Persistence of Lactobacilli and Streptococci in the Bovine Rumen During Penicillin Administration[1]

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At the 1957 Conference on Rumen Function, Barrentine *et al.* (1958) and Emery *et al.* (1958) presented reports supporting the use of penicillin for the control of bloat in cattle. At the same conference, Johnson *et al.* (1958) and Smith and Emery (1958) reported that the administration of 50 to 100 mg of penicillin daily reduced the incidence of pasture bloat, but this control was not effective after 7 to 9 days of treatment. Johns (1958) found that, after penicillin had been given every 2 days for some 12 doses, the inhibiting effect on rumen fermentation disappeared. Little information is available to explain the mode of action of this prophylactic measure or the disappearance of the inhibiting effect after continued administration. Smith and Emery (1958) detected a "penicillin destroying principle" in Seitz-filtered rumen fluid of penicillin-treated cattle after 1 week of treatment.

The present study is an attempt to determine the effect of penicillin upon the lactobacilli, streptococci, and coliform bacteria in the rumens of fistulated steers grazed on Ladino clover. The first two of the bacterial groups were selected for their possible role in the bloat process, in view of the report of Perry and Briggs (1957) that the "lactobacilli constitute a major component of the bovine ruminal microflora . . . " and that of Dain *et al.* (1956) that *Streptococcus bovis* may be important in the production of the slime often associated with bloat.

The coliform bacteria that were isolated were assayed for their possible role in the destruction of penicillin since Harper (1943) has reported that sterile filtrates of coliform bacteria were capable of penicillin inactivation. Bondi and Dietz (1944) designated the penicillin destroying agent produced by paracolon bacilli as penicillinase.

Experimental Methods

From September 29, 1958 through November 21, 1958, three fistulated steers (No. 50, 22, and 42) were grazed twice daily on a Ladino clover pasture at the University of Kentucky Agricultural Experiment Station farm. At intervals of 7 days during the first month of study and at intervals of 1 to 2 days during the period of penicillin administration, samples of rumen contents were obtained via the fistula for bacteriological analysis. A Colorado tube placed in several sites of the rumen, with the aid of slight vacuum, was employed to obtain the samples. Except for the first three sampling periods, collections were made in the morning prior to placing the animals on pasture.

After sampling on October 7, steer 50 was given 3 oz of penicillin-sodium chloride mixture,[2] which contained 0.18 per cent or 150 mg of penicillin daily, through November 5, 1958. Steer 22 was given 1 oz of the penicillin-salt mixture (50 mg of penicillin) daily during this period. Steer 50 was given four additional daily doses of 250,000 units each of crystalline penicillin-G (potassium) between November 6 and November 9. All administrations were made through the fistula immediately after sampling. Steer 42 served as an untreated control.

Samples for bacteriological analysis were strained through cheesecloth, and a 1-ml aliquot of each strained sample was decimally diluted in sterile tap water. Enumeration and identification of the coliform bacteria were made by the screening procedure of Wiseman and Sarles (1956). Numbers of lactobacilli were determined by plating selected dilutions of the samples in LBS agar, a dehydrated product prepared by the Baltimore Biological Laboratories according to the formula of Rogosa *et al.* (1951). The rumen streptococci were counted by plating in the azide agar of Harrison and Hansen (1950). Frequent Gram stains were made of colonies in the azide plates to check the selectivity of the medium. Plates containing LBS and azide agar were incubated from 2 to 4 days at 37 C to insure the counting of "pin-point" colonies. Coliform bacteria were counted after 2 days' incubation at 37 C.

After examination of the plates that were made during the final period of sampling, isolates from the azide medium were subcultured into a similar medium without the azide and agar. Lactobacilli isolates were subcultured in a modified LBS broth (Rogosa *et al.*, 1953). All coliform cultures were maintained in nutrient broth. Penicillin resistance was determined by culturing the isolates in subculture-broth containing 1, 10, 50, and 100 μg of penicillin G per ml. Penicillin inactivation was determined *in vitro* by the addition of one drop of a mixture of ruminal paracolon bacteria to

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[2] Supplied by the Morton Salt Co., Chicago, Illinois.

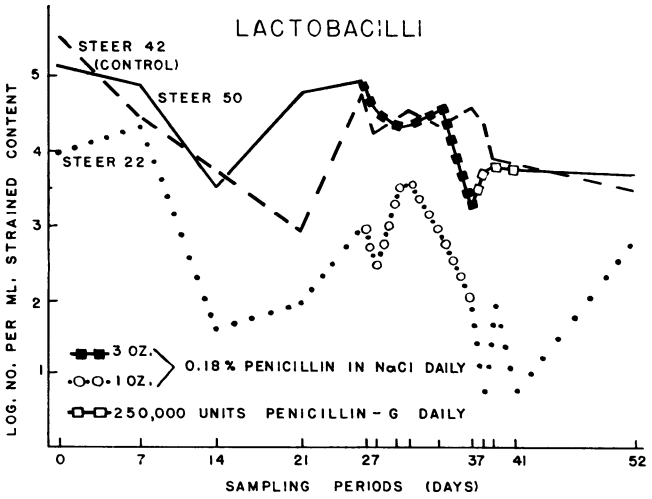

Figure 1. Effect of daily penicillin administration upon numbers of lactobacilli in the rumens of fistulated steers grazed on Ladino clover.

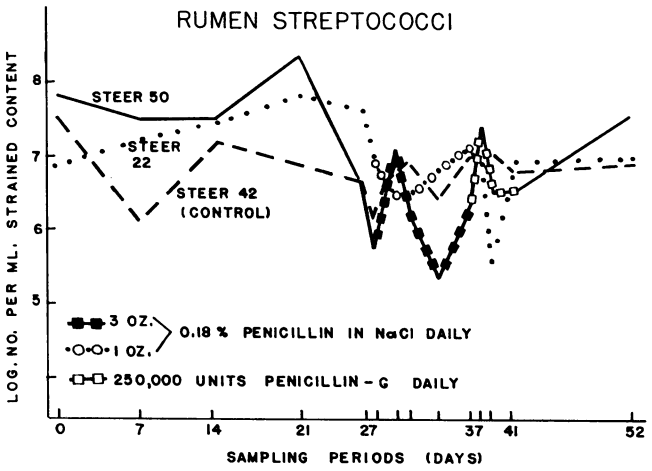

Figure 2. Effect of daily penicillin administration upon numbers of rumen streptococci in the rumens of fistulated steers grazed on Ladino clover.

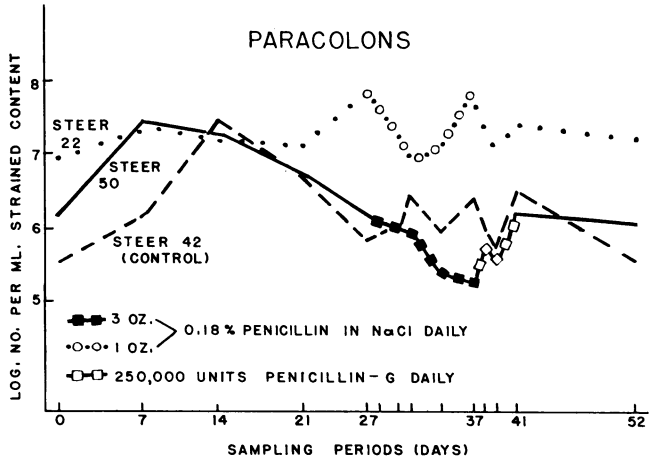

Figure 3. Effect of daily penicillin administration upon numbers of paracolon bacteria in the rumens of fistulated steers grazed on Ladino clover.

each tube of streptococcal subculture broth containing penicillin, before incubation with the streptococci isolates. Survival of the streptococci in penicillin-containing broth was determined by Gram stain and by subculturing into azide broth. Penicillin inactivation by filtrates of cultures of the ruminal paracolon bacteria was determined by the method of Harper (1943). The results are shown in graphic form in figures 1, 2, and 3.

Results

The daily administration of 50 mg and 150 mg of penicillin to Ladino-fed fistulated steers produced no persistent changes in the numbers of ruminal lactobacilli, streptococci, or coliform bacteria during a 2-week period of daily treatment. The numbers of lactobacilli in the rumen of steer 50 which received 150 mg of penicillin daily paralleled the count of lactobacilli from the rumen of the control steer. Except for the first two periods of study, steer 22 consistently had a low lactobacilli count (100 to 1000 per ml of content); yet treatment with 50 mg of penicillin daily had no apparent effect upon these numbers. The administration of penicillin did not markedly alter the numbers of ruminal streptococci in the test steers. The apparent decrease and subsequent increase in numbers of streptococci in the rumen of steer 50 during the first 10 days of penicillin treatment suggest the development of penicillin-resistant strains.

Penicillin resistance studies with 10 streptococcal isolates taken from azide medium, prepared from rumen contents from each of the three animals on the 37th day, revealed that all isolates were sensitive to 1 μg per ml of penicillin. Assays with the isolates of *Lactobacillus* revealed that this bacterial group was also sensitive to 1 μg of penicillin per ml of culture medium. These results indicate that the persistence of numbers of streptococci and lactobacilli in rumen of penicillin-treated steers was not the result of the development of resistant strains. This finding is in contrast to earlier findings (Wiseman, 1956) in which penicillin-resistant strains of *Lactobacillus* developed in the intestinal tract of chickens fed dietary penicillin.

Figure 3 shows a constancy in the numbers of ruminal coliform bacteria (*ca* 1,000,000 per ml of content). The nonlactose-fermenting paracolons (primarily *Paracolobactrum coliforme* and *P. aerogenoides*) were the predominating coliform bacteria. This confirms the findings of other studies in this laboratory, using cattle fed on diets of clover, hay, and grain. Steer 22 had a relatively higher paracolon count during the last 5 weeks of study and had its lowest *Lactobacillus* count during this same period, suggesting that a balance may exist between these two groups of bacteria.

Experiments with paracolon bacteria recovered from

the rumen fluid from the two test steers and from the steer which had not received penicillin showed that all of the paracolon isolates were capable of growing in nutrient broth containing 100 μg of penicillin per ml. The "penicillin inactivation" trials revealed that the addition of rumen paracolon bacteria to broth containing 100 μg of penicillin per ml would permit the growth of enterococcal strains which were shown to be sensitive in pure culture to 1 μg of penicillin per ml.

Discussion

The *in vitro* destruction of penicillin by rumen paracolon bacteria suggests that this group of organisms may have inactivated the penicillin which had been administered to the steers in this study. This inactivation could then be the factor responsible for the persistence of the penicillin-sensitive lactobacilli and rumen streptococci. It is possible that the "penicillin-destroying principle" present in Seitz-filtered rumen content as reported by Smith and Emery (1958) is penicillinase produced *in vivo* by rumen paracolon bacteria. These authors indicated that in cattle receiving 100 mg of penicillin daily for 3 weeks, the antibiotic "was not detectable in most cases at 3 hr after ingestion."

The constancy of numbers of lactobacilli, streptococci, and paracolon bacteria in the rumen of fistulated steers fed Ladino clover suggests that a microbial balance exists in the rumen of cattle as it does in the intestinal tract of other species. In the bovine rumen this balance is apparently well stabilized, even the administration of penicillin failed to alter the equilibrium. This is in contrast to the findings of Wiseman *et al.* (1956) in studies of the effect of dietary antibiotics upon the intestinal flora of chickens. They showed that the balance between the numbers of intestinal lactobacilli and the numbers of intestinal coliform bacteria could be altered by feeding low levels of a penicillin-bacitracin mixture. The reduction in numbers of lactobacilli was followed by an immediate increase in the numbers of lactose-fermenting coliform bacteria. Since paracolon bacteria are the predominate coliform group in the bovine rumen and the lactose-fermenting bacteria of this group often predominates in the intestines of chickens, the penicillin inactivation which occurs in the rumen may not occur in the intestinal tract of chickens. The greater potential for penicillin inactivation by paracolon bacteria was reported by Harper (1943) who noted that the Seitz filtrate of a paracolon bacillus destroyed penicillin, whereas similar filtrates of lactose-fermenting coliform bacteria "showed no such complete destruction." Bondi and Dietz (1944) reported that phenolized cultures of paracolon bacteria diluted 1:128 were still capable of inactivating penicillin, whereas similar inactivation by *Escherichia coli* did not occur beyond a dilution of 1:32.

That a balance exists between groups of microorganisms in the rumen has also been reported by Briggs (1956), who states " . . ., we have found a surprising constancy of results . . ., the alteration in the numbers of bacteria being quite small even with marked changes in diet." Several reported attempts to alter the balance by the addition of bacteria foreign to the rumen have been unsuccessful. Hobson *et al.* (1958) were unable to establish in the rumen of a sheep a gram-negative coccus isolated from a calf. In our laboratory, attempts to establish in the bovine rumen a slime-producing *Aerobacter* isolated from Ladino clover were not successful (Wiseman *et al.*, 1959).

Since penicillin is an effective temporary control for bloat in Ladino-fed cattle, the results of this study suggest that preceding or during bloating some alteration may occur among the ruminal microflora which permits the antibiotic to remain active. Our findings suggest that the numbers of penicillinase-producing bacteria may be reduced during the early stages of bloat, thus permitting the antibiotic to be active against microorganisms which are important to the bloat process. An increase in numbers of penicillinase-producing ruminal flora after about 1 week of treatment could then be responsible for the loss of antibiotic activity. Since bloating did not occur in the steers used during this experiment, these postulates could not be verified.

It should be emphasized that this experiment was designed in line with the recommended practice of using 50 to 100 mg of penicillin per day for the control of bloat in cattle. Although a level of approximately twice the therapeutic dose was used in this trial, it is not suggested that higher levels would not markedly alter the numbers or penicillin resistance of bacteria in the rumen. It is estimated that with the administration of 150 mg of penicillin to steer 50 the final concentration of penicillin that remained after the complete admixture of the antibiotic with the rumen content was at least 2 μg per ml. This level (if active) should have been effective in reducing the numbers of ruminal lactobacilli and streptococci, since isolates of these bacterial groups were shown to be sensitive to 1 μg of penicillin per ml of culture medium. It is assumed that significant changes in numbers or penicillin resistance of these bacterial groups did not occur during the 24-hr period between administration of the antibiotic and sampling. It is possible that, if an effective level of penicillin had not been reached in the rumen, the "natural" resistance of lactobacilli and rumen streptococci could have accounted for their persistence. Fitzgerald and Jordan (1953) noted that some of their strains of oral lactobacilli were not capable of growing in a medium containing more than 0.2 units of sodium penicillin G per ml, whereas other strains resisted a concentration of 2 units per ml. White and Sherman (1944) reported that most of the enterococcal species

grew quantitatively in a selective medium which contained 0.325 units of penicillin per ml, but that *Streptococcus durans* was partially inhibited by this level of antibiotic.

Summary

Two Ladino-fed fistulated steers were given daily doses of penicillin (50 and 150 mg per dose) for a period of about 2 weeks. Bacteriological analyses revealed that no pronounced alteration in numbers of rumen lactobacilli, streptococci, or paracolon bacteria occurred during the period of penicillin administration. This apparent lack of penicillin activity was not correlated with the appearance of penicillin-resistant lactobacilli or streptococci. The ability of the paracolon bacteria to inactivate relatively high concentrations of penicillin *in vitro* suggests that these bacteria may be responsible for the inactivation of the penicillin in the rumen.

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