.021 | 0.017 | 0 | 0 | 0 | 0 |
| 459 | 5083 | 52 | 0 | 0.046 | 0.052 | 0.031 | 0.029 | 0.033 | 0.021 | 0 | 0 | 0 | 0 |
| 463 | 5201 | 63 | 0 | 0.045 | 0.036 | 0.028 | 0.020 | 0.018 | 0.017 | 0 | 0 | 0 | 0 |
| 514 | 5429 | 48 | 0 | 0.039 | 0.031 | 0.035 | 0.035 | 0.041 | 0.026 | 0.023 | 0.021 | 0 | 0 |
| 440 | 5600 | 56 | 0 | 0.035 | 0.025 | 0.035 | 0.029 | 0.035 | 0.028 | 0.029 | 0.026 | 0 | 0 |
| 2168 | 4922 | 40 | 0 | 0.033 | 0.026 | 0.018 | 0.023 | 0.025 | 0.023 | 0.022 | 0.022 | 0 | 0 |
| Ave. | 5136 | | 0 | 0.048 | 0.035 | 0.026 | 0.022 | 0.020 | 0.016 | 0.008 | 0.007 | 0 | 0 |

*Single injection

The response of the individual cows to the administration of penicillin, as shown by the concentration of this drug in the milk, varied considerably. The milk from 7 of 12 cows of this series was free from penicillin 36 hours after the last injection, whereas the milk from the 5 remaining cows required 60 hours to reach this stage. On an average basis the antibiotic could be detected in the milk 48 hours after the last injection.

Maximum concentrations of penicillin occurred in the milk from the cows of this series as follows: three, after the first injection; seven, after the second injection; and two, after the third injection.

The highest concentration of penicillin, 0.52 units per milliliter, occurred in the milk from cow 455, 12 hours after the initial injection. This was much higher than observed in the milk from any other animal. Penicillin concentrations that followed the second and third injections were relatively low, 0.086 and 0.08 units respectively.

The next higher concentration of penicillin, 0.33 units per milliliter, occurred in the milk from cow 2208 following the second injection. The penicillin curve of the milk from this animal declined rather than increased following the third injection.

The penicillin concentrations of the milk from cows 2162, 461 and 437 were relatively low throughout the period of observation. Maximum concentrations observed were 0.115, 0.013 and 0.095 units per milliliter respectively.

The Food and Drug Administration method of Arret and Kirschbaum (1) would have detected penicillin in the milk from the individual cows of this series most of the time during the period of observation. However, with only a few cows undergoing treatment within a herd, the influence of dilution would greatly increase the probability of negative tests on composite samples, such as farm tank samples.

To meet a zero tolerance for penicillin the results of this study indicate a withholding period of at least 60 hours following the last treatment when procaine penicillin G, aqueous suspension is administered intramuscularly at the level of 5000 u/lb. of body weight.

Data in Table 2 and Figure 2 show penicillin levels in the milk from the 12 cows that received the oil base type of procaine penicillin G.

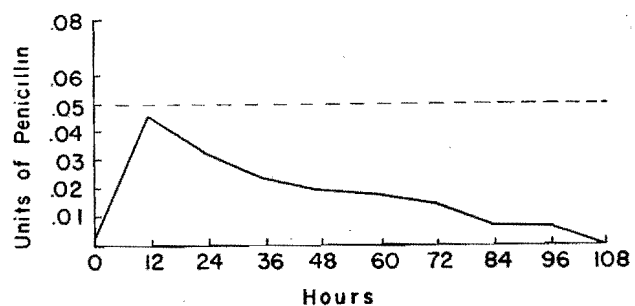


Figure 2. Penicillin levels in milk following the intramuscular injection of procaine penicillin G in oil with aluminum monostearate. Single injection of approximately 5000 u/lb. of body weight. Average of 12 cows.

The highest average level of 0.048 units of penicillin per ml. of milk was observed twelve hours after the injection of the antibiotic. This concentration gradually declined and penicillin was not detectable at the 108-hour period. The cows varied in their response to the drug. However, most penicillin levels of the milk were of the same general pattern, with a maximum concentration in 12 hours followed by a steady decline up to 84 to 96 hours. Variations in concentrations and duration of time that penicillin was present occurred in the milk from the respective cows of this series.

The penicillin concentration of the milk from cow 460 was very low at all times, and it persisted in the milk for only 36 hours. This cow was the heaviest producing cow of the series, with a daily average of 65 pounds during the observation period. Cows 429 and 463 produced almost as much milk, 63 pounds each. The milk from these animals, however, retained measurable amounts of penicillin for a period of 72 hours.

Cow 459 served as a test animal twice in this series, immediately after freshening and again 4 weeks later. Penicillin persisted in the milk of the first trial up to 96 hours as compared with 72 hours for the second trial.

The greatest concentration of penicillin was found in the milk from cow 309. However, measurable amounts of penicillin were not present after 48 hours. The milk production level of this cow was low, only ten pounds per day.

The penicillin concentration of the milk from cows 514, 440 and 2168 remained at rather constant levels up to 96 hours after the injection. Then it dropped to negative values.

Only 4 of 12 cows of this series produced milk in which the penicillin content was high enough to be detected by use of the F.D.A. 2-1/2 hour test. However, in order to meet a zero tolerance for penicillin in the milk from individual cows the results of this study indicate a withholding period of at least 108 hours following the intramuscular injection of procaine penicillin G, oil base with aluminum monostearate.

Figure 3, constructed from the data of Hollister *et al.* (7) illustrates the average penicillin curve in the milk from a series of cows, each of which was given a single intramuscular injection of benzathine penicillin V at the rate of 6,000,000 units. Average penicillin levels at all observation periods were too low for detection with the Food and Drug Administration 2-1/2 hour method (1). This is the penicillin preparation for which the Food and Drug Administration (4) has designated a 7-day withholding period

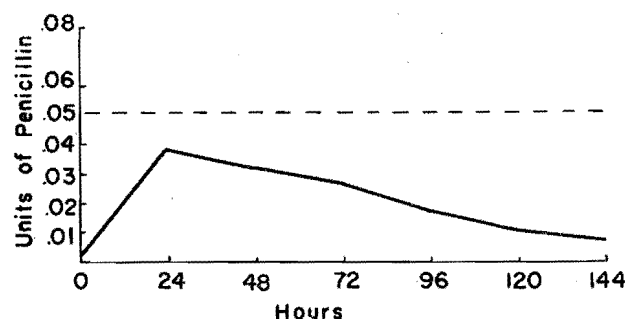


Figure 3. Penicillin levels in milk following the intramuscular injection of benzathine penicillin V. Single injection of 6,000,000 u/cow. Average of 6 cows. From data of Hollister *et al.* (7).

of the milk following the intramuscular injection of the antibiotic.

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